Dissertation zur Erlangung des Doktorgrades der
Naturwissenschaften an der Fakultät für Biologie der
Ludwig-Maximilians-Universität München

ANT/PLANT SYMBIOSES: EVOLUTION, SPECIALIZATION, AND BREAKDOWN

Guillaume Chomicki

Munich, 4 July 2016

| Preface |

Statutory declaration

Erklärung

Diese Dissertation wurde im Sinne von §12 der Promotionsordnung von Prof. Dr. Susanne S. Renner betreut. Ich erkläre hiermit, dass die Dissertation nicht einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe.

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt wurde.

Guillaume Chomicki, 4 July 2016
(Unterschrift)

1. Gutachter: Prof. Dr. Susanne S. Renner
2. Gutachter: Prof. Dr. Dirk Metzler

Datum der mündlichen Prüfung: Mittwoch, den 12. Oktober 2016

| Table of contents |

Note

In this thesis, I present the results from my doctoral research, carried out in Munich from July 2013 to July 2016 under the supervision of Prof. Dr. Susanne S. Renner. My thesis resulted in eight manuscripts, presented in Chapters 1 to 8, of which four have been published (Chapters 1, 2, 3 and 4), one is in accepted pending revisions (Chapter 5), and three are still unpublished. I also gave the conference talks listed below. I generated all data and conducted all analyses myself, except for the DNA matrix of *Pseudomyrmex* (part of Chapter 2), which was built by Prof. Dr. Philip S. Ward (University of California, Davis), the CT scanning of floral buds (part of Chapter 4), which was done in collaboration with Dr. Yannick M. Staedler (University of Vienna), the metabolomics measurements, which were done by Dr. Martin Lehmann in the lab of Prof. Dr. Peter Geigenberger (Ludwig-Maximilians-University of Munich), and the probabilistic modeling (part of Chapter 7), which was done by Prof. Dr. Dirk Metzler (Ludwig-Maximilians-University of Munich), in a collaborative framework. Writing and discussion involved collaboration with Prof. Dr. Susanne S. Renner.

Guillaume Chomicki	Prof. Dr. Susanne S. Renner
(Signature)	(Signature)

List of publications

Peer-reviewed articles

Chomicki G., Renner S.S. (2015). Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. ***New Phytologist*** 207: 411-424.

Chomicki G., Ward P.S., Renner S.S. (2015). Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. ***Proceedings of the Royal Society of London B: Biological Sciences*** 282: 20152200.

Chomicki G., Renner S.S. (2016). Evolutionary relationships and history of the ant-epiphytic genus *Squamalleria* (Rubiaceae: Psychotrieae) and their taxonomic implications. ***PLoS ONE*** 11: e0151317.

Chomicki G., Staedler Y., Schönenberger J., Renner S.S. (2016). Partner choice through concealed floral sugar rewards evolved with the specialization of ant/plant mutualisms. ***New Phytologist*** (DOI: 10.1111/nph.13990). Published online 9 May 2016

Chomicki G., Renner S.S. Active and exclusive planting and fertilization by ants of their hosts' offspring. ***Nature Plants*** (Submitted 21 May 2016, accepted pending revisions, 21 June 2016).

Unpublished manuscripts

Chomicki G., Renner S.S. Altitude drives mutualism breakdown, leading to accelerated morphological evolution.

Chomicki G., Metzler D., Renner S.S. Domatium 3D structure maximizes benefits in facultative and obligate nutritional mutualisms.

Chomicki G., Janda M., Renner S.S. The assembly of Australasian ant gardens: specialization via host broadening. Invited manuscript in ***Proceedings of the Royal Society of London B: Biological Sciences*** special feature on 'Ants in their biotic environment'.

Oral presentations

2016 **Chomicki G.** *Specialization and loss of mutualism between the Hydnophytinae and ants*. Talk at the “Ants 2016” conference, May 5-8th, Munich.

2016 **Chomicki G.** *Regulation of the plant microtubule cortical array*. Invited seminar Lecture, Systematic Botany and Mycology, University of Munich, November.

2015 **Chomicki G.** *Evolution and maintenance of symbioses between ants and plants*. Invited seminar Lecture, School of Biological Sciences, University of Sydney, December.

2015 **Chomicki G.** *Evolution and maintenance of symbioses between ants and Epiphytic Rubiaceae*. Invited seminar Lecture, Systematic Botany and Mycology, University of Munich, November.

2015 **Chomicki G.** *Mutualism evolution, maintenance and dissolution in ant/plant symbioses.* Contributed talk at the Systematics Association Biennial 2015 meeting, 26-29 August, Oxford.

2015 **Chomicki G.** *The evolution and functional specialization in ant-plant symbioses*. Invited lecture at the Natural History Museum, Paris and CNRS, UMR ISYEB. February 3rd.

2014 **Chomicki G.** *Phylogenetic distribution and specialization in ant/plant symbioses*. Invited seminar lecture, University of Vienna, December.

2014 **Chomicki G.** *The evolution of plant ants and ant plants*. Invited seminar Lecture, Systematic Botany and Mycology, University of Munich, October.

2014 **Chomicki G.** *Root photoprotection in epiphytic orchids: a new function for the velamen*. Invited seminar Lecture, Systematic Botany and Mycology, Munich, May 2014.

Posters

2015 **Chomicki G.** and Renner S. (Jan 2015). *Biogeographic patterns underlying the specialization or loss of symbiotic association between ant and plants*. Poster presented at the 7the biennial conference of the International society of biogeography, Bayreuth, Germany.

2014 **Chomicki G.** and Renner S. *The staggered evolution of ant-plant symbioses in the World’s intertropical regions during the last 15 million years*. Poster presented at the Radiation meeting, Zurich, Switzerland, June.

Herbaria visited

- Munich herbarium (M), Germany, 2013-2016.
- Sydney Herbarium (NSW), Australia, December 2015.
- Paris Herbarium (P), France, February 2015.
- Dublin Herbarium (DUB), Ireland, April 2015.
- Leiden Herbarium (L), Netherlands, November 2014.
- Oxford Herbarium (OXF and FHO), England, October 2013.
- British Museum Herbarium (BM), England, October 2013.
- Kew Herbarium (K), October, England, 2013.

Fieldwork

- Fiji: Viti Levu, Vanua Levu and Taveuni, April 2015
- Fiji: Viti Levu, Vanua Levu and Taveuni, March/April 2014

Funding

The studies presented here were financially supported by the following organizations:

- German Research Foundation (DFG), grant RE 603/20.
- Graduate student research award, Society of Systematic Biologists.
- Graduate Research Award, American Association of Plant Taxonomy.

| Summary |

This doctoral thesis focuses on the evolution of ant/plant symbioses, a conspicuous form of mutualism involving some 113 species of ants and 684 species of vascular plants and occurring throughout the World's tropical zones. My thesis addresses the following questions: (*i*) When, how often, and where did ant-plant symbioses evolve? (*ii*) By which steps did ant/plant symbioses evolve and which biotic or abiotic traits have favored them? (*iii*) How do ant/plant symbioses negotiate the tradeoff between specialization and stabilization? (*iv*) How often and under which conditions do ant/plant symbioses break down? (*v*) Are obligate epiphytic ant/plant symbioses dispersed by their ant symbionts? And (*vi*) how do related species of facultative and obligate ant-plants maximize benefits from the mutualism? To address these questions, I chose a clade of Australasian Rubiaceae that includes species with facultative, obligate or no ant symbioses and inferred its species relationships and geographic history, the precondition for studying the evolution of species' interactions with ants.

To answer question (*i*), I performed a literature survey of ant-plants and used capture-release models to estimate the expected number of ant-plants worldwide. I found that Australasia contains about 289 ant-plants, making it equally rich in ant-plants as the Neotropics (Chapter 1). Using a 1,140 species tree with ant-plants and their non-ant-plant relatives, I estimated a minimum of 158 origins of ant domatia in vascular plants (Chapter 1). I then employed molecular clock-dated phylogenies for 56% of the World's known ant/plant lineages and found that the extant ant/plant symbioses in the Neotropics and Australasia date back to the Middle Miocene, while those in Africa only date back to 5-10 million years (Chapter 1).

To answer question (*ii*), I used a phylogenetic framework for the ant genus with the largest number of obligate plant-ants (*Pseudomyrmex*) as well as phylogenies for its main plant host lineages (Chapter 2). I showed that host and symbiont broadening, meaning one partner increases the number of partners with which it interacts, is a dominant process in the evolution of ant/plant symbioses, even in the most specialized lineages such as the Central American ant/acacia mutualism

(Chapter 2). Such increased host use led to the recruitment of new ant-plant lineages by plant-nesting ants; symbiont broadening in some instances appears to have resulted in complete partner replacement (Chapter 2). Another empirical finding is that parasites (i.e., ant species benefitting from plant rewards without reciprocating) originated from free-living generalists ant species, not from mutualists evolving into cheaters as predicted by theory. Host broadening apparently also was frequent in Australasian ant-gardens and seems to have favored the evolution of domatia once plants regularly 'find themselves' in ant-gardens (Chapter 8).

Before going to the field in Fiji, I examined the relevant collections of Australasian in several herbaria (OXF, FHO, SUVA, DUB, K, L, M, BM, P), in addition to online databases and photos from other herbaria (in particular A, GH, FI, US, BISH). I discovered three new species in Fiji, resulting in now nine species of *Squamellaria* in the archipelago. By generating DNA sequences from relevant type material, I enlarged the (natural, monophyletic) genus *Squamellaria* from three species in the last revision (Jebb, 1991) to twelve species (Chapter 3). This taxonomic framework was essential to address all subsequent questions.

To answer question (*iii*), I performed experiments and observations during eight weeks of fieldwork in September-October 2014 and March 2015 on all nine species of *Squamellaria*. By using DNA and morphological traits from herbarium material, I was able to place the *Squamellaria* data into a much larger comparative evolutionary framework (Chapter 4). Mutualism specialization requires more investment from each partner to increase levels of rewarding and partner fidelity, which increases the exploitation potential by opportunists. I showed that obligate ant-plants negotiated this tradeoff by evolving exclusive food rewards that can only be accessed by the obligate ant mutualist (Chapter 4).

To answer question (*iv*), I generated a phylogeny for my focal clade that includes 76 of its 102 species, including several that I discovered during my fieldwork (above). Using this phylogeny and ancestral state reconstructions, I inferred ten losses of facultative symbiosis with ants, making this system well suited to study the ecological context of mutualism breakdown. In Hydnophytinae, mutualism breakdown has been driven by shifts to montane habitats (>1500 m alt.) where ants are scarce (Chapter 6). The evolution of a key mutualistic trait – entrance hole size – tightly tracked mutualistic strategies, with obligate ant-plants undergoing little evolutionary change in hole diameter, while species that lost mutualisms were free to rapidly change this trait. This indicates that mutualistic strategies, by determining the level of stabilizing selection, drive morphological evolution in mutualism-associated traits (Chapter 6; see also Discussion).

To answer question (*v*), I used Fijian *Squamellaria* to study how facultative versus obligate ant-plants are dispersed, again relying on my own field observations and experiments. Facultative ant-plants are bird-dispersed, but obligate ant-plants are dispersed by their ant symbiont, the Dolichoderinae species *Philidris nagasau* (Chapter 5). Obligate ant-plant species of *Squamellaria* and *P. nagasau* ants engage in a type of ant-plant mutualism that is new to science, wherein the ants farm their hosts, planting the seeds inside tree bark of preferred host tree species and fertilizing the seedlings by defecating in their tiny domatia (before these are large enough to house any ant nest) (Chapter 5).

To answer question (*vi*), I again used the Fijian *Squamellaria* system and designed experiments with stable isotopes (^{15}N) to determine how ants fertilize hosts and how nitrogen uptake differs between facultative and obligate hosts. I also used Computed-Tomography Scanning to build 3D models of ant domatia. The domatia of *Squamellaria* attain rugby ball to pumpkin size, and their inner structure was essentially unknown. I discovered that in the obligate symbiosis, there is a single large cavity with small (ca. 2-3 mm in diameter) hyper-absorptive structures, termed 'warts', that are recognized by *P. nagasau* ants, which exclusively defecate on them, thus maximizing nitrogen benefits to the plants (Chapter 7). By contrast, facultative hosts have several unlinked cavities and lack absorptive warts. Because there is a high ant nest turnover in the facultative ant-plant species of *Squamellaria*, the modular domatium limits competition between inhabitants and maximizes the time any individual plant spends with nitrogen-providing ants (Chapter 7).

| General introduction |

General introduction

“*The history of evolution and biodiversity is fundamentally a history of the evolution of species interactions. Species in pure isolation simply do not make sense*”
John N. Thompson (1999)

Species interactions have shaped the history of life. A number of major events in this history are the direct consequences of new species interactions (Margulis and Fester, 1991; Szathmáry and Maynard Smith, 1995). Mitochondria paved the way to the origin of the eukaryotic cell, chloroplasts to that of Glaucophytes, Chlorophytes and red algae, symbiosis with dinoflagellates led to coral reefs, and mycorrhiza were the basis for the conquest of land by plants. Species interactions can be mutualistic if they are beneficial to both partners (+/+), parasitic if they benefit one partner but harm the other (-/+), competitive if each partner has a negative effect on the other (-/-), commensal if one partner benefits from the other who in turn is not affected by the interaction (+/0), amensal if one partner harms the other but is not affected by the interaction (-/0), or neutral if the interaction has no effect on either partner (0/0). Whether the latter can still be considered an interaction is unclear. At least in theory, the net effect of these interactions can grade into one another (Haskell, 1949; Fig. 1). In many interactions, the net effect of the interaction can also change over time and space (Herre et al., 1999; Hojo et al., 2015). This is especially true for mutualisms, which show strong context-dependency in their net outcomes (Chamberlain et al., 2014). Nevertheless, a number of obligate mutualisms have been maintained over many million years, such as the *Buchnera*/aphid nutritional mutualism that may be 200 million years old (Clark et al., 2000; Hosokawa et al., 2006; Jousselin et al., 2009). This suggests that despite fluctuation of benefits, (+/+) interactions can be maintained over long time periods.

Mutualistic interactions may represent up to half of all species interactions occurring in a community (Stone and Roberts, 1991). They play an essential role in ecosystem functioning, with for instance 80% of all nitrogen acquired by plants in boreal and temperate forests from mycorrhizal fungi and nitrogen-fixing bacteria and up to 75% of phosphorus across biomes (van der Heijden et al., 2008), and with some 92% of all angiosperms being animal-pollinated (Renner, 2006). Yet, mutualism only became a popular focus of research in the 1980s, with the appearance of several seminal publications (Boucher et al., 1982; Thompson, 1982; Futuyma and Slatkin, 1983; Harley and Smith, 1983; Boucher, 1985).

In the *Origin of Species*, Darwin (1859: 126) considered that “Natural selection cannot possibly produce any modification in any one species for the good of another species (…). If it could be proved that any part of the structure in any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection”

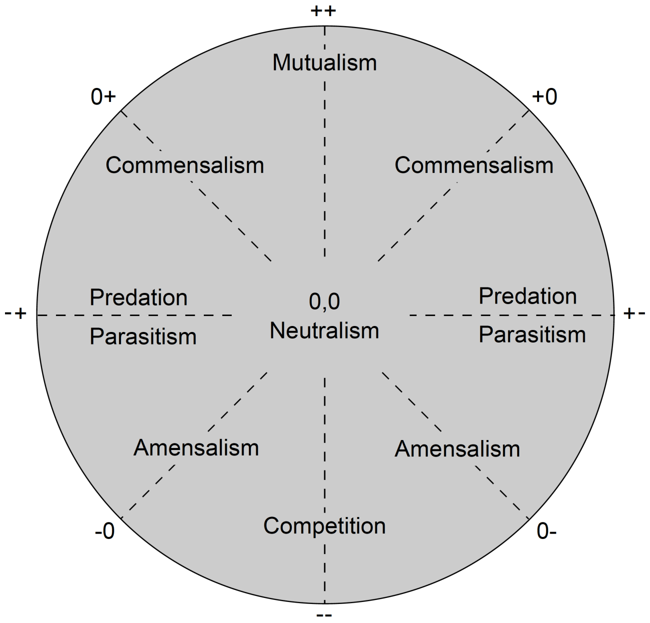


Figure 1. The coaction compass. Initially proposed by Haskell (1949), it highlights how species interactions can grade into one another. Each interaction is denoted by its net effect (positive, neutral, or negative) on each of the two partners. Moving along the radius of the circle changes the intensity but not the sign of the interaction, while moving around the circumference shifts the direction of the net effects for one or both species. Adapted from Bronstein (2015).

Theory predicts that natural selection should favor cheaters that reap the benefits of cooperation without its costs, thus implying shifts to parasitism (Trivers, 1971; Axelrod and Hamilton, 1981). One important source of conflict between partners is reproduction. When the reproduction of one partner is unlinked to that of the other, investment in reproduction reduces selection for mutualistic traits. This is especially evident in ant/plant symbioses where plant investment in flowering and fruiting reduces resource allocation to domatia and food rewards, and led to the evolution of castration behavior (the destruction of inflorescences by ant workers) in both the New and Old World tropics (Stanton et al., 1999; Frederickson, 2009).

This introduction sets the background for the following chapters. First, I will define the concepts of mutualism, symbiosis, coevolution and cospeciation. Then, I develop the conceptual framework around mutualism evolution, stability, breakdown and specialization, which will be used in subsequent chapters. This will be followed by a brief overview of the diversity of mutualisms in which ants engage, and I then end on a presentation of the main biological 'systems' used in my research.

Definitions and concepts: mutualism, symbiosis, coevolution and cospeciation

Before delving into the specifics, I will briefly define some important terms. This is necessary because there is a lot of terminological confusion present in the literature. As noted above, mutualism refers to a (+/+) species interaction, and was first coined by Pierre van Beneden in 1873 to refer to "mutual aid among species." Six years latter, Anton de Bary described symbiosis as "the living together of distinct organisms" (de Bary, 1879). Mutualism is thus defined by the sign of the interaction while symbiosis refers to the persistent living together (physically very close or even attached during some stages), and can have any interaction outcome (Fig. 1). Conversely, mutualism can be symbiotic or non-symbiotic. Despite these clear and distinct definitions, confusion spread only a few years after de Bary's work (Lewin, 1982), perhaps spawned by de Bary (1879) focusing his inquiry of symbioses on mutualistic examples (Goff, 1982). Whatever the reasons, the confusion became so widespread that in 1937, the committee of the American Society of Parasitologists was given the responsibility to ensure the proper use of the terms mutualism and symbiosis (Hertig et al., 1937; Bronstein, 2015). Even today, some researchers refuse to use de Bary's definition of symbiosis, arguing that it is something of a 'catch-all category' that is "not accepted by most general biologists or non biologists today" (Douglas, 2010: 5); Douglas instead prefers to refer to symbiosis as 'a persistent mutualism.' In this thesis, I stick to the original definitions of mutualism and symbiosis of van Beneden (1873) and de Bary (1879).

Another problematic term is coevolution. Coevolution was perceived early on by Charles Darwin when, after examining the long nectar spurs of the Madagascan orchid *Angraecum sesquipedale*, he predicted that a hawkmoth pollinator with a 30-cm tongue would be found. Darwin (1862: pp. 198-203) posited that natural selection on moths “in relation to their general conditions of life, either in the larval or mature state” (p. 202) would have favored moths with longer tongues that in turn selected for longer nectar spurs in the orchid, resulting in “a race in gaining length between the nectary of the *Angræcum* and the proboscis of certain moths” (p. 202-203), thus formulating the first mechanistic model for a coevolutionary process. While the term coevolution was coined by Charles Mode (1958), who provided the first model of coevolution based on gene-for-gene interactions of parasites and their hosts, it became widely popular only after a seminal paper by Ehrlich and Raven (1964), who scrutinized the food plant specialization of butterfly larvae, concluding that overcoming plant defenses allowing larvae to feed on them, or vice-versa evolving chemical defense protecting from attacks of phytophagous animals, both opened up new adaptive zones. They posited that coevolutionary processes lead to evolutionary arm races between butterflies and plants that can potentially increase species’ number, a process now referred to as ‘escape-and radiate coevolution’ (Thompson, 1989). Ehrlich and Raven (1964) however did not define coevolution, and this led to a proliferation of studies in the 1970’s that assumed coevolution based on apparent trait matches between interacting partners (Anderson, 2015), when the matching might simply have resulted from ecological fitting. This led Janzen (1980:1) to stress that coevolution is “an evolutionary change in a trait of the individuals in one population in response to a trait of the individuals of a second population [of a distinct species], followed by an evolutionary response in the first population in response to the change in the first.” This definition implies that interacting organisms must have shared a long-enough history so that reciprocal evolutionary changes can have occurred. Such a definition makes coevolution hard to demonstrate empirically, and few studies have managed to quantify reciprocal selection acting on both partners (Anderson, 2015). Another difficulty with the concept of coevolution is more recent and consists in the misconception that coevolution gives rise to matching phylogenies between interacting groups (Hafner and Nadler, 1988; Smith et al., 2008; Cuthill and Charleston, 2012). As noted before, coevolution refers to a series of *processes* involving the reciprocal evolutionary change of interacting species, driven by natural selection. By contrast, phylogenetic congruence is a *pattern*, which is often the result of cospeciation, the synchronous speciation pattern of interacting species (Kellog, 1913; Fahrenholz, 2013).

Not surprisingly, patterns of phylogenetic congruence have been found in a number of interacting lineages, both mutualistic, such as leaf-cutter ants and fungi (Hinkle et al., 1994; Mikheyev et al., 2010), squid and bioluminescent bacteria (Nishiguchi et al., 1998), *Buchnera* bacteria and aphids (Clark et al., 2000), fig/fig-wasp (Cruaud et al., 2012), and parasitic, such as gophers and lice (Hafner and Nader, 1988), birds and avian parasites (Ricklefs et al., 2004), seabirds and lice (Page et al., 2004), and bats and mites (Bruyndonckx et al., 2009).

All of the cited studies also found extensive host switching, which may contribute to speciation by promoting the interruption of gene flow among conspecific populations. It is important to keep in mind that current inferential tools for phylogeny-based diversification studies tend to overestimate cospeciation (de Vienne et al., 2013). Even in host/parasite interactions, where the parasites were deemed to reflect the host phylogeny (Fahrenholz' rule, Fahrenholz, 1913), work that relies on molecular phylogenies has shown that host-shift is the likely predominant mode of speciation and that cospeciation is rare (de Vienne et al., 2013). The most convincing cases of cospeciation come from symbioses with vertical transmission (parental inheritance of the symbiont), such as the *Buchnera*/aphid nutritional mutualism (Clark et al., 2000; Hosokawa et al., 2006; Jousselin et al., 2009). Clearly, however, phylogenetic congruence can occur independent of whether an interaction is coevolved (Anderson, 2015), and a causal link between coevolution and cospeciation would imply that coevolution acts on reproductive isolation, which may rarely be the case (Moe and Weiblen, 2012), being restricted to a few pollination mutualisms such as the fig and fig-wasp (Cruaud et al., 2012) and the *Glochidion* (Phyllanthaceae) and *Epicephala* moths (Kawakita et al., 2004).

Origin and maintenance of cooperation among species

Their evolutionary and ecological importance, together with their paradoxical existence, make mutualisms an important research topic. This thesis deals with several key aspects of the ecology and evolution of mutualisms (Chapters 1-8).

Before the 1960's, cooperative phenomena were largely dismissed among evolutionary biologists (Axelrod and Hamilton, 1981), probably at least partly because of their apparent lack of accordance with Darwinian evolution. Integration of cooperative phenomena into evolutionary theory came with the development of genetic kinship theory by Hamilton (1964) and with reciprocation theory (Trivers, 1971; Boorman and Levitt, 1980; Chase, 1980; Axelrod and Hamilton, 1981). I here focus on the influential paper by Axelrod and Hamilton (1981), which continues to be a dominant framework for understanding how cooperation can evolve and be maintained in natural populations. What is known as the 'prisoner's dilemma' in game theory has been central to the understanding of cooperation (Trivett, 1971; Axelrod and Hamilton, 1981; Fig. 2). In this game, each of the two players can either cooperate or defect. If player B cooperates, player A can either cooperate, which yields a rewards from the mutual cooperation (R), or defect, which leads to a greater payoff because the gains from partner B's cooperation are taken without the costs of cooperating, hence T > R. This 'temptation to defect' illustrates the prediction that cheating should be pervasive in mutualism. Thus, it pays to defect no matter what the other partner does. But the dilemma comes if both partners defect, which leads to lower payoff (punishment, P) than if they had both cooperated (R > P).

	Player B	
Player A	C Cooperation	D Defection
C Cooperation	R=3 Reward for mutual cooperation	S=0 Sucker's payoff
D Defection	T=5 Temptation to defect	P=1 Punishment for mutual defection

Figure 2. The prisoner's dilemma. The payoff to player A is shown numerically. The game is defined by the following inequalities: T > R > P > S (1) and R > (S + T)/2 (2). Modified from Axelrod and Hamilton (1981).

Always defecting ("ALL D") thus appeared to be an evolutionary stable strategy in the single round game (Axelrod and Hamilton, 1981). But if the game is repeated and the participants can remember the outcome of the previous game and identify their partner, the array of solutions changes drastically. Axelrod and Hamilton (1981) performed a series of computer tournaments with human players, and a distinct strategy emerged as best: TIT FOR TAT. This strategy is based on reciprocity: one partner should cooperate on the first round and thus each partner's move mirrors the other partner's move in the preceding round. They showed that cooperation based on reciprocity could emerge as an evolutionary stable strategy and go to fixation, and the robustness of the strategy was attributed to three parameters: (i) partners should never defect first; (ii) retaliation can occur by the defect of the other partner, and (iii) partners forgive after a single act of retaliation (Axelrod and Hamilton, 1981). But how can TIT FOR TAT evolve in a world of ALL D? The authors provided two solutions: (i) altruism, wherein an individual has some interest in the partner's gain, thus eliminating the inequality $T > R$ or (ii) spatial clustering of cooperating individuals, which leads to a drift in the frequencies of cooperators, limiting their interaction with non-cooperators. This early insight about clustering led to the development of two mechanisms, both able to lead to the evolution of cooperation: network reciprocity, wherein cooperators succeed by network clusters in which they help one another (Novak and May; 1992; Novak, 2006) and group selection wherein a group of cooperators can be more successful than a group of defectors (Traulsen and Novak, 2006, Novak, 2006).

The exploitation of mutualism

Even though mutualism theory predicts instability and that the temptation to defect should lead to cheating, instances of cheating are reduced to a few well-documented examples, such as in pollination mutualisms that involve oviposition and larval feeding, for example, *Yucca*-moths or fig-wasps, which sometimes over-oviposit and then damage their host (Pellmyr et al., 1996; Machado et al., 2001), Monotropoideae (Ericaceae) parasitic plants that obtain carbon from their mycorrhizal associates (Bidartondo and Bruns, 2001), or *Maculinea* butterflies that parasitize ants (Als et al., 2004). The exploitation of mutualisms is common, but recent evidence suggests that phylogenetically unrelated 'parasites of mutualism' dominate the market, while 'cheater mutants' evolving from mutualists that shifted to parasitism are rare (Yu, 2001; Sachs and Simms, 2006; Frederickson, 2013; Sachs, 2015; Chapter 2 [Chomicki et al., 2015]).

The scarcity of cheaters could be due to three non-mutually exclusive reasons. Firstly, it could be an artefact of the methods used to detect cheaters (in particular, quantifying costs and benefits for both partners is hard and has been done for very few mutualisms in conjunction with the necessary phylogenetic framework). Secondly, cheating could lead to unstable states fostering rapid mutualism breakdown (reversion to autonomy in facultative mutualism and extinction in obligate ones) such that we are not able to track them. Thirdly, the scarcity could reflect an early misconception about mutualisms, namely that mutualism does not favor cheating (Sachs and Simms, 2006; Sachs, 2015). This latter idea is based on several lines of evidence. First, a key prediction of mutualism theory is that cheaters select for retaliations (Axelrod and Hamilton, 1981). Examination of retaliation mechanisms, such as fig trees shedding figs that have been cheated (over-oviposited relative to the number of pollinated flowers), domatium-bearing plants shedding leaf domatia that have been attacked by herbivorous insects, or reduced resource allocation to nodules inhabited by inefficient *Rhizobium* strains, strongly suggests that these strategies evolved for reasons unrelated to retaliation (Frederickson, 2013; Chapter 4 [Chomicki et al., 2016]). Thus this contrasts with the idea that mutualists are constantly trying to maximize benefits and reduce costs through cheating, defence and counter-defence (Herre et al., 1999; Egger and Hibbett, 2004; Douglas, 2008; Sachs, 2015). If partners engaged in mutualism are in a constant war to pull the greatest part of benefits, one should expect intense coevolutionary arms race between mutualistic partners. Molecular evolutionary work has revealed, however, that genes mediating mutualisms show evolutionary stasis compared to genes involved in parasitic interactions that show signs of high positive selection (Jiggins et al., 2002; Kimbrel et al., 2013). This supports the idea that conflict between mutualistic partners is largely resolved. Given the meagre field data and experimental results, we should refrain from over-generalizing and consider the possibility that selection for cheating is present in some mutualisms, as evidenced by cases where parasites indeed evolved from mutualistic ancestors (Pellmyr et al., 1996; Bidartondo and Bruns, 2001; Machado et al., 2001; Als et al., 2004). In other mutualisms – perhaps representing a larger proportion – there is insufficient conflict to drive selection for cheating. In any case, there is a great need for empirical investigations of mutualism stability and (re-) considerations of the partner's interests.

The evolution and stability of mutualism

Independently of whether mutualists are dealing with disloyalties of their partners or from third parties, exploitation should be limited or rapidly quenched to prevent mutualism breakdown. Three main pathways have been put forward to explain the origin and maintenance of mutualism: by-product mutualism, partner-fidelity feedback, and partner choice (Sachs et al., 2004; Frederickson, 2013). By-product mutualism implies that the benefit to the other partner is the consequence of a selfish action and thus has no cost; hence selection for cheating cannot arise, nor can opportunists exploit such mutualisms. Three subtypes of by-product mutualisms are distinguished (Sachs et al., 2004): (i) one-way by-product, wherein A benefits B as a consequence of a selfish action (West-Eberhard, 1975; Brown, 1983; Connor, 1995); (ii) two-way by-product, wherein both A and B benefit from each other as consequences of their selfish actions (Hamilton, 1971; Queller, 1985; Connor, 1995) and (iii) by-product reciprocity wherein A evolves to enhance the benefits of B, which in turn increases by-product benefits back to A (Connor, 1986). An example of one-way by-product benefits is alpha predators incidentally providing food for carrion feeders. Müllerian mimicry is an example of two-way by-product mutualism. Facultative epiphytic ant-plants also are good examples of by-product benefits since the plants evolved domatia that benefit the ants, and this increases their trophic resources via ant faecal material and detritus brought into the nest (see Chapter 7).

The other two pathways explaining the origin and maintenance of mutualism are part of the same framework of directed reciprocity and relate directly to the iterated Prisoner's dilemma game described above. Partner-fidelity feedback occurs when the increase in the fitness of one partner increases the fitness of the other partner, which implies that partners interact long enough so that an automatic feedback can occur (Sachs et al., 2004). Plastids and mitochondria, for instance, form partner-fidelity feedbacks with their hosts. A pre-condition for partner-fidelity feedback to occur is the coupling of partner's fitnesses, which can occur for instance via vertical transmission (Bull and Rice, 1991) or via mechanisms increasing the population viscosity, for instance by limiting the dispersal ability of the partner (Nowak and May 1992; Doebeli and Knowlton 1998). Partner-fidelity feedback prevents cheating since it would decrease a partner's own fitness by failing to maintain the other partner fitness.

The third pathway to the origin and maintenance of mutualism is partner choice, which is based on the differential or exclusive rewarding of a preferred partner(s). While the choice of a cooperative species B by species A benefits only A, this interaction promotes the evolution of cooperation in species B. However, a number of mechanisms mediating partner choice use rewards (such as food) for the selected partner (Heil et al., 2005; Orona-Tamayo et al., 2013; Chapter 4 [Chomicki et al., 2016]), thus making partner choice mechanisms efficient in mutualism stabilization. The efficiency of partner choice is deemed to decrease when the number of alternative partners decreases, since it limits the choice and decreases outside options for the selecting partner (Noë and Hammerstein, 1994; Noë, 2001; Akçay, 2015). The mechanisms for 'chosing' cooperative partners vary, with animals resorting to behavioural or image scoring means (Noë, 1990; Bshary, 2002) and plants using chemical or physical means (Federle et al., 1997; Heil et al., 2005; Orona-Tamayo et al., 2013; Chapter 4 [Chomicki et al., 2016]). The decision rules resulting from the choice can be relative, 'take the most cooperative individuals', or absolute, 'take any individual above a threshold value' (West et al., 2002).

Breakdown of mutualism

Mutualism can break down when the costs outweigh the benefits for one or both partners. This can happen if cheating arises in a mutualism, resulting in shifts to parasitism, or if unrelated exploiters disrupt it (Bronstein et al., 2003; Wilson et al., 2003; Sachs and Simms, 2006). The two other possible outcomes of mutualism breakdown are reversion to autonomy, which is typically expected in facultative mutualisms; and extinction, which is expected in obligate mutualisms (Vandermeer and Boucher, 1978; Keeler, 1985; Schemske and Lande, 1985; Holland et al., 2004; Fosters and Wenseleers, 2006). Mutualism reversion to autonomy can occur when the benefits can be obtained cheaply from the environment (Allen, 1991; Sprent, 2001) or are no longer needed (Palmer et al., 2010). Decrease in partner's abundance can also drive mutualism back to autonomy or to extinction. Tracing mutualism breakdown requires densely sampled phylogenies, but only reversion to autonomy or shift to parasitism can be recovered. Determining the context for mutualism breakdowns, for instance a decrease in partner abundance or a change in environmental conditions affecting the costs and benefits of the mutualisms, requires extensive comparative data for both partners (Chapter 6).

Diversity of mutualisms involving ants

Mutualisms can be broadly divided into three categories: *transportation mutualisms*, such as pollination and seed dispersal; *protection mutualisms*, such as protective ant/plant mutualisms or cleaner-fish/fish mutualisms, and *nutritional mutualisms*, such as legume-*Rhizobium*, mycorrhizal associations, coral-algae symbioses or gut microbiomes (Bronstein, 2015). Ants (family Formicidae) are the largest and most abundant group of eusocial insects and engage in all three kinds of mutualisms. They do so with many groups, including plants, insects (including other ant species), bacteria, and fungi. Ants engage in nutritional mutualisms with endosymbionts to enrich their diet; an example is the obligate mutualism between carpenter ants (*Camponotus*) and *Blochmannia* bacteria that live in bacteriocytes and synthesize essential amino acids for its host (Feldhaar et al., 2007). As territorial and often aggressive organisms, ants engage in defense mutualisms where they provide protection for insects, such as honeydew-producing homopterans (Way, 1963) or plants that offer extrafloral nectar (EFN) (Bentley, 1977). These mutualisms likely opened new niches for ants in canopies by adapting to feed on sugar-based (herbivorous) diet from initially ground-dwelling predators (Wilson and Hölldobler, 2005) and partly explain how tropical rainforest canopies sustain a density of ants that vastly exceeds what could be sustained if they only fed on insect prey (Davidson et al., 2003).

A particularly outstanding mutualism involving ants is that between attine ants and fungi. Attine ants obligately depend on mutualistic fungi for food. The higher attines (leafcutter ants) grow their obligate symbiotic fungi on freshly cut leaves, which provide them with a virtually unlimited food resource and allows them to achieve enormous colonies of over 5000 gardens and 10 million workers (Mueller, 2015). Fungi symbionts (*Attamyces*) are vertically transmitted by founding queens, which fertilize the inoculum with their own feces and manipulate a complex microbiota to optimize garden growth (Mueller et al., 2005; Mueller, 2012). Leafcutter ants harvest the specialized hyphal swellings (gongylidia) and sterilize the fungi, limiting them to clonal propagation, which results in large monocultures

(Mueller et al., 2010). A novel type of ant agriculture involving plant cultivation is described in chapter 5.

Besides the aforementioned EFN-based defense mutualism, ants engage in an important transportation mutualism with thousands of angiosperms in 77 families in which they collect and carry seeds to feed on the lipid-rich and protein-rich seed appendages (elaiosomes) before abandoning the seeds outside the nest (Sernander, 1906; Lengyel et al., 2010), typically within 0.1 to 77 meters from where the seeds were produced (Gomez and Espadaler, 1998). A unique type of dispersal and planting by ants (myrmecochory) is referred to as ant-gardens and involves ants that sow seeds of certain epiphytes inside carton nests to solidify it; these ants often obtain additional nutritional rewards from the epiphytes in their gardens (Ule, 1901, Davidson, 1988; Kaufmann, 2002). The evolutionary history of Australasia ant-gardens is analyzed with molecular clock-dated phylogenies in Chapter 8.

Ants also engage in symbioses with plants in the tropics, and this comes in two flavors, not necessarily mutually exclusive: (*i*) defense mutualisms involving the ant housing for protection against herbivores and sometimes encroaching vegetation (Davidson and McKey, 1993; Renner and Ricklefs, 1998; Frederickson et al., 2005); and (*ii*) nutritional mutualism wherein the host benefits from ant-derived nitrogen (and phosphorus) (Benzing, 1970; Huxley, 1978; Rickson, 1979; Rico-Gray et al., 1989; Gay, 1993; Treseder et al., 1995; Gegenbauer et al., 2012). Plants involved in defense mutualism with ants are typically terrestrial, while those involved in nutritional mutualisms are epiphytes (Chomicki and Renner, 2015) – largely because epiphytes are under strong pressure for obtaining nitrogen and phosphorus (Zotz and Hietz, 2001). However, there is evidence that at least some ant/plant protection symbioses also benefit the plant hosts nutritionally (Sagers et al., 2000) and likewise that some epiphytic ant/plant nutritional mutualisms also have a defensive function (Huxley, 1978). Ant/plant symbioses are the focus of this thesis.

Many ant species engage in several types of mutualisms, such as protective ant/plant symbiosis and homoptera tending. The number of mutualisms that one species engages in appears to be limited by the conditions required by different partners and the tradeoff between the net benefits versus the matching of partner requirements (Oliver et al., 2008).

Study systems

In this thesis, I address both large-scale evolutionary questions (Chapters 1, 2 and 8) and smaller scale ecological and evolutionary questions (Chapters 3, 4, 5, 6, 7). I used a phylogenetic framework for all ant-plants (Chapter 1), one for *Pseudomyrmex* ants and their plant hosts (Chapter 2), and a third for the ant genus *Philidris* and its associated plant hosts with which it forms ant-gardens (Chapter 8). For other research questions, I focused on the Rubiaceae subtribe Hydnophytinae (Chapter 6), and a Fijian clade of the genus *Squamellaria* with nine species (Chapters 3, 4, 5, 7), which I extensively investigated during two fieldtrips to Fiji. The Hydnophytinae forms a subtribe of 102 species and five genera: *Hydnophytum* with 50 species (Jebb and Huxley, unpublished; Chomicki and Renner, 2016); *Myrmecodia* with 26 species (Huxley and Jebb, 1993); *Squamellaria* with 12 species (Chomicki and Renner, 2016); *Anthorrhiza* with 9 species (Huxley and Jebb, 1991a; Jebb, 1993) and *Myrmephytum* with 5 species (Huxley and Jebb, 1991b). They are distributed in South-East Asia, all around Indonesia and as far as the Fiji Islands, but their diversity peaks in Papua New Guinea (Fig. 3).

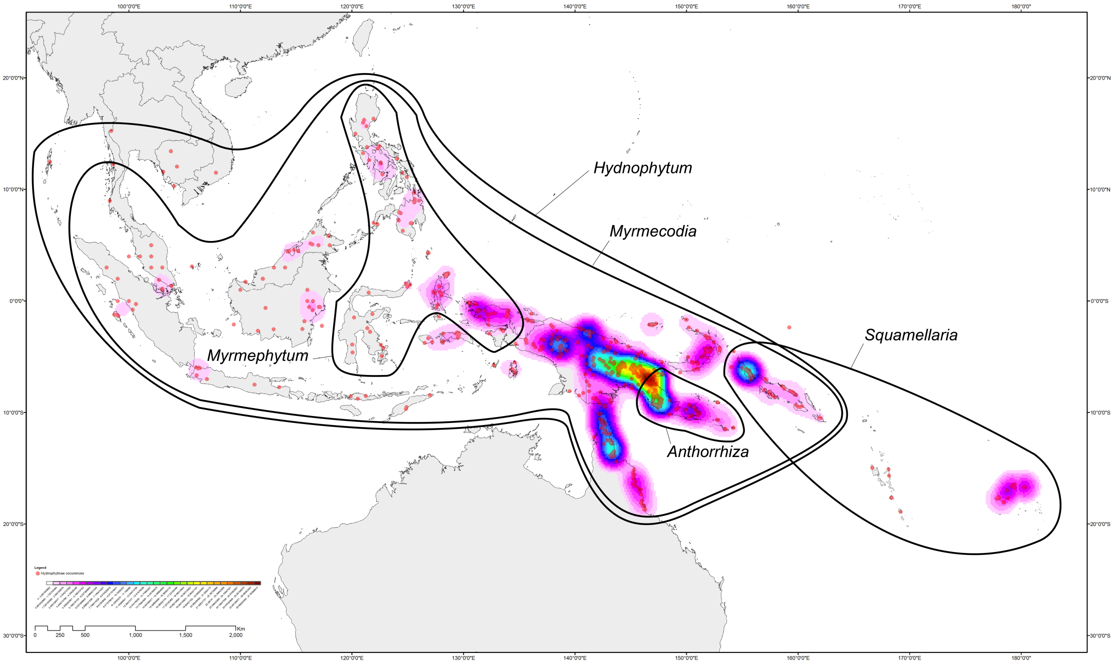


Figure 3. Density map for the Hydnophytinae generated using ArcMap (ESRI, 1999-2008) from over 1,000 occurrence data points for all 102 Hydnophytinae species.

A common feature of all Hydnophytinae is the tuber that derives from a modified hypocotyl and functions as domatium in most species (Huxley and Jebb, 1991c). The structure of the tuber varies tremendously (Jebb, 1985; 1991) and has been central to two chapters of this thesis (Chapters 6 and 7), where I used CT-scanning to generate 3D models of the cavities.

Thesis structure

This thesis is divided into eight chapters. **Chapter 1** (G. Chomicki and S. S. Renner. *New Phytologist* 227: 411-424) provides a phylogenetic framework for all ant-plant symbioses, asking how many times, when and where did ant domatium evolved. **Chapter 2** (G. Chomicki, P. S. Ward, S. S. Renner. *Proceedings of the Royal Society B: Biological Sciences* 282: 20152200; featured on the front cover) focuses on Neotropical ant/plant symbioses with *Pseudomyrmex* ants, using phylogenetic, biogeography and ancestral state approaches to determine the stages in coevolution between *Pseudomyrmex* and its plant hosts. **Chapter 3** (G. Chomicki, S. S. Renner. *PLoS ONE* 11: e0151317) describes four new species of epiphytic ant-plants and a re-circumscription of the ant-epiphytic genus *Squamellaria* (Rubiaceae), which is essential to the establishment of this genus as a system to study mutualism specialization. **Chapter 4** (G. Chomicki, Y. M, Staedler, J. Schönenberger, S. S. Renner. *New Phytologist*, in press, DOI: 10.1111/nph.13990, featured on the front cover) describes a novel food reward in obligate ant-epiphytic *Squamellaria* and demonstrates that it acts as an efficient partner choice mechanism, as well as revealing the evolutionary origin of this mechanism. **Chapter 5** (G. Chomicki and S. S. Renner. *Nature Plants*, accepted with revisions 21 June 2016) demonstrates a novel type of plant farming by ants wherein the dolichoderine ant *Philidris nagasau* plant seeds of its obligate *Squamellaria* hosts inside tree bark, and fertilize the seedlings by defecating inside their tiny domatia that as yet cannot sustain an ant colony. **Chapter 6** (G. Chomicki, S. S. Renner. Unpublished manuscript) provides a solid phylogenetic framework for the Hydnophytinae (Rubiaceae) and uses it to trace the recurrent breakdown of mutualism, using niche modeling, CT scanning and a number of phylogenetic comparative methods to uncover the underlying mechanism behind mutualism breakdown. **Chapter 7** (G. Chomicki, D. Metzler, S. S. Renner, unpublished manuscript) combines field experiments, CT scanning and mathematical modeling (the latter performed by D. Metzler) to ask how do related facultative and obligate hosts maximize benefits under stable or fluctuating symbiont pools. **Chapter**

8 (G. Chomicki, M. Janda, S. S. Renner. Invited manuscript for *Proceedings of the Royal Society B*, special feature edited by S. S. Renner and G. Chomicki) traces the evolutionary origins of South-East Asian ant-gardens, using phylogenetic frameworks for the most important ant genus and plants, biogeography and trait analyses.

References

Akçay E. (2015). Evolutionary models of mutualism. In *Mutualism*. Ed. J.L. Bronstein. *Oxford University Press, Oxford, UK*, pp. 57-76.

Allen M.F. (1991). *The Ecology of Mycorrhizae*. Cambridge University Press.

Als T.D, Vila R., Kandul N.P., Nash D.R., Yen S.H., Hsu Y.F., Mignault A.A., Boomsma J.J., Pierce N.E. (2004). The evolution of alternative parasitic life histories in large blue butterflies. *Nature* 432: 386-390.

Anderson B. (2015). Coevolution in mutualism. In *Mutualism. Ed. J.L. Bronstein. Oxford University Press, Oxford, UK*, pp.107-130.

Axelrox R.M., Hamilton W.D. (1981). The evolution of cooperation. *Science* 211: 1390-1396.

Bentley B.L. (1977). Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* 8: 407-427.

Benzing, D.H. (1970). An investigation of two bromeliad myrmecophytes: *Tillandsia butzii* Mez, *T. caput-medusae* E. Morren, and their ants. *Bulletin of the Torrey Botanical Club* 97: 109-115.

Bidartondo M.I., Bruns T.D. (2001). Extreme specificity in epiparasitic Monotropoideae (Ericaceae): widespread phylogenetic and geographical structure. *Molecular Ecology* 10: 2285-2295.

Boorman S.A., Levitt, P.R. (1980). The genetics of altruism. *New York: Academic*.

Boucher D.H., James S., Keeler K.H. (1982). The ecology of mutualism. *Annual Review of Ecology and Systematics* 13: 315–347.

Boucher D.H., ed. (1985). *The biology of mutualism: Ecology and evolution*. Oxford University Press, New York, USA.

Bronstein J.L. (2015). The study of mutualism. In *Mutualism.Ed. J.L. Bronstein. Oxford University Press, Oxford, UK*, pp. 3-19.

Bronstein J.L., Wilson W.G., Morris W.F. (2003). Ecological dynamics of mutualist/antagonist communities. *The American Naturalist* 162: S24-S39.

Brown J.L. (1983). Cooperation—a biologist's dilemma. *Advances in the Study of Behavior* 13: 1–37.

Bruyndonckx N., Dubey S., Ruedi M., Christe P. (2009). Molecular cophylogenetic relationships between European bats and their ectoparasitic mites (Acari, Spinturnicidae). *Molecular Phylogenetics and Evolution* 51: 227-237.

Bshary R. (2002). Biting cleaner fish use altruism to deceive image–scoring client reef fish. *Proceedings of the Royal Society of London B: Biological Sciences* 269: 2087-2093.

Bull J.J., Rice W.R. (1991). Distinguishing mechanisms for the evolution of co-operation. *Journal of Theoretical Biology* 149: 63–74.

Chamberlain S.A., Bronstein J.L., Rudgers J.A. (2014). How context dependent are species interactions? *Ecology Letters* 17: 881-890.

Chase I.D. (1980). Cooperative and noncooperative behavior in animals. *The American Naturalist* 115: 827-857.

Chomicki G., Renner S.S. (2015). Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist* 207: 411-424.

Chomicki G., Renner S.S. (2016). Evolutionary Relationships and Biogeography of the ant-epiphytic genus *Squamellaria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *PloS one* 11: e0151317.

Chomicki G., Staedler Y.M., Schönenberger J., Renner S.S. (2016). Partner choice through concealed floral sugar rewards evolved with the specialization of ant–plant mutualisms. *New Phytologist*. In press. DOI: 10.1111/nph.13990.

Chomicki G., Ward P.S., Renner S.S. (2015). Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. *Proceedings of the Royal Society B: Biological Sciences* 282: 20152200.

Clark M.A., Moran N.A., Baumann P., Wernegreen J.J. (2000). Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* 54: 517-525.

Connor R.C. (1995). The benefits of mutualism: a conceptual framework. *Biological Reviews 70*: 427–457.

Connor R.C. (1986). Pseudo-reciprocity: investing in mutualism. *Animal Behaviour* 34: 1562–1584.

Cruaud A., Rønsted N., Chantarasuwan B., Chou L.S., Clement W.L., Couloux A., Cousins B., Genson G., Harrison R.D., Hanson P.E., Hossaert-Mckey M. (2012). An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Systematic Biology* 61: 1029–1047.

Cuthill J.H., Charleston M. (2012). Phylogenetic codivergence supports coevolution of mimetic *Heliconius* butterflies. *PLoS One* 7: e36464.

Darwin C. (1862). *On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing*. London: John Murray.

Darwin CR. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. Murray, London.

Davidson D.W., McKey D. (1993). The evolutionary ecology of symbiotic ant/plant relationships. *Journal of Hymenopteran Research* 2: 13–83.

Davidson D.W. (1988). Ecological studies of Neotropical ant gardens. *Ecology* 69: 1138-1152.

Davidson D.W., Cook S.C., Snelling R.R., Chua T.H. (2003). Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300: 969-972.

De Bary A. (1879). *Die Erscheinungen der Symbiose*. Strassburg: Karl J. Trübner.

Doebeli M., Knowlton N. (1998). The evolution of interspecific mutualisms. *Proceedings of the National Academy of Sciences of the USA* 95: 8676-8680.

Douglas A.E. (2008). Conflict, cheats and the persistence of symbioses. *New Phytologist* 177: 849-858.

Douglas A.E. (2010). *The symbiotic habit*. Princeton University Press, Princeton, New Jersey, USA.

Egger K.N., Hibbett D.S. (2004). The evolutionary implications of exploitation in mycorrhizas. *Canadian Journal of Botany* 82: 1110-1121.

Ehrlich P.R., Raven P.H. (1964). Butterflies and plants: a study in coevolution. *Evolution* 18: 586-608.

ESRI E. (1999-2008) ArcMap 9.3. ArcGIS 9, 1999-2008.

Fahrenholz H. (1913). Ectoparasiten und abstammungslehre. *Zoologischer Anzeiger*, 41: 371-374.

Federle W., Maschwitz U., Fiala B., Riederer M., Hölldobler B. (1997). Slippery ant-plants and skilful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). *Oecologia* 112: 217-224.

Feldhaar H., Straka J., Krischke M., Berthold K., Stoll S., Mueller M.J., Gross R., (2007). Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC biology* 5: 48.

Foster K.R., Wenseleers T. (2006). A general model for the evolution of mutualisms. *Journal of Evolutionary Biology* 19: 1283-1293.

Frederickson M.E. (2009). Conflict over reproduction in an ant-plant symbiosis: why *Allomerus octoarticulatus* ants sterilize *Cordia nodosa* trees. *The American Naturalist* 173: 675-681.

Frederickson M.E. (2013). Rethinking mutualism stability: cheaters and the evolution of sanctions. *The Quarterly review of biology* 88: 269-295.

Frederickson M.E., Greene M.J., Gordon D.M. (2005). Ecology:'Devil's gardens' bedevilled by ants. *Nature* 437: 495-496.

Futuyma D.J., Slatkin M. (1983). *Coevolution.* Sinauer. Sunderland, Massachusetts.

Gay H. (1993). Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Biological Journal of the Linnean Society* 50: 221-233.

Gegenbauer C., Mayer V.E., Zotz G., Richter A. (2012). Uptake of ant-derived nitrogen in the myrmecophytic orchid *Caularthron bilamellatum*. *Annals of Botany* 110: 757-766.

Goff L.J. (1982). Symbiosis and parasitism: another viewpoint. *BioScience 32*: 255-256.

Gomez C., Espadaler X. (1998). Myrmecochorous dispersal distances: a world survey. *Journal of Biogeography* 25: 573–580.

Hafner, M.S., Nadler S.A. (1988). Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* 332: 258-259.

Hamilton W.D. (1971). Geometry of the selfish herd. *Journal of Theoretical Biology* 31: 295–311.

Harley J.L., Smith S.E. (1983). *Mycorrhizal symbiosis*. Academic Press, Inc.

Haskell E.F. (1949). A clarification of social science. *Main Currents in Modern Thought* 7: 45-51.

Heil M., Rattke J., Boland W. (2005). Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308: 560-563.

Herre E.A., Knowlton N., Mueller U.G., Rehner S.A. (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14: 49-53.

Hertig M., Taliaferro W.H., Schwartz B. (1937). Report of the committee on terminology. *Journal of Parasitology 23*: 325-329.

Hinkle G., Wetterer J.K., Schultz T.R., Sogin M.L. (1994). Phylogeny of the attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. *Science* 266: 1695-1697.

Hojo M.K., Pierce N.E., Tsuji K. (2015). Lycaenid caterpillar secretions manipulate attendant ant behavior. *Current Biology* 25: 2260-2264.

Holland J.N., DeAngelis D.L., Schultz S.T. (2004). Evolutionary stability of mutualism: interspecific population regulation as an evolutionary stable strategy. *Proceedings of the Royal Society B: Biological Sciences* 271: 1807–1814.

Hosokawa T., Kikuchi Y., Nikoh N., Shimada M., Fukatsu T. (2006). Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biology* 4: p.e337.

Huxley C.R., Jebb M.H.P. (1991a). The tuberous epiphytes of the Rubiaceae 2: the new genus *Anthorrhiza*. *Blumea* 36: 21-41.

Huxley C.R., Jebb M.H.P. (1991b). The tuberous epiphytes of the Rubiaceae 3: a revision of *Myrmephytum* to include *Myrmedoma*. *Blumea* 36: 43-52.

Huxley C.R., Jebb M.H.P. (1991c). The tuberous epiphytes of the Rubiaceae 1: a new subtribe, the Hydnophytinae. *Blumea* 36: 1-20.

Huxley C.R., Jebb M.H.P. (1993). The tuberous epiphytes of the Rubiaceae, 5. A revision of *Myrmecodia. Blumea* 37: 271-334.

Huxley C.R. (1978). The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* 80: 231-268.

Janzen D.H. (1980). When is it coevolution. *Evolution* 34: 611-612.

Jebb M.H.P., Huxley C.R. The Tuberous Epiphytes of the Rubiaceae 7: a revision of the genus *Hydnophytum* Jack. Unpublished manuscript.

Jebb M.H.P. (1985). *Taxonomy and tuber morphology of the rubiaceous Ant-Plants*. Doctoral dissertation, University of Oxford.

Jebb M.H.P. (1991). Cavity structure and function in the tuberous Rubiaceae. pp. 374-390. In Huxley CR and Cutler DF eds. *Ant-plant interactions*. Oxford: Oxford University Press.

Jebb M.H.P. (1993). *Anthorrhiza camilla*. *Blumea* 37: 341-344.

Jiggins F.M., Hurst G.D., Yang Z. (2002). Host-symbiont conflicts: positive selection on an outer membrane protein of parasitic but not mutualistic Rickettsiaceae. *Molecular Biology and Evolution* 19: 1341-1349.

Jousselin E., Desdevises Y., Coeur d'Acier A. (2009). Fine-scale cospeciation between *Brachycaudus* and *Buchnera aphidicola*: bacterial genome helps define species and evolutionary relationships in aphids. *Proceedings of the Royal Society of London B: Biological Sciences* 276: 187-196.

Kaufmann E. (2002). Southeast Asian Ant-Gardens: Diversity, ecology, ecosystematic significance, and evolution of mutualistic ant-epiphyte associations. Doctoral dissertation, Johann Wolfgang Goethe-Universität, Frankfurt.

Kawakita A., Takimura A., Terachi T., Sota T., Kato M. (2004). Cospeciation analysis of an obligate pollination mutualism: have *Glochidion* trees (Euphorbiaceae) and pollinating *Epicephala* moths (Gracillariidae) diversified in parallel? *Evolution* 58: 2201-2214.

Keeler K.H. (1985). Cost:benefit models of mutualism. In *The Biology of Mutualism, Ecology and Evolution* (Boucher, D.H., ed.), pp. 100–127, Oxford University Press.

Kellog V.L. (1913). Distribution and species-forming of ecto-parasites. *The American Naturalist* 47: 129–158.

Kimbrel J.A., Thomas W.J., Jiang Y., Creason A.L., Thireault C.A., Sachs J.L., Chang J.H. (2013). Mutualistic co-evolution of type III effector genes in *Sinorhizobium fredii* and *Bradyrhizobium japonicum*. *PLoS Pathogens* 9: e1003204.

Lengyel S., Gove A.D., Latimer A.M., Majer J.D., Dunn R.R. (2010). Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: a global survey. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 43-55.

Lewin R.A. (1982). Symbiosis and parasitism—definitions and evaluations. *BioScience* 32: 254-260.

Machado C.A., Jousselin E., Kjellberg F., Compton S.G., Herre E.A. (2001). Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proceedings of the Royal Society B: Biological Scienes* 268: 685-694.

Margulis L., Fester R. (1991). *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. MIT Press, Boston, Massachusetts, USA.

Mikheyev A.S., Mueller U.G., Abbot P. (2010). Comparative dating of attine ant and lepiotaceous cultivar phylogenies reveals coevolutionary synchrony and discord. *The American Naturalist* 175: E126-E133.

Mode C.J. (1958). A mathematical model for the co-evolution of obligate parasites and their hosts. *Evolution* 12: 158-165.

Moe A.M., Weiblen G.D. (2012). Pollinator-mediated reproductive isolation among dioecious fig species (*Ficus*, Moraceae). *Evolution* 66: 3710-3721.

Mueller U.G. (2015). The attine ant-fungus mutualism. In *Mutualism. Ed. J.L. Bronstein. Oxford University Press, Oxford, UK*, pp.78-79.

Mueller U.G. (2012). Symbiont recruitment versus ant-symbiont co-evolution in the attine ant–microbe symbiosis. *Current opinion in microbiology* 15: 269-277.

Mueller U.G., Gerardo N.M., Aanen D.K., Six D.L., Schultz T.R. (2005). The evolution of agriculture in insects. *Annual Review of Ecology, Evolution, and Systematics* 36: 563-595.

Mueller U.G., Scott J.J., Ishak H.D., Cooper M., Rodrigues A. (2010). Monoculture of leafcutter ant gardens. *PLoS One* 5: e12668.

Nishiguchi M.K., Ruby E.G., McFall-Ngai M.J. (1998). Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in sepiolid squid-vibrio symbioses. *Applied and environmental microbiology* 64: 3209-3213.

Noë R. (1990). A veto game played by baboons: a challenge to the use of the prisoner's dilemma as a paradigm for reciprocity and cooperation. *Animal Behaviour* 39: 78–90.

Noë R. (2001). Biological markets: partner choice as the driving force behind the evolution of mutualisms. Pages 93–118 in *Economics in Nature: Social Dilemmas, Mate Choice and Biological Markets*. edited by R. Noë Ronald Noë, J. A.R.A.M. Van Hooff, P. Hammerstein. Cambridge University Press, Cambridge, UK.

Noë R., Hammerstein P. (1994). Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. *Behavioral ecology and sociobiology 35*: 1-11.

Nowak M.A., May R.M. (1992). Evolutionary games and spatial chaos. *Nature* 359: 826-829.

Nowak M.A. (2006). Five rules for the evolution of cooperation. *Science* 314: 1560-1563.

Oliver T.H., Leather S.R., Cook J.M. (2008). Macroevolutionary patterns in the origin of mutualisms involving ants. *Journal of Evolutionary Biology* 21: 1597-1608.

Orona-Tamayo D., Wielsch N., Blanco-Labra A., Svatos A., Farías-Rodríguez R., Heil M. (2013). Exclusive rewards in mutualisms: ant proteases and plant protease inhibitors create a lock–key system to protect *Acacia* food bodies from exploitation. *Molecular Ecology* 22: 4087-4100.

Page R.D., Cruickshank R.H., Dickens M., Furness R.W., Kennedy M., Palma R.L., Smith V.S. (2004). Phylogeny of “*Philoceanus* complex” seabird lice (Phthiraptera: Ischnocera) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 30: 633-652.

Palmer T.M., Stanton M.L., Young T.P., Goheen J.R., Pringle R.M., Karban R. (2008). Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. *Science* 319:192-195.

Pellmyr O., Leebens-Mack J., Huth C.J. (1996). Non-mutualistic yucca moths and their evolutionary consequences. *Nature* 380: 155-156.

Queller D.C. (1985). Kinship, reciprocity and synergism in the evolution of social behavior. *Nature* 318: 366– 367.

Renner S.S. (2006). Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. *In* N. M. Waser and J. Ollerton (eds.), *Plant-Pollinator Interactions: From Specialization to Generalization*, pp. 123-144. Univ. of Chicago Press, Chicago, USA.

Renner S.S., Ricklefs R.E. (1998). Herbicidal activity of domatia-inhabiting ants in patches of *Tococa guianensis* and *Clidemia heterophylla*. *Biotropica* 30: 324-327.

Ricklefs R.E., Fallon S.M., Bermingham E. (2004). Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology* 53: 111-119.

Rickson F.R. (1979). Absorption of animal tissue breakdown products into a plant stem – the feeding of a plant by ants. *American Journal of Botany* 66: 87-90.

Rico-Gray V., Barber J.T., Thien L.B., Ellgaard E.G., Toney J.J. (1989). An unusual animal-plant interaction: feeding of *Schomburgkia tibicinis* (Orchidaceae) by ants. *American Journal of Botany* 76: 603-608.

Sachs J.L. (2015). The exploitation of mutualism. In *Mutualism. Ed. J.L. Bronstein. Oxford University Press, Oxford, UK*, pp. 93-106.

Sachs J.L., Simms E.L. (2006). Pathways to mutualism breakdown. *Trends in Ecology and Evolution* 21: 585-592.

Sachs J.L., Mueller U.G., Wilcox T.P., Bull J.J. (2004). The evolution of cooperation. *The Quarterly Review of Biology* 79: 135-160.

Sagers C.L., Ginger S.M., Evans R.D. (2000). Carbon and nitrogen isotopes trace nutrient exchange in an ant-plant mutualism. *Oecologia* 123: 582-586.

Schemske D.W., Lande R. (1985). The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39: 41-52.

Sernander R. (1906). *Entwurf einer Monographie der europäischen Myrmekochoren* (Vol. 41, No. 7). Almqvist & Wiksells.

Smith C.I., Godsoe W.K., Tank S., Yoder J.B., Pellmyr O. (2008). Distinguishing coevolution from covicariance in an obligate pollination mutualism: asynchronous divergence in Joshua tree and its pollinators. *Evolution* 62: 2676-2687.

Sprent J.I. (2001). *Nodulation in Legumes*. Royal Botanic Gardens.

Stanton M.L., Palmer T.M., Young T.P., Evans A., Turner M.L. (1999). Sterilization and canopy modification of a swollen thorn acacia tree by a plant-ant. *Nature* 401: 578-581.

Stone L., Roberts A. (1991). Conditions for a species to gain advantage from the presence of competitors. *Ecology* 72: 1964-1972.

Szathmáry E.. Maynard Smith J. (1995). The major evolutionary transitions. *Nature* 374: 227-232.

Thompson J.N. (1982). *Interaction and Coevolution*. John Wiley & Sons, New York, USA. 179 pp.

Thompson J.N. (1999). The evolution of species interactions. *Science* 284: 2116-2118.

Thompson J.N. (1989). Concepts of coevolution. *Trends in Ecology and Evolution* 4: 179-183.

Traulsen A., Nowak M.A. (2006). Evolution of cooperation by multilevel selection. *Proceedings of the National Academy of Sciences of the USA* 103: 10952-10955.

Treseder K.K., Davidson D.W., Ehleringer J.R. (1995). Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* 375: 137-139.

Trivers R.L. (1971). The evolution of reciprocal altruism. *Quarterly review of biology* 46: 35-57.

Ule E.H.G. (1901). *Ameisengärten im Amazonasgebiet*. Wilhelm Engelmann.

Van Beneden P.J. (1873). Un mot sur la vie sociale des animaux inférieurs. *Bulletins de l'Academie Royale des Sciences, des Lettres et des Beaux Arts de Belgique* 2: 779-796.

Van Der Heijden M.G., Bardgett R.D., Van Straalen N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters* 11: 296-310.

Vandermeer J.H., Boucher D.H. (1978). Varieties of mutualistic interaction in population models. *Journal of Theoretical Biology* 74:549–558.

Vienne D.M., Refrégier G., López-Villavicencio M., Tellier A., Hood M.E., Giraud T. (2013). Cospeciation vs. host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist* 198: 347-385.

Way M.J. (1963). Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology* 8: 307-344.

West S.A., Kiers E.T., Simms E.L., Denison R.F. (2002b). Sanctions and mutualism stability: why do rhizobia fix nitrogen? *Proceedings of the Royal Society of London B*: *Biological Sciences* 269: 685–694.

West S.A., Pen I., Griffin A.S. (2002a). Cooperation and competition between relatives. *Science* 296: 72–75.

West-Eberhard M.J. (1975). Evolution of social behavior by kin selection. *Quarterly Review of Biology* 50: 1– 33.

Wilson E.O., Hölldobler B. (2005). The rise of the ants: a phylogenetic and ecological explanation. *Proceedings of the National Academy of Sciences of the USA* 102: 7411-7414.

Wilson W.G., Morris W.F., Bronstein J.L. (2003). Coexistence of mutualists and exploiters on spatial landscapes. *Ecological Monographs* 73: 397-413.

Yu D.W. (2001). Parasites of mutualisms. *Biological Journal of the Linnean Society* 72: 529-546.

Zotz G., Hietz P. (2001). The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of Experimental Botany* 52: 2067-2078.

| Chapter 1 |

Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics

Guillaume Chomicki and Susanne S. Renner

New Phytologist 207: 411-424 (2015).

New Phytologist

Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics

Guillaume Chomicki and Susanne S. Renner

Systematic Botany and Mycology, Department of Biology, University of Munich (LMU), Munich 80638, Germany

Author for correspondence:
Guillaume Chomicki
Tel: +49 89 17861 285
Email: guillaume.chomicki@gmail.com

Received: *1 August 2014*
Accepted: *4 December 2014*

New Phytologist (2015) **207:** 411–424
doi: 10.1111/nph.13271

Key words: ant–plant symbioses, domatia, extrafloral nectaries (EFNs), mutualism, myrmecophytes, radiations, symbioses.

Summary

- Ant–plant symbioses involve over 110 ant species in five subfamilies that are facultative or obligate occupants of stem, leaf or root domatia formed by hundreds of ant-plant species. The phylogenetic distribution and geological ages of these associations, and the frequency of gains or losses of domatium, are largely unknown.
- We compiled an up-to-date list of ant domatium-bearing plants, estimated their probable true number from model-based statistical inference, generated dated phylogenies that include *c*. 50% of ant-plant lineages, and traced the occurrence of domatia and extrafloral nectaries on a 1181-species tree, using likelihood and Bayesian methods.
- We found 681 vascular plants with domatia (159 genera in 50 families) resulting from minimally 158 inferred domatium origins and 43 secondary losses over the last 19 Myr. The oldest African ant–plant symbioses are younger than those in Australasia and the Neotropics. The best statistical model suggests that the true number of myrmecophytes may approach 1140 species.
- The phylogenetic distribution of ant-plants shows that domatia evolved from a range of pre-adapted morphological structures and have been lost frequently, suggesting that domatia have no generalizable effect on diversification. The Miocene origin of ant–plant symbioses is consistent with inferred changes in diet and behaviour during ant evolution.

Introduction

The fossil record and molecular clock dating show that ants and plants have been coexisting for at least 120 Myr (Brady *et al.*, 2006; Moreau *et al.*, 2006; Bell *et al.*, 2010; Magallón *et al.*, 2013; Moreau & Bell, 2013). Traits that support a long history of ant–plant interactions include elaiosomes, fatty appendages on seeds meant for ant dispersers that may have occurred as early as 75 Myr ago (Ma) (Dunn *et al.*, 2007). Extrafloral nectaries (EFNs), involving a defence mutualism through sugar secretion recruiting ant mutualists, are known from Oligocene fossils (Pemberton, 1992) and evolved over 450 times in vascular plants (Weber & Keeler, 2013). A third type of ant–plant mutualism involves ants living in myrmecophytes, plants with modified structures to host ants (domatia). No fossil ant domatia are known, nor has there been a phylogenetic analysis focusing on these structures and the geological times when they arose or were lost. For the other two ant-related plant traits, namely elaiosomes and EFNs, recent analyses suggest that they fostered diversification, implying that mutualistic interaction with insects may have impacted macroevolutionary patterns (Lengyel *et al.*, 2009; Weber & Agrawal, 2014). In the absence of a phylogenetic framework, it is unclear whether domatia also favoured diversification.

Domatia occur in numerous plant species with modified leaves, stems or roots that provide cavities occupied by ants (Fig. 1). Some plants with domatia in addition possess specialized food bodies or EFNs. The domatium-living ants in return provide their plant hosts with protection against herbivores, with extra nutrients, or with the physical or chemical removal of competing plant species (Janzen, 1967, 1969; Davidson & McKey, 1993; Jolivet, 1996; Renner & Ricklefs, 1998). At least 113 species of ants from five subfamilies – Myrmicinae, Formicinae, Dolichoderinae, Pseudomyrmecinae, and Ponerinae – occasionally or obligatorily nest in plants (McKey & Davidson, 1993). Examples of facultative (opportunistic) ant–plant symbioses (involving domatia) are species of *Tillandsia* that can host over 30 arboreal ant species in their interlocked leaf bases (Benzing, 1970; Dejean *et al.*, 1995). Examples of obligate ant–plant symbioses are those between Central American species of *Vachellia* (formerly placed in *Acacia*) and *Pseudomyrmex* ants of the *ferrugineus* group (Heil *et al.*, 2005, 2009; Orona-Tamayo & Heil, 2013). Despite a large amount of data on aspects of chemical ecology, food webs and feedback mechanisms between plants and ant symbionts (reviewed by Orona-Tamayo & Heil, 2013; Mayer *et al.*, 2014), little is known about the evolution of the traits that may have facilitated domatium-based symbioses and about the frequency of their evolutionary turnover in the tropical regions of Australasia, Africa and the New World where most ant–plant symbioses occur.

Phylogenetic frameworks for both the plants and the ants have so far been developed for three ant–plant symbioses, one from Africa, one from Southeast Asia and one from the Neotropics. In the African *Leonardoxa africana*, two of four subspecies have specialized domatia that were colonized in parallel by older, pre-adapted ant species (Chenuil & McKey, 1996; Brouat *et al.*, 2004). Species of the Southeast Asian *Crematogaster* subgenus *Decacrema* independently colonized three groups of *Macaranga* species: the *Pachystemon* group *c.* 12 Ma, a smooth-stemmed group *c.* 5 Ma and the *M. pruinosa* group *c.* 4.5 Ma (Quek *et al.*, 2004). As in *Leonardoxa*, colonization of plant hosts required pre-adaptations, such as the ability to adhere to slippery stems or excavation behaviour linked to specific morphological features of their hosts (Federle *et al.*, 1997, 2000; Markstädter *et al.*, 2000; Quek *et al.*, 2004). Lastly, a subgroup of Mesoamerican *Vachellia* co-diversified with *Pseudomyrmex* ants, following a single colonization event c. 5 Ma and subsequent host broadening within the myrmecophytic *Vachellia* (Gómez-Acevedo *et al.*, 2010). Phylogenetic studies of *Macaranga*, *Piper* section *Macrostachys*, *Neonauclea* and *Barteria* (without phylogenies of the relevant ants) have shown independent evolution of domatia within these genera, followed by secondary losses (Blattner *et al.*, 2001; Davies *et al.*, 2001; Tepe *et al.*, 2004; Razafimandimbison *et al.*, 2005; Peccoud *et al.*, 2013). Because of the need to re-associate at each generation, ant–plant symbioses likely involve little or no co-speciation but rather co-diversification, where the interacting groups

Fig. 1 Diversity of ant domatia. (a) *Myrmephytum arfakianum* (Rubiaceae), Arfak Mountains, Papua. The domatium is a swollen hypocotyle with a system of internal galleries. (b) *Hoya imbricata* (Apocynaceae), Indonesia. These so-called 'external' domatia are formed by leaves pressed against the host tree. (c) *Maieta guianensis* (Melastomataceae), Seringalzinho, Rio Jau, Amazonas, Brazil. The domatia consist of leaf pouches at the base of the lamina. (d) *Macaranga indistincta* (Euphorbiaceae) with *Crematogaster* (Myrmicinae) ants, Sabah, Borneo. Note the Beccarian bodies and the entrance holes. The inset shows a longitudinal section of an *M. pearsonii* stem domatium, showing the cultivation of scale insects by *Crematogaster* ants. Photos: (a, b) Andreas Wistuba; (c) Nigel Smith; (d) Eduard Linsenmair; inset, Brigitte Fiala.

diversify by host broadening or switching (Ehrlich & Raven, 1964; Cruaud *et al.*, 2012; de Vienne *et al.*, 2013).

Domatia might be selectively favoured in plants living in nutrient-poor habitats, such as epiphytes (Janzen, 1974), plants that already have EFNs patrolled by nectar-foraging ants, or plants patrolled by ants tending scale insects (Ward, 1991; Davidson & McKey, 1993). Wilson & Hölldobler's (2005) dynastic-succession hypothesis moreover posits that the transition from a diet involving predation on ground-dwelling insects to one involving secretions from tended hemipterans or from nectar glands, associated with aboveground living, occurred relatively late in the history of ants, coinciding with the evolution of angiosperm-dominated tropical forests that provided complex habitats. If such transitions in diet and habitat indeed evolved recently, that is, no earlier than the Eocene, then myrmecophytes inhabited by arboreal ants might be relatively young, something that can be tested with clock-dated phylogenies for relevant plant clades.

By assembling a new list of domatium-bearing vascular plant species worldwide, a large phylogenetic framework for ant-plants, and dated phylogenies for half of all myrmecophyte lineages, we address the following questions about the evolution of ant–plant symbioses.: (1) How often have domatia been gained or lost (a question answerable with minimal estimates from trait reconstructions on phylogenies)? Where in the land plants do we find the highest concentrations of myrmecophyte origins and the largest clades with myrmecophytic species and how clustered are they? (2) Where are these clades located geographically? (3) How old are they? And (4) are there significant differences in the ages of myrmecophyte lineages in the Neotropics, Australasia and Africa? Such age differences might be expected because the African forests were more affected by Miocene and Pliocene climate oscillations than were Australasian and Amazonian forests (van Zinderen Bakker & Mercer, 1986; Jacobs, 2004).

Materials and Methods

Known ant-plants, types of domatia and inference of the likely total ant-plant number

In order to assemble a species-level list of ant-plants we conducted a literature search in Google Scholar (http://scholar.google.com) using the terms 'myrmecophytes', 'domatia', 'ant plants' and 'ant/plant symbiosis'; we also searched monographs of relevant genera, such as *Cecropia*, *Myrmecodia*, *Neonauclea*, *Triplaris* and *Ruprechtia*. We incorporated the genus-level myrmecophyte lists of Davidson & McKey (1993), McKey & Davidson (1993) and Jolivet (1996), and an unpublished list provided by Camilla Huxley-Lambrick in November 2013. The taxonomic assignment of species to genera and families was updated following recent literature and during GenBank (http://www.ncbi.nlm.nih.gov) searches for DNA sequences of myrmecophytes. We define a myrmecophyte as a plant species that has a structure to host ants (a myrmecodomatium); this includes external domatia (Fig. 1b), but excludes plant structures used by ants to make a nest (e.g. the root system of *Coryanthes*, Orchidaceae).

We classified domatia into eight types: (1) stem domatia, any hollow stem or twig, independent of the order or number of shoot axes transformed into domatia; (2) leaf pouches, all pouch domatia formed on the petiole and/or lamina; (3) hollow rachis, the leaf rachis axis is swollen and hollow, as in *Tachigali*; (4) leaf base domatia, a cavity formed into the spaces of interlocked leaf bases, as in *Tillandsia*; (5) stipular domatia, which include stipular thorns, stipular pouches, either closed or open; (6) root tuber domatia, for a transformed root tuber; (7) external domatia, for domatia formed by epiphytes with a structure pressed against the host tree which can be a leaf (Fig. 1b) or a modified stem; (8) hypocotyle with galleries, for the unique domatia of the Hydnophytinae (*Myrmecodia*, *Hydnophytum* and related genera).

Our list of ant-plant species is almost certainly incomplete due to overlooked literature and as yet unrecorded ant–plant symbioses. To estimate the true number of myrmecophytes, we used the model comparison framework implemented in CatchAll (Bunge, 2011). By using the same search terms ('myrmecophytes', 'domatia', 'ant plants' and 'ant/plant symbiosis') and each genus or species name from our list (Supporting Information Table S1) in Google Scholar (as of 1 September 2014), we obtained the frequency of publications per myrmecophyte species and used this as input in CatchAll. We compared five nonparametric models (Good-Turing, Chao1, ACE, ACE1 and Chao–Bunge gamma-Poisson) and five parametric models (Poisson, single exponential mixed Poisson, and mixtures of two, three and four exponentials mixed Poisson) to find the best-fitting estimate (Bunge, 2011).

Alignments and phylogenetic analyses

In order to infer the minimal numbers of gains and losses of domatia in angiosperms, we searched GenBank for the 681 myrmecophytes in our species-level list. For the 323 species present, we searched for their closest relatives, using previously published phylogenies, by including other congeneric species when genera were small, or by similarity based on the 100 highest-scoring BLAST hits of the myrmecophyte target sequence. We also included a representative sample of domatium-lacking families of angiosperms, gymnosperms and ferns, typically with one species per family except for the largest angiosperm families where one species per subfamily was included. The resulting matrix consisted of 1181 species and 3958 sequences downloaded from GenBank (http://www.ncbi.nlm.nih.gov), comprising the nuclear *18S* rDNA and ITS regions, the plastid genes *rbcL*, *matK*, *ndhF* and *atpB*, and the plastid spacers *trnL-trnF* and *atpB-rbcL*. The final matrix comprised 1181 species and 38 080 aligned nucleotides, with 57% missing data (cells in the matrix filled with 'nnn' or '—'), including *rbcL* (799 sequences; 32% missing data), *matK* (752; 36%), *ndhF* sequences (532; 55%), *atpB* sequences (358; 69%), *18S* rDNA sequences (304; 74%), ITS sequences (600; 49%), *trnL-trnF* sequences (488; 60%) and *atpB-rbcL* (135; 88%). Accession numbers are in Table S2.

Tips naming was automated with Phyutility (Smith & Dunn, 2008), and sequences were aligned with MAFFT v7 (Katoh & Standley, 2013). The five genes (*rbcL, matK, atpB, ndhF, 18S* rDNA) were aligned using standard settings. For the more rapidly evolving spacer regions (ITS, *trnL-trnF, atpB-rbcL*), we selected the option 'leave gappy regions unaligned', with a similarity threshold of 0.8. This approach allowed us to align complete ITS sequences across land plants. Minor alignment errors were manually corrected in Mesquite v2.75 (Maddison & Maddison, 2011) and the matrices were concatenated in Geneious v5.4 (Drummond *et al.*, 2011).

Maximum-likelihood (ML) inference relied on RAxML v7.0 (Stamatakis *et al.*, 2008) with 100 ML bootstrap replicates and the analysis partitioned by gene region, all under the GTR + Γ substitution model, as selected under the AIC criterion by jmodeltest2 (Darriba *et al.*, 2012), with six rate categories. The tree was rooted on *Selaginella moellendorfii*. The tree with all tip names is presented in Fig. S1.

Molecular clock dating of myrmecophyte groups

In order to infer absolute divergence times for myrmecophyte lineages, we generated local phylogenies that were more densely sampled than our higher-level vascular plant tree (previous section). For this, we used published datasets representing nearly half of all myrmecophyte-containing lineages: namely *Barteria* (Peccoud *et al.*, 2013), *Clerodendrum*, *Leonardoxa* (Brouat *et al.*, 2001) in Africa; *Cecropia*, *Cordia* (Weeks *et al.*, 2010), Miconiaeae (Melastomataceae; Michelangeli *et al.*, 2004), *Piper* (Tepe *et al.*, 2004), *Platymiscium* (Saslis-Lagoudakis *et al.*, 2008), *Ruprechtia*, *Triplaris* (Sanchez & Kron, 2008) and *Vachellia* (Gómez-Acevedo *et al.*, 2010) from the Neotropics; and *Dischidia*, *Hoya* (Wanntorp *et al.*, 2006), the Hydnophytinae (*Myrmecodia*, *Hydnophytum*, *Myrmephytum*, *Squamellaria*, *Anthorrhiza*), *Macaranga* (Blattner *et al.*, 2001; Davies *et al.*, 2001) and *Neonauclea* (Razafimandimbison *et al.*, 2005) from Australasia. Accession numbers are either in Table S2 or appear next to the respective species name in Figs S2–S15. Alignment and phylogenetic analyses were performed as described above for the 1181-species tree, except that the Q-INS-i approach was selected in MAFFT to take into account RNA secondary structure when aligning the ITS region, as recommended for this marker when aligning fewer than 200 sequences (Katoh & Standley, 2013).

Dating for all data matrices relied on BEAST v1.8 (Drummond *et al.*, 2012) and the GTR + Γ substitution model with six rate categories. The tree prior was a pure-birth (Yule) tree, with MCMC chain lengths between 20 and 60 million generations, sampling every 10 000th generation, with the chain length depending on convergence as determined by examining the log files in Tracer v1.5 (Rambaut & Drummond, 2009) after removal of a burn-in proportion of 10% of the trees. Unless otherwise stated below, we used uncorrelated log-normal (UCLN) clock models. For calibration, we used either secondary constraints from other dated phylogenies or nucleotide substitution rates. Secondary constraints were assigned normal distribution priors with a standard deviation (SD) matching the 95% confidence interval from the original study when presented or otherwise a 20% SD. Specifically, the secondary calibrations were: for *Piper,* the split between *Piper* and *Peperomia* was assigned an age of 91.2 ± 10 Myr (Smith *et al.*, 2008). For *Macaranga*, the split between *Blumeodendron* and the *Hancea* (*Mallotus* (*Macaranga*)) clade was assigned an age of 86.4 ± 5 Myr, the *Mallotus* plus *Macaranga* clade an age of 59 ± 10 Myr, and the *Macaranga* crown an age of 33.5 ± 12 Myr (van Welzen *et al.*, 2014). For *Triplaris*/*Ruprechtia*, the split between Brunnichieae and its sister clade was assigned an age of 69.1 ± 25 Myr (Schuster *et al.*, 2013). For *Platymiscium*, we set the split between *Riedellia* and its sister clade to 47.2 ± 5 Myr (node 47 in Lavin *et al.*, 2005). For *Vachellia*, we assigned the split between the (*Vachellia constricta* (*V. schottii* (*V. neovernicosa*)) clade and its sister group, which includes a myrmecophyte clade, an age of 12.3 ± 3 Myr (Gómez-Acevedo *et al.*, 2010). In the Boraginales, the relationships between the main clades were constrained to match the topology found by Weigend *et al.* (2013) with denser sampling of taxa and genes. We assigned the split between the (*Nama* (*Eriodictyon* (*Wigandia*)) clade and the rest of the Boraginales, including Cordiaceae, an age of 60.4 ± 10 Myr (Weeks *et al.*, 2010), which resulted in an age of 52 Myr for the *Ehretia* stem group, consistent with Eocene *Ehretia* fossil fruits (Gottschling *et al.*, 2002). For the Hydnophytinae (*Squamellaria, Hydnophytum*, *Myrmephytum*, *Anthorrhiza*, *Myrmecodia*), we assigned 14.5 ± 6 Myr to the crown group node of the sister group of Hydnophytinae (Barrabé *et al.*, 2014). For *Neonauclea*, we assigned an age of 40 ± 10 Myr to the root, corresponding to the crown group of the Cinchonoideae (Bremer & Eriksson, 2009). For *Barteria*, we assigned the split of *Barteria* and *Passiflora* to 39 ± 10 Myr using the *Passiflora* stem group age (Hearn, 2006).

For clades that lack fossils and have not been clock-dated in other studies, we used published substitution rates for calibration and strict or relaxed clock models following analyses of the extent of rate heterogeneity in Tracer. Because rates can vary greatly and may correlate with generation time (Kay *et al.*, 2006; Smith & Donoghue, 2008), we used three rates for each phylogeny, spanning the range of plausible rates. For *Leonardoxa* and the Miconieae, we used rates of 1×10^{-9}, 2×10^{-9}, or 3×10^{-9} substitutions per site per year, representative of ITS in woody species (Kay *et al.*, 2006), with a strict clock model for *Leonardoxa* and UCLN relaxed clocks for *Neonauclea* and the Miconieae. For *Clerodendrum*, we used a strict clock model and rates of 1×10^{-9}, 2×10^{-9}, or 3×10^{-9} substitutions per site per year for both ITS and the *trnL-F* region (Chase *et al.*, 1993; Richardson *et al.*, 2001; Kay *et al.*, 2006). To calibrate the Apocynaceae matrix of Wanntorp *et al.* (2006), which consists of two plastid spacer regions and nuclear ITS, we used a strict clock and rates of 2.5×10^{-9}, 3.5×10^{-9}, or 4.5×10^{-9} substitutions per site per year, consistent with noncoding plastid and ITS substitution rates in other herbaceous perennials (Manen & Natali, 1995; Richardson *et al.*, 2001; Kay *et al.*, 2006) For *Cecropia*, we built a combined *trnL-F, rbcL* and *matK* matrix and used a strict clock with substitution rates of 0.8×10^{-9}, 1.2×10^{-9} or 2×10^{-9} substitutions per site per year, based on rates for these loci in other woody groups (Chase *et al.*, 1993; Richardson *et al.*, 2001; Lavin *et al.*, 2005).

We cross-validated age estimates against those from published studies with overlapping taxon sampling. The trees obtained from each clock run were summarized with TreeAnnotator v1.8.0, with a 10% burn-in and showing only nodes ≥ 0.98 posterior probability. Time-calibrated trees are shown in Figs S2–S15.

Sister-group geographic mapping

We selected 20 sister clade pairs from our 1181-species tree or published phylogenies and then downloaded the geographic ranges of these closest relatives from the Global Biodiversity Information Facility (GBIF) (http://www.gbif.org/species). The closest relatives were *Acacia cochliacantha*, *Adenia cynachifolia*, *Androsiphon adenostegia*, *Conceveiba pleistemona*, *Cordia collococa*, *Cordia ecalyculata*, *Cuviera subuliflora*, *Euphronia guianensis*, *Henriettea succosa*, *Korthalsia jala*, *Leucosyke australis*, *Ludekia borneensis*, *Mallotus brachythyrsus*, *M. nudiflorus*, *M. ficifolius*, *Macbridenia peruviana*, *Microsorum linguiforme*, *Piper aequale*, *Psychotria hawaiensis* and *Ruprechtia triflora*. The distributions were plotted on a world map using DIVA-GIS (Hijmans *et al.*, 2005). The mean annual temperatures were downloaded from WorldClim (http://www.worldclim.org/).

Ancestral state reconstructions

In order to reconstruct gains and losses of domatia, we scored domatium absence (0) and presence (1) for all 1181 species in our tree based on our World myrmecophyte list (Table S1). Ancestral reconstruction relied on maximum likelihood (ML) implemented in Mesquite using the highest scoring likelihood tree and the Markov two-parameter model (Lewis, 2001), which allows for different forward and backward change frequencies. Domatium presence in a common ancestor was assumed if the ML probability was ≥ 70%. We added a single gain for genera with domatium-bearing species (Table S1) that were not included in our 1181-species matrix.

We also inferred the evolution of extrafloral nectaries on our 1181-species tree, using the same approach. We scored EFN-bearing species as 1, and EFN-lacking species as 0, according to the World List of plants with extrafloral nectaries (Keeler, 2008). We also mapped EFNs onto the *Macaranga* and the *Vachellia* chronograms. In addition to the ML approach implemented in Mesquite, we inferred ancestral states (both for EFNs and domatia) in *Vachellia* and *Macaranga* using the Bayesian reversible-jump MCMC approach for discrete characters implemented in BayesTraits (Pagel & Meade, 2007) on a sample of 2000 trees from BEAST (burn-in excluded), thereby taking into account topological uncertainty. The chain was run for 50×10^6 generations, and rate coefficients and ancestral states were sampled every 1000th generation. We ensured that the acceptance rate was between 20% and 40% as recommended in the manual.

Results

Frequency and geography of ant–plant symbioses, and the distribution of domatium types and growth forms

Our world list of myrmecophytes includes 681 species in 159 genera and 50 families (Table S1, which also provides information on geographic ranges). Our modelling approach to estimate the true number of myrmecophytes (including ones not yet documented or missed in our literature search) yielded 1139 species under the best-fit model (1-exponential mixed Poisson, Table 1).

Ant–plant symbioses are almost exclusively tropical. Exceptions are species of *Vachellia* ranging into South Texas and African *Vachellia drepanolobium* south of the Tropic of Capricorn. There are strong diversity asymmetries in absolute species numbers, with overall *c.* 7 times more ant-plant species than plant-ant species. This asymmetry is present in all three biogeographic regions (Fig. 2) and may be strongest in Australasia, although that might be an artefact of the lack of taxonomic knowledge of Australasian ants and cryptic species complexes (personal communications from M. Janda, Czech Academy of Sciences, October 2013, and V. Witte, University of Munich, June 2014). Closest relatives of ant-plant clades for which we could evaluate geographic ranges were all distributed in the tropics and absent from temperate regions (Fig. 2).

Domatium-bearing plants are present in one family of ferns, absent in gymnosperms, and generally widespread in angiosperms, although they are absent in basal eudicots. The higher eudicots, however, contain the majority of myrmecophytes, with Rubiaceae having the highest number (162 species, Table S1), followed by Melastomataceae (144 species, Table S1). The ancestral reconstruction implies 158 independent origins and 43 losses of domatia (Fig. 3). In some genera, such as *Cecropia*, *Dischidia*,

Table 1 Predicted total number of ant domatium-bearing species from model estimates

Model		Tau	Estimated total species	SE	Lower CB	Upper CB
Best model	1 exponential mixed Poisson	8	1139	40	1067	1224
Model 2a	Poisson	5	805	16	778	840
Model 2b	2 exponential mixed Poisson	10	1160	72.8	1037	1325
Model 2c	2 exponential mixed Poisson	40	1159	49.5	1072	1267
Non-P1	Chao1	2	842	28.4	795	908
Non-P2	ACE1	10	1070	59.6	969	1205

Tau is the upper frequency cut-off; SE, the standard error of the estimate; Lower and Upper CB, the 95% confidence bound. The best model (first line) is followed by the three next best-fit parametric models (Models 2a–c) and the two best-fitting nonparametric models (non-P1, P2).

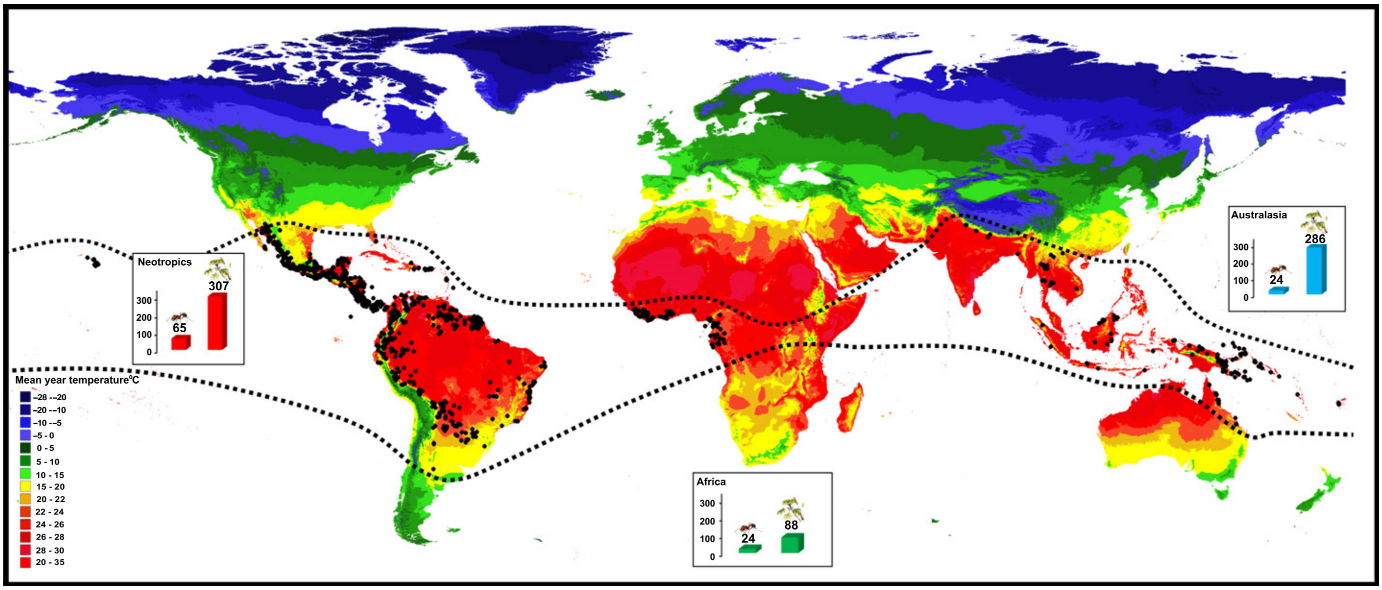


Fig. 2 Distribution of myrmecophyte sister groups on a map showing the mean annual temperature (WorldClim). The dots show the distribution of 20 species sister to over half of all 681 myrmecophytes. The dashed lines show the northern and southern borders of myrmecophyte sister group distribution. The inset shows the numbers of myrmecophytes and plant-ants in the three biogeographic regions based on our species list (Supporting Information Table S1).

Macaranga, *Neonauclea*, *Piper*, *Platymiscium* and *Tococa*, domatia evolved several times (Figs 3, S3, S6, S9–S13). In the Miconiaeae (*Tococa* and relatives; Fig. S10), leaf pouch domatia have been gained at least eight times and lost seven times, whereas in *Barteria*, *Myrmecodia* and *Triplaris* domatia appear to have evolved but once (Figs 3, S2, S7, S14). Overall, gains were over three times more frequent than losses, which may partly be due to under-sampling of myrmecophyte-rich genera that might include additional losses (e.g. *Cecropia*, *Hydnophytum*).

Stem domatia make up more than half of all domatium types (354 of 681) and were the most common type in all three biogeographic regions. Stem domatia also evolved and were lost most often (Fig. 4c). Leaf pouches are found in the Neotropics and in Africa, but are almost absent in Australasia. Hollow rachis and leaf base domatia are the particular domatium types of *Tachigali* and *Tillandsia*, respectively, and hence are restricted to the Neotropics. Australasian epiphytes, such as the ferns *Platycerium* and *Lecanopteris mirabilis* or the Apocynaceae *Hoya imbricata* (Fig. 1b), typically have external domatia, whereas Hydnophytinae (*Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, *Myrmephytum* (Fig. 1a), *Squamellaria*) have swollen hypocotyls with ant galleries (Fig. 4).

Most Neotropical and African ant-plants are trees or shrubs with 79% (239 species) and 95% (83 species), respectively (Fig. 4b). By contrast, trees and shrubs make up only 40% (113 species) of the Australasian ant-plants, whereas epiphytes contribute 47% (131 species). In Africa, epiphytic ant-plants are lacking, and in the Neotropics they represent < 15%. Lianas are a rare growth form among myrmecophytes, especially in the Neotropics (0.3%), but also in Africa (3%) and Australasia (10%). Herbs are also infrequent, with the Neotropics having the highest percentage (6%), especially due to species of *Piper* (Tepe *et al.*, 2004).

Ages of domatium-bearing plant clades

In order to infer when the oldest extant domatia may have originated, we generated dated phylogenies for clades that together include 56% of the 681 known domatium-bearing species. These clock-dated lineages represent 45 independent acquisitions of domatia (Figs S2–S15); their ages are plotted in Fig. 5(a). Regardless of whether 'slow', 'standard' or 'high' substitution rates were used for calibration (see the Materials and Methods section), no ant-plant crown age (or stem age for single myrmecophyte species) is older than 19 Myr, and the oldest domatium-bearing species or species groups in Africa date only to the last 6 Myr. An exception is the split of *Clerodendrum rotundifolium* from its nondomatium-bearing sister group, which was dated to 10.5 Myr using the slowest of the three employed substitution rates (Figs 5a, S8). The recent origin of African myrmecophytes matches the significantly smaller species numbers in African myrmecophyte radiations (maximal radiation size evaluated by the sum of myrmecophytes per genus, Fig. 5b) as compared to Australasian and New World radiations (Fig. 5b; *t*-test, $P < 0.01$).

Discussion

Recurrent entry into a new adaptive zone by plants with ant domatia

Ant–plant symbioses are an almost exclusively tropical phenomenon (Fig. 2). That the sister groups of myrmecophyte lineages also occur in the tropics confirms that these symbioses evolved there, rather than arising by immigration of facultative ant–plant mutualisms from higher latitudes. The only extratropical domatium-occupying ants are species in the *Pseudomyrmex ferrugineus*

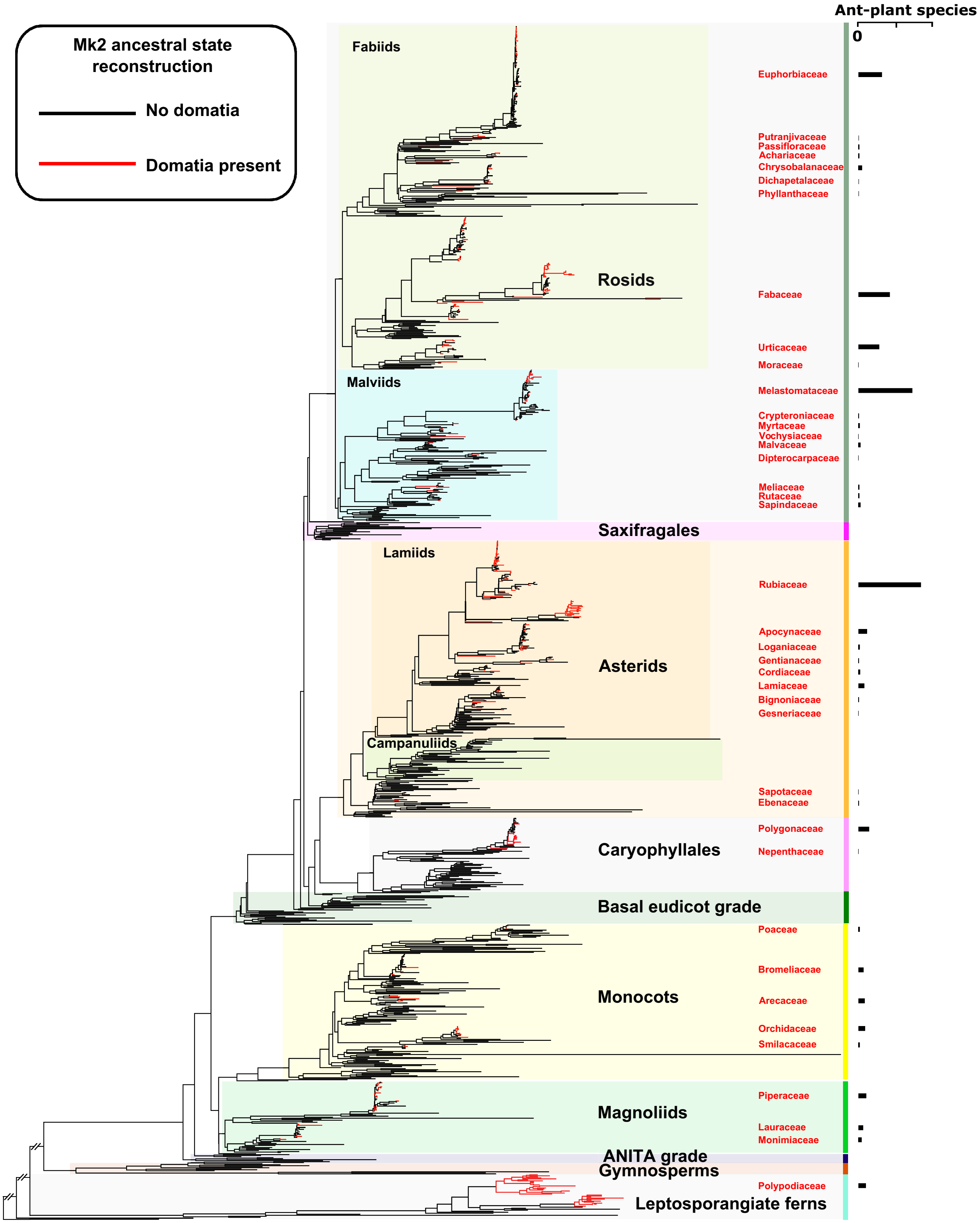


Fig. 3 Gains and losses of domatia in plants, inferred using a 2-parameter Markov model on a maximum likelihood 8-gene tree of 1181 species oversampled for myrmecophytes. Families with ant-plants in red, with the number of their ant-plant species on the right. Species names at tips are shown in Fig. S1.

group and some *Crematogaster*. The still scarce DNA sequence data for ants precluded detailed phylogenetic evaluation of the origins of plant-living ants, but the 31 ant genera known to nest in domatia provide a minimum bound (Fig. S16). In the subfamily Pseudomyrmecinae, in which *c.* 40 of *c.* 230 species nest in domatia, Ward & Downie (2005, with a sampling of 49 of 230

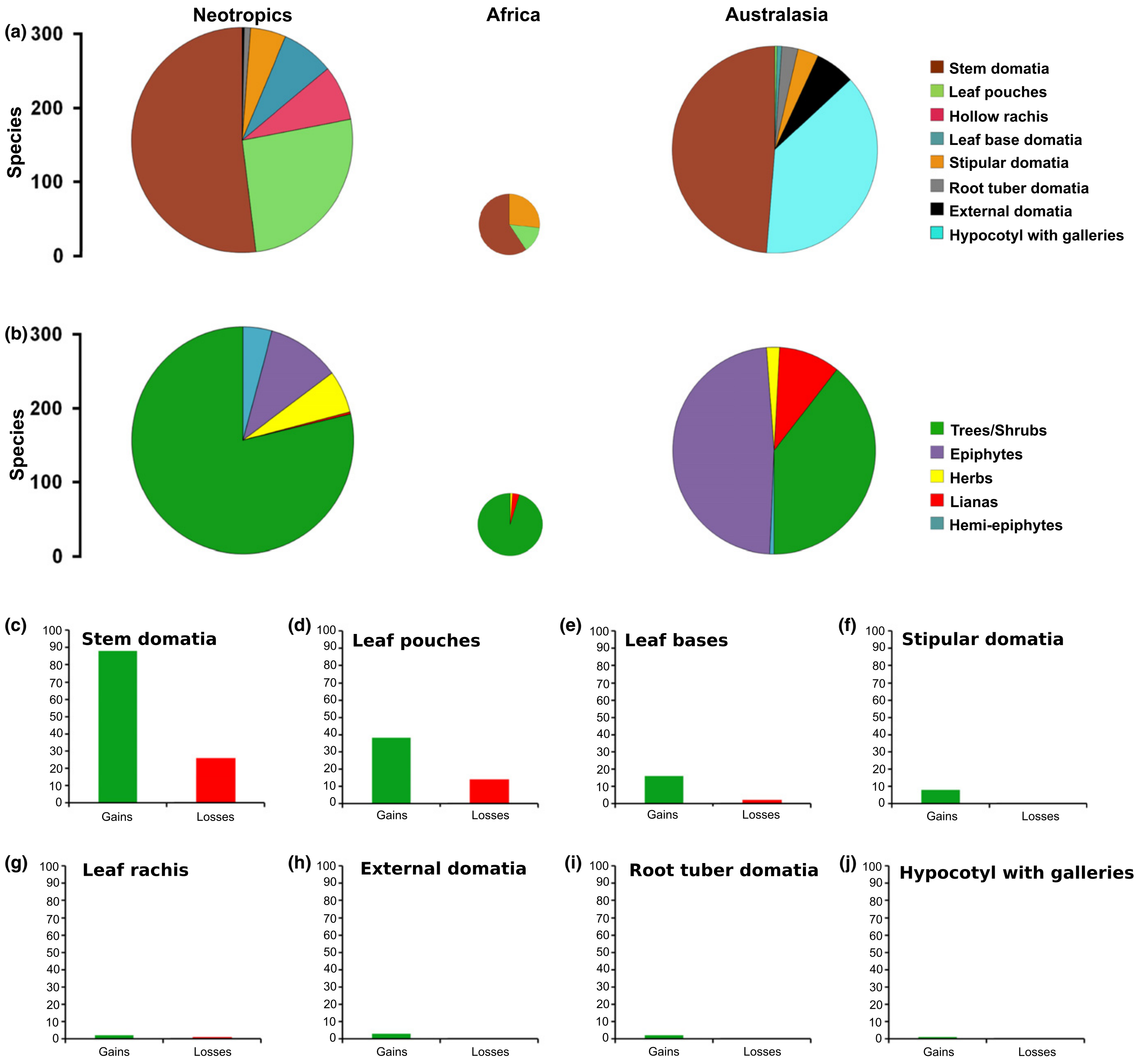


Fig. 4 Distributions and evolution of domatium types, and growth habit of myrmecophytes. (a) Distribution of domatium types in the different biogeographic regions. (b) Myrmecophyte habit. (c–j) Evolutionary gains and losses of domatium types, as inferred from the maximum likelihood (ML) reconstruction on the large tree (Fig. 3).

species) inferred 10 independent transitions to obligate domatium nesting. Together with the 31 ant genera, this yields *c.* 40 independent transitions towards obligate nesting in domatia.

The roughly three-fold higher number of domatium gains (158) than plant-ant origins (40–60) implies that recruitment of new myrmecophyte lineages through expansion of ants to non-myrmecophyte plants that subsequently evolved domatia is a driver for the evolution of additional myrmecophytes. Host switching or broadening had been inferred between species of *Macaranga* (Quek *et al.*, 2004) and *Vachellia* (Gómez-Acevedo *et al.*, 2010), but not between phylogenetically distant plant groups. Whatever the predominant modes of new host recruitment, ant–plant symbioses do not appear to have led to particular species richness in the involved plant or ant lineages (although we conducted no formal tests). In the few ant-plant clades with high species number (Hydnophytinae, *Neonauclea*, *Macaranga*), it is possible to envisage higher diversification rates, but the global scatter of domatium evolution suggests that presence of this trait did not significantly enhance plant diversity. Of the 158 origins of domatia inferred in this study, most are associated with small radiations (species-poor clades) or no radiation (Fig. 5b). This implies that specialized ant–plant symbioses either represent an evolutionary 'dead-end' and go extinct, or that domatia are lost as readily as they are gained (Peccoud *et al.*, 2013). Domatium loss has been inferred in *Macaranga* (Blattner *et al.*, 2001; Davies *et al.*, 2001; Fig. S9), *Neonauclea* (Razafimandimbison *et al.*,

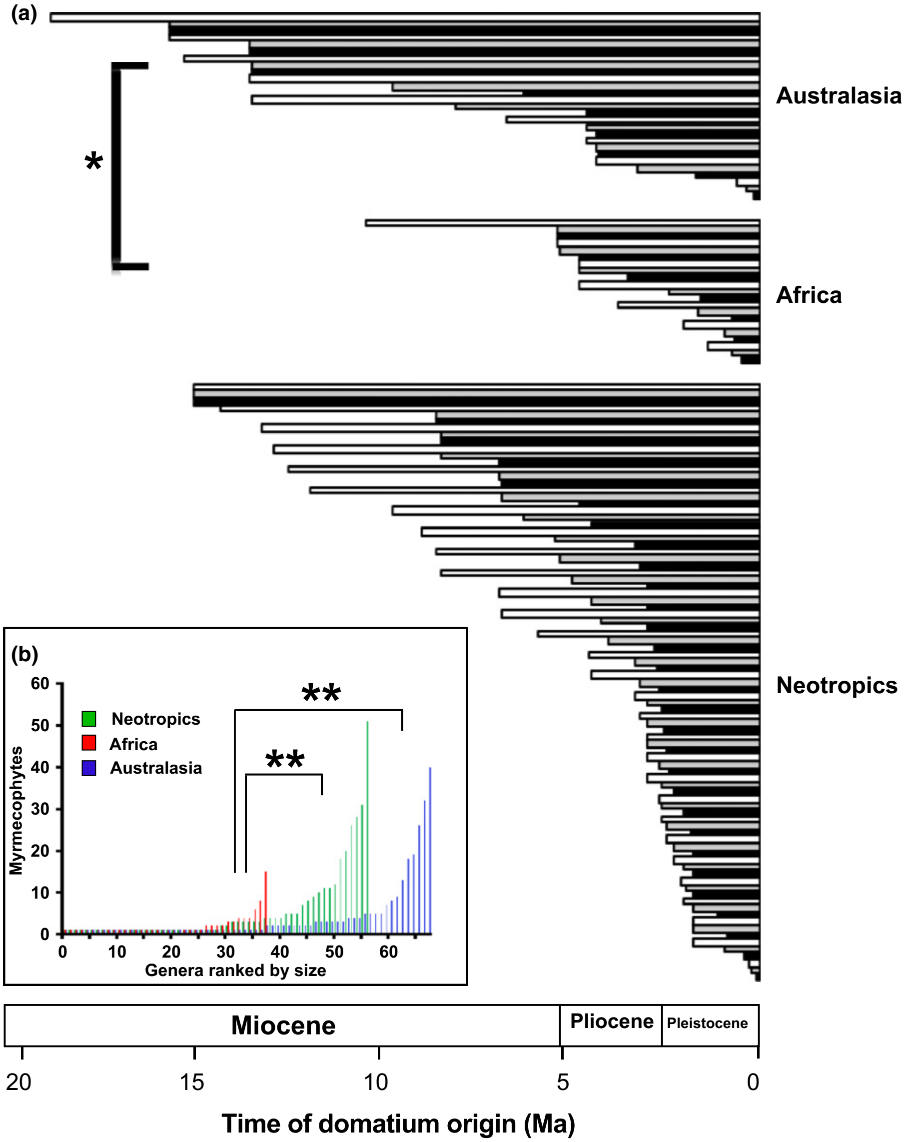


Fig. 5 Temporal build-up of myrmecophytes and maximal size of ant plant radiations in the Neotropics, Africa, and Southeast Asia/ Australia. (a) Oldest inferred appearances of domatia in the three regions. Each bar represents a crown age for a node inferred to represent independent evolution of domatia. Black, grey and white shows the 'slow', 'standard' and 'high' substitution rates approaches, respectively, for lineages lacking fossil or secondary calibrations (see the Materials and Methods section). Ma, Myr ago. (b) Number of myrmecophyte species per genus in the three regions, suggesting the maximal sizes of ant-plant radiations. Asterisks refer to statistical differences in *t*-tests in (a) and (b): *, $P < 0.05$; **, $P < 0.01$.

2005; Fig. S11), *Tococa* (Michelangeli *et al.*, 2004; Fig. S10) and *Barteria* (Peccoud *et al.*, 2013), and we here inferred 43 further losses. Nevertheless, the minimally 158 gains of domatia show the recurrent entry of plant lineages into a new adaptive zone *sensu* Ehrlich & Raven (1964). The apparent evolutionary lability of domatia, with for instance minimally eight gains and seven losses in the Miconieae alone (Figs 1, S10), matches their phenotypic plasticity. For example, in *Tococa*, the same individual can possess or lack leaf pouch domatia (Renner, 1997), suggesting that domatium development, at least in the Miconieae, may depend on labile gene expression switches.

Scattered phylogenetic pattern of ant plants

An important result of this study is the phylogenetic scatter of domatium-bearing species. This differs from other findings of the homoplasy of traits relating to ant–plant interaction such as EFNs (Marazzi & Sanderson, 2010; Weber & Agrawal, 2014) or the epiphytic habit (Chomicki *et al.*, 2015), which both show more clustered phylogenetic patterns. A pattern first revealed here is the parallel (homoplasic) evolution of similar domatium types among closely related species, for example in the Miconieae. The frequent domatium loss that we also detected might relate to antagonistic interactions among closely related species competing for plant-ants.

Traits that may have facilitated domatium evolution

Traits that may facilitate the evolution of ant–plant symbioses have long been discussed (Benson, 1985; Davidson & McKey, 1993). One such trait might be extrafloral nectaries, in which case one would expect that many myrmecophyte lineages would ancestrally have EFNs. Our trait reconstructions, however, revealed that although 14 myrmecophyte lineages have EFNs (African *Vachellia*, *Barteria*, *Callicarpa japonica*, *Fagraea*, *Ficus obscura*, *Hirtella*, *Humboldtia*, *Leonardoxa*, *Macaranga*, *Nepenthes bicalcarata*, *Pometia*, *Shorea acuminata*, Mesoamerican *Vachellia*, *Zanthoxylum*; Fig. S17), only in Mesoamerican *Vachellia* did domatium-bearing species clearly evolve from EFN-bearing ancestors (Fig. S17b).

Many different plant organs have been transformed into domatia (Fig. 1). Stipular thorns and large bud-protecting stipules likely facilitated the evolution of stipular domatia (Davidson & McKey, 1993). Palmate venation, found for instance in Neotropical Melastomataceae and African Malvaceae, may have fostered the evolution of leaf pouches, whether for mites or for ants (Schnell & Grout de Beaufort, 1966; O'Dowd & Willson, 1989; Davidson & McKey, 1993). Stem domatia require a minimal diameter of primary stem, and thus seem to have evolved primarily in thick-stemmed plants. Another trait that may have facilitated domatium evolution is seed dispersal by ants, and indeed Australasian epiphytic myrmecophytes (e.g. *Dischidia*, *Hoya*, *Hydnophytum*, *Myrmecodia*) often form ant gardens and are also dispersed by ants (Huxley, 1978, 1980; Kaufmann, 2002; Kaufmann & Maschwitz, 2006).

The growth form spectrum of myrmecophytes

Figure 4 shows that among ant-plants, lianas and herbs are rare growth forms and that the Neotropics have very few epiphytic myrmecophytes. Given that *c.* 19% of tropical vascular plants species are climbers and 41% are herbs (Gentry & Dodson, 1987; Gentry, 1991), the 4.5% climbers and 3.1% herbs found among ant-plants are far fewer than expected (Fig. 4). The underrepresentation of herbs may relate to the minimum plant size and generation time needed to bear and maintain domatia. In the Neotropics, all 20 herbaceous ant-plants are *Piper* species from section *Macrostachys* that form large perennial herbs. The remaining herbaceous ant plants also are perennials with sufficiently large stems (Jolivet, 1973, 1996; Champluvier, 1994; Kaufmann *et al.*, 2001; Tepe *et al.*, 2004; our Table S1). The underrepresentation of climbers may relate to their narrow stems and numerous contact points with surrounding vegetation, increasing the likelihood of invasion by nonmutualistic ants. In the African climbing ant-plant *Vitex thyrsiflora,* occupied by the specialized ant *Tetraponera tessmanii*, morphological and behavioural filters prevent other ants from entering the domatia (Djiéto-Lordon *et al.*, 2005).

Recent ages of domatium-based symbioses compared to seed dispersal and nectar-feeding mutualisms

A major finding of this study is that ant plant lineages in Africa may not be much older than 5 Myr and those in the Neotropics and Australasia not much older than 15 Myr (Fig. 5). The younger age of African myrmecophytes, associated with a three times lower species richness than found in the other two regions (Fig. 2b), suggests that the climate oscillations in tropical Africa during the late Miocene and Pliocene (van Zinderen Bakker & Mercer, 1986; Jacobs, 2004) either limited diversification or drove older myrmecophytes to extinction. During the middle Miocene, starting from *c.* 16 Ma onwards, the African continent underwent gradual cooling and uplift in the east and south, leading to an expansion of woodlands and savannas, and reducing lowland rain forests (van Zinderen Bakker & Mercer, 1986; Jacobs, 2004), which harbour most African myrmecophytes. The mid-Miocene ages of Neotropical *Pseudomyrmex* (Pseudomyrmecinae) and *Azteca* (Dolichoderinae) inferred in other studies (Gómez-Acevedo *et al.*, 2010; Pringle *et al.*, 2012) also fit with a relatively recent evolution of ant–plant symbioses (Fig. 5).

Because ants and plants have been coexisting for at least 120 Myr (Brady *et al.*, 2006; Bell *et al.*, 2010; Moreau & Bell, 2013), it has been suggested that ant–plant symbioses in general are old, notwithstanding a few young ant–plant symbioses (Davidson & McKey, 1993). For example, Jolivet (1996: 169) suggested that 'Myrmecodomy must be very old, i.e. since the Cretaceous, and it is at that period that we find the first fossil ants.' Yet none of the 46 myrmecophyte lineages for which we estimated crown group ages predates the mid-Miocene. Seed dispersal by ants (myrmechochory) apparently had arisen by 75 Ma (Dunn *et al.*, 2007; Lengyel *et al.*, 2009), whereas EFNs have arisen over the past 50 Myr (*Passiflora*: 322 species with EFNs, 40 Myr, Hearn, 2006; *Inga*: 294 species with EFNs, 10 Myr, Richardson *et al.*, 2001; *Senna*: *c.* 250 species with EFN; 40 Myr, Marazzi & Sanderson, 2010), and no EFN fossils pre-date the Oligocene (*Populus*: Pemberton, 1992; *Macaranga*, *Mallotus*: Nucete *et al.*, 2012). Today, EFNs are known from 3941 vascular plants, and they evolved at least 457 times (Weber & Keeler, 2013), whereas domatia are known from 681 vascular plants and evolved minimally 158 times (this study). Ant domatia so far have no fossil record, different from mite domatia (O'Dowd *et al.*, 1991).

From these data, the evolutionary sequence of ant–plant mutualisms may have begun with seed dispersal in the Late Cretaceous, followed by EFNs in the Eocene, and the evolution of domatium nesting during the Miocene. In their dynastic-succession hypothesis, Wilson & Hölldobler (2005) emphasized the importance of complex habitats provided by angiosperms for the transition of ants from a diet based on insect predation to harvesting hemipteran secretions and EFN nectar. Sugary secretions as a diet supplement are key to arboreal ant diets because there are not enough canopy-dwelling insects to sustain large ant colonies hunting for insect prey (Davidson *et al.*, 2003; Wilson & Hölldobler, 2005). The Miocene origin of many epiphyte domatia inferred in our study is consistent with Wilson & Hölldobler's scenario because these domatia are inhabited by arboreal ants, often tending scale insects and collecting nectar from EFNs. Because nesting space is commonly a limiting resource for ants (Philpott & Foster, 2005), the evolution of domatia as additional ant nesting sites may have been in part driven by Miocene ant radiations in tropical canopies (Brady *et al.*, 2006; Moreau *et al.*, 2006; Moreau & Bell, 2013). At the same time, canopy epiphytes clearly gain from the additional nutrients coming from symbiotic ants (Benzing, 1970; Huxley, 1978; Rickson, 1979; Rico-Gray *et al.*, 1989; Gay, 1993; Treseder *et al.*, 1995; Gegenbauer *et al.*, 2012).

Conclusions

There now are 681 known ant-plant species (Table S1), but their true number may be as high as 1139 species. The absence of the ancestral trait ‘EFN’ in most domatium-bearing lineages suggests that ant-plant symbioses rarely evolved from pre-existing defence mutualisms. More commonly, they may have evolved from parasitic relationships with scale insects, a trait we did not analyse *per se*, but which is common in domatium-living ants. Among the largest ant-plant groups (all molecular-clock dated here), none are older than 19 Myr, that is early Miocene, with African ant–plant symbioses apparently not pre-dating the late Miocene. Radiations of domatium-bearing lineages have produced few sizeable clades (Hydnophytinae, *c.* 100 species, 80 of them ant-plants; *Cecropia*, 61 species, 48 ant-plants), losses of domatia are frequent, and radiations in the associated plant-ants are also species-poor, resulting in a scattered phylogenetic distribution (Fig. S16). There is thus no straightforward effect of ant–plant symbioses on diversification rates.

Acknowledgements

We thank Matthew Jebb for his unpublished revision of *Hydnophytum* and Camilla Huxley-Lambrick for an unpublished list of ant-plants; William J. Baker, Milan Janda, Kathleen Keeler, Conrad Labandeira, Marjorie Webber and Volker Witte for discussion; Brigitte Fiala, Eduard Linsenmair, Nigel Smith and Andreas Wistuba for the photographs used in Fig. 1; and Jeremy Aroles for helping compile the species list in Table S1. We thank Martin Heil, Doyle McKey and an anonymous reviewer for critical comments that helped improve the manuscript.

References

Barrabé L, Maggia L, Pillon Y, Rigault F, Mouly A, Davis AP, Buerki S. 2014. New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Molecular Phylogenetics and Evolution* 71: 15–35.

Bell CD, Soltis DE, Soltis PS. 2010. The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97: 1296–1303.

Benson WW. 1985. Amazon ant/plants. In: Prance GT, Lovejoy TE, eds. *Amazonia.* Oxford, UK: Pergamon Press, 239–266.

Benzing DH. 1970. An investigation of two bromeliad myrmecophytes: *Tillandsia butzii* Mez, *T. caput-medusae* E. Morren, and their ants. *Bulletin of the Torrey Botanical Club* 97: 109–115.

Blattner FR, Weising K, Bänfer G, Maschwitz U, Fiala B. 2001. Molecular analysis of phylogenetic relationships among myrmecophytic *Macaranga* species (Euphorbiaceae). *Molecular Phylogenetics and Evolution* 19: 331–344.

Brady SG, Schultz TR, Fisher BL, Ward PS. 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences, USA* 103: 18172–18177.

Bremer B, Eriksson T. 2009. Time tree of Rubiaceae: phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Sciences* 170: 766–793.

Brouat C, Gielly L, McKey D. 2001. Phylogenetic relationships in the genus *Leonardoxa* (Leguminosae: Caesalpinioideae) inferred from chloroplast trnL intron and trnL-trnF intergenic spacer sequences. *American Journal of Botany* 88: 143–149.

Brouat C, McKey D, Douzery EJP. 2004. Differentiation in a geographical mosaic of plants coevolving with ants: phylogeny of the *Leonardoxa africana* complex (Fabaceae: Caesalpinioideae) using amplified fragment length polymorphism markers. *Molecular Ecology* 13: 1157–1171.

Bunge J. 2011. Estimating the number of species with CatchAll. *Pacific Symposium on Biocomputing* 2011: 121–130.

Champluvier D. 1994. *Brachystephanus myrmecophilus* (Acanthaceae), espèce nouvelle du Zaïre oriental: un cas intéressant de myrmécophilie. *Belgian Journal of Botany* 127: 45–60.

Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price A, Hills HG, Qiu Y-L *et al.* 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden* 80: 528–580.

Chenuil A, McKey DB. 1996. Molecular phylogenetic study of a myrmecophyte symbiosis: did *Leonardoxa*/ant associations diversify via cospeciation? *Molecular Phylogenetics and Evolution* 6: 270–286.

Chomicki G, Bidel LPR, Ming F, Coiro M, Zhang X, Wang Y, Baissac Y, Jay-Allemand C, Renner SS. 2015. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. *New Phytologist* 205: 1330–1341.

Cruaud A, Rønsted N, Chantarasuwan B, Chou LS, Clement WL, Couloux A, Savolainen V. 2012. An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Systematic Biology* 61: 1029–1047.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Davidson DW, Cook SC, Snelling RR, Chua TH. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300: 969–972.

Davidson DW, McKey D. 1993. The evolutionary ecology of symbiotic ant/plant relationships. *Journal of Hymenopteran Research* 2: 13–83.

Davies SJ, Lum SK, Chan R, Wang LK. 2001. Evolution of myrmecophytism in western Malesian *Macaranga* (Euphorbiaceae). *Evolution* 55: 1542–1559.

Dejean A, Olmsted I, Snelling RR. 1995. Tree-epiphyte–ant relationships in the low inundated forest of Sian Ka’an Biosphere Reserve, Quintana Roo, Mexico. *Biotropica* 27: 57–70.

Djiéto-Lordon C, Dejean A, Ring RA, Nkongmeneck BA, Lauga J, McKey D. 2005. Ecology of an improbable association: the pseudomyrmecine plant-ant *Tetraponera tessmanni* and the myrmecophytic liana *Vitex thyrsiflora* (Lamiaceae) in Cameroon. *Biotropica* 37: 421–430.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M. 2011. *Geneious, version 5.4.* Auckland, New Zealand: Geneious.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.

Dunn RR, Gove AD, Barraclough TG, Givnish TJ, Majer JD. 2007. Convergent evolution of an ant-plant mutualism across plant families, continents, and time. *Evolutionary Ecology Research* 9: 1349.

Ehrlich PR, Raven PH. 1964. Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.

Federle W, Maschwitz U, Fiala B, Riederer M, Hölldobler B. 1997. Slippery ant-plants and skilful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). *Oecologia* 112: 217–224.

Federle W, Rohrseitz K, Hölldobler B. 2000. Attachment forces of ants measured with a centrifuge: better ‘wax-runners’ have a poorer attachment to a smooth surface. *Journal of Experimental Biology* 203: 505–512.

Gay H. 1993. Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern Lecanopteris Reinw. (Polypodiaceae). *Biological Journal of the Linnean Society* 50: 221–233.

Gegenbauer C, Mayer VE, Zotz G, Richter A. 2012. Uptake of ant-derived nitrogen in the myrmecophytic orchid *Caularthron bilamellatum. Annals of Botany* 110: 757–766.

Gentry AH. 1991. The distribution and evolution of climbing plants. In: Putz FE, Mooney HA, eds. *The biology of vines.* Cambridge, UK: Cambridge University Press.

Gentry AH, Dodson CH. 1987. Diversity and biogeography of neotropical vascular epiphytes. *Annals of the Missouri Botanical Garden* 74: 205–233.

Gómez-Acevedo S, Rico-Arce L, Delgado-Salinas A, Magallón S, Eguiarte LE. 2010. Neotropical mutualism between *Acacia* and *Pseudomyrmex*: phylogeny and divergence times. *Molecular Phylogenetics and Evolution* 56: 393–408.

Gottschling M, Mai DH, Hilger HH. 2002. The systematic position of *Ehretia* fossils (Ehretiaceae, Boraginales) from the European Tertiary and implications for character evolution. *Review of Palaeobotany and Palynology* **121**: 149–156.

Hearn DJ. 2006. *Adenia* (Passifloraceae) and its adaptive radiation: phylogeny and growth form diversification. *Systematic Botany* **31**: 805–821.

Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Bueno JCS. 2009. Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences, USA* **106**: 18091–18096.

Heil M, Rattke J, Boland W. 2005. Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* **308**: 560–563.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965–1978.

Huxley CR. 1978. The ant-plants Myrmecodia and Hydnophytum Rubiaceae, and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* **80**: 231–268.

Huxley CR. 1980. Symbiosis between ants and epiphytes. *Biological Reviews* **55**: 321–340.

Jacobs BF. 2004. Palaeobotanical studies from tropical Africa: relevance to the evolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society B* **359**: 1573–1583.

Janzen DH. 1967. Interaction of the bull's-horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. *University of Kansas Science Bulletin* **47**: 315–558.

Janzen DH. 1969. Allelopathy by myrmecophytes: the ant *Azteca* as an allelopathic agent of *Cecropia*. *Ecology* **50**: 147–153.

Janzen DH. 1974. Epiphytic myrmecophytes in Sarawak: mutualism through the feeding of plants by ants. *Biotropica* **6**: 237–259.

Jolivet P. 1973. Les plantes myrmecophiles du Sud Est Asiatique. *Cahiers du Pacifique* **17**: 41–69.

Jolivet P. 1996. *Ants and plants: an example of coevolution.* Leiden, the Netherlands: Backhuys.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kaufmann E. 2002. *Southeast Asian ant-gardens: diversity, ecology, ecosystematic significance, and evolution of mutualistic ant–epiphyte associations.* PhD thesis, University of Frankfurt, Frankfurt, Germany.

Kaufmann E, Maschwitz U. 2006. Ant-gardens of tropical Asian rainforests. *Naturwissenschaften* **93**: 216–227.

Kaufmann E, Weissflog A, Hashim R, Maschwitz U. 2001. Ant-gardens on the giant bamboo *Gigantochloa scortechinii* (Poaceae) in West-Malaysia. *Insectes Sociaux* **48**: 125–133.

Kay KM, Whittall JB, Hodges SA. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evolutionary Biology* **6**: 36.

Keeler KH. 2008. *World list of angiosperms with extrafloral nectaries.* [WWW document] URL http://biosci-labs.unl.edu/Emeriti/keeler/extrafloral/worldlistfamilies.htm [accessed 1 June 2014].

Lavin M, Herendeen PS, Wojciechowski MF. 2005. Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Systematic Biology* **54**: 575–594.

Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2009. Ants sow the seeds of global diversification in flowering plants. *PLoS ONE* **4**: e5480.

Lewis PO. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* **50**: 913–925.

Maddison WP, Maddison DR. 2011. *Mesquite 2.75: a modular system for evolutionary analysis.* [WWW document] URL http://mesquiteproject.org [accessed 1 June 2014].

Magallón S, Hilu KW, Quandt D. 2013. Land plant evolutionary timeline: gene effects are secondary to fossil constraints in relaxed clock estimation of age and substitution rates. *American Journal of Botany* **100**: 556–573.

Manen JF, Natali A. 1995. Comparison of the evolution of ribulose-1, 5-biphosphate carboxylase (rbcL) and atpB-rbcL noncoding spacer sequences in a recent plant group, the tribe Rubieae (Rubiaceae). *Journal of Molecular Evolution* **41**: 920–927.

Marazzi B, Sanderson MJ. 2010. Large-scale patterns of diversification in the widespread legume genus Senna and the evolutionary role of extrafloral nectaries. *Evolution* **64**: 3570–3592.

Markstädter C, Federle W, Jetter R, Riederer M, Hölldobler B. 2000. Chemical composition of the slippery epicuticular wax blooms on *Macaranga* (Euphorbiaceae) ant-plants. *Chemoecology* **10**: 33–40.

Mayer VE, Frederickson ME, McKey D, Blatrix R. 2014. Current issues in the evolutionary ecology of ant–plant symbioses. *New Phytologist* **202**: 749–764.

McKey D, Davidson DW. 1993. Ant–plant symbioses in Africa and the Neotropics: history, biogeography and diversity. In: Goldblatt P, ed. *Biological relationships between Africa and South America.* Yale, CT, USA: Yale University Press, 568–606.

Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skean JD. 2004. A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. *Taxon* **53**: 279.

Moreau CS, Bell CD. 2013. Testing the 'museum versus cradle' tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* **67**: 2240–2257.

Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE. 2006. Phylogeny of the ants: diversification in the age of angiosperms. *Science* **312**: 101–104.

Nucete M, Van Konijnenburg-van Cittert JHA, Van Welzen PC. 2012. Fossils and palaeontological distributions of *Macaranga* and *Mallotus* (Euphorbiaceae). *Palaeogeography, Palaeoclimatology, Palaeoecology* **353**: 104–115.

O'Dowd DJ, Brew CR, Christophel DC, Norton RA. 1991. Mite-plant associations from the Eocene of southern Australia. *Science* **252**: 99–101.

O'Dowd DJ, Willson MF. 1989. Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. *Biological Journal of the Linnean Society* **37**: 191–236.

Orona-Tamayo D, Heil M. 2013. Stabilizing mutualisms threatened by exploiters: new insights from ant–plant research. *Biotropica* **45**: 654–665.

Pagel M, Meade A. 2007. *BayesTraits.* Computer program and documentation. [WWW document] URL http://www.evolution.rdg.ac.uk/BayesTraits.html [accessed 1 June 2014].

Peccoud J, Piatscheck F, Yockteng R, Garcia M, Sauve M, Djiéto-Lordon C, Harris DJ, Blatrix R. 2013. Multi-locus phylogenies of the genus *Barteria* (Passifloraceae) portray complex patterns in the evolution of myrmecophytism. *Molecular Phylogenetics and Evolution* **66**: 824–832.

Pemberton RW. 1992. Fossil extrafloral nectaries, evidence for the ant-guard antiherbivore defense in an Oligocene *Populus*. *American Journal of Botany* **79**: 1242–1246.

Philpott SM, Foster PF. 2005. Nest-site limitation in coffee agroecosystems: artificial nests maintain diversity of arboreal ants. *Ecological Applications* **15**: 1478–1485.

Pringle EG, Ramirez SR, Bonebrake TC, Gordon DM, Dirzo R. 2012. Diversification and phylogeographic structure in widespread Azteca plant-ants from the northern Neotropics. *Molecular Ecology* **21**: 3576–3592.

Quek SP, Davies SJ, Itino T, Pierce NE. 2004. Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in Crematogaster (Formicidae: Myrmicinae) inhabitants of Macaranga (Euphorbiaceae). *Evolution* **58**: 554–570.

Rambaut A, Drummond AJ. 2009. *Tracer v1.5. Edinburgh (UK).* [WWW document] URL http://tree.bio.ed.ac.uk/software/tracer/ [accessed 1 June 2014].

Razafimandimbison SG, Moog J, Lantz H, Maschwitz U, Bremer B. 2005. Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea* s.s. (Rubiaceae). *Molecular Phylogenetics and Evolution* **34**: 334–354.

Renner SS. 1997. *Tococa caryophyllaea* (DC.) Renner (Melastomataceae): a climbing *Tococa. BioLlania* **6** (Wurdack Festschrift): 497–500.

Renner SS, Ricklefs RE. 1998. Herbicidal activity of domatia-inhabiting ants in patches of *Tococa guianensis* and *Clidemia heterophylla. Biotropica* **30**: 324–327.

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001. Rapid diversification of a species-rich genus of neotropical rain forest trees. *Science* **293**: 2242–2245.

Rickson FR. 1979. Absorption of animal tissue breakdown products into a plant stem-the feeding of a plant by ants. *American Journal of Botany* **66**: 87–90.

Rico-Gray V, Barber JT, Thien LB, Ellgaard EG, Toney JJ. 1989. An unusual animal-plant interaction: feeding of *Schomburgkia tibicinis* (Orchidaceae) by ants. *American Journal of Botany* **76**: 603–608.

Sanchez A, Kron KA. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Systematic Botany* **33**: 87–96.

Saslis-Lagoudakis C, Chase MW, Robinson DN, Russell SJ, Klitgaard BB. 2008. Phylogenetics of neotropical *Platymiscium* (Leguminosae: Dalbergieae): systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *American Journal of Botany* **95**: 1270–1286.

Schnell R, Grout de Beaufort F. 1966. Contribution à l'étude des plantes à myrmécodomaties de l'Afrique intertropicale. *Mémoire de l'Institut Fondamental d'Afrique Noire* **75**: 1–66.

Schuster TM, Setaro SD, Kron KA. 2013. Age estimates for the buckwheat family Polygonaceae based on sequence data calibrated by fossils and with a focus on the Amphi-Pacific *Muehlenbeckia*. *PLoS ONE* **8**: e61261.

Smith SA, Donoghue MJ. 2008. Rates of molecular evolution are linked to life history in flowering plants. *Science* **322**: 86–89.

Smith JF, Stevens AC, Tepe EJ, Davidson C. 2008. Placing the origin of two species-rich genera in the late cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (Piperaceae). *Plant Systematics and Evolution* **275**: 9–30.

Smith SA, Dunn CW. 2008. Phyutility: a phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* **24**: 715–716.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**: 758–771.

Tepe EJ, Vincent MA, Watson LE. 2004. Phylogenetic patterns, evolutionary trends, and the origin of ant-plant associations in *Piper* section *Macrostachys*: Burger's hypotheses revisited. In: Dayer LA, Palmer AND, eds. *Piper: a model genus for studies of phytochemistry, ecology, and evolution.* New York, NY, USA: Springer, 156–178.

Treseder KK, Davidson DW, Ehleringer JR. 1995. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* **375**: 137–139.

de Vienne DM, Refrégier G, López-Villavicencio M, Tellier A, Hood ME, Giraud T. 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist* **198**: 347–385.

Wanntorp L, Kocyan A, Renner SS. 2006. Wax plants disentangled: a phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* **39**: 722–733.

Ward PS. 1991. Phylogenetic analysis of pseudomyrmecine ants associated with domatia-bearing plants. In: Huxley CR, ed. *Ant–plant interactions.* Oxford, UK: Oxford University Press, 335–352.

Ward PS, Downie DA. 2005. The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. *Systematic Entomology* **30**: 310–335.

Weber MG, Agrawal AA. 2014. Defense mutualisms enhance plant diversification. *Proceedings of the National Academy of Sciences, USA* **111**: 16442–16447.

Weber MG, Keeler KH. 2013. The phylogenetic distribution of extrafloral nectaries in plants. *Annals of Botany* **111**: 1251–1261.

Weeks A, Baird KE, McMullen CK. 2010. Origin and evolution of endemic Galápagos *Varronia* species (Cordiaceae). *Molecular Phylogenetics and Evolution* **57**: 948–954.

Weigend M, Luebert F, Gottschling M, Couvreur TL, Hilger HH, Miller JS. 2013. From capsules to nutlets—phylogenetic relationships in the Boraginales. *Cladistics* **30**: 508–518.

van Welzen PC, Strijk JS, van Konijnenburg-van Cittert JH, Nucete M, Merckx VS. 2014. Dated phylogenies of the sister genera *Macaranga* and *Mallotus* (Euphorbiaceae): congruence in historical biogeographic patterns? *PLoS ONE* **9**: e85713.

Wilson EO, Hölldobler B. 2005. The rise of the ants: a phylogenetic and ecological explanation. *Proceedings of the National Academy of Sciences, USA* **102**: 7411–7414.

van Zinderen Bakker EM, Mercer JH. 1986. Major late Cenozoic climatic events and palaeoenvironmental changes in Africa viewed in a world wide context. *Palaeogeography, Palaeoclimatology, Palaeoecology* **56**: 217–235.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 1181-species tree from Fig. 3 with species names.

Fig. S2 Dated phylogeny of *Barteria* (Passifloraceae).

Fig. S3 Dated phylogeny of *Cecropia* (Urticaceae).

Fig. S4 Dated phylogeny of *Clerodendrum* (Lamiaceae).

Fig. S5 Dated phylogeny of *Cordia* (Cordiaceae).

Fig. S6 Dated phylogeny of *Dischidia* (Apocynaceae).

Fig. S7 Dated phylogeny of the Hydnophytinae (*Anthorrhiza, Hydnophytum, Myrmecodia*, *Myrmephytum*, *Squamellaria*; Rubiaceae).

Fig. S8 Dated phylogeny of *Leonardoxa* (Fabaceae – Caesalpinioideae).

Fig. S9 Dated phylogeny of *Macaranga* (Euphorbiaceae).

Fig. S10 Dated phylogeny of the Miconieae (*Clidemia*, *Maieta*, *Tococa* and *Topobea* (the latter, Blakeeae); Melastomataceae).

Fig. S11 Dated phylogeny of *Nauclea* and *Neonauclea* (Rubiaceae).

Fig. S12 Dated phylogeny of *Piper* (Piperaceae).

Fig. S13 Dated phylogeny of *Platymiscium* (Fabaceae – Faboideae).

Fig. S14 Dated phylogeny of *Ruprechtia* and *Triplaris* (Polygonaceae).

Fig. S15 Dated phylogeny of *Vachellia* (Fabaceae – Mimosoideae).

Fig. S16 Number of plant-ants per genus in the six subfamilies containing plant–ants.

Fig. S17 Comparison of domatium and EFN evolution on the 1181 taxa tree.

Table S1 The World list of ant domatium-bearing plants

Table S2 Genbank accession numbers for phylogenies generated in this study (Figs 1, S1–S15)

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**

© 2015 The Authors
New Phytologist © 2015 New Phytologist Trust

Supplementary Information Figs S1-S17

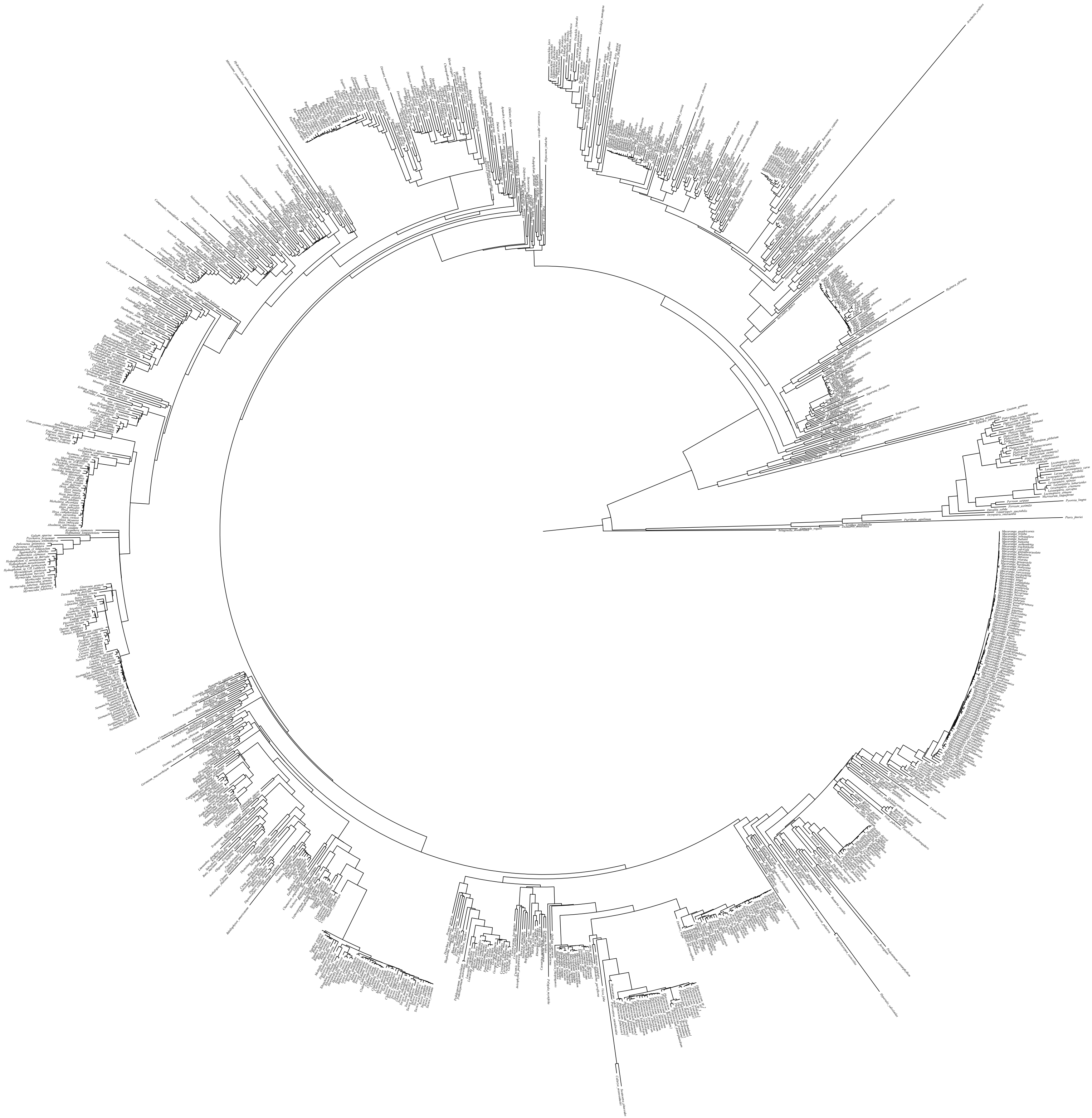


Fig. S1. 1,181 taxon tree shown in Fig. 3. with all tips labelled.

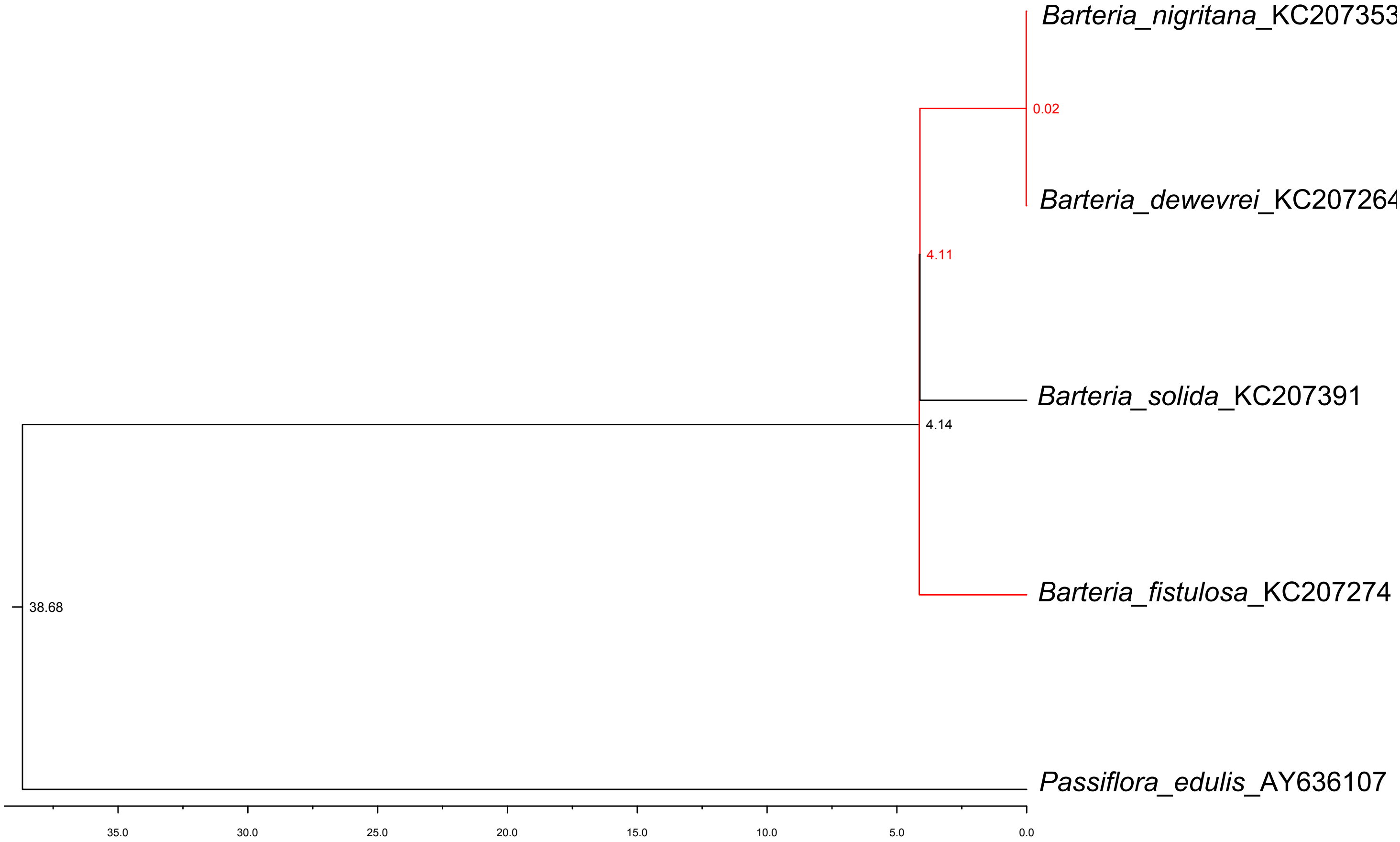


Fig. S2. Dated phylogeny of *Barteria* (Passifloraceae).

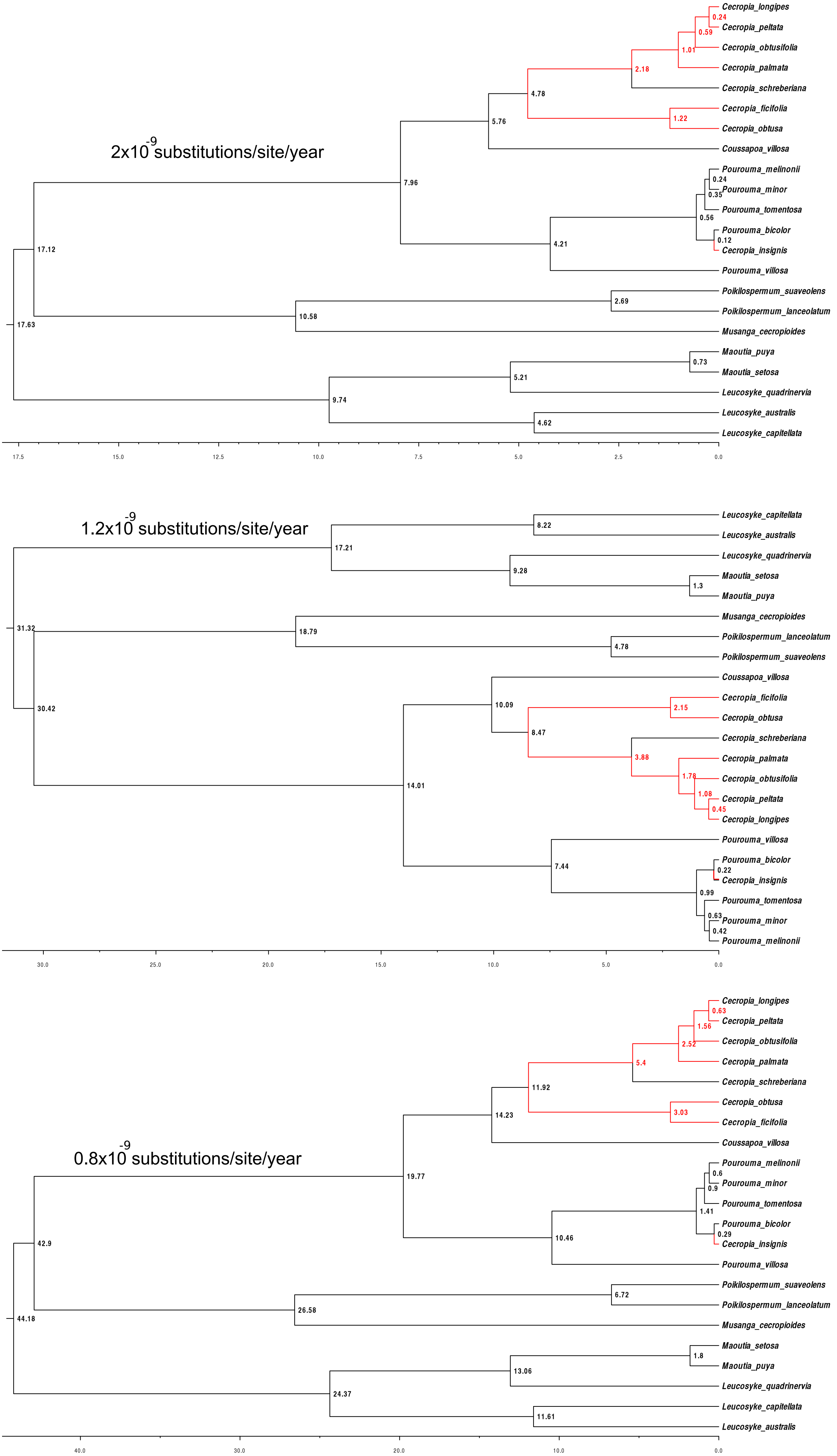


Fig. S3. Dated phylogeny of *Cecropia* (Urticaceae).

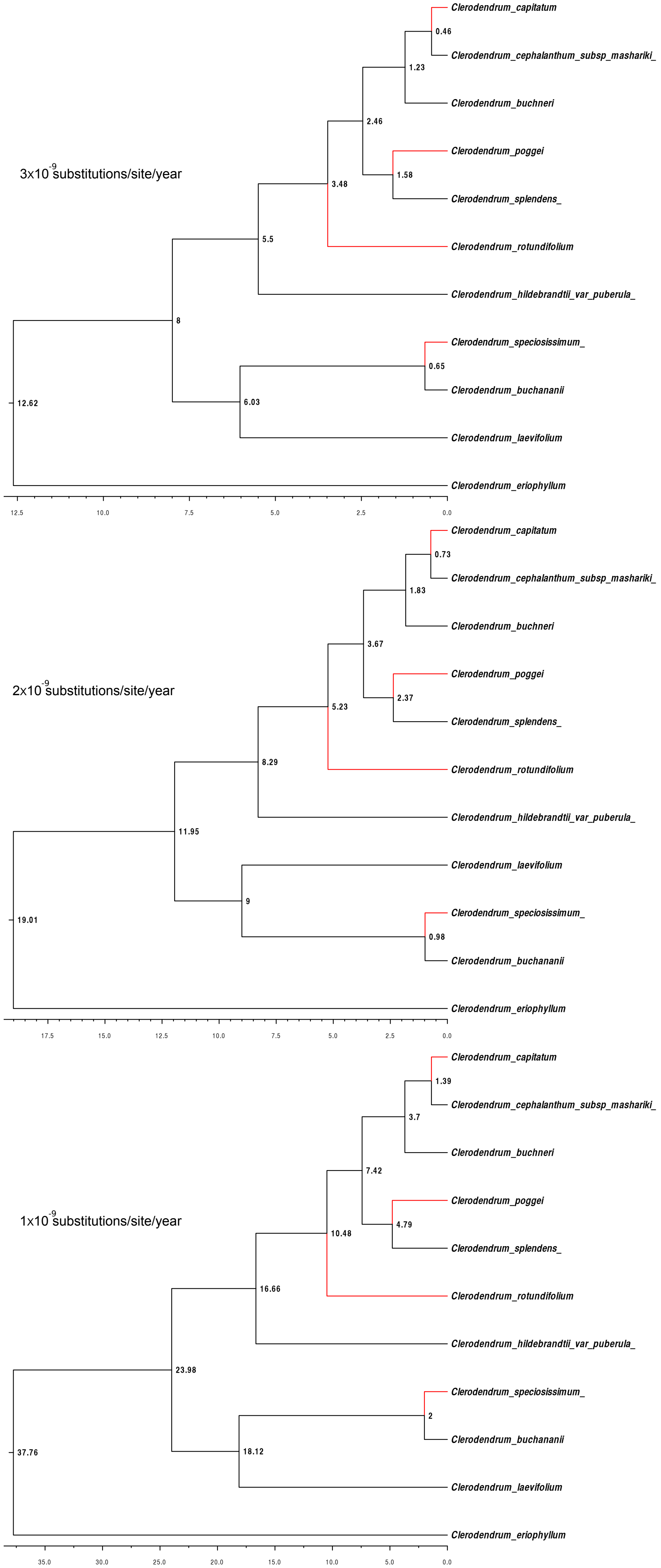


Fig. S4. Dated phylogeny of *Clerodendrum* (Lamiaceae)

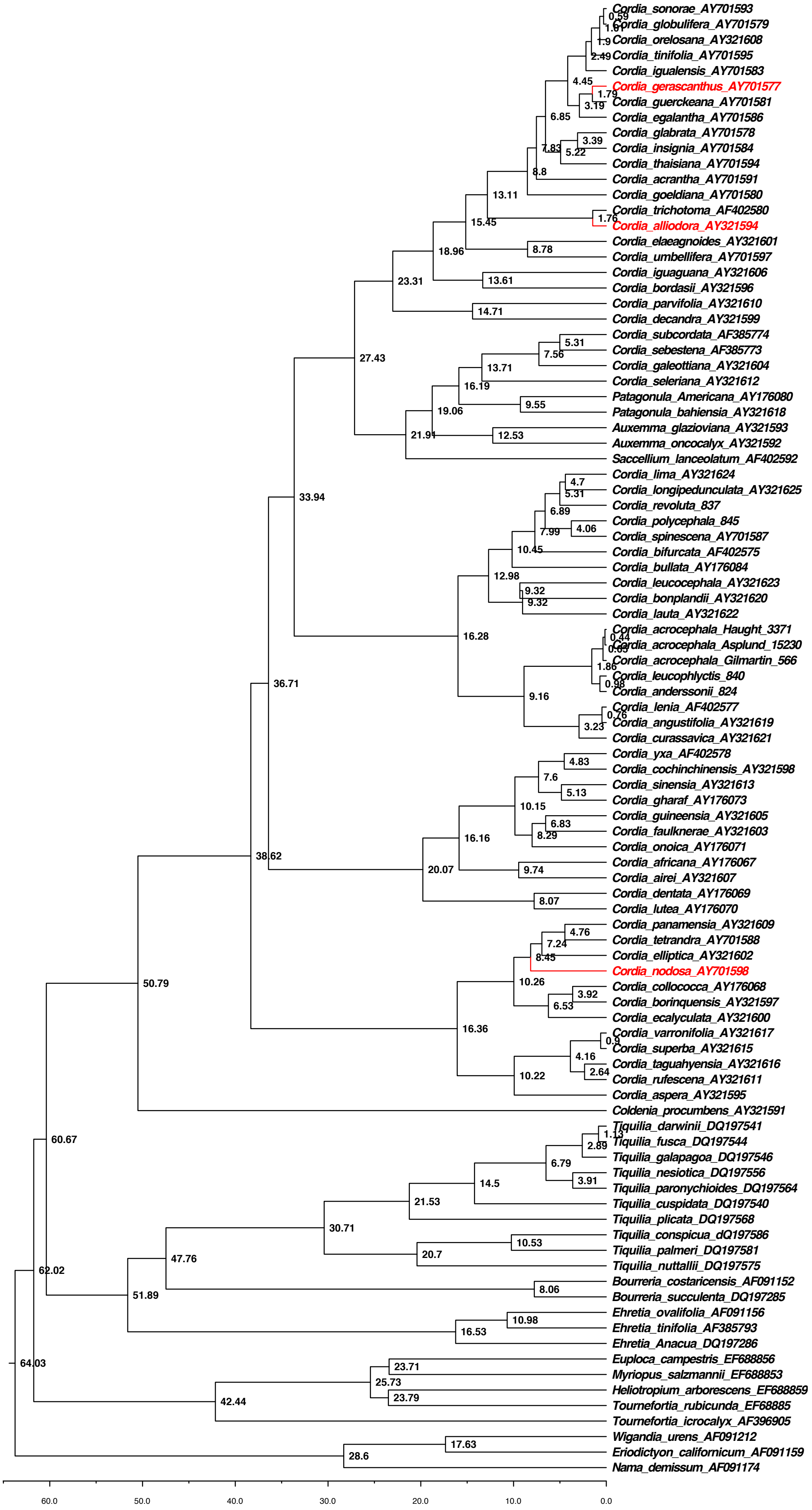
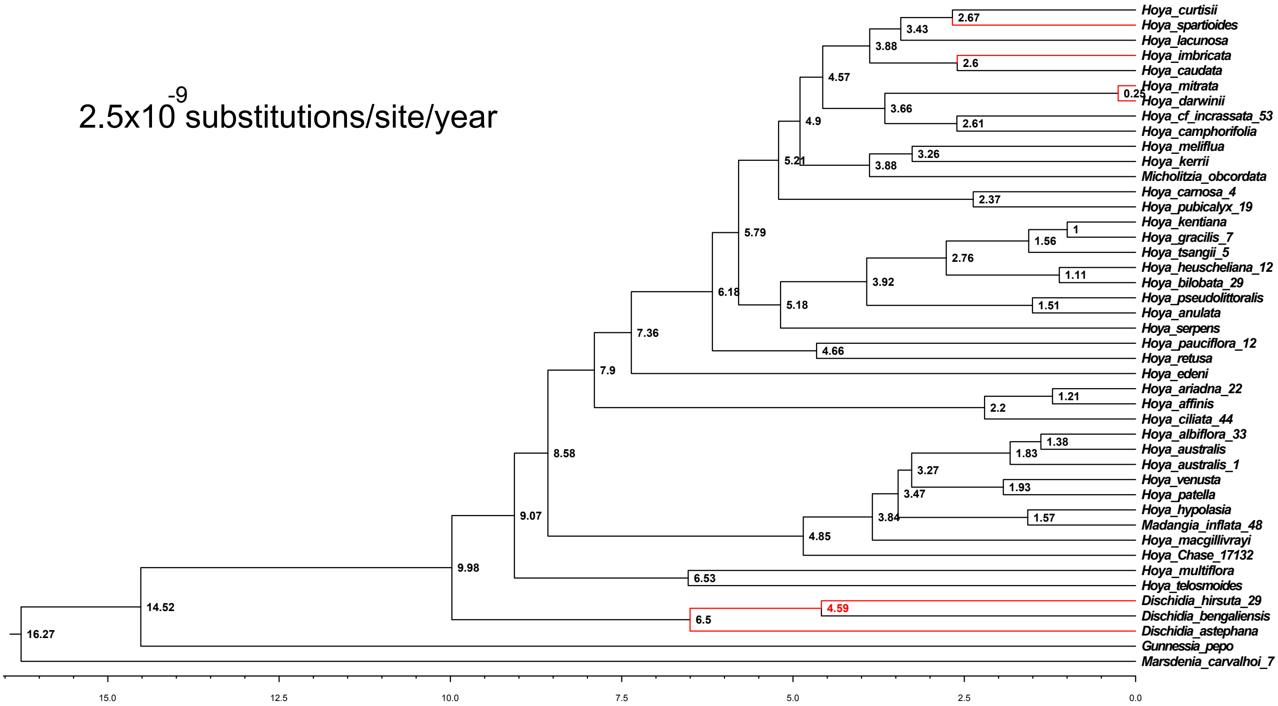
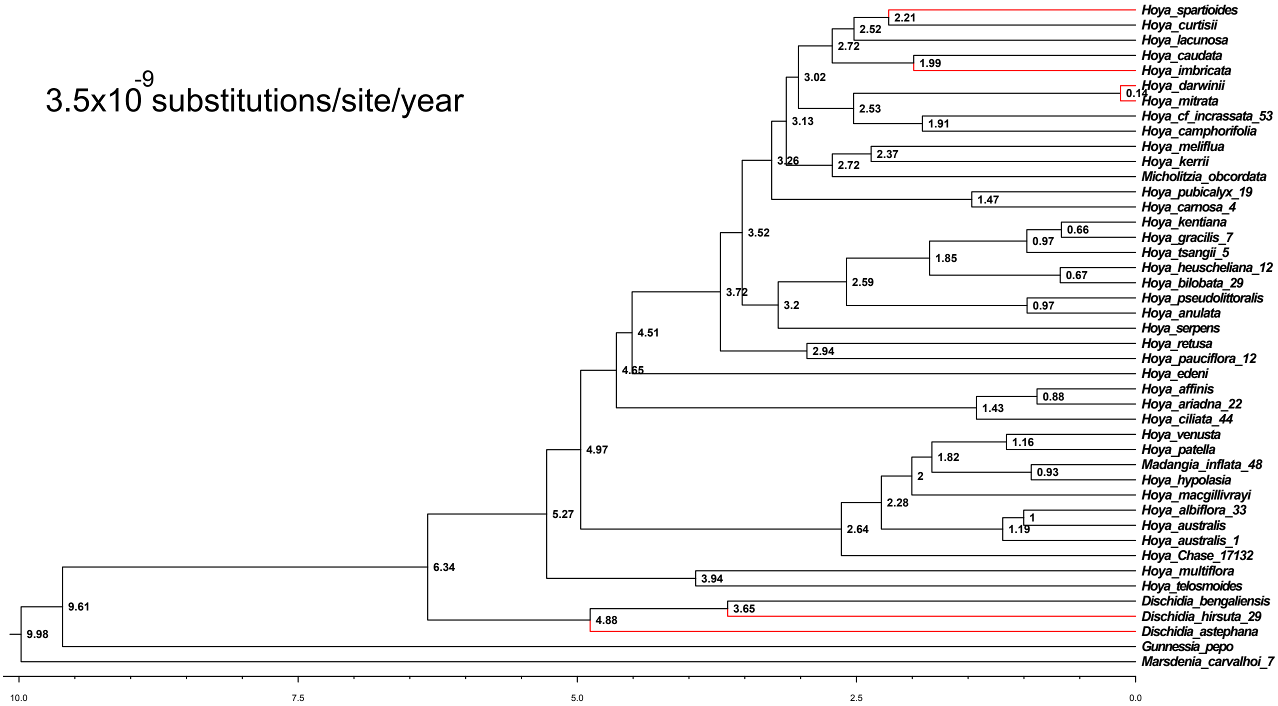


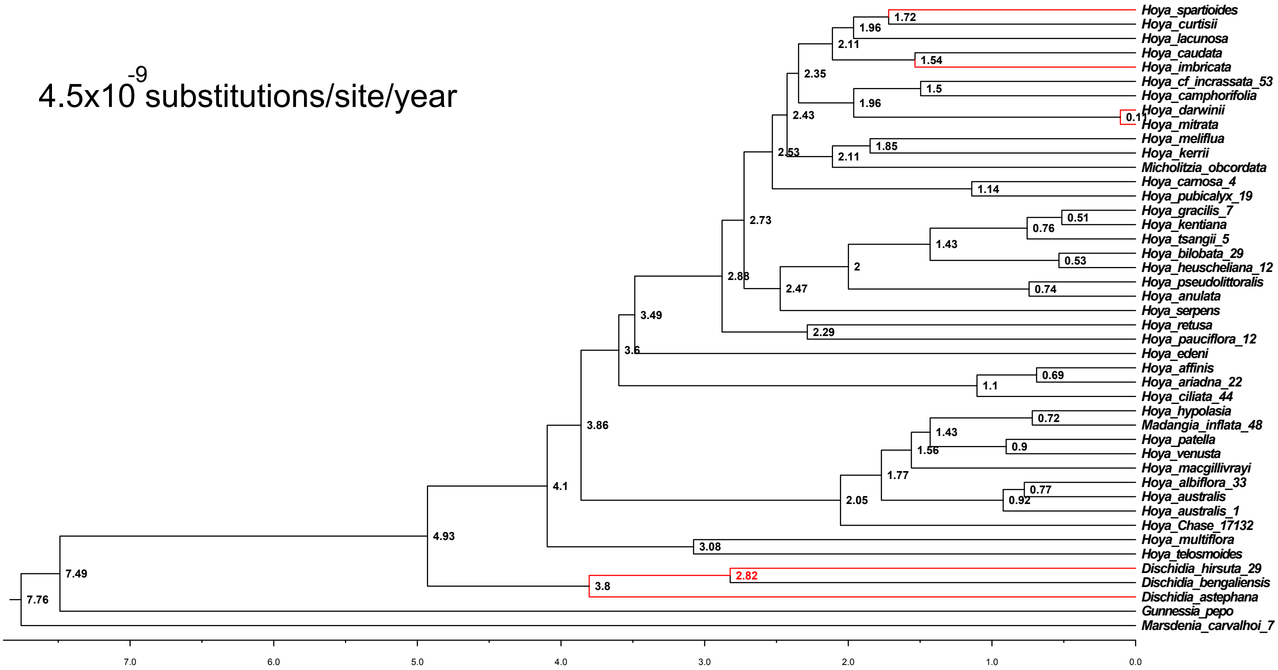
Fig. S5. Dated phylogeny of Cordia (Cordiaceae).



2.5×10^{-9} substitutions/site/year



3.5×10^{-9} substitutions/site/year



4.5×10^{-9} substitutions/site/year

Fig. S6. Phylogeny of Hoya and Dischidia (Apocynaceae).

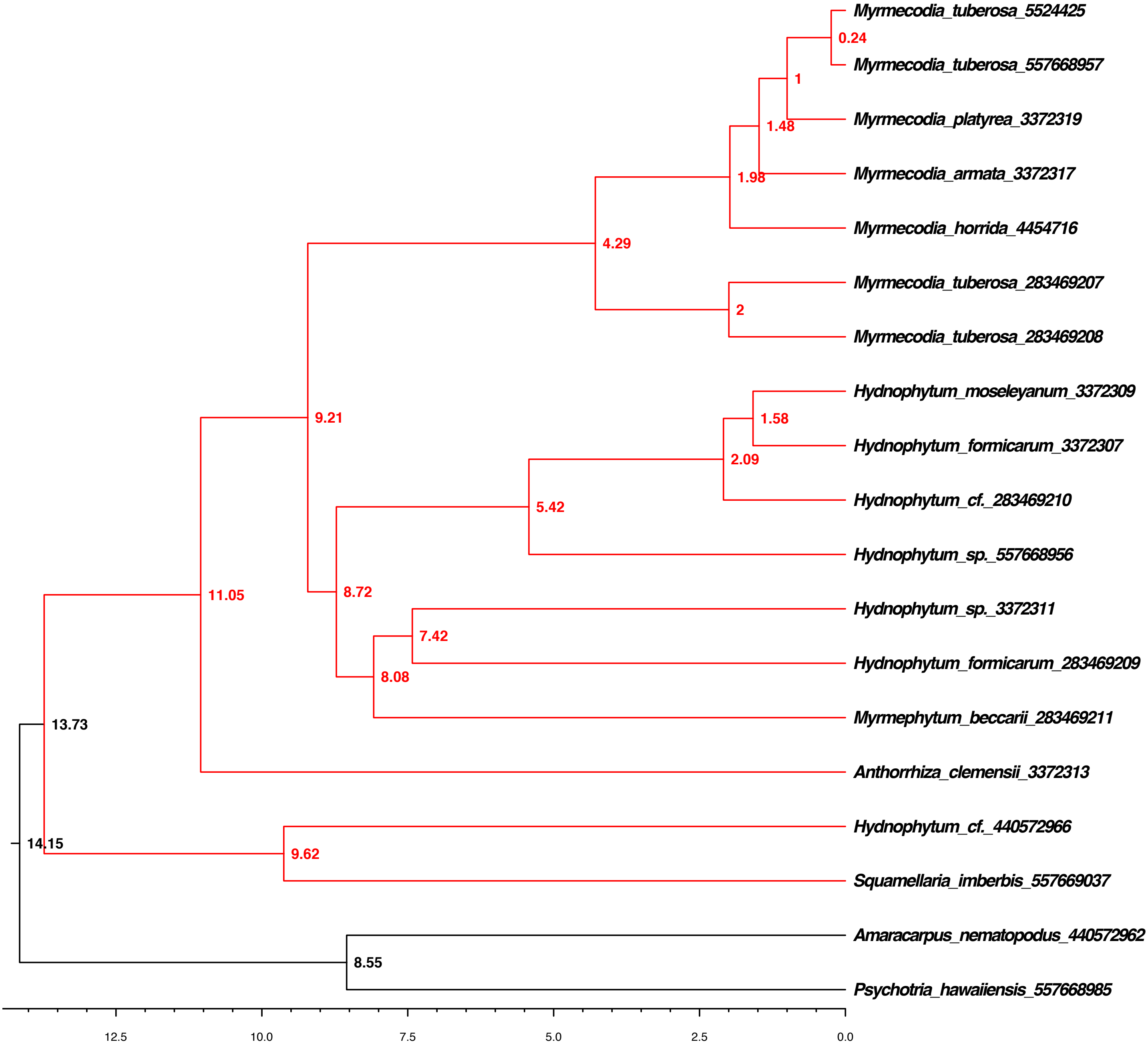


Fig. S7. Dated phylogeny of the Hydnophytinae.

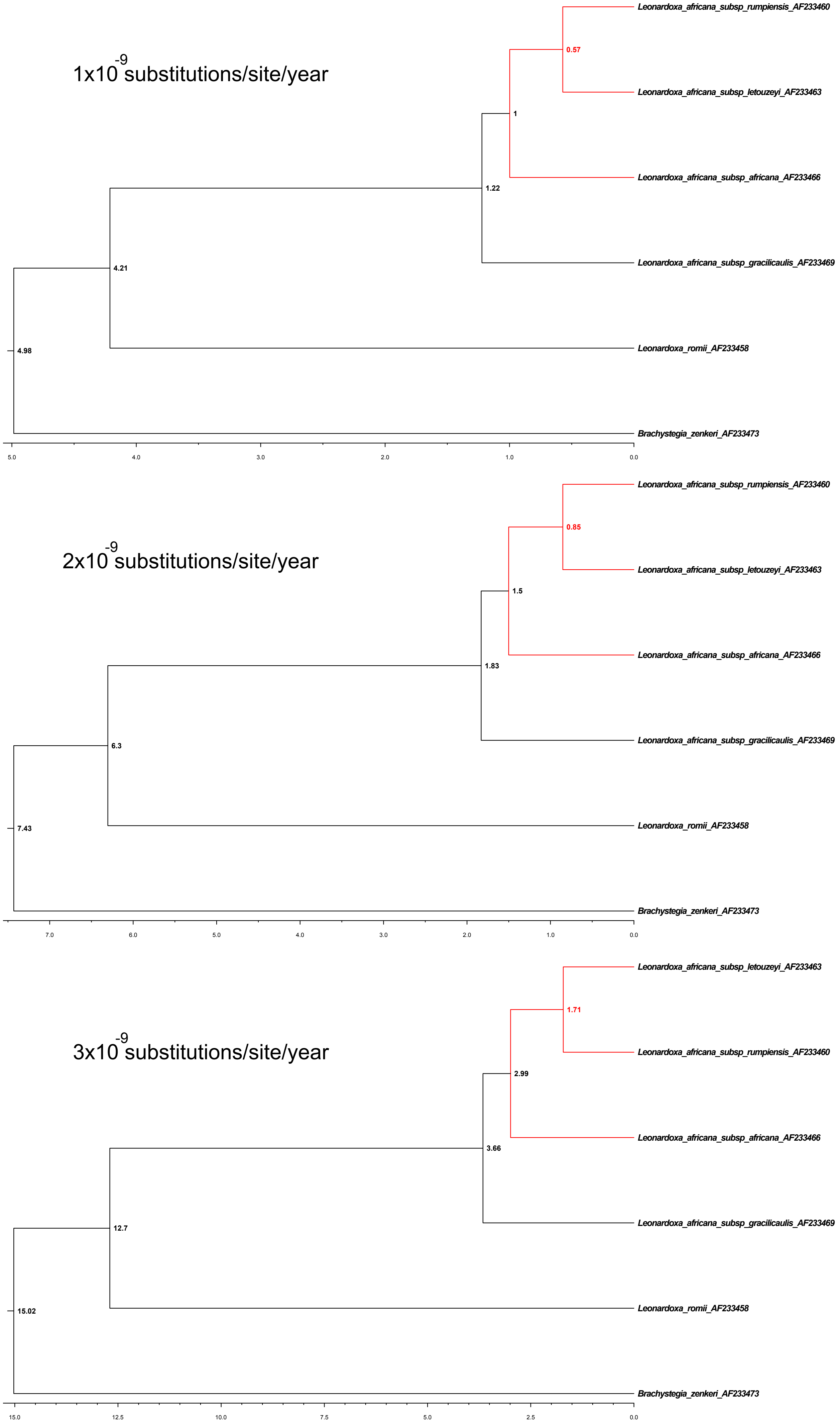


Fig. S8. Dated phylogeny of *Leonardoxa* (Fabaceae).

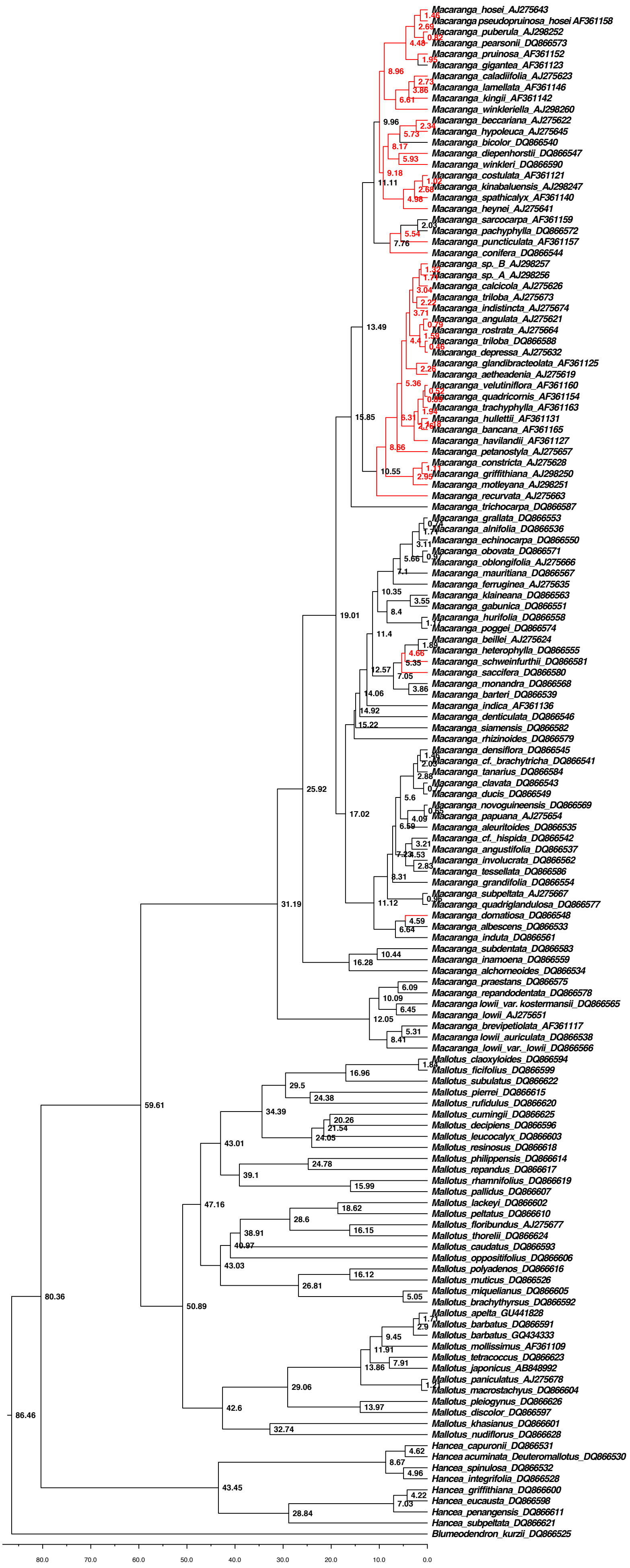


Fig. S9. Dated phylogeny of Macaranga (Euphorbiaceae).

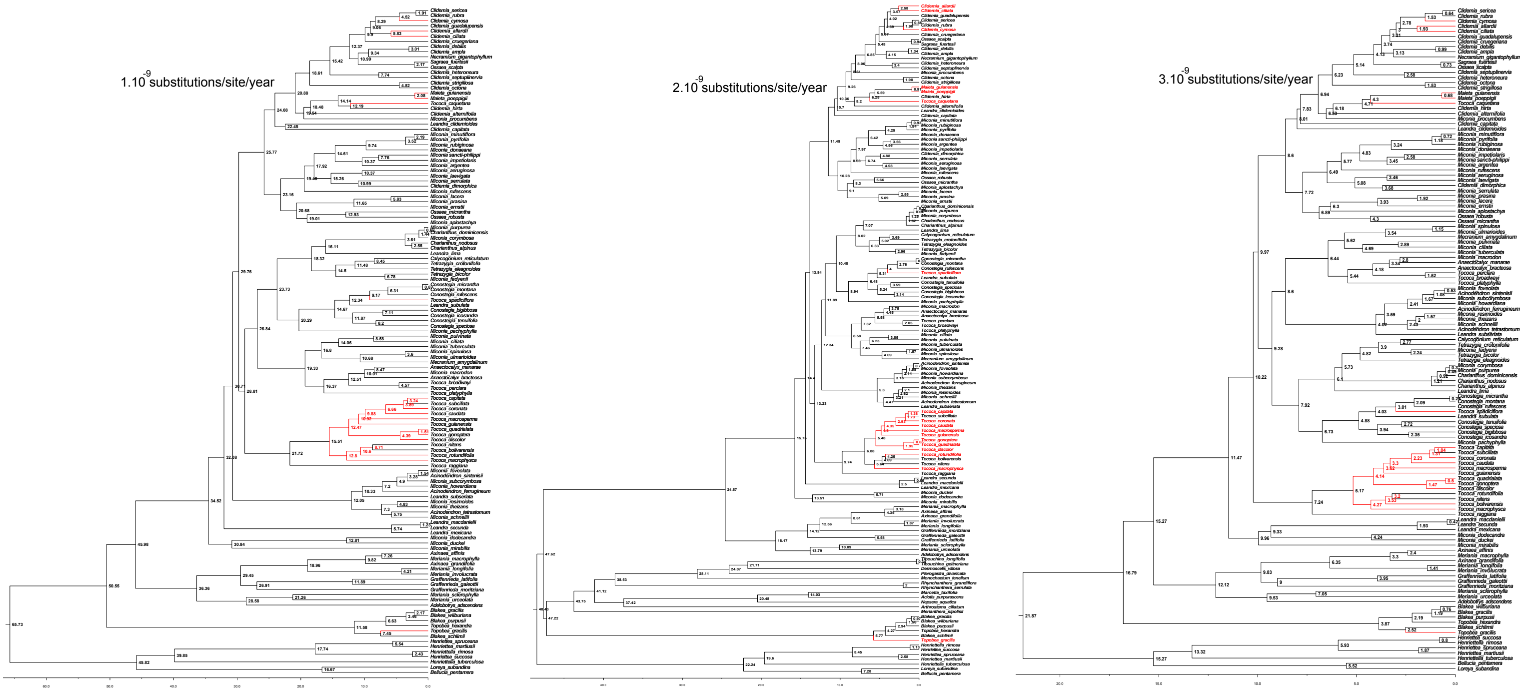


Fig. S10. Dated phylogeny of the Miconieae (Melastomataceae).

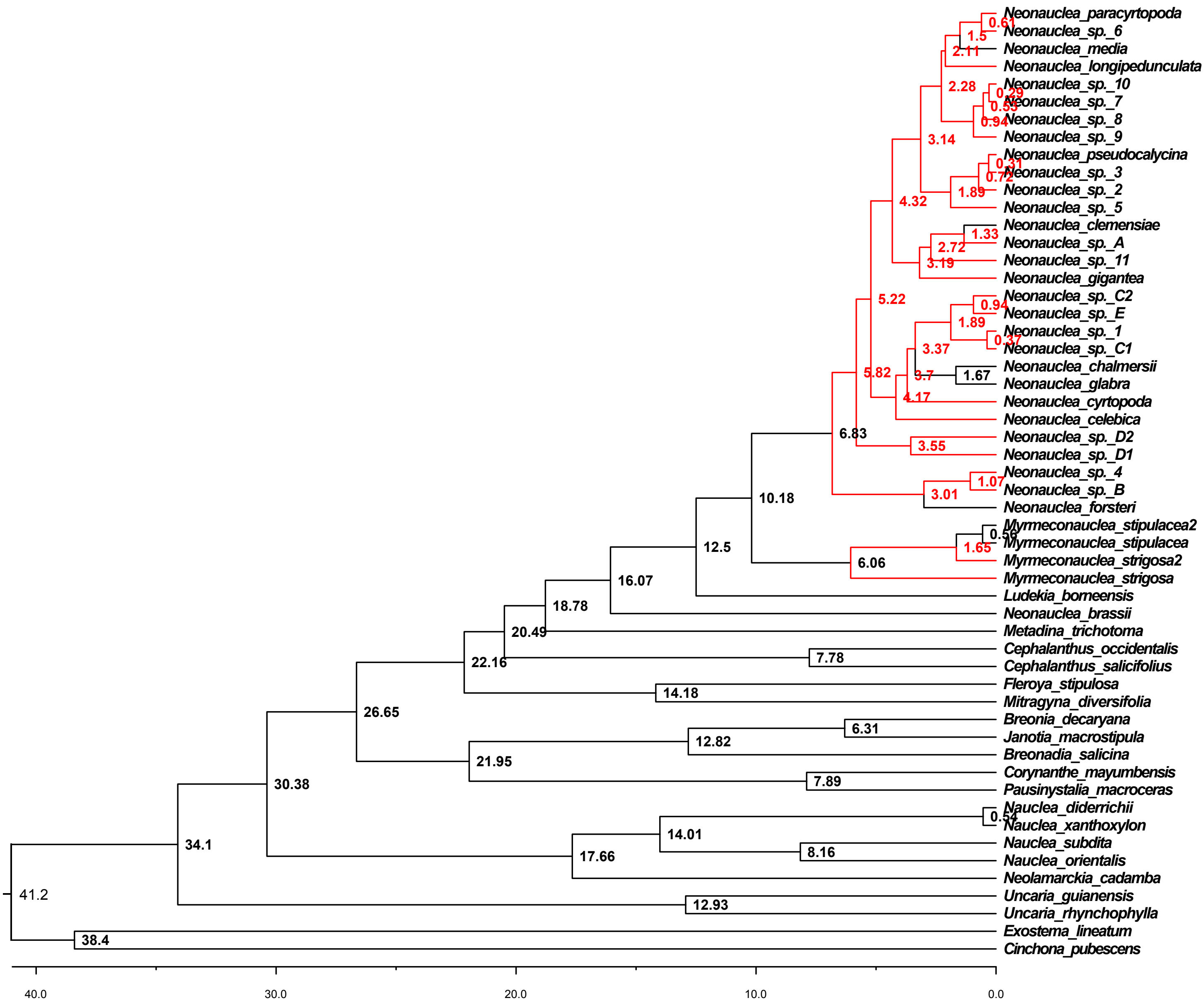


Fig. S11. Dated phylogeny of *Neonauclea* (Rubiaceae).

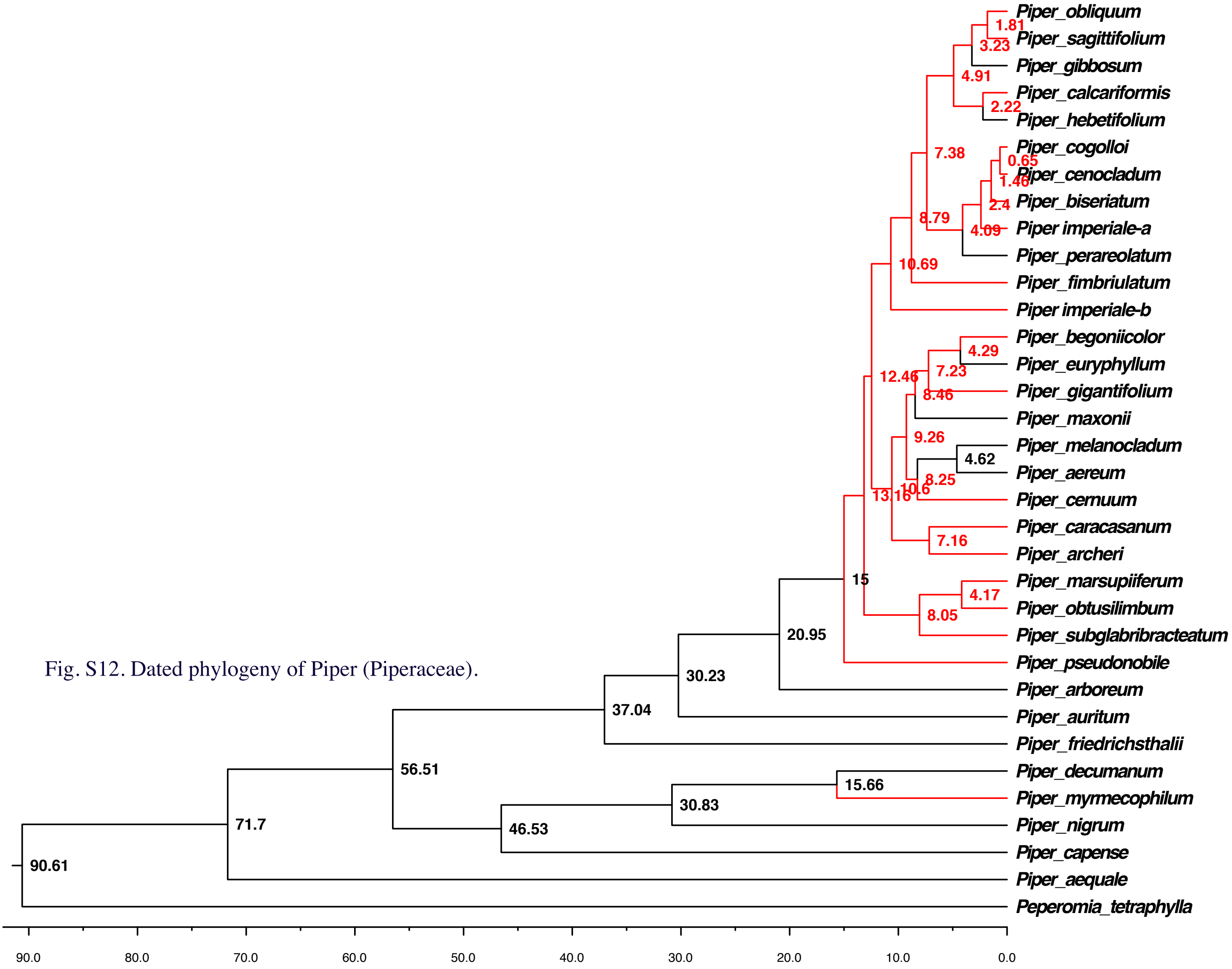


Fig. S12. Dated phylogeny of Piper (Piperaceae).

Fig. S13. Dated phylogeny of Platymiscium (Fabaceae)

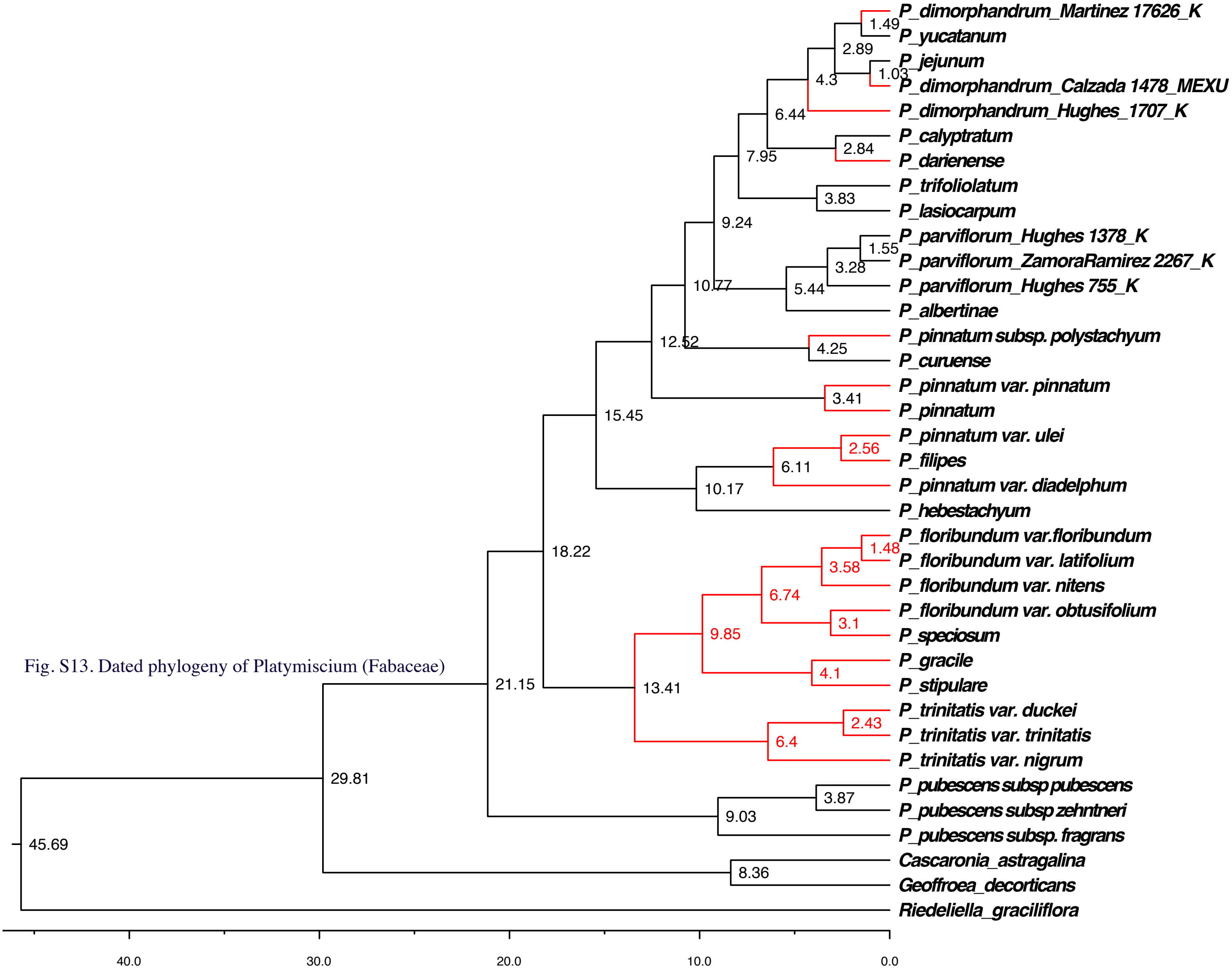


Fig. S14. Dated phylogeny of Ruprechtia and Triplaris (Polygonaceae).

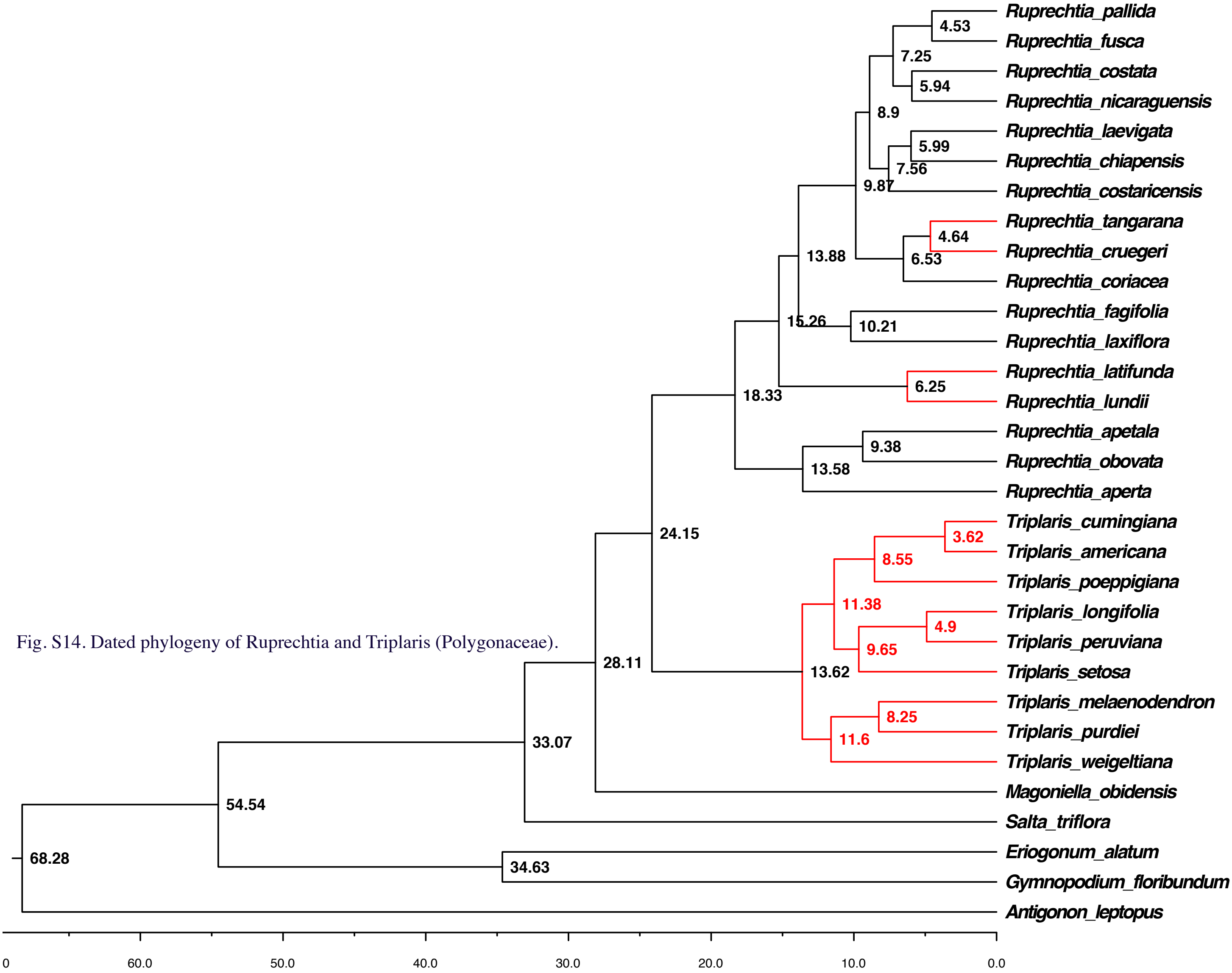
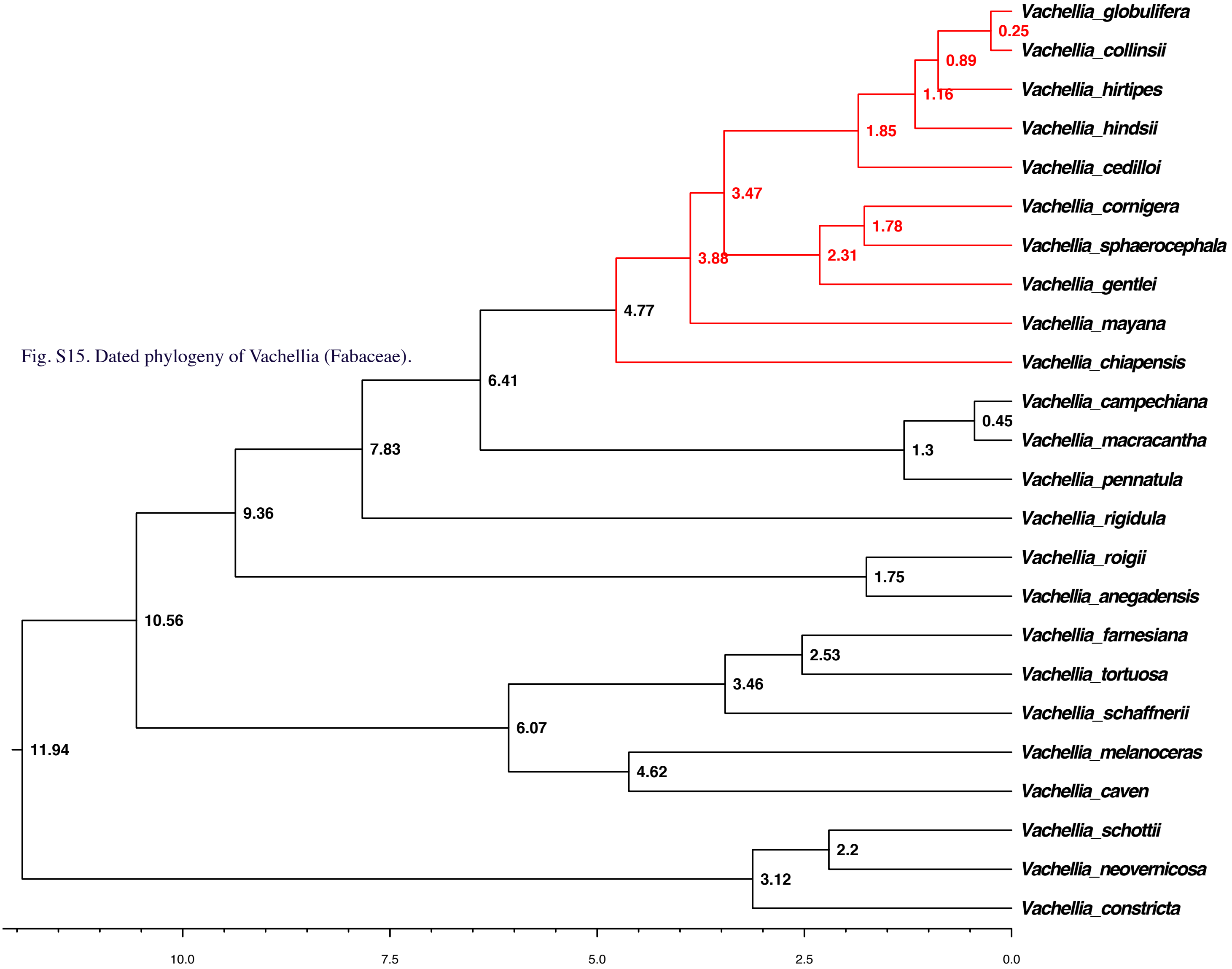


Fig. S15. Dated phylogeny of Vachellia (Fabaceae).



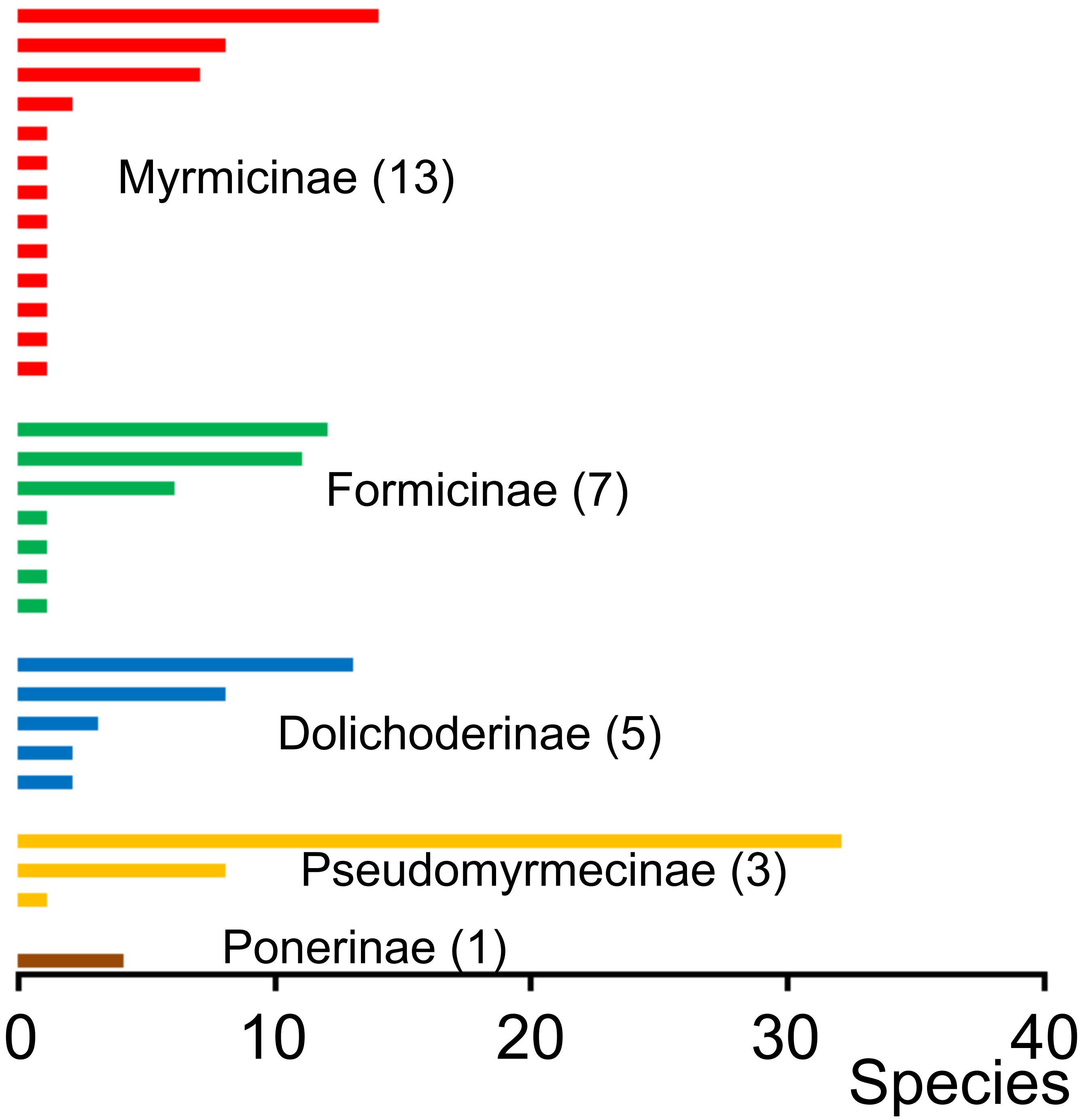


Fig. S16. Number of plant-ants per genus in the six subfamilies containing plant-ants, providing an estimate of the size of plant ant radiations. Numbers of domatium-nesting species from McKey & Davidson (1993); total species per genus from AntCat (http://www.antcat.org/). Each bar corresponds to a genus.

McKey D, Davidson DW. 1993. Ant–plant symbioses in Africa and the Neotropics: history, biogeography and diversity. *Biological Relationships Between Africa and South America*, Ed. P. Goldblatt, Yale University Press, Yale, Connecticut, p.p. 568-606.

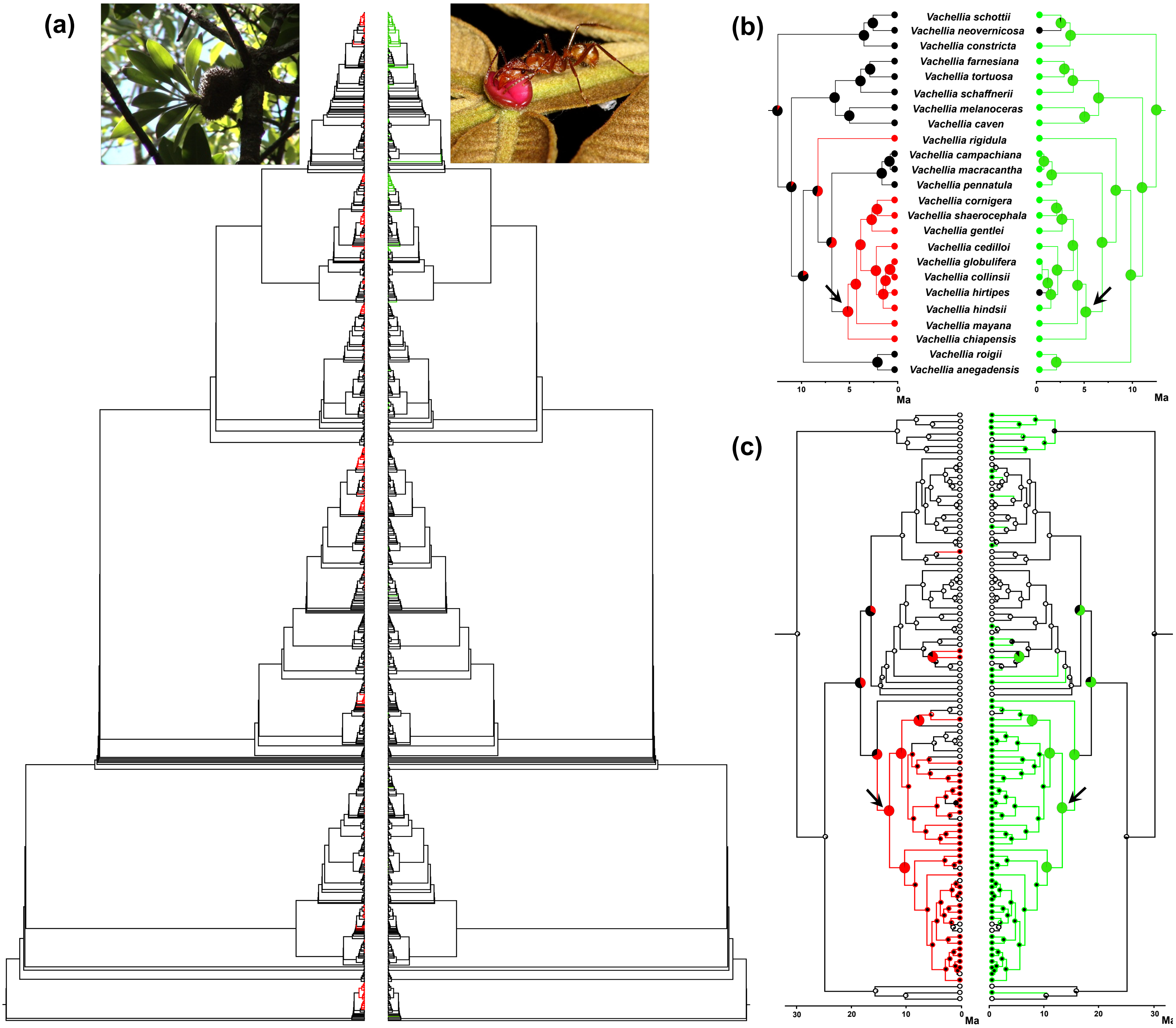


Fig. S17. Comparison of domatium and EFN evolution on the 1,181 taxa tree. (a) Maximum likelihood ancestral state reconstruction of domatia (left, red) and EFNs (right, green) under a Markov 2-parameters model on the 1181-species tree. (b-c) Ancestral state reconstruction of domatia (left, red) and EFNs (right, green) using a Markov 2-parameters model (small pies) and a reverse jump MCMC approach (big pies) on *Vachellia* (b) and *Macaranga* (c). Tree tips can be seen in Fig. S9. Photos: left, *Myrmecodia beccarii* in Queensland, Australia, photo G. Chomicki. Right, extrafloral nectary of an *Inga* species visited by an ant, Neotropics, photo A. Kay.

Supporting Information Table S1
Chomicki G, Renner SS. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist*.

Table S1 The world list of plants with ant domatium

Ant domatium-bearing species	**Family**	**References**	**Geography**
Absolmsia spartoides (Benth.) Kuntze	Apocynaceae	Wannthorp *et al*. (2006)	Borneo
Acacia bullockii Brenan	Fabaceae-Mimosoideae	Hocking (1970)	Africa
Acacia burttii Baker *f*.	Fabaceae-Mimosoideae	Hocking (1970)	East Africa, Tanzania
Acacia bussei Sjostedt	Fabaceae-Mimosoideae	Wheeler (1942); Jolivet (1996)	East Africa
Acacia (Vachellia) cedilloi L. Rico	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Neotropics
Acacia (Vachellia) chiapensis Saff.	Fabaceae-Mimosoideae	Heil *et al*. (2009)	Mexico
Acacia (Vachellia) collinsii Saff.	Fabaceae-Mimosoideae	Heil *et al*. (2009)	Mexico
Acacia (Vachellia) cornigera (L.) Willd.	Fabaceae-Mimosoideae	Janzen (1966)	Neotropics
Acacia drepanolobium Sjostedt	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Africa
Acacia elatior Brenan	Fabaceae-Mimosoideae	Hocking (1970)	East Africa, Tanzania
Acacia erythrophloea	Fabaceae-	Hocking (1970)	East Africa, Tanzania

Brenan	Mimosoideae		
Acacia (Vachellia) gentlei Standl.	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Neotropics
Acacia (Vachellia) globulifera Saff.	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Neotropics
Acacia (Vachellia) hindsii Benth.	Fabaceae-Mimosoideae	Heil *et al*. (2009); Heil *et al*. (2010)	Neotropics
Acacia (Vachellia) hirtipes Saff.	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Neotropics
Acacia horrida (L.) Willd.	Fabaceae-Mimosoideae	Ward Blog (http://wardlab.wordpress.com/research/pseudomyrmecinae/ant-plants/); Hocking (1970)	India
Acacia horrida subsp. benadirensis (Chiov.) Hillc. & Brenan	Fabaceae-Mimosoideae	Hocking (1970)	East Africa
Acacia leucophloea (Roxb.) Willd.	Fabaceae-Mimosoideae	Mani (1964); Jolivet (1996)	India
Acacia luederitzii Engl.	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Africa
Acacia malacocephala Harms	Fabaceae-Mimosoideae	Wheeler (1942); Jolivet (1996); Hocking (1970)	Africa
Acacia (Vachellia) mayana Lundell	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Neotropics
Acacia mbuluensis	Fabaceae-	Hocking (1970)	East Africa

Brenan	Mimosoideae		
Acacia (Vachellia) melanoceras Beurl.	Fabaceae-Mimosoideae	Seiber & Erbinger (1995)	Neotropics
Acacia myrmecophila R.Vig.	Fabaceae-Mimosoideae	Paulian (1949)	Madagascar, South of the Island
Acacia pseudofistula Harms	Fabaceae-Mimosoideae	Brown (1960); Hocking (1970)	Tropical Africa
Acacia (Vachellia) rigidula Benth.	Fabaceae-Mimosoideae	Seigler *et al*. (1982); Jolivet (1996)	Texas
Acacia seyal Delile	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Africa
Acacia (Vachellia) sphaerocephala Schltdl. & Cham.	Fabaceae-Mimosoideae	Gomez-Acevedo *et al*. (2010)	Neotropics
Acacia zanzibarica (S.Moore) Taub.	Fabaceae-Mimosoideae	Wheeler (1942); Jolivet (1996)	Africa
Actinodaphne sesquipedalis Hook.f. & Thomson ex Meisn.	Lauraceae	Moog *et al* (2003)	SE Asia - Malaysia
Aiouea vexatrix van der Werff	Lauraceae	Van der Werff (1988); Jolivet (1996)	Panama
Alexa cowanii Yakovlev	Fabaceae-Faboideae	McKey (1989)	Neotropics
Allomaieta grandiflora Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Andira sapindoides (DC.)	Fabaceae -	C. R. Huxley-Lambrick (pers. comm.)	Neotropics

Benth. (= *Pterocarpus sapindoides*)	Faboideae		
Anthocleista nobilis G.Don	Gentianaceae	Belin-Depoux (1993)	Neotropics
Anthorrhiza areolata C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza bracteosa C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza caerulea C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza camilla Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza chrysacantha C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza echinella C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza mitis C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza recurvispina C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Anthorrhiza stevensii C.R.Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991a)	Papua New Guinea
Aphanamixis polystachya (Wall.) R.Parker	Meliaceae	Bequaert (1922); Mabberley (1985); Gullan *et al*. (1993); Jolivet (1996)	Papua New Guinea
Barteria dewevrei De Wild. & T.Durand	Passifloraceae	Bequaert (1922); Peccoud *et al*. (2013)	Tropical Africa
Barteria fistulosa Mast.	Passifloraceae	Bequaert (1922); Jolivet (1996) ; Peccoud *et al*. (2013)	Tropical Africa
Barteria nigritana Hook.f.	Passifloraceae	McKey & Davidson (1993)	Tropical Africa, Central forest block
Bertiera bicarpellata (K.Schum.) N.Hallé ***(= B. simplicaulis)***	Rubiaceae	McKey & Davidson (1993)	Tropical Africa, Central forest block

Besleria formicaria Nowicke	Gesneriaceae	Windsor & Jolivet (1996)	Panama
Blakea austin-smithii Standl.	Melastomataceae	Michelangeli (2010)	Neotropics
Blakea chlorantha Almeda	Melastomataceae	Michelangeli (2010)	Neotropics
Blakea formicaria Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Blakea involvens Markgr.	Melastomataceae	Michelangeli (2010)	Neotropics: Ecuador
Blakea jativae Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics : Ecuador
Blakea perforate Almeda	Melastomataceae	Michelangeli (2010)	Neotropics: Panama
Blakea podagrica Triana	Melastomataceae	Michelangeli (2010)	Neotropics: Colombia
Blakea polyantha Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics : Ecuador
Blakea punctulata (Triana) Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics: Ecuador, Colombia
Blakea subconnata O. Berg ex Triana	Melastomataceae	Michelangeli (2010)	Neotropics
Blakea subvaginata Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics: Ecuador (cloud forest)
Brachystephanus myrmecophilus Champl.	Acanthaceae	Champluvier (1994)	Africa, Zaire
Buchnerodendron speciosum Gürke	Achariaceae	Jolivet (1996); McKey & Davidson (1993)	Tropical Africa, Central forest block
Calamus javensis Blume	Arecaceae	Moog *et al* (2003); Jolivet (1996)	SE Asia - Malaysia
Calamus laevigatus Mart.	Arecaceae	Moog *et al* (2003)	SE Asia - Malaysia
Calamus polystachys Becc.	Arecaceae	Moog *et al* (2003)	SE Asia - Malaysia
Callerya nieuwenhuisii (J.J.Sm.) Schot ***(=Millettia nieuwenhuisii)***	Fabaceae	Maschwitz *et al*. (1989); Agosti *et al*. (1999)	SE Asia, Sabah

Callicarpa saccata Steenis	Lamiaceae	Van Stenis (1969)	SE Asia
Caloncoba welwitschii (Oliv.) Gilg	Achariaceae	Jolivet (1996)	Africa
Calpocalyx cauliflorus Hoyle	Fabaceae - Mimosoideae	McKey & Davidson (1993)	Tropical Africa, Central forest block
Canthium yangambiense Louis	Rubiaceae	C. R. Huxley-Lambrick (pers. obs.)	SE Asia
Caularthron amazonicum (Schltr.) H.G.Jones	Orchidaceae	Bequaert (1922); Gegenbauer *et al*. (2012)	South America
Caularthron bicornutum (Hook.) Raf.	Orchidaceae	Bequaert (1922); Gegenbauer *et al*. (2012)	South America
Caularthron bilamellatum (Rchb.f.) R.E.Schult.	Orchidaceae	Bequaert (1922); Gegenbauer *et al*. (2012)	South America
Caularthron kraenzlinianum H.G.Jones	Orchidaceae	Gegenbauer *et al*. (2012)	South America
Cecropia albicans Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia angulata I.W.Bailey	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia angustifolia Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia annulata C.C.Berg & P.Franco	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia concolor Willd.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia distachya Huber	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia elongate Rusby	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia engleriana Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia ficifolia Warb. ex Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics

Cecropia garciae Standl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia glaziovii Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia goudotiana Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia granvilleana C.C.Berg	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia herthae Diels	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia heterochroma C.C.Berg & P.Franco	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia hispidissima Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia idroboi Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia insignis Liebm.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia kavanayensis Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia latiloba Miq.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia litoralis Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia longipes Pittier	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia marginalis Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia maxima Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia megastachya Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia membranacea Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia metensis Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia Montana Warb. ex Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia multisecta P.Franco & C.C.Berg	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia mutisiana	Urticaceae	Berg *et al*. (2005)	Neotropics

Mildbr.			
Cecropia obtusa Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia obtusifolia Bertol.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia pachystachya Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia palmate Willd.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia pastasana Diels	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia peltata L.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia plicata Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia polystachya Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia purpurascens C.C. Berg	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia putumayonis Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia reticulatea Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia sararensis Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia saxatilis Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia silvae C.C.Berg	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia strigosa Trécul	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia subintegra Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia telenitida Cuatrec**.**	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia ulei Snethl.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia utcubambana Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics
Cecropia virgusa Cuatrec.	Urticaceae	Berg *et al*. (2005)	Neotropics

Chisocheton lasiocarpus (Miq.) Valeton	Meliaceae	Schumann & Lauterbach (1901); Bequaert (1922); Stevens (1975); Mabberley (1979)	SE Asia: Indonesia, New Guinea and Solomons
Chisocheton tomentosus (Roxb.) Mabb.	Meliaceae	Moog *et al*. (2003)	Malaysia
Clerodendrum breviflorum Ridl.	Lamiaceae	Moog *et al* (2003)	SE Asia - Malaysia
Clerodendrum capitatum (Willd.) Schumach. & Thonn.	Lamiaceae	Schnell (1966)	Tropical Africa
Clerodendrum deflexum Wall.	Lamiaceae	Moog *et al* (2003)	SE Asia - Malaysia
Clerodendrum excavatum De Wild.	Lamiaceae	Bequaert (1922); Jolivet (1996)	Tropical Africa
Clerodendrum fistulosum Becc.	Lamiaceae	Blatter (1928); Uphof (1942); Maschwitz *et al*. (1994)	SE Asia
Clerodendrum formicarum Gürke	Lamiaceae	Jolivet (1996)	Tropical Africa
Clerodendrum phyllomega var. myrmecophila (Ridl.) Moldenke	Lamiaceae	Moog *et al* (2003)	SE Asia - Malaysia
Clerodendrum poggei Gürke (= ***C. angolense***)	Lamiaceae	Jolivet (1996)	Tropical Africa
Clerodendrum rotundifolium Oliv. (= ***C. guerkei*** Baker)	Lamiaceae	Schnell (1966)	Tropical Africa
Clerodendrum speciosissimum Drapiez	Lamiaceae	Schnell (1966)	Tropical Africa
Clidemia acostae Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia allardii Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia ayangannensis Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics

Clidemia ciliata Pav. ex D. Don ***var. elata*** and ***var testiculata***	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia collina Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia crenulata Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia ferox Gleason	Melastomataceae	Michelangeli (2010)	Neotropics: Brazil
Clidemia foliosa Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia folsomii Almeda	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia heptamera Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia heterophylla (Desr.) Gleason	Melastomataceae	Renner & Rickleys (1998)	Neotropics; Peru - Ecuador
Clidemia inobsepta Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia juruensis (Pilg.) Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia killipii Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia lanuginosa Almeda	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia myrmecina Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia neblinae Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia pilosa Pav. ex D. Don	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia pubescens Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia rodriguezii Almeda	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia setosa (Triana) Gleason	Melastomataceae	Michelangeli (2010)	Neotropics

Clidemia sp. ***A***	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia spectabilis Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia sprucei Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia taurina Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia tenebrosa Almeda	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia tococoidea (DC.) Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Clidemia ventricosa Almeda	Melastomataceae	Michelangeli (2010)	Neotropics
Coccoloba excelsa Benth**.** ***(=Coccoloba parimensis)***	Polygonaceae	Bequaert (1922); Wheeler & Britton (1908); Spruce (1908)	Central america
Cola caricifolia (G.Don) K.Schum. (= C. dewevrei)	Malvaceae - Sterculioideae	Bequaert (1922); Jolivet (1996)	Africa
Cola marsupium K.Schum.	Malvaceae - Sterculioideae	Bequaert (1922); Schnell (1966, 1970); Jolivet (1996)	Tropical Africa
Conceveiba martiana Baill.	Euphorbiaceae	Benson (1983); Jolivet (1996)	Tropical America
Conostegia dentata Triana	Melastomataceae	Michelangeli (2010)	Neotropics
Conostegia inusitata Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics: Peru
Conostegia setosa Triana	Melastomataceae	Alonso *et al*. (1998); Micheangeli (2010)	Neotropics
Conostegia sp***. A***	Melastomataceae	Michelangeli (2010)	Neotropics: Peru
Cordia alliodora (Ruiz & Pav.) Oken	Boraginaceae	Ward (1999)	Costa Rica
Cordia gerascanthus L.	Boraginaceae	Bailey(1924)	Neotropics
Cordia glabrata (Mart.) A.DC.	Boraginaceae	Chodat (1920)	Neotropics
Cordia longituba Chodat & Vischer	Boraginaceae	Chodat (1920); Forel (1920)	Neotropics

Cordia nodosa Lam.	Boraginaceae	Bailey(1924)	Neotropics
Coussapoa asperifolia Trécul	Urticaceae	Berg *et al*. (1990)	Neotropics
Crypteronia griffithii C.B.Clarke	Crypteroniaceae	Agosti *et al*. (1999); Jolivet (1996); Maschwitz *et al*. (1991)	SE Asia, Malaysia, Sumatra, Borneo
Crypteronia macrophylla Beus.-Osinga	Crypteroniaceae	Agosti *et al*. (1999)	SE Asia, Central part of Sarawak only
Cupaniopsis anacardioides (A.Rich.) Radlk.	Sapindaceae	Ward (1991)	North Queensland
Cuviera acutiflora DC.	Rubiaceae	C. R. Huxley-Lambrick (pers. obs.)	Tropical Africa
Cuviera latior Wernham	Rubiaceae	Onana (2008)	Tropical Africa
Cuviera ledermannii K.Krause	Rubiaceae	Bequaert (1922); Schumann (1888)	Tropical Africa
Cuviera letestui Pellegr.	Rubiaceae	Onana (2008)	Tropica Africa
Cuviera longiflora Hiern ***(= C. angolensis)***	Rubiaceae	Uphof (1942); Robbrecht (1979)	Tropical Africa
Cuviera macroura K.Schum.	Rubiaceae	C. R. Huxley-Lambrick (pers. obs.)	Tropical Africa
Cuviera physinodes K.Schum.	Rubiaceae	Schumann (1888); Bequaert (1922)	Tropical Africa
Cuviera pierrei N.Hallé	Rubiaceae	Onana (2008)	Tropica Africa
Dactyladenia floretii Breteler	Chrysobalanaceae	Breteler (2000)	Gabon
Dactyladenia jongkindii Breteler	Chrysobalanaceae	Breteler (2000)	Gabon
Daemonorops jenkinsiana (Griff.) Mart.	Arecaceae	Bequaert (1922); Jolivet (1996)	Indo-Maleysian region
Daemonorops macrophylla Becc.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Daemonorops oligophylla Becc.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia

Daemonorops sabut Becc.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Daemonorops verticillaris (Griff.) Mart.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Delpydora macrophylla Pierre	Sapotaceae	McKey & Davidson (1993)	Tropical Africa, Central forest block
Dichapetalum gassitae Breteler	Dichapetalaceae	Breteler & Nzabi (1995)	Gabon
Diospyros andamanica (Kurz) Bakh.	Ebenaceae	Wong & Puff (1995)	SE Asia - Malaysia
Diospyros conocarpa Gürke ex K.Schum.	Ebenaceae	White (1977); McKey & Davidson (1993)	Tropical Africa
Diplectria stipularis Kuntze (=Dissochaeta)	Melastomataceae	Clausing (1998)	SE Asia
Dischidia albiflora Griff.	Apocynaceae	Kaufmann *et al*. (2001); Moog *et al*. (2003)	Java
Dischidia astephana Scort. ex King & Gamble	Apocynaceae	Weir & Kiew (1986); Kaufmann *et al*. (2001)	SE Asia
Dischidia cochleata Blume	Apocynaceae	Moog *et al*. (2003)	SE Asia
Dischidia complex Griff.	Apocynaceae	Rintz (1980)	Malaysia
Dischidia digitiformis Becc.	Apocynaceae	Beccari (1877)	Sulawesi
Dischidia diphylla Elmer	Apocynaceae	Elmer (1938)	SE Asia
Dischidia imbricata (Blume) Steud.	Apocynaceae	Kaufmann *et al*. (2001) ; Moog *et al*. (2003)	Java
Dischidia longipedunculata Ridl.	Apocynaceae	Kerr (1912); Ridley (1912); Moog *et al*. (2003)	SE Asia
Dischidia major (Vahl) Merr.	Apocynaceae	Griffith & Solly (1851); Groom (1893); Scott & Sargant (1893); Treseder *et al*. (1995)	SE Asia: Java, Thailand, Vietnam and Laos
Dischidia milnei Hemsl.	Apocynaceae	Baker (1891); Brown (1895)	SE Asia
Dischidia platyphylla Schltr.	Apocynaceae	Rintz (1980)	SE Asia
Dischidia purpurea Merr.	Apocynaceae	Rintz (1980)	SE Asia

Dischidia reniformis Schltr.	Apocynaceae	Rintz (1980)	SE Asia
Dischidia saccata Warb.	Apocynaceae	Perkins (1904)	SE Asia
Dischidia sorsogonensis Elmer	Apocynaceae	C. R. Huxley-Lambrick (pers. obs.); Elmer 15809 (BM)	Philippines
Dischidia vidalii Becc. (= ***D. pectenoides***)	Apocynaceae	Bequaert (1922)	SE Asia
Dischidia wallichii Wight	Apocynaceae	C. R. Huxley-Lambrick (pers. obs.)	SE Asia
Dischidiopsis papuana (Warb.) Schltr.	Apocynaceae	Hellwigianae (1894)	SE Asia (PNG)
Drypetes longifolia (Blume) Pax & K.Hoffm.	Euphorbiaceae	Agosti *et al*. (1999)	SE Asia, Sabah
Duroia hirsuta (Poepp.) K.Schum.	Rubiaceae	Wheeler & Bequaert (1929)	Neotropics, Amazonia
Duroia petiolaris Spruce ex K.Schum.	Rubiaceae	Wheeler & Bequaert (1929)	Neotropics, Amazonia
Duroia saccifera (Mart. ex Schult. & Schult.f.) K.Schum.	Rubiaceae	Wheeler & Bequaert (1929); Dattilo *et al*. (2013)	Neotropics, Amazonia
Empogona crepiniana (De Wild. & T.Durand) Tosh & Robbr.	Rubiaceae	Robbrecht (1979)	Tropical Africa
Encyclia belizensis subsp. ***parviflora*** (Regel) Dressler & G.E.Pollard	Orchidaceae	Damon & Perez-Soriano (2005)	Belize, Central America
Endospermum labios *Schodde*	Euphorbiaceae	Letourneau *et al*. (1993); Jolivet (1996); C. R. Huxley-Lambrick (pers. comm.)	Papua New Guinea, Madang area
Endospermum medullosum L.S.Sm.	Euphorbiaceae	Jolivet (1996)	SE Asia, Malaysia Region
Endospermum moluccanum (Teijsm. & Binn.) Kurz	Euphorbiaceae	Arias-Guerrero & Van Welzen (2011)	SE Asia
Endospermum	Euphorbiaceae	Arias-Guerrero & Van Welzen (2011)	SE Asia

myrmecophilum L.S.Sm.			
Epitaberna myrmoecia K. Schum.	Apocynaceae	Bequaert (1922)	Cameroon
Erythrina variegata L. (= *E. lithosperma*)	Fabaceae	Green (1904); Bequaert (1922)	SE Asia
Fagraea auriculata Jack	Loganiaceae	Bequaert (1922); Burch (1891); Jolivet (1996)	Oriental
Fagraea borneensis Scheffer	Loganiaceae	Bequaert (1922); Burch (1891); Jolivet (1996)	Borneo
Fagraea imperialis Miquel	Loganiaceae	Bequaert (1922); Burch (1891); Jolivet (1996)	Sumatra
Ficus obscura Blume ***var. borneensis*** (Miq.) Corner	Moraceae	Moog *et al*. (2003)	SE Asia - Malaysia
Gardenia imperialis K.Schum.	Rubiaceae	Schnell (1963); Schnell (1966); McKey & Davidson (1993)	Tropical Africa, West African forest block
Gardenia imperialis subsp. physophylla (K.Schum.) L.Pauwels	Rubiaceae	Bequaert (1922); Jolivet (1996)	Tropical Africa
Gertrudia amplifolia K. Schum.	Flacourtiaceae	Schumann & Laut (1901); Jolivet (1996)	SE Asia
Gigantochloa ligulata Gamble	Poaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Gigantochloa scortechinii *Gamble*	Poaceae	Davidson & McKey (1993); Kaufmann *et al*. (2001)	SE Asia
Gigantochloa thoi K.M.Wong	Poaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Gleasonia uaupensis Ducke	Rubiaceae	Kempf (1975); Benson (1983, 1985); Rogers (1984); Ferdandez (2007); Jolivet (1996)	Neotropics: Brazil
Grammatophyllum speciosum Blume	Orchidaceae	Bequaert (1922); Moog *et al*. (2003)	SE Asia - Malaysia
Heinsia myrmoecia (K.Schum.) N.Hallé	Rubiaceae	Jolivet (1996); McKey & Davidson (1993)	Tropical Africa, Central forest block
Helicia sp.	Proteaceae	Camilla Lambrick (Huxley) (pers. comm.)	Papua New Guinea
Henriettella cuneata	Melastomataceae	Michelangeli (2010)	Neotropics

(Standl.) Gleason (=*Henriettea cuneata*)			
Hirtella dorvalii Prance	Chrysobalanaceae	Prance (1972)	Neotropics
Hirtella duckei Huber	Chrysobalanaceae	Prance (1972)	Neotropics
Hirtella guainiae Spruce ex Hook.f.	Chrysobalanaceae	Prance (1972)	Neotropics
Hirtella myrmecophila Pilg.	Chrysobalanaceae	Dattillo *et al*. (2013); Prance (1972)	Neotropics
Hirtella physophora Mart. & Zucc.	Chrysobalanaceae	Dattilo *et al*. (2013); Prance (1972)	Neotropics
Hirtella revillae Prance	Chrysobalanaceae	McKey & Davidson (1993)	Neotropics
Hirtella vesiculosa Suess.	Chrysobalanaceae	Prance (1972)	Neotropics
Hoffmannia vesiculifera Standl.(*H. kirkbridei* and *H. morii* are synonymes and are reported by Jolivet (1996) to have smaller pouches, pointing to intraspecific variation)	Rubiaceae	Windsor & Jolivet (1996); Jolivet (1996)	Panama
Hoya darwinii Loher	Apocynaceae	Wanntorp *et al*. (2006)	Papua New Guinea
Hoya imbricata Decne.	Apocynaceae	Jolivet (1996)	Philippines
Hoya mitrata Kerr	Apocynaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Humboldtia brunonis Wall.	Fabaceae: Caesalpinioideae	Dev *et al*. (2010)	India, Western Ghats
Humboldtia decurrens Oliv.	Fabaceae: Caesalpinioideae	Dev *et al*. (2010)	India
Humboldtia laurifolia M.Vahl	Fabaceae: Caesalpinioideae	Bower (1887); Schimper (1903); Escherich (1906, 1911); Ridley (1910); Wheeler (1910); Jolivet (1996); Dev *et al*. (2010);	In Ceylon
Hydnophytum acuminicalyx Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia

Hydnophytum albertisii Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum buxifolium Merr. & L. M. Perry	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum confertifolium Merr. & L. M. Perry	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum cordifolium Valeton	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum decipiens Merr. & L. M. Perry	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum ferrugineum P. I. Forst.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum formicarum Jack	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum grandiflorum Becc.	Rubiaceae	Jebb and Huxley (in press)	Australasia
Hydnophytum grandifolium Valeton	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum guppyanum Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum hellwigii Warb.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum heterophyllum Merr. & L. M. Perry	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum kebarense Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum lauterbachii Valeton	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum linearifolium Valeton	Rubiaceae	Jebb & Huxley (in press)	Australasia

Hydnophytum longiflorum A.Gray	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum lucidulum Valeton	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum magnifolium Merr. & L. M. Perry	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum mamberamoense Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum mayuense Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum microphyllum Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum minirubrum Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum morotaiense Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum moseleyanum Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum multituberosum Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum orichalcum Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum ovatum Miq.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum pauper Valeton ex Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia

Hydnophytum perangustum Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum petiolatum Becc. var. ***petiolatum***	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum radicans Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum ramispinum Merr. & L. M. Perry	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum reevii Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum spathulatum Valeton	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum terrestris Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum tetrapterum Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum tortuosum Becc.	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum trichomanes Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Hydnophytum vanuatuense Jebb & C. R. Huxley	Rubiaceae	Jebb & Huxley (in press)	Australasia
Ixora hippoperifera Bremek.	Rubiaceae	McKey & Davidson (1993)	Tropical Africa, Central forest block
Keetia venosa (Oliv.) Bridson	Rubiaceae	Camilla Lambrick (Huxley) (pers. comm.)	Tropical Africa
Kibara archboldiana A.C.Sm.	Monimiaceae	Philipson (1985)	S. Papua New Guinea
Kibara carrii Philipson	Monimiaceae	Philipson (1985)	Malaysia Central Province

Kibara ferox Philipson	Monimiaceae	Philipson (1985)	E. Papua New Guinea
Kibara formicarum Becc.	Monimiaceae	Beccari (1877); Bequaert (1922); Jolivet (1996)	SE Asia, Malaysia
Kibara latifolia Philipson	Monimiaceae	Philipson (1985)	Moluccas
Korthalsia angustifolia Blume	Arecaceae	Beccari (1884-6); Emery (1888); Forel (1902); Ridley (1907, 1910), Shelford (1916); Bequaert (1922); Jolivet (1996)	SE Asia
Korthalsia chab Becc.	Arecaceae	Huth (1886)	SE Asia
Korthalsia echinometra Becc.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Korthalsia hispida Becc.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Korthalsia horrida Becc.	Arecaceae	Huth (1886)	SE Asia
Korthalsia rostrata Blume	Arecaceae	Beccari (1884); Jolivet (1996); Moog *et al*. (2003)	SE Asia - Malaysia
Korthalsia scaphigera Mart.	Arecaceae	Ridley (1910)	SE Asia
Korthalsia scortechinii Becc.	Arecaceae	Moog *et al*. (2003); Jolivet (1996)	SE Asia - Malaysia
Lecanopteris balgooyi Hennipman	Polypodiaceae	Haufler *et al*. (2003)	Sulawesi (Celebes)
Lecanopteris carnosa (Reinw.) Blume	Polypodiaceae	Haufler *et al*. (2003)	Sulawesi (Celebes)
Lecanopteris celebica Hennipman	Polypodiaceae	Haufler *et al*. (2003)	Sulawesi (Celebes)
Lecanopteris crustacea Copel.	Polypodiaceae	Haufler *et al*. (2003)	Western Malesia
Lecanopteris curtisii Baker	Polypodiaceae	Haufler *et al*. (2003)	SE Asia
Lecanopteris deparioides (Ces.) Baker	Polypodiaceae	Haufler *et al*. (2003)	Java, Western Malesia, Philippines
Lecanopteris holttumii Hennipman	Polypodiaceae	Haufler *et al*. (2003)	SE Asia
Lecanopteris lomarioides (Kunze ex Mett.) Copel.	Polypodiaceae	Haufler *et al*. (2003)	Sulawesi (Celebes), Philippines

Lecanopteris luzonensis Hennipman	Polypodiaceae	Haufler *et al*. (2003)	Philippines
Lecanopteris mirabilis (C. Chr.) Copel.	Polypodiaceae	Haufler *et al*. (2003)	New Guinea and Moluccas
Lecanopteris pumila Blume	Polypodiaceae	Haufler *et al*. (2003)	Western Malesia
Lecanopteris sinuosa (Hook.) Copel.	Polypodiaceae	Janzen (1974); Jolivet (1996); Haufler *et al*. (2003)	Western Malesia, Sulawesi, Philippines, New Guinea and Moluccas, also Indochina and Vanuatu
Lecanopteris spinosa Jermy & Walker	Polypodiaceae	Haufler *et al*. (2003)	Sulawesi (Celebes)
Leonardoxa africana (Baill.) Aubrév. subsp. ***africana;*** subsp**.** ***rumpiensis*** and subsp**.** ***letouzeyi***	Fabaceae-Caesalpinioideae	Chenuil & McKey (1996); McKey (2000) Three of the four subspecies have domatia, albeit with different level of specialization cf. McKey (2000).	Africa
Lepisanthes alata (Blume) Leenh.	Sapindaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Lepisanthes amoena (Hassk.) Leenh.	Sapindaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Leptactina mannii Hook.f.	Rubiaceae	Breteler & Nzabi (1995)	Gabon
Leptospermum polygalifolium **subsp.** ***polygalifolium (=Leptospermum flavescens*** Smith***)***	Myrtaceae	Weir & Kew (1985); Moog *et al*. (2003); Jolivet (1996)	SE Asia - Malaysia
Luvunga **sp.**	Rutaceae	Agosti *et al*. (1999)	SE Asia, Pahang, Fraser's hill, 800m
Macaranga aetheadenia Airy Shaw	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga beccariana Merr.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia

Macaranga caladiifolia Becc.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga calcicola Airy Shaw	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga constricta Whitemore & Airy Shaw	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga depressa Muell. Arg.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga formicarum Pax and O Hoffmann	Euphorbiaceae	Bequaert (1922); Beccari (1884); Ridley (1910); Pax (1914); Shelford (1916)	SE Asia
Macaranga glandibracteolata S.J.Davies	Euphorbiaceae	Fiala & Maschwitz (1992)	SE Asia
Macaranga havilandii Airy Shaw	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga hosei King ex. Hk. Fil.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga hulletii King ex Hook. f.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga hullettii King ex Hook.f.	Euphorbiaceae	Moog *et al* (2003)	Malaysia
Macaranga hypoleuca Muell. Arg.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga indistincta Whitmore	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga kingii Hook. f.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga lamellata Whitmore	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga maingayi (Miq.) Müll.Arg.	Euphorbiaceae	Bake & So (1934)	SE Asia
Macaranga motleyana Muell. Arg. subsp. ***griffithiana*** (Muell. Arg.)	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia

Whitemore			
Macaranga pearsonii Merr.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga petanostyla Airy Shaw	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga pruinosa (Miq.) Muell. Arg.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga puberula Heine	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga puncticulata Gage	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga puncticulata Gage	Euphorbiaceae	Moog *et al* (2003)	Malaysia
Macaranga quadricornis Ridley	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga recurvata Gage	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga rostrata Heine	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga saccifera Pax	Euphorbiaceae	Bequaert (1922); Schnell & Grout de Beaufort (1966); McKey & Davidson (1993)	Tropical Africa, Central forest block
Macaranga schweinfurthii Pax	Euphorbiaceae	Bequaert (1922)	Tropical Africa, Central forest block
Macaranga trachyphylla Airy Shaw	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga triloba (BL.) Muell. Arg.	Euphorbiaceae	Smith W (1903); Takahashi (1951); Rickson (1980); Fiala & Maschwitz (1992)	Malaysia
Macaranga winkleri Pax & Hoffm.	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia
Macaranga winkleriella Whitemore	Euphorbiaceae	Fiala & Maschwitz (1992)	Malaysia

Macrolobium acaciifolium (Benth.) Benth.	Fabaceae-cesalpinioideae	Ward (1999)	Ecuador
Magnistipula bimarsupiata Letouzey	Chrysobalanaceae	Letouzey & White (1978); McKey & Davidson (1993)	Tropical Africa, Gabon
Maieta guianensis Aubl.	Melastomataceae	Dattilo *et al*. (2013)	Neotropics
Maieta neblinensis Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics
Maieta poeppigii Mart. ex Cogn.	Melastomataceae	Michelangeli (2010)	Neotropics
Markea ciliata Benth.	Solanaceae	Spruce (1908)	South America
Markea formicarum Dammer	Solanaceae	Spruce (1908); Weber (1943)	Neotropics
Markea ulei (Dammer) Cuatrec.	Solanaceae	Spruce (1908); Davidson & Epsein (1989); Spruce (1908)	Neotropics
Medinilla loheri* Merrill/*Medinilla disparifolia C.B.Rob.	Melastomataceae	Bequaert (1922); Jolivet (1996); Solereder (1920)	Luzon, Philippines
Merianthera burlemarxii Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics: Brazil
Miconia bailloniana J.F. Macbr.	Melastomataceae	Michelangeli (2010)	Neotropics: Peru
Miconia expansa Gleason	Melastomataceae	Michelangeli (2010)	Neotropics: Peru
Miconia flaccida Gleason	Melastomataceae	Michelangeli (2010)	Neotropics: Peru
Miconia hospitalis Wurdack	Melastomataceae	Michelangeli (2010)	Neotropics: Peru
Mischocarpus paradoxus Radlk.	Sapindaceae	M. Jebb (pers. obs.); C. R. Huxley-Lambrick (pers. comm.)	Papua New Guinea
Myrcia madida McVaugh	Myrtaceae	Ferreira & de Vasconcelos (2010); Vicente *et al*. (2012)	Neotropics -Amazone - Brazil, Mato Grosso
Myristica euryocarpa Warburg	Myristicaceae	Warburg (1897); Halle (1968)	SE Asia

Myristica heterophylla K. Schumm.	Myristicaceae	Warburg (1897); Halle (1968)	SE Asia
Myristica subalulata Miq. ***(= M. Myrmecophila)***	Myristicaceae	Huth (1886); Bequaert (1922); Warburg (1897); Halle (1968); Beccari (1884-6); Gullan *et al*. (1993); Jolivet (1996)	New Guinea
Myrmecodia alata Becc.	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia albertisii Becc.	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia angustifolia Valeton	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia archboldiana Merr. & L.M.Perry	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia aureospina Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia beccarii Hook.f.	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia brassii Merr. & L.M.Perry	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia erinacea Becc.	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia ferox Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia gracilispina Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia horrida Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia jobiensis Becc.	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia kutubuensis Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia lamii Merr. & L.M.Perry	Rubiaceae	Huxley & Jebb (1993)	SE Asia

Myrmecodia longifolia Valeton	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia longissima Valeton	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia melanacantha Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia oblongata Valeton	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia oksapminensis Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia paradoxa Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia pendens Merr. & L.M.Perry	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia platytyrea Becc.	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia pteroaspida Huxley & Jebb	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia schlechteri Valeton	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia sterrophylla Merr. & L.M.Perry	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmecodia tuberosa Jack	Rubiaceae	Huxley & Jebb (1993)	SE Asia
Myrmeconauclea strigosa (Korth.) Merr.	Rubiaceae - Naucleeae	Bequaert (1922); Haviland (1887); Merrill (1920); Razafimandimbison *et al*. (2005)	Borneo
Myrmecophila × laguna-guerrerae Carnevali, L.Ibarra & J.L.Tapia	Orchidaceae	Bequaert (1922)	Neotropics
Myrmecophila albopurpurea (H.Strachan ex Fawc.) Nir	Orchidaceae	Bequaert (1922)	Neotropics

Myrmecophila brysiana (Lem.) G.C.Kenn.	Orchidaceae	Jones (1968)	Guatemala, Honduras, Trinidad, Cuba
Myrmecophila christinae Carnevali & Gómez-Juárez	Orchidaceae	Rico-Gray *et al*. (2004)	Mexico, Central America
Myrmecophila exaltata (Kraenzl.) G.C.Kenn.	Orchidaceae	Jones (1968)	Guatemala
Myrmecophila galeottiana (A.Rich.) Rolfe	Orchidaceae	Jones (1968)	Mexico
Myrmecophila grandiflora (Lindl.) Carnevali & J.L.Tapia & I.Ramírez	Orchidaceae	Jones (1968)	Honduras
Myrmecophila humboldtii (Rchb.f.) Rolfe	Orchidaceae	Bequaert (1922)	Neotropics
Myrmecophila thomsoniana (Rchb.f.) Rolfe	Orchidaceae	Jones (1968)	Guatemala, Honduras, Trinidad, Barbados, Caymans
Myrmecophila tibicinis (Bateman ex Lindl.) Rolfe (=*Schomburgkia tibicinis*)	Orchidaceae	Mayr (1862); Huth (1886); Ross (1909); Step (1913); Bequaert (1922); Rico-Gray *et al*. (1989)	Mexico, Central America, Cuba, Hispaniola
Myrmecophila wendlandii (Rchb.f.) G.C.Kenn.	Orchidaceae	Jones (1968)	Guatemala, Honduras, Nicaragua
Myrmephytum arfakianum (Becc.) Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991b)	SE Asia
Myrmephytum beccarii Elmer	Rubiaceae	Huxley & Jebb (1991b)	SE Asia
Myrmephytum moniliforme Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991b)	SE Asia

Myrmephytum naumannii (Warb.) Huxley & Jebb	Rubiaceae	Huxley & Jebb (1991b)	SE Asia
Myrmephytum selebicum (Becc.) Becc.	Rubiaceae	Huxley & Jebb (1991b)	SE Asia
Nauclea vanderguchtii (De Wild.) E.M.A.Petit	Rubiaceae - Naucleeae	Razafimandimbison *et al*. (2005)	Tropical Africa, Central forest block
Neonauclea artocarpoides Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	North Borneo
Neonauclea borneensis Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	East-Central Borneo
Neonauclea butonensis Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	Celebes
Neonauclea calcarea Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	East Borneo
Neonauclea celebica (Havil.) Merr.	Rubiaceae - Naucleeae	Ridsdale (1989); Razafimandimbison *et al*. (2005)	Celebes (scattered collections from North to South of Sulawesi)
Neonauclea cyrtopoda (Miq.) Merr.	Rubiaceae - Naucleeae	Ridsdale (1989)	North and West Sumatra, Small Island close to West Sumatra
Neonauclea excelsioides Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	North and east Borneo
Neonauclea formicaria (Elmer) Merr.	Rubiaceae - Naucleeae	Ridsdale (1989)	Philippines, from Center to South (no record in the North)
Neonauclea gigantean (Valeton) Merr.	Rubiaceae - Naucleeae	Ridsdale (1989); Razafimandimbison *et al*. (2005)	Borneo (many collections in the North, and SW, none in south east)
Neonauclea havilandii Koord. ex Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	Celebes (scattered collections from North to South of Sulawesi)
Neonauclea kentia (Merr.) Merr.	Rubiaceae - Naucleeae	Ridsdale (1989)	Extreme South West Celebes
Neonauclea lanceolata (Blume) Merr.	Rubiaceae	C. R. Huxley-Lambrick (pers. comm.)	SE Asia
Neonauclea longipedunculata Merr.	Rubiaceae - Naucleeae	Razafimandimbison *et al*. (2005)	SE Asia

Neonauclea paracyrtopoda Bakh.f. & Ridsdale	Rubiaceae - Naucleeae	Razafimandimbison *et al*. (2005)	North - NW Borneo
Neonauclea pseudocalycina Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	North and Central Borneo, Small island close to North (east) Borneo
Neonauclea rupestris Bakh.f. & Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	Extreme South West Celebes
Neonauclea superba (S.Moore) S.Moore	Rubiaceae - Naucleeae	Ridsdale (1989)	Sumatra (scattered collections from North to South Sumatra)
Neonauclea unicapitulifera Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	South East Celebes, including small island in the south)
Neonauclea ventricosa Ridsdale	Rubiaceae - Naucleeae	Ridsdale (1989)	Celebes
Nepenthes bicalcarata Hook.f.	Nepenthaceae	Beccari (1884); Shelford (1916); Bequaert (1922); Thompson (1981)	Malaysian region
Notopleura epiphytica (K.Krause) C.M.Taylor (=***Uragoga epiphytica***)	Rubiaceae	Bequaert (1922); C. R. Huxley (pers. comm.)	West Africa
Ocotea atirrensis Mez & Donn.Sm. (= *O. pedalifolia*)	Lauraceae	Van der Werff (1988); Stout (1979)	Neotropics
Ocotea dendrodaphne Mez	Lauraceae	Van der Werff (1988)	Neotropics
Ocotea javitensis (Kunth) Pittier	Lauraceae	Van der Werff (1988)	Neotropics
Ormosia macrophylla Benth.	Fabaceae - Faboideae	Benson (1985); Jolivet (1996)	Neotropics
Ossaea bullifera (Pilg.) Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Pachycentria constricta Blume	Melastomataceae	Kaufmann *et al*. (2001)	Malaya, Borneo
Pachycentria glauca Triana	Melastomataceae	Kaufmann *et al*. (2001)	Borneo
Pachycentria	Melastomataceae	Beccari (1884); Ridley (1910); Bequaert (1922)	SE Asia

macrorrhiza Becc.			
Pachycentria maingayi J.F.Maxwell	Melastomataceae	Kaufmann *et al*. (2001)	Malaya
Pachycentria microstyla Becc.	Melastomataceae	Beccari (1884); Ridley (1910); Shelford (1916); Bequaert (1922)	SE Asia
Pachycentria tuberculata Korth. (=*P. tuberosa*)	Melastomataceae	Beccari (1884); Ridley (1910); Bequaert (1922); Kaufmann *et al*. (2001)	SE Asia, Borneo
Palicourea corymbifera (Müll.Arg.) Standl.	Rubiaceae	Dattilo *et al*. (2013)	Neotropics
Patima formicaria Johnson	Rubiaceae	Schnell & Grout de Beaufort (1966); Delprete *et al*. (2005)	South America
Patima guianensis Aubl. (= ***Sabicea guianensis***)	Rubiaceae	Schnell & Grout de Beaufort (1966); Delprete *et al*. (2005)	Guyanas and Northern Brazil
Pavetta owariensis P. Beauv.	Rubiaceae	C. R. Huxley-Lambrick (pers. comm.)	Tropical Africa, Cameroon
Philodendron myrmecophilum Engl.	Araceae	C.R. Huxley-Lambrick (pers. comm.)	Neotropics
Picrolemma sp.	Simaroubiaceae	W. W. Benson (pers. comm.) to C. R. Huxley-Lambrick	Neotropics
Piper archeri Trel. & Yunck.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper auritum Kunth.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper begoniicolor Trel. & Yunck.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper biseriatum C.DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper calceolatum C. DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics

Piper caracasanum Bredem. ex Link	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper cenocladum C.DC.	Piperaceae - Piper subgenus - Macrostachys	Rish *et al*. (1977)	Neotropics
Piper cernuum Vell.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper cogolloi Callejas	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper fimbriulatum C. DC.	Piperaceae - Piper subgenus - Macrostachys	Jolivet (1996)	Neotropics
Piper gigantifolium C.DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper imperiale (Miq.) C.DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper longispicum C.DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper marsupiatum Trel. & Yunck.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper myrmecophilum C.DC.	Piperaceae	Jolivet (1996)	Philippines
Piper nobile C.DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper obliquum Ruiz & Pav.	Piperaceae - Piper subgenus -	Rish *et al*. (1977)	Neotropics

	Macrostachys		
Piper obtusilimbum C. DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper pseudonobile C. DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Piper sagittifolium C. DC.	Piperaceae - Piper subgenus - Macrostachys	Rish *et al*. (1977)	Neotropics
Piper subglabribracteatum C. DC.	Piperaceae - Piper subgenus - Macrostachys	Tepe *et al*. (2004)	Neotropics
Pithecellobium saman (Jacq.) Benth.	Fabaceae	Ward (1991)	Neotropics
Platycerium coronarium (Mull.) Desv.	Polypodiaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Platycerium ridleyi Christ	Polypodiaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Platymiscium darienense Dwyer	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium dimorphandrum Donn.Sm.	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium filipes Benth.	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium floribundum Vogel	Fabaceae - Faboideae - Dalbergieae	Bequaert (1922); Klitgaard (2005)	Neotropics
Platymiscium gracile Benth.	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics

Platymiscium pinnatum (Jacq.) Dugand	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium speciosum Vogel	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium stipulare Benth.	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium trinitatis Benth.	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Platymiscium ulei Harms	Fabaceae - Faboideae - Dalbergieae	Klitgaard (2005)	Neotropics
Plectronia laurentii De Wild.	Rubiaceae	de Wildeman (1906); Kohl (1909); lUphof (1942); Jolivet (1996)	Tropical Africa
Pleurothyrium cuneifolium Nees	Lauraceae	Van der Werff (1988)	Brazil, Ecuador, Colombia
Pleurothyrium obovatum van der Werff	Lauraceae	Van der Werff (1988)	Ecuador, altitude up to 2800 m
Pleurothyrium parviflorum Ducke	Lauraceae	Schumann (1888); Bequaert (1922); Van der Werff (2008)	Western Amazon (Ecuador, Peru) and Central Amazon (Brazil)
Pleurothyrium poeppigii Nees	Lauraceae	Van der Werff (2008)	Bolivia, Brazil, Colombia, Ecuador, Peru
Pogonotium ursinum (Becc.) J.Dransf.	Arecaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Polypodium schomburgkianum Kunze	Polypodiaceae	Spruce (1908); Huxley (1980)	Neotropics
Pometia pinnata J.R. Forst. & G. Forst.	Sapindaceae	Moog *et al*. (2003); Moog *et al*. (2008)	SE Asia - Malaysia
Pourouma formicarum Ducke	Urticaceae	Berg *et al*. (1990)	Neotropics
Pourouma guianensis	Urticaceae	Penzing (1892); Bequaert (1922); O' Dowd (1982)	Neotropics

Aubl.			
Pourouma myrmecophila Ducke	Urticaceae	Berg *et al*. (1990)	Neotropics
Psychotria bidentata (Benth.) Hiern ***(formerly Uragoga)***	Rubiaceae	Bequaert (1922); C. R. Huxley (pers. comm.)	West Africa
Psychotria cyanopharynx K.Schum. ***(=Grumilea refractistipula Wildeman)***	Rubiaceae	Bequaert (1922); Wildeman (1923); Jolivet (1996); C. R. Huxley (pers. comm.)	Africa
Psychotria myrmecophila K.Schum. & Lauterb.	Rubiaceae	Schumann & Lant (1901); Bequaert (1922); Heckroth *et al*. (2004); Jolivet (1996)	New Guinea
Psychotria venosa (Hiern) E.M.A.Petit (= ***Grumilea venosa or Uragoga venosa***)	Rubiaceae	Bequaert (1922)	West Africa
Psydrax bridsoniana Cheek & Sonké	Rubiaceae	Bridson (1985)	Tropical Africa, West African forest block
Psydrax dunlapii (Hutch. & Dalziel) Bridson	Rubiaceae	Bridson (1985)	Tropical Africa, West African forest block
Psydrax paradoxa (Virot) Mouly	Rubiaceae	Mouly (2006)	New Caledonia
Psydrax parviflora (Afzel.) Bridson	Rubiaceae	C. R. Huxley-Lambrick (pers. comm.)	Tropical Africa
Psydrax subcordata (DC.) Bridson	Rubiaceae	Jolivet (1996)	Africa
Psydrax subcordata var. ***connata*** (De Wild. & T.Durand) Bridson	Rubiaceae	Jolivet (1996)	Tropical Africa, West Africanto central African forest block
Psydrax subcordata var. ***subcordata*** (DC.) Bridson (= ***Canthium glabriflorum***)	Rubiaceae	Schnell & Grout de Beaufort (1966); McKey & Davidson (1993)	Tropical Africa, West of the Dahomet gap
Pterocarpus amazonum (Benth.) Amshoff (= *P.*	Fabaceae-Faboideae	Rojo (1972); Spruce (1908)	Neotropics

ancylocalyx Benth.)			
Andira sapindoides (DC.) Benth. (= *Pterocarpus sapindoides*)	Fabaceae - Faboideae	C. R. Huxley (pers. comm.)	Neotropics
Remijia glomerata Huber	Rubiaceae	Benson (1985); Jolivet (1996)	Tropical America, Amazon
Remijia physophora Benth. ex K.Schum.	Rubiaceae	Spruce (1908); Bequaert (1922); Robbrecht (1988)	Neotropics
Rothmannia libisa N.Hallé	Rubiaceae	Somers & Robbrecht (1991)	Tropical Africa
Rothmannia lujae (De Wild.) Keay	Rubiaceae	de Wildeman (1904); McKey & Davidson (1993); Jolivet (1996)	Tropical Africa, Central forest block
Rothmannia macrocarpa (Hiern) Keay ***(= Randia myrmecophylla)***	Rubiaceae	Somers & Robbrecht (1991); Jolivet (1996); Bequaert (1922)	Tropical Africa, Central forest block
Rothmannia munsae (Schweinf. ex Hiern) E.M.A.Petit	Rubiaceae	Somers & Robbrecht (1991)	Tropical Africa
Ruprechtia cruegeri Griseb. ex Lindau	Polygonaceae	Pendry (2004); Sanchez (2011)	Neotropics
Ruprechtia jamesonii Meisn.	Polygonaceae	Spruce (1908); Bequaert (1922)	Neotropics
Ruprechtia latifunda Pendry	Polygonaceae	Sanchez (2011)	Neotropics
Ruprechtia laurifolia (Schltdl. & Cham.) C.A. Mey.	Polygonaceae	Pendry (2004)	Neotropics
Ruprechtia lundii Meisn.	Polygonaceae	Pendry (2004)	Neotropics
Ruprechtia maracensis Brandbyge	Polygonaceae	Pendry (2004)	Neotropics
Ruprechtia obidensis Huber	Polygonaceae	Pendry (2004)	Neotropics
Ruprechtia tangarana Standl.	Polygonaceae	Pendry (2004)	Neotropics

Ryparosa fasciculata King	Achariaceae	Moog *et al*. (1997); Agosti *et al*. (1999)	SE Asia
Rytigynia bridsoniae Verdc. subsp. ***kahuzica***	Rubiaceae	Verdcourt (1987)	Tropical Africa
Sapium laurifolium (A.Rich.) Griseb. (=***Sapium utile***)	Euphorbiaceae	Ward (1999)	Ecuador
Saraca thaipingensis Prain	Fabaceae	Moog *et al*. (1997); Agosti *et al*. (1999)	SE Asia, Malaysia
Saurania sp.	Malvaceae - Sterculioideae	Jolivet (1973); Jolivet (1996)	SE Asia
Scaphopetalum dewevrei De Wild. & T.Durand	Malvaceae - Sterculioideae	Bequaert (1922); Uphof (1942)	Tropical Africa
Scaphopetalum thonneri De Wild. & T.Durand	Malvaceae - Sterculioideae	Bequaert (1922); De Wildeman (1897) ; McKey & Davidson (1993)	Tropical Africa, Central forest block; Zaire, Gabon, Cameroon, and Congo
Schizostachyum sp.	Poaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Schlerolobium odoratissimum Benth.	Fabaceae - Faboideae	Spruce (1908); Bequaert (1922)	Brazil
Sclerolobium odoratissimum Benth.	Fabaceae - Faboideae	Jolivet (1996)	Tropical America
Semecarpus sp.	Anacardiaceae	C. R. Huxlex-Lambrick (pers. comm.)	Papua New Guinea
Shorea acuminata Dyer	Dipterocarpaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Siparuna sp.	Monimiaceae	Benson (1983); Jolivet (1996)	
Sloanea sp.	Elaeocarpaceae	Benson (1983); Jolivet (1996)	Tropical America
Smilax borneensis A.DC.	Smilacaceae	Heckroth *et al*. (2004)	Borneo, Malaysia
Smilax gigantean Merr.	Smilacaceae	Heckroth *et al*. (2004)	Borneo
Smilax ocreata A.DC.	Smilacaceae	Heckroth *et al*. (2004)	India: Assam, Thailand
Smilax perfoliata Lour.	Smilacaceae	Heckroth *et al*. (2004)	India, Thailand
Solanopteris bifrons (Hook.) Copel.	Polypodiaceae	Hagemann (1969)	Costa Rica to Peru
Solanopteris bismarckii Rauh	Polypodiaceae	Hagemann (1969)	Costa Rica to Peru

Solanopteris brunei (H. Christ) Wagner	Polypodiaceae	Hagemann (1969); Wagner (1972)	Costa rica to Columbia
Solanopteris tuberosum (Maxon) Rauh	Polypodiaceae	Hagemann (1969)	Ecuador
Spatholobus bracteolatus King	Fabaceae - Faboideae	Agosti *et al*. (1999)	SE Asia, Malaysia
Spatholobus oblongifolius Merr.	Fabaceae - Faboideae	Maschwitz et al. (1989); Agosti *et al*. (1999)	SE Asia, Sarawak
Squamellaria imberbis (A.Gray) Becc.	Rubiaceae	Jebb (1991)	Fiji
Squamellaria major A.C.Sm.	Rubiaceae	Jebb (1991)	Fiji
Squamellaria thekii Jebb	Rubiaceae	Jebb (1991)	Fiji
Steganthera hospitans (Becc.) Kaneh. & Hatus. (= ***Amthobembix hospitans***)	Monimiaceae	Beccari (1877); Jolivet (1996)	SE Asia, PNG
Steganthera moszkowskii (Perkins) Kaneh. & Hatus.	Monimiaceae	Philipson (1984)	Papua New Guinea - West Irian
Steganthera royenii Philipson	Monimiaceae	Philipson (1984)	Papua New Guinea - West Irian
Stereospermum kunthianum Cham.	Bignonaceae	Davidson & McKey (1993)	Tropical Africa, Central forest block
Stereospermum tetragonum DC. (= ***S. personatum***)	Bignonaceae	Ward Blog (http://wardlab.wordpress.com/research/pseudomyrmecinae/ant-plants/)	India
Strychnos vanprukii Craib	Loganiaceae	Agosti *et al*. (1999); Moog *et al* (2003)	SE Asia, Malaysia
Symmeria paniculata Benth.	Polygonaceae	Spruce (1908); Jolivet (1996)	Guiana, Northern Brazil, Sierra Lara
Syzygium cormiflorum (F.Muell.) B.Hyland	Myrtaceae	Jolivet (1996); Montheith (pers. comm.) to C. R. Huxley-Lambrick July 1984; Hyland (1983)	
Syzygium erythrocalyx	Myrtaceae	Jolivet (1996); Montheith (pers. comm.) to C. R. Huxley-	

(C.T.White) B.Hyland		Lambrick July 1984; Hyland (1983)	
Tachia guianensis Aubl. (= ***Myrmecia***)	Gentianaceae	Schumann (1888); Huth (1887); Bequaert (1922)	Neotropics
Tachigali argyrophylla Ducke	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali candelabrum van der Werff	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali catingae Ducke	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali cavipes (Benth.) J.F.Macbr.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali cenepensis van der Werff	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali chrysophylla (Poepp.) Zarucchi & Herend.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali dwyeri (R.S. Cowan) Zarucchi & Herend.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali formicarum Harms	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali fusca van der Werff	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali glauca Tul.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali grandistipulata Harms	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali longiflora Ducke	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali loretensis van der Werff	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali macbridei Zarucchi & Herend.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics

Tachigali macrostachya Huber	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali myrmecophila (Ducke) Ducke	Fabaceae-cesalpinioideae	Bequaert (1922);Passmore *et al*. (2012)	Neotropics
Tachigali odoratissima (Spruce ex Benth.) Zarucchi & Herend.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali paniculata Aubl.	Fabaceae-cesalpinioideae	Bailey (1923)	Neotropics
Tachigali physophora (Huber) Zarucchi & Herend.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali plumbea Ducke	Fabaceae-cesalpinioideae	Passmore *et al*. (2012)	Neotropics
Tachigali poeppigiana Tul.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali ptychophysca Benth.	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali rigida Ducke	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali schultesiana Dwyer	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali vaupesiana van der Werff	Fabaceae-cesalpinioideae	Van der Werf (2008)	Neotropics
Tachigali venusta Dwyer	Fabaceae-cesalpinioideae	Passmore *et al*. (2012)	Neotropics
Teijsmanniodendron pteropodum (Miq.) Bakh.	Lamiaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Tetrathylacium macrophyllum Poepp.	Salicaceae	Schmidt (2001); Jolivet (1996)	Peru to Costa Rica
Tillandsia balbisiana Schult. & Schult.f.	Bromeliaceae	Jolivet (1996); Dejean *et al*. (1995)	Neotropics: Mexico
Tillandsia bulbosa Hook.	Bromeliaceae	Baker (1889); Adams (1972); Benzing (pers. comm.) to C. R. Huxley-Lambrick (1978)	Neotropics

Tillandsia butzii Mez	Bromeliaceae	Benzing (1970)	Neotropics: Mexico
Tillandsia caput-medusae E.Morren	Bromeliaceae	Benzing (1970)	Neotropics: Mexico
Tillandsia flexuosa Sw.	Bromeliaceae	Baker (1889); Adams (1972); Benzing in letter July 1978 to C. R. Huxley-Lambrick	Neotropics
Tillandsia paraensis Mez	Bromeliaceae	C. R. Huxley-Lambrick (pers. comm.)	Neotropics
Tillandsia paucifolia Baker	Bromeliaceae	Jolivet (1996)	Neotropics
Tillandsia pseudobaileyi C.S.Gardner	Bromeliaceae	Jolivet (1996)	Neotropics
Tillandsia streptophylla Scheidw. ex E.Morren	Bromeliaceae	Schimper (1888); Benzing (1978) (pers. comm.) to C. R. Huxley-Lambrick	Central America
Tococa aristata Benth.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa bullifera Mart. & Schrank ex DC.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa capitata Trail ex Cogn.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa caquetana Sprague	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa carolensis Gleason	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa caudate Markgr.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa ciliate Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa cordata O. Berg ex Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa coronata Benth.	Melastomataceae	Michelangeli (2000)	Neotropics
Tococa costoides Michelangeli	Melastomataceae	Michelangeli (2006)	Brazil (Amazonas state)
Tococa filiformis (Gleason) K. Wurdack	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa gonoptera Gleason	Melastomataceae	Michelangeli (2005)	Neotropics

Supporting Information Table S1
Chomicki G, Renner SS. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist*.

Tococa guianensis Aubl.	Melastomataceae	Schumann (1888); Spruce (1908); Bequaert (1922); Renner & Rickleys (1998)	Neotropics; Peru - Ecuador
Tococa hirta O. Berg ex Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa lancifolia Spruce ex Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa leticiana Michelangeli	Melastomataceae	Michelangeli (2006)	Colombia (Leticia only)
Tococa macrophysca Spruce ex Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa macroptera Naudin	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa macrosperma Mart.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa obovata Gleason subsp. ***nebliensis***	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa pachystachya Wurdack	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa parviflora Spruce ex Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa pauciflora Spruce ex Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa quadrialata (Naudin) J.F. Macbr.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa racemifera Wurdack	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa rotundifolia (Triana) Wurdack	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa spadiciflora Triana	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa stellata Gleason	Melastomataceae	Michelangeli (2010)	Neotropics
Tococa stenoptera Gleason	Melastomataceae	Michelangeli (2005)	Neotropics

Tococa stephanotricha Naudin	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa symphyandra (Triana) Cogn.	Melastomataceae	Michelangeli (2005)	Neotropics
Tococa undabunda J.F. Macbr.	Melastomataceae	Michelangeli (2010)	Neotropics
Topobea gracilis Triana	Melastomataceae	Michelangeli (2010)	Neotropics: Colombia
Topobea inflata Triana	Melastomataceae	Michelangeli (2010)	Neotropics: Colombia
Topobea pluvialis Standl.	Melastomataceae	Michelangeli (2010)	Neotropics: Panama
Tricalysia pangolina N.Hallé	Rubiaceae	Robbrecht (1979)	Tropical Africa
Triplaris americana L.	Polygonaceae	Oliveira *et al*. (1987); Weddell (1849); Ward (1999); Sanchez (2011)	Venezuela, Peru, Colombia, Brazil
Triplaris caracasana Cham.	Polygonaceae	Sanchez (2011)	Neotropics
Triplaris cumingiana Fisch. & C.A.Mey.	Polygonaceae	Oliveira *et al*. (1987); Sanchez (2011)	Neotropics
Triplaris dugandii Brandbyge	Polygonaceae	Sanchez (2011)	Peru, Brazil, Ecuador
Triplaris felipensis Wedd.	Polygonaceae	Oliveira *et al*. (1987); Ward (1999)	Venezuela
Triplaris fulva Huber	Polygonaceae	Sanchez (2011)	Neotropics
Triplaris gardneriana Wedd.	Polygonaceae	Sanchez (2011)	Neotropics
Triplaris longifolia Huber	Polygonaceae	Oliveira *et al*. (1987); Sanchez (2011)	Peru
Triplaris melaenodendron (Bertol.) Standl. & Steyerm.	Polygonaceae	Sanchez (2011)	Colombia, Costa Rica
Triplaris moyobambensis Brandbyge	Polygonaceae	Sanchez (2011)	Neotropics
Triplaris peruviana Fisch. & Meyer ex C.A. Meyer	Polygonaceae	Sanchez (2011)	Peru

Triplaris poeppigiana Wedd.	Polygonaceae	Sanchez (2011)	Peru, Brazil
Triplaris punctata Standl.	Polygonaceae	Sanchez (2011)	Peru
Triplaris purdiae Meisn.	Polygonaceae	Sanchez (2011)	Neotropics
Triplaris purdiei Meisn.	Polygonaceae	Sanchez (2011)	Colombia
Triplaris setosa Rusby	Polygonaceae	Sanchez (2011)	Peru
Triplaris vestita Rusby	Polygonaceae	Sanchez (2011)	Neotropics
Triplaris weigeltiana (Rchb.) Kuntze	Polygonaceae	Ward (1999)	Venezuela, Guyana
Uapaca staudtii Pax	Phyllanthaceae	Bretler & Nzabi (1995)	Cameroun, Gabon, Nigeria
Uncaria africana G. Don	Rubiaceae	de Wildeman (1919); Bequaert (1922); Uphof (1942); Jolivet (1996)	Tropical Africa
Uncaria sp. 1	Rubiaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Uncaria sp. 2	Rubiaceae	Moog *et al*. (2003)	SE Asia - Malaysia
Vitex agelaeifolia Mildbr. ex Piep.	Lamiaceae	Schnell (1966); Jolivet (1996)	Tropical Africa
Vitex grandiflora Turcz.	Lamiaceae	Schnell (1966); Jolivet (1996)	Tropical Africa
Vitex thyrsiflora Baker	Lamiaceae	Schnell (1966); Djiero-Lordon *et al*. (2005)	Cameroun
Vitex yaundensis Gürke	Lamiaceae	Engler (1903)	Cameroon (endemic)
Vochysia vismiifolia Spruce ex Warm.	Vochysiaceae	Blüthgen & Wesenberg (2001)	Amazone
Wightia borneensis Hook.f.	Scrophulariaceae	Jolivet (1973); Moog *et al*. (2003)	SE Asia - Malaysia
Zanthoxylum limonella (Dennst.) Alston	Rutaceae	Moog *et al*. (2003)	SE Asia
Zanthoxylum myriacanthum Wall. ex Hook. f.	Rutaceae	Moog *et al*. (2002) ; Moog *et al*. (2003)	SE Asia

Supporting Information Table S1
Chomicki G, Renner SS. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist*.

References

Adams, C.D. (1972) *Flowering plants of Jamaica*. Univ of the West Indies, Mona Jamaica.

Agosti, D., Moog, J., & Maschwitz, U. (1999). Revision of the oriental plant-ant genus Cladomyrma. American Museum novitates; no. 3283.

Alonso, L. E. (1998). Spatial and Temporal Variation in the Ant Occupants of a Facultative Ant Plant. *Biotropica*, *30*(2), 201-213.

Bailey, I. W. (1923). Notes on Neotropical Ant-Plants. II. Tachigalia paniculata Aubl. *Botanical Gazette* 75: 27-41.

Bailey, I. W. (1924). Notes on Neotropical Ant-Plants. III. Cordia nodosa Lam. *Botanical Gazette* 77: 32-49.

Bake, J. A., & So, B. (1934). Notes on the biology of Macaranga spp. Gard. Bull. S. S. Vol. VIII.

Baker, G. (1889) *Handbook of the Bromeliaceae*. George Bell and Sons London.

Baker, J. G. (1891). A summary of the new ferns which have been discovered or described since 1874. Annals of Botany (4), 455-500.

Beccari, O. (1877). La Specie di Palme Raccolte alla Nuova Guinea da O. Beccari e dal Medesimo Adesso Descritte, con Note sulle Specie dei Paesi Circonvicini. Malesia 1: 8 – 102.

Beccari, O. (1884). *Piante ospitatrici, ossia piante formicarie della Malesia e della Papuasia. Malesia), 2*, Genoa, p.p. 1-340.

Beccari, O. MalesiaI, II and III, raccolta d'osservazioni lese e papuano (1877–1889).

Belin-Depoux, M. (1993). Importance des nectaires extra—floraux dans les interactions plantes-fourmis. *Acta Botanica Gallica*, *140*(2), 183-205.

Benson, W. W. (1985). Amazon ant-plants. *Prance, G, T,, Lovejoy, T, E ed (s). Amazonia. Pergamon Press: Oxford, etc*, 239-266.

Benzing, D. H. (1970). An investigation of two bromeliad myrmecophytes: Tillandsia butzii Mez, T. caput-medusae E. Morren, and their ants. *Bulletin of the Torrey Botanical Club*, 109-115.

Bequaert (1922). Ants and their diverse relations to the plant world. Bull. Amer. Mus. Nat. Hist. 45, 333-583.

Berg, C. C., Akkermans, R. W. A. P., & Van Heusden, E. C. H. (1990). Cecropiaceae: Coussapoa and Pourouma, with an introduction to the family. *Flora Neotropica* 51: 1-208.

Berg, C. C., Rosselli, P. F., & Davidson, D. W. (2005). Cecropia. *Flora Neotropica*, 1-230.

Supporting Information Table S1
Chomicki G, Renner SS. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist*.

Blatter, E. (1928). Myrmecosymbiosis in the Indo-Malayan flora. *J. Ind. Bot. Soc*. *7*(3-4), 176-185.

Blüthgen, N., & Wesenberg, J. (2001). Ants Induce Domatia in a Rain Forest Tree (Vochysia vismiaefolia) 1. *Biotropica*, *33*(4), 637-642.

Bower, F. O. (1887). On Humboldtia laurifolia, Vahl, as a myrmekophilous plant. *Proceedings of the Philosophical Society of Glasgow*, *18*, 320-326.

Breteler, F. J. (2000). Novitates Gabonenses 38. Two new species of Dactyladenia (Chrysobalanaceae) from Gabon. *Adansonia*, *22*(1), 117-122.

Breteler, F. J., & Nzabi, T. (1995). Novitates gabonenses 23. Delpydora (Sapotaceae), its taxonomy and myrmecophily. *Bulletin du Muséum national d'histoire naturelle. Section B, Adansonia*, *17*(1-2), 29-38.

Bridson, D. M. (1985). The reinstatement of Psydrax (Rubiaceae, subfam. Cinchonoideae tribe Vanguerieae) and a revision of the African species. *Kew Bulletin*, 687-725.

Brown, N. E. (1895). Diagnoses Africanæ, VIII. Asclepiadeæ. Bulletin of Miscellaneous Information (Royal Gardens, Kew), 247-265.

Brown, W. L. (1960). Ants, acacias and browsing mammals. *Ecology* 41: 587-592.

Burch, W. (1891). Beitrage zur kenntniss der mymecophilen pflanzen und der bedeutung der extranuptialen nectarien (Vol. 10). EJ Brill.

Champluvier, D. (1994). Brachystephanus myrmecophilus (Acanthaceae), espèce nouvelle du Zaïre oriental: un cas intéressant de myrmécophilie. *Belgian journal of botany*, 45-60.

Chenuil, A., & McKey, D. B. (1996). Molecular Phylogenetic Study of a Myrmecophyte Symbiosis: Did *Leonardoxa* /Ant Associations Diversify via Cospeciation?. *Molecular Phylogenetics and Evolution*, *6*(2), 270-286.

Chodat R. (1920). La myrmécophilie des *Cordia* de la Section Gerascanthus. Bulletin de la Societe Botanique de Geneve 172-200.

Clausing, G. (1998). Observations on ant-plant interactions in Pachycentria and other genera of the Dissochaeteae (Melastomataceae) in Sabah and Sarawak. *Flora*, *193*(4), 360-368.

Damon, A., & Pérez-Soriano, M. A. (2005). Interaction between ants and orchids in the Soconusco region, Chiapas, Mexico. *Entomotropica* 20: 59-65.

Dáttilo, W., Izzo, T. J., Vasconcelos, H. L., & Rico-Gray, V. (2013). Strength of the modular pattern in Amazonian symbiotic ant–plant networks. *Arthropod-Plant Interactions*, *7*(4), 455-461.

Davidson, D. W., & Epstein, W. W. (1989). Epiphytic associations with ants. In *Vascular plants as epiphytes* (pp. 200-233). Springer Berlin Heidelberg.

Davidson, D.W., McKey, D.B. (1993). The evolutionary ecology of symbiotic ant–plant relationships. *J. Hym. Res*. 2, 13–83.

De Wildeman E. (1897). Une plante myrmecophile nouvelle. Bull Herb. Boiss. V. 521-526.

De Wildeman E. (1919). La myrmecophilie dans le genre *Uncaria* en Afrique. C. R. Soc. Biol. Paris 82: 1076-1078.

De Wildeman, E. (1904). Sur le *Randia lujae* De Wild. nov. sp., plante myrmecophyte et acarophyte, nouvelle de la famille des Rubiacees. *CR Acad. Sci., Paris,.(138)*, 913-14.

De Wildeman, E. (1906). New or noteworthy plants. Gard. Chron., ser, 3(39), 380.

Dejean, A., Olmsted, I., & Snelling, R. R. (1995). Tree-epiphyte-ant relationships in the low inundated forest of Sian Ka'an Biosphere Reserve, Quintana Roo, Mexico. *Biotropica*, 57-70.

Delprete, P. G., Nee, M., & Koek-Noorman, J. (2005). Resurrection and revision of Patima (Rubiaceae, Cinchonoideae, Hamelieae). Annals of the Missouri Botanical Garden, 103-112.

Delprete, P. G., Nee, M., & Koek-Noorman, J. (2005). Resurrection and revision of Patima (Rubiaceae, Cinchonoideae, Hamelieae). *Annals of the Missouri Botanical Garden*, 103-112.

Dev, S. A., Shenoy, M., & Borges, R. M. (2010). Genetic and clonal diversity of the endemic ant-plant Humboldtia brunonis (Fabaceae) in the Western Ghats of India. *Journal of biosciences*, *35*(2), 267-279.

Djiéto-Lordon, C., Dejean, A., Ring, R. A., Nkongmeneck, B. A., Lauga, J., & McKey, D. (2005). Ecology of an Improbable Association: The Pseudomyrmecine Plant-ant *Tetraponera tessmanni* and the Myrmecophytic Liana *Vitex thyrsiflora* (Lamiaceae) in Cameroon1. Biotropica, 37(3), 421-430.

Duviard, D., & Segeren, P. (1974). La colonisation d'un Myrmécophyte, le Parasolier, parCrematogaster spp.(Myrmicinae) en Côte-d'Ivoire forestière. *Insectes Sociaux*, *21*(2), 191-212.

Elmer, A. D. E. (1938). Notes on Apocynaceae. Leafl. Philipp. Bot. 10: 3543–3599.

Emery, C. (1888). Über den sogenannten Kaumagen einiger Ameisen. Zeitschrift für Wissenschaftliche Zoologie 46 378-412.

Engler, A. (1903). Über die Vegetationsformen Ost-Afrikas auf Grund einer Reise durch Usambara zum Kilimandscharo. Zeitschrift der Gesellschaft für Erdkunde Berlin, 4, 254-279.

Escherich, K. (1906). Die Ameise. F. Vieweg und Sohn.

Escherich, K. (1911). Zwei Beitrage zum Kapitel: Ameisen und Pflanzen. Biol. Centralbl, 31, 44-51.

Fernández, F. (2007). The myrmicine ant genus Allomerus Mayr (Hymenoptera: Formicidae). Caldasia, 29(1), 159-175.

Ferreira, L. V., & Vasconcelos, H. L. D. (2010). On a poorly known Amazonian ant-plant association: Myrcia madida McVaugh (Myrtaceae) and Myrcidris epicharis Ward (Hymenoptera: Formicidae: Pseudomyrmecinae). Bol. Mus. Para. Emílio Goeldi. Cienc. Nat., Belém, v. 5, n. 3, p. 363-367, set.- dez.

Fiala, B., & Maschwitz, U. (1992). Domatia as most important adaptations in the evolution of myrmecophytes in the paleotropical tree genusMacaranga (Euphorbiaceae). *Plant Systematics and Evolution*, *180*(1-2), 53-64.

Fiala, B., & Maschwitz, U. (1992). Food bodies and their significance for obligate ant- association in the tree genus Macaranga (Euphorbiaceae). *Botanical Journal of the Linnean Society*, *110*(1), 61-75.

Forel, A. (1902). Fourmis nouvelles d'Australie. Revue Suisse de Zoologie, 10, 405-548.

Forel, A. (1920). Fourmis trouvées dans des galles de Cordia et d'Agonandra, etc. *Bulletin de la Société Botanique de Genève*, *2*(12), 201-208.

Gegenbauer, C., Mayer, V. E., Zotz, G., & Richter, A. (2012). Uptake of ant-derived nitrogen in the myrmecophytic orchid Caularthron bilamellatum. *Annals of botany*, *110*(4), 757-766.

Gómez-Acevedo, S., Rico-Arce, L., Delgado-Salinas, A., Magallón, S., & Eguiarte, L. E. (2010). Neotropical mutualism between *Acacia* and *Pseudomyrmex*: Phylogeny and divergence times. *Molecular Phylogenetics and Evolution*, *56*(1), 393-408.

Green, E. E. (1904). On some Javanese Coccidae: with descriptions of new species. Entomologist's Monthly Magazine, 40, 204-210.

Griffith, W., & Solly, R. H. (1851). XXI. On the Structure of the Ascidia and Stomata of *Dischidia Rafflesiana*, Wall. Transactions of the Linnean Society of London, 20(3), 387-390.

Groom, P. (1893). On Dischidia rafflesiana (Wall). *Annals of Botany* (2), 223-241.

Guerrero, S. A., & van Welzen, P. C. (2011). Revision of Malesian Endospermum (Euphorbiaceae) with notes on phylogeny and historical biogeography. *Edinburgh Journal of Botany*, 68(03), 443-482.

Gullan, P. J., Buckley, R. C., & Ward, P. S. (1993). Ant-tended scale insects (Hemiptera: Coccidae: Myzolecanium) within lowland rain forest trees in Papua New Guinea. *Journal of tropical ecology*, *9*(01), 81-91.

Gullan, P. J., Buckley, R. C., & Ward, P. S. (1993). Ant-tended scale insects (Hemiptera: Coccidae: Myzolecanium) within lowland rain forest trees in Papua New Guinea. *Journal of tropical ecology*, *9*(01), 81-91.

Hagemann, W. (1969). Zur Morphologie der Knolle von Polypodium bifrons Hook. und P. brunei Werckle. *Mem. Soc. bot. Fr*, 17-27.

Haufler, C. H., Grammer, W. A., Hennipman, E., Ranker, T. A., Smith, A. R., & Schneider, H. (2003). Systematics of the ant-fern genus Lecanopteris (Polypodiaceae): testing phylogenetic hypotheses with DNA sequences. *Systematic Botany*, *28*(2), 217-227.

Haviland, E. (1887). Flowering seasons of Australian plants. In Proceedings of the Linnean Society of New South Wales (Vol. 2, No. 348).

Heckroth, H. P., Moog, J., Jankal, H. I., Fialal, B., & Chung, A. Y. C. (2004). Smilax borneensis (Smilacaceae), an unspecific climbing ant-plant from Borneo and myrmecophytic traits in other Asiatic Smilax species. *Sandakania*, 14: 33-50.

Heil, M., González-Teuber, M., Clement, L. W., Kautz, S., Verhaagh, M., & Bueno, J. C. S. (2009). Divergent investment strategies of Acacia myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences*, *106*(43), 18091-18096.

Heil, M., Orona-Tamayo, D., Eilmus, S., Kautz, S., & González-Teuber, M. (2010). Chemical communication and coevolution in an ant–plant mutualism. *Chemoecology*, *20*(2), 63-74.

Hellwigianae, Plantae. "Beitrag zur Flora von Kaiser Wilhelms-Land." Bot. Jahrb 18 (1894): 184-212.

Hocking, B. (1970). Insect associations with the swollen thorn acacias. *Transactions of the Royal Entomological Society of London*, *122*(7), 211-255.

Huth, E., & als Pflanzenschutz, A. (1886). Verzeichnis der bisher bekannten myrmekophilen Pflanzen.

Huxley, C. (1980). Symbiosis between ants and epiphytes. *Biological Reviews*, *55*(3), 321-340.

Huxley, C. R., & Jebb, M. H. P. (1991a). The tuberous epiphytes of the Rubiaceae 2: the new genus Anthorrhiza. *Blumea*, 36(1), 21-41.

Huxley, C. R., & Jebb, M. H. P. (1991b). The tuberous epiphytes of the Rubiaceae: 3. A revision of Myrmephytum to include Myrmedoma. Blumea, 36(1), 43-52.

Huxley, C. R., & Jebb, M. H. P. (1993). The tuberous epiphytes of the Rubiaceae 5: A revision of Myrmecodia. *Blumea*, 37, 271-334.

Hyland, B. P. M. (1983). A revision of Syzygium and allied genera (Myrtaceae)in Australia. *Aust. J. Bot. Suppl. Series* No. 9: 1-164.

Janzen, D. H. (1966). Coevolution of mutualism between ants and acacias in Central America. *Evolution*, 249-275.

Jebb, M. H. P. (1991). The tuberous epiphytes of the Rubiaceae: 4. A revision of *Squamellaria*. *Blumea*, 36(1), 53-61.

Jebb, M. P. H. & Huxley, C. R. (In press). The Tuberous Epiphytes of the Rubiaceae 7: a revision of the genus *Hydnophytum* Jack. *Blumea*.

Jolivet, P. (1973). Essai d'Analyse Ecologique de la Faune Chrysomelidienne de la Coree. *Cahiers du Pacifique*, *17*, 253-288.

Jolivet, P. (1986). Les fourmis et les plantes. *Societé Nouvelle des Editions Boubée, Paris, 254p.*

Jones, H. G. (1968). The genus Schomburgkia in Mexico and Central America. *Ceiba*, *14*, 27-37.

Kaufmann, E., Weissflog, A., Hashim, R., & Maschwitz, U. (2001). Ant-gardens on the giant bamboo *Gigantochloa scortechinii* (Poaceae) in West-Malaysia. *Insectes Sociaux*, *48*(2), 125-133.

Kempf, W. W. (1975). Miscellaneous Studies on Neotropical Ants. Ill.(Hymenoptera, Formicidae).

Kerr, A. F. G. (1912). Notes on Dischidia rafflesiana Wall., and Dischidia nummularia, Blume. In Sci Proc R Dublin Soc (Vol. 13, pp. 292-315).

Klitgaard, B. B. (2005). Ptatymiscium (Leguminosae: Dalbergieae): Biogeography Systematics, Morphology, Taxonomy and Uses. *Kew Bulletin*, 321-400.

Kohl, H. (1909). Die Ameisenpflanzen des tropischen Afrika mit besonderer Berücksichtigung ihrer biologischen Verhältnisse. Natur und Offenbarung, 55, 89-111.

Letourneau, D. K., Feynner Arias, G., & Jebb, M. (1993). Coping with enemy-filled space: herbivores on Endospermum in Papua New Guinea. *Biotropica*, 95-99.

Letouzey, R., & White, F. (1976). Chrysobalanacees nouvelles du Cameroun et du Gabon. *Adansonia*, *16*(2), 229-243.

Letouzey, R., & White, F. (1978). *Flore du Gabon* (Vol. 24). Muséum national d'histoire naturelle, Laboratoire de phanérogamie.

Mabberley, D. J. (1979). The species of Chisocheton (Meliaceae). *Bull. Brit. Mus.(Nat. Hist.), Bot 6*(4). 386 p.p.

Mabberley, D. J. (1985). Florae Malesianae Praecursores: 67. Meliaceae divers genera. *Blumea*, *31*(1), 129-152.

Mani, M. S. (1964). *Ecology of Plant Galls*, Springer Netherlands.

Maschwitz, U., Fiala, B., & Linsenmair, K. E. (1994). Clerodendrum fistulosum (Verbenaceae), an unspecific myrmecophyte from Borneo with spontaneously opening domatia. *Blumea*, *39*(1-2), 143-150.

Maschwitz, U., Fiala, B., Lee, Y. F., CHEy, V. K., & Tan, F. L. (1989). New and little-known myrmecophytic associations from Bornean rain forests. Malayan Nat. J, 43, 106-115.

Maschwitz, U., Fiala, B., Lee, Y. F., CHEy, V. K., & Tan, F. L. (1989). New and little-known myrmecophytic associations from Bornean rain forests. *Malayan Nat. J*, *43*, 106-115.

Maschwitz, U., Fiala, B., Moog, J., & Saw, L. G. (1991). Two new myrmecophytic associations from the Malay Peninsula: Ants of the genusCladomyrma (Formicidae, Camponotinae) as partners ofSaraca thaipingensis (Caesalpiniaceae) andCrypteronia griffithii (Crypteroniaceae). *Insectes Sociaux*, *38*(1), 27-35.

Mayr G. (1862). Myrmecologische Studien. — Verh. K.K. Zool. Bot. Ges. Wien 12: 649-776.

McKey, D. (1989). Interactions between ants and leguminous plants. *Stirton, C, H,, Zarucchi, J, L ed (s). Advances in legume biology*. *Mon. Syst. Bot. Missouri bot. Gard*, *29*, 673-718.

McKey, D. B. (2000). Leonardoxa africana (Leguminosae: Caesalpinioideae): a complex of mostly allopatric subspecies. *Adansonia*, *22*(1), 71-109.

McKey, D., & Davidson, D. W. (1993). Ant–plant symbioses in Africa and the Neotropics: history, biogeography and diversity. Biological Relationships Between Africa and South America, 568-606.

Merrill, E. D. (1920). Myrmeconauclea, a new genus of Rubiaceous plants from Palawan and Borneo. Phillipines Journal of Sciences, 17, 375-376.

Michelangeli, F. A. (2000). A cladistic analysis of the genus Tococa (Melastomataceae) based on morphological data. *Systematic Botany*, *25*(2), 211-234.

Michelangeli, F. A. (2005). Tococa (Melastomataceae). *Flora Neotropica*, 1-114.

Michelangeli, F. A. (2006). Two new species of myrmecophytic Tococa (Melastomataceae) with unusually large floral bracts. *Brittonia*, *58*(2), 151-155.

Michelangeli, F. A. (2010). Neotropical myrmecophilous Melastomataceae: An annotated list and key. *Proceedings of the California Academy of Sciences*, *61*(7), 409.

Moog J, Fiala B, Werner M, Weissflog A, Saw LG and Maschwitz U, 2003. Ant-plant diversity in Peninsular Malaysia, with special reference to the Pasoh Forest Reserve. In: *Pasoh: ecology of a lowland rain forest in southeast Asia* (Okuda T, Manokaran N, Matsumoto Y, Niiyama K, Thomas SC, Ashton PS, eds), Springer Press, Tokyo, 459- 494.

Moog, J., Atzinger, K., Hashim, R., & Maschwitz, U. (2008). Do tenants always pay their rent? The Asian ant-plant Pometia pinnata (Sapindaceae) and its leaf domatia provide free access to generalist ants. *Asian Myrmecology*, *2*, 17-32

Moog, J., Drude, T., Maschwitz, U., & Agosti, D. (1997). Flood control by ants: water-bailing behaviour in the Southeast asian plant-ant genus Cladomyrma Wheeler (Formicidae, Formicinae). *Naturwissenschaften*, *84*(6), 242-245.

Moog, J., Feldhaar, H., & Maschwitz, U. (2002). On the Caulinary Domatia of the SE-Asian Ant-Plant Zanthoxylum myriacanthum Wall. ex Hook. f.(Rutaceae) and the Protection Against Herbivory. *Sociobiology*, *40*(3) : 1-28.

Mouly, A. (2006). Statut de Plectmnia paradoxa Virot, Rubiaceae myrmécophile de Nouvelle-Calédonie. *Adansonia*, *28*(1), 161-166.

O'Dowd, D. J. (1982). Pearl bodies as ant food: an ecological role for some leaf emergences of tropical plants. *Biotropica*, 40-49.

Oliveira PS, Oliveira-Filho AT, Cintra R (1987). Ant foraging on ant-inhabited Triplaris (Polygonaceae) in western Brazil: a Field experiment using live termite-baits. *J Trop Ecol 3*:193-200

Onana, J. M. (2008). A new combination and key to the species of Cuviera subgenus Globulostylis (Rubiaceae: Vanguerieae) from Central Africa. *Kew Bulletin*, *63*(3), 401-403.

Passmore, H. A., Bruna, E. M., Heredia, S. M., & Vasconcelos, H. L. (2012). Resilient networks of ant-plant mutualists in Amazonian forest fragments. *PloS one*, *7*(8), e40803.

Paulian, R. (1949). A naturalist in the Ivory Coast. *Un naturaliste en Cote d'Ivoire*. 217 p.p.

Pax, F. (1914). Euphorbiaceae--Acalypheae--Mercurialinae. (Vol. 63).

Peccoud, J., Piatscheck, F., Yockteng, R., Garcia, M., Sauve, M., Djiéto-Lordon, C., & Blatrix, R. (2013). Multi-locus phylogenies of the genus *Barteria* (Passifloraceae) portray complex patterns in the evolution of myrmecophytism. *Molecular phylogenetics and evolution*, *66*(3), 824-832.

Pendry, C. A. (2004). Monograph of Ruprechtia (Polygonaceae). *Systematic Botany Monographs*, 1-113.

Penzig,0 (1892). Ueber die Perldriisen des Weinstockes und anderer Pflanzen. *Atti. Del Congr .Bot. Intern.(*Genoa): 237-245.

Perkins, J. R. (1904). Fragmenta florae philippinae: contributions to the flora of the Philippine Islands. Gebrüder Borntraeger.

Philipson, W. R. (1985). A synopsis of the Malesian species of Kibara (Monimiaceae). *Blumea*, *30*(2), 389-415.

Philipson, W. R. (1985). Faika and Parakibara: two new genera of Monimiaceae from Malesia. Blumea 30: 417–423.

Prance, G. T. (1972). Chrysobalanaceae. *Flora Neotropica* 9: 1-409.

Razafimandimbison, S. G., Moog, J., Lantz, H., Maschwitz, U., & Bremer, B. (2005). Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea* ss (Rubiaceae). *Molecular phylogenetics and evolution*, *34*(2), 334-354.

Renner, S. S., & Ricklefs, R. E. (1998). Herbicidal Activity of Domatia- Inhabiting Ants in Patches of Tococa guianensis and Clidemia heterophylla1. *Biotropica*, *30*(2), 324-327.

Rickson, F. R. (1980). Developmental anatomy and ultrastructure of the ant food bodies (Beccarian bodies) of *Macaranga triloba* and *M. hypoleuca* (Euphorbiaceae). *Am J Bot* 67:285-292.

Rico-Gray, V., Barber, J. T., Thien, L. B., Ellgaard, E. G., & Toney, J. J. (1989). An unusual animal-plant interaction: feeding of Schomburgkia tibicinis (Orchidaceae) by ants. *American Journal of Botany* 76: 603-608.

Rico-Gray, V., Oliveira, P. S., Parra-Tabla, V., Cuautle, M., & Díaz-Castelazo, C. (2004). Ant–plant interactions: their seasonal variation and effects on plant fitness. In *Coastal Dunes* (pp. 221-239). Springer Berlin Heidelberg.

Ridley, H. N. (1907). *Materials for a Flora of the Malayan Peninsula*. Singapore.

Ridley, H. N. (1910). Symbiosis of ants and plants. *Annals of Botany* 2: 457-483.

Ridley, H. N. (1912). New and rare Malayan plants (Series VI). Journal of the Straits Branch of the Royal Asiatic Society, 1-43.

Ridsdale, C. E. (1989). A revision of Neonauclea (Rubiaceae). *Blumea*, *34*(1), 177-275.

Rintz, R. E. (1980). The peninsular Malayan species of Dischidia (Apocynaceae). *Blumea*, *26*(1), 81-126.

Risch, S., McClure, M., Vandermeer, J., & Waltz, S. (1977). Mutualism between three species of tropical Piper (Piperaceae) and their ant inhabitants. American Midland Naturalist 98: 433-444.

Robbrecht, E. (1979). The African genus Tricalysia A. Rich.(Rubiaceae-Coffeeae). 1. A revision of the species of subgenus Empogona. *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie* 49: 239-360.

Robbrecht, E. (1988). Tropical woody Rubiaceae. *Opera Botanica Belgica, 1*(272), 599-602.

Rogers, G. K. (1984). Gleasonia, Henriquezia, and Platycarpum (Rubiaceae). *Flora Neotropica* 39: 1-134.

Rojo, J. P. (1972). Pterocarpus (Leguminosae-Papilionaceae) revised for the world. *Lehre J: Cramer viii, 119p.(Phanerogamarum Monographiae, tomus 5). Illustrations, maps, dot maps*, 4-6.

Ross. (1909) Pflanzen und ameisen in tropischen Mexico Naturwiss. Wochenschr. NF. 8: 822-830.;

Sanchez, A. (2011). Evolutionary relationships in Polygonaceae with emphasis on *Triplaris*. Wake Forest University, PhD Thesis.

Schimper, A. F. W. (1888). Die epiphytische Vegetation Amerikas. Bot. Mitt. Tropen II. Fischer Vlg., Jena.

Schimper A. F. W. (1903). Plant-geography upon a physiological basis. Clarendon Press, Oxford, UK.

Schmidt, M. R. (2001). Interactions between Tetrathylacium macrophyllum (Flacourtiaceae) and its live-stem inhabiting ants. *Austria: University of Vienna*.

Schnell, R. (1970). Introduction à la phytogéographie des pays tropicaux. Paris, Gauthier-Villars, 1971, 4-7.

Schnell, R., Cusset, G., & Quenum, M. (1963). Contribution a l'etude des glandes extra-florales chez quelques groupes de plantes tropicales. Rev. gen. bot, 70, 269-342.

Schnell, R., & Grout de Beaufort, F. (1966). Contribution à l'étude des plantes à myrmécodomaties de l'Afrique intertropicale. Mém. Inst. Fond. Afr. Noire (75), 1-66.

Schnell, R., & Grout de Beaufort, F. (1966). Contribution à l'étude des plantes à myrmécodomaties de l'Afrique intertropicale. *Mém. Inst. Fond. Afr. Noire*, (75), 1-66.

Schumann, K. (1888). *Einige neue Ameisenpflanzen*. G. Bernstein

Schumann, K., & Lauterbach, K. (1901). Die Florader Deutschen Schutzgebeite inder Sudsee. Borntraeger, Leipzig.

Scott, D. H., & Sargant, E. (1893). On the Pitchers of Dischidia rafflesiana (Wall). Annals of Botany, (2), 243-268.

Seigler, D. S., & Ebinger, J. E. (1995). Taxonomic revision of the ant-acacias (Fabaceae, Mimosoideae, Acacia, series Gummiferae) of the New World. *Annals of the Missouri Botanical Garden*, 117-138.

Seigler, D., Saupe, S. G., Young, D. A., & Richardson, P. M. (1982). *Acacia rigidula*: A New Ant-Acacia. *The Southwestern Naturalist*, 364-365.

Shelford, R. 1916. A naturalist in Borneo. T. Fisher Unwin, London, England. 331 pp.

Smith, W. (1903). *Macaranga triloba*: a new myrmecophilous plant. *New Phytologist* 2: 79-83.

Solereder, H. (1920). Über eine heterophylle philippinische Ameisenpflanze aus der Familie der Melastomaceae, nebst Bemerkungen über das Auftreten von Amylodextrinkörner in den sog. Perldrusen. Naturw. Wochenschr. NF, 35, 689-691.

Somers, C., & Robbrecht, E. (1991). A Precursor to the Treatment of Rothmannia (Rubiaceae-Gardenieae) in" Flore d'Afrique Centrale". *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie*, 295-304.

Spruce, R. (1908). Notes of a Botanist on the Amazon & Andes: Being Records of Travel on the Amazon and Its Tributaries, the Trombetas, Rio Negro, Uaupés, Casiquiari, Pacimoni, Huallaga and Pastasa: as Also to the Cataracts of the Orinoco, Along the Eastern Side of the Andes of Peru and Ecuador, and the Shores of the Pacific, During the Years 1849-1864 (Vol. 2). Macmillan and Company, limited.

Step, E. (1913). *Messmates. A book of strange companionship in Nature*. London.

Stevens, P. F. (1975). Review of Chisocheton (Meliaceae) in Papuasia. *Contributions from Herbarium Australiense*, *1975*(11), 1-55.

Stout, J. (1979). An association of an ant, a mealy bug, and an understory tree from a Costa Rican rain forest. *Biotropica* 11: 309-311.

Takahashi, R. (1951). On the myrmecophyte, Macaranga triloba, and its ant in Malaya. Bull. biogeogr. Soc. Jap. 15: 11-12.

Tepe, E. J., Vincent, M. A., & Watson, L. E. (2004). Phylogenetic Patterns, Evolutionary Trends, and the Origin of Ant–Plant Associations in Piper section Macrostachys: Burger's Hypotheses Revisited. In *Piper: a model genus for studies of phytochemistry, ecology, and evolution* (pp. 156-178). Springer US.

Thompson, J. N. (1981). Reversed animal- plant interactions: the evolution of insectivorous and ant- fed plants. *Biological Journal of the Linnean Society*, *16*(2), 147-155.

Treseder, K. K., Davidson, D. W., & Ehleringer, J. R. (1995). Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature*, *375*(6527), 137-139.

Uphof, J. T. (1942). Ecological relations of plants with ants and termites. *The Botanical Review*, *8*(9), 563-598.

Van Der Werff, H. (1988). Eight new species and one new combination of Neotropical Lauraceae. *Annals of the Missouri Botanical Garden*, 402-419.

Van der Werff, H. (2008). A Synopsis of the Genus Tachigali (Leguminosae: Caesalpinioideae) in Northern South America 1. *Annals of the Missouri Botanical Garden*, *95*(4), 618-661.

Steenis, C. V. (1969). Plant speciation in Malesia, with special reference to the theory of non-adaptive saltatory evolution. Biological journal of the Linnean society, 1: 97-133.

Verdcourt, B. (1987). Notes on African Rubiaceae: Vanguerieae. *Kew bulletin* 42: 123-199.

Vicente, R. E., Dáttilo, W., & Izzo, T. J. (2012). New record of a very specialized interaction: *Myrcidris epicharis* Ward 1990 (Pseudomyrmecinae) and its myrmecophyte host Myrcia madida McVaugh (Myrtaceae) in Brazilian Meridional Amazon. *Acta Amazonica*, 42(4), 567-570.

Wagner, W. H. (1972). Solanopteris brunei, a little-known fern epiphyte with dimorphic stems. *American Fern Journal* 62: 33-43.

Wanntorp, L., Kocyan, A., & Renner, S. S. (2006). Wax plants disentangled: A phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Molecular phylogenetics and evolution*, *39*(3), 722-733.

Warburg, O. 1897. Monographieder Myristic-aceen. *Nova Acta Acad. Leop*.-Carol.68: 1-680.

Ward Blog (http://wardlab.wordpress.com/research/pseudomyrmecinae/ant-plants/)

Ward, P. S. (1991). Phylogenetic analysis of pseudomyrmecine ants associated with domatia-bearing plants. *Ant–plant interactions*, 335-352.

Ward, P. S. (1999). Systematics, biogeography and host plant associations of the Pseudomyrmex viduus group (Hymenoptera: Formicidae), Triplaris- and Tachigali inhabiting ants. *Zoological Journal of the Linnean Society*, *126*(4), 451-540.

Weber, N. A. (1943). Parabiosis in Neotropical" Ant Gardens". *Ecology*, *24*(3), 400-404.

Weddell, H. A. (1849). Histoire naturelle des Quinquinas: ou monographie du genre Cinchona suivie d'une description du genre Cascarilla et de quelques autres plantes de la même tribu. V. Masson.

Weir, J. S., & Kiew, R. (1986). A reassessment of the relations in Malaysia between ants (*Crematogaster*) on trees (*Leptospermum* and *Dacrydium*) and epiphytes of the genus *Dischidia* (Apocynaceae) including ‘ant-plants’. *Biological Journal of the Linnean Society*, *27*(2), 113-132.

Wheeler, W. M. (1910). *Ants: their structure, development and behavior* (Vol. 9). Columbia University Press.

Wheeler, W. M. (1942). Studies of Neotropical ant-plants and their ants. *Bull. Amer. Mus. Comp. Zool. Harvard* 90: 1- 25l.

Wheeler, W. M., & Bequaert, J. C. (1929). Amazonian myrmecophytes and their ants. *Zool. Anz, 82*, 10-39.

Wheeler, W. M., & Britton, N. L. (1908). The ants of Porto Rico and the Virgin Islands. Bulletin of the AMNH; v. 24, article 6.

White, F. (1979). The Guineo-Congolian Region and its relationships to other phytochoria. *Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie*, 11-55.

Wildeman E. (1923). Quelques mots sur la myrmecophylie chez *Grumilea refrastipula*, *Ann Soc Sc Brux* 42.

Windsor, D. M., & Jolivet, P. (1996). Aspects of the morphology and ecology of two Panamanian ant-plants, *Hoffmannia vesciculifera* (Rubiaceae) and *Besleria formicaria* (Gesneriaceae). *Journal of Tropical Ecology*, *12*(06), 835-842.

Wong, K. M., & Puff, C. (1995). Notes on a myrmecophytic heterophyllous Diospyros (Ebenaceae). *Sandakania* 6: 55-62.

Table S2. Genbank accession numbers for the large myrmecophyte phylogeny.

Family	**Species**	**rbcL**	**matK**	**ITS**	**trnL-trnF**	**atpB-rbcL**	**ndhF**	**atpB**	**18S**
Asclepiadaceae	*Absolmsia spartioides*	-	-	-	DQ334549	DQ334591	-	-	-
Fabaceae	*Acacia anegadensis*	-	HM020706	-	HM020796	-	-	-	-
Fabaceae	*Acacia berlandieri*	-	HM020707	-	HM020797	-	-	-	-
Fabaceae	*Acacia caven*	-	AF274131	-	AF522967	-	-	-	-
Fabaceae	*Acacia cedilloi*	-	HM020708	-	HM020798	-	-	-	-
Fabaceae	*Acacia chiapensis*	.	HM020709	-	HM020799	-	-	-	-
Fabaceae	*Acacia cochliacantha*	-	HM020710	-	HM020800	-	-	-	-
Fabaceae	*Acacia collinsii*	JQ592077	HM020711	-	HM020801	-	-	-	-
Fabaceae	*Acacia constricta*	-	HM020712	EF638217	HM020802	-	EU811927	-	-
Fabaceae	*Acacia cornigera*	JQ592080	HM020713	EF638218	HM020803	-	EU811920	-	-
Fabaceae	*Acacia depanolobium*	-	HM020714	-	HM020804	-	-	-	-
Fabaceae	*Acacia dolichostachya*	-	DQ371892	-	DQ371873	-	-	-	-
Fabaceae	*Acacia farnesiana*	FJ716673	HM020715	AF360728	HM020805	-	-	-	-
Fabaceae	*Acacia gentlei*	-	HM020717	-	HM020807	-	-	-	-
Fabaceae	*Acacia globulifera*	-	HM020718	.	HM020808	-	-	-	-
Fabaceae	*Acacia hindsii*	-	HM020719	-	HM020809	-	-	-	-
Fabaceae	*Acacia hirtipes*	-	HM020720	-	HM020810	-	-	-	-
Fabaceae	*Acacia karroo*	AM235003	AF274137	JQ265837	HM020811	-	-	-	-
Fabaceae	*Acacia leucophloea*	JX195515	-	JX139100	-	-	-	-	-
Fabaceae	*Acacia luederitzii* var. *luederitzii*	JX572207	AF523186	-	-	-	-	-	-
Fabaceae	*Acacia luederitzii* var. *retinens*	JX572208	JX517653	JQ265849	-	-	-	-	-
Fabaceae	*Acacia macracantha*	-	HM020721	EF638220	HM020812	-	-	-	-
Fabaceae	*Acacia mayana*	-	HM020722	-	HM020813	-	-	-	-
Fabaceae	*Acacia mearnsii*	-	HM020723	-	AF195694	-	-	-	-
Fabaceae-Mimosoideae	*Acacia melanoxylon*	HM849736	AF274166	-	-	-	EU811932	-	-
Fabaceae	*Acacia neovernicosa*	JN796927	AF523113	-	AF522970	-	-	-	-

Fabaceae	*Acacia pennatula*	-	HM020724	-	HM020814	-	-	-	-
Fabaceae	*Acacia rigidula*	-	AF523188	-	-	-	-	-	-
Fabaceae	*Acacia schaffneri*	-	AF274132	-	HM020818	-	-	-	-
Fabaceae	*Acacia schottii*	-	AF274136	-	AF522971	-	-	-	-
Fabaceae	*Acacia seyal*	-	HM020728	-	HM020820	-	-	-	-
Fabaceae	*Acacia sphareocephala*	-	HM020729	-	HM020821	-	-	-	-
Fabaceae	*Acacia tortilis*	EU213442	AF274140	KC733803	HM020822	-	-	-	-
Fabaceae	*Acacia tortuosa*	-	HM020730	-	HM020823	-	-	-	-
Fabaceae	*Acaciella kamelensis*	-	HM020734	-	HM020827	-	-	-	-
Polemoniaceae	*Acanthogilia gloriosa*	AY725863	L48562	-	-	-	AY725875	AY725926	L49271
Calyceraceae	*Acicarpha tribuloides*	EU841134	EU841361	EU841173	EU841093	-	AJ429129	AJ318963	-
Acoraceae	*Acorus calamus*	FJ875015	AB040154	DQ008852	EU814691	-	EU814664	-	-
Rubiaceae	*Acranthera siamensis*	EU145450	-	-	-	-	EU145399	-	-
Actinidiaceae	*Actinidia chinensis*	L01882	U61324	-	-	-	-	AJ235382	AF419792
Lauraceae	*Actinodaphne sesquipedalis*	-	-	AF272247	AF268695	-	-	-	-
Malvaceae	*Adansonia digitata*	GU981721	AY321168	-	-	-	AF111720	GU981688	-
Euphorbiaceae	*Adenocline acuta*	AB233874	AB233770	-	-	-	-	-	-
Bromeliaceae	*Aechmea bromeliifolia*	JN202118	GU475466	-	-	-	HQ895727	-	-
Aextoxicaceae	*Aextoxicon punctatum*	X83986	DQ182342	-	AY145362	HE651123	-	AJ235384	AF206839
Amaryllidaceae	*Agapanthus africanus*	HM640485	HM640599	-	AF508516	AY699127	JX903309	JX903728	HM640715
Asparagaceae	*Agave americana*	JX903133	JX903544	U23997	AB817544	-	JX903310	JX903729	HM991824
Opiliaceae	*Agonandra racemosa*	DQ790130	DQ790169	-	-	-	-	-	L24079
Simaroubaceae	*Ailanthus altissima*	U02726	EF489111	-	-	-	EU002194	AF035895	AF206842
Asteraceae	*Ainsliaea apiculata*	EU384944	EU385321	AB288429	EU385036	-	EU385130	-	-
Lauraceae	*Aiouea dubia*	-	AJ247143	-	-	-	-	-	-
Fabaceae	*Albizia kalkora*	HQ427141	AF523083	JF708202	AF522945	-	-	-	-
Fabaceae	*Albizia saman*	JQ591998	JQ587828	JX870770	JX870886	-	-	-	-
Fabaceae	*Alexa grandiflora*	-	JF491262	-	-	-	-	-	-
Amaryllidaceae	*Allium cepa*	D38294	JQ276391	FJ664287	AF184337	AJ299093	JQ276779	JQ273601	JQ283941
Melastomataceae	*Allomaieta pancurana*	JF831993	-	-	-	-	JF831967	-	-
Alseuosmiaceae	*Alseuosmia macrophylla*	X87377	AJ429378	EU331126	AJ430965	-	AJ238334	AJ236198	AF206844
Picramniaceae	*Alvaradoa amorphoides*	AF123277	-	-	-	-	-	AJ235387	-
Alzateaceae	*Alzatea verticillata*	U26316	AY151567	-	-	-	AF215591	-	AM235484

Amaryllidaceae	*Amaryllis belladona*	JQ273899	JX903555	JX464257	JX464334	-	JQ276782	JX903750	JQ283909
Orchidaceae	*Amblostoma armeniacum*	AF518058	AF263748	AF260165	AF266993	-	-	-	-
Amborellaceae	*Amborella trichopoda*	L12628	DQ185522	-	AY145324	-	AF235046	-	-
Ancistrocladaceae	*Ancistrocladus korupensis*	Z97636	GQ470536	GQ443549	-	-	-	AF209526	AF206846
Haemodoraceae	*Anigozanthos flavidus*	AJ404843	AB088796	-	GU223397	-	EF422987	AF387600	AF069214
Anisophylleaceae	*Anisophyllea purpurascens*	AY973486	AY973464	AY973452	AY973428	-	AY973475	AY973439	-
Dipterocarpaceae	*Anisoptera marginata*	Y15144	AJ581409	-	-	-	EU002197	AF035918	AF206849
Poaceae	*Anomochloa marantoidea*	AF021875	AF164381	-	-	-	U21992	EF422977	-
Basellaceae	*Anredera baselloides*	HQ621333	HQ620830	-	-	-	HQ620942	HQ620741	-
Plantaginaceae	*Anthirrhinum majus*	L11688	AF375189	-	-	-	GQ996983	GQ996968	AJ236047
Gentianaceae	*Anthocleista grandiflora*	L14389	JX518238	-	-	-	AJ235829	-	AJ236026
Gentianaceae	*Anthocleista nobilis*	-	-	-	-	DQ131695	-	-	-
Rubiaceae	*Anthorrhiza caerulea*	-	-	-	-	AB044146	-	-	-
Rubiaceae	*Anthorrhiza clemensii*	-	-	AF034915	-	-	-	-	-
Polygonaceae	*Antigonon leptopus*	AF297146	GU135098	FJ154462	-	-	EF438027	-	-
Meliaceae	*Aphanamixis polystachya*	AY128213	AY128178	AY695584	KF211844	-	-	-	-
Aphanopetalaceae	*Aphanopetalum resinosum*	AF274596	EF179066	-	-	-	EF207459	AF274675	AF274600
Aphloiaceae	*Aphloia theiformis*	AF206735	HQ680692	JF804888	JF804888	-	-	HQ680705	AF206851
Asparagaceae	*Aphyllanthes monspeliensis*	JQ273903	HM640614	-	-	AY147754	JX903370	JX903788	HM640729
Aponogetonaceae	*Aponogeton distachyos*	U80684	HQ456482	AY926320	AY290825	-	HQ901552	-	-
Orchidaceae	*Apostasia wallichii*	HM640552	AY557212	AY557228	AY557219	-	HQ181103	-	-
Aptandraceae	*Aptandra tubicina*	DQ790141	DQ790178	-	-	-	-	-	DQ790105
Brassicaceae	*Arabidopsis thaliana*	U91966	AF144348	AJ232900	AY122452	-	HM120263	AJ971661	X16077
Corsiaceae	*Arachnitis uniflora*	-	-	-	-	-	-	-	GQ497571
Araucariaceae	*Araucaria araucana*	U96467	AF456373				-	DQ646109	FJ179543
Sapotaceae	*Argania spinosa*	EU980805	DQ924090	-	-	-	AY230664	DQ923981	-
Poaceae	*Aristida adscensionis*	AM849349	HE573938	-	-	-	AM849125	EF422970	-
Aristolochiaceae	*Aristolochia gigantea*	AB205591	DQ882187	-	-	-	DQ356468	AY572284	AY572306
Araceae	*Arum maculatum*	GU067582	GU067608	GU067558	GU067633	-	-	-	-
Poaceae	*Arundo donax*	U13226	AF164408	-	-	-	GU222718	-	-

Xanthorrhoeaceae	*Asphodelus aestivus*	HM640527	HM640645	-	AJ290257	-	JX903424	JX903841	HM640760
Asteliaceae	*Astelia alpina*	Z77261	AY368372	-	HM459530	AY147739	AY147775	-	-
Asteropeiaceae	*Asteropeia micraster*	AF206737	JQ844149	-	-	-	HQ843266	AF209533	AF206857
Atherospermataceae	*Atherosperma moschatum*	AF121362	DQ401343	-	-	-	JF268468	-	-
Garryaceae	*Aucuba japonica*	AY725858	GQ997060	-	-	-	GQ997066	GQ997051	U42522
Austrobaileyaceae	*Austrobaileya scandens*	L12632	DQ185523	EF210562	AY145326	HE651090	AF238052	AJ235403	AF206858
Oxalidaceae	*Averrhoa carambola*	L14692	JX661928	-	-	-	JX662737	JX663789	AY929366
Balanopaceae	*Balanops pachyphylla*	JX664033	JX661929	-	-	-	JX662738	JX663790	-
Barbeuiaceae	*Barbeuia madagascariensis*	GQ497673	AY042552	-	-	-	-	-	-
Barbeyaceae	*Barbeya oleoides*	U60314	JF317418	-	-	-	JF317437	AF209535	AF206862
Lecythidaceae	*Barringtonia asiatica*	EU980812	DQ924095	-	-	-	AF421044	DQ923986	-
Passifloraceae	*Barteria dewevrei*	KC207151	KC207225	KC207264	KC207130	-	-	-	-
Passifloraceae	*Barteria fistulosa*	DQ123392	AB536620	KC207274	KC207138	-	DQ123199	-	-
Passifloraceae	*Barteria nigritana*	KC207156	KC207235	KC207353	KC207131	-	-	-	-
Passifloraceae	*Barteria solida*	KC207147	KC207246	KC207391	KC207142	-	-	-	-
Bataceae	*Batis maritima*	L22438	AY483219	-	-	-	EU002199	AF209538	U42504
Cytinaceae	*Bdallophyton americanum*	-	-	-	-	-	-	-	AY739089
Asparagaceae	*Beaucarnea recurvata Lem.*	HM640473	HM640586	U24027	-	-	JX903308	AF168888	HM640703
Begoniaceae	*Begonia cucullata*	GU135205	GU135043	-	-	-	EU002200	-	-
Melastomataceae	*Bellucia pentamera*	KF781624	-	KF781576	-	-	AF215578	-	-
Berberidopsidaceae	*Berberidopsis corallina*	EU002274	EU002171	-	-	-	EU002201	EU002158	AF206866
Elatinaceae	*Bergia texana*	AY380344	JX661930	-	-	-	JX662739	FJ707527	AY674577
Rubiaceae	*Bertiera bicarpellata*	-	-	-	DQ180572	-	-	-	-
Rubiaceae	*Bertiera laxissima*	-	-	-	DQ180573	-	-	-	-
Bruniaceae	*Berzelia lanuginosa*	L14391	AY490955	-	GQ984070	-	AJ236241	AF095731	U42508
Gesneriaceae	*Besleria aggregata*	-	-	DQ070479	GQ166796	-	-	-	-
Gesneriaceae	*Besleria formicaria*	-	-	EF445705	EF445756	-	-	-	-
Amaranthaceae	*Beta vulgaris*	DQ067450	AY514832	AY858597	HE577473	DQ074969	HM630042	DQ067451	FJ669720
Biebersteinaceae	*Biebersteinia orphanidis*	AF035920	-	-	-	-	-	AF035921	GQ497568
Bixaceae	*Bixa orellana*	AF022128	FM179929	-	-	-	EU077547	AF035897	AF206868

Melastomataceae	*Blakea gracilis*	JF831999	-	AY460445	-	-	JF831974	-	-
Melastomataceae	*Blakea purpusii*	-	-	AY460440	-	-	-	-	-
Melastomataceae	*Blakea schlimii*	EU711386	-	AY460441	-	-	EU711373	-	-
Melastomataceae	*Blakea wilburiana*	-	-	AY460442	-	-	-	-	-
Blandfordiaceae	*Blandfordia punicea*	Z73694	AY557206	-	HM459535	AY147740	AY147776	-	-
Euphorbiaceae	*Blumeodendron kurzii*	-	EF582623	DQ866525	DQ899181	-	-	-	-
Euphorbiaceae	*Blumeodendron kurzii*	-	EF582623	DQ866525	DQ899181	-	-	-	-
Altroemeriaceae	*Bomarea bolivarensis*	AY120368	JQ404827	-	-	-	JQ404589	-	-
Bonnetiaceae	*Bonnetia sessilis*	HQ332010	EF135509	-	-	-	HQ331849	FJ707526	FJ707523
Boryaceae	*Borya septentrionalis*	AF206741	HM640651	-	-	-	AY225059	-	-
Malvaceae	*Brachychiton acerifolius*	GU981722	JX495761	-	-	-	AY795594	GU981689	-
Acanthaceae	*Brachystephanus africanus* S.Moore	-	-	DQ372469	-	-	-	-	-
Cabombaceae	*Brasenia schreberi*	M77031	AF092973	-	-	-	-	AF209544	AF206874
Akaniaceae	*Bretschneidera sinensis*	M95753	AY483220	AF254758	JF448517	JF448483	AY483247	AF209546	-
Rhizophoraceae	*Bruguiera gymnorhiza*	U26320	AF105088	-	-	-	EU002204	AB233719	AF206875
Brunelliaceae	*Brunellia acutangula*	FJ707536	EF135512	-	-	-	FJ670136	FJ669993	FJ669718
Scrophulariaceae	*Buddleja davidii*	L14392	HQ384530	-	-	-	HQ384835	HQ384734	AF107581
Burmanniaceae	*Burmannia capitata*	AY149347	-	EU816733	-	-	-	AY147596	DQ786065
Burseraceae	*Bursera simaruba*	GU246028	JQ587169	-	-	-	EU002206	GU246056	-
Butomaceae	*Butomus umbellatus*	U80685	AY952416	JF780965	DQ786413	-	AF546997	-	-
Buxaceae	*Buxus sempervivens*	DQ182333	AF543728	AF245429	AY145357	HE651113	AJ236280	AF092110	L54065
Byblidaceae	*Byblis gigantea*	AB546628	AF531774	-	-	-	JN686614	AJ236181	U42509
Arecaceae	*Calamus viminalis*	JQ042062	JQ435566	-	-	-	-	-	-
Arecaceae	*Calamus viminalis*	JQ042062	JQ435566	-	-	-	-	-	-
Calceolariaceae	*Calceolaria tripartita*	HM849833	HM851030	-	-	-	-	-	-
Fabaceae	*Callerya nieuwenhuisii*	-	-	AF467030	-	-	-	-	-
Lamiaceae	*Callicarpa japonica*	JQ618479	FM163257	-	-	-	AF130148	-	-
Lamiaceae	*Callicarpa japonica*	JQ618479	FM163257	-	-	-	AF130148	-	-
Achariaceae	*Caloncoba echinata*	-	-	-	-	-	AY757113	-	-
Achariaceae	*Caloncoba welwitschii*	-	-	-	-	-	AY757114	JX663794	-
Fabaceae	*Calpocalyx dinklagei*	AM234257	AY944551	-	-	-	-	-	-
Fabaceae	*Calpocalyx dinklagei* Harms	AM234257	AY944551	-	-	-	-	-	-

Calycanthaceae	*Calycanthus occidentalis*	AY642860	AJ627917	-	-	-	-	-	DQ490922
Theaceae	*Camellia sinensis*	X69732	AF380077	-	-	-	AF421077	AY725933	AB120309
Campanulaceae	*Campanula rotundiflolia*	EU713443	JN571958	DQ304615	EF213153	EF213355	-	EU437668	-
Campynemataceae	*Campynema lineare*	JN417505	JN417414	-	-	-	AY224997	AJ417573	GQ497570
Annonaceae	*Cananga odorata*	FJ976123	AY220438	-	-	-	AY841403	DQ401322	AF469770
Canellaceae	*Canella winterana*	AJ131928	DQ882240	L03844	AY145348	-	AY218191	AF528847	AF206879
Cannaceae	*Canna indica*	AM110251	AF434862	-	-	-	FJ861155	JX903948	D29785
Rubiaceae	*Canthium armatum*	AM117259	JF270895	JQ957994	JQ958229	-	-	-	-
Capparaceae	*Capparis spinosa*	AY167985	EU371772	-	AY122422	-	EU373694	AF035900	EU090942
Caricaceae	*Carica papaya*	M95671	AY483221	AY461547	JX091823	-	EU002209	AF035901	U42514
Carlemanniaceae	*Carlemannia tetragona*	DQ673316	HQ384548	-	-	-	DQ673290	HQ384757	-
Cyclanthaceae	*Carludovica palmata*	AF197596	AB088793	-	-	-	DQ355787	AF293861	KF264478
Betulaceae	*Carpinus betulus*	AY263928	AF297392	-	-	-	-	AY263943	AY263898
Juglandaceae	*Carya glabra*	AF119186	KF201333	-	-	-	-	AF209555	AF206880
Caryocaraceae	*Caryocar glabrum*	JQ626035	EF135515	-	-	-	AY425039	AF209556	AF206881
Rutaceae	*Casimiroa edulis*	EU042975	EU042837	-	-	-	-	AF066837	-
Casuarinaceae	*Casuarina equisetifolia*	AY263930	AY191701	-	-	-	-	AY263947	U42515
Orchidaceae	*Caularthron bicornutum*	-	-	AY008518	-	-	-	-	-
Orchidaceae	*Caularthron bilamellatum*	AF518059	AF263780	AF260173	AF267001	-	-	-	-
Urticaceae	*Cecropia ficifolia*	KF138133	-	KF137825	KF138296	-	-	-	KF137749
Urticaceae	*Cecropia insignis*	JQ594309	JQ589383	-	-	-	AY289264	-	-
Urticaceae	*Cecropia longipes*	GQ981693	GQ981957	-	-	-	-	-	-
Urticaceae	*Cecropia obtusa*	JQ626251	JQ626552	-	-	-	-	-	-
Urticaceae	*Cecropia obtusifolia*	KF138134	JQ589386	-	DQ179377	-	AY289263	-	-
Urticaceae	*Cecropia palmata.*	AF061196	GU135054	-	AF501615	-	AY289262	-	-
Urticaceae	*Cecropia peltata*	JQ594320	JQ589389	-	-	-	AY289265	-	-
Urticaceae	*Cecropia schreberiana*	HM446770	HM446666	-	-	-	-	-	-
Centrolepidaceae	*Centrolepis monogyna*	DQ257505	-	-	-	-	-	HQ180449	-
Centroplacaceae	*Centroplacus glaucinus*	JX664039	FJ670002	-	-	-	FJ670066	JX663796	FJ669687
Cephalotaceae	*Cephalotus follicularis*	L01894	FJ670045	-	-	-	-	AY788265	-
Cercidiphyllaceae	*Cercidiphyllum japonicum*	JN541237	KC737246	-	-	-	KC737263	JN541233	D29783

Arecaceae	*Ceroxylon quindiuense*	AJ404781	AM114607	AJ242151	AJ241284	-	EU186212	-	-
Chenopodiaceae	*Chenopodium quinoa*	JX570803	HE855648	KF709219	HE577571	-	-	-	-
Asteraceae	*Chionopappus benthamii*	-	JN837459	AB355524	AB355593	-	JN837355	-	-
Meliaceae	*Chisocheton ceramicus*	GQ248566	GQ248095	-	AB057512	-	-	-	-
Meliaceae	*Chisocheton lasiocarpus*	-	-	-	AB057528	-	-	-	-
Meliaceae	*Chisocheton macrophyllus*	AY128221	AY128183	DQ861613	AB057521	-	-	-	-
Meliaceae	*Chisocheton tomentosus*	-	-	-	AB057527	-	-	-	-
Chrysobalanaceae	*Chrysobalanus icaco*	JX664040	JX661935	-	-	-	JX662745	JX663797	U42519
Circaesteraceae	*Circaeaster agrestis*	FJ626607	GU266594	-	-	-	-	AF092116	AF094538
Cistaceae	*Cistus monspelliensis*	FJ225881	DQ093012	-	-	-	-	-	-
Montiaceae	*Claytonia perfoliata*	AF132093	AY764091	AY764040	-	-	AF194831	-	GQ497578
Cleomaceae	*Cleome hassleriana*	M95755	AY491649	AY662284	-	-	-	AF209565	U42511
Lamiaceae	*Clerodendrum buchanani*	-	-	U77742	FJ952040	-	AY310124	-	-
Lamiaceae	*Clerodendrum buchneri*	-	-	U77743	-	-	-	-	-
Lamiaceae	*Clerodendrum capitatum*	-	-	-	FJ952036	-	-	-	-
Lamiaceae	*Clerodendrum cephalanthum*	-	-	U77745	FJ952037	-	-	-	-
Lamiaceae	*Clerodendrum eriophyllum*	JX572413	JX517512	U77747	-	-	-	-	-
Lamiaceae	*Clerodendrum hildebrandtii*	-	-	U77749	FJ952039	-	-	-	-
Lamiaceae	*Clerodendrum laevifolium*	-	-	U77754	-	-	-	-	-
Lamiaceae	*Clerodendrum poggei*	-	-	-	FJ952034	-	-	-	-
Lamiaceae	*Clerodendrum rotundifolium*	-	-	U77766	FJ952035	-	-	-	-
Lamiaceae	*Clerodendrum speciosissimum*	-	-	U77769	FJ952041	-	-	-	-
Lamiaceae	*Clerodendrum splendens*	JQ618469	-	U77770	FJ952027	-	AY310126	-	-
Clethraceae	*Clethra arborea*	AF421088	HM850891	-	-	-	AF421046	AF420965	-
Melastomataceae	*Clidemia allardii*	-	-	AY460468	-	-	EU055904	-	-
Melastomataceae	*Clidemia ciliata*	-	-	AY460472	-	-	EU055907	-	-

Melastomataceae	*Clidemia cruegeriana*	-	-	AY460473	-	-	EU055911	-	-
Melastomataceae	*Clidemia cymosa*	-	-	AY460474	-	-	-	-	-
Melastomataceae	*Clidemia dimorphica*	-	-	AY460476	-	-	-	-	-
Melastomataceae	*Clidemia hirta*	-	-	AY460479	-	-	-	-	-
Melastomataceae	*Clidemia octona*	JQ592655	-	AY460480	-	-	-	-	-
Melastomataceae	*Clidemia petiolaris*	AM235643	-	-	-	-	AM235410	AJ235777	AM235516
Melastomataceae	*Clidemia rubra*	AF215535	-	AY460481	-	-	AF215579	-	-
Melastomataceae	*Clidemia sericea*	-	-	AY460483	-	-	EU055926	-	-
Cyrillaceae	*Cliftonia monophylla*	AY082697	AF380079	-	-	-	AY082694	AY082692	-
Clusiaceae	*Clusia rosea*	JX664041	JX661936	-	-	-	JX662746	AB233641	AB233537
Polygonaceae	*Coccoloba swartzii*	AF297150	EF437995	FJ154469	-	-	EF438034	-	-
Menispermaceae	*Cocculus orbiculatus*	L12642	DQ478611	-	-	-	JN051704	AF197614	AF197581
Arecaceae	*Cocos nucifera*	AY012507	HQ265564	HQ265515	AM113647	-	AY044566	AY012450	AY012393
Codonaceae	*Codon schenckii*	KF158091	HQ384576	-	GQ285270	-	KF158022	-	-
Rubiaceae	*Coffea arabica*	X83631	DQ401346	-	-	-	AJ236290	AJ235441	-
Malvaceae	*Cola acuminata*	AY082353	AY321179	-	-	-	AF111759	-	-
Malvaceae	*Cola acuminata*	AY082353	AY321179	-	-	-	AF111759	-	-
Gentianaceae	*Comastoma cyananthiflorum*	-	KC861250	KC861320	AJ407999	GU250970	-	-	-
Euphorbiaceae	*Conceveiba guianensis*	JQ626138	JQ626450	FJ037823	AY794791	-	-	-	-
Euphorbiaceae	*Conceveiba martiana*	AY788170	FJ670011	DQ006004	AY794789	-	FJ670080	-	-
Euphorbiaceae	*Conceveiba maynasensis*	-	-	DQ006005	-	-	-	-	-
Euphorbiaceae	*Conceveiba pleiostemona*	JQ591422	-	-	AY794794	-	-	-	-
Melastomataceae	*Conostegia micrantha*	-	-	AY460487	-	-	-	-	-
Lophiocarpaceae	*Corbichonia decumbens*	FN824475	FN825760	-	-	-	-	GQ497648	GQ497577
Boraginaceae	*Cordia alliodora*	GQ981712	JQ587067	JF332103	EU861992	-	-	-	-
Boraginaceae	*Cordia borinquensis*	HM446784	HM446678	AY321597	-	-	-	-	-
Boraginaceae	*Cordia gerascanthus*	JQ590899	JQ587094	JF332100	EU862003	-	-	-	-
Boraginaceae	*Cordia glabrata*	-	-	JF332097	-	-	-	-	-
Boraginaceae	*Cordia guerkeana*	-	-	AY701581	-	-	-	-	-
Boraginaceae	*Cordia insignis*	-	-	JF332098	-	-	-	-	-
Boraginaceae	*Cordia megalantha*	-	-	JF332101	-	-	-	-	-

Boraginaceae	*Cordia nodosa .*	-	-	JF332072	HQ286269	-	AF047808	-	-
Boraginaceae	*Cordia trichotoma*	EU599827	EU599651	JF332099	EU599915	-	-	-	-
Coriariaceae	*Coriaria myrtifolia*	L01897	AF542600	KC796598	AM397179	HE651128	AY968493	AF092117	AF206891
Cornaceae	*Cornus alterifolia*	AF190432	U96889	DQ340526	JF321138	-	EU373581	EU373560	-
Argophyllaceae	*Corokia cotoneaster*	L11221	AY491646	EF635466	-	-	AF130182	AJ235445	U42523
Caryophyllaceae	*Corrigiola litoralis*	FN868311	FN825767	AJ310980	FJ404979	-	-	-	-
Asteraceae	*Corymbium enerve*	GU817751	GU817445	GU818532	-	-	GU817848	-	-
Corynocarpaceae	*Corynocarpus laevigatus*	AF148994	AY968448	AF149004	AY968565	-	AY968497	AJ235446	-
Arecaceae	*Corypha umbraculifera*	AJ404761	AM114595	-	AJ241267	AM903164	HQ720624	-	-
Urticaceae	*Coussapoa villosa*	JQ594323	-	-	-	-	AY289261	-	-
Crassulaceae	*Crassula marnierana*	L01899	AF115600	-	-	-	-	AJ235447	U42525
Crossosomataceae	*Crossosoma bigelovii*	AY101844	HQ680694	DQ307116	DQ307148	-	DQ307082	HQ680707	AF193942
Crypteroniaceae	*Crypteronia borneensis*	AM235622	-	-	-	-	AM235389	-	-
Crypteroniaceae	*Crypteronia glabriflora*	AM235623	-	-	-	-	AM235390	-	-
Crypteroniaceae	*Crypteronia griffithii*	AJ605087	-	-	-	-	AJ605098	-	-
Crypteroniaceae	*Crypteronia paniculata*	AY078153	AY151566	-	-	AY151690	EU002217	-	-
Ctenolophonaceae	*Ctenolophon engelianus*	AJ402940	EF135524	-	-	-	FJ670074	AY788215	AY674589
Cucurbitaceae	*Cucumis sativus*	L21937	AB735562	AY833602	HM597036	-	-	AF209572	AF206894
Sapindaceae	*Cupaniopsis anacardioides*	L13182	AY724283	EU720438	EU721199	-	EU002218	-	-
Sapindaceae	*Cupaniopsis petiolulata*	-	JN681368	JN681309	JN681481	-	-	-	-
Cupressaceae	*Cupressus sempervirens*	L12571	HM023994	-	-	-	-	-	-
Curtisiaceae	*Curtisia dentata*	L11222	U96901	-	JF321164	-	JF321074	JF298838	L16007
Rubiaceae	*Cuviera acutiflora*			JQ957973	JQ958270	-	-	-	-
Rubiaceae	*Cuviera longiflora*	-	-	JQ957974	AJ620134	-	-	-	-
Rubiaceae	*Cuviera physinodes*	-	-	JQ957977	JQ958274	-	-	-	-
	Cuviera subuliflora	-	-	JQ957980	JQ958275	-	-	-	-
Cyatheaceae	*Cyathea podophylla*	JF303964	JF303907	-	-	-	-	-	-
Cycadaceae	*Cycas revoluta*	AY056556	JQ512413	-	-	-	AF469695	AF469657	-
Cynnomoraceae	*Cynnomorium coccineum*	-	-	-	-	-	-	-	AY957442
Orchidaceae	*Cypripedium passerinum*	JN965462	JQ182200	Z78516	JF796858	-	AY147779	-	-
Chrysobalanaceae	*Dactyladenia staudtii*	KC628484	JQ898829	-	-	-	-	-	-
Chrysobalanaceae	*Dactyladenia staudtii*	KC628484	JQ898829	-	-	-	-	-	-
Arecaceae	*Daemonorops*	-	-	AJ242070	-	-	EU186190	-	-

	didymophylla								
Arecaceae	*Daemonorops fissa*	-	-	AJ242075	-	-	EU186189	-	-
Poaceae	*Danthonia californica*	HE573372	HE573983				GU222712	EF422972	-
Daphnophyllaceae	*Daphniphyllum macropodum*	AM183400	AB445379	-	-	-	FJ670160	-	-
Datiscaceae	*Datisca glomerata*	L21940	AY968449	AF485250	AY968567	-	AY968499	AY968432	U42426
Apiaceae	*Daucus carota*	HM849948	JN894953	AY552527	FJ490764	-	-	-	-
Davalliaceae	*Davallia solida*	AY096193	-	-	-	-	-	EF452029	-
Degeneriaceae	*Degeneria vitensis*	L12643	AB055549	-	-	-	AY394736	AJ235451	AF206898
Fabaceae	*Delonix regia*	AY904419	KF379238	-	-	-	-	-	-
Sapotaceae	*Delpydora gracilis*	-	-	DQ246674	DQ344291	DQ344354	AY230679	-	-
Sapotaceae	*Delpydora macrophylla*	-	-	DQ246675	DQ344292	DQ344355	AY230680	-	-
Loranthaceae	*Dendropemon bicolor*	EU544469	EU544422	-	-	-	-	-	AF039075
Columelliaceae	*Desfontainia spinosa*	Z29670	AJ429363	-	AJ430950	-	AJ011988	AJ419677	GQ983565
Diapensiaceae	*Diapensia lapponica*	L12612	AJ429283	-	-	-	AY725881	AF420967	AF419794
Dichapetalaceae	*Dichapetalum macrocarpum*	AF089764	EF135527	-	-	-	AY425044	-	AF206902
Dicksoniaceae	*Dicksonia antartica*	U05919	EU223820	-	-	-	-	U93829	U18624
Didiereaceae	*Didierea madagascariensis*	HQ621335	HQ620831	-	AF095917	-	HQ620943	HQ620743	-
Didymelaceae	*Didymeles perrieri*	AF061994	DQ401354	AF245432	-	-	AF241603	AF092119	AF094541
Dilleniaceae	*Dillenia indica*	GQ997181	GQ997143	AY096030	KF953925	-	GQ997149	GQ997134	-
Costaceae	*Dimerocostus strobilaceus*	AF243838	AY994612	-	-	-	AY124997	AF168909	AF168839
	Dioicodendron dioicum	HM164163	FJ905349	FJ984975	AF102411	-	FJ871952	-	-
Droseraceae	*Dionaea muscipula*	L01904	AF204847	JN388078	-	FJ764815	-	AY096112	AY096116
Dioscoreaceae	*Dioscorea bulbifera*	AY904791	JF705347	-	JQ733841	-	AY007652	AF187059	AF069203
Ebenaceae	*Diospyros andamanica*	EU980645	-	-	DQ924208	-	DQ924101	-	-
Ebenaceae	*Diospyros kaki*	EU980698	DQ924028	JQ975107	DQ924245	X91004	DQ924138	DQ923921	-
Dipentodontaceae	*Dipentodon sinicus*	AF375609	AJ429397	-	-	-	AJ429131	-	AF375610
Melastomataceae	*Diplectria divaricata*	AF270746	-	-	-	-	AF215556	-	-
Melastomataceae	*Diplectria divaricata*	AF270746	-	-	-	-	AF215556	-	-
Dipterocarpaceae	*Dipterocarpus alatus*	-	KC568466	-	AB246603	DQ157299	-	-	-

Dipterocarpaceae	*Dipterocarpus costatus*	-	KC708340	-	DQ157281	DQ157300	-	-	-
Dirachmaceae	*Dirachma socotrana*	AJ225789	JF317423	-	-	-	JF317442	-	JF317364
Asclepiadaceae	*Dischidia astephana*	-	HQ327603	DQ334459	DQ334534	DQ334576	-	-	-
Asclepiadaceae	*Dischidia bengalensis*	-	-	-	AF214343	-	-	-	-
Asclepiadaceae	*Dischidia chinensis*	EU196273	-	KC878572	-	-	-	-	-
Asclepiadaceae	*Dischidia hirsuta*	-	HQ327590	DQ334455	DQ334531	DQ334573	-	-	-
Asclepiadaceae	*Dischidia* sp. *1 LW-2011*	-	HQ327547	-	-	-	-	-	-
Euphorbiaceae	*Ditta myricoides*	AY794871	AB268043	-	AY794675	-	-	-	-
Malvaceae	*Dombeya calantha*	GU981733	-	-	-	-	-	GU981692	-
Stylidiaceae	*Donatia fascicularis*	AF307913	AJ429384	AF451599	AJ430972	-	AJ225074	-	-
Asteraceae	*Doniophyton anomalum*	AY874430	EU385348	EU841164	EF530302	-	EU385156	-	-
Doryanthaceae	*Doranthes palmeri*	JQ273911	HM640653	-	GQ423737	AY699128	JQ276793	JQ273616	HM640767
Winteraceae	*Drimys winteri*	AF093734	AY437816	AY004126	AY145347	-	AF123806	AF093425	U42823
Drosophyllaceae	*Drosophyllum lusitanicum*	L01907	AY514860	HM204890	-	-	-	AY096113	AY096119
Dryopteridaceae	*Dryopteris intermedia*	KF186510	JQ941642	-	-	-	-	-	-
Euphorbiaceae	*Drypetes roxburghii*	JX664068	EF135530	-	-	-	JX662771	AF209578	U42534
Olacaceae	*Dulacia candida*	DQ790137	DQ790174	-	-	-	-	-	DQ790109
Malvaceae	*Durio zibethinus*	AF022119	AY321188	-	-	-	AF111749	AF209580	AF206905
Rubiaceae	*Duroia aquatica*	JQ625889	-	AF183782	-	-	-	-	-
Rubiaceae	*Duroia eriopila*	JQ626024	JQ626449	AF183784		-	-	-	-
Rubiaceae	*Duroia hirsuta*	AJ286696	-	AF183786	AF152666	-	-	-	-
Rubiaceae	*Duroia longiflora*	JQ626252	-	-	-	-	-	-	-
Ecdeiocoleaceae	*Ecdeiocolea monostachya*	HQ182426	DQ257528	-	-	-	HQ181111	HQ180455	GQ497573
Poaceae	*Echinochloa crus-galli*	HM849963	HE574054	-	-	-	AM849149	-	-
Boraginaceae	*Echium vulgare*	KF158087	FJ789898	FJ763247	FJ763301	-	KF158018	AJ504819	-
Ehretiaceae	*Ehretia acuminata*	GQ997264	EU599656	AF385799	EU600008	-	GQ997232	GQ997217	HQ384690
Poaceae	*Ehrharta calycina*	AM235057	EU434288	-	-	-	GU222719	-	-
Pontederiaceae	*Eichhornia crassipes*	HM849967	AB040212	-	-	-	FJ861142	EF422981	AF069215
Restionaceae	*Elegia fenestrata*	AY123238	AY881508	-	-	-	AF547016	AY465536	-
Hydrocharitaceae	*Elodea nuttallii*	U80696	AB002568	EF526378	JF703282	EF529713	KC812637	-	-
Emblingiaceae	*Emblingia calceoliflora*	AJ402949	-	-	-	-	AY483256	-	-
Rubiaceae	*Empogona kirkii*	-	-	-	JF916965	-	-	-	-

Orchidaceae	*Encyclia tampensis*	-	AY396116	-	-	-	-	-	-
Euphorbiaceae	*Endospermum labios*	JF739115	-	-	-	-	AY374313	-	-
Euphorbiaceae	*Endospermum medullosum*	JF738624	-	-	-	-	-	-	-
Euphorbiaceae	*Endospermum moluccanum*	AJ402950	EF135533	-	AY794671	-	AY425051	-	-
Ephedraceae	*Ephedra intermedia*	AY056566	EF053131	-	-	-	-	-	AB453786
Onagraceae	*Epilobium angustifolium*	L10217	JN894188	-	-	-	AF495784	AF209582	AF206907
Polygonaceae	*Eriogonum alatum*	EF437977	EF437998	FJ154472	-	-	EF438038	-	-
Fabaceae	*Erythrina crista-galli*	Z70170	AY386869	FN825780	-	-	-	-	AF525296
Erythroxyalaceae	*Erythroxylum confusum*	L13183	-	-	-	-	-	AJ235466	AF206909
Escalloniaceae	*Escallonia rubra*	AJ419692	AJ429365	-	-	-	AJ277383	AJ318974	-
Myrtaceae	*Eucalyptus globulus*	HM849985	AY521535	AY615679	HQ287710	HQ287598	HQ287661	-	-
Eucommiaceae	*Eucommia ulmoides*	L01917	AJ429317	-	-	-	AJ429113	AJ235469	HQ384682
Cunoniaceae	*Eucryphia lucida*	L01918	GU266609	-	-	-	EU002223	AF209584	U42533
Euphorbiaceae	*Euphorbia pulcherrima*	AY794819	-	-	-	-	JQ750842	-	L37582
Euphroniaceae	*Euphronia guianensis*	AF089762	JX661941	-	-	-	AY425052	AB233637	AB233533
Eupomatiaceae	*Eupomatia bennettii*	L12644	DQ401341	-	-	-	AY218175	AJ235473	AF469771
Eupteleaceae	*Euptelea polyandra*	L12645	DQ401348	-	-	-	JN051737	AF528850	L75831
Loganiaceae	*Fagraea auriculata*	-	-	JX283363	JX217757	-	JX283422	-	-
Loganiaceae	*Fagraea elliptica*	-	AJ388158	JX283358	JX217752	-	JX283417	-	-
Loganiaceae	*Fagraea fragrans*	-	-	JX283360	JX217754	-	JX283419	-	-
Loganiaceae	*Fagraea imperialis*	-	-	JX283369	JX217763	-	JX283428	-	-
Loganiaceae	*Fagraea racemosa*	-	-	FJ232578	JX217774	-	JX283439	-	-
Moraceae	*Ficus benjamina*	AF500350	JQ773506	JN117620	AF501605	GQ504358	AF500377	-	-
Moraceae	*Ficus obscura*	-	-	EU091676	-	-	-	-	-
Flagellariaceae	*Flagellaria indica*	L12678	DQ257515	-	-	-	AY465643	AJ419141	AF168845
Fouquieriaceae	*Fouquieria columnaris*	AY725861	EU628508	-	-	-	AF207961	AJ235501	AF003961
Francoaceae	*Francoa sonchifolia*	L11184	-	-	-	-	-	-	L28137
Frankeniaceae	*Frankenia pulverulenta*	Z97638	HM851067	-	-	-	HQ843268	AJ235476	AF206914
Himantandraceae	*Galbulimima belgraveana*	L12646	AY220441	-	-	-	AY218176	AJ235478	AF206916
Rubiaceae	*Galium aparine*	X81091	HQ384560	-	-	-	-	-	HQ384689
Rubiaceae	*Gardenia imperialis*	-	-	-	-	DQ131737		-	-

Rubiaceae	*Gardenia taitensis*	-	-	KC576963	AF102426	AJ233988	-	-	-
Geissolomataceae	*Geissoloma marginatum*	AJ403022	HQ680697	-	-	-	-	HQ680710	-
Gelsemiaceae	*Gelsemium sempervivens*	L14397	AJ429322	-	-	-	AF130170	AJ236193	AJ236025
Geraniaceae	*Geranium macrorrhizum*	L14696	EU922243	-	-	-	EU922249	EU922236	-
Gerrardinaceae	*Gerrardina foliosa*	AY757086	FM179924	-	-	-	AY757130	AY757085	-
Poaceae	*Gigantochloa albociliata*	-	HM448946	DQ270130	DQ137353	-	KF365015	-	-
Poaceae	*Gigantochloa levis*	-	KF364976	GQ464820	-	-	KF365012	-	-
Poaceae	*Gigantochloa ligulata*	-	EU434257	-	EU434065	EU434129	KF365023	-	-
Poaceae	*Gigantochloa scortechinii*	-	EU434258	-	EU434066	EU434130	-	-	-
Ginkgoaceae	*Ginkgo biloba*	AJ235804	AF456370	-	-	-	EU016965	DQ069344	D16448
Gisekiaceae	*Gisekia africana*	-	JQ844144	JX232575	HE585086	-	HQ843269	-	-
Rubiaceae	*Gleasonia prancei*	-	-	-	AF152682	-	-	-	-
Zingiberaceae	*Globba curtisii*	L05449	AB088797	-	-	-	AY125001	AF168913	AF168846
Bromeliaceae	*Glomeropitcairnia penduliflora*	L19975	AY614030	-	-	-	L75864	AF168914	AF168847
Colchicaceae	*Gloriosa superba*	D28867	JQ024964	-	-	-	EU044626	-	-
Gnetaceae	*Gnetum gnemon*	L12680	AY449621	-	-	-	-	AF187060	-
Asteraceae	*Gochnatia hypoleuca*	EU384978	EU385357	-	EU385071	-	EU385165	-	-
Gomortegaceae	*Gomortega keule*	AF206773	-	-	-	-	-	D89560	D89562
Caryopteridaceae	*Gonocaryum litorale*	AJ235779	GQ983654	-	GQ984057	-	AJ400889	-	-
Orchidaceae	*Goodyera pubescens*	FJ571329	AF263663	FJ473326	FJ571279	-	-	-	-
Goupiaceae	*Goupia glabra*	AJ235780	EF135544	-	-	-	AY425054	AJ235485	AF206920
Anacampserotaceae	*Grahamia bracteata*	AY875217	AY015273	-	-	-	AF194846	-	-
Orchidaceae	*Grammatophyllum papuanum*	-	EF079262	-	-	-	-	-	-
Orchidaceae	*Grammatophyllum speciosum*	AF074176	AF239510	AF470488	AF239606	-	-	-	-
Malvaceae	*Grewia occidentalis*	AJ233152	JX517699	-	-	-	-	AJ233105	AF206921
Greyiaceae	*Greyia radlkoferi*	L11185	AF542592	-	-	-	-	AF209594	U43151
Griseliniaceae	*Griselinia littoralis*	AF307916	AJ429372	-	AJ430958	-	-	-	-
Grubbiaceae	*Grubbia tomentosa*	Z83141	AF323184	-	JF321165		AJ400890	JF298839	
Guamatelaceae	*Guamatela tuerckheimii*	DQ443463	DQ443460	-	-	-	-	DQ443453	-

Gunneraceae	*Gunnera manicata*	EU002279	EU002179	-	-	-	EU002226	EU002162	U43787
Asclepiadaceae	*Gunnessia pepo*	-	-	DQ334446	DQ334528	DQ334570	-	-	-
Asteraceae	*Gymnarrhena micrantha*	EU384983	EU385362	-	EU385076	-	EU385170	-	-
Polygonaceae	*Gymnopodium floribundum*	HM137379	GQ206197	JQ352534	-	-	GQ206282	-	-
Hernandiaceae	*Gyrocarpus americanus*	KF381159	DQ401370	-	-	-	-	AJ235487	AF206923
Gyrostemonaceae	*Gyrostemon tepperi*	L22440	AY483237	-	-	-	AY483253	-	AF070971
Stilbaceae	*Halleria lucida*	AF026828	AF375188	-	-	-	AF188185	HQ384732	-
Halophytaceae	*Halophytum ameghinoi*	AJ403024	AY514852	EU410352	-	-	HQ843270	GQ497647	GQ497574
Hamamelidaceae	*Hamamelis japonica*	AY263940	AF248617	-	-	-	EF207467	AY263960	AY147113
Euphorbiaceae	*Hancea eucausta*	-	EF582624	DQ866598	DQ899256	-	-	-	-
Hanguanaceae	*Hanguana maleyana*	AJ417896	JQ435569	KF933719	AM113698	-	AY007654	AF387602	AF387604
Asteraceae	*Hecastocleis shockleyi*	EU384984	EU385363	AY190282	EU385077	-	EU385171	-	-
Monimiaceae	*Hedycarya arborea*	L12648	AM396509	-	-	-	AY394738	AJ235490	AF206924
Rubiaceae	*Heinsia crinita*	Y11849	KC627834	-	-	-	HM164372	-	-
Rubiaceae	*Heinsia crinita*	Y11849	HM119540	-	-	-	HM164372	-	-
Erythropalaceae	*Heisteria parvifolia*	DQ790163	DQ790199	-	-	-	-	AJ235492	-
Heliconiaceae	*Heliconia rostrata*	AF378767	JQ435568	-	-	-	FJ861145	AF168921	AF168850
Heliotropiaceae	*Heliotropium arborescens*	L14399	HQ384570	HQ286112	HQ286140	-	EF688911	HQ384780	-
Helwingiaceae	*Helwingia japonica*	L11226	AJ430195	AF200593	AJ430963	X94941	AF130207	-	-
Xanthorrhoeaceae	*Hemerocallis middendorffii*	KC704811	KC704541	-	AB097833	AB095544	-	-	-
Melastomataceae	*Henriettea succosa*	KF781628	-	KF781570	-	-	GU968815	-	-
Melastomataceae	*Henriettella rimosa*	-	-	AY460567	-	-	GU968818	-	-
Melastomataceae	*Henriettella tuberculosa*	KF781629	-	KF781571	-	-	GU968816	-	-
Saxifragaceae	*Heuchera micrantha*	L01925	-	-	-	-	AF130222	-	L28139
Malvaceae	*Hibiscus rosa-sinensis*	AY082359	AY321160	-	-	-	AY589075	-	FJ665614
Chrysobalanaceae	*Hirtella americana*	GQ981763	-	-	-	-	-	-	-
Chrysobalanaceae	*Hirtella araguariensis*	JQ626071	JQ898846	FJ037809	-	-	-	-	-
Chrysobalanaceae	*Hirtella bicornis*	AF089756	JQ898856	JQ898984	-	-	AY425055	AY788225	-
Chrysobalanaceae	*Hirtella ciliata*	JQ898748	JQ898853	JQ899024	-	-	-	-	-
Chrysobalanaceae	*Hirtella davisii*	JQ898732	JQ898881	JQ898982	-	-	-	-	-
Chrysobalanaceae	*Hirtella glandistipula*	JQ898731	-	JQ898985	-	-	-	-	-
Chrysobalanaceae	*Hirtella glandulosa*	FJ038001	-	FJ037810	FJ039267	-	-	-	-

Chrysobalanaceae	*Hirtella hebeclada*	KF981211	JQ898843	JQ899020	-	-	-	-	-
Chrysobalanaceae	*Hirtella macrosepala*	JQ625795	JQ898840	JQ898989	-	-	-	-	-
Chrysobalanaceae	*Hirtella physophora*	JQ898737	-	JQ898981	-	-	-	-	-
Chrysobalanaceae	*Hirtella racemosa*	JQ591068	-	-	-	-	-	-	-
Chrysobalanaceae	*Hirtella rugosa*	HM446812	-	-	-	-	-	-	-
Chrysobalanaceae	*Hirtella silicea*	JQ898751	JQ898871	JQ899014	-	-	-	-	-
Chrysobalanaceae	*Hirtella suffulta*	JQ625956	JQ898858	FJ037811	FJ039264	-	-	-	-
Chrysobalanaceae	*Hirtella tenuifolia*	JQ898730	JQ898845	JQ898987	-	-	-	-	-
Chrysobalanaceae	*Hirtella triandra*	GQ424481	JQ898854	GQ424461	-	-	-	-	-
Chrysobalanaceae	*Hirtella zanzibarica*	JX572679	JX518073	-	-	-	-	-	-
Rubiaceae	*Hoffmannia longipetiolata*	JQ593696	JQ589615	-	-	-	-	-	-
Asclepiadaceae	*Hoya affinis*	-	HQ327536	DQ334481	DQ334546	DQ334588	-	-	-
Asclepiadaceae	*Hoya albiflora*	-	HQ327567	DQ334496	DQ334555	DQ334597	-	-	-
Asclepiadaceae	*Hoya ariadna*	-	HQ327535	DQ334506	DQ334559	DQ334602	-	-	-
Asclepiadaceae	*Hoya bilobata*	-	HQ327599	DQ334492	DQ334554	DQ334596	-	-	-
Asclepiadaceae	*Hoya camphorifolia*	-	HQ327564	DQ334473	DQ334539	DQ334581	-	-	-
Asclepiadaceae	*Hoya carnosa*	EU196279	HQ327586	DQ334464	DQ334535	DQ334577	-	-	-
Asclepiadaceae	*Hoya caudata*	-	HQ327606	DQ334483	DQ334548	DQ334590	-	-	-
Asclepiadaceae	*Hoya* cf. *darwinii Chase 17135*	-	-	DQ334477	DQ334542	DQ334584	-	-	-
Asclepiadaceae	*Hoya* cf. *incrassata Chase 17136*	-	-	DQ334518	DQ334561	DQ334604	-	-	-
Asclepiadaceae	*Hoya ciliata*	-	HQ327537	DQ334514	DQ334562	DQ334605	-	-	-
Asclepiadaceae	*Hoya curtisii*	-	HQ327608	DQ334479	DQ334544	DQ334586	-	-	-
Asclepiadaceae	*Hoya darwinii*	-	HQ327591	-	-	-	-	-	-
Asclepiadaceae	*Hoya imbricata*	-	HQ327605	DQ334480	DQ334545	DQ334587	-	-	-
Asclepiadaceae	*Hoya incrassata*	-	HQ327556	-	-	-	-	-	-
Asclepiadaceae	*Hoya lacunosa*	-	HQ327601	DQ334499	DQ334557	DQ334599	-	-	-
Asclepiadaceae	*Hoya mitrata*	-	HQ327604	DQ334500	DQ334558	DQ334600	-	-	-
Asclepiadaceae	*Hoya patella*	-	HQ327553	DQ334498	DQ334556	DQ334598	-	-	-
Asclepiadaceae	*Hoya pauciflora*	-	HQ327541	DQ334468	DQ334536	DQ334578	-	-	-
Asclepiadaceae	*Hoya pubicalyx*	JX104129	HQ327588	DQ334450	DQ334530	DQ334572	-	-	-

Asclepiadaceae	*Hoya retusa*	EU196280	HQ327552	DQ334457	DQ334532	DQ334574	-	-	-
Asclepiadaceae	*Hoya serpens*	JQ933365	HQ327575	DQ334482	DQ334547	DQ334589	-	-	-
Asclepiadaceae	*Hoya telosmoides*	-	HQ327592	DQ334486	DQ334551	DQ334593	-	-	-
Huaceae	*Hua gabonii*	FJ670185	FJ670056	-	-	-	EU002230	FJ669995	AY929345
Fabaceae	*Humboldtia bourdillonii*	JX163311	-	-	-	-	-	-	-
Fabaceae	*Humboldtia brunonis*	JX163310	-	-	AF365214	-	-	-	-
Fabaceae	*Humboldtia laurifolia*	-	-	-	AF365211	-	-	-	-
Fabaceae	*Humboldtia unijuga*	-	-	-	AF365213	-	-	-	-
Fabaceae	*Humboldtia vahliana*	-	-	-	AF365212	-	-	-	-
Humiriaceae	*Humiria balsamifera*	L01926	JX661945	-	-	-	EU002231	AB233681	AB233577
Cannabaceae	*Humulus lupulus*	AF206777	AF345318	-	-	-	AY289251	AF209599	AF206931
Rubiaceae	*Hydnophytum* cf. *longistylum* McPherson 19437	-	-	JX155078	JX155030	-	JX155123	-	-
Rubiaceae	*Hydnophytum* cf. *moseleyanum* GDA-2008	-	-	AM980870	-	-	-	-	-
Rubiaceae	*Hydnophytum formicarum*	X83645	-	AF034912	JN643391	X76480	-	-	-
Rubiaceae	*Hydnophytum moseleyanum*	-	-	AF034913	JN643392	-	-	-	-
Rubiaceae	*Hydnophytum* sp. Barrabée & Rigault 1041 voucher Barrabe & Rigault 1041 (NOU)	-	-	KF675912	KF676176	-	KF676000	-	-
Rubiaceae	*Hydnophytum* sp. *C.H. Lambrick 132/83 18S*	-	-	AF034914	-	-	-	-	-
Hydnoraceae	*Hydnora africana*	-	-	-	-	-	-	-	L25681
Hydrangeaceae	*Hydrangea macrophylla*	L11187	AB038178	GU983033	JN226774	-	AF130218	AF528852	U42781
Ranunculaceae	*Hydrastis canadensis*	L75849	AB069849	-	-	-	AY145146	AF093382	L75828
Hydroleaceae	*Hydrolea ovata*	L14293	AJ429356	-	-	-	AF013999	AJ236184	AJ236014
Hydrostachyaceae	*Hydrostachys imbricata*	JF308653	FN811268	-	JF321169	-	JF321077	AJ236230	AJ235983
Papaveraceae	*Hypecoum imberbe*	U86628	GU266596	-	-	-	-	U86398	L75836
Molluginaceae?	*Hypertelis salsoloides*	FN824478	AJ582918	-	FN825763	-	-		
Hypoxidaceae	*Hypoxis villosa*	JX903208	JX903619	-	HM459513	-	JX903456	JX903873	-

Geraniaceae	*Hypseocharis pimpinellifolia*	DQ317048	-	-	-	-	-	-	DQ317014
Aquifoliaceae	*Ilex cornuta*	GQ997347	GQ997309	AF200591	FJ394743	AF471624	GQ997315	-	-
Balsaminaceae	*Impatiens capensis*	Z83142	AJ429280	AY712664	AF396205	DQ147823	AF130210	-	-
Fabaceae	*Inga edulis*	-	HM020738	JX870764	HM020836	-	-	-	-
Convolvulaceae	*Ipomoea batatas*	AY100962	AJ429355	-	-	-	AF130177	AY100753	HM053485
Iridaceae	*Iris missouriensis*	AY149365	AY596648	-	EU939488	AY147745	HM574454	AY147620	-
Stemonuraceae	*Irvingbaileya australis*	AF156733	-	-	KC428544	-	AJ236255	-	-
Irvingiaceae	*Irvingia malayana*	JX664054	JX661948	-	-	-	JX662757	JX663807	AB233580
Poaceae	*Isachne arundinacea*	AM849337	HE573992	-	-	-	AY847119	-	-
Iteaceae	*Itea virginica*	L11188	EF456732	-	-	-	EF207469	-	U42545
Ixerbaceae	*Ixerba brexioides*	AF084476	HQ680698	-	-	-	EU002235	-	AF084476
Ixioliriaceae	*Ixiolirion tataricum*	Z73704	AJ579965	KF261068	AJ290280	AY147746	AY147781	AJ235507	AF206940
Rubiaceae	*Ixora foliosa*	-	-	HG315466	HG315203	-	-	-	-
Rubiaceae	*Ixora hippoperifera*	KC628561	KC627857	-	HG315213	-	-	-	-
Petrosaviaceae	*Japonolirion osense*	JQ068978	JQ068952	-	-	-	AY147764	JQ068977	AF206942
Joinvilleaceae	*Joinvillea australis*	HQ182433	DQ257534	-	-	-	EU832891	EU832855	AF168855
Juncaceae	*Juncus effusus*	L12681	HQ180871	-	-	-	HQ181118	HQ180463	AF206944
Rubiaceae	*Keetia venosa*	-	-	JQ957992	KF488286	-	-	-	-
Monimiaceae	*Kibara rigidifolia*	AF050221	-	-	-	-	-	-	-
Bignonaceae	*Kigelia africana*	AF102648	AF051988	JN115030	EF105072	-	AF102632	-	-
Achariaceae	*Kiggelaria africana*	AF206786	EF135555	-	-	-	AY757144	AB233635	AF206945
Dasypogonaceae	*Kingia australis*	HQ182435	AM114718	-	-	-	HQ181119	HQ180464	-
Kirkiaceae	*Kirkia acuminata*	JX572707	JX517399	-	-	-	-	HE588084	-
Myristicaceae	*Knema latericia*	L12653	-	-	-	-	AY394740	AF209611	AF206946
Koeberliniaceae	*Koeberlinia spinosa*	L14600	AY483222	-	-	-	AY483249	AF209612	U42512
Arecaceae	*Korthalsia cheb*	AM110188	AM114546	AJ242101	AM113613	-	EU186184	-	-
Arecaceae	*Korthalsia debilis*	AJ829877	-	-	-	-	-	-	-
Arecaceae	*Korthalsia jala*	-	-	AJ242104	-	-	-	-	-
Krameriaceae	*Krameria ixine*	EU644679	EU604050	-	-	-	-	AJ235514	AF206948
Eriocaulaceae	*Lachnocaulon anceps*	Y13019	-	-	-	-	EF394003	AJ419137	-
Lacistemataceae	*Lacistema robustum*	JX664056	JX661950	-	-	-	JX662759	JX663809	-
Lactoridaceae	*Lactoris fernandeziana*	L08763	AF543739	-	-	-	AF123809	AJ235515	U42783
Lamiaceae	*Lamium purpureum*	HM850103	HQ384493	-	-	-	U78694	HQ384696	HQ384694

Lanariaceae	*Lanaria lanata*	Z77313	AY368376	-	HM459536	AY147747	AY147782	-	-
Lardizabalaceae	*Lardizabala biternata*	L37919	AY437809	-	-	-	-	DQ401326	L37910
Zygophyllaceae	*Larrea tridentata*	Y15022	AM396502	-	-	-	AY968516	AY935860	AY929372
Lauraceae	*Laurus nobilis*	AF197593	AF244407	-	-	-	-	AJ235518	AF197580
Polypodiaceae	*Lecanopteris balgooyi*	AF470328	-	-	AY083631	-	-	-	-
Polypodiaceae	*Lecanopteris carnosa*	AF470322	-	-	AY083625	-	-	-	-
Polypodiaceae	*Lecanopteris celebica*	AF470323	-	-	AY083626	-	-	-	-
Polypodiaceae	*Lecanopteris crustacea*	AF470329	-	-	AY083632	-	-	-	-
Polypodiaceae	*Lecanopteris deparioides*	AF470324	-	-	AY083627	-	-	-	-
Polypodiaceae	*Lecanopteris lomarioides*	AF470326	-	-	AY083629	-	-	-	-
Polypodiaceae	*Lecanopteris luzonensis*	AF470325	-	-	AY083628	-	-	-	-
Polypodiaceae	*Lecanopteris mirabilis*	AF470330	-	-	AY083633	-	-	-	-
Polypodiaceae	*Lecanopteris pumila*	AF470331	-	-	AY083634	-	-	-	-
Polypodiaceae	*Lecanopteris sarcopus*	EU482935	-	-	EU483030	-	-	-	-
Polypodiaceae	*Lecanopteris sinuosa*	AF470321	-	-	AY083624	-	-	-	-
Polypodiaceae	*Lecanopteris spinosa*	AF470327	-	-	AY083630	-	-	-	-
Fabaceae	*Leonardoxa africana*	-	EU361992	-	AF365118	-	-	-	-
Fabaceae	*Leonardoxa africana* subsp. *africana*	-	-	-	AF233466	-	-	-	-
Fabaceae	*Leonardoxa africana* subsp. *gracilicaulis*	-	-	-	AF233469	-	-	-	-
Fabaceae	*Leonardoxa africana* subsp. *letouzeyi*	-	-	-	AF233463	-	-	-	-
Fabaceae	*Leonardoxa africana* subsp. *rumpiensis*	-	-	-	AF233460	-	-	-	-
Fabaceae	*Leonardoxa bequaertii*	-	-	-	AF233457	-	-	-	-
Fabaceae	*Leonardoxa romii*	-	-	-	AF233458	-	-	-	-
Cyperaceae	*Lepironia articulata*	AB369976	FR832787	-	-	-	AB373104	-	-
Sapindaceae	*Lepisanthes alata*	AY724360	AY724312	EU720450	EU721217	-	-	-	-
Sapindaceae	*Lepisanthes rubiginosa*	-	EU720614	EU720446	EU721212	-	-	-	-
Rubiaceae	*Leptactina delagoensis*	AM117240	JF265502	-	-	-	-	-	-
Rubiaceae	*Leptactina delagoensis*	AM117240	JF270849	-	-	-	-	-	-
Myrtaceae	*Leptospermum polygalifolium* subsp.			EU850668				-	-

	polygalifolium								
Myrtaceae	*Leptospermum scoparium*	HM850121	HM851051	AY772398	AY740905	-	AM235423	-	-
Urticaceae	*Leucosyke australis*	JF739090	-	-	-	-	-	-	-
Urticaceae	*Leucosyke capitellata*	FJ976144	-	-	FJ432258	-	-	-	-
Urticaceae	*Leucosyke quadrinervia*	KF138190	KF138019	KF137876	KF138354	-	-	-	-
Magnoliaceae	*Liliodendron tulipifera*	AY008947	AF123480	-	-	-	AF107997	AJ235522	AF206954
Limeaceae	*Limeum africanum*	-	JQ844143	AJ532594	AJ558034	AJ532610	HQ843272	-	-
Limnanthaceae	*Limnanthes douglasii*	L14700	-	FJ895960	FJ895969	-	-	AF209619	-
Orobranchaceae	*Lindenbergia philippinensis*	AF123664	AF051990	-	-	-	AF123686	HQ384708	-
Linderniaceae	*Lindernia crustacea*	AB259807	FR728405	-	-	-	-	-	-
Linaceae	*Linum perenne*	FJ169582	AB038182	-	-	-	FJ160802	AJ235521	L24401
Altingiaceae	*Liquidambar styraciflua*	AF119181	AF133219	-	-	-	EU002239	EU002164	U42553
Sapindaceae	*Litchi chinensis*	AY724361	EU720564	-	-	-	-	JQ349005	JF759906
Ericaceae	*Loiseleuria procumbens*	U49288	U61352	-	-	-	JX890298	-	-
Lomariopsidaceae	*Lomariopsis spectabilis*	AB232401	JF303952	-	-	-	-	-	-
Caprifoliaceae	*Lonicera japonica*	GQ997430	GQ997392	KF160908	HM228585	-	GQ997398	GQ997383	-
Lophopyxidaceae	*Lophopyxis maingayi*	AY663643	EF135560	-	-	-	FJ670105	AY788235	AY674614
Melastomataceae	*Loreya subandina*	-	-	AY460563	-	-	GU968824	-	-
Rubiaceae	*Ludekia borneensis*	AJ346983	-	AJ346870	AJ346962	GQ852043	-	-	-
Anarthriaceae	*Lyginia imberbis*	GQ408937	DQ257524	-	-	-	EU832883	-	-
Primulaceae-Myrsinoideae	*Lysimachia europaea*	U96655	-	-	-	-	AY856439	AF213790	-
Lythraceae	*Lythrum salicaria*	AF421496	HQ593354	-	-	-	AF495775	AF209621	AF206955
Euphorbiaceae	*Macaranga aetheadenia*	-	-	AJ275618	-	DQ358340	-	-	-
Euphorbiaceae	*Macaranga albescens*	-	-	DQ866533	DQ899189	-	-	-	-
Euphorbiaceae	*Macaranga alchorneoides*	-	-	DQ866534	DQ899190	-	-	-	-
Euphorbiaceae	*Macaranga aleuritoides*	AB267922	AB268026	DQ866535	DQ899191	-	AY374319	AB267974	-
Euphorbiaceae	*Macaranga alnifolia*	-	-	DQ866536	DQ899192	-	-	-	-
Euphorbiaceae	*Macaranga andamanica*	HQ415215	HQ415380	-	-	-	-	-	-
Euphorbiaceae	*Macaranga angulata*	-	-	AJ275621	-	DQ358370	-	-	-
Euphorbiaceae	*Macaranga angustifolia*	-	-	DQ866537	DQ899193	-	-	-	-
Euphorbiaceae	*Macaranga ashtonii*	-	-	-	-	DQ358377	-	-	-
Euphorbiaceae	*Macaranga auriculata*	-	-	DQ866538	DQ899194	-	-	-	-

Euphorbiaceae	*Macaranga bancana*	-	-	AF361114	-	DQ358311	-	-	-
Euphorbiaceae	*Macaranga barteri*	-	-	DQ866539	DQ899195	-	-	-	-
Euphorbiaceae	*Macaranga beccariana*	-	-	AJ275622	-	DQ358281	-	-	-
Euphorbiaceae	*Macaranga beillei*	-	-	AJ275624	-	-	-	-	-
Euphorbiaceae	*Macaranga bicolor*	-	-	DQ866540	DQ899196	-	-	-	-
Euphorbiaceae	*Macaranga bifoveata*	JF739038	-	-	DQ899197	-	-	-	-
Euphorbiaceae	*Macaranga brachytricha*	-	-	-	-	-	FJ976108	-	-
Euphorbiaceae	*Macaranga brevipetiolata*	-	-	AF361117	-	-	-	-	-
Euphorbiaceae	*Macaranga caladiifolia*	-	-	AJ275623	-	DQ358286	-	-	-
Euphorbiaceae	*Macaranga calcicola*	-	-	AJ275626	-	DQ358347	-	-	-
Euphorbiaceae	*Macaranga clavata*	-	-	DQ866543	DQ899200	-	FJ976109	-	-
Euphorbiaceae	*Macaranga conifera*	-	-	AF361119	-	DQ358252	-	-	-
Euphorbiaceae	*Macaranga constricta*	-	-	AJ275628	-	-	-	-	-
Euphorbiaceae	*Macaranga costulata*	-	-	AF361121	-	-	-	-	-
Euphorbiaceae	*Macaranga curtisii*	-	-	AJ275629	-	DQ358302	-	-	-
Euphorbiaceae	*Macaranga densiflora*	-	-	DQ866545	DQ899202	-	AY374317	-	-
Euphorbiaceae	*Macaranga denticulata*	-	-	DQ866546	DQ899203	-	-	-	-
Euphorbiaceae	*Macaranga depressa*	-	-	AJ275632	-	DQ358365	-	-	-
Euphorbiaceae	*Macaranga diepenhorstii*	-	-	AJ275634	DQ899204	DQ358253	-	-	-
Euphorbiaceae	*Macaranga domatiosa*	-	-	DQ866548	DQ899205	-	-	-	-
Euphorbiaceae	*Macaranga ducis*	-	-	DQ866549	DQ899206	-	AY374321	-	-
Euphorbiaceae	*Macaranga echinocarpa*	-	-	DQ866550	DQ899207	-	-	-	-
Euphorbiaceae	*Macaranga fallacina*	JF738793	-	-	-	-	FJ976110	-	-
Euphorbiaceae	*Macaranga ferruginea*	-	-	AJ275635	-	-	-	-	-
Euphorbiaceae	*Macaranga gabunica*	-	-	DQ866551	DQ899208	-	-	-	-
Euphorbiaceae	*Macaranga gigantea*	-	-	DQ866552	DQ899209	DQ358262	-	-	-
Euphorbiaceae	*Macaranga glandibracteolata*	-	-	AJ275639	-	DQ358354	-	-	-
Euphorbiaceae	*Macaranga grallata*	-	-	DQ866553	DQ899210	-	-	-	-
Euphorbiaceae	*Macaranga grandifolia*	AY794935	-	DQ866554	DQ899211	-	AY674730	-	-
Euphorbiaceae	*Macaranga griffithiana*	-	-	AJ298249	-	DQ358314	-	-	-
Euphorbiaceae	*Macaranga havilandii*	-	-	AJ275640	-	DQ358334	-	-	-
Euphorbiaceae	*Macaranga heterophylla*	-	-	DQ866555	DQ899212	-	-	-	-
Euphorbiaceae	*Macaranga heynei*	-	-	AJ275641	DQ899213	DQ358250	-	-	-

Euphorbiaceae	*Macaranga hispida*	-	-	-	-	-	FJ976111	-	-
Euphorbiaceae	*Macaranga hosei*	-	-	AJ275643	-	DQ358257	-	-	-
Euphorbiaceae	*Macaranga hullettii*	-	-	AJ275644	DQ899215	DQ358308	-	-	-
Euphorbiaceae	*Macaranga hurifolia*	-	-	DQ866558	DQ899216	-	-	-	-
Euphorbiaceae	*Macaranga hypoleuca*	-	EF582627	AJ275645	-	DQ358277	-	-	-
Euphorbiaceae	*Macaranga inamoena*	-	EF582628	DQ866559	DQ899217	-	-	-	-
Euphorbiaceae	*Macaranga indica*	-	-	AJ275646	DQ899218	-	-	-	-
Euphorbiaceae	*Macaranga indistincta*	-	-	AJ275647	-	DQ358359	-	-	-
Euphorbiaceae	*Macaranga induta*	-	-	DQ866561	DQ899219	-	-	-	-
Euphorbiaceae	*Macaranga involucrata*	-	-	DQ866562	DQ899220	-	-	-	-
Euphorbiaceae	*Macaranga kinabaluensis*	-	-	AJ298247	-	-	-	-	-
Euphorbiaceae	*Macaranga kingii*	-	-	AJ275649	-	-	-	-	-
Euphorbiaceae	*Macaranga klaineana*	-	-	DQ866563	DQ899221	-	-	-	-
Euphorbiaceae	*Macaranga kurzii*	-	EF582629	-	-	-	-	-	-
Euphorbiaceae	*Macaranga lamellata*	-	-	AJ298248	DQ899222	DQ358288	-	-	-
Euphorbiaceae	*Macaranga lowii* var. *kostermanii*	-	-	DQ866565	DQ899223	-	-	-	-
Euphorbiaceae	*Macaranga mauritiana*	-	-	DQ866567	DQ899225	-	-	-	-
Euphorbiaceae	*Macaranga monandra*	KC628123	-	DQ866568	DQ899226	-	-	-	-
Euphorbiaceae	*Macaranga motleyana*	-	-	AJ275653	-	DQ358345	-	-	-
Euphorbiaceae	*Macaranga neobritannica*	JF738873	-	-	-	-	-	-	-
Euphorbiaceae	*Macaranga novaguineensis*	-	-	DQ866569	DQ899227	-	AY374320	-	-
Euphorbiaceae	*Macaranga oblongifolia*	-	-	DQ866570	DQ899228	-	-	-	-
Euphorbiaceae	*Macaranga obovata*	-	-	DQ866571	DQ899229	-	-	-	-
Euphorbiaceae	*Macaranga pachyphylla*	-	-	DQ866572	DQ899230	-	-	-	-
Euphorbiaceae	*Macaranga papuana*	-	-	AJ275654	-	-	-	-	-
Euphorbiaceae	*Macaranga pearsonii*	-	-	DQ866573	DQ899231	DQ358267	-	-	-
Euphorbiaceae	*Macaranga petanostyla*	-	-	AJ275656	-	DQ358382	-	-	-
Euphorbiaceae	*Macaranga poggei*	-	-	DQ866574	DQ899232	-	-	-	-
Euphorbiaceae	*Macaranga praestans*	-	-	DQ866575	DQ899233	-	-	-	-
Euphorbiaceae	*Macaranga pruinosa*	-	-	AJ275659	-	DQ358263	-	-	-
Euphorbiaceae	*Macaranga*	-	-	AF361153	-	-	-	-	-

	pseudopruinosa (=M. hosei)								
Euphorbiaceae	*Macaranga puberula*	-	-	AJ298252	-	DQ358269	-	-	-
Euphorbiaceae	*Macaranga punctata*	JF738734	-	-	-	-	-	-	-
Euphorbiaceae	*Macaranga puncticulata*	-	-	DQ866576	DQ899234	DQ358297	-	-	-
Euphorbiaceae	*Macaranga quadricornis*	-	-	AJ275661	-	-	-	-	-
Euphorbiaceae	*Macaranga quadriglandulosa*	JF739042	-	DQ866577	DQ899235	-	AY374318	-	-
Euphorbiaceae	*Macaranga recurvata*	-	-	AJ298253	-	DQ358244	-	-	-
Euphorbiaceae	*Macaranga repandodentata*	-	-	DQ866578	DQ899236	-	-	-	-
Euphorbiaceae	*Macaranga rhizinoides*	-	-	DQ866579	DQ899237	-	-	-	-
Euphorbiaceae	*Macaranga rostrata*	-	-	AJ298254	-	DQ358366	-	-	-
Euphorbiaceae	*Macaranga rufescens*	-	-	-	-	DQ358264	-	-	-
Euphorbiaceae	*Macaranga saccifera*	-	-	DQ866580	DQ899238	-	-	-	-
Euphorbiaceae	*Macaranga sampsonii*	HQ415216	HQ415381	-	-	-	-	-	-
Euphorbiaceae	*Macaranga sarcocarpa*	-	-	AJ275665	-	DQ358296	-	-	-
Euphorbiaceae	*Macaranga schweinfurthii*	-	-	DQ866581	DQ899239	-	-	-	-
Euphorbiaceae	*Macaranga siamensis*	-	-	DQ866582	DQ899240	-	-	-	-
Euphorbiaceae	*Macaranga sp. A* (cf *Blattner et al., 2001)*	-	-	AJ298256	-	DQ358384	-	-	-
Euphorbiaceae	*Macaranga* sp. *B*	-	-	AJ298257	-	DQ358353	-	-	-
Euphorbiaceae	*Macaranga* sp. *C*	-	-	-	-	DQ358331	-	-	-
Euphorbiaceae	*Macaranga* sp. *D*	-	-	-	-	DQ358335	-	-	-
Euphorbiaceae	*Macaranga spathicalyx*	-	-	AF361140	-	-	-	-	-
Euphorbiaceae	*Macaranga subdentata*	-	-	DQ866583	DQ899241	-	-	-	-
Euphorbiaceae	*Macaranga subpeltata*	-	-	AJ275667	-	-	-	-	-
Euphorbiaceae	*Macaranga tanarius*	AB233866	EF582630	DQ866584	DQ899242	AY159469	-	AB233658	-
Euphorbiaceae	*Macaranga tessellata*	-	-	DQ866586	DQ899244	-	-	-	-
Euphorbiaceae	*Macaranga trachyphylla*	-	-	AJ275672	-	DQ358333	-	-	-
Euphorbiaceae	*Macaranga trichocarpa*	-	EF582631	DQ866587	DQ899245	-	-	-	-
Euphorbiaceae	*Macaranga triloba*	-	-	DQ866588	DQ899246	DQ358318	-	-	-
Euphorbiaceae	*Macaranga umbrosa*	-	-	DQ866589	DQ899247	DQ358293	-	-	-

Euphorbiaceae	*Macaranga velutina*	-	-	-	-	DQ358339	-	-	-
Euphorbiaceae	*Macaranga velutiniflora*	-	-	AF361160	-	-	-	-	-
Euphorbiaceae	*Macaranga winklerella*	-	-	AJ298260	-	DQ358249	-	-	-
Euphorbiaceae	*Macaranga winkleri*	-	-	AJ275675	DQ899248	DQ358248	-	-	-
Unresolved Caryophyllales	*Macarthuria australis*	FN824479	FN825765	-	-	-	-	-	-
Rubiaceae	*Macbrideina peruviana*	-	FJ905366	FJ984992	AF152635	-	FJ871957	-	-
Fabaceae	*Macrolobium acaciifolium*	U74191	KF794163	-	EU361820	-	-	-	-
Fabaceae	*Macrolobium bifolium*	JQ625745	EU361996	FJ817498	AF365200	-	-	-	-
Fabaceae	*Macrolobium campestre*	-	-	FJ817499	FJ817551	-	-	-	-
Fabaceae	*Macrolobium ischnocalyx*	-	EU361997	-	AF365201	-	-	-	-
Fabaceae	*Macrolobium longipes*	-	-	FJ817501	FJ817553	-	-	-	-
Maesoideae-Primulaceae	*Maesa tenera*	-	AJ429288	-	-	-	AF213750	AF213781	-
Chrysobalanaceae	*Magnistipula conrauana*	GQ424485	-	-	-	-	-	-	-
Polygonaceae	*Magoniella obidensis*	-	-	HQ693103	-	-	HQ693214	-	-
Melastomataceae	*Maieta guianensis*	AF215537	-	AY460498	-	-	AF215581	-	-
Melastomataceae	*Maieta poeppigii*	-	-	AY460499	-	-	-	-	-
Euphorbiaceae	*Mallotus cumingii*	-	EF582642	DQ866625	DQ899284	-	-	-	-
Euphorbiaceae	*Mallotus discolor*	-	EF582645	DQ866597	DQ899255	-	-	-	-
Euphorbiaceae	*Mallotus nudiflorus*	AY663648	EF582667	DQ866627	AY794763	-	-	-	-
Euphorbiaceae	*Mallotus paniculatus*	HQ415220	EF582671	DQ866608	DQ899268	-	-	-	-
Malpighiaceae	*Malpighia glabra*	HQ247544	AB233796	-	-	-	HQ246876	AB233692	AB233588
Calophyllaceae	*Mammea americana*	JX664058	JX661952	-	-	-	JX662761	JX663811	-
Piperaceae	*Manekia naranjoana*	AY572256	DQ882239	-	-	-	-	AY572280	AY572302
Anacardiaceae	*Mangifera indica*	GU935433	AY594472	-	-	-	-	GU943767	GU647220
Urticaceae	*Maoutia puya*	KF138192	KF138020	KF138356	-	-	-	-	-
Urticaceae	*Maoutia setosa*	KF138192	KF138020	-	KF138356	-	-	-	-
Marantaceae	*Maranta bicolor*	AF378768	AY140302	-	-	-	AY656094	AF168927	AF069225
Marcgraviaceae	*Marcgravia rectiflora*	Z83148	-	-	-	-	AJ236263	AJ235529	-
Solanaceae	*Markea panamensis*	-	-	-	EU581028	-	EU580884	-	-

Solanaceae	*Markea ulei*	-	-	-	EU581028	-	-	-	-
Asclepiadaceae	*Marsdenia carvalhoi*	JQ933400	HQ327531	DQ334421	DQ334521	DQ334563	-	-	-
Martinyaceae	*Martynia annua*	HQ384889	HQ384524	-	-	-	JN686624	HQ384730	-
Mayacaceae	*Mayaca fluviatilis*	HQ182438	-	-	-	-	AY147770	AF168929	AF168859
Mazaceae	*Mazus reptans*	HQ384872	HQ384502	-	-	-	HQ384817	HQ384705	-
Melastomataceae	*Medinilla humbertiana*	AF215517	-	-	-	-	AF215557	-	-
Ochnaceae	*Medusagyne oppositifolia*	JX664059	JX661953	-	-	-	JX662762	JX663812	AF206959
Melianthaceae	*Melianthus major*	AJ403027	GU266598	-	-	-	-	AJ235532	-
Loasaceae	*Mentzelia lindleyi*	JF308669	AF503307	-	JF321199	-	AJ236261	AJ236235	AJ235987
Menyanthaceae	*Menyanthes trifoliata*	EF173093	EF173062	EF173025	GQ245111	-	L39388	AJ235533	AJ236009
Melastomataceae	*Merianthera sipolisii*	JQ899100	-	AY460459	-	-	JQ899126	-	-
Aizoaceae	*Mesembryanthemum cristallinum*	HM850175	HM850877	AM162360	KC834529	AM161298	-	-	-
Metteniusaceae	*Metteniusa tessmanniana*	AM421128	-	-	-	-	-	AM421129	AM421127
Asclepiadaceae	*Micholitzia obcordata*	AJ419750	-	DQ334501	AF214381	DQ334601	-	-	-
Melastomataceae	*Miconia macrodon*	-	-	AY460516	-	-	EU056058	-	-
Melastomataceae	*Miconia sancti-philippi*	-	-	AY460527	-	-	-	-	-
Polypodiaceae	*Microsorum linguiforme*	AF470334	-	-	AY083637	-	-	-	-
Microteaceae	*Microtea debilis*	-	FN597632	JX232577	-	-	-	-	-
Coulaceae	*Minquartia guianensis*	DQ790148	DQ790185	-	-	-	-	-	L24396
Nyctaginaceae	*Mirabilis jalapa*	M62565	FN868307	AF212009	-	AJ532611	AF194826	AF209629	U42788
Misodendraceae	*Misodendrum linearifolium*	L26074	DQ787438	-	-	-	-	-	L24397
Mitrastemonaceae	*Mitrastema yamamotoi*	-	-	-	-	-	-	-	AY739090
Molluginaceae	*Mollugo verticillata*	HQ621337	FN825740	EU434728	FJ405003	-	HQ620945	HQ620745	HQ620945
Poaceae	*Monanthochloe littoralis*	JN681664	AF312349	-	-	-	EF561674	-	-
Montiniaceae	*Montinia caryophyllacea*	L11194	HQ384569	-	-	-	AF130178	AY100852	U42808
Myricaceae	*Morella cerifera*	AF119179	U92857	-	-	-	EU002243	AJ235537	AF206967
Moringaceae	*Moringa oleifera*	L11359	AY483223	AF378588	JX091843	-	AY122405	AF209633	U42786
Melastomataceae	*Mouriri cyphocarpa*	U26327	-	-	-	-	-	AF209634	AF206965
Muntingiaceae	*Muntingia calabura*	AY328197	FM179930	-	-	-	AF111781	AJ233068	U42539
Musaceae	*Musa acuminata*	EU017045	EU016987	-	-	-	EU016989	AF168931	U42083
Urticaceae	*Musanga cecropioides*	KC628289	KC627656	-	-	-	-	-	-
Haloragaceae	*Myriophyllum sibiricum*	L11195	FJ870928	-	-	-	-	AJ235538	U42551

Myristicaceae	*Myristica fragrans*	AF206798	AJ966803				AY218188	AF209636	AF206968
Myristicaceae	*Myristica fragrans*	AF206798	AJ966803	-	-	-	AY218188	AF209636	AF206968
Rubiaceae	*Myrmecodia armata*	-	-	AF034917	-	-	-	-	-
Rubiaceae	*Myrmecodia horrida*	-	-	AF071988	JN053649	-	-	-	-
Rubiaceae	*Myrmecodia longifolia*	-	-	-	-	AB044150	-	-	-
Rubiaceae	*Myrmecodia platyrea*	X87147	-	AF034918	-	-	-	-	-
Rubiaceae	*Myrmecodia platytyrea*	-	-	-	-	AB044151	-	-	-
Rubiaceae	*Myrmecodia tuberosa*	-	-	AF149313	-	-	-	-	-
Rubiaceae	*Myrmecodia tuberosa* voucher *Andreasen 341 (UPS)*	-	-	KF675913	KF676177	-	KF676001	-	-
Rubiaceae	*Myrmecodia tuberosa 2*	-	-	AM980867	-	-	-	-	-
Rubiaceae	*Myrmeconauclea stipulacea*	KC737721	-	AJ821880	KC737824	-	-	-	-
Rubiaceae	*Myrmeconauclea strigosa*	AJ346989	-	AJ821881	AJ346934	GQ852052	-	-	-
Orchidaceae	*Myrmecophila brysiana*	-	-	EF065700	-	-	-	-	-
Orchidaceae	*Myrmecophila christinae*	-	-	EF065701	-	-	-	-	-
Orchidaceae	*Myrmecophila exaltata*	-	-	AY008582	-	-	-	-	-
Orchidaceae	*Myrmecophila galeottiana*	-	-	AY008580	-	-	-	-	-
Orchidaceae	*Myrmecophila grandiflora*	-	-	EF065702	-	-	-	-	-
Orchidaceae	*Myrmecophila tibicinis*	-	AY396099	EF065703	AY422402	-	-	-	-
Orchidaceae	*Myrmecophila wendlandii*	-	-	AY008579	-	-	-	-	-
Rubiaceae	*Myrmephytum beccarii*	-	-	AM980871	-	AB044152	-	-	-
Rubiaceae	*Myrmephytum selebicum*	-	-	AF034916	JN643396	-	-	-	-
Myrothamnaceae	*Myrothamnus flabellifolius*	AF060707	AM396507	-	-	-	JF268476	AF093386	AF094555
Boraginaceae	*Nama demissa*	KF158101	HQ384572	AF091174	HQ412980	-	AF047767	HQ384782	-
Narcethiaceae	*Narthecium ossifragum*	AY149348	AB679482	-	EU186251	EU186226	AY147763	AY147597	AB679370
Rubiaceae	*Nauclea vanderguchtii*	KC737737	-	KC737631	-	-	-	-	-
Nelumbonaceae	*Nelumbo lutea*	DQ182337	EU642710	-	-	-	EU642680	AF093387	L75835
Rubiaceae	*Neonauclea brassii*	AJ346991	-	AJ346879	-	-	GQ852244	-	-

Rubiaceae	*Neonauclea celebica*	KC737722	-	AJ821870	KC737825	-	KC737661	-	-
Rubiaceae	*Neonauclea chalmersii*	-	-	AJ821856	-	-	-	-	-
Rubiaceae	*Neonauclea clemensiae*	AJ318450	-	AJ346898	AJ346940	GQ852055	GQ852246	-	-
Rubiaceae	*Neonauclea cyrtopoda*	KC737723	-	AJ821869	-	-	KC737692	-	-
Rubiaceae	*Neonauclea forsteri*	AJ346992	-	AJ346880	AJ346941	-	-	-	-
Rubiaceae	*Neonauclea gigantea*	KC737724	-	AJ821867	KC737826	-	KC737663	-	-
Rubiaceae	*Neonauclea gigantea*	KC737724	-	AJ821867	KC737826	-		-	-
Rubiaceae	*Neonauclea glabra*	KC737725	-	AJ821863	-	-	KC737693	-	-
Rubiaceae	*Neonauclea longipedunculata*	AJ346993	-	AJ821882	AJ346942	-	KC737664	-	-
Rubiaceae	*Neonauclea media*	-	-	AJ821864	-	-		-	-
Rubiaceae	*Neonauclea paracyrtopoda*	KC737726	-	AJ821865	KC737827	-	KC737665	-	-
Rubiaceae	*Neonauclea pseudocalycina*	KC737727	-	AJ821855	-	-	KC737694	-	-
Rubiaceae	*Neonauclea* sp. *1* Moog AMO-114 (L)	-	-	AJ821859	-	-	-	-	-
Rubiaceae	*Neonauclea* sp. *10* Moog AMO-088 (L)	-	-	AJ821858	-	-		-	-
Rubiaceae	*Neonauclea* sp. 11 Moog	-	-	AJ821866	-	-		-	-
Rubiaceae	*Neonauclea* sp. *2* Moog AMO118 (L)	-	-	AJ821861	-	-	-	-	-
Rubiaceae	*Neonauclea* sp. *3* Moog AMO-119 (L)	-	-	AJ821877	-	-		-	-
Rubiaceae	*Neonauclea* sp. *4* Moog AMO-087 (L)	-	-	AJ821853	-	-		-	-
Rubiaceae	*Neonauclea* sp. *5* Moog AMO-098 (L)	-	-	AJ821874	-	-		-	-
Rubiaceae	*Neonauclea* sp. *6* Moog AMO-127 (L)	-	-	AJ821876	-	-		-	-
Rubiaceae	*Neonauclea* sp. *7* Moog AMO-124 (L)	-	-	AJ821862	-	-		-	-
Rubiaceae	*Neonauclea* sp. *8* Moog AMO-042 (L)	-	-	AJ821857	-	-		-	-

Rubiaceae	*Neonauclea* sp. *9* Moog AMO-112 (L)	-	-	AJ821860	-	-		-	-
Rubiaceae	*Neonauclea* sp. *A* Moog	-	-	AJ821852	-	-		-	-
Rubiaceae	*Neonauclea* sp. *C1* Moog AMO-066 (L)	-	-	AJ821872	-	-	-	-	-
Rubiaceae	*Neonauclea* sp. *C2* Moog AMO-080 (L)	-	-	AJ821873	-	-	-	-	-
Rubiaceae	*Neonauclea* sp. *D1* Moog AMO-015 (L)	-	-	AJ821868	-	-	-	-	-
Rubiaceae	*Neonauclea* sp. *D2* Moog AMO-085 (L)	-	-	AJ821878	-	-	-	-	-
Rubiaceae	*Neonauclea* sp. *E* Moog AMO-074 (L)	-	-	AJ821875	-	-	-	-	-
Nepenthaceae	*Nepenthes alata*	L01936	AY042618	-	-	-	-	-	-
Nepenthaceae	*Nepenthes alata*	L01936	AF315891	HM204891	-	-	HQ843276	AF093388	-
Nepenthaceae	*Nepenthes bicalcarata*	-	DQ007089	-	-	-	-	-	-
Nepenthaceae	*Nepenthes gracilis*	-	AF315937	JX042555	JX042567	-	-	-	-
Neuradaceae	*Neurada procumbens*	U06814	-	-	-	-	EU002245	AJ233069	AF206970
Nothofagaceae	*Nothofagus solandri*	L13362	AB015464	-	-	-	-	AY605517	-
Rubiaceae	*Notopleura anomothyrsa*	JQ593713	JQ589619	-	-	-	-	-	-
Asteraceae	*Nouelia insignis*	EU385000	EU385378	-	EU385093	-	EU385187	-	-
Nymphaeaceae	*Nymphaea odorata*	M77034	AF092988	-	-	-	AY394742	AJ235544	AF206973
Arecaceae	*Nypa fruticans*	JX903253	JX903670	-	AY145339	AM903146	EU186217	AY012414	AY012357
Nyssaceae	*Nyssa yunnanensis*	AF384106	JQ280870	JQ280774	EU734506	-	-	-	-
Ixonanthaceae	*Ochthocosmus longipedicellatus*	FJ707535	EF135573	-	-	-	FJ670101	FJ707529	AY674621
Lauraceae	*Ocotea argyrophylla*	JQ626098	JQ626566	-	-	-	-	-	-
Lauraceae	*Ocotea atirrensis*	JQ592352	JQ588105	-	-	-	-	-	-
Lauraceae	*Ocotea bullata*	AM235002	JQ024978	AF272298	AF268734	-	-	-	-
Lauraceae	*Ocotea dendrodaphne*	JQ594769	JQ589756	-	-	-	-	-	-
Lauraceae	*Ocotea floribunda*	HM446841	EU153866	-	-	-	-	-	-
Lauraceae	*Ocotea guianensis*	-	EU153867	GQ480381	AF268737	-	-	-	-
Lauraceae	*Ocotea oblonga*	GQ981817	GQ982053	-	-	-	-	-	-
Lauraceae	*Ocotea odorifera*	-	-	GQ480388	AF268738	-	-	-	-

Lauraceae	*Ocotea subterminalis*	JQ625984	JQ626427	-	FJ039249	-	-	-	-
Lauraceae	*Ocotea whitei*	JQ594820	JQ589832	-	-	-	-	-	-
Octoknemaceae	*Octoknema* sp. *D. Nickrent 4560*	DQ790139	DQ790176	-	-	-	-	-	DQ790117
Oleaceae	*Olea europea*	DQ673304	AJ429335	-	-	-	AF130163	AJ236163	L49289
Resedaceae	*Oligomeris linifolia*	FJ212215	AY483240	FJ212193	DQ987039	-	AY483255	-	-
Penaeaceae	*Olinia emarginata*	AJ605089	JX970901	-	-	-	AJ605102	GQ497640	AM235487
Oncothecaceae	*Oncotheca balansae*	AJ131950	AJ429320	-	-	-	AJ429114	AJ235549	AF206976
Lowiaceae	*Orchidantha fimbriata*	L05456	AY952417	-	-	-	AY656109	AF168933	AF168865
Fabaceae	*Ormosia stipularis*	JQ626235	JX295882	-	-	-	-	-	-
Fabaceae	*Ormosia stipularis*	JQ626235	JX295882	-	-	-	-	-	-
Osmundaceae	*Osmunda regalis*	EF588705	HF585137	-	-	-	-	-	-
Melastomataceae	*Ossaea angustifolia*	-	-	-	-	-	GQ139324	-	-
Melastomataceae	*Ossaea angustifolia*	-	-	-	-	-	GQ139324	-	-
Melastomataceae	*Pachycentria constricta*	-	-	-	-	-	AF289381	-	-
Melastomataceae	*Pachycentria glauca*	-	-	-	-	-	AF289382	-	-
Melastomataceae	*Pachycentria pulverulenta*	-	-	-	-	-	AF289383	-	-
Paeoniaceae	*Paeonia suffruticosa*	AJ402982	JN712203	-	-	-	-	AF093389	JN712207
Rubiaceae	*Palicourea corymbifera*	-	-	AF149320	-	-	-	-	-
Rubiaceae	*Palicourea guianensis*	GQ981825	GQ982058	AY635552	AF152615	-	-	-	-
Araliaceae	*Panax japonicus*	D44580	AB088000	AY271918	-	JN700296	-	-	-
Pandaceae	*Panda oleosa*	AY663644	FJ670032	-	-	-	FJ670111	AY788242	AY788153
Pandanaceae	*Pandanus tectorius*	AY952439	AY952418	-	-	-	-	AF308043	AY952391
Paracryphiaceae	*Paracryphia alticola*	AJ402983	AJ429367	-	-	-	AJ429121	AJ419679	GQ497580
Celastraceae	*Parnassia palustris*	JF942903	KC475150	JF811095	DQ860576	-	EU002249	AJ235552	AY929353
Passifloraceae	*Passiflora quadrangularis*	L01940	FM179937	AY636107	AY636106	-	-	-	-
Paulowniaceae	*Paulownia tomentosa*	L36447	AF051997	-	-	-	L36406	AJ236174	AJ236039
Nitrariaceae	*Pegalum harmala*	DQ267164	AY177667	-	-	-	-	-	-
Tetrameristaceae	*Pelliciera rhizophorae*	AF206804	AJ429303	-	-	-	AY725871	AF209647	AF206983
Pennantiaceae	*Pennantia corymbosa*	AJ494842	AJ494844	EF635468	GQ984043	-	AJ312949	-	-
Malvaceae	*Pentace polyantha*	AJ233156	AY321183	-	-	-	AF111758	AJ233109	-

Pentadiplandraceae	*Pentadiplandra brazzeana*	U38533	AY483239	-	AY122463	-	AY483254	-	AF070972
Pentaphragmataceae	*Pentaphragma ellipiticum*	AJ419699	AJ429387	-	AJ430975	-	AF130183	AJ318980	-
Penthoraceae	*Penthorum sedoides*	L11197	EF179063	-	-	-	EF207474	-	-
Piperaceae	*Peperomia serpens*	AY572270	DQ212747	-	-	-	EU519667	AF093390	AY572314
Peraceae	*Pera bicolor*	AY794968	EF135578	-	-	-	AY425075	AY788244	AY674624
Cactaceae	*Pereskia aculeata*	M97888	AY875355	JF508526	HM041338	-	JF508673	AF209648	AF206986
Peridiscaceae	*Peridiscus lucidus*	AY380356	DQ411570	-	-	-	AY425076	AY372816	AY372815
Petenaeaceae	*Petenaea cordata*	FN677367	-	-	-	-	-	FN677366	-
Petermanniaceae	*Petermannia cirrosa*	AY465714	JQ435572	-	-	-	AY225001	AY465558	-
Hydrophyllaceae	*Phacelia campanularia*	KF158107	-	AF091188	KF158200	-	KF158038	-	-
Orchidaceae	*Phalaenopsis equestris*	AF074211	AF263677	AY273748	AY273651	AY389430	-	-	-
Poaceae	*Pharus latifolius*	AY357724	AF164388	-	-	-	GU222698	-	-
Achatocarpaceae	*Phaulothamnus spinescens*	M97887	AY514846	-	FJ405007	JQ407842	AY858610	HQ843259	HQ843433
Phellinaceae	*Phelline comosa*	X69748	-	-	-	-	AJ238342	AJ235557	-
Philesiaceae	*Philesia magellanica*	HQ901562	JN417369	-	-	-	AF276014	AY465551	-
Philydraceae	*Philydrum lanuginosum*	U41596	DQ401369	-	-	AY147734	U41622	AY147607	AY952390
Phrymaceae	*Phryma leptostachya*	U28881	AJ429341	-	-	-	AJ617586	HQ384710	HQ384688
Phyllanthaceae	*Phyllanthus talbotii*	KC514097	KC514101	KC414630	KC514099	-	-	-	-
Phyllanthaceae	*Phyllanthus urinaria*	JX664064	JX661958	-	-	-	JX662767	JX663817	-
Phyllonomaceae	*Phyllonoma laticuspis*	L11201	-	-	-	AF471727	AF130208	-	-
Poaceae	*Phyllostachys bambusoides*	AB088833	AB088805	-	-	-	KC020505	-	-
Physenaceae	*Physena madagascariensis*	-	-	-	KC479292	-	HQ843280	HQ843260	HQ843434
Phytolaccaceae	*Phytollaca americana*	M62567	JQ844139	DQ317076	-	-	AF130229	AF528855	AF094557
Apodanthaceae	*Pilostyles berteroi*	-	-	-	-	-	-	-	HM592263
Pinaceae	*Pinus sylvestris*	JF701589	AB084492				-	-	-
Piperaceae	*Piper aereum*	-	-	EF056220	-	-	-	-	-
Piperaceae	*Piper arboreum*	GQ981830	-	EF056223	EU519615	-	EU519704	-	-
Piperaceae	*Piper archeri*	-	-	AF275178	-	-	-	-	-
Piperaceae	*Piper begoniicolor*	-	-	EF056226	-	-	-	-	-

Piperaceae	*Piper biseriatum*	-	-	EF056230	-	-	-	-	-
Piperaceae	*Piper calcariformis*	-	-	EF056233	-	-	-	-	-
Piperaceae	*Piper caracasanum*	-	-	EF056238	-	-	-	-	-
Piperaceae	*Piper cenocladum*	JQ593202	JQ588636	EF056239	-	-	-	-	-
Piperaceae	*Piper cernuum*	-	-	EF056242	-	-	-	-	-
Piperaceae	*Piper cogolloi*	-	-	EF056244	-	-	-	-	-
Piperaceae	*Piper decumanum*	-	DQ882212	AF275203	-	-	-	-	-
Piperaceae	*Piper euryphyllum*	-	-	EF056250	-	-	-	-	-
Piperaceae	*Piper filistilum*	-	-	AF275155	-	-	-	-	-
Piperaceae	*Piper fimbriulatum*	-	-	EF056254	-	-	-	-	-
Piperaceae	*Piper gibbosum*	-	-	EF056256	-	-	-	-	-
Piperaceae	*Piper gigantifolium*	-	-	EF056259	-	-	-	-	-
Piperaceae	*Piper hebetifolium*	-	-	EF056262	-	-	-	-	-
Piperaceae	*Piper hispidum*	AY572249	DQ882219	AM901423	EU519811	-	EU519721	AY572273	AY572295
Piperaceae	*Piper imperiale – a*	JQ593213	JQ588642	EF056263	-	-	EU519732	-	-
Piperaceae	*Piper imperiale – b*	-	-	EF056264	-	-	-	-	-
Piperaceae	*Piper marsupiiferum*	-	-	EF056269	-	-	-	-	-
Piperaceae	*Piper maxonii*	-	-	EF056270	-	-	-	-	-
Piperaceae	*Piper melanocladum*	-	-	EF056271	-	-	-	-	-
Piperaceae	*Piper myrmecophilum*	-	-	AY572328	-	-	-	-	-
Piperaceae	*Piper nigrum*	EF591363	AB040153	-	-	-	EU519689	-	-
Piperaceae	*Piper obliquum*	-	-	EF056273	EU519807	-	EU519717	-	-
Piperaceae	*Piper obtusilimbum*	-	-	EF056278	-	-	-	-	-
Piperaceae	*Piper perareolatum*	-	-	EF056279	-	-	-	-	-
Piperaceae	*Piper pseudonobile*	-	-	EF056280	-	-	-	-	-
Piperaceae	*Piper sagittifolium*			EF056284			-	-	-
Piperaceae	*Piper subglabribracteatum*	-	-	AY326220	-	-	-	-	-
Pittosporaceae	*Pittosporum tobira*	HM850261	HQ619824	-	FJ490806	-	-	-	-
Platanaceae	*Platanus occidentalis*	AF081073	GU266600	-	-	-	EU642681	EU642741	U42794
Polypodiaceae	*Platycerium alcicorne*	DQ164445	-	-	DQ164509	-	-	-	-
Polypodiaceae	*Platycerium andinum*	DQ164446	-	-	DQ164510	-	-	-	-
Polypodiaceae	*Platycerium bifurcatum*	AF470341	-	-	DQ164511	-	-	-	-
Polypodiaceae	*Platycerium coronarium*	DQ164448	-	-	DQ164512	-	-	-	-

Polypodiaceae	*Platycerium elephantotis*	DQ164449	-	-	DQ164513	-	-	-	-
Polypodiaceae	*Platycerium ellisii*	DQ164450	-	-	DQ164514	-	-	-	-
Polypodiaceae	*Platycerium grande*	DQ164451	-	-	DQ164515	-	-	-	-
Polypodiaceae	*Platycerium hillii*	DQ164452	-	-	DQ164516	-	-	-	-
Polypodiaceae	*Platycerium holltumi*	DQ164453	-	-	DQ164517	-	-	-	-
Polypodiaceae	*Platycerium madagascariense*	DQ164454	-	-	DQ164518	-	-	-	-
Polypodiaceae	*Platycerium quadridichotomum*	DQ164455	-	-	DQ164519	-	-	-	-
Polypodiaceae	*Platycerium ridleyi*	DQ164456	-	-	DQ164520	-	-	-	-
Polypodiaceae	*Platycerium stemaria* var. *laurentii*	DQ164458	-	-	DQ164522	-	-	-	-
Polypodiaceae	*Platycerium stemaria* var. *stemaria*	DQ164457	-	-	DQ164521	-	-	-	-
Polypodiaceae	*Platycerium superbum*	DQ164459	-	-	DQ164523	-	-	-	-
Polypodiaceae	*Platycerium veitchii*	DQ164460	-	-	DQ164524	-	-	-	-
Polypodiaceae	*Platycerium wallichii*	DQ164461	-	-	DQ164525	-	-	-	-
Polypodiaceae	*Platycerium wandae*	DQ164462	-	-	DQ164526	-	-	-	-
Polypodiaceae	*Platycerium willinckii*	DQ164463	-	-	DQ164527	-	-	-	-
Fabaceae	*Platymiscium albertinae*	-	EU735932	EU735870	EU735989	-	-	-	-
Fabaceae	*Platymiscium calyptratum*	-	EU735933	EU735872	EU735990	-	-	-	-
Fabaceae	*Platymiscium curuense*	-	EU735934	EU735873	EU735991	-	-	-	-
Fabaceae	*Platymiscium darienense*	-	EU735935	EU735874	-	-	-	-	-
Fabaceae	*Platymiscium dimorphandrum*	-	EU735937	EU735878	EU736052	-	-	-	-
Fabaceae	*Platymiscium filipes*	-	EU735940	EU735879	EU735996	-	-	-	-
Fabaceae	*Platymiscium floribundum* var. *floribundum*	-	EU735941	EU735880	EU736054	-	-	-	-
Fabaceae	*Platymiscium floribundum* var. *latifolium*	-	EU735942	EU735881	EU736055	-	-	-	-
Fabaceae	*Platymiscium floribundum* var. *nitens*	-	EU735943	EU735882	EU736056	-	-	-	-

Fabaceae	*Platymiscium floribundum* var. *obtusifolium*	-	EU735946	EU736059	EU735885	-	-	-	-
Fabaceae	*Platymiscium gracile*	-	EU735947	EU735886	EU736003	-	-	-	-
Fabaceae	*Platymiscium hebestachyum*	-	EU735948	EU735887	EU736004	-	-	-	-
Fabaceae	*Platymiscium jejunum*	-	EU735949	EU735888	EU736005	-	-	-	-
Fabaceae	*Platymiscium lasiocarpum*	-	EU735950	EU735889	EU736006	-	-	-	-
Fabaceae	*Platymiscium parviflorum*	JQ591985	JQ587818	EU735895	EU736011	-	-	-	-
Fabaceae	*Platymiscium pinnatum*	JQ626063	JQ626473	EU735909	EU736075	-	-	-	-
Fabaceae	*Platymiscium pinnatum* subsp. *polystachyum*	-	EU735963	EU735906	EU736021	-	-	-	-
Fabaceae	*Platymiscium pinnatum* var. *diadelphum*	-	EU735957	EU735898	EU736014	-	-	-	-
Fabaceae	*Platymiscium pinnatum var. pinnatum*	-	EU735959	EU735900	EU736016	-	-	-	-
Fabaceae	*Platymiscium pinnatum* var. *ulei*	-	EU735961	EU735902	EU736018	-	-	-	-
Fabaceae	*Platymiscium pubescens* subsp. *fragrans*	JN083775	EU735968	EU735910	EU736025	-	-	-	-
Fabaceae	*Platymiscium pubescens* subsp. *pubescens*	-	EU735971	EU735914	EU736029	-	-	-	-
Fabaceae	*Platymiscium pubescens* subsp. *zehntneri*	-	EU735974	EU735916	EU736031	-	-	-	-
Fabaceae	*Platymiscium* sp. Mostacedo & Mostacedo 3283	-	EU735975	EU735917	EU736032	-	-	-	-
Fabaceae	*Platymiscium* sp. *Nee 41760*	-	EU735976	EU735918	EU736033	-	-	-	-
Fabaceae	*Platymiscium* sp. *Pennington 761*	-	EU735977	EU735919	EU736034	-	-	-	-
Fabaceae	*Platymiscium speciosum*	-	EU735978	EU735920	EU736035	-	-	-	-

Fabaceae	*Platymiscium stipulare*	-	EU735980	EU735921	EU736036	-	-	-	-
Fabaceae	*Platymiscium trifoliolatum*	KF436469	EU735981	EU735923	EU736038	-	-	-	-
Fabaceae	*Platymiscium trinitatis var. duckei*	-	EU735983	EU735925	EU736040	-	-	-	-
Fabaceae	*Platymiscium trinitatis var. nigrum*	-	-	EU735927	EU736042	-	-	-	-
Fabaceae	*Platymiscium trinitatis var. trinitatis*	-	EU735986	EU735928	EU736043	-	-	-	-
Fabaceae	*Platymiscium yucatanum*	-	EU735988	EU735930	EU736045	-	-	-	-
Lauraceae	*Pleurothyrium costanense*	-	AJ247185	-	-	-	-	-	-
Lauraceae	*Pleurothyrium costanense*	-	AJ247185	-	-	-	-	-	-
Plocospermataceae	*Plocosperma buxifolium*	HQ384904	AJ429315	-	-	-	AJ011985	HQ384756	HQ384684
Plumbaginaceae	*Plumbago auriculata*	EU002283	EU002187	JF831220	JF831319	-	EU002252	EU002166	U42795
Poaceae	*Poa pratensis*	JX848506	AF164402	-	-	-	U21980	-	-
Picrodendraceae	*Podocalyx loranthoides* Klotzsch	JX664066	JX661960	-	-	-	JX662769	AY788248	AY674629
Podocarpaceae	*Podocarpus macrophyllus*	AF249616	AF228111	-	-	-	AF469699	AF469661	-
Berberidaceae	*Podophyllum peltatum*	AF093716	AB069843	-	-	-	AY145155	AF092109	L24413
Podostemaceae	*Podostemum ceratophyllum*	HM470438	AB038201	-	-	-	JX662770	JX663820	-
Arecaceae	*Pogonotium ursinum*	AJ829901	-	AJ242079	-	-	EU186198	-	-
Urticaceae	*Poikilospermum lanceolatum*	KF138231	KF138053	KF137912	DQ179374	-	-	-	KF137786
Urticaceae	*Poikilospermum suaveolens*	KF138233	KF138054	KF137914	FJ432260	-	-	-	-
Polygalaceae	*Polygala myrtifolia*	AJ829699	EU604043	-	-	-	-	-	-
Polygonaceae	*Polygonum aviculare*	EF653761	HM357913	HM357902	EF653787	JN234937	EF438059	-	-
Polypodiaceae	*Polypodium plebeium*	EU650116	-	-	-	-	-	-	-
Tetrachondraceae	*Polypremum procumbens*	AJ011989	AJ429351	-	-	-	AJ011986	HQ384749	-
Sapindaceae	*Pometia pinnata*	FJ976161	EU720638	EU720471	EU721154	-	-	-	-
Sapindaceae	*Pometia tomentosa*	-	AF314802	-	-	-	-	-	-
Portulacaceae	*Portulaca oleacera*	AY875249	AY875349	GQ478103	-	-	HQ620949	HQ620749	HQ843437

Posidoniaceae	*Posidonia oceanica*	U80719	GQ927729	GQ927725	GQ927752	-	-	-	-
Potamogetonaceae	*Potamogeton distinctus*	AB004901	AB088780	DQ840282	AB871508	AB871483	-	-	-
Rapateaceae	*Potarophytum riparium*	HQ182446	HQ180882	-	-	-	AF207627	HQ180475	-
Urticaceae	*Pourouma bicolor*	JQ626107	FJ514767	FJ037804	FJ039334	-	-	-	-
Urticaceae	*Pourouma melinonii*	JQ625760	JQ626349	-	-	-	-	-	-
Urticaceae	*Pourouma minor*	JQ625720	JQ589400	-	-	-	-	-	-
Urticaceae	*Pourouma tomentosa*	JQ626115	FJ514760	-	FJ039338	-	-	-	-
Urticaceae	*Pourouma villosa*	JQ626293	JQ626574	-	-	-	-	-	-
Primulaceae-Primuloideae	*Primula veitchiana*	AF213802	-	-	-	-	AF213759	AF213788	-
Thurniaceae	*Prionium serratum*	U49223	-	-	-	-	EU832896	EU832861	-
Fabaceae	*Prosopis laevigata*	-	HM020742	-	HM020840	-	-	-	-
Rosaceae	*Prunus persica*	AF411493	AF288117	-	-	-	JQ776897	AF209660	L28749
Rubiaceae	*Psychotria ficigemma*	JQ625868	JQ626366	-	-	-	-	-	-
Rubiaceae	*Psydrax paradoxa*	-	-	-	EF205634	-	-	-	-
Rubiaceae	*Psydrax parviflora*	-	-	JQ957999	JQ958218	-	-	-	-
Dennstaedtiaceae	*Pteridium aquilinum*	AY300097	EU223824	-	-	-	-	U93835	U18628
Pteridaceae	*Pteris fauriei*	U05647	JF303919	-	-	-	-	-	-
Fabaceae	*Pterocarpus rohrii*	JN083747	JN083564	EF451061	EF451101	-	-	-	-
Fabaceae	*Pterocarpus santalinoides*	JN083756	JN083571	JN083514	JN083681	-	-	-	-
Pterostemonaceae	*Pterostemon rotundifolius*	L11203	AF274630	-	-	-	EF207475	AJ235573	U42547
Poaceae	*Puelia olyriformis*	HQ604036	HQ604000	-	-	-	HQ604006	HQ603992	-
Putranjiviaceae	*Putranjiva roxburghii*	JX664068	EF135530	-	-	-	JX662771	AF209578	U42534
Polypodiaceae	*Pyrrosia assimilis*	DQ164464	-	-	DQ164528	-	-	-	-
Polypodiaceae	*Pyrrosia lingua*	AF470343	-	-	AY083646	-	-	-	-
Polypodiaceae	*Pyrrosia serpens*	EF463260	-	-	DQ164535	-	-	-	-
Vochysiaceae	*Qualea albiflora*	JQ626202	JQ626501	-	-	-	AM235431	-	AM235539
Fagaceae	*Quercus rubra*	M58391	AB125043	-	-	-	AF130226	AF209663	AF132892
Quillajaceae	*Quillaja saponaria*	U06822	AY386843	-	-	-	EU002255	GQ497659	-
Rafflesiaceae	*Rafflesia keithii*	-	-	-	-	-	-	-	AY739084
Rubiaceae	*Randia aculeata*	Z68832	HM119563	-	-	-	HM164390	-	-
Rhabdodendraceae	*Rhabdodendron*	Z97649	JQ844136	EU410359	-	-	HQ843284	AJ235578	AF207007

	amazonicum								
Rhamnaceae	*Rhamnus cathartica*	L13189	AY257533	-	-	-	-	AJ235579	AJ235979
Rhipogonaceae	*Rhipogonum elseyanum*	GQ497672	JN417372	-	-	-	AF276016	AF168941	GQ497583
Cactaceae	*Rhipsalis baccifera*	FR853379	FN669738	HQ727784	HM041350	-	-	-	-
Rhoipteleaceae	*Rhoiptelea chiliantha*	AF017687	-	-	-	-	-	-	-
Sphaerosepalaceae	*Rhopalocarpus lucidus*	AF022130	-	-	-	-	AF111780	-	-
Grossulariaceae	*Ribes fasciculatum* var. *chinense*	KC737394	KC737242	-	-	-	KC737259	-	-
Roridaceae	*Roridula gorgonias*	L01950	AJ429294	-	-	-	AJ236270	AJ236180	AF207010
Lamiaceae	*Rotheca incisa*	-	-	U77750	-	-	L49158	-	-
Rubiaceae	*Rothmannia capensis*	AM117266	JX517592	-	-	-	HM164394	-	-
Connaraceae	*Rourea minor*	FJ707537	EF135591	-	-	-	FJ670137	FJ669994	EF135603
Rousseaceae	*Roussea simplex*	AJ235792	AJ429389	-	AJ430977	-	-	AJ235586	U42548
Polygonaceae	*Ruprechtia aperta*	-	-	HQ693104	-	-	HQ693215	-	-
Polygonaceae	*Ruprechtia apetala*	-	FN597637	HQ693105	-	-	HQ693216	-	-
Polygonaceae	*Ruprechtia chiapensis*	FJ154456	FJ154495	AY256524	-	-	FJ154506	-	-
Polygonaceae	*Ruprechtia coriacea*	HM137381	AY042648	HM137442	-	-	HM137423	-	-
Polygonaceae	*Ruprechtia costaricensis*	-	-	HQ693106	-	-	HQ693217	-	-
Polygonaceae	*Ruprechtia costata*	JQ593537	JQ588854	HQ693107	-	-	HQ693218	-	-
Polygonaceae	*Ruprechtia cruegeri*	HM137382	HM137402	AY256549	-	-	HM137424	-	-
Polygonaceae	*Ruprechtia fagifolia*	-	HQ693203	AY256536	-	-	HQ693219	-	-
Polygonaceae	*Ruprechtia fusca*	FJ154457	FJ154496	AY256529	-	-	FJ154507	-	-
Polygonaceae	*Ruprechtia laevigata*	-	-	AY256525	-	-	HQ693220	-	-
Polygonaceae	*Ruprechtia latifunda*	-	-	HQ693110	-	-	-	-	-
Polygonaceae	*Ruprechtia laxiflora*	EF437987	EF438024	HM137444	-	-	EF438063	-	-
Polygonaceae	*Ruprechtia lundii*	-	-	HQ693111	-	-	HQ693221	-	-
Polygonaceae	*Ruprechtia nicaraguensis*	-	-	HQ693112	-	-	HQ693222	-	-
Polygonaceae	*Ruprechtia obovata*	-	HQ693208	HQ693113	-	-	HQ693223	-	-
Polygonaceae	*Ruprechtia pallida*	HM137383	HM137403	JQ352581	-	-	HM137425	-	-
Polygonaceae	*Ruprechtia tangarana*	GQ206233	-	AY256543	-	-	-	-	-
Lepidobotryaceae	*Ruptiliocarpon caracolito*	AJ402997	AY935918	-	FJ670055	-	FJ670153	AY788275	AY929361
Achariaceae	*Ryparosa javanica*	AJ418802	-	-	-	-	-	-	-
Sabiaceae	*Sabia swinhoei*	FJ626616	GU266603	-	-	-	AJ236276	AF093395	L75840

Alismataceae	*Sagittaria trifolia*	JF944158	JF781083	AY395991	-	EU247785	-	-	-
Gesneriaceae	*Saintpaulia ionantha*	HQ384898	-	-	-	-	HQ384841	HQ384744	-
Salicaceae	*Salix reticulata*	AJ235793	EF135592	-	-	-	AY425082	AJ235590	AF207011
Polygonaceae	*Salta triflora*	GQ206234	-	AY256560	-	-	GQ206299	-	-
Salvadoraceae	*Salvadora angustifolia*	U38532	-	-	KC479309	-	-	-	GQ497584
Santalaceae	*Santalum macgregorii*	EF584607	EF584631	-	-	-	-	-	EF584585
Euphorbiaceae	*Sapium ellipticum*	KC628624	JX517498	-	-	-	-	-	-
Fabaceae	*Saraca thaipingensis*	-	-	-	AF365155	-	-	-	-
Chloranthaceae	*Sarcandra glabra*	HQ336522	AF543733	JN407441	AF329948	HE651087	-	AF092114	AF094536
Sarcobataceae	*Sarcobatus vermiculatus*	AF132088	JQ844135	EF079501	-	-	HQ843286	GQ497660	GQ497586
Sarcolaenaceae	*Sarcolaena oblongifolia*	U26337	-	-	-	-	-	-	-
Sarraceniaceae	*Sarracenia flava*	L01952	JQ619000	-	-	-	-	AJ235594	-
Saururaceae	*Saururus chinensis*	AB205611	DQ212713	-	-	-	-	AJ235596	D29782
Asteraceae	*Saussurea involucrata*	GQ436481	AB118148	FJ980351	AY328115	-	AY466424	-	-
Goodeniaceae	*Scaevola aemula*	EU017199	EU385394	AY102728	JQ682795	-	EU017145	EU017162	AJ236008
Malvaceae	*Scaphopetalum amoenum*	-	-	-	-	-	AF287925	-	-
Malvaceae	*Scaphopetalum thonneri*	-	-	-	-	-	AF287937	-	-
Scheuchzeriaceae	*Scheuchzeria palustris*	U03728	GQ452338	DQ786414	-	-	AF547007	-	-
Illiciaceae	*Schisandra chinensis*	AF238061	DQ185526	AB558158	DQ342262	-	AF238062	AF239790	AF094561
Schlegeliaceae	*Schlegelia fuscata*	HQ384880	HQ384514	-	-	-	HQ384828	HQ384718	-
Schoepfiaceae	*Schoepfia schreberi*	L11205	DQ787447	-	-	-	-	AF209671	AF207017
Sciadopityaceae	*Sciadopitys verticillata*	AB645804	AB023994	-	-	-	AF469700	AB645771	D85292
Triuridaceae	*Sciaphila* sp. *Dransfield 7345*	FN870930	-	-	-	-	-	-	-
Apocynaceae	*Secamone elliptica*	DQ660665	DQ660541	-	-	-	-	-	-
Selaginellaceae	*Selaginella doederleinii*	AB574643	AY826400	-	-	-	-	-	-
Pedaliaceae	*Sesamum indicum*	HQ384882	AJ429340	-	-	-	L36413	AJ236176	AJ236041
Setchellanthaceae	*Setchellanthus caeruleus*	U41455	-	-	KC778758	-	AF209674	-	-
Elaeagnaceae	*Shepherdia canadensis*	U17039	KC475874	-	-	-	-	AF209675	AF207020
Dipterocarpaceae	*Shorea acuminata*	-	AB246440	-	AB368855	-	-	-	-
Caryophyllaceae	*Silene vulgaris*	EF646883	EF547245	AY594309	FN821374	-	DQ841746	-	HM562728
Simmondsiaceae	*Simmondsia chinensis*	AF093732	AY514854	-	-	-	-	AF093401	AF094562
Adoxaceae	*Sinadoxa corydalifolia*	AF446929	AF446899	AF248611	AF366926	AF446989	AF447019	GQ983638	-

Siparunaceae	*Siparuna decipiens*	JQ626097	AJ966808	-	-	-	-	-	DQ007411
Sladeniaceae	*Sladenia celastrifolia*	AF320784	AJ429297	-	-	-	AF421081	AF420988	AF320782
Elaeocarpaceae	*Sloanea berteroana*	HM446872	FJ670047	-	-	-	FJ670140	AJ235603	-
Smilacaceae	*Smilax aspera*	GU945049	GU945042	JF978644	GU945057	-	-	-	-
Smilacaceae	*Smilax lasioneura*	GU945055	JF461392	AY775251	GU945069	-	-	-	-
Smilacaceae	*Smilax ocreata*	GQ436283	KC511369	JF978757	-	-	-	-	-
Smilacaceae	*Smilax perfoliata*	JF944423	JF461403	JF978763	-	-	-	-	-
Solanaceae	*Solanum tuberosum*	M76402	EF438886	-	-	-	L76287	AM233351	-
Fabaceae	*Spatholobus parviflorus*	AB045825	-	-	-	-	-	-	-
Sphenocleaceae	*Sphenoclea zeylanica*	L18798	AJ429360	-	-	-	AJ429119	GQ497656	GQ497585
Rubiaceae	*Squamellaria imberbis*	-	-	KF675993	KF676258	-	KF676079	-	-
Stachyuraceae	*Stachyurus praecox*	AJ235794	DQ443457	DQ307115	DQ307147	-	DQ307081	AJ235609	AF207025
Staphyleaceae	*Staphylea colchica*	EU002285	EU002189	-	-	-	EU002261	EU002168	-
Monimiaceae	*Steganthera hirsuta*	AF121368	-	-	-	-	-	-	-
Stegnospermataceae	*Stegnosperma halimifolium*	M62571	HQ878442	-	-	-	HQ843287	-	-
Stemonaceae	*Stemona japonica*	AB088826	AB040210	-	-	-	AF547009	AF308037	AF207028
Proteaceae	*Stenocarpus sinuatus*	U79174	EU169665	-	-	-	JQ257235	JQ257304	-
Bignonaceae	*Stereospermum chelonoides*	-	-	KF199892	-	-	-	-	-
Bignonaceae	*Stereospermum euphorioides*	-	-	-	AY500409	-	AY500453	-	-
Bignonaceae	*Stereospermum kunthianum*	JX572995	JX517630	-	-	-	-	-	-
Bignonaceae	*Stereospermum nematocarpum*	JQ933491	-	-	EF105085	-	EF105025	-	-
Bignonaceae	*Stereospermum tetragonum*	-	-	KF199893	-	-	-	-	-
Asteraceae	*Stevia rebaudiana*	AY215182	AY215865	AB457301	AY215991	-	-	-	-
Asteraceae	*Stifftia chrysantha*	EU385020	EU385399	-	JF920296	-	EU385208	-	-
Strasburgeria	*Strasburgeria robusta*	AJ403007	HQ680701	-	-	-	EU002262	AF502597	AF502596
Strelitziaceae	*Strelitzia reginae*	AM235157	JQ027251	-	-	-	AY465646	AY465540	-
Strombosiaceae	*Strombosia grandifolia*	DQ790156	DQ790192	-	-	-	-	-	DQ790123
Loganiaceae	*Strychnos minor*	DQ660669	AB636279	-	-	-	-	-	-

Styracaceae	*Styrax officinalis*	EU980810	DQ924099	-	-	-	AF421084	AF420984	-
Surianaceae	*Suriana maritima*	U07680	AY386950	-	-	-	EU002264	-	-
Fabaceae	*Swainsona phacoides*	-	-	GQ246028	-	-	-	-	-
Meliaceae	*Swietenia macrophylla*	AY128241	EF489114	-	-	-	EU002265	AF066857	AF207031
Polygonaceae	*Symmeria paniculata*	GQ206235	GQ206209	-	-	-	GQ206300	-	-
Symplocaceae	*Symplocos paniculata*	L12624	AF440433	-	-	-	-	-	U43297
Myrtaceae	*Syzygium cormiflorum*	-	DQ088572	AY187184	KC428617	-	DQ088494	-	-
Myrtaceae	*Syzygium cumini*	GU135161	GU135062	JN115051	JF804935	-	AY498814	-	-
Myrtaceae	*Syzygium erythrocalyx*	-	DQ088576	AY187187	-	-	-	-	-
Myrtaceae	*Syzygium jambos*	JX856783	DQ088583	KC815991	-	-	DQ088502	-	-
Taccaceae	*Tacca chantrieri*	AJ235810	AY973837	JF978861	FJ194472	JQ733659	AY007659	AF308025	DQ786086
Fabaceae	*Tachigali bracteolata*	JQ625782	-	-	-	-	-	-	-
Fabaceae	*Tachigali guianensis*	JQ626001	-	-	-	-	-	-	-
Fabaceae	*Tachigali melinonii*	JQ626276	-	-	-	-	-	-	-
Fabaceae	*Tachigali myrmecophila*	AY904394			AY899706	-	-	-	-
Fabaceae	*Tachigali paniculata*	JQ625944	-	-	AF430790	-	-	-	-
Fabaceae	*Tachigali paniculata*	JQ625944	-	-	AF430790	-	-	-	-
Fabaceae	*Tachigali paraensis*	JQ625892	-	-	-	-	-	-	-
Fabaceae	*Tachigali* sp. *Clarke 7212*	-	-	-	AF365113	-	-	-	-
Fabaceae	*Tachigali* sp. *Klitgaard 687*	AM234242	-	-	AF365111	-	-	-	-
Fabaceae	*Tachigali versicolor*	GQ981893	-	-	-	-	-	-	-
Winteraceae	*Takhtajania perrieri*	AY572264	DQ401371	AY004129	AY004146	-	-	AY572287	AF207032
Talinaceae	*Talinum paniculatum*	AY875214	AY015274	EU410357	-	-	AF194830	HQ843263	HQ843439
Tamaricaceae	*Tamarix aphylla*	KC505173	KC840667	JN115052	-	-	-	-	AY099936
Tapisciaceae	*Tapiscia sinensis*	AF206825	FM179925	-	-	-	EU002266	AF209685	AF207034
Taxaceae	*Taxus baccata*	AF456388	AF457109	-	-	-	-	AJ235619	AB107896
Bignoniaceae	*Tecoma capensis*	HM850394	HM850767	-	-	-	DQ222642	-	-
Tecophilaeaceae	*Tecophilaea cyanocrocus*	Z73709	HM640661	-	AJ290276	KC161457	JX903500	AJ235620	HM640781
Lamiaceae	*Teijsmanniodendron bogoriense*	JF738613	-	FM200106	-	-	FM200148	-	-
Lamiaceae	*Teijsmanniodendron pteropodum*	-	-	FM200107	-	-	FM200149	-	-
Combretaceae	*Terminalia catappa*	U26338	GU135057	-	-	-	EU002267	AF209686	AF207037

Pentaphylacaceae	*Ternstroemia gymnanthera*	AF380064	AF380109	-	-	-	AF421076	HQ437935	-
Tetracarpaeaceae	*Tetracarpaea tasmanica*	L11207	L34154	-	-	-	EF207481	AF209688	U42549
Tetrameleaceae	*Tetrameles nudiflora*	L21943	AY968458	AF280105	AY091831	-	AY968511	AF209689	U41502
Salicaceae	*Tetrathylacium macrophyllum*	-	-	-	AY756910	-	AY757192	-	-
Salicaceae	*Tetrathylacium macrophyllum*	-	-	-	AY756910	-	AY757192	-	-
Malvaceae	*Theobroma cacao*	AF022125	AY321195	-	-	-	AF287916	AJ233090	AF207040
Theophrastoideae-Primulaceae	*Theophrasta americana*	AF213819	AJ429307	-	-	-	AF213762	AF213792	-
Thismiaceae	*Thismia rodwayi*	AY939892	FR832844	-	-	-	-	AF308034	KF692536
Thomandersiaceae	*Thomandersia laurifolia*	AY919280	HQ384515	-	-	-	HQ384829	HQ384719	-
Acanthaceae	*Thunbergia alata*	HQ384878	AF531811	-	-	-	U12667	HQ384716	AF107569
Thymelaeaceae	*Thymelaea hirsuta*	Y15151	EU002191	-	-	-	-	-	AF207041
Melastomataceae	*Tibouchina longifolia*	JQ592706	-	JQ730204	-	-	AF215572	-	-
Melastomataceae	*Tibouchina urvilleana*	U26339	-	FJ628141	GQ465904	-	AF272820	-	-
Ticodendraceae	*Ticodendron incognitum*	AF061197	U92855	-	-	-	-	AY147103	AY147110
Malvaceae	*Tilia americana*	AF022127	AY321191	-	-	-	AF111760	GU981686	AF207042
Bromeliaceae	*Tillandsia achyrostachys*	FM211062	FM210788	FJ666959	FM211662	FM210799	-	-	-
Bromeliaceae	*Tillandsia baileyi*	-	-	EU126835	-	-	-	-	-
Bromeliaceae	*Tillandsia balbisiana*	-	-	FJ666960	-	-	-	-	-
Bromeliaceae	*Tillandsia botteri*	-	-	DQ870646	-	-	-	-	-
Bromeliaceae	*Tillandsia bulbosa*	JN202174	JN202271	FJ666943	-	-	-	-	-
Bromeliaceae	*Tillandsia butzii*	-	-	-	-	-	-	-	-
Bromeliaceae	*Tillandsia caput-medusae*	AY614464	AY614098	FJ666955	AY614342	-	-	-	-
Bromeliaceae	*Tillandsia concolor*	-	-	EU126837	-	-	-	-	-
Bromeliaceae	*Tillandsia eizii*	-	-	FJ666947	-	-	-	-	-
Bromeliaceae	*Tillandsia erubescens*	-	FM956447	FJ666953	-	-		-	-
Bromeliaceae	*Tillandsia filifolia*	-	FM956446	FJ666944	-	-	-	-	-
Bromeliaceae	*Tillandsia flabellata*	-	-	FJ666949	-	-	-	-	-
Bromeliaceae	*Tillandsia heterophylla*	AY614477	AY614111	FJ666946	AY614355	-	-	-	-
Bromeliaceae	*Tillandsia ionantha*	AY614465	AY614099	FJ666951	AY614343	-	-	-	-

Bromeliaceae	*Tillandsia juncea*	AY614463	FM956443	EU126836	AY614341	-	-	-	-
Bromeliaceae	*Tillandsia magnusiana*	-	-	FJ666963	-	-	-	-	-
Bromeliaceae	*Tillandsia makoyana*	-	FM956439	FJ666957	-	-	-	-	-
Bromeliaceae	*Tillandsia palmasolana*	-	-	DQ870649	-	-	-	-	-
Bromeliaceae	*Tillandsia paucifolia*	FN550874	FN550871	DQ870648	FN550872	FN550870	-	-	-
Bromeliaceae	*Tillandsia paucifolia*	FN550874	FN550871	DQ870648	FN550872	FN550870	-	-	-
Bromeliaceae	*Tillandsia pruinosa*	-	-	EU126838	-	-	-	-	-
Bromeliaceae	*Tillandsia pseudobaileyi*	-	-	DQ870651	-	-	-	-	-
Bromeliaceae	*Tillandsia punctulata*	AY614453	AY614087	FJ666950	AY614331	-	-	-	-
Bromeliaceae	*Tillandsia schiedeana*	-	FM956434	DQ870647	-	-	-	-	-
Bromeliaceae	*Tillandsia streptophylla*	-	-	DQ870650	-	-	-	-	-
Boraginaceae	*Tiquilia hispidissima*	-	DQ197240	DQ197527	KF673285	-	DQ197268	-	-
Melastomataceae	*Tococa bolivarensis*	-	-	AY460547	-	-	-	-	-
Melastomataceae	*Tococa broadwayi*	-	-	AY460548	-	-	EU056134	-	-
Melastomataceae	*Tococa capitata*	-	-	AY460549	-	-	-	-	-
Melastomataceae	*Tococa caquetana*	-	-	AY460550	-	-	EU056135	-	-
Melastomataceae	*Tococa caudata*	-	-	AY460551	-	-	-	-	-
Melastomataceae	*Tococa coronata*	-	-	AY460552	-	-	-	-	-
Melastomataceae	*Tococa discolor*	-	-	EU055895	-	-	-	-	-
Melastomataceae	*Tococa filiformis*	JQ899106	-	-	-	-	JQ899131	-	-
Melastomataceae	*Tococa gonoptera*	-	-	AY460553	-	-	-	-	-
Melastomataceae	*Tococa guianensis*	AM235650	-	AY460554	-	-	AY498834	-	AM235525
Melastomataceae	*Tococa macrophysca*	-	-	AY460555	-	-	-	-	-
Melastomataceae	*Tococa macrosperma*	-	-	AY460556	-	-	-	-	-
Melastomataceae	*Tococa nitens*	-	-	AY460557	-	-	-	-	-
Melastomataceae	*Tococa perclara*	-	-	AY460558	-	-	EU056137	-	-
Melastomataceae	*Tococa platyphylla*	-	-	EU055896	-	-	EU056138	-	-
Melastomataceae	*Tococa quadrialata*	-	-	EF418922	-	-	-	-	-
Melastomataceae	*Tococa raggiana*	-	-	AY460559	-	-	-	-	-
Melastomataceae	*Tococa rotundifolia*	AF215539	-	AY460560	-	-	-	-	-
Melastomataceae	*Tococa spadiciflora*	-	-	EU055897	-	-	EU056139	-	-
Melastomataceae	*Tococa subciliata*	-	-	AY460561	-	-	-	-	-

Tofieldaceae	*Tofieldia coccinea*	HQ901585	AB746435	AB746449	AB451586	-	HQ901558	-	-
Melastomataceae	*Topobea gracilis*	-	-	AY460443	-	-	-	-	-
Melastomataceae	*Topobea hexandra*	-	-	AY460444	-	-	-	-	-
Torricelliaceae	*Torricellia tiliifolia*	AF299089	AJ429375	-	AJ430961	-	AJ429127	-	-
Tovariaceae	*Tovaria pendula*	M95758	AY483242	FJ212196	AY122465	-	AY122407	-	-
Commelinaceae	*Tradescanthia ohiensis*	HQ182454	HQ180889	-	KC512074	-	HQ181138	AF168950	AF069213
Rubiaceae	*Tricalysia sonderiana*	HM164184	JX517841	-	-	-	HM164401	-	-
Juncaginaceae	*Triglochin maritimum*	U80714	AB088782	HQ456455	GQ245623	-	AF546998	-	-
Trigoniaceae	*Trigonia nivea*	AF206830	EF135598	-	-	-	AY425084	AF209691	AF207047
Trimeniaceae	*Trimenia moorei*	AY116658	DQ401360	-	-	-	AY116655	AY116653	-
Dioncophyllaceae	*Triphyophyllum peltatum*	Z97637	AF315940	HM204913	-	-	-	-	AF207049
Polygonaceae	*Triplaris americana*	Y16910	AY042668	FJ154486	AJ312251	-	FJ154508	-	-
Polygonaceae	*Triplaris cumingiana*	GQ981906	GQ206210	GQ206269	-	-	GQ206301	-	-
Polygonaceae	*Triplaris longifolia*	-	-	HQ693114	-	-	HQ693224	-	-
Polygonaceae	*Triplaris melaenodendron*	JQ593542	JQ588857	HQ693115	-	-	HQ693225	-	-
Polygonaceae	*Triplaris peruviana*	-	-	HQ693116	-	-	HQ693226	-	-
Polygonaceae	*Triplaris poeppigana*	AF297137	FJ154497	FJ154487	-	-	FJ154509	-	-
Polygonaceae	*Triplaris purdiei*	-	-	HQ693117	-	-	HQ693227	-	-
Polygonaceae	*Triplaris setosa*	FJ154458	FJ154498	FJ154488	-	-	FJ154510	-	-
Polygonaceae	*Triplaris weigeltiana*	HM137384	HM137405	HM137446	-	-	HM137426	-	-
Hydatellaceae	*Trithuria cowieana*	JQ284224	JQ284111	-	-	-	JQ284074	JQ284187	-
Trochodendraceae	*Trochodendron aralioides*	L01958	GQ998807	-	-	-	EU002269	EU002169	DQ008738
Tropaeolaceae	*Tropaeolum majus*	L14706	FM179931	JN115053	AB043665	-	AJ236281	-	L28750
Liliaceae	*Tulipa kolpakowskiana*	Z77292	HF953073	-	-	-	-	AJ235633	-
Typhaceae	*Typha latifolia*	DQ069503	DQ069587	-	-	-	-	AF168951	AF168880
Phyllanthaceae	*Uapaca guineensis*	AY830390	AY830287	-	-	-	AY830356	-	-
Phyllanthaceae	*Uapaca staudtii*	KC628129	-	-	-	-	-	-	-
Ulmaceae	*Ulmus macrocarpa*	JF317495	JF317435	-	-	-	JF317455	JF317416	JF317377
Rubiaceae	*Uncaria africana*	AJ347006	-	AJ414545	AJ346951	-		-	-
Rubiaceae	*Uncaria lancifolia*	KC737740	-	KF881264	KC737836	-	KC737687	-	-
Lentibulariaceae	*Utricularia biflora*	L13190	-	-	-	-	-	AJ235636	-

Ericaceae	*Vaccinium macrocarpon*	L12625	U61316	-	-	-	AF419754	-	AF419808
Fabaceae	*Vachellia melanoceras*	GQ981912	GQ982124	-	-	-	-	-	-
Vahliaceae	*Vahlia capensis*	L11208	AJ429316	-	-	-	AJ429112	AJ236217	U42813
Caprifoliaceae	*Valeriana arborea*	AY362484	AY362526	AY360096	AY360116	AY362505	AY362463	-	-
Orchidaceae	*Vanilla planifolia*	JN005701	AJ310079	AF391786	AY557223	-	-	-	-
Melanthiaceae	*Veratrum oxysepalum*	JN417478	JN417387	-	-	-	-	-	-
Verbenaceae	*Verbena officinalis*	HM850444	HM853866	-	-	-	HM216789	-	-
Piperaceae	*Verhuellia lunaria*	KC862247	-	-	-	-	-	KC862244	KC862240
Fabaceae-Faboideae	*Vicia faba*	JN661200	JX505862	-	-	-	M36832	-	AJ851227
Apocynaceae	*Vinca minor*	HQ384908	DQ660553	-	-	-	HQ384848	HQ384764	-
Violaceae	*Viola pubescens*	JX664075	JX661966	-	-	-	JX662778	FJ669992	FJ669717
Hypericaceae	*Vismia baccifera* subsp. *ferruginea*	JX664076	JX661967	-	-	-	JX662779	JX663828	-
Lamiaceae	*Vitex agnus-castus*	U78716	AB284182	-	-	-	U78707	HQ384699	-
Vitaceae	*Vitis vinifera*	AJ635355	AJ429274	-	-	-	AJ429103	AM083947	GQ849399
Vivianiaceae	*Viviania marifolia*	L14707	-	-	-	-	EU002272	AF209696	AF207054
Vochysiaceae	*Vochysia guianensis*	JQ625791	JQ626355	-	-	-	-	-	-
Wellstediaceae	*Wellstedia dinteri*	HQ384928	HQ384575	-	HQ412983	-	KF158027	HQ384786	-
Welwitschiaceae	*Welwitschia mirabilis*	AJ235814	AF280996	-	-	-	-	AF239795	AF207059
Xanthorrhoeaceae	*Xanthorrhoea preissii*	JQ273935	JQ276430	-	-	-	JQ276817	JQ273640	JQ283930
Xeronemaceae	*Xeronema callistemon*	HM640547	JQ276431	-	-	AY147752	JX903505	JX903924	HM640784
Velloziaceae	*Xerophyta elegans*	AJ131946	JX286710	-	-	-	AF547011	AY147609	KF197080
Ximeniaceae	*Ximenia americana*	GQ997898	GQ997871	-	-	-	-	GQ997862	L24428
Xyridaceae	*Xyris jupicai*	AY465698	-	-	-	-	AF547021	AY465541	-
Zamiaceae	*Zamia furfuracea*	JQ770263	AF410170	-	-	-	AF469702	JQ770253	-
Rutaceae	*Zanthoxylum acuminatum*	JQ594535	JQ589550	HM851472	HM851508	-	-	-	-
Rutaceae	*Zanthoxylum myriacanthum*	HQ415108	HQ415287	-	-	-	-	-	-
Rutaceae	*Zanthoxylum setulosum*	GQ981923	GQ982131	-	-	-	-	-	-
Zosteraceae	*Zostera marina*	AB125349	AB125355	AF102274	-	-	-	-	-

| Chapter 2 |

Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics

Guillaume Chomicki, Philip S. Ward and Susanne S. Renner

Proceedings of the Royal Society of London B: Biological Sciences 282: 20152200 (2015).

PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Chomicki G, Ward PS, Renner SS. 2015 Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. *Proc. R. Soc. B* **282**: 20152200. http://dx.doi.org/10.1098/rspb.2015.2200

Received: 12 September 2015
Accepted: 26 October 2015

Subject Areas:
evolution, ecology

Keywords:
ant/plant coevolution, co-radiation, secondary colonization, molecular clocks, *Pseudomyrmex*, symbiosis

Author for correspondence:
Guillaume Chomicki
e-mail: guillaume.chomicki@gmail.com

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2015.2200 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY
PUBLISHING

Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics

Guillaume Chomicki[1], Philip S. Ward[2] and Susanne S. Renner[1]

[1]Systematic Botany and Mycology, Department of Biology, University of Munich (LMU), 80638 Munich, Germany
[2]Department of Entomology and Nematology, University of California, Davis, CA 95616, USA

Symbioses include some of the clearest cases of coevolution, but their origin, loss or reassembly with different partners can rarely be inferred. Here we use ant/plant symbioses involving three plant clades to investigate the evolution of symbioses. We generated phylogenies for the big-eyed arboreal ants (Pseudomyrmecinae), including 72% of their 286 species, as well as for five of their plant host groups, in each case sampling more than 61% of the species. We show that the ant-housing *Vachellia* (Mimosoideae) clade and its ants co-diversified for the past 5 Ma, with some species additionally colonized by younger plant-nesting ant species, some parasitic. An apparent co-radiation of ants and *Tachigali* (Caesalpinioideae) was followed by waves of colonization by the same ant clade, and subsequent occupation by a younger ant group. Wide crown and stem age differences between the ant-housing genus *Triplaris* (Polygonaceae) and its obligate ant inhabitants, and stochastic trait mapping, indicate that its domatium evolved earlier than the ants now occupying it, suggesting previous symbioses that dissolved. Parasitic ant species evolved from generalists, not from mutualists, and are younger than the mutualistic systems they parasitize. Our study illuminates the macroevolutionary assembly of ant/plant symbioses, which has been highly dynamic, even in very specialized systems.

1. Introduction

The origin, maintenance, and breakdown of mutualisms are key questions in ecology and evolutionary biology [1–3]. Mapping traits of the mutualists and non-mutualist relatives on time-calibrated phylogenies has proved a powerful approach to unveil the temporal and geographical origin of mutualisms. A finding of co-phylogenetic studies of mutualisms is that co-speciation is rare (reviewed in [4]) and restricted to a few symbioses, especially those with vertical transmission, such as *Buchnera* bacterial endosymbionts and aphids [5–7]. Co-speciation in mutualistic partnerships that do not involve vertical transmission may exist in some obligate systems—for instance figs and their wasp pollinators as suggested by matching divergence times, although occasional wasp switches to other figs have been documented [8]. Other obligate mutualisms, such as the *Yucca*/yucca moth pollination mutualism, were found to have evolved multiple times [9,10]. Non-specialized mutualisms can exist between partners of highly discordant ages, for example, between introduced plants and native insect or bird pollinators [11]. Only species-dense molecular clock-dated phylogenies of both partner lineages therefore can elucidate the evolution of mutualistic systems. Such analyses over the past few years have revealed that cheaters rarely evolve from mutualists, contrary to theory [3].

Ant/plant symbioses involve plants with modified structures (domatia) that house ants, in return for protection or extra nutrients and sometimes also the physical or chemical removal of competing plant species [12–14]. Ant/plant symbioses appear to be younger than seed dispersal by ants or extrafloral nectary-mediated plant defence by ants, with no extant domatium-bearing clade older than 20 Myr [14]. Few co-phylogenetic studies of ant/plant systems

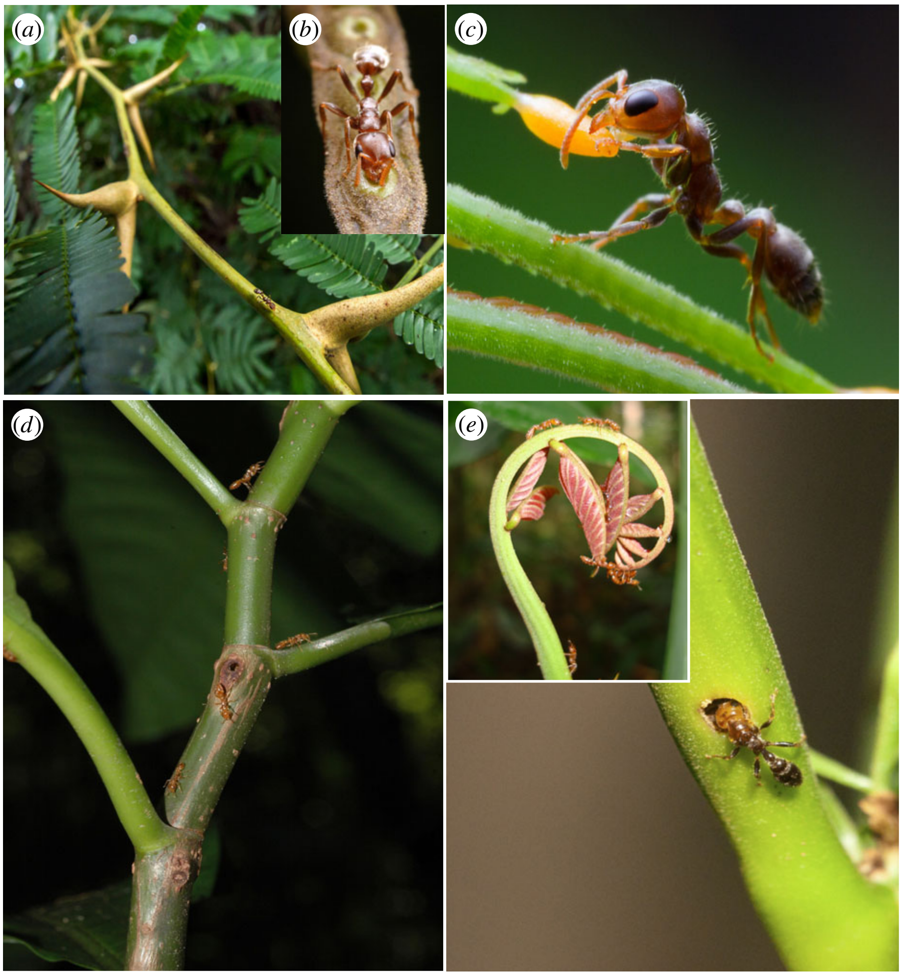


Figure 1. Examples of *Pseudomyrmex*/plant symbioses. (*a–c*) *Vachellia*/*Pseudomyrmex peperi* symbiosis. (*a*) *Vachellia* habit with stipular thorn domatia. (*b*) *Pseudomyrmex peperi* worker feeding on the large *Vachellia* extrafloral nectaries. (*c*) *Pseudomyrmex peperi* collecting a protein-rich Beltian body from the *Vachellia* leaflet tips. (*d*) *Triplaris americana* domatium inhabited by *Pseudomyrmex triplarinus*. (*e*) *Tachigali myrmecophila*/*Pseudomyrmex concolor*-group symbiosis. (*e*, inset) *P. concolor* entering in a *Tachigali myrmecophila* leaf domatium. (*e*) *Pseudomyrmex penetrator* entering the leaf rachis domatium, where an entrance hole has been chewed. Photo credits: (*a–c*) Alexander Wild, (*d*) Fabian Michelangeli, (*e*, inset), Heraldo Vasconcelos, (*e*) Ricardo Solar. (Online version in colour.)

have been conducted. In the African *Leonardoxa africana*, two of four subspecies have specialized domatia that were colonized in parallel by pre-adapted ant species [15,16]. Species of the Southeast Asian *Crematogaster borneensis*-group (former subgenus *Decacrema*) independently colonized three species groups of *Macaranga*, with an apparent matching of plant stem morphology and associated ant behaviour [17]. Co-radiation has been inferred in *Pseudomyrmex* and Mesoamerican *Vachellia* [18].

Pseudomyrmecinae comprise 230 described species in three genera [19–22], with 32 of the species living in plant domatia [14,19], making Pseudomyrmecinae the most diverse plant-occupying ant group worldwide [14]. Of the three genera, *Myrcidris* includes two species (one undescribed) from northern South America, *Pseudomyrmex* has 134 species, also confined to the New World, and *Tetraponera* comprises 95 species in Africa and Australasia [23]. Most species nest in dead hollow twigs of living plants, others nest only in the domatia of particular species that they protect against herbivores (figure 1), and some are parasites of other ant/plant symbioses [19,21,24,25]. Obligate domatium-nesting big-eyed ants have entered into more or less tight symbioses with species of the Fabaceae genera *Vachellia*, *Tachigali* and *Platymiscium*, and the Polygonaceae genera *Triplaris* and *Ruprechtia* [18,20,26,27]. This system is therefore ideal to study the evolution of ant/plant symbioses.

We had three expectations concerning the evolution of big-eyed ant/plant symbioses: (i) co-radiation (co-diversification) would be seen only in relatively young clades because of the increasing probability of partner loss over time, (ii) non-mutualistic domatium-nesting big-eyed ant species (i.e. parasites of existing symbioses) would be younger than mutualistic species, and (iii) highly age-discrepant partners would be rare in specialized symbioses. To evaluate geographical range shifts in both partners, we rely on a statistical biogeographic approach that allows comparing models with and without the assumption of speciation-with-dispersal [28,29]. With respect to geographical evolution, we expected that for specialized symbioses, ancestral areas of plant–ant clades should match those of their plant hosts.

2. Material and methods

(a) Taxon sampling, DNA isolation and amplification

The most important myrmecophyte genera associated with *Pseudomyrmex* ants are: *Vachellia* (Fabaceae: Mimosoideae), *Platymisicum* (Fabaceae: Faboideae), *Tachigali* (Fabaceae: Caesalpinoideae), *Triplaris* (Polygonaceae: Eriogonoideae) and *Ruprechtia*, the latter two being sister groups [30]. Our plant sampling ranged from 61 to 75% (see the electronic supplementary material, Material and Methods for details).

We sampled 64% of Pseudomyrmecinae including 78% of *Pseudomyrmex* species. Ten non-pseudomyrmecine ant species, including representatives of the sister-group (Myrmeciinae), were used as outgroups. Building on previous studies [22], we compiled or newly generated sequences from 10 nuclear markers, namely 28S rRNA, Wg, AbdA, LW Rh, EF1αF2, ArgK, Enolase, CAD, Top1 and Ubx. Out of 2150 sequences in the Pseudomyrmecinae matrix, 1990 are new (GenBank accession no. KR828817–KR830806). Taxon names, permanent voucher numbers with linked geographical information, and GenBank accession numbers are listed in the electronic supplementary material, table S1. The aligned data matrix for Pseudomyrmecinae has been deposited in TreeBase (study accession S17550). Primer sequences are given in the electronic supplementary material, table S2.

For *Vachellia*, *Platymiscium* and the *Triplaris*/*Ruprechtia* clades, we used sequences from published studies [18,30,31]; markers and alignment length are described in the electronic supplementary material, Material and Methods. For *Tachigali*, we sequenced ITS1 (nuclear) and *matK*, *trnL* intron, *trnL–trnF* and *trnH-PsbA* spacers (plastid) for 36 specimens. DNA isolation, purification and amplification followed standard methods [32]. Taxon names, vouchers, geographical information and GenBank accession numbers are listed in the electronic supplementary material, tables S3 (*Vachellia*), S4 (*Platymiscium*), S5 (*Triplaris*/*Ruprechtia*) and S6 (*Tachigali*). For more details see the electronic supplementary material, Materials and methods.

(b) DNA sequence alignment and phylogenetic analyses

Sequence alignments were performed in MAFFT v. 7 [33] (plants) or CLUSTAL X v. 2.1 [34] (Pseudomyrmecinae), manually edited and concatenated in MESQUITE v. 2.75 [35] (plants) or MacClade v. 4.08 [36] (Pseudomyrmecinae). Maximum-Likelihood tree inference relied on RAxML v. 8.1 [37] (plants) or GARLI v. 2.0 [38] (Pseudomyrmecinae), with 100 ML bootstrap replicates. For all plant analyses, we used the GTR + Γ substitution model in RAxML, while Pseudomyrmecinae were analysed using the partition scheme identified by PartitionFinder [39] (electronic supplementary material, table S7). For *Tachigali* and the Pseudomyrmecinae, we also conducted Bayesian analyses in MRBAYES v. 3.2 [40], with partitioning by gene region for *Tachigali*, using the best-fitting models identified by jModelTest2 [41], and using the scheme identified by PartitionFinder for the Pseudomyrmecinae (electronic supplementary material, table S7). Further details are provided in the electronic supplementary material, Materials and Methods.

(c) Molecular clock dating

Molecular clock dating relied on BEAST v. 2 [42] and the GTR + Γ substitution model with empirical nucleotide frequencies and six rate categories. In all cases, we used the uncorrelated lognormal relaxed clock model, since its standard deviation was always more than 0.5. We used Yule tree priors, with Markov chain Monte Carlo (MCMC) chain lengths between 20 and 60 million generations, sampling every 10 000th generation with chain length depending on convergence as determined by examining the log files in TRACER v. 1.5 [43] after removal of an initial burn-in proportion of 10% of the trees. Fossil and secondary calibrations for all five DNA matrices are explained in detail in the electronic supplementary material, Material and methods.

(d) Ancestral state reconstructions

We coded Pseudomyrmecinae as (0) 'ground nesting', for species nesting in the ground; (1) 'arboreal generalist', for unspecialized arboreal species nesting in dead twigs or branches of various plants, but not usually in domatia; (2) 'domatium mutualist', for plant-ants nesting obligately in domatia and presenting aggressive behaviour, and (3) 'domatium parasite', for species obligately living in domatia but with a timid behaviour that results in the absence of defense payback to their host. Species assignments to these categories are based on published studies [19,20,27,44–47] and personal observations by P.S.W. over the past 30 years. To infer the ancestral states of nesting habits, we used the ML approach implemented in MESQUITE v. 2.75 [35] with the MK1 model and the R package Ape (Ace function) [48], using as input trees both the maximum clade credibility tree from BEAST and the best ML tree from GARLI. To take into account topological uncertainties, we used two approaches: we ran MK1 reconstructions on a sample of 1000 Bayesian trees from the BEAST MCMC runs, and we used the Bayesian reversible jump MCMC approach in BayesTraits [49], which allows transition rates between character states to vary. The chain was run for 50×10^6 generations, and rate coefficients and ancestral states were sampled every 1000th generation. We ensured that the acceptance rate was between 20 and 40%, as recommended in the manual, and reconstructed the nodes of interest using the command 'addnode'.

To reconstruct the evolutionary gains and losses of domatia in *Vachellia*, *Tachigali*, *Triplaris*/*Ruprechtia* and *Platymiscium*, we coded each tip for domatium absence (0) or domatium presence (1), using the world list of domatium-bearing plants [14]. We performed ancestral state reconstructions using the same approaches as for the Pseudomyrmecinae. These reconstructions, including the assumptions of the model used, are described further in the electronic supplementary material, Material and methods.

(e) Historical biogeography and range sizes

We coded the geographical ranges of all Pseudomyrmecinae and of all plant species in the phylogenies as: A = USA, B = Mexico to Panama including the Caribbean, C = Northern and Central Andes (Venezuela, Colombia, Ecuador, Peru, Bolivia), D = Brazil and the Guianas, E = Chile, Argentina, Paraguay and Uruguay, F = Afrotropics and G = India, southeast Asia and Australia. The coded Neotropical regions are shown in figure 2. To infer whether (i) ancestral areas of Pseudomyrmecinae clades match those of their plant hosts and whether (ii) our focal symbioses coincide with geographical range shifts, we used ancestral range reconstruction (back to 33.7 Ma) using the multimodel approach implemented in the R package BioGeoBEARS [28,29] on the BEAST chronograms.

To determine whether increased *Pseudomyrmex* specialization (here obligate nesting in a particular plant species) coincides with range narrowing or broadening, we evaluated the range size of each plant-ant species and compared it to that of its sister group based on occurrence data from a database of vouchered material compiled by P.S.W. (electronic supplementary material, table S8). We calculated range sizes as the extent of occurrence using the software DIVA-GIS [50], following an approximate minimum convex polygon. Given the dense geographical sampling of Pseudomyrmecinae (electronic supplementary material, table S8), this approach reduces the risk of overestimating range sizes. Range size calculation and sister group taxonomic composition are described in detail in the electronic supplementary material, Material and methods.

(f) Interactions

We searched the literature to obtain information about the types of interactions between the plant and ant species sampled in our study. Data for *Triplaris* and the *Pseudomyrmex triplarinus* group come mainly from [51], those for *Vachellia* and the *P. ferrugineus* group from [20] (summarized in fig. 73) and [18]. Apart from these two groups, species-level information is scarce since botanists at best note the ant genus and entomologists the plant genus. We thus included indirect data from morphological traits and notes on genera (without species names), as long as there was a geographical overlap. All inferred links are depicted as dotted lines in the respective figures.

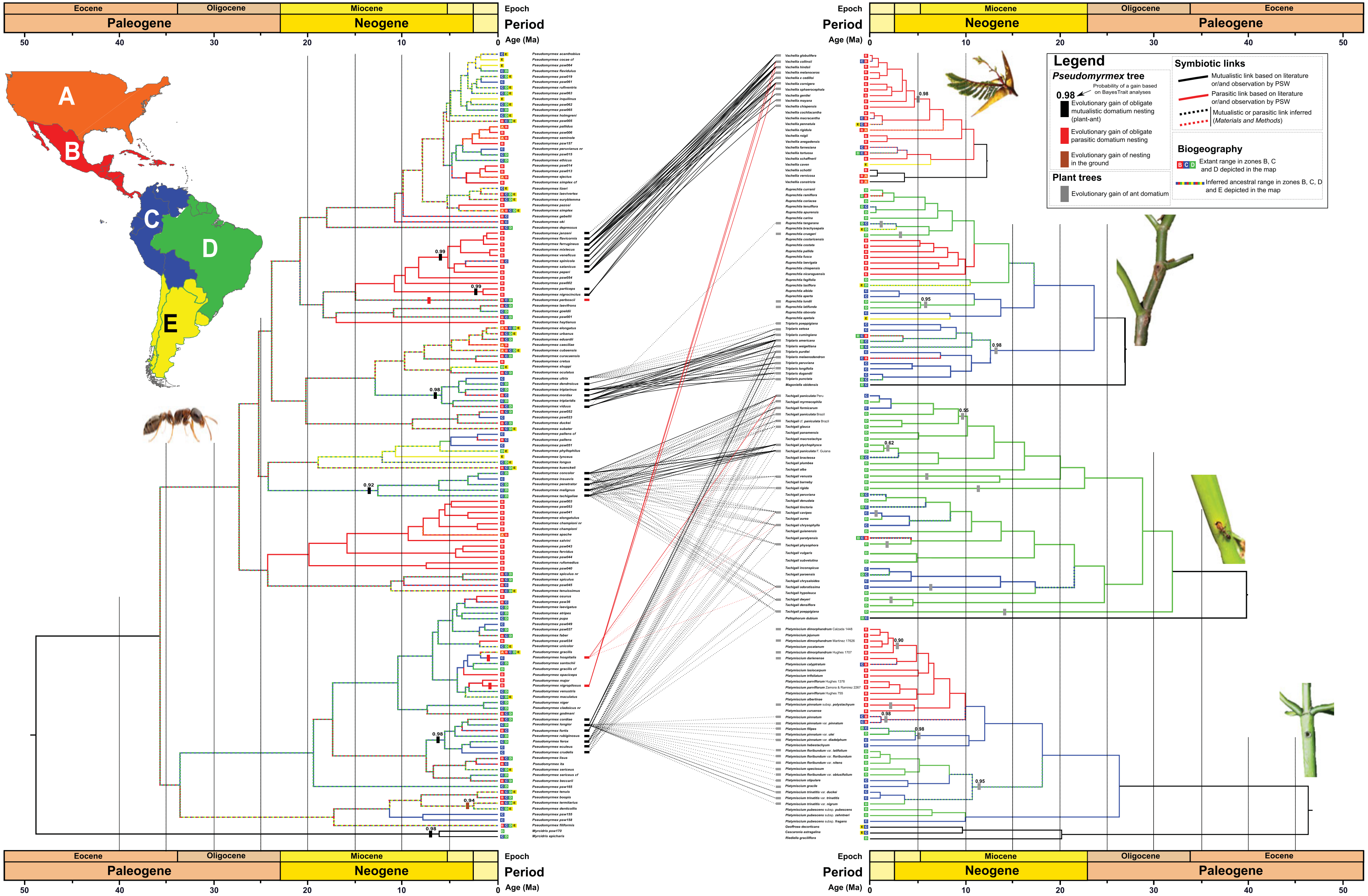


Figure 2. Dated phylogenies of *Pseudomyrmex* and its five main plant host groups: *Vachellia*, *Triplaris*/*Ruprechtia*, *Tachigali* and *Platymiscium*. Colour coding (map) shows the ancestral range resulting from the best-fit model (§2). Links between ant and plant species are solid black for documented mutualistic interactions, red for documented parasitic interactions, and dotted for inferred interactions. Black rectangles mark the evolutionary gain of mutualistic obligate plant nesting; red rectangles mark parasitic obligate plant nesting; and brown rectangles indicate ground nesting. The remaining *Pseudomyrmex* species are generalist arboreal ants. Grey rectangles mark the evolutionary gain of ant domatia. Numbers above rectangles refer to the probability of an inferred gain based on BayesTraits analyses (this cannot be inferred for single species; see §2). A rectangle positioned next to a crown group means that the trait originated at that node, while the rectangle position for branches leading to single species is arbitrary. Ancestral state reconstructions are shown in the electronic supplementary material, figures S5–S10 and S12. (Online version in colour.)

3. Results

(a) Phylogenetics of Neotropical Pseudomyrmecinae and their plant hosts

Both ML and Bayesian phylogenetic inference showed a well-supported Neotropical *Myrcidris* + *Pseudomyrmex* clade and four maximally supported *Pseudomyrmex* plant-ant groups (*P. ferrugineus* group, *P. concolor* group, *P. triplarinus* group and *P. sericeus* group; electronic supplementary material, figures S1 and S2). An unexpected result is that the *Vachellia* ('ant-*Acacia*') ants are not monophyletic, but instead form two clades separated by two species of arboreal generalists from Central America (figure 2), extending a previous finding [47].

Phylogenetic relationships in *Triplaris*/*Ruprechtia*, *Platymiscium* and *Vachellia* are as found in previous studies [18,30,31]. The monophyly of the newly investigated genus *Tachigali* is maximally supported in ML and Bayesian analyses (electronic supplementary material, figure S3), and the sister species relationships involving the position of myrmecophytes relevant to this study are well to moderately supported (electronic supplementary material, figure S3).

(b) Times of origin of Pseudomyrmecinae and their plant hosts

The most recent common ancestor (MRCA) of Pseudomyrmecinae dates to 71.7 ± 7 Ma, significantly older than found in chronograms that focused on all ants and therefore included only a few Pseudomyrmecinae [52,53]. The stem age of *Pseudomyrmex* is 49.0 ± 4 Ma, its crown age 35.8 ± 4 Ma (figure 2; electronic supplementary material, figure S4). The main clade of *Vachellia*-inhabiting species in the *P. ferrugineus* species group—here referred to as the *P. ferrugineus* subgroup—dates to 5.1 ± 1.5 Ma, matching the age of the MRCA of the Mesoamerican *Vachellia* clade, 4.7 ± 2 Ma. Two related *Vachellia*-inhabiting *Pseudomyrmex* species, *P. nigrocinctus* and *P. particeps*, forming the *P. nigrocinctus* subgroup, however, evolved 1.5 ± 1 Ma, after the radiation of the *Vachellia* species that they currently inhabit (figures 2 and 3*a*). Similarly, the *P. triplarinus* group evolved 5.7 ± 2 Ma, after the radiation of its obligate host clade, *Triplaris* (18 species, 61% sampled), here dated to 13 ± 2 Ma (figures 2 and 3*c*). The *P. concolor* species group dates to 12.2 ± 3 Ma, overlapping the age range inferred for the *Tachigali* clade that it inhabits (the *T. paniculata* group, 9.3 ± 5 Ma; figures 2 and 3*b*). Other ant-housing *Tachigali* species originated between 9.3 and 1.5 Ma, but the origin of domatia in single species cannot be dated (figure 2). Lastly, the *P. fortis* subgroup, a clade of myrmecophyte-inhabiting species within the *P. sericeus* species group, whose species nest in *Tachigali*, *Triplaris*, *Platymiscium* and other ant-plants, dates to 5.5 ± 1.5 Ma, and does not show any obvious crown matching with any of its hosts. Within this clade is a subgroup of strict *Tachigali* specialists, the *P. crudelis* complex, originating 3.7 ± 1 Ma, well after the *P. concolor* group.

(c) Biogeography of plant-nesting *Pseudomyrmex* and their plant hosts

BioGeoBEARS model comparison yielded the BAYAREA + J model as best fitting the ant data, significantly better than DEC + J (Lnl = −451.87 versus −608.91; electronic supplementary material, table S9a shows all statistics of BioGeoBEARS runs). DEC + J was the best-fit model for *Triplaris*/*Ruprechtia* and *Platymiscium*, while for *Vachellia* and *Tachigali* DEC had the same likelihood as DEC + J (electronic supplementary material, table S9b-e). Because many Pseudomyrmecinae species are widespread, the inferred ancestral ranges are also wide (figure 2). The ancestral area of *Pseudomyrmex* includes Central and Northern South America (ML probability = 0.8), and the ancestral ranges of the *P. ferrugineus* and the *P. nigrocinctus* subgroups are Central America (ML prob. = 1 and = 0.95, respectively), matching the inferred ancestral range of their *Vachellia* host plants (ML prob. = 0.99). The *P. triplarinus* group originated in Northern South America (ML prob. = 0.8), matching the ancestral range of its host, *Triplaris* (ML prob. = 0.8), and the same holds for the *P. concolor* group (ML prob. = 0.9) and its host *Tachigali* (ML prob. = 0.75–1 depending on lineage). The *P. fortis* species group within the *P. sericeus* group evolved in Northern South America (ML prob. = 0.9), where some of its hosts also arose (*Triplaris* and some myrmecophytic lineages of *Platymiscium*). In *Pseudomyrmex*, the evolution of obligate plant nesting correlates with a reduction in the number of ancestral areas (figure 2), which is partially confirmed by range size analysis (electronic supplementary material, figure S11).

(d) Gains or losses of plant nesting in Pseudomyrmecinae and of domatia in their major plant hosts

Our ML and Bayesian reconstructions (electronic supplementary material, Material and Methods; and figures S5 and S6) of plant nesting in Pseudomyrmecinae strongly support 10 origins of obligate domatium living in this subfamily, including five in the genus *Pseudomyrmex* alone. This result was highly supported across methodological approaches (figure 2; electronic supplementary material, figures S5, S6 and S12; and also electronic supplementary material). Two independent origins of domatium living are supported within the *P. ferrugineus* group, one in the *P. ferrugineus* subgroup and one in the *P. nigrocinctus* subgroup (Bayesian prob. 0.98, ML prob. 0.97–0.99; electronic supplementary material, figures S5, S6 and S12). No loss of obligate plant nesting was detected. In the plants, we inferred single gains of domatia in *Vachellia* (electronic supplementary material, figures S7 and S12) and *Triplaris* (electronic supplementary material, figures S8 and S12), confirming previous results [14], and three gains of domatia in *Ruprechtia* (electronic supplementary material, figures S8 and S12), at least nine in *Tachigali* (electronic supplementary material, figures S9 and S12) and five in *Platymiscium* (electronic supplementary material, figure S10 and S12). No domatium loss was inferred. Stochastic trait mapping (electronic supplementary material, Materials and Methods; and electronic supplementary material, figure S12) confirmed the results obtained with other methods.

4. Discussion

(a) Macroevolutionary assembly of ant/plant symbioses

The expectation that highly age-discrepant partners would be rare turned out to be wrong, while our expectations that co-diversification would be seen only in relatively young

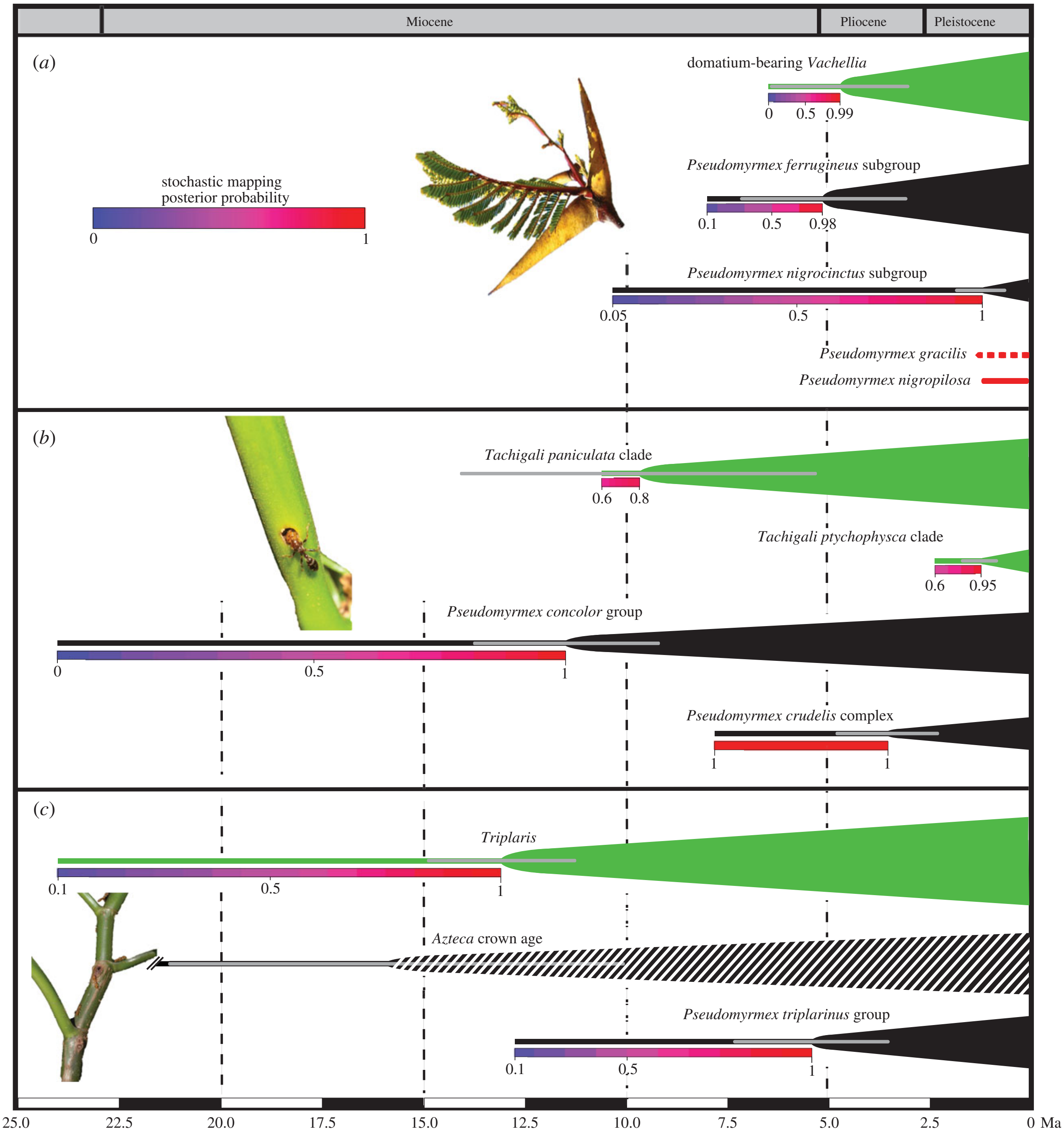


Figure 3. Macroevolutionary patterns of age and trait matching of interacting *Pseudomyrmex* ants and domatium-bearing plant lineages and hypothetical-associated evolutionary processes. (*a*) Co-radiation of *Vachellia* and the *P. ferrugineus* subgroup, followed by secondary colonization by mutualistic species of the *P. nigrocinctus* species complex, parasitic *P. nigropilosus* and the generalist *P. gracilis*. (*b*) Potential initial co-radiation of *Tachigali* and the *P. concolor* species group, followed by host broadening to other *Tachigali* lineages and secondary colonization of *Tachigali* by members of the *P. crudelis* species complex. (*c*) In domatium-bearing *Triplaris*, crown and stem ages and ancestral state reconstruction suggest that the ant mutualists (the *P. triplarinus* group) that currently nest in *Triplaris* domatia are younger by approximately 8 Myr than is domatium-presence in *Triplaris*, suggesting possible symbioses with other (earlier) ant species, such as *Azteca* whose crown age (banded) matches *Triplaris* and which sometimes forms symbioses with the latter (see §4). Grey error bars show the 95% CI from BEAST. Black (ants) or green (plant) bars depict stem branches. Colour gradient along the stem branch shows the posterior probability of a density plot summarizing 1000 stochastic simulations of trait evolution. See also the associated electronic supplementary material, figure S12. Below the arbitrary threshold of 0.5, the traits (domatium or domatium-nesting) are unlikely to have been present. (Online version in colour.)

clades and that parasitic species would be younger than mutualistic species were both met. Temporally matched radiation (co-radiation) of interacting clades has occurred in the *P. ferrugineus* group and its *Vachellia* host species in Central America (figure 3*a*). Most ant species in this group can nest in several *Vachellia* species, with the exception of *P. satanicus*, which seems restricted to *V. melanoceras* ([20,24]; figure 2). We found no obvious matching of the DNA tree topologies, suggesting the absence of co-speciation, and the branching times of interacting species are not always temporally matched (figure 2), further pointing to host broadening and host switching. The limited dispersal ability of symbiotic ants and plants and their typically low specificity probably hamper co-speciation in ant/plant symbioses [12,14]. Reciprocally matching traits in *Vachellia* and their big-eyed ant symbionts include protein-rich food bodies (Beltian bodies) that are more effectively digested by *P. ferrugineus* ants than by generalist species [54], enlarged extrafloral nectaries

(EFNs) with post-secretory nectar sucrose hydrolysis and the ants' ability to feed on sucrose-poor nectar [55]. A novel finding of this study is that *P. nigrocinctus* and *P. particeps* form a distinct lineage much younger than the remaining *Vachellia* ants, which apparently colonized already domatium-possessing *Vachellia* species (figure 3*a*; electronic supplementary material, figures S5–S7). *Pseudomyrmex nigrocinctus* is widespread, occupying several *Vachellia* species, while *P. particeps* is known only from *V. allenii*, a species that can also be inhabited by *P. spinicola*, a member of the *P. ferrugineus* subgroup [20]. *Vachellia allenii* thus represents a clear case of symbiont broadening, with the younger ant species *P. particeps* now competing with *P. spinicola* for domatia to live in. Both *P. nigrocinctus* and *P. particeps* patrol their host plants aggressively and gather Beltian bodies and extrafloral nectar ([56]; P.S.W., personal observation), but the extent to which they have adapted nutritionally to *Vachellia*, perhaps with traits similar to those found in the *P. ferrugineus* subgroup [54,55], remains to be investigated.

Tachigali domatia, which evolved at least nine times (figure 2), arise from an enlargement of the leaf rachis (and in some cases also the inflorescence stem), which may be developmentally 'easy' and happen readily under selection pressure from domatium-nesting ants, in this case ants of the *P. concolor* group (figure 2), as long as founder queens can cover the distance between domatium-bearing and non-domatium-bearing species occurring sympatrically [57]. The repeated evolution of domatia in related *Tachigali* species provides a striking example of parallel evolution that results from recurrent colonization by *P. concolor* group. The significantly younger age of the *P. crudelis* species group (3.7 Ma versus 12.2 for the *P. concolor* group and 8 Ma for the main domatium-bearing *Tachigali* lineage) strongly suggests that it secondarily colonized *Tachigali* (figures 2, 3*b*; electronic supplementary material, figures S5, S6 and S9). Secondary colonization, such as that of *Vachellia* by the *P. nigrocinctus* species complex and of *Tachigali* by the *P. crudelis* species complex, results in symbiont broadening for the plants and enables entry into 'new adaptive zones' represented by the myrmecophytes. The *P. triplarinus* group is 5–8 Myr younger than its obligate host *Triplaris* (figures 2 and 3; electronic supplementary material, figures S5, S6, S8 and S12). Wide crown and stem age differences between the ant-housing genus *Triplaris* (Polygonaceae) and its obligate ant inhabitants, and stochastic trait mapping (figures 2 and 3; electronic supplementary material, figure S12), indicate that its domatium evolved earlier than the ants now occupying it, suggesting previous symbioses that dissolved. *Triplaris* might thus represent a later stage in the evolution of coevolution as envisioned by Ehrlich & Raven [58], namely the complete switching to a new partner. Partner replacement could come about through colonization of domatia by generalist plant-ants [59,60]. A potential candidate for an earlier symbiosis with *Triplaris* is *Azteca*, a clade whose crown age matches that of *Triplaris* (figure 3*c*, [61]) and which contains both *Triplaris* specialists [46,62] and infrequent occupants of *Triplaris* domatia [51]. Alternatively, the inferred domatium trait might be an exaptation that would only have been converted later into a domatium, or *Triplaris* might have been associated with (now extinct) stem lineages species of the *P. triplarinus* group.

(b) Recent colonization of mutualistic symbioses by parasitic ant species

Our time-calibrated phylogenetic framework for the evolution of big-eyed ants and their plant host groups reveals that specialized mutualist species form well-defined clades, while parasite species consist of singletons (figure 2). Although the time of origin of a parasitic lifestyle in single species cannot be inferred, the relevant sister species divergence times imply that parasites evolved later than mutualists: *P. nigropilosus*, a specialist ant species that obligately nests in *Vachellia* and feeds on its food bodies and extrafloral nectar but does not protect it against herbivores or encroaching vegetation [27], split from its sister species *P. major* only 1.5 Ma. Similarly, *P. gracilis*, an arboreal generalist that occasionally occupies *Vachellia* and prevents queens of mutualistic ants from founding a new colony [63], split from the related species, *P. hospitalis*, only 1.7 Ma. Younger ages of ant parasites compared to mutualists are expected since mutualistic selection pressure must first have led to the evolution of domatia before parasitic ants could exploit these nesting structures. In all cases, we found that parasites evolved from generalists and not from mutualists, contrary to a common prediction in mutualism models [3], but consistent with previous phylogenetic analyses with less dense species sampling [47].

5. Conclusion

Our study reveals macroevolutionary patterns that may represent different stages in the evolution of ant/plant symbioses. Based on crown ages, we inferred co-diversification in the *Vachellia*/*Pseudomyrmex ferrugineus*-group system over a few million years and secondary and parallel colonizations of *Vachellia*, *Tachigali* and *Platymiscium* by other ant groups that entered new 'adaptive zones' (mutualistic or parasitic). In *Triplaris*, we present evidence that the current *Pseudomyrmex* partners are secondary colonists that displaced earlier symbiont species, possibly as a consequence of locally reduced abundances and competition among plant-ants for nesting sites. The repeated evolution of domatia in *Tachigali* (26 of its 54 species have domatia; figure 2) may provide an example of a guild, namely the *Pseudomyrmex concolor* species group, imposing selection pressures on related plant species. Altogether, our study reveals that assemblage of ant/plant symbioses has been highly dynamic, even in very specialized systems, such as the iconic Central American *Vachellia* and its thorn-domatium-nesting *P. ferrugineus* group.

Data accessibility. Data are available in TreeBase accessions S17550 (Pseudomymecinae) and Dryad accession http://dx.doi.org/10.5061/dryad.5p51r (plants).

Competing interests. We declare we have no competing interests.

Funding. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20 and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to G.C. Research on Pseudomyrmecinae ants was supported by a series of grants to P.S.W. from the US National Science Foundation, most recently DEB-1354996.

Acknowledgements. We thank Alex Wild, Heraldo Vasconcelos, Fabian Michelangeli and Ricardo Solar for photos, and curators of the herbaria of the Missouri Botanical Garden and the Catholic University of Quito for permission to isolate DNA from specimens under their care. We thank Rumsais Blatrix for a *Tachigali* leaf sample, Rosa Elena Andrade and Christian Feregrino for help in the lab, and Jeremy Aroles for proofreading the manuscript. P.S.W. extends gratitude to numerous collectors and curators who provided access to ant specimens, especially Gary Alpert, Woody Benson, Roberto Brandão, Stefan Cover, Fernando Fernández, Brian Fisher, John Lattke, Jack Longino, Bill MacKay, Roy Snelling and Jim Wetterer. We are grateful to the associate editor Megan Frederickson and three anonymous reviewers for critical comments that improved the manuscript.

References

1. Bronstein JL, Alarcón R, Geber M. 2006 The evolution of plant–insect mutualisms. *New Phytol*. **172**, 412–428. (doi:10.1111/j.1469-8137.2006.01864.x)
2. Frederickson ME. 2013 Rethinking mutualism stability: cheaters and the evolution of sanctions. *Q. Rev. Biol*. **88**, 269–295. (doi:10.1086/673757)
3. Sachs JL, Simms EL. 2006 Pathways to mutualism breakdown. *Trends Ecol. Evol*. **21**, 585–592. (doi:10.1016/j.tree.2006.06.018)
4. de Vienne DM, Refrégier G, López-Villavicencio M, Tellier A, Hood ME, Giraud T. 2013 Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytol*. **198**, 347–385. (doi:10.1111/nph.12150)
5. Clark MA, Moran NA, Baumann P, Wernegreen JJ. 2000 Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* **54**, 517–525. (doi:10.1111/j.0014-3820.2000.tb00054.x)
6. Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T. 2006 Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biol*. **4**, e337. (doi:10.1371/journal.pbio.0040337)
7. Jousselin E, Desdevises Y, Coeur d'Acier A. 2009 Fine-scale cospeciation between *Brachycaudus* and *Buchnera aphidicola*: bacterial genome helps define species and evolutionary relationships in aphids. *Proc. R. Soc. B* **276**, 187–196. (doi:10.1098/rspb.2008.0679)
8. Cruaud A *et al.* 2012 An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Syst. Biol*. **61**, 1029–1047. (doi:10.1093/sysbio/sys068)
9. Bogler DJ, Neff JL, Simpson BB. 1995 Multiple origins of the yucca-yucca moth association. *Proc. Natl Acad. Sci. USA* **92**, 6864–6867. (doi:10.1073/pnas.92.15.6864)
10. Smith CI, Pellmyr O, Althoff DM, Balcazar-Lara M, Leebens-Mack J, Segraves KA. 2008 Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. *Proc. R. Soc. B* **275**, 249–258. (doi:10.1098/rspb.2007.1405)
11. Gu L, Luo Z, Zhang D, Renner SS. 2010 Pollination of *Rhodoleia championii* (Hamamelidaceae) in subtropical China. *Biotropica* **42**, 336–341. (doi:10.1111/j.1744-7429.2009.00585.x)
12. Davidson DW, McKey D. 1993 The evolutionary ecology of symbiotic ant/plant relationships. *J. Hymenopt. Res*. **2**, 13–83.
13. Frederickson ME, Greene MJ, Gordon DM. 2005 Ecology: 'Devil's gardens' bedevilled by ants. *Nature* **437**, 495–496. (doi:10.1038/437495a)
14. Chomicki G, Renner SS. 2015 Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytol*. **207**, 411–424. (doi:10.1111/nph.13271)
15. Chenuil A, McKey D. 1996 Molecular phylogenetic study of a myrmecophyte symbiosis: did *Leonardoxa*/ant associations diversify via cospeciation? *Mol. Phylogenet. Evol*. **6**, 270–286. (doi:10.1006/mpev.1996.0076)
16. Brouat C, McKey D, Douzery EJP. 2004 Differentiation in a geographical mosaic of plants coevolving with ants: phylogeny of the *Leonardoxa africana* complex (Fabaceae: Caesalpinioideae) using amplified fragment length polymorphism markers. *Mol. Ecol*. **13**, 1157–1171. (doi:10.1111/j.1365-294X.2004.02113.x)
17. Quek SP, Davies SJ, Itino T, Pierce NE. 2004 Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). *Evolution* **58**, 554–570. (doi:10.1111/j.0014-3820.2004.tb01678.x)
18. Gómez-Acevedo S, Rico-Arce L, Delgado-Salinas A, Magallón S, Eguiarte LE. 2010 Neotropical mutualism between *Acacia* and *Pseudomyrmex*: phylogeny and divergence times. *Mol. Phylogenet. Evol*. **561**, 393–408. (doi:10.1016/j.ympev.2010.03.018)
19. Ward PS. 1991 Phylogenetic analysis of pseudomyrmecine ants associated with domatia-bearing plants. In *Ant-plant interactions* (eds CR Huxley, DF Cutler), pp. 335–352. New York, NY: Oxford University Press.
20. Ward PS. 1993 Systematic studies on *Pseudomyrmex* acacia-ants Hymenoptera: Formicidae: Pseudomyrmecinae. *J. Hym. Res*. **2**, 117–168.
21. Ward PS. 1999 Systematics, biogeography and host plant associations of the *Pseudomyrmex viduus* group Hymenoptera: Formicidae, *Triplaris*- and *Tachigali*-inhabiting ants. *Zool. J. Linn. Soc*. **126**, 451–540. (doi:10.1111/j.1096-3642.1999.tb00157.x)
22. Ward PS, Downie DA. 2005 The ant subfamily Pseudomyrmecinae Hymenoptera: Formicidae: phylogeny and evolution of big-eyed arboreal ants. *Syst. Entomol*. **30**, 310–335. (doi:10.1111/j.1365-3113.2004.00281.x)
23. AntCat. 2015 AntCat. An online catalog of the ants of the world. See http://antcat.org (accessed 24 April 2015).
24. Janzen DH. 1974 *Swollen-thorn* Acacias *of Central America*. Washington, DC: Smithsonian Institution Press.
25. Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Silva Bueno JC. 2009 Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proc. Natl Acad. Sci. USA* **106**, 18 091–18 096. (doi:10.1073/pnas.0904304106)
26. Hocking B. 1970 Insect associations with the swollen thorn acacias. *Trans. R. Entomol. Soc. Lond*. **122**, 211–255. (doi:10.1111/j.1365-2311.1970.tb00532.x)
27. Janzen DH. 1975 *Pseudomyrmex nigropilosa*: a parasite of a mutualism. *Science* **188**, 936–937. (doi:10.1126/science.188.4191.936)
28. Matzke NJ. 2012 Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in dispersal–extinction–cladogenesis DEC analyses. *Front. Biogeogr*. **4**, 210.
29. Matzke NJ. 2014 Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol*. **63**, 951–970. (doi:10.1093/sysbio/syu056)
30. Sanchez A, Kron KA. 2008 Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Syst. Bot*. **33**, 87–96. (doi:10.1600/036364408783887456)
31. Saslis-Lagoudakis C, Chase MW, Robinson DN, Russell SJ, Klitgaard BB. 2008 Phylogenetics of neotropical *Platymiscium* Leguminosae: Dalbergieae: systematics, divergence times, and biogeography inferred from nuclear ribosomal and plastid DNA sequence data. *Am. J. Bot*. **95**, 1270–1286. (doi:10.3732/ajb.0800101)
32. Chomicki G, Renner SS. 2015 Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. *New Phytol*. **205**, 526–532. (doi:10.1111/nph.13163)
33. Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol*. **30**, 772–780. (doi:10.1093/molbev/mst010)
34. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997 The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res*. **24**, 4876–4882. (doi:10.1093/nar/25.24.4876)
35. Maddison WP, Maddison DR. 2011 Mesquite 2.75: a modular system for evolutionary analysis. See http://mesquiteproject.org (accessed 1 April 2015).
36. Maddison DR, Maddison WP. 2005 *MacClade 4: analysis of phylogeny and character evolution*. Version 4.08. Sunderland, MA: Sinauer Press.
37. Stamatakis A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **309**, 1312–1313. (doi:10.1093/bioinformatics/btu033)
38. Bazinet AL, Zwickl DJ, Cummings MP. 2014 A gateway for phylogenetic analysis powered by grid computing featuring GARLI 2.0. *Syst. Biol*. **63**, 812–818. (doi:10.1093/sysbio/syu031)
39. Lanfear R, Calcott B, Ho SY, Guindon S. 2012 PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol*. **296**, 1695–1701. (doi:10.1093/molbev/mss020)
40. Ronquist F *et al.* 2012 MrBayes 3.2: efficient Bayesian phylogenetic inference and model

choice across a large model space. *Syst. Biol*. **613**, 539–542. (doi:10.1093/sysbio/sys029)

41. Darriba D, Taboada GL, Doallo R, Posada D. 2012 jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**, 772. (doi:10.1038/nmeth.2109)
42. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014 BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comp. Biol.* **104**, e1003537. (doi:10.1371/journal.pcbi.1003537)
43. Rambaut A, Drummond AJ. 2007 Tracer—MCMC trace analysis tool version v1.5. See http://beast.bio.ed.ac.uk.
44. Janzen DH. 1966 Coevolution of mutualism between ants and acacias in Central America. *Evolution* **20**, 249–275. (doi:10.2307/2406628)
45. Wheeler WM, Bailey IW. 1920 The feeding habits of pseudomyrmine and other ants. *Trans. Am. Philos. Soc*. **22**, 235–279. (doi:10.2307/1005485)
46. Wheeler WM. 1942 Studies of Neotropical ant-plants and their ants. *Bull. Mus. Comp. Zool.* **90**, 1–262.
47. Kautz S, Lumbsch HT, Ward PS, Heil M. 2009 How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners. *Evolution* **63**, 839–853. (doi:10.1111/j.1558-5646.2008.00594.x)
48. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)
49. Pagel M, Meade A. 2013 *BayesTraits v. 2.0*. Reading, MA: University of Reading. See http://www.evolution.rdg.ac.uk/BayesTraits.html.
50. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005 Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol*. **25**, 1965–1978. (doi:10.1002/joc.1276)
51. Sanchez A. 2011 Evolutionary relationships in Polygonaceae with an emphasis on *Triplaris*. PhD thesis, Wake Forest University, NC, USA
52. Moreau CS, Bell CD, Villa R, Archibald SB, Pierce N. 2006 Phylogeny of ants: diversification in the age of angiosperms. *Science* **312**, 101–104. (doi:10.1126/science.1124891)
53. Moreau CS, Bell CD. 2013 Testing the museum versus cradle tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* **67**, 2240–2257. (doi:10.1111/evo.12105)
54. Orona-Tamayo D, Wielsch N, Blanco-Labra A, Svatos A, Farías-Rodríguez R, Heil M. 2013 Exclusive rewards in mutualisms: ant proteases and plant protease inhibitors create a lock–key system to protect *Acacia* food bodies from exploitation. *Mol. Ecol*. **2215**, 4087–4100. (doi:10.1111/mec.12320)
55. Heil M, Rattke J, Boland W. 2005 Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* **308**, 560–563. (doi:10.1126/science.1107536)
56. Beulig ML, Janzen DH. 1969 Variation in behavior among obligate acacia-ants from the same colony (*Pseudomyrmex nigrocincta*). *J. Kansas Entomol. Soc.* **42**, 58–67.
57. van der Werff H. 2008 A synopsis of the genus *Tachigali* (Leguminosae: Caesalpinioideae) in Northern South America. *Ann. Miss. Bot Gard*. **95**, 618–661. (doi:10.3417/2007159)
58. Ehrlich PR, Raven PH. 1964 Butterflies and plants: a study in coevolution. *Evolution* **18**, 586–608. (doi:10.2307/2406212)
59. McKey D. 1984 Interaction of the ant-plant *Leonardoxa africana* Caesalpiniaceae with its obligate inhabitants in a rainforest in Cameroon. *Biotropica* **16**, 81–99. (doi:10.2307/2387840)
60. Maschwitz U, Fiala B, Davies SJ, Linsenmair KE. 1996 A South-East Asian myrmecophyte with two alternative inhabitants: *Camponotus* or *Crematogaster* as partners of *Macaranga lamellata*. *Ecotropica* **2**, 29–40.
61. Pringle EG, Ramirez SR, Bonebrake TC, Gordon DM, Dirzo R. 2012 Diversification and phylogeographic structure in widespread *Azteca* plant-ants from the northern Neotropics. *Mol. Ecol*. **21**, 3576–3592. (doi:10.1111/j.1365-294X.2012.05618.x)
62. Longino JT. 2007 A taxonomic review of the genus *Azteca* (Hymenoptera: Formicidae) in Costa Rica and a global revision of the aurita group. *Zootaxa* **1491**, 1–63.
63. Clement LW, Köppen S, Brand WA, Heil M. 2008 Strategies of a parasite of the ant–*Acacia* mutualism. *Behav. Ecol. Sociobiol*. **26**, 953–962. (doi:10.1007/s00265-007-0520-1)

Supplementary online material

(a) Additional information on sampling, DNA extraction and amplification

The most important myrmecophyte genera associated with *Pseudomyrmex* ants are: *Vachellia* (Fabaceae: Mimosoideae), *Platymisicum* (Fabaceae: Faboideae), *Tachigali* (Fabaceae: Caesalpinoideae), *Triplaris* (Polygonaceae: Eriogonoideae) and *Ruprechtia*, the latter two being sister groups [30]. The domatia of *Vachellia* are modified stipules (hollow thorns), those of *Platymiscium* swollen stems, those of *Tachigali* swollen petioles, rachises, or inflorescence stems, in *Triplaris* hollow internodes, and those of *Ruprechtia* hollow stems [14]. Domatia are reported in 26 of the 54 species of *Tachigali* [57], all 18 species of *Triplaris* [64], 24 of the 37 species of *Ruprechtia* [65], 15 of the 32 species of *Vachellia* [26] and 10 of the 29 species of *Platymiscium* [31,66]. For plants, we sampled 37 (68%) of the species of *Tachigali* including 13 (50%) domatium-bearing species, 11 (61%) species of *Triplaris*, 24 (65%) species of *Ruprechtia* including 4 (67%) of its domatium-bearing species; 24 (75%) species of *Vachellia* including 10 (67%) of its domatium-bearing species; and 19 (65%) of the species of *Platymiscium* including 9 (90%) of its domatium-bearing species. Of Pseudomyrmecinae, we sampled 64 (59%) of *Tetraponera* species including 15 undescribed species, two species of *Myrcidris* (one undescribed), and 139 *Pseudomyrmex* species (78%) including 41 undescribed species, resulting in a dataset of 205 Pseudomyrmecinae species. Our ant sampling includes 28 of the 32 described plant-ant species found in Pseudomyrmecinae. Markers for the published plant matrices are as following: for *Vachellia*, three plastid markers were available (*matK* gene, *psaB-rps14* and *trnL-trnF* spacers); for *Platymiscium*, one nuclear region (ITS) and three plastid regions (*matK*, *trnL* intron, *trnL-trnF* spacer); for *Triplaris*/*Ruprechtia*, one nuclear region (ITS) and four plastid regions (*matK*, *ndhF*, *ndhC-trnV*, *rps16-trnK*). To generate a phylogeny for *Tachigali*, we studied herbarium specimens in Munich (M) and Missouri (MO), and images of specimens from Quito (QCA), and then selected suitable specimens for destructive sampling of c. 20 mg of leaf fragments. The *Tachigali* herbarium material proved recalcitrant, but we obtained sequences from our four markers (ITS1, matK, trnL-trnF and psbA-trnH) from 36 specimens representing 34 species. Primers used for Pseudomyrmecinae are described in Table S2. For *Tachigali*, we designed a new primer for ITS1 (ITS1-T-

Fw: CCCATCGATCACGAAAGAACGACC; ITS2-T_R: GATATCCGTTGCCGAGAGTC) using FastPCR v. 6.5.13 [67]. To amplify *Tachigali trnL-trnF* region that comprises the *trnL* intron and the *trnL-trnF* spacer, we used C/D (C (Fw): CGAAATCGGTAGACGCTACG; D (Rev): GGGGATAGAGGGACTTGAAC) and E/F primers, (E (Fw): GGTTCAAGTCCCTCTATCCC; F (Rev): ATTTGAACTGGTGACACGAG): respectively [68]. To amplify *matK*, we used the F1/R2 primers (F1(Fw): GGTTTGCACTCATTGTGGAAATTCC; R2(Rev): AAGATGTTAATCGTAAATGAGAAG) [69]. To amplify the *psbA-trnH* spacer, we used PsbA (PsbA(Fw): GTTATGCATGAACGTAATGCTC) primer [70] and trnH2 (trnH2(Rev): (CGCGCATGGTGGATTCACAATCC) [71]. All sequences were manually edited in Sequencher v. 4.1 [72] for *Tachigali* and in Sequencher v5.2.2 [73] for Pseudomyrmecinae sequences. All sequences of *Tachigali* or Pseudomyrmecinae generated were BLAST-searched to check for potential contamination before incorporation into a DNA matrix.

(b) Additional information on DNA sequence alignment and phylogenetic analyses

For the five plant datasets, sequence alignments were performed in MAFFT vs. 7 [33], under standard settings except for the ITS region aligned using the Q-INS-S option, which takes into consideration RNA secondary structure and which is recommended for this marker. Each of the ten individual Pseudomyrmecinae DNA fragments were aligned in ClustalX v.2.1 [34]. All individual alignments were manually screened for minor alignment errors and edited in Mesquite v. 2.75 [35] for the plant datasets and in MacClade v. 4.08 [36] for the Pseudomyrmecinae. To detect potential statistically supported incongruence, we inferred gene trees for each markers individually using RAxML v. 8.1 [37] for the plant datasets and in GARLI v. 2.0 [38] for the Pseudomyrmecinae. In the absence of statistically supported incongruence (i.e. ML BS>70%), we concatenated the datasets manually in Mesquite v. 2.75 (plants) or MacClade v. 4.08 (Pseudomyrmecinae). Combined alignment lengths were 7293 nucleotides for the Pseudomyrmecinae, 5301 for the *Triplaris/Ruprechtia* clade, 4197 for *Vachellia*, 2675 for *Platymiscium*, and 2181 for *Tachigali*. For the Pseudomyrmecinae, introns of protein-coding genes and two hypervariable regions of

28S were excluded, yielding unproblematic alignment of the remaining ant sequences. Implementation of AIC criterion in jmodeltest2 [41] selected HKY as for *matK*, trnL-trnF spacer and for *psbA-trnH*, JC for *ITS* and trnL intron. We used this five partitions scheme in MrBayes 3.2 [40] (nst =1 for JC and nst =2 for HKY), but the topology and support were equivalent when all five markers were partitioned under GTR+G. The two main *Tachigali* clades recovered are further supported by flower morphology [57].

(c) Additional information on dating analyses

Data were not partitioned because the assumption of independence among loci may cause spurious precision in divergence time estimates [74-75]. Our species sampling of 61-78% of all involved clades is sufficiently dense to guard against biases due to undersampling of extant species, especially given that we used Bayesian dating [76].

To calibrate the Pseudomyrmecinae phylogeny, we used four fossil constraints. (i) *Pseudomyrmex baros* from Dominican amber (15-25 Ma) [77-78] resembles extant *P. haytianus* [79], and we therefore used this fossil to calibrate the node representing the most recent common ancestor of *P. haytianus* and its sister group (the *goeldii* group), assigning it a gamma prior (offset = 15 Ma; shape $\alpha = 3$; scale $\beta = 3.8$, median = 25 Ma). (ii) *Pseudomyrmex antiquus*, a fossil from Dominican amber, is morphologically similar to the extant *P. rufomedius* [79]. We therefore used *P. antiquus* to calibrate the stem node of the *P. rufomedius* clade assigning it the same gamma prior as for *P. baros*. (iii) To calibrate the crown node of the Pseudomyrmecinae, we used their oldest available fossils, a series of *Tetraponera* from Oise amber (53-65 Ma, [80]), thought to represent the Pseudomyrmecinae crown group (Cédric Aria, University of Toronto, pers. comm. to PSW on 29 April 2015; [81]), and we assigned it a normal prior (mean = 55 Ma; stdev = 5, truncated to 55 Ma) that allowed the ages to fall between 55 and 70 Ma. (iv) The oldest known *Pseudomyrmex* is an impression fossil, *P. saxalum*, from the Middle Eocene (ca. 46 Ma) Kishenehn formation of Northwestern Montana (USA) [82]. This taxon cannot be assigned to any extant species group, however, so we used it to calibrate the *Pseudomyrmex* stem group. We gave this node a broad gamma prior (offset = 46 Ma; shape $\alpha = 2$; scale $\beta = 5$, median = 54 Ma) so that the ages fall between 46 and 70 Ma.

To calibrate the *Triplaris*/*Ruprechtia* tree, we constrained the root node, the split between Brunnichieae and its sister clade, to an age of 69.1 ± 25 Ma based on [83] using a normal distribution (mean = 69.1 Ma, stdev = 5). In addition, we used an 8-12 Ma *Ruprechtia* fossil fruit from the Cuenca basin in Southern Ecuador that is similar to *R. aperta* [65,84]. We used this fossil to constrain the age of the clade containing *R. aperta*, *R. albida*, *R. latifunda* and *R. lundii*, using a gamma prior (offset = 8 Mry, $\alpha = 2$; $\beta = 2.4$). For *Platymiscium*, we constrained the split between *Riedellia* and its sister clade to 47.2 ± 5 Ma based on [85] using a normal distribution (mean = 47.2 Ma, stdev = 3). To calibrate *Vachellia*, we constrained the split between the (*Vachellia constricta* (*V. schottii* (*V. vernicosa*)) clade and its sister group, which includes the myrmecophyte clade, to an age of 12.3 ± 3 Ma based on [18] using a normal distribution (mean = 12.3 Ma, stdev = 2). To calibrate the *Tachigali* tree, we first searched an appropriate outgroup by inserting our *Tachigali matK* sequences into a large Fabaceae matrix [86] and conducting a tree search in RAxML. Based on the results, we selected *Peltophorum dubium* as an outgroup for *Tachigali* and then constrained the corresponding node to 40.3 ± 10 Ma based on [85] using a wide normal prior (mean = 40.3 Ma; stdev =5).

(d) Additional information on ancestral state reconstructions

We generated ancestral state reconstructions of nesting habit in Pseudomyrmecinae and domatium evolution in our five focal plant groups using three approaches: (i) the ML approach implemented in Mesquite v. 2.75 [35], (i) the Bayesian reverse jump MCMC approach implemented in BayesTraits [49] and (i) the discrete ML approach implemented in the function Ace of the R package Ape [48]. We moreover used as three types of imput trees: (i) the maximum credibility tree from BEAST, (ii) a sample of 1000 bayesian trees from BEAST and (iii) additionally, for Pseudomyrmecinae only, the best ML tree from GARLI. This array of methods allowed us to test for the robustness of our ancestral state inferences in a wide array of model complexity, across the topological uncertainty, and in the case of Pseudomyrmecinae, to test if ultrametric and ML trees gave similar results. The Maximum Likelihood approach implemented in Mesquite relied on the Markov one-parameter model (MK1), which assumes that all character state transition rates are equal [87]; as well as the MK2 model (for plant clade only, since only two character states are allowed in this model), which allow backward and forward rates to be different [87]. The ML approach implemented in the Ace function of Ape included three models: (i) Equal rates (ER) model, wherein all rates are equal, as in the MK1 model; (ii) Symmetrical rate model (SYM), wherein the backward and forward character state transition rates are equal for each combination of character states, but each distinct state combination can have a distinct rate; (iii) All rate different (ARD), wherein all rates are allowed to vary. The Ace function of Ape moreover gives a general statistic for each model, which can be compared. In all cases, we obtained the best likelihood for the ARD model, although the differences were in most cases subtle. For the Pseudomyrmecinae only, we further tested whether reconstruction on the chronogram was similar to that on the ML tree, since it has been suggested that the branch length model can sometimes affect the results [88], and our results were entirely comparable. Our results were consistent across this array of ancestral state model complexity, tree topological uncertainties and branch length model (Fig. S5-S10).

We used stochastic character mapping to infer possible histories of domatium evolution and obligate domatium nesting not only at nodes but also along branches in the phylogenies. We used the function make.simmap in the phytools package (v. 04-60) [89], which implements the stochastic character mapping approach developed by [90]. We estimated ancestral states using an equal rate model, and then simulated 1,000 character histories on the maximum clade credibility trees form BEAST. For the Pseudomyrmecinae, we used a binary coding with plant-ant (obligate mutualistic domatium nesting, coded ‘1’) opposed to all other nesting strategies (arboreal generalist, parasitic, or ground-nesting, coded ‘0’). We summarized the 1,000 simulated character histories using the function densityMap (also in phytools). All density plots are shown in Fig. S12.

(d) Additional information on biogeography and range size determination

To infer whether (i) ancestral areas of Pseudomyrmecinae clades match those of their plant hosts and whether (ii) our focal symbioses coincide with geographic range shifts, we used ancestral range reconstruction (back to 33.7 Ma) using the R package BioGeoBEARS [28-29] on the BEAST chronograms. BioGeoBEARS infers ancestral geographic ranges and permits comparison of three biogeographic models, namely dispersal-extinction-cladogenesis (DEC), dispersal-vicariance (DIVALIKE), and

BAYAREA (BAYAREALIKE). Founder-event speciation is modeled via a speciation parameter j that can be added to each of the models. We selected the best model based on LogLikelihood values as well as the Akaike Information Criterion (ΔAICc). For the DEC model, we defined a dispersal matrix wherein two adjacent (physically connected) regions have a dispersal probability of 1, whereas non-connected areas have a probability of 0.001.

The sister group comparisons consisted of the following taxa: (i) for the *P. ferrugineus* group, we used *P. perboscii*, *P. haytianus* and the 3 species from the *P. goeldii* group (5 species) as sister in order to test for significant difference in range size; removing the two generalists that belong to the *P. ferrugineus* group (*P. psw0054* and *P. psw002*) does not change the results; (ii) for the *P. triplarinus* group, the sister group consisted of the *P. oculatus* group (9 species) and (iii) for the *P. concolor* group, the sister consisted of *P. kuenckeli* and the *P. pallens* group. Since we inferred range contractions associated with the evolution of specialized plant-ants in our ancestral area reconstruction in BioGeoBEARS, we determined range size based on occurrence data to validate these potential range contractions. There are two basic measures of range size, the extent of occurrence (EOO), which can be thought as “a minimum convex polygon or convex hull embracing the occurrences of a species” [91] and the area of occupancy (AOO), which is calculated as the sum of grid cells where a species is present. Because the AOO is more sensitive to sampling density, but also because we are interested in the maximal extent of species occurrences, we here used the EOO. The occurrence data used comes from a database of Pseudomyrmecinae occurrence assembled over the last decades by PSW; the data used for the analyses is shown in Table S8. We calculated the EOO in DIVA-GIS using the ‘points to convex polygon’ function to obtain a shapefile with the EOO, then convert it in a gridfile using the ‘polygon to grid’ function, and finally measured the polygon using the describe grid function. The number of cells obtained was converted in km^2 by multiplying by the grid resolution used (2.5’). Significance for differences among sister groups was probed using Mann-Whitney Wilcoxon test in R (the R project for statistical computing).

Supplementary references

64. Brandbyge J. 1986 A revision of the genus *Triplaris* (Polygonaceae). *Nord. J. Bot.* **6**, 545-570.

65. Pendry CA. 2004 Monograph of *Ruprechtia* Polygonaceae. *Syst. Bot. Monographs* 67, 1-113.

66. Klitgaard BB. 2005 *Platymiscium* (Leguminosae: Dalbergieae): Biogeography, systematics, morphology, taxonomy and uses. *Kew Bull.* **60**, 321-400.

67. Kalendar R, Lee D, Schulman AH. 2011 Java web tools for PCR, *in silico* PCR, and oligonucleotide assembly and analysis. *Genomics* **98,** 137-144.

68. Taberlet P, Gielly L, Pautou G, Bouvet J. 1991 Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **17**, 1105-1109.

69. Yokoyama J, Suzuki M, Iwatsuki K, Hasebe M. 2000 Molecular phylogeny of *Coriaria*, with special emphasis on the disjunct distribution. *Mol. Phylogenet. Evol.* **14**, 11-19.

70. Sang T, Crawford D, Stuessy T. 1997 Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *Am. J. Bot.* **84**, 1120-1120.

71. Tate JA, Simpson BB 2003 Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Syst. Bot.* **28**, 723-737.

72. Codes G. 2000 Sequencher: Version 4.1.2. Gene Codes Corporation. *Ann Arbor*.

73. Codes G. 2013 Sequencher: Version 5.2.2. Gene Codes Corporation. *Ann Arbor*.

74. Dos Reis M, Zhu T, Yang Z. 2014 The impact of the rate prior on Bayesian estimation of divergence times with multiple loci. *Syst. Biol.* **63**, 555-565 doi: 10.1093/sysbio/syu020.

75. Zhu T, dos Reis M, Yang Z. 2015 Characterization of the uncertainty of divergence time estimation under relaxed molecular clock models using multiple loci. *Syst. Biol.* **64**, 267-280 doi: 10.1093/sysbio/syu109.

76. Linder HP, Hardy CR, Rutschmann F. 2005 Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Mol. Phylogenet. Evol.* **35**: 569-582.

77. Lambert JB, Frye JS, Poinar GO. 1985 Amber from the Dominican Republic: analysis by nuclear magnetic resonance spectroscopy. *Archaeometry* **27**, 43-51.

78. Iturralde-Vincent MA, MacPhee RDE. 1996 Age and paleogeographical origin of Dominican amber. *Science* **273**, 1850-1852.

79. Ward PS. 1992 Ants of the genus *Pseudomyrmex* (Hymenoptera: Formicidae)

from Dominican amber, with a synopsis of the extant Antillean species. *Psyche* **99**, 55-85.

80. Nel A, Brasero N. 2010 Oise amber. In D. Penneys ed. *Biodiversity of Fossils in Amber from the Major World Deposits*, pp. 137-148, Siri Scientific Press, Rochdale, UK.
81. Aria C, Perrichot V, Nel, A. 2011 Fossil Ponerinae (Hymenoptera: Formicidae) in Early Eocene amber of France. *Zootaxa* **2870**, 53-62.
82. Lapolla JS, Greenwalt DE. 2015 Fossil Ants (Hymenoptera: Formicidae) of the Middle Eocene Kishenehn Formation. *Sociobiology* **62**, 163-174.
83. Schuster TM, Setaro, S. D., & Kron, K. A. 2013 Age estimates for the buckwheat family Polygonaceae based on sequence data calibrated by fossils and with a focus on the Amphi-Pacific *Muehlenbeckia*. *PLoS ONE*, *8*(4), e61261.
84. Burnham RJ, Graham A. 1999 The history of Neotropical vegetation: new developments and status. *Ann. Mo. Bot. Gard.* **86**, 546-589.
85. Lavin M, Herendeen PS, Wojciechowski MF. 2005 Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Syst. Biol.* **54**, 575-594.
86. Wojciechowski MF, Lavin M, Sanderson MJ. 2004 A phylogeny of legumes (Leguminosae) based on analysis of the plastid matK gene resolves many well-supported subclades within the family. *Am. J. Bot.* **9111**, 1846-1862.
87. Lewis PO. 2001 A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* **50**, 913-925.
88. Cusimano N, Renner SS. 2014 Ultrametric trees or phylograms for ancestral state reconstruction: does it matter? *Taxon* **63**, 721-726.
89. Revell LJ. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217-223.
90. Bollback, J. P. (2006). SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC bioinformatics* **7**, 88.
91. Gaston KJ, Fuller RA. 2009 The sizes of species' geographic ranges. *J. Appl. Ecology*, *46*(1), 1-9.

Figure S1. Maximum likelihood phylogeny of the ant subfamily Pseudomyrmecinae estimated from a partitioned analysis using GARLI 2.0. This tree has -lnL = 79457.54. Values above branches or at nodes show the ML bootstrap from 100 replicates. Values are shown for the ingroup when there are greater than 50%.

Figure S2. Phylogeny of the ant subfamily Pseudomyrmecinae estimated from a partitioned Bayesian analsysis using MrBayes 3.2.3. Node support is given as a Bayesian posterior probability at nodes (see Materials and methods for MrBayes parameters).

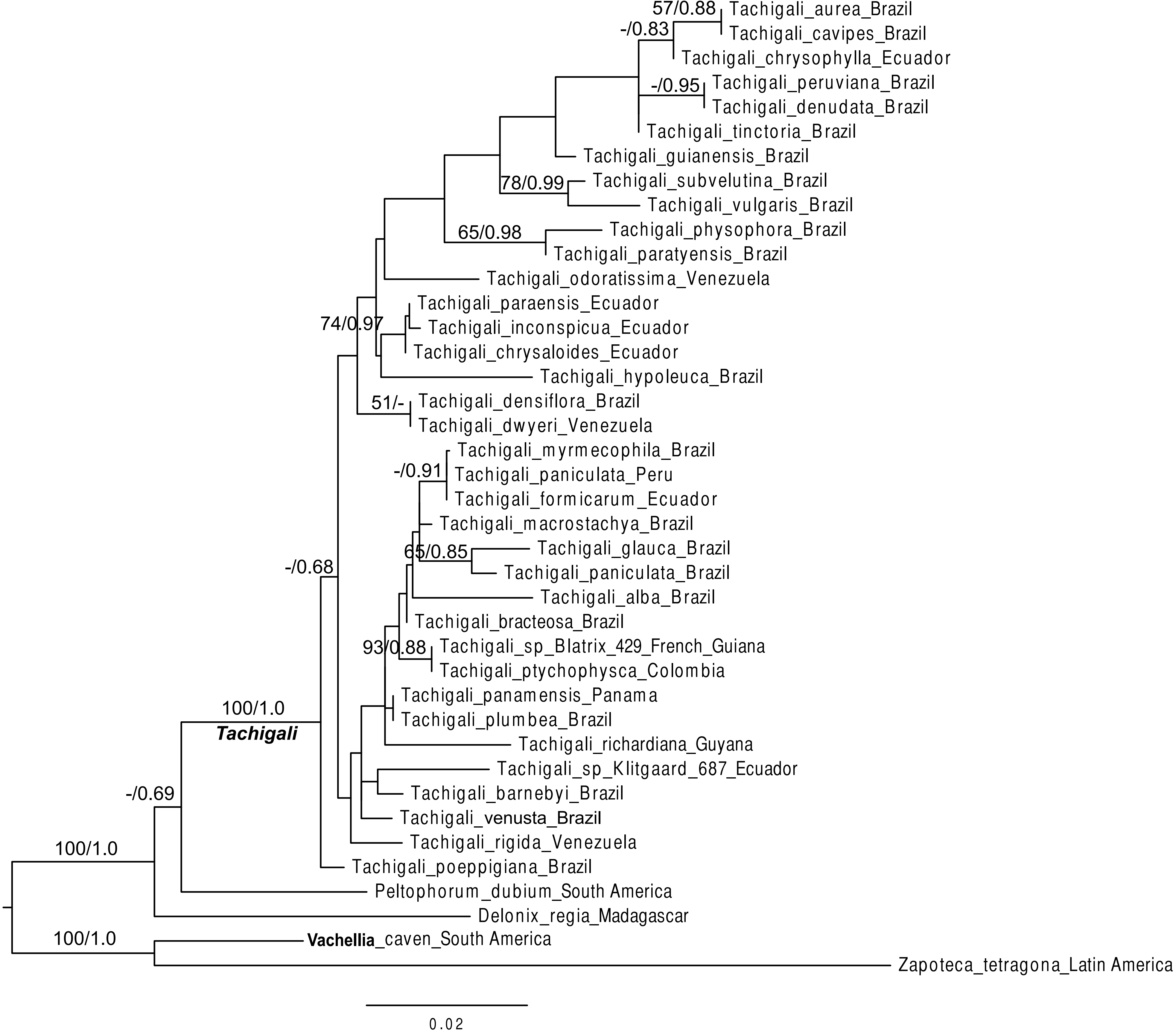


Figure S3: Maximum likelihood tree for *Tachigali* (Fabaceae). Number above branches shows bootstrap support based on 100 replicates in RAxML and posterior probability based on MrBayes runs.

Figure S4: Pseudomyrmecinae molecular clock-dated tree.

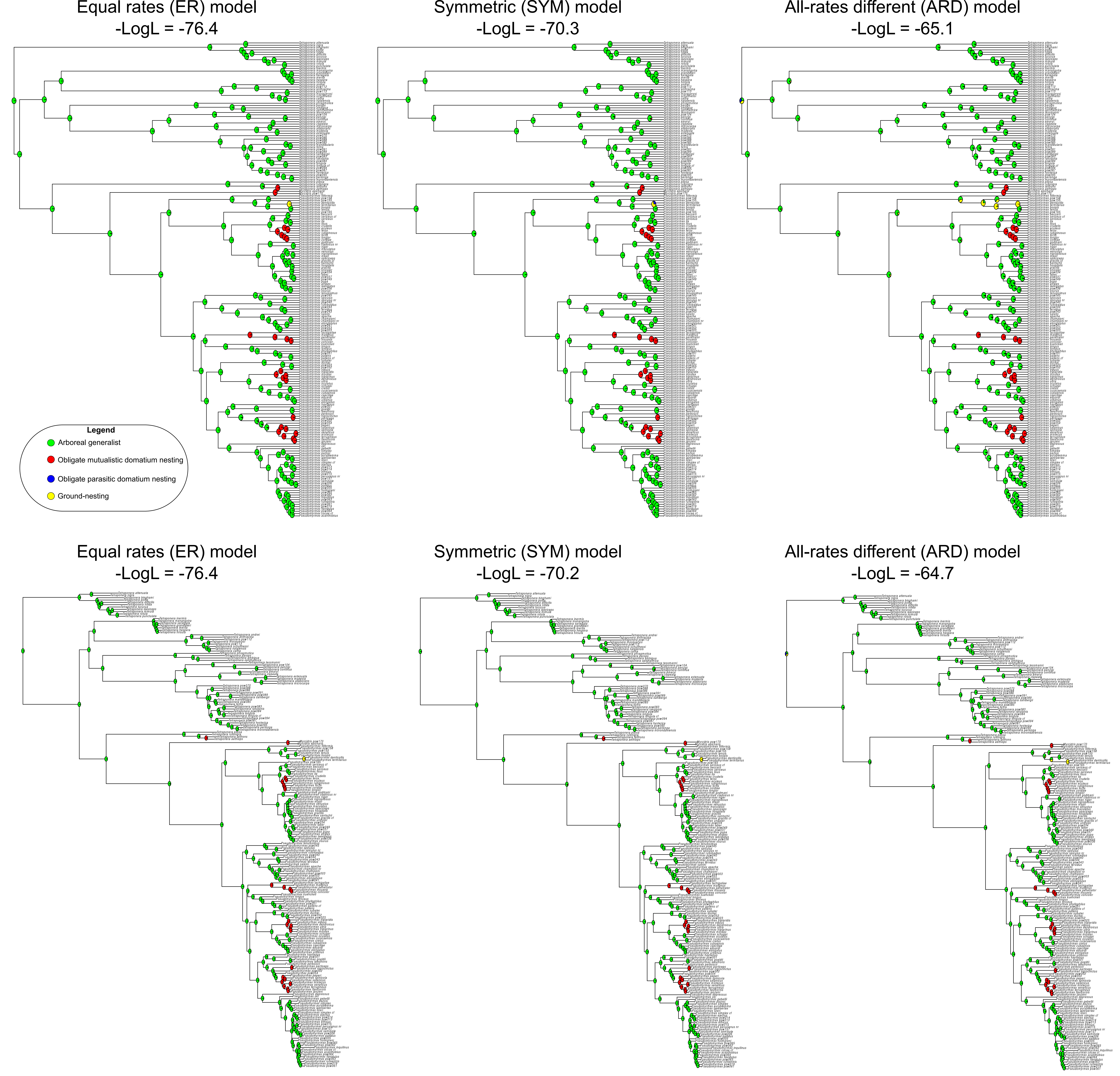


Figure S5: Reconstruction of Pseudomyrmecinae nesting behavior using the Ace function in Ape.

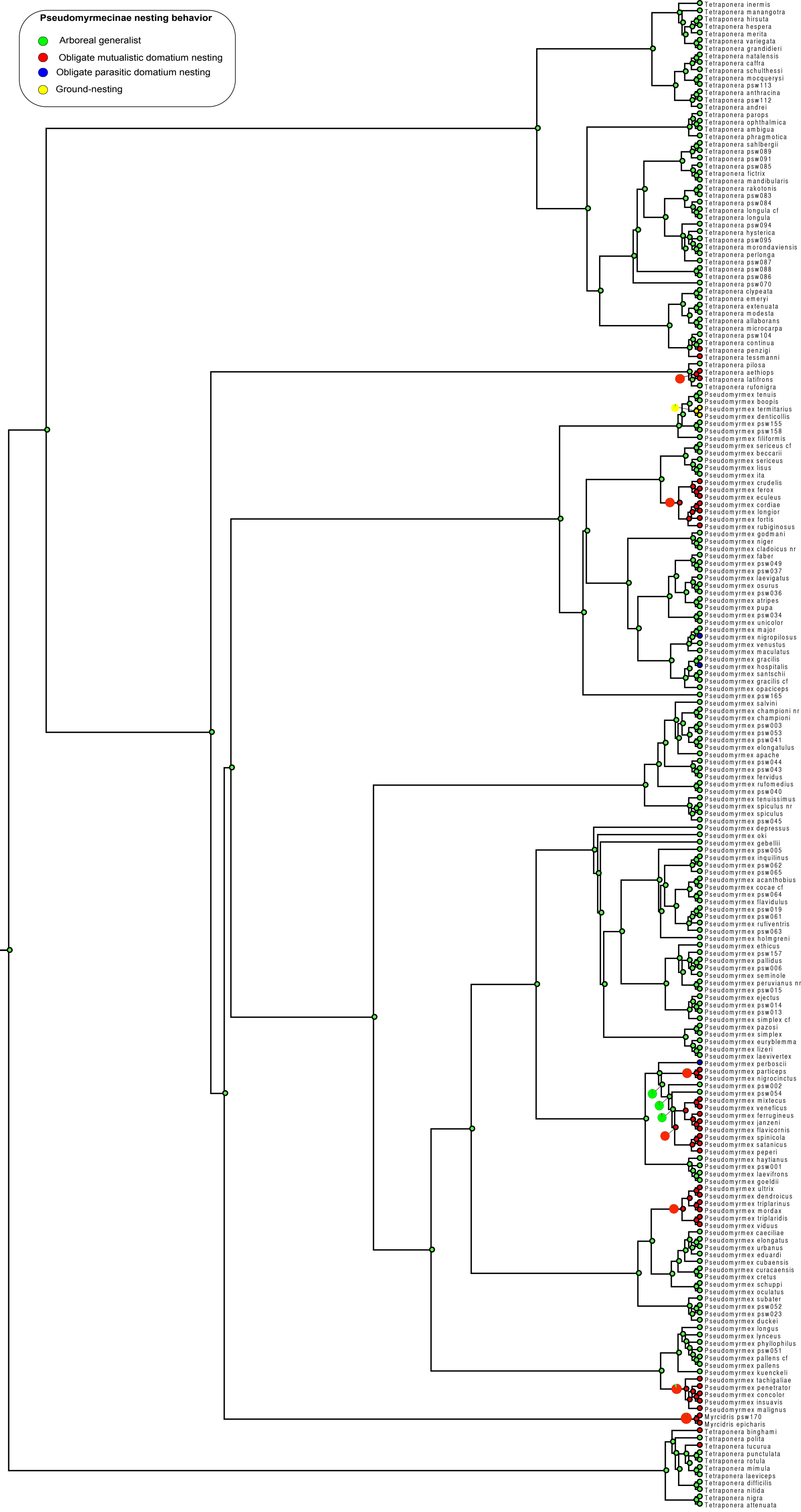
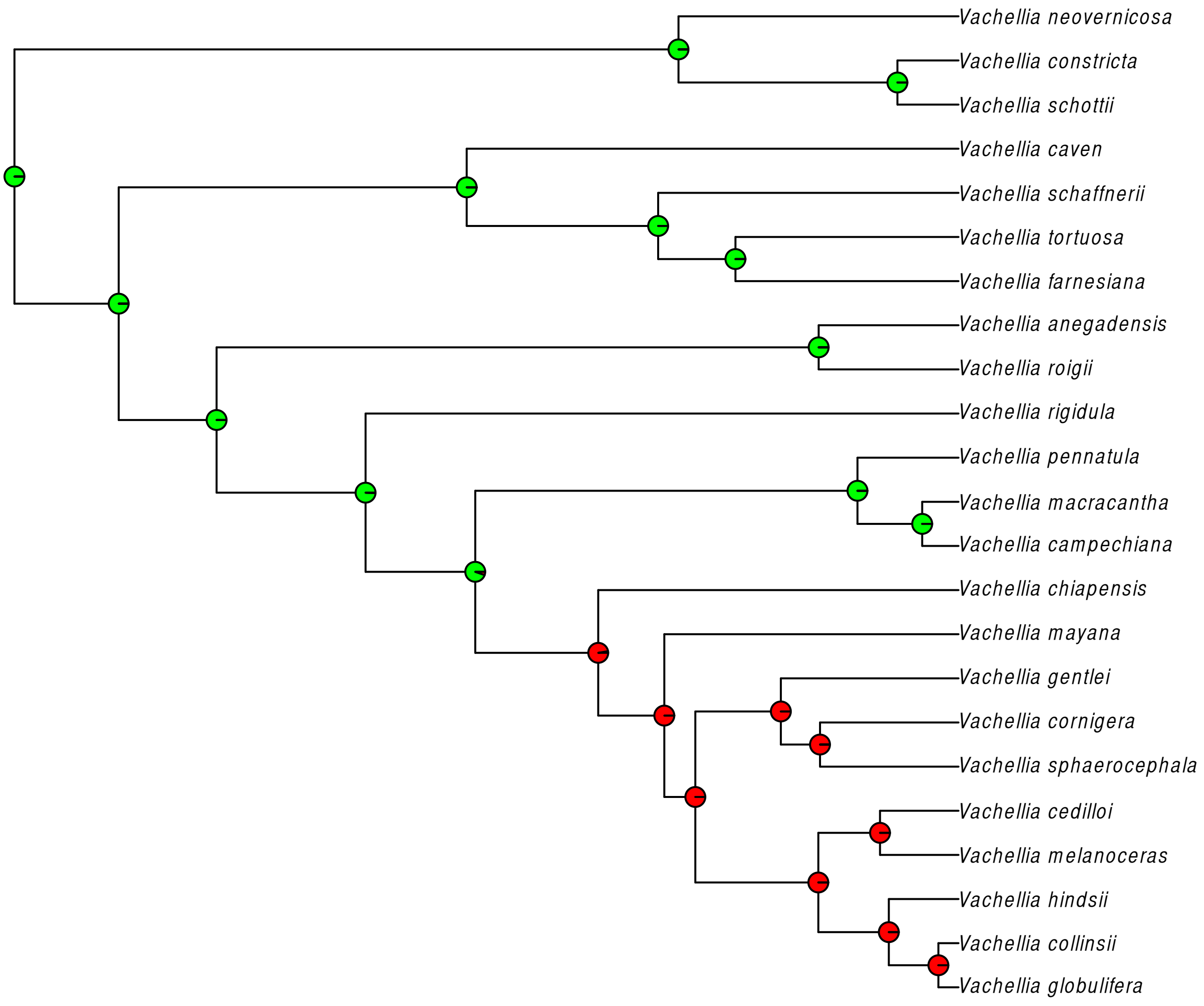
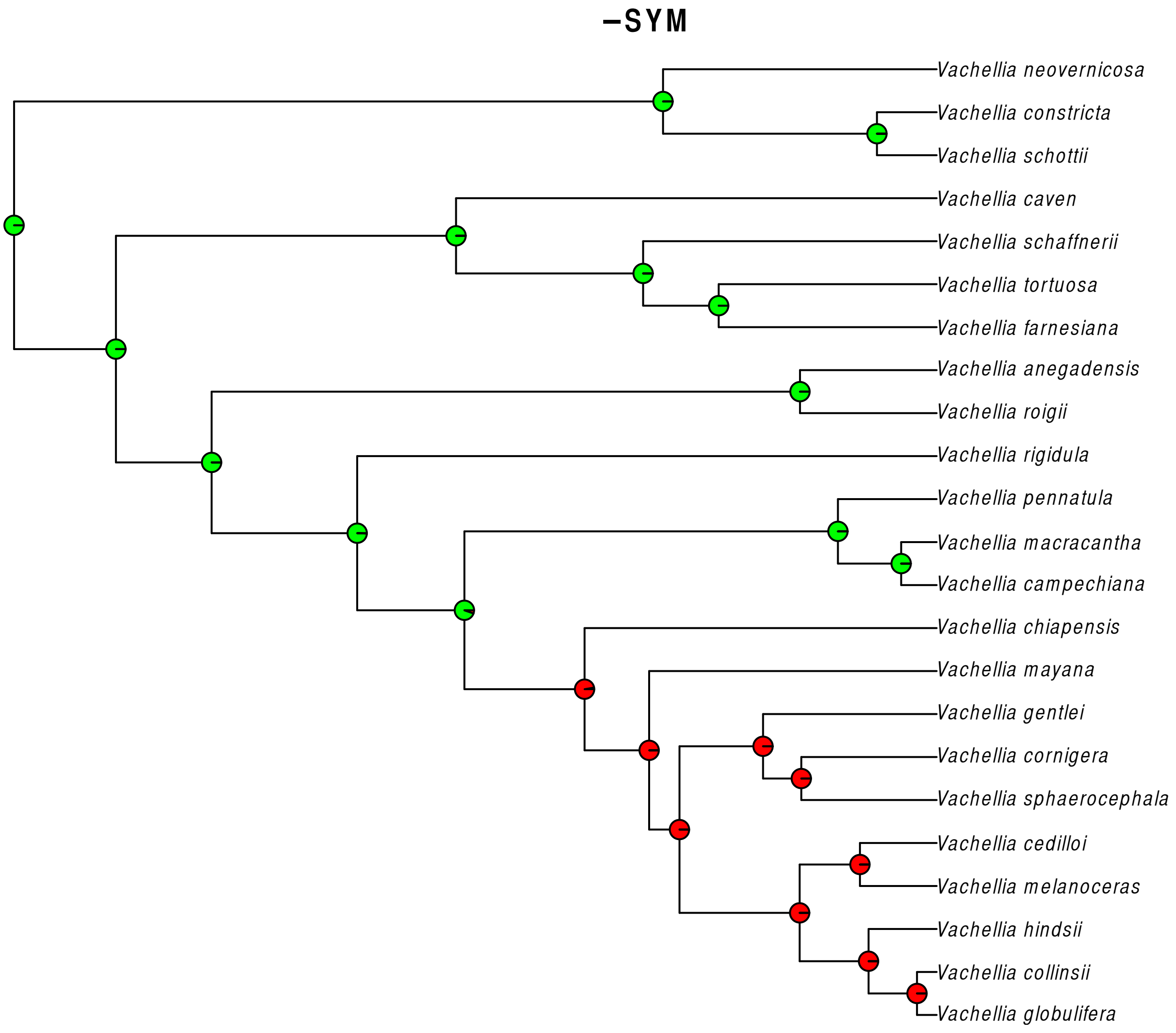


Figure S6. Ancestral state reconstruction of nesting habit using the MK1 model in Mesquite and a reverse MCMC approach in BayesTraits (big pies).

Equal rate model (ER)
LnL = -5.04



Symmetric rate model (SYM)
LnL = -5.04



All rates different model (ARD)
LnL = -4.81

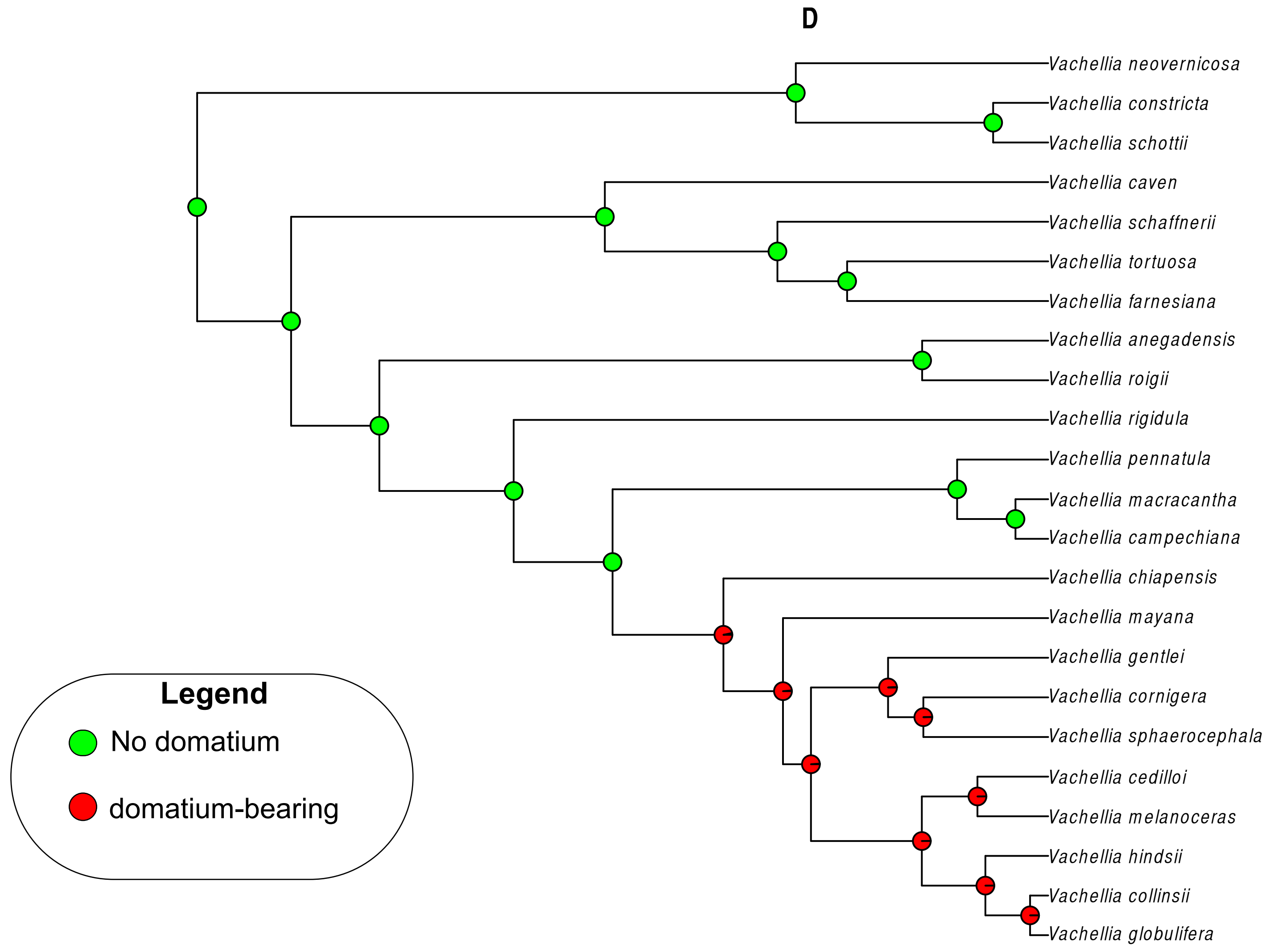
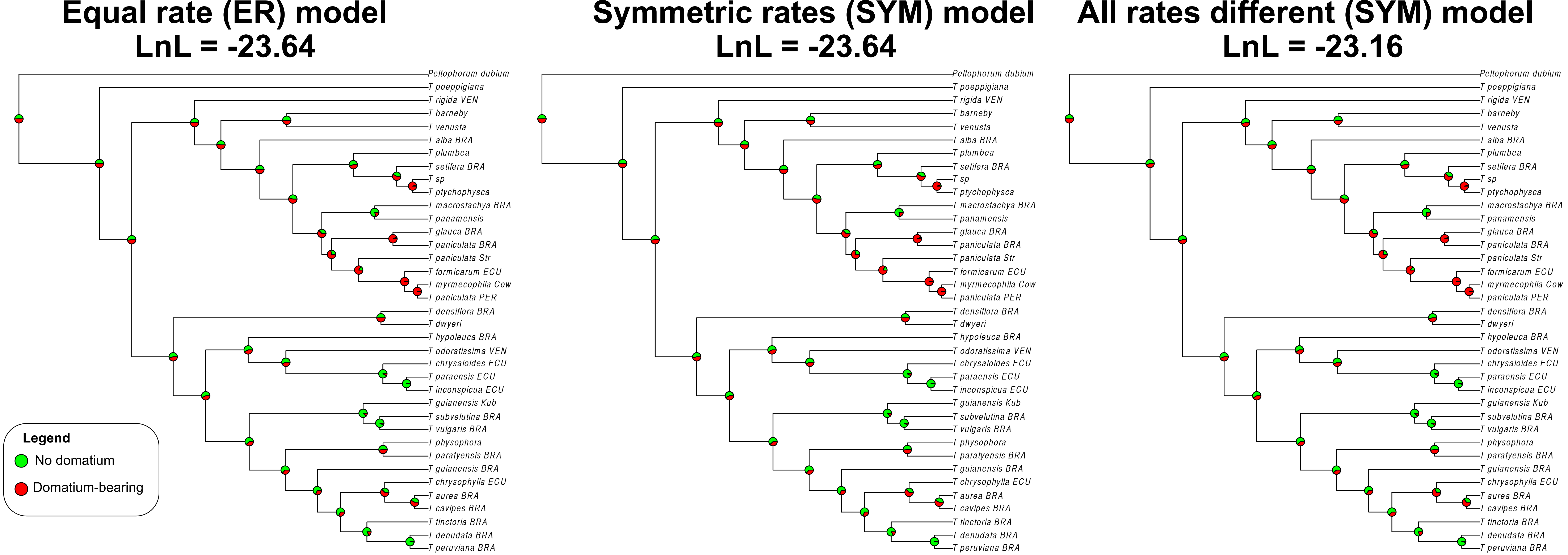


Figure S7: *Vachellia* ancestral state reconstruction of domatium evolution using the Ace function in Ape.

Figure S8: *Triplaris/Ruprechtia* ancestral state reconstruction of domatium evolution using the Ace function in Ape.

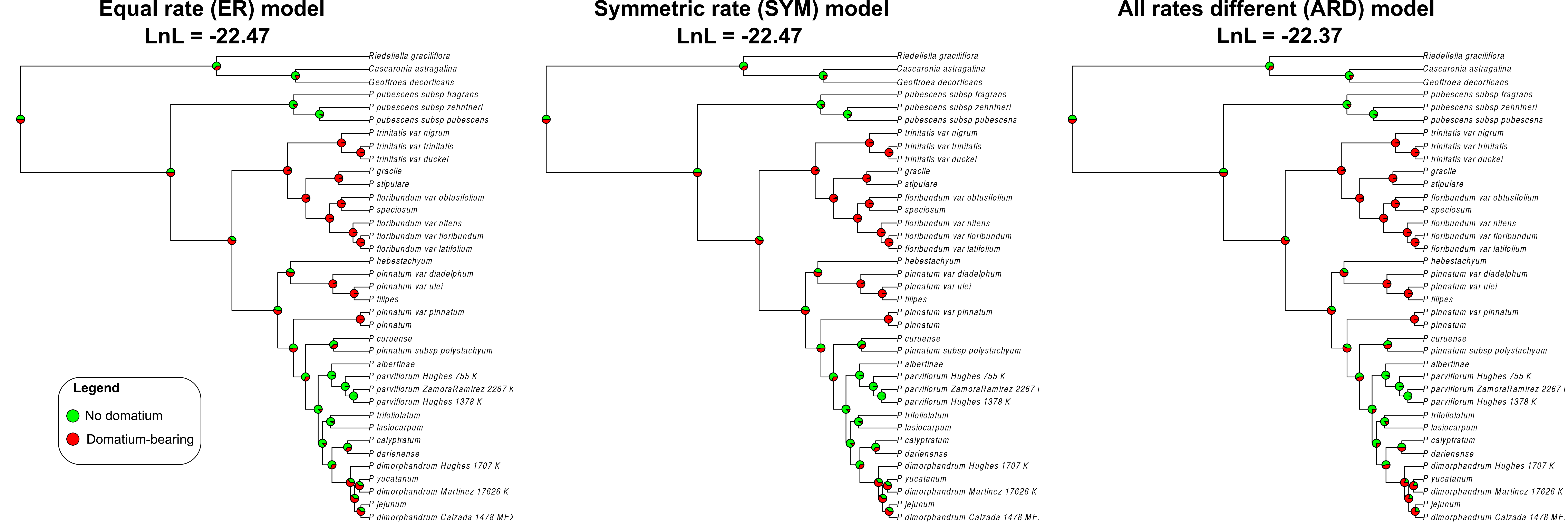


Equal rate (ER) model
LnL = -23.64

Symmetric rates (SYM) model
LnL = -23.64

All rates different (SYM) model
LnL = -23.16

Figure S9: *Tachigali* ancestral state reconstruction of domatium evolution using the Ace function in Ape.


Equal rate (ER) model
LnL = -22.47

Symmetric rate (SYM) model
LnL = -22.47

All rates different (ARD) model
LnL = -22.37

Figure S10: *Platymiscium* ancestral state reconstruction of domatium evolution using the Ace function in Ape.

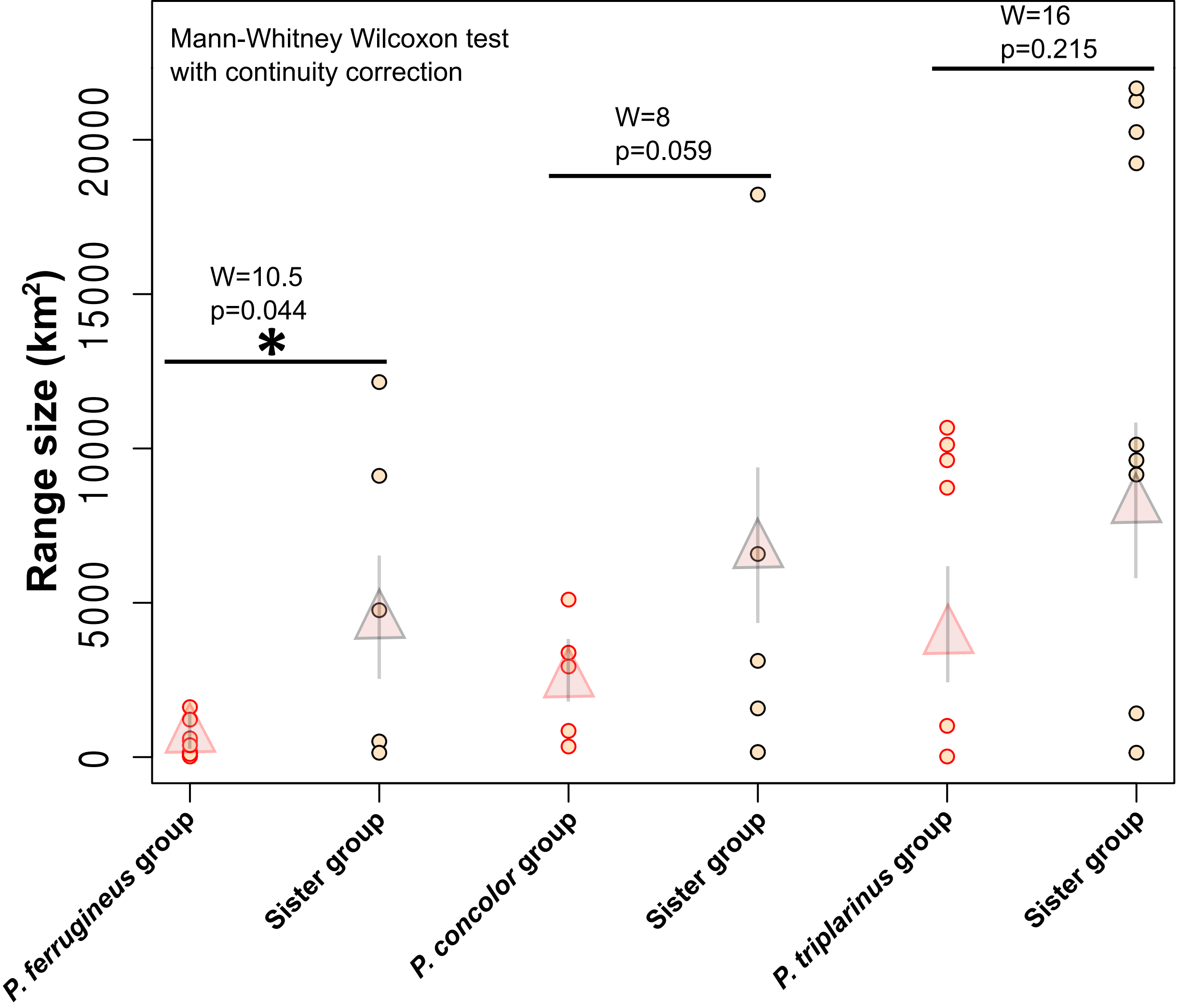


Figure S11: Range sizes in *Pseudomyrmex* plant-ants and their sister groups. The triangle indicates the mean for each group. (a) *Pseudomyrmex ferrugineus* group. (b) *P. triparinus* group, excluding *P. viduus* which is not a *Triplaris* specialist. (c) *P. concolor* group. The "sister group" of the *P. ferrugineus* group includes the immediate sister taxon (a single species, *P. perboscii*) and the next most closely related clade (see Supplementary Materials and Methods).

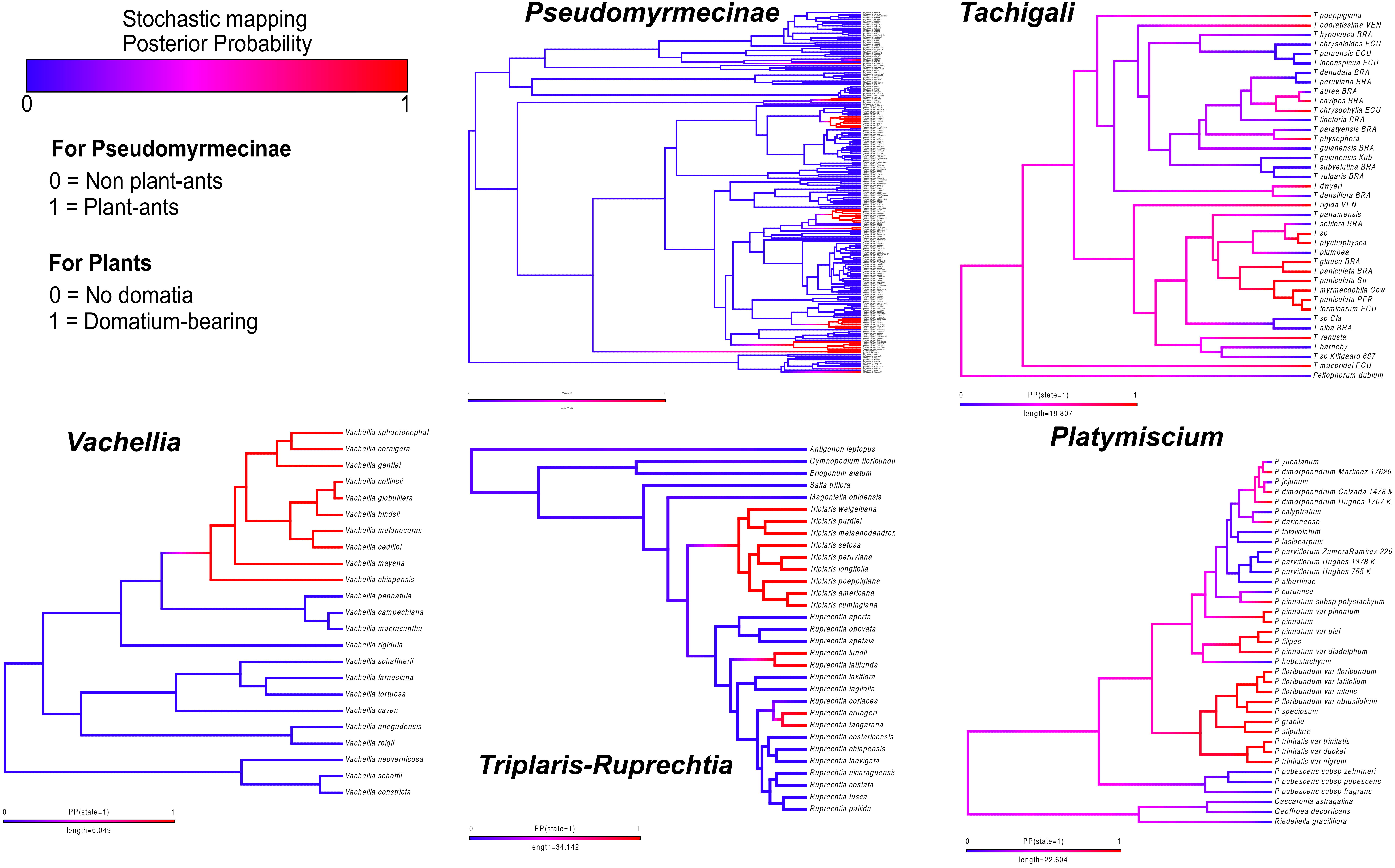


Figure S12: Stochastic trait mapping probability density maps for the traits ‘plant nesting behavior’ and ‘domatium presence’.

Table S1. Taxon names, voucher specimen codes, and GenBank accession numbers for the Pseudomyrmecinae and outgroups. Full collection data for each sample can be obtained by searching on the voucher specimen code in AntWeb (www.antweb.org).

Taxon	Voucher	28S	Wg	Abd-A	LW Rh	EF1aF2	ArgK	Enolase	CAD	Top1	Ubx
Myrcidris_epicharis	CASENT0106096	AY703584	KR828975	AY703718	KR829348	EF013453	KR829767	KR829974	KR830182	KR830598	KR830389
Myrcidris_psw170	CASENT0220647	KR828817	KR828976	KR829190	KR829349	KR829563	KR829768	KR829975	KR830183	KR830599	KR830390
Pseudomyrmex_acanthobius	CASENT0173746	KR828818	KR828977	KR829191	KR829350	KR829564	KR829769	KR829976	KR830184	KR830600	KR830391
Pseudomyrmex_apache	CASENT0220657	AY703585	KR828978	AY703719	KR829351	KR829565	KR829770	KR829977	KR830185	KR830601	KR830392
Pseudomyrmex_atripes	CASENT0220665	KR828819	KR828979	KR829192	KR829352	KR829566	KR829771	KR829978	KR830186	KR830602	KR830393
Pseudomyrmex_beccarii	CASENT0220713	KR828820	KR828980	KR829193	KR829353	KR829567	KR829772	KR829979	KR830187	KR830603	KR830394
Pseudomyrmex_boopis	CASENT0220727	AY703586	KR828981	AY703720	KR829354	KR829568	KR829773	KR829980	KR830188	KR830604	KR830395
Pseudomyrmex_caeciliae	CASENT0103315	KR828821	KR828982	KR829194	KR829355	KR829569	KR829774	KR829981	KR830189	KR830605	KR830396
Pseudomyrmex_championi	CASENT0220658	KR828822	KR828983	KR829195	KR829356	KR829570	KR829775	KR829982	KR830190	KR830606	KR830397
Pseudomyrmex_championi_nr	JTLC000010310	KR828823	KR828984	KR829196	KR829357	KR829571	KR829776	KR829983	KR830191	KR830607	KR830398
Pseudomyrmex_cladoicus_nr	CASENT0106336	KR828824	KR828985	KR829197	KR829358	KR829572	KR829777	KR829984	KR830192	KR830608	KR830399
Pseudomyrmex_cocae_cf	CASENT0220686	KR828825	KR828986	KR829198	KR829359	KR829573	KR829778	KR829985	KR830193	KR830609	KR830400
Pseudomyrmex_concolor	CASENT0220648	AY703587	KR828987	AY703721	KR829360	KR829574	KR829779	KR829986	KR830194	KR830610	KR830401
Pseudomyrmex_cordiae	CASENT0106768	AY703588	KR828988	AY703722	KR829361	KR829575	KR829780	KR829987	KR830195	KR830611	KR830402
Pseudomyrmex_cretus	CASENT0220674	KR828826	KR828989	KR829199	KR829362	KR829576	KR829781	KR829988	KR830196	KR830612	KR830403
Pseudomyrmex_crudelis	CASENT0902863	KR828827	KR828990	KR829200	KR829363	KR829577	KR829782	KR829989	KR830197	KR830613	KR830404
Pseudomyrmex_cubaensis	CASENT0220675	AY703589	KR828991	AY703723	KR829364	KR829578	KR829783	KR829990	KR830198	KR830614	KR830405
Pseudomyrmex_curacaensis	CASENT0220676	KR828828	KR828992	KR829201	KR829365	KR829579	KR829784	KR829991	KR830199	KR830615	KR830406
Pseudomyrmex_dendroicus	CASENT0220733	AY703590	KR828993	AY703724	KR829366	KR829580	KR829785	KR829992	KR830200	KR830616	KR830407
Pseudomyrmex_denticollis	CASENT0220728	KR828829	KR828994	KR829202	KR829367	KR829581	KR829786	KR829993	KR830201	KR830617	KR830408
Pseudomyrmex_depressus	CASENT0221016	KR828830	KR828995	KR829203	KR829368	KR829582	KR829787	KR829994	KR830202	KR830618	KR830409
Pseudomyrmex_duckei	CASENT0220653	KR828831	KR828996	KR829204	KR829369	KR829583	KR829788	KR829995	KR830203	KR830619	KR830410
Pseudomyrmex_eculeus	CASENT0220714	KR828832	KR828997	KR829205	KR829370	KR829584	KR829789	KR829996	KR830204	KR830620	KR830411
Pseudomyrmex_eduardi	CASENT0005873	KR828833	KR828998	KR829206	KR829371	KR829585	KR829790	KR829997	KR830205	KR830621	KR830412
Pseudomyrmex_ejectus	CASENT0220687	KR828834	KR828999	KR829207	KR829372	KR829586	KR829791	KR829998	KR830206	KR830622	KR830413
Pseudomyrmex_elongatulus	CASENT0217577	AY703592	KR829000	AY703726	KR829373	KR829587	KR829792	KR829999	KR830207	KR830623	KR830414
Pseudomyrmex_elongatus	CASENT0220677	KR828835	KR829001	KR829208	KR829374	KR829588	KR829793	KR830000	KR830208	KR830624	KR830415
Pseudomyrmex_ethicus	CASENT0106770	KR828836	KR829002	KR829209	KR829375	KR829589	KR829794	KR830001	KR830209	KR830625	KR830416
Pseudomyrmex_euryblemma	CASENT0220688	KR828837	KR829003	KR829210	KR829376	KR829590	KR829795	KR830002	KR830210	KR830626	KR830417
Pseudomyrmex_faber	CASENT0005836	KR828838	KR829004	KR829211	KR829377	KR829591	KR829796	KR830003	KR830211	KR830627	KR830418
Pseudomyrmex_ferox	CASENT0220715	KR828839	KR829005	KR829212	KR829378	KR829592	KR829797	KR830004	KR830212	KR830628	KR830419
Pseudomyrmex_ferrugineus	CASENT0220557	KR828840	KR829006	KR829213	KR829379	KR829593	KR829798	KR830005	KR830213	KR830629	KR830420
Pseudomyrmex_fervidus	CASENT0220663	KR828841	KR829007	KR829214	KR829380	KR829594	KR829799	KR830006	KR830214	KR830630	KR830421
Pseudomyrmex_filiformis	CASENT0220738	AY703593	KR829008	AY703727	KR829381	KR829595	KR829800	KR830007	KR830215	KR830631	KR830422
Pseudomyrmex_flavicornis	CASENT0005786	AY703594	KR829009	AY703728	KR829382	KR829596	KR829801	KR830008	KR830216	KR830632	KR830423
Pseudomyrmex_flavidulus	CASENT0220689	KR828842	KR829010	KR829215	KR829383	KR829597	KR829802	KR830009	KR830217	KR830633	KR830424
Pseudomyrmex_fortis	CASENT0220716	KR828843	KR829011	KR829216	KR829384	KR829598	KR829803	KR830010	KR830218	KR830634	KR830425
Pseudomyrmex_gebellii	CASENT0221020	KR828844	KR829012	KR829217	KR829385	KR829599	KR829804	KR830011	KR830219	KR830635	KR830426
Pseudomyrmex_godmani	CASENT0220646	AY703595	KR829013	AY703729	KR829386	KR829600	KR829805	KR830012	KR830220	KR830636	KR830427
Pseudomyrmex_goeldii	CASENT0221019	KR828845	KR829014	KR829218	KR829387	KR829601	KR829806	KR830013	KR830221	KR830637	KR830428

Pseudomyrmex_gracilis	CASENT0106097	AY703596	KR829015	AY703730	KR829388	EF013502	KR829807	KR830014	KR830222	KR830638	KR830429
Pseudomyrmex_gracilis_cf	CASENT0220666	KR828846	KR829016	KR829219	KR829389	KR829602	KR829808	KR830015	KR830223	KR830639	KR830430
Pseudomyrmex_haytianus	CASENT0221014	AY703597	KR829017	AY703731	KR829390	KR829603	KR829809	KR830016	KR830224	KR830640	KR830431
Pseudomyrmex_holmgreni	CASENT0220690	AY703598	KR829018	AY703732	KR829391	KR829604	KR829810	KR830017	KR830225	KR830641	KR830432
Pseudomyrmex_hospitalis	CASENT0005839	KR828847	KR829019	KR829220	KR829392	KR829605	KR829811	KR830018	KR830226	KR830642	KR830433
Pseudomyrmex_inquilinus	CASENT0902926	KR828848	KR829020	KR829221	KR829393	KR829606	KR829812	KR830019	KR830227	KR830643	KR830434
Pseudomyrmex_insuavis	CASENT0220649	KR828849	KR829021	KR829222	KR829394	KR829607	KR829813	KR830020	KR830228	KR830644	KR830435
Pseudomyrmex_ita	CASENT0220717	AY703599	KR829022	AY703733	KR829395	KR829608	KR829814	KR830021	KR830229	KR830645	KR830436
Pseudomyrmex_janzeni	CASENT0221012	KR828850	KR829023	KR829223	KR829396	KR829609	KR829815	KR830022	KR830230	KR830646	KR830437
Pseudomyrmex_kuenckeli	CASENT0220739	AY703600	KR829024	AY703734	KR829397	KR829610	KR829816	KR830023	KR830231	KR830647	KR830438
Pseudomyrmex_laevifrons	CASENT0221017	KR828851	KR829025	KR829224	KR829398	KR829611	KR829817	KR830024	KR830232	KR830648	KR830439
Pseudomyrmex_laevigatus	CASENT0005840	KR828852	KR829026	KR829225	KR829399	KR829612	KR829818	KR830025	KR830233	KR830649	KR830440
Pseudomyrmex_laevivertex	CASENT0220691	KR828853	KR829027	KR829226	KR829400	KR829613	KR829819	KR830026	KR830234	KR830650	KR830441
Pseudomyrmex_lisus	CASENT0220718	KR828854	KR829028	KR829227	KR829401	KR829614	KR829820	KR830027	KR830235	KR830651	KR830442
Pseudomyrmex_lizeri	CASENT0220692	KR828855	KR829029	KR829228	KR829402	KR829615	KR829821	KR830028	KR830236	KR830652	KR830443
Pseudomyrmex_longior	CASENT0220719	KR828856	KR829030	KR829229	KR829403	KR829616	KR829822	KR830029	KR830237	KR830653	KR830444
Pseudomyrmex_longus	CASENT0220680	KR828857	KR829031	KR829230	KR829404	KR829617	KR829823	KR830030	KR830238	KR830654	KR830445
Pseudomyrmex_lynceus	CASENT0220681	KR828858	KR829032	KR829231	KR829405	KR829618	KR829824	KR830031	KR830239	KR830655	KR830446
Pseudomyrmex_maculatus	CASENT0005841	KR828859	KR829033	KR829232	KR829406	KR829619	KR829825	KR830032	KR830240	KR830656	KR830447
Pseudomyrmex_major	CASENT0220667	KR828860	KR829034	KR829233	KR829407	KR829620	KR829826	KR830033	KR830241	KR830657	KR830448
Pseudomyrmex_malignus	CASENT0220650	KR828861	KR829035	KR829234	KR829408	KR829621	KR829827	KR830034	KR830242	KR830658	KR830449
Pseudomyrmex_mixtecus	CASENT0221008	KR828862	KR829036	KR829235	KR829409	KR829622	KR829828	KR830035	KR830243	KR830659	KR830450
Pseudomyrmex_mordax	CASENT0220734	KR828863	KR829037	KR829236	KR829410	KR829623	KR829829	KR830036	KR830244	KR830660	KR830451
Pseudomyrmex_niger	CASENT0106334	KR828864	KR829038	KR829237	KR829411	KR829624	KR829830	KR830037	KR830245	KR830661	KR830452
Pseudomyrmex_nigrocinctus	CASENT0005795	AY703601	KR829039	AY703735	KR829412	KR829625	KR829831	KR830038	KR830246	KR830662	KR830453
Pseudomyrmex_nigropilosus	CASENT0005846	AY703602	KR829040	AY703736	KR829413	KR829626	KR829832	KR830039	KR830247	KR830663	KR830454
Pseudomyrmex_oculatus	CASENT0220678	AY703603	KR829041	AY703737	KR829414	KR829627	KR829833	KR830040	KR830248	KR830664	KR830455
Pseudomyrmex_oki	CASENT0220693	AY703604	KR829042	AY703738	KR829415	KR829628	KR829834	KR830041	KR830249	KR830665	KR830456
Pseudomyrmex_opaciceps	CASENT0220668	KR828865	KR829043	KR829238	KR829416	KR829629	KR829835	KR830042	KR830250	KR830666	KR830457
Pseudomyrmex_osurus	CASENT0220669	KR828866	KR829044	KR829239	KR829417	KR829630	KR829836	KR830043	KR830251	KR830667	KR830458
Pseudomyrmex_pallens	CASENT0220682	KR828867	KR829045	KR829240	KR829418	KR829631	KR829837	KR830044	KR830252	KR830668	KR830459
Pseudomyrmex_pallens_cf	CASENT0220683	KR828868	KR829046	KR829241	KR829419	KR829632	KR829838	KR830045	KR830253	KR830669	KR830460
Pseudomyrmex_pallidus	CASENT0220694	AY703605	KR829047	AY703739	KR829420	KR829633	KR829839	KR830046	KR830254	KR830670	KR830461
Pseudomyrmex_particeps	CASENT0220618	KR828869	KR829048	KR829242	KR829421	KR829634	KR829840	KR830047	KR830255	KR830671	KR830462
Pseudomyrmex_pazosi	CASENT0220695	AY703607	KR829049	AY703741	KR829422	KR829635	KR829841	KR830048	KR830256	KR830672	KR830463
Pseudomyrmex_penetrator	CASENT0220651	KR828870	KR829050	KR829243	KR829423	KR829636	KR829842	KR830049	KR830257	KR830673	KR830464
Pseudomyrmex_peperi	CASENT0220623	KR828871	KR829051	KR829244	KR829424	KR829637	KR829843	KR830050	KR830258	KR830674	KR830465
Pseudomyrmex_perboscii	CASENT0221015	KR828872	KR829052	KR829245	KR829425	KR829638	KR829844	KR830051	KR830259	KR830675	KR830466
Pseudomyrmex_peruvianus_nr	CASENT0220696	KR828873	KR829053	KR829246	KR829426	KR829639	KR829845	KR830052	KR830260	KR830676	KR830467
Pseudomyrmex_phyllophilus	CASENT0220684	AY703608	KR829054	AY703742	KR829427	KR829640	KR829846	KR830053	KR830261	KR830677	KR830468
Pseudomyrmex_psw001	CASENT0221018	KR828874	KR829055	KR829247	KR829428	KR829641	KR829847	KR830054	KR830262	KR830678	KR830469
Pseudomyrmex_psw002	CASENT0106120	KR828875	KR829056	KR829248	KR829429	KR829642	KR829848	KR830055	KR830263	KR830679	KR830470
Pseudomyrmex_psw003	CASENT0220659	KR828876	KR829057	KR829249	KR829430	KR829643	KR829849	KR830056	KR830264	KR830680	KR830471
Pseudomyrmex_psw005	CASENT0220697	KR828877	KR829058	KR829250	KR829431	KR829644	KR829850	KR830057	KR830265	KR830681	KR830472
Pseudomyrmex_psw006	CASENT0220698	KR828878	KR829059	KR829251	KR829432	KR829645	KR829851	KR830058	KR830266	KR830682	KR830473

Pseudomyrmex_psw013	CASENT0220699	KR828879	KR829060	KR829252	KR829433	KR829646	KR829852	KR830059	KR830267	KR830683	KR830474
Pseudomyrmex_psw014	CASENT0220700	KR828880	KR829061	KR829253	KR829434	KR829647	KR829853	KR830060	KR830268	KR830684	KR830475
Pseudomyrmex_psw015	CASENT0220701	KR828881	KR829062	KR829254	KR829435	KR829648	KR829854	KR830061	KR830269	KR830685	KR830476
Pseudomyrmex_psw019	CASENT0220702	KR828882	KR829063	KR829255	KR829436	KR829649	KR829855	KR830062	KR830270	KR830686	KR830477
Pseudomyrmex_psw023	CASENT0220654	KR828883	KR829064	KR829256	KR829437	KR829650	KR829856	KR830063	KR830271	KR830687	KR830478
Pseudomyrmex_psw034	CASENT0005864	KR828884	KR829065	KR829257	KR829438	KR829651	KR829857	KR830064	KR830272	KR830688	KR830479
Pseudomyrmex_psw036	INBIOCRI001238150	KR828885	KR829066	KR829258	KR829439	KR829652	KR829858	KR830065	KR830273	KR830689	KR830480
Pseudomyrmex_psw037	CASENT0005866	KR828886	KR829067	KR829259	KR829440	KR829653	KR829859	KR830066	KR830274	KR830690	KR830481
Pseudomyrmex_psw040	CASENT0220711	KR828887	KR829068	KR829260	KR829441	KR829654	KR829860	KR830067	KR830275	KR830691	KR830482
Pseudomyrmex_psw041	CASENT0220660	KR828888	KR829069	KR829261	KR829442	KR829655	KR829861	KR830068	KR830276	KR830692	KR830483
Pseudomyrmex_psw043	INB0003239081	KR828889	KR829070	KR829262	KR829443	KR829656	KR829862	KR830069	KR830277	KR830693	KR830484
Pseudomyrmex_psw044	CASENT0220664	KR828890	KR829071	KR829263	KR829444	KR829657	KR829863	KR830070	KR830278	KR830694	KR830485
Pseudomyrmex_psw045	CASENT0106335	KR828891	KR829072	KR829264	KR829445	KR829658	KR829864	KR830071	KR830279	KR830695	KR830486
Pseudomyrmex_psw049	CASENT0005867	KR828892	KR829073	KR829265	KR829446	KR829659	KR829865	KR830072	KR830280	KR830696	KR830487
Pseudomyrmex_psw051	CASENT0220685	KR828893	KR829074	KR829266	KR829447	KR829660	KR829866	KR830073	KR830281	KR830697	KR830488
Pseudomyrmex_psw052	CASENT0220655	KR828894	KR829075	KR829267	KR829448	KR829661	KR829867	KR830074	KR830282	KR830698	KR830489
Pseudomyrmex_psw053	CASENT0220661	KR828895	KR829076	KR829268	KR829449	KR829662	KR829868	KR830075	KR830283	KR830699	KR830490
Pseudomyrmex_psw054	CASENT0106121	KR828896	KR829077	KR829269	KR829450	KR829663	KR829869	KR830076	KR830284	KR830700	KR830491
Pseudomyrmex_psw061	CASENT0220703	KR828897	KR829078	KR829270	KR829451	KR829664	KR829870	KR830077	KR830285	KR830701	KR830492
Pseudomyrmex_psw062	CASENT0220704	KR828898	KR829079	KR829271	KR829452	KR829665	KR829871	KR830078	KR830286	KR830702	KR830493
Pseudomyrmex_psw063	CASENT0220705	KR828899	KR829080	KR829272	KR829453	KR829666	KR829872	KR830079	KR830287	KR830703	KR830494
Pseudomyrmex_psw064	CASENT0220706	KR828900	KR829081	KR829273	KR829454	KR829667	KR829873	KR830080	KR830288	KR830704	KR830495
Pseudomyrmex_psw065	CASENT0220707	KR828901	KR829082	KR829274	KR829455	KR829668	KR829874	KR830081	KR830289	KR830705	KR830496
Pseudomyrmex_psw155	CASENT0220729	KR828902	KR829083	KR829275	KR829456	KR829669	KR829875	KR830082	KR830290	KR830706	KR830497
Pseudomyrmex_psw157	CASENT0173025	KR828903	KR829084	KR829276	KR829457	KR829670	KR829876	KR830083	KR830291	KR830707	KR830498
Pseudomyrmex_psw158	CASENT0220730	KR828904	KR829085	KR829277	KR829458	KR829671	KR829877	KR830084	KR830292	KR830708	KR830499
Pseudomyrmex_psw165	CASENT0220720	KR828905	KR829086	KR829278	KR829459	KR829672	KR829878	KR830085	KR830293	KR830709	KR830500
Pseudomyrmex_pupa	CASENT0220670	KR828906	KR829087	KR829279	KR829460	KR829673	KR829879	KR830086	KR830294	KR830710	KR830501
Pseudomyrmex_rubiginosus	CASENT0220721	KR828907	KR829088	KR829280	KR829461	KR829674	KR829880	KR830087	KR830295	KR830711	KR830502
Pseudomyrmex_rufiventris	CASENT0220708	KR828908	KR829089	KR829281	KR829462	KR829675	KR829881	KR830088	KR830296	KR830712	KR830503
Pseudomyrmex_rufomedius	CASENT0220712	KR828909	KR829090	KR829282	KR829463	KR829676	KR829882	KR830089	KR830297	KR830713	KR830504
Pseudomyrmex_salvini	CASENT0220662	KR828910	KR829091	KR829283	KR829464	KR829677	KR829883	KR830090	KR830298	KR830714	KR830505
Pseudomyrmex_santschii	CASENT0220671	KR828911	KR829092	KR829284	KR829465	KR829678	KR829884	KR830091	KR830299	KR830715	KR830506
Pseudomyrmex_satanicus	CASENT0220626	KR828912	KR829093	KR829285	KR829466	KR829679	KR829885	KR830092	KR830300	KR830716	KR830507
Pseudomyrmex_schuppi	CASENT0220679	KR828913	KR829094	KR829286	KR829467	KR829680	KR829886	KR830093	KR830301	KR830717	KR830508
Pseudomyrmex_seminole	CASENT0220709	KR828914	KR829095	KR829287	KR829468	KR829681	KR829887	KR830094	KR830302	KR830718	KR830509
Pseudomyrmex_sericeus	CASENT0220722	AY703609	KR829096	AY703743	KR829469	KR829682	KR829888	KR830095	KR830303	KR830719	KR830510
Pseudomyrmex_sericeus_cf	CASENT0220723	KR828915	KR829097	KR829288	KR829470	KR829683	KR829889	KR830096	KR830304	KR830720	KR830511
Pseudomyrmex_simplex	CASENT0220710	AY703610	KR829098	AY703744	KR829471	KR829684	KR829890	KR830097	KR830305	KR830721	KR830512
Pseudomyrmex_simplex_cf	CASENT0221980	KR828916	KR829099	KR829289	KR829472	KR829685	KR829891	KR830098	KR830306	KR830722	KR830513
Pseudomyrmex_spiculus	CASENT0220724	AY703611	KR829100	AY703745	KR829473	KR829686	KR829892	KR830099	KR830307	KR830723	KR830514
Pseudomyrmex_spiculus_nr	CASENT0220725	KR828917	KR829101	KR829290	KR829474	KR829687	KR829893	KR830100	KR830308	KR830724	KR830515
Pseudomyrmex_spinicola	CASENT0005800	KR828918	KR829102	KR829291	KR829475	KR829688	KR829894	KR830101	KR830309	KR830725	KR830516
Pseudomyrmex_subater	CASENT0220656	AY703612	KR829103	AY703746	KR829476	KR829689	KR829895	KR830102	KR830310	KR830726	KR830517
Pseudomyrmex_tachigaliae	CASENT0220652	AY703613	KR829104	AY703747	KR829477	KR829690	KR829896	KR830103	KR830311	KR830727	KR830518

Pseudomyrmex_tenuis	CASENT0220731	AY703614	KR829105	AY703748	KR829478	KR829691	KR829897	KR830104	KR830312	KR830728	KR830519
Pseudomyrmex_tenuissimus	CASENT0220726	AY703615	KR829106	AY703749	KR829479	KR829692	KR829898	KR830105	KR830313	KR830729	KR830520
Pseudomyrmex_termitarius	CASENT0220732	AY703616	KR829107	AY703750	KR829480	KR829693	KR829899	KR830106	KR830314	KR830730	KR830521
Pseudomyrmex_triplaridis	CASENT0220735	KR828919	KR829108	KR829292	KR829481	KR829694	KR829900	KR830107	KR830315	KR830731	KR830522
Pseudomyrmex_triplarinus	CASENT0220736	KR828920	KR829109	KR829293	KR829482	KR829695	KR829901	KR830108	KR830316	KR830732	KR830523
Pseudomyrmex_ultrix	CASENT0902890	KR828921	KR829110	KR829294	KR829483	KR829696	KR829902	KR830109	KR830317	KR830733	KR830524
Pseudomyrmex_unicolor	CASENT0220672	KR828922	KR829111	KR829295	KR829484	KR829697	KR829903	KR830110	KR830318	KR830734	KR830525
Pseudomyrmex_urbanus	CASENT0106772	KR828923	KR829112	KR829296	KR829485	KR829698	KR829904	KR830111	KR830319	KR830735	KR830526
Pseudomyrmex_veneficus	CASENT0220644	KR828924	KR829113	KR829297	KR829486	KR829699	KR829905	KR830112	KR830320	KR830736	KR830527
Pseudomyrmex_venustus	CASENT0220673	KR828925	KR829114	KR829298	KR829487	KR829700	KR829906	KR830113	KR830321	KR830737	KR830528
Pseudomyrmex_viduus	CASENT0220737	AY703617	KR829115	AY703751	KR829488	KR829701	KR829907	KR830114	KR830322	KR830738	KR830529
Tetraponera_aethiops	CASENT0415286	AY703569	KR829116	AY703703	KR829489	KR829702	KR829908	KR830115	KR830323	KR830739	KR830530
Tetraponera_allaborans	CASENT0220740	AY703570	KR829117	AY703704	KR829490	KR829703	KR829909	KR830116	KR830324	KR830740	KR830531
Tetraponera_ambigua	CASENT0220749	AY703571	KR829118	AY703705	KR829491	KR829704	KR829910	KR830117	KR830325	KR830741	KR830532
Tetraponera_andrei	CASENT0220751	KR828926	KR829119	KR829299	KR829492	KR829705	KR829911	KR830118	KR830326	KR830742	KR830533
Tetraponera_anthracina	CASENT0415393	KR828927	KR829120	KR829300	KR829493	KR829706	KR829912	KR830119	KR830327	KR830743	KR830534
Tetraponera_attenuata	CASENT0220757	KR828928	KR829121	KR829301	KR829494	KR829707	KR829913	KR830120	KR830328	KR830744	KR830535
Tetraponera_binghami	CASENT0220758	KR828929	KR829122	KR829302	KR829495	KR829708	KR829914	KR830121	KR830329	KR830745	KR830536
Tetraponera_caffra	CASENT0220752	AY703572	KR829123	AY703706	KR829496	KR829709	KR829915	KR830122	KR830330	KR830746	KR830537
Tetraponera_clypeata	CASENT0220741	AY703573	KR829124	AY703707	KR829497	KR829710	KR829916	KR830123	KR830331	KR830747	KR830538
Tetraponera_continua	CASENT0220742	KR828930	KR829125	KR829303	KR829498	KR829711	KR829917	KR830124	KR830332	KR830748	KR830539
Tetraponera_difficilis	CASENT0220759	KR828931	KR829126	KR829304	KR829499	KR829712	KR829918	KR830125	KR830333	KR830749	KR830540
Tetraponera_emeryi	CASENT0220743	KR828932	KR829127	KR829305	KR829500	KR829713	KR829919	KR830126	KR830334	KR830750	KR830541
Tetraponera_extenuata	CASENT0220744	KR828933	KR829128	KR829306	KR829501	KR829714	KR829920	KR830180	KR830335	KR830751	KR830542
Tetraponera_fictrix	CASENT0012854	KR828934	KR829129	KR829307	KR829502	KR829715	KR829921	KR830127	KR830336	KR830752	KR830543
Tetraponera_grandidieri	CASENT0170349	AY703574	KR829130	AY703708	KR829503	KR829716	KR829922	KR830128	KR830337	KR830753	KR830544
Tetraponera_hespera	CASENT0489492	KR828935	KR829131	KR829308	KR829504	KR829717	KR829923	KR830129	KR830338	KR830754	KR830545
Tetraponera_hirsuta	CASENT0170369	KR828936	KR829132	KR829309	KR829505	KR829718	KR829924	KR830130	KR830339	KR830755	KR830546
Tetraponera_hysterica	CASENT0012842	KR828937	KR829133	KR829310	KR829506	KR829719	KR829925	KR830131	KR830340	KR830756	KR830547
Tetraponera_inermis	CASENT0106765	KR828938	KR829134	KR829311	KR829507	KR829720	KR829926	KR830132	KR830341	KR830757	KR830548
Tetraponera_laeviceps	CASENT0220760	KR828939	KR829135	KR829312	KR829508	KR829721	KR829927	KR830133	KR830342	KR830758	KR830549
Tetraponera_latifrons	CASENT0403378	KR828940	KR829136	KR829313	KR829509	KR829722	KR829928	KR830134	KR830343	KR830759	KR830550
Tetraponera_longula	CASENT0012843	KR828941	KR829137	KR829314	KR829510	KR829723	KR829929	KR830135	KR830344	KR830760	KR830551
Tetraponera_longula_cf	CASENT0012844	KR828942	KR829138	KR829315	KR829511	KR829724	KR829930	KR830136	KR830345	KR830761	KR830552
Tetraponera_manangotra	CASENT0120025	KR828943	KR829139	KR829316	KR829512	KR829725	KR829931	KR830137	KR830346	KR830762	KR830553
Tetraponera_mandibularis	CASENT0012845	KR828944	KR829140	KR829317	KR829513	KR829726	KR829932	KR830138	KR830347	KR830763	KR830554
Tetraponera_merita	CASENT0404433	KR828945	KR829141	KR829318	KR829514	KR829727	KR829933	KR830139	KR830348	KR830764	KR830555
Tetraponera_microcarpa	CASENT0220745	KR828946	KR829142	KR829319	KR829515	KR829728	KR829934	KR830140	KR830349	KR830765	KR830556
Tetraponera_mimula	CASENT0220761	KR828947	KR829143	KR829320	KR829516	KR829729	KR829935	KR830141	KR830350	KR830766	KR830557
Tetraponera_mocquerysi	CASENT0220753	KR828948	KR829144	KR829321	KR829517	KR829730	KR829936	KR830142	KR830351	KR830767	KR830558
Tetraponera_modesta	CASENT0220746	KR828949	KR829145	KR829322	KR829518	KR829731	KR829937	KR830181	KR830352	KR830768	KR830559
Tetraponera_morondaviensis	CASENT0012835	AY703575	KR829146	AY703709	KR829519	KR829732	KR829938	KR830143	KR830353	KR830769	KR830560
Tetraponera_natalensis	CASENT0220754	AY703576	KR829147	AY703710	KR829520	KR829733	KR829939	KR830144	KR830354	KR830770	KR830561
Tetraponera_nigra	CASENT0220762	AY703577	KR829148	AY703711	KR829521	KR829734	KR829940	KR830145	KR830355	KR830771	KR830562
Tetraponera_nitida	CASENT0220763	KR828950	KR829149	KR829323	KR829522	KR829735	KR829941	KR830146	KR830356	KR830772	KR830563

Tetraponera_ophthalmica	CASENT0091881	KR828951	KR829150	KR829324	KR829523	KR829736	KR829942	KR830147	KR830357	KR830773	KR830564
Tetraponera_parops	CASENT0106133	AY703578	KR829151	AY703712	KR829524	KR829737	KR829943	KR830148	KR830358	KR830774	KR830565
Tetraponera_penzigi	CASENT0220747	AY703579	KR829152	AY703713	KR829525	KR829738	KR829944	KR830149	KR830359	KR830775	KR830566
Tetraponera_perlonga	CASENT0012838	KR828952	KR829153	KR829325	KR829526	KR829739	KR829945	KR830150	KR830360	KR830776	KR830567
Tetraponera_phragmotica	CASENT0220750	KR828953	KR829154	KR829326	KR829527	KR829740	KR829946	KR830151	KR830361	KR830777	KR830568
Tetraponera_pilosa	CASENT0220765	AY703580	KR829155	AY703714	KR829528	KR829741	KR829947	KR830152	KR830362	KR830778	KR830569
Tetraponera_polita	CASENT0220764	KR828954	KR829156	KR829327	KR829529	KR829742	KR829948	KR830153	KR830363	KR830779	KR830570
Tetraponera_psw070	CASENT0012860	KR828955	KR829157	KR829328	KR829530	KR829743	KR829949	KR830154	KR830364	KR830780	KR830571
Tetraponera_psw083	CASENT0012847	KR828956	KR829158	KR829329	KR829531	KR829744	KR829950	KR830155	KR830365	KR830781	KR830572
Tetraponera_psw084	CASENT0012846	KR828957	KR829159	KR829330	KR829532	KR829745	KR829951	KR830156	KR830366	KR830782	KR830573
Tetraponera_psw085	CASENT0012853	KR828958	KR829160	KR829331	KR829533	KR829746	KR829952	KR830157	KR830367	KR830783	KR830574
Tetraponera_psw086	CASENT0012855	KR828959	KR829161	KR829332	KR829534	KR829747	KR829953	KR830158	KR830368	KR830784	KR830575
Tetraponera_psw087	CASENT0220748	KR828960	KR829162	KR829333	KR829535	KR829748	KR829954	KR830159	KR830369	KR830785	KR830576
Tetraponera_psw088	CASENT0012848	KR828961	KR829163	KR829334	KR829536	KR829749	KR829955	KR830160	KR830370	KR830786	KR830577
Tetraponera_psw089	CASENT0012852	KR828962	KR829164	KR829335	KR829537	KR829750	KR829956	KR830161	KR830371	KR830787	KR830578
Tetraponera_psw091	CASENT0012857	KR828963	KR829165	KR829336	KR829538	KR829751	KR829957	KR830162	KR830372	KR830788	KR830579
Tetraponera_psw094	CASENT0012840	KR828964	KR829166	KR829337	KR829539	KR829752	KR829958	KR830163	KR830373	KR830789	KR830580
Tetraponera_psw095	CASENT0012836	KR828965	KR829167	KR829338	KR829540	KR829753	KR829959	KR830164	KR830374	KR830790	KR830581
Tetraponera_psw104	CASENT0527209	KR828966	KR829168	KR829339	KR829541	KR829754	KR829960	KR830165	KR830375	KR830791	KR830582
Tetraponera_psw112	CASENT0409791	KR828967	KR829169	KR829340	KR829542	KR829755	KR829961	KR830166	KR830376	KR830792	KR830583
Tetraponera_psw113	CASENT0220755	KR828968	KR829170	KR829341	KR829543	KR829756	KR829962	KR830167	KR830377	KR830793	KR830584
Tetraponera_punctulata	CASENT0106098	AY703581	KR829171	AY703715	KR829544	EF013523	KR829963	KR830168	KR830378	KR830794	KR830585
Tetraponera_rakotonis	CASENT0012841	KR828969	KR829172	KR829342	KR829545	KR829757	KR829964	KR830169	KR830379	KR830795	KR830586
Tetraponera_rotula	CASENT0902834	KR828970	KR829173	KR829343	KR829546	KR829758	KR829965	KR830170	KR830380	KR830796	KR830587
Tetraponera_rufonigra	CASENT0106099	AY703582	KR829174	AY703716	KR829547	EF013524	FJ939892	FJ940128	FJ940011	KJ523718	KJ523635
Tetraponera_sahlbergii	CASENT0012851	KR828971	KR829175	KR829344	KR829548	KR829759	KR829966	KR830171	KR830381	KR830797	KR830588
Tetraponera_schulthessi	CASENT0220756	KR828972	KR829176	KR829345	KR829549	KR829760	KR829967	KR830172	KR830382	KR830798	KR830589
Tetraponera_tessmanni	CASENT0415600	AY703583	KR829177	AY703717	KR829550	KR829761	KR829968	KR830173	KR830383	KR830799	KR830590
Tetraponera_tucurua	CASENT0106767	KR828973	KR829178	KR829346	KR829551	KR829762	KR829969	KR830174	KR830384	KR830800	KR830591
Tetraponera_variegata	CASENT0122581	KR828974	KR829179	KR829347	KR829552	KR829763	KR829970	KR830175	KR830385	KR830801	KR830592
Aneuretus_simoni	CASENT0007014	EF012961	KR829180	EF013089	KR829553	EF013382	FJ939840	FJ940076	FJ939894	KJ523645	KJ523562
Dolichoderus_pustulatus	CASENT0106164	FJ939792	KR829181	FJ939824	KR829554	FJ939963	FJ939859	FJ940095	FJ939913	KJ523685	KJ523602
Leptomyrmex_erythrocephalus	CASENT0106077	AY703561	KR829182	AY703695	KR829555	EF013437	FJ939871	FJ940107	FJ939925	KR830802	KR830593
Liometopum_occidentale	CASENT0106078	AY867465	KR829183	AY867481	KR829556	EF013441	FJ939875	FJ940111	FJ939929	KR830803	KR830594
Myrmecia_fulvipes	CASENT0220766	AY703564	KR829184	AY703698	KR829557	KR829764	KR829971	KR830176	KR830386	KR830804	KR830595
Myrmecia_picta	USNMENT00923933	AY703565	KR829185	AY703699	KR829558	KR829765	KR829972	KR830177	KR830387	KR830805	KR830596
Myrmecia_pilosula	USNMENT00923934	AY703566	KR829186	AY703700	KR829559	KR829766	KR829973	KR830178	KR830388	KR830806	KR830597
Myrmecia_pyriformis	CASENT0106088	AY703567	KR829187	AY703701	KR829560	EF013454	FJ939878	FJ940114	FJ939932	KJ523699	KJ523616
Myrmica_tahoensis	CASENT0106091	AY703562	KR829188	AY703696	KR829561	EF013459	GU085791	KR830179	KJ860798	KJ861640	KJ860369
Nothomyrmecia_macrops	CASENT0106089	AY703568	KR829189	AY703702	KR829562	EF013466	FJ939880	FJ940116	FJ939934	KJ523703	KJ523620

Table S2. Primers used for amplifying ten nuclear gene fragments in Pseudomyrmecinae. The most frequently used primers are in bold font. Other primers were employed for amplifying shorter stretches of DNA and/or for sequencing across (or within) expanded introns. Coordinates are based on the indicated GenBank sequence. Primers within introns cannot be precisely mapped with these coordinates. Primer exon sequence is given as codon triplets.

Gene	Primer	Sequence (5' - 3')	Coordinates	GenBank # for coordinates	Reference
28S	**28S-3318F**	CCCCCTGAATTTAAGCATAT	3318-3337	M21017 (Dros. melanogaster)	Schmitz & Moritz (1994)
28S	**28S-3635F**	ACCGTGAGGGAAAGTTGAAAAG	3635-3657	M21017 (Dros. melanogaster)	This study
28S	**28S-3665F**	AGAGAGAGTTCAAGAGTACGTG	3665-3686	M21017 (Dros. melanogaster)	Belshaw & Quicke (1997)
28S	28S-D2aF	CGTCGTCGTGCACTTCTCCC	n/a	n/a	This study (ant-specific, in D2a region)
28S	**28S-3706R**	GGTTTACCCCTGAACGGTT	3706-3688	M21017 (Dros. melanogaster)	Ward & Downie (2005)
28S	**28S-4068R**	TTGGTCCGTGTTTCAAGACGGG	4068-4047	M21017 (Dros. melanogaster)	Belshaw & Quicke (1997)
28S	**28S-4078R**	GTTAGACTCCTTGGTCCGTGTT	4078-4056	M21017 (Dros. melanogaster)	This study
28S	28S-D2cR	GSGCCAGGAYCGGGGACGGC	n/a	n/a	This study (ant-specific, in D2c region)
Wg	Wg254F	CGA GAG ACC GCK TTY RTC TAY GC	254-276	AY101369.1 (Pheidole morrisi)	This study
Wg	**Wg290F**	GCW GTR ACT CAC AGY ATC GC	290-309	AY101369.1 (Pheidole morrisi)	This study
Wg	Wg367F	A CGC GCA TCA TCC ACC GTT CG	367-387	AY101369.1 (Pheidole morrisi)	This study (customized for Myrmeciinae)
Wg	Wg398F	TGG GGT GGT TGC TCG GAT AAC ATC G	398-422	AY101369.1 (Pheidole morrisi)	This study (customized for Myrmeciinae)
Wg	Wg463F	C GAR CGC GGY CGA AAT CTR CG	463-483	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	Wg524EF	GCAG CAC GTR TCY TCV GAR ATG CG	~524-543	AY101369.1 (Pheidole morrisi)	This study
Wg	**Wg542F**	CGY CAG GAR TGY AAR TGY CAY GGY	542-570	AY101369.1 (Pheidole morrisi)	This study
Wg	**Wg575F**	TCG TGC GCR GTC AAR ACY TGC TGG AT	575-600	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	Wg578F	TGC ACN GTG AAR ACY TGC TGG ATG CG	578-603	AY101369.1 (Pheidole morrisi)	Ward & Downie (2005)
Wg	Wg716F	AGC AAY TCG GCS AGC AAY TCB GTG C	716-740	AY101369.1 (Pheidole morrisi)	This study
Wg	Wg737F	GTG CAY CAY ATT CGC GAG GG	737-756	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	Wg523ER	GTGCTTAC TRB TCT GCC VGC TTC	~523-509	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	**Wg636R**	AG ATT GTC BCC RAC CAC GCG	636-617	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	**Wg645R2**	CG RTC CTT GAG ATT GTC BCC	645-626	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	Wg795R	GG CTT CAR YTG GAA RTT GTA DCG	795-773	AY101369.1 (Pheidole morrisi)	This study
Wg	Wg822R	CC GGG YGG CTT RTG YTC CGG RTT	822-800	AY101369.1 (Pheidole morrisi)	This study
Wg	Wg846R	TC CAT RTA RAC GAG GTC YTT YGG	846-824	AY101369.1 (Pheidole morrisi)	This study (customized for Pseudomyrmecinae)
Wg	**Wg1032R**	AC YTC GCA GCA CCA RTG GAA	1032-1013	AY101369.1 (Pheidole morrisi)	Abouheif & Wray (2002)
Wg	Wg1054R	CGT YCT GCA YAS YTG RCA YTT	1054-1034	AY101369.1 (Pheidole morrisi)	This study
Wg	**Wg1083R**	CT CTA YAG RCA YGT GTG TAC CRT CTT	1083-1058	AY101369.1 (Pheidole morrisi)	This study
LW Rh	**LR116F**	GGC GGA TTY GGY AAY CAR ACV GT	116-138	U26026 (Apis mellifera)	This study
LW Rh	**LR125F**	GGY AAY CAR ACV GTR GTB GAC AAR GT	125-150	U26026 (Apis mellifera)	This study
LW Rh	LR128F	AAY CAA ACM GTR GTD GAC AAA GT	128-150	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR134F	ACM GTR GTD GAC AAA GTK CCA CC	134-156	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	**LR143F**	GAC AAA GTK CCA CCR GAR ATG CT	143-165	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR182F	CAC TGG TAT CAR TTC GCA CCS AT	182-204	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR188F	TAY CAR TWY CCR CCR TTR AAY CC	188-210	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR398F	AAT TGC TAT TAY GAR ACN TGG GT	398-420	U26026 (Apis mellifera)	Ward & Downie (2005)

LW Rh	LR398F2	AAT TGC TAT TAC GAG ACG TGG GT	398-420	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR455F	GCG GGY TCC CTG TTC GGA TGT G	455-476	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	**LR482F**	ATW TGG ACR ATG ACR ATG ATY GC	482-504	U26026 (Apis mellifera)	This study
LW Rh	**LR503F**	GCA TTY GAY AGR TAY AAY GTA ATC GT	503-528	U26026 (Apis mellifera)	This study
LW Rh	LR560F	AAY GGC GCD CTT CTT CGY ATA	560-580	U26026 (Apis mellifera)	This study (customized for myrmeciomorphs)
LW Rh	LR644F	GTR CCS GAR GGY AAC ATG ACY GC	644-666	U26026 (Apis mellifera)	This study
LW Rh	LR794F	GCH GCY CAY GAG AAG AAY ATG CG	794-816	U26026 (Apis mellifera)	This study
LW Rh	LR391ER	ACGYAC CAT RGG YGG RGA CAT	~391-377	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR480R	GA GCC ACA TCC RAA CAG RGA ACC	480-458	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR508R	GAA YGC RAT CAT CGT CAT YGT CCA	508-485	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR543R	GC GGA CAR DCC YTT RAC RAT YAC	543-521	U26026 (Apis mellifera)	This study
LW Rh	**LR639ER**	YTTAC CG RTT CCA TCC RAA CA	~639-624	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	**LR672R**	CC RCA MGC VGT CAT GTT RCC TTC	672-650	U26026 (Apis mellifera)	Ward & Downie (2005)
LW Rh	LR672R2	CC RCA RGC RGT CAT GTT RCC YTC	672-650	U26026 (Apis mellifera)	This study
LW Rh	LR683R	TC YTT GYT CAR GTA RTC RGT ACC	683-671	U26026 (Apis mellifera)	This study (customized for most Pseudomyrmex)
LW Rh	LR718R	CAA AAT GTA CGA TCT GGA GAA CCA	718-695	U26026 (Apis mellifera)	This study (customized for Pseudomyrmex oculatus- and triplarinus-groups)
LW Rh	LR769R	GCT RTA RAT RAT GAG GAA CAR CGG	769-746	U26026 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
LW Rh	LR855R	GA TCG YAR VGA AGC RAC GTT CAT	855-833	U26026 (Apis mellifera)	This study
LW Rh	LR946R	ATA TGG AGT CCA NGC CAT RAA CCA	946-923	U26026 (Apis mellifera)	This study
LW Rh	**LR1047R**	GG ATT RTA YAC RGC RTT GGC TTT BGC	1047-1022	U26026 (Apis mellifera)	This study
LW Rh	**LR1065ER**	AC CT RAT RCC RTA TAC RAT VGG ATT	~1065-1043	U26026 (Apis mellifera)	This study
Abd-A	**AA1167F**	GGCA CCGA CMGG CGAT ATG AG	1167-1194	AF332515 (Myrmica rubra)	This study
Abd-A	AA1172F	CA CATC GGCA CCGG CGAT ATG AG	1172-1194	AF332515 (Myrmica rubra)	Ward & Downie (2005)
Abd-A	**AA1182F**	CCGG CGAT ATG AGT ACG AAA TTC	1182-1204	AF332515 (Myrmica rubra)	Ward & Downie (2005)
Abd-A	AA1317F	TA RAA TCG AGT YTA TCC GCG GCT	1317-1339	AF332515 (Myrmica rubra)	This study
Abd-A	AA1317F2	TA GAA TCG AGT YTA TCC GCG GCT GC	1317-1341	AF332515 (Myrmica rubra)	This study
Abd-A	AA1457F	TCC AGY ATG TAT CCK TAC GTR TC	1457-1480	AF332515 (Myrmica rubra)	This study
Abd-A	AA1607F	GCT GCT GTA GAC GCC GCT ACT GC	1607-1629	AF332515 (Myrmica rubra)	Ward & Downie (2005)
Abd-A	AA1573R	GA CCN GAA RCC RGG BAC CAT GCT	1573-1550	AF332515 (Myrmica rubra)	This study
Abd-A	AA1659R	CT RGC YGT RTA ACG GCA GGA TTT	1659-1637	AF332515 (Myrmica rubra)	This study
Abd-A	AA1743R2	GG CGT AGC GCC GTT TTG ATG ATG	1743-1721	AF332515 (Myrmica rubra)	Ward & Downie (2005)
Abd-A	**AA1824R**	TA GAA YTG TGC CGC CGC TGC CAT	1824-1802	AF332515 (Myrmica rubra)	Ward & Downie (2005)
Abd-A	**AA1881R**	GG TTG TTG GCA GGA TGT CAA AGG	1881-1859	AF332515 (Myrmica rubra)	Ward & Downie (2005)
Abd-A	**AA1890R**	CC DGT RGT RGG YTG YTG GCA RGA	1890-1868	AF332515 (Myrmica rubra)	This study
EF1aF2	F2-499F	TTTTTAG ATG GGC AAA GGW TCC	499-520	AF015267 (Apis mellifera)	This study
EF1aF2	**F2-515F**	GGT TCC TTC AAR TAY GCY TGG GT	515-537	AF015267 (Apis mellifera)	This study
EF1aF2	F2-557F	GAA CGT GAA CGT GGT ATY ACS AT	557-579	AF015267 (Apis mellifera)	Brady et al. (2006)
EF1aF2	**F2-557F2**	GAR CGT GAG CGT GGT ATC ACS AT	557-579	AF015267 (Apis mellifera)	This study
EF1aF2	F2-587F	GCY YTG TGG AAR TTY GAA AC	587-606	AF015267 (Apis mellifera)	This study
EF1aF2	F2-629F	ATY GAY GCY CCY GGA CAY AGR GA	629-651	AF015267 (Apis mellifera)	This study
EF1aF2	F2-806F	ATY GTY GGH GTY AAC AAR ATG GAY TC	806-831	AF015267 (Apis mellifera)	This study
EF1aF2	F2-812F	GGW GTY AAC AAG ATG GAY TCY AC	812-834	AF015267 (Apis mellifera)	This study
EF1aF2	F2-933R	AC RAA BGC RAC MGC RGC YGG	933-914	AF015267 (Apis mellifera)	This study

EF1aF2	F2-1062R	GC RTC SAG RGC TTC RAT RAG RCA	1062-1040	AF015267 (Apis mellifera)	This study
EF1aF2	F2-1118R	TTAC CTG AAG GGG AAG ACG RAG	1118-1097	AF015267 (Apis mellifera)	Brady et al. (2006)
EF1aF2	**F2-1118R2**	TTAC CTG RAG CGG AAG ACG CAA	1118-1097	AF015267 (Apis mellifera)	This study (customized for most Pseudomyrmecinae)
EF1aF2	F2-1118R4	TTAC CTG KAG RGG KAR ACG MAG	1118-1097	AF015267 (Apis mellifera)	This study (customized for Tetraponera ambigua-group)
EF1aF2	F2-1368R	AT CTT RTA YAC GTC CTGCAAT	1368-~1348	AF015267 (Apis mellifera)	This study
EF1aF2	**F2-1371R**	CC RAT CTT RTA YAC GTC CTGC	1371-1351	AF015267 (Apis mellifera)	This study
ArgK	**AK1F2**	ATG GTT GAY GCY GCY GTT YTG GA	1-23	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	**AK1F7**	ATG GTR GAY GCA GCD GTT YTG GAY AA	1-26	AF023619 (Apis mellifera) (cds)	This study
ArgK	AK1F8	AAGRGYAACAAY ATG GTR GAY GCA GC	~1-14	AF023619 (Apis mellifera) (cds)	This study
ArgK	**AK4F2**	GTT GAY GCY GCY GTT YTG GAY AA	4-26	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK61F2	GAC AGC AAR TCR CTG YTG AAG AA	61-83	AF023619 (Apis mellifera) (cds)	Modified from Kawakita et al. (2003)
ArgK	AK106F	GAT CAR CTS AAG ACN AGV AAG AC	106-128	AF023619 (Apis mellifera) (cds)	This study
ArgK	**AK244F**	GAY CCC ATY ATY GAC GAY TAY CA	244-266	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK286F	GAY AAR CAY CCG CCM AAR GAY TT	286-308	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK346EF6	GATTTGATTTTATGTAG RGT GAA TAC	~346-354	AF023619 (Apis mellifera) (cds)	This study (customized for Tetraponera ambigua-group)
ArgK	AK346EF7	TACAG RGY GAR TAC ATT GTG	~346-360	AF023619 (Apis mellifera) (cds)	This study (customized for some Tetraponera taxa)
ArgK	AK346EF8	CATGCAG GGY GAR TAC ATT G	~346-358	AF023619 (Apis mellifera) (cds)	This study (customized for Pseudomyrmex)
ArgK	**AK346EF**	AG GGT GAR TAC ATC GTR TCH ACT CG	~346-368	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK421F	GAR GCS CAR TAY AAR GAR ATG GA	421-443	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK218R	GC YTC RGC RTC RGG NGC RTA	218-199	AF023619 (Apis mellifera) (cds)	This study
ArgK	AK308R	AA RTC YTT KGG CGG RTG YTT RTC	308-286	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK345ER	ACTYAC VGT VGG RTC RAG RTT	~345-331	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	**AK345ER2**	ACTYAC MGT YGG RTC RAG ATT	~345-331	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	**AK389R**	AG RGA GCG ACC GCA TCG CAC	389-370	AF023619 (Apis mellifera) (cds)	This study (customized for Pseudomyrmecinae)
ArgK	AK392R	TC CAA RGA GCG RCC GCA TC	392-374	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK446R	TC YTC CAT YTC YTT RTA YTG IGC	446-424	AF023619 (Apis mellifera) (cds)	This study
ArgK	**AK461R**	GT GCT RGA YAC YTT CTC YTC CAT	461-439	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK533R	TG YTG YAC YTC CTT GCT CAT RCC	533-511	AF023619 (Apis mellifera) (cds)	This study
ArgK	AK638R	TC GTT RTG RAA GAT RCC RCG YCC	638-616	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	**AK720ER**	AC CTG YCC RAG RTC ACC RCC CAT	~720-700	AF023619 (Apis mellifera) (cds)	Ward et al. (2010)
ArgK	AK960R	RCC VCC YTC AGC CTC KGT GTG CTC	960-937	AF023619 (Apis mellifera) (cds)	http://www.danforthlab.entomology.cornell.edu/files/all/argk_primers.pdf
ArgK	AKiF3	TGTTGCAACGTTYCGACCAATGG	within intron	n/a	This study (customized for Tetraponera ambigua-group)
ArgK	AKiF4	AGATCTTTGTATTTGGTGAGTG	within intron	n/a	This study (customized for Tetraponera ambigua-group)
ArgK	AKiR1	GACACTTTTACTGTTAGTTTGC	within intron	n/a	This study (customized for Tetraponera ambigua-group)
ArgK	AKiR2	GTTAGTGGAAAAGGTCAATGRAAAGC	within intron	n/a	This study (customized for Tetraponera ambigua-group)
Enolase	EL154F	CAA ATC TTC GAC TCT CGT GGN AAY CC	154-179	XM_625053 (Apis mellifera)	Alex Wild (pers. comm.)
Enolase	EL190EF	TTAG GTC GAT HTT GTC ACT GA	~190-206	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	EL229F	GTA CCA TCA GGN GCN TCY ACY GG	229-251	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	**EL229F2**	GTK CCA TCY GGY GCA TCY ACY GG	229-251	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	EL298F	CAT GGA AAR TCY GTT TTC AAR GC	298-320	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	EL364F	GCC AAC TTR GAA GTT ACR CAG CAA GA	364-389	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	EL427F	CCG AAT AAA TCS AAA CTT GGN GCR AAY	427-455	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	EL454F	GCA ATT TTG GGY GTY TCY TTG GC	454-476	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)

Enolase	EL583F	ATY AAY GGW GGH TCH CAY GCT GG	583-605	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	EL363ER	C CTT YAA YAA TTC AGG AGC AAT RAT	~363-337	XM_625053 (Apis mellifera)	This study (customized for myrmeciomorphs)
Enolase	EL438R	GGA TTT ATT TGG AGT ACC RTC CA	438-416	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	EL488R	GC CTT GCA DAY WGC YAR RGA GAC ACC	488-463	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	EL519R	GTA CAR AGG CAV YTT YTT CTT GGC	519-496	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	EL545R	TT KCC AGC YAA YTC RGC RAT ATA	545-523	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	EL644R	GT WGG HAR RAT CAT RAA YTC YTG CAT	644-619	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	EL692R	TA AAC YTC ACT GCC CAT TTT CAT RGC	692-667	XM_625053 (Apis mellifera)	This study (customized for myrmeciomorphs)
Enolase	**EL794R**	GC YTC YTT GTT CTC YAR AAT RTT YGG	794-766	XM_625053 (Apis mellifera)	Ward et al. (2010)
Enolase	EL822ER	AYTTAC TTT AGT AGC NTC TAT GAT CA	~822-803	XM_625053 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Enolase	**EL885R**	CTT GTA GAA CTC NGA NGC NGC NAC RTC	885-856	XM_625053 (Apis mellifera)	Wild & Maddison (2008)
Enolase	ELiF	CAGGACGTGATAAAAATAACTAAGCC	within intron	n/a	This study (customized for Tetraponera natalensis-group)
Enolase	ELi2F	CAACTTGCTACAATTTTGCTCAGTGTGC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELi2F2	TCTTTCCTGACACCAATTCAGCCCG	within intron	n/a	This study (customized for Tetraponera rufonigra-group)
Enolase	ELi2F3	CGTGAACTTTGTGAGTCTCAAACATGC	within intron	n/a	This study (customized for Tetraponera rufonigra-group)
Enolase	ELi2F4	GCTTAACGGTTCTGYATTTAACTGC	within intron	n/a	This study (customized for Tetraponera rufonigra-group)
Enolase	ELi2F5	GGTATTGGGAGTAAATTGGAGAACTCC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELi2F6	GAGAGAATGAAGATGCTGTTGGAAGC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELi2F7	AGGCWTTTATAGCARCTAATAAACTGAC	within intron	n/a	This study (customized for Pseudomyrmex goeldii-group)
Enolase	ELi3F	AGAGTACAATTGTTTTTGTATTTTATAC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELiR	CAGAAGAGAAACTCATYACACGATCTA	within intron	n/a	This study (customized for Tetraponera natalensis-group)
Enolase	ELi2R	CTTCCTCTTCATTCCCACATACTCTA	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELi2R2	CACTTTTATTGACACTTCATGCAGCGC	within intron	n/a	This study (customized for Tetraponera rufonigra-group)
Enolase	ELi2R3	TGTAAGWGACAGCACGCGATGTTGC	within intron	n/a	This study (customized for Tetraponera rufonigra-group)
Enolase	ELi2R4	TAACGCATCTCAGAAGTAACACAGTC	within intron	n/a	This study (customized for Tetraponera rufonigra-group)
Enolase	ELi2R5	AAATTTCTTGCCCTTTGTCCCGC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELi2R6	ACATACTATACTTAAAACTACCTTAGCC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
Enolase	ELi3R	ATAMAGTTATCAGATATATTTAC	within intron	n/a	This study (customized for Pseudomyrmex pallidus-group)
CAD	CD852EF2	CAG V TAY GGY AAY CGV GGY CAY AA	~852-872	XM_393888 (Apis mellifera)	This study
CAD	CD892F	GGY ACC GGR CGT TGY TAY ATG AC	892-914	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	**CD892F2**	GGH ACC GRA CGT TGC TAC ATG AC	892-914	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
CAD	CD1028F	CG TAC TTY TCC GTB CAR TTY CAY CCR G	1028-1054	XM_393888 (Apis mellifera)	This study
CAD	CD1090F	TTC GAY GTG TTY YTG GAR AGY GT	1090-1112	XM_393888 (Apis mellifera)	This study
CAD	CD1117F	GCC GAA GTC GAG GRT TCT CGA ATT	1117-1140	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmex ferrugineus- and goeldii-groups)
CAD	CD1238F	CG GGW GGD CTY AGY ATY GGY CAR GC	1238-1262	XM_393888 (Apis mellifera)	This study
CAD	CD1258F	CAG GCS GGA GAR TTY GAY TAY TCR GG	1258-1283	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1258F4	CAG GCY GGW GAR TTY GAY TAY TCD	1258-1286	XM_393888 (Apis mellifera)	This study
CAD	CD1276F	TAY TCR GGY TCG CAR GCS ATH AAR GC	1276-1301	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1351F	ACG GTR CAG ACV TCV AAR GGH ATG GC	1351-1376	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1369F	GGH ATG GCY GAY AAR GTR TAT TTY TTR	1369-1397	XM_393888 (Apis mellifera)	This study
CAD	CD1402F	ACA CCR GAH TAT GTY GAG CAG GT	1402-~1422	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
CAD	**CD1423EF**	AG GTR ATA CRA TCG GAR AGR CCD GA	~1423-1445	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1423EF3	AG GTR ATA CAA TCC GAR AGR CCG GA	~1423-1445	XM_393888 (Apis mellifera)	Ward (unpubl.). Customized for Tetraponera nigra group.
CAD	CD1423F	GTR ATA SAA TCR GAR AGR CCR GAY GGC	1423-1449	XM_393888 (Apis mellifera)	Ward (unpubl.). Customized for Pseudomyrmecinae

CAD	CD1540F	CTR GGW ACR CCR ATY GAR TCY ATH AT	1540-1565	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1597F	CGT ATY AGC GAG ATA AAY GAA ARA	1597-1625	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1597F2	CGT ATT AGC GAG ATA AAT GAA AAA GTT	1597-1625	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmex)
CAD	CD1630F	AGY GCT GCC GTG TAY TCY GTT CAA GA	1630-1655	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1657EF2	TTCTAAACAG GCR TTR GAA GC	~1657-1667	XM_393888 (Apis mellifera)	Ward (unpubl.). Customized for Pseudomyrmecinae
CAD	CD1106R	TC CAR RAA YAC RTC RAA RAG RCA YTC	1106-1081	XM_393888 (Apis mellifera)	This study
CAD	CD1250R	CT GAG YCC WCC BGA DCC YAR AAT YA	1250-1226	XM_393888 (Apis mellifera)	This study
CAD	CD1288R	G YGA RCC YGA RTA RTC RAA YTC KCC	1288-1264	XM_393888 (Apis mellifera)	This study
CAD	CD1316R	GA YTC YTC YTT YAR YGC YTT DAT SGC	1316-1291	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1325R	TG YTG TAY SGA TTC YTC YTT CAA CGC	1325-1300	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
CAD	CD1388R	TA YAC YTT RTC RGC CAT DCC YTT BGA	1388-1363	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1422ER	AC CTG CTC RAC ATA DTC YGG TGT	~1422-1402	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
CAD	CD1469R	CC GCC GAA TGT TAA TAG TAC GCC G	1469-1446	XM_393888 (Apis mellifera)	This study (customized for most Pseudomyrmex)
CAD	CD1478R	GC RGT CTG YCC RCC RAA YGT TAA YAR	1478-1450	XM_393888 (Apis mellifera)	This study
CAD	**CD1491R**	GCC GCA RTT NAG RGC RGT YTG YCC	1491-1468	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1491R2	GCC ACA RTT RAG AGM GGT YTG TCC	1491-1468	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
CAD	**CD1592R2**	GC GAA CAR YTT TCT RTC YTC VGT TTG	1592-1567	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
CAD	**CD1592R**	GC RAA YAT YTT YCT RTC YTC RGT	1592-1570	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1656ER	TTAC YTC TTG AAC RGA RTA SAC RGC	~1656-1636	XM_393888 (Apis mellifera)	Ward et al. (2010) (customized for Pseudomyrmecinae)
CAD	CD1656ER2	TTAC YTC TTG AAC RGA RTA YAC GGC	~1656-1636	XM_393888 (Apis mellifera)	This study (customized for Tetraponera nigra group)
CAD	CD1703R	GC RCG YGC CAT WAC RGG RTA RCC	1703-1681	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1703R2	GC RCG CGC CAT TAC RGG ATA YCC	1703-1681	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmex)
CAD	CD1721R	CC DCC RAG NGA RAA YGC RGC RCG	1721-1699	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1721R2	CC ACC RAG TGA RAA CGC RGC RCG	1721-1699	XM_393888 (Apis mellifera)	This study (customized for Pseudomyrmex)
CAD	CD1730R	GA TCC RAG RCC WCC RAG YGA RAA YGC	1730-1705	XM_393888 (Apis mellifera)	This study
CAD	CD1847R	AC YTC RTA YTC BAC YTC YTT CCA RCC	1847-1822	XM_393888 (Apis mellifera)	This study
CAD	**CD1910R**	CC GAG RGG RTC RAC RTT YTC CAT RTT	1910-1879	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CD1934R	AC RAT YGA YTC RCC DGT RTG RAT RCC	1934-1909	XM_393888 (Apis mellifera)	This study
CAD	**CD1955R**	AG TGT YTG ACT CGG HGC DAC VAC RAT	1955-1930	XM_393888 (Apis mellifera)	Ward et al. (2010)
CAD	CDi5F	TGCTTCGTGGTTCATAATTACTATTAGA	within intron	n/a	This study (customized for Tetraponera nigra group)
CAD	CDi5F3	CTATATTGCATCGGGTCATATTGTTGC	within intron	n/a	This study (customized for Tetraponera nigra group)
CAD	CDi5F6	GAACATTTTTCCAATTATTTATTTCAG	within intron	n/a	This study (customized for Tetraponera nigra group)
CAD	CDi6F	CAGACTCACAGCTCACCTTTTAAAGTGC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6F3	GCATCTTTTAAAACTCYCTTAAAMAATCTG	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6F4	CTTATACGTGATAAGTATAAATATRCGATG	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6F5	ATTTAYTGCGCACTRATGTTATACTG	within intron	n/a	This study (customized for Tetraponera nigra-group)
CAD	CDi6F6	CTAACTTCAGATTTGAAATCAGCGATCC	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6F7	CTGTTCAGTACTAAGATTAAGATATTGTT	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6F8	TACTTTTAAACCTAAAAGAAAGAGTTAG	within intron	n/a	This study (customized for Pseudomyrmex concolor-group)
CAD	CDi6F9	GGACTTCACCTTGGTTTAAGTTGTCG	within intron	n/a	This study (customized for Pseudomyrmex tachigaliae)
CAD	CDi6F10A	GTTAAGAGGATGCTAGACCGTCCCG	within intron	n/a	This study (customized for Tetraponera ambigua-group)
CAD	CDi6F10B	GGACGAAATGGAAAACGCTTTTCTAG	within intron	n/a	This study (customized for Tetraponera ambigua-group)
CAD	CDi6F11	CTCTGTAAATAAATTTCTTTCTAAACAG	within intron	n/a	This study (customized for Pseudomyrmex, in part)
CAD	CDi6F12	GTCCAACATCCTCTTAACGTTGAT	within intron	n/a	This study (customized for Tetraponera ambigua-group)
CAD	CDi6F14	AGAGAGAGACTTTTCTATGAACTAACTA	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)

CAD	CDi6F15	GAAAACATTTCTACCGTCATTGCGG	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6F17	GTCACAGCGATAAATGGTYACATGAC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi5R	CTCTTAATACAGAAGAAACTAATTATTG	within intron	n/a	This study (customized for Tetraponera nigra group)
CAD	CDi5R3	ATTGCGTTTTGTTGGCACAAAATTCGAC	within intron	n/a	This study (customized for Tetraponera nigra group)
CAD	CDi5R4	CCCGATGCAATATAGTATTAGTTACGC	within intron	n/a	This study (customized for Tetraponera nigra group)
CAD	CDi5R5	TGTTAATTACGAAGCAGTAAGTTCAC	within intron	n/a	This study (customized for Pseudomyrmex pallidus group)
CAD	CDi5R6	GTTTTAATRTAGAGAAGATTATTGGC	within intron	n/a	This study (customized for Pseudomyrmex pallidus group)
CAD	CDi6R2	GTCTAACATGTTCTGTTGTTTATTTAGC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R3	ATCAACGGCACATTAGTACYGAAAAATATC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R4	CTTGACGCTATAACGCGGKTGCGC	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6R5	TCTATMCTTAATAGTTTCCGAGAAATCG	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6R6	CTGCCAAATTAGGTTTTTTCAGCAGATC	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6R7	GTACAAAGAAAACAAGTAGTAAGTATACG	within intron	n/a	This study (customized for Tetraponera laeviceps-complex)
CAD	CDi6R8	TTACAGAGATAATAACAATAACACATCA	within intron	n/a	This study (customized for Pseudomyrmex concolor-group)
CAD	CDi6R9	GTTTGAGTTGAACCTCGCTTTCCGCG	within intron	n/a	This study (customized for Pseudomyrmex tachigaliae)
CAD	CDi6R10	ATGTAATTTCTTCATCTATTTACATCAG	within intron	n/a	This study (customized for Tetraponera ambigua-group)
CAD	CDi6R11	CAATGGATTAATTTACAAAAATAAAGAG	within intron	n/a	This study (customized for Pseudomyrmex, in part)
CAD	CDi6R12	GCAGCTTCTAACGCCTGTTCAGAAAG	within intron	n/a	This study (customized for Tetraponera ambigua-group)
CAD	CDi6R14	CGTACGTGGTCAAAATTCAATACTG	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R15	GATCATTTATCGCTGTGACAAGCAAC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R16	CGTTGAACACTGAGATGAGAACTGC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R17	CACATAAAAAGGATTAACGATCCGTTGC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R18	GTCAAACGACTTGTTTGAACAGTAACG	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R19	GAACARTAACGGATTTTGACYAYGTACG	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R20	ATCAARGTATCACGAGTGAACTTGCC	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
CAD	CDi6R21	CGTCATGTRACCATTTATCGCTGTG	within intron	n/a	Ward et al. (2010) (customized for Tetraponera rufonigra-group)
Top1	**TP1293EF**	TKCAG G TGG GAR GAR GAR AAG AA	~1293-1310	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1303F	GAG AAG AAR ARY GAY GGH ACR AAR	1303-1329	XM_396203.3 (Apis mellifera)	This study
Top1	**TP1339F**	GAR CAY AAR GGA CCK GTR TTY GCA CC	1339-1364	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1506F	C AAC TTY TTC CAY GAY TGG CGR GA	1506-1529	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1525F	CGA GAR GTG ATG ACY GAR TCD GAR CG	1525-1550	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1705F	ATC GAC GGB CAY AAR GAR AAR ATH GG	1705-1730	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1729F2	GGY AAC TTY AAR ATY GAG CCD CCV GG	1729-1754	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1765F	GGY CGT GGY GAG CAY CCB AAR ATG GG	1765-1790	XM_396203.3 (Apis mellifera)	This study
Top1	TP1901F2	CY AAT GTY ACD TGG CTH GCR TCH TGG	1901-1928	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1906F	GTC ACR TGG CTC GCR TCM TGG AC	1906-1928	XM_396203.3 (Apis mellifera)	This study (customized for Pseudomyrmecinae)
Top1	TP1987F	GGH GAA AAR GAY TGG CAR AAR TAY GA	1987-2012	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP2065F	GAR GAY TGG AAR AGY AAR GAR ATG CG	2065-2090	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1598R	GC RTG CAT CTC YTT RAA GTT RCA	1598-1576	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1793R	TT RCC CAT YTT RGG RTG CTC RCC RCG	1793-1768	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1805R	CG CYT CTT YAR YTT RCC CAT YTT RGG	1805-1780	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP1892R	CG YAC YTC YTT CCA YTT RTG RCC	1892-1870	XM_396203.3 (Apis mellifera)	This study
Top1	TP2012R	TC RTA YTT YTG CCA RTC YTT YTC DCC	2012-1987	XM_396203.3 (Apis mellifera)	This study
Top1	TP2043R	GTC RAT RGA CTG YGC CAR CTT BCG	2043-2020	XM_396203.3 (Apis mellifera)	This study

Top1	TP2167R	G ATC YTC RTC CTT YTC RTT RCC RGC	2167-2143	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	TP2192R	GA RCA RCA RCC YAC DGT RTC HGC YTG	2192-2167	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	**TP2266ER2**	GTTAC C TAA RAA RTC RAA YAC RAC BAC	~2266-2245	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Top1	**TP2266ER3**	GTYAC C TAA RAA RTC RAA BAC RAC	~2266-2248	XM_396203.3 (Apis mellifera)	Ward & Sumnicht (2012)
Ubx	**UB1EF**	GGRTA ATG AAC TCG TAY TTY GAR CAG	~1-21	NM_001168700.1 (Apis	Ward & Sumnicht (2012) (as UB1F)
Ubx	**UB1EF6**	GATYCAARRTACCCGGGRTA ATG AAC	~1-6	NM_001168700.1 (Apis	Ward & Sumnicht (2012) (as UB1F6)
Ubx	UB214F	CCR CCY CAR GAY TCR CCR TAY GAY GC	214-239	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB259F	AAG CTT TAY TCG ACG ACR CCH GAR GC	259-284	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB289F	GGT CAY ACY ACR TCV TCR TAY TCR AC	289-314	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB323F	CR AAR GAC TGT AAR CAR CAR GAY CA	323-347	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB278R	C DGG YGT CGT YGA RTA VAG YTT RCA	278-256	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB314R	GT YGA RTA YGA DGA YGT RGT RTG RCC	314-289	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB389R	GC TGC CAT YAC CGC VGC RTA DCC	389-367	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB412R	G CCA MAC GTC YTT GAC SGC YGC	412-391	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	**UB671R**	AT RGC CAT CCA RGG RTA GAA SGT RTG	671-646	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	**UB676ER**	CTYAC C TGC TAT RGC CAT CCA RGG	~676-658	NM_001168700.1 (Apis	Ward & Sumnicht (2012)
Ubx	UB676ER2	GCAAACTCAC C TGC TAT RGC	~676-667	NM_001168700.1 (Apis	This study

References

Abouheif E, Wray GA. 2002. Evolution of the gene network underlying wing polyphenism in ants. *Science* **297**, 249-252.

Belshaw R, Quicke DLJ. 1997. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Mol. Phylogenet. Evol.* **7**, 281–293.

Brady, SG, Schultz TR, Fisher BL, Ward PS. 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proc. Natl. Acad. Sci. USA* **103**, 18172-18177.

Kawakita A, Sota T, Ascher JS, Ito M, Tanaka H, Kato M. 2003. Evolution and phylogenetic utility of alignment gaps within intron sequences of three nuclear genes in bumble bees (*Bombus*). *Mol. Biol. Evol.* **20**, 87–92.

Schmitz J, Moritz RFA. 1994. Sequence analysis of the D1 and D2 regions of 28S rDNA in the hornet (*Vespa crabro*) (Hymenoptera, Vespinae). *Insect Mol. Biol.* **3**, 273–277.

Ward PS, Brady SG, Fisher BL, Schultz TR. 2010. Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Syst. Biol.* **59**, 342-362.

Ward PS, Downie DA. 2005. The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. *Syst. Entomol.* **30**, 310-335.

Ward PS, Sumnicht TP. 2012. Molecular and morphological evidence for three sympatric species of *Leptanilla* (Hymenoptera: Formicidae) on the Greek island of Rhodes. *Myrmecol. News* **17**, 5-11.

Wild AL, Maddison DR. 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Mol. Phylogenet. Evol.* **48**, 877–891.

Table S3. *Vachellia* plant material included in this study, with GenBank accession numbers.

Taxon	matK	trnL-F	psaB-rps14
Vachellia anegadensis **(Britton) Seigler & Ebinger**	HM020706	HM020796	HM020743
Vachellia campechiana **(Mill.) Seigler & Ebinger**	HM020710	HM020800	HM020747
Vachellia caven **(Molina) Seigler & Ebinger**	AF274131	AF522967	-
Vachellia **x** ***cedilloi*** **(L. Rico) Seigler & Ebinger**	HM020708	HM020798	HM020745
Vachellia chiapensis **(Saff.) Seigler & Ebinger**	HM020709	HM020799	HM020746
Vachellia collinsii **(Saff.) Seigler & Ebinger**	HM020711	HM020801	HM020748
Vachellia constricta **(Benth.) Seigler & Ebinger**	HM020712	HM020802	HM020749
Vachellia cornigera **(L.) Seigler & Ebinger**	HM020713	HM020803	HM020750
Vachellia farnesiana **(L.) Wight & Arn.**	HM020715	HM020805	HM020752
Vachellia gentlei **(Standl.) Seigler & Ebinger**	HM020717	HM020807	HM020754
Vachellia globulifera **(Saff.) Seigler & Ebinger**	HM020718	HM020808	HM020755
Vachellia hindsii **(Benth.) Seigler & Ebinger**	HM020719	HM020809	HM020756
Vachellia macracantha **(Humb. & Bonpl. ex Willd.) Seigler & Ebinger**	HM020721	HM020812	HM020759
Vachellia mayana **(Lundell) Seigler & Ebinger**	HM020722	HM020813	HM020760
Vachellia melanoceras **(Beurl.) Seigler & Ebinger Voucher STRI:BCI 132160**	GQ982124	-	-
Acacia neovernicosa **Isely**	AF523113	AF522970	-
Vachellia pennatula **(Schltdl. & Cham.) Seigler & Ebinger**	HM020724	HM020814	HM020762
Vachellia roigii **(León) Seigler & Ebinger**	-	-	HM020765
Vachellia rigidula **(Benth.) Seigler & Ebinger**	AF523188	-	
Vachellia schaffneri **(S. Watson) Seigler & Ebinger**	AF274132	HM020818	HM020767
Vachellia schottii **(Torr.) Seigler & Ebinger**	AF274136	AF522971	-
Vachellia sphaerocephala **(Cham. & Schltdl.) Seigler & Ebinger**	HM020729	HM020821	HM020769
Vachellia tortuosa **(L.) Seigler & Ebinger**	HM020730	HM020823	HM020771

Table S4. *Platymisicum* plant material included in this study, with species authors, vouchers and their geographic origin, and GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	Voucher	Geographic origin	*ITS*	*mat*K	*trnL* intron	*trnL-F* spacer
***Platymiscium albertinae* Standl. & L. O. Williams**	R. A. Molina 3197 **(GH)**	Honduras	EU735870	EU735932	EU735989	EU736046
***Platymiscium calyptratum* M. Sousa & Klitgaard**	L. Tenorio & M. Hernadéz 126 **(MEXU)**	Mexico, Hildalgo	EU735872	EU735933	EU735990	EU736047
***Platymiscium curuense* N. Zamora & Klitgaard**	L.D. Gómez et al. 23357 **(K)**	Costa Rica, San Jose	EU735873	EU735934	EU735991	EU736048
***Platymiscium darienense* Dwyer**	N. Garwood 2718A **(BM)**	Panama	EU735874	EU735935	-	-
***Platymiscium dimorphandrum* Donn. Sm.**	J.I. Calzada 14786 **(MEXU)**	Mexico, Oaxaca	EU735876	EU735937	EU735993	EU736050
***Platymiscium dimorphandrum* Donn. Sm.**	C. Hughes et al. 1707 **(K)**	Honduras	EU735875	EU735936	EU735992	EU736049
***Platymiscium dimorphandrum* Donn. Sm.**	E. Martinez S. 17626 **(K)**	Mexico, Chiapas	EU735878	EU735939	EU735995	EU736052
***Platymiscium filipes* Benth.**	B. Klitgaard 35 **(K)**	Brazil, Amazonas	EU735879	EU735940	EU735996	EU736053
***Platymiscium floribundum* Vogel *var. latifolium* (Benth.) Benth.**	B. Klitgaard 49 **(K)**	Brazil, Sao Paulo	EU735881	EU735942	EU735998	EU736055
***Platymiscium floribundum* Vogel *var. nitens* (Vogel) Klitgaard**	B. Klitgaard 6 **(K)**	Brazil, Rio de Janeiro	EU735882	EU735943	EU735999	EU736056
***Platymiscium floribundum* Vogel *var. obtusifolium* (Harms) Klitgaard**	B. Klitgaard 71 **(K)**	Brazil, Bahia	EU735884	EU735945	EU736001	EU736058
Platymiscium floribundum* Vogel *var.floribundum	B. Klitgaard 1 **(K)**	Brazil, Rio de Janeiro	EU735880	EU735941	EU735997	EU736054
***Platymiscium gracile* Benth.**	T.B. Croat 51099 **(MO)**	Peru	EU735886	EU735947	EU736003	-
***Platymiscium hebestachyum* Benth.**	M.T. Dawe 38 **(K)**	Colombia	EU735887	EU735948	EU736004	-
***Platymiscium jejunum* Klitgaard**	B. Cortes et al. 829 **(MEXU)**	Mexico, Oaxaca	EU735888	EU735949	EU736005	EU736060

***Platymiscium lasiocarpum* Sandwith**	H. Ireland 4 **(K)**	Mexico, Oaxaca	EU735889	EU735950	EU736006	EU736061
***Platymiscium parviflorum* Benth.**	C. Hughes 1378 **(K)**	Nicaragua, Esteli	EU735893	EU735953	EU736009	EU736063
***Platymiscium parviflorum* Benth.**	C. Hughes 755 **(K)**	Honduras	EU735891	EU735951	EU736007	EU736062
***Platymiscium parviflorum* Benth.**	N. Zamora & S. Ramirez 2267 **(K)**	Costa Rica, Guanacaste	EU735895	EU735954	EU736011	EU736065
***Platymiscium pinnatum* (Jacq.) Dugand**	T.D. Pennington 15017 **(K)**	Ecuador, Esmeraldas	EU735907	EU735965	EU736022	EU736074
Platymiscium pinnatum* (Jacq.) Dugand *var. diadelphum	G. Aymard 2365 **(NY)**	Venezuela	EU735896	EU735955	EU736012	EU736066
Platymiscium pinnatum* (Jacq.) Dugand subsp. *pinnatum* var. *pinnatum	T.D. Pennington et al. 14945 **(K)**	Ecuador, Esmeraldas	EU735900	EU735959	EU736016	EU736068
***Platymiscium pinnatum* (Jacq.) Dugand subsp. *pinnatum* var. *ulei* (Harms ex Harms) Klitgaard**	T.D. Pennington 15030 **(K)**	Ecuador, Napo	EU735902	EU735961	EU736018	EU736070
***Platymiscium* pinnatum (Jacq.) Dugand subsp. *polystachyum* (Benth.) Klitgaard**	R.T. Pennington & N. Zamora 591 (K)	Costa Rica, Puntarenas	EU735904	EU735962	EU736019	EU736071
***Platymiscium pubescens* Micheli subsp. *fragrans* (Rusby) Klitgaard**	M. Nee 47866 **(MEXU)**	Bolivia, Santa Cruz	EU735911	EU735969	EU736026	EU736078
Platymiscium pubescens* Micheli subsp. *pubescens	B. Klitgaard 16 **(AAU)**	Brazil, Minas Gerais	EU735914	EU735972	EU736029	EU736081
***Platymiscium pubescens* Micheli subsp. *zehntneri* (Harms) Klitgaard**	B. Klitgaard 72 **(AAU)**	Brazil, Bahia	EU735915	EU735973	EU736030	EU736082
***Platymiscium speciosum* Vogel**	B. Klitgaard 26 **(K)**	Brazil, Espirito Santo	EU735920	EU735978	EU736035	EU736087
***Platymiscium stipulare* Benth.**	B. Klitgaard 623 **(AAU)**	Ecuador, Napo	EU735922	EU735980	EU736036	EU736088
***Platymiscium trifoliolatum* Benth.**	Calzada et al. 19160 **(K)**	Mexico, Nayarit	EU735923	EU735981	EU736038	EU736089
***Platymiscium trinitatis* Benth. var. *duckei* (Huber) Klitgaard**	B. Klitgaard 38 **(K)**	Brazil, Amazonas	EU735925	EU735983	EU736040	EU736091
***Platymiscium trinitatis* Benth. var. *nigrum* (Ducke) Klitgaard**	L.A. Ferreira 9526 **(K)**	Brazil, Para	EU735927	-	EU736042	EU736093
Platymiscium trinitatis* Benth. var. *trinitatis	B. Klitgaard 31 **(K)**	Brazil, Amazonas	EU735928	EU735985	EU736043	EU736094

***Platymiscium yucatanum* Standl.**	N. Aguirre s.n. **(K)**	Mexico	EU735930	EU735987	EU736045	EU736095
***Cascaronia astragalina* Griseb.**	B. Klitgaard 100 (**K**)	Argentina	AF204235	AF272072	AF208958	EU735858
***Geoffroea decorticans* (Hook. & Arn.) Burkart**	M. Lavin 750 (**MONT**)	USA	AF189057	AF270880	-	-
***Riedeliella graciliflora* Harms**	Ratter et al. 7494 (**E**)	Brazil	-	AH009910	AF208949	-

Table S5. *Triplaris* and *Ruprechtia* plant material included in this study, with species authors, vouchers and their geographic origin, and GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp). Vouchers and sequences marked with an asterisk belong together; some GenBank sequences are unvouchered.

Taxon	Voucher	Geographic origin	ITS	matK	ndhF	ndhC-trnV	rps16-trnK
***Antigonon leptopus* Hook. & Arn.**	*M. Luckow 4630 (BH)	?	FJ154462	EF437988	EF438027	*HQ693163	*HQ693194
***Eriogonum alatum* Torr.**	*J.L. Reveal 8515 (MARY)	USA	FJ154472	EF437998	EF438038	*HQ693165	*HQ693196
***Gymnopodium floribundum* Rolfe**	*J.M. Burke 48 (BH)	?	GQ206251	GQ206197	GQ206282	*HQ693166	*HQ693197
***Magoniella obidensis* (Huber) Adr. Sanchez**	L. Cayola et al. 107 (MO)	Bolivia	HQ693103	HQ693198	HQ693214	HQ693151	HQ693167
***Ruprechtia aperta* Pendry**	T. Sarkinen et al. 2192 (FHO)	Peru, Amazonas	HQ693104	HQ693199	HQ693215	HQ693139	HQ693169
***Ruprechtia apetala* Wedd.**	M.H. Nee & R. Flores 54796 (NY)	Bolivia	HQ693105	HQ693200	HQ693216	HQ693140	HQ693170
***Ruprechtia chiapensis* Lundell**	*J.M. Burke s.n. (BH)	?	FJ154482	FJ154495	FJ154506	*HQ693141	*HQ693171
***Ruprechtia coriacea* (H.Karst.) Benth. & Hook.f. ex B.D. Jacks.**	*A. Sanchez 104 (WFU)	Venezuela?	HM137442	HM137401	HM137423	*HQ693142	*HQ693172
***Ruprechtia costaricensis* Pendry**	A. Sanchez 400 (WFU)	Costa Rica	HQ693106	HQ693201	HQ693217	HQ693143	HQ693173
***Ruprechtia costata* Meisn.**	J.J.M. Castillo 2119 (MO)	Costa Rica	HQ693107	HQ693202	HQ693218	-	HQ693174
***Ruprechtia cruegeri* Griseb. ex Lindau**	*M. Luckow 4587 (BH)	Venezuela	HM137443	HM137402	HM137424	*HQ693144	*HQ693175
***Ruprechtia fagifolia* Meisn.**	W. Thomas et al. 9638 (NY)	Brazil	HQ693108	HQ693203	HQ693219	HQ693145	HQ693176
***Ruprechtia fusca* Fernald**	*C.A. Pendry 868 (E)	Mexico	FJ154483	FJ154496	FJ154507	*HQ693146	*HQ693177
***Ruprechtia laevigata* Pendry**	R.C. Torres et al. 9084 (MO)	Mexico	HQ693109	HQ693204	HQ693220	HQ693147	HQ693178
***Ruprechtia latifunda* Pendry**	A. Sanchez 164 (WFU)	Brazil	HQ693110	HQ693205	-	-	-
***Ruprechtia laxiflora* Meisn.**	*D.E. Prado s.n. (E)	Argentina	FJ154484	EF438024	EF438063	*Q693148	*HQ693179

Ruprechtia lundii **Meisn.**	A. Sanchez 160 (WFU)	Brazil	HQ693111	HQ693206	HQ693221	HQ693149	HQ693180
Ruprechtia nicaraguensis **Pendry**	R.M. Rueda et al. 2444 (MO)	Nicaragua	HQ693112	HQ693207	HQ693222	HQ693150	HQ693181
Ruprechtia obovata **Pendry**	T. Sarkinen et al. 2221 (FHO)	Peru, Cajamarca	HQ693113	HQ693208	HQ693223	HQ693152	HQ693182
Ruprechtia pallida **Standl.**	J.B. Burke 129 (BH)	Mexico	HM137445	HM137403	HM137425	-	*HQ693183
Ruprechtia tangarana **Standl.**	M.R. Silman s.n. (WFU)	Peru	-	-	-	HQ693153	HQ693184
Salta triflora **(Griseb.) Adr. Sanchez**	M.H. Nee et al. 53657 (MO)	Bolivia	*GQ206267	*HQ693213	GQ206299	*HQ693154	*HQ693168
Triplaris americana **L.**	M. Luckow 4635 (BH)	Colombia	FJ154486	AY042668	FJ154508	*HQ693155	*HQ693185
Triplaris cumingiana **Fisch. & C.A. Mey.**	A. Sanchez 100 (WFU)	Colombia	GQ206269	GQ206210	GQ206301	HQ693156	HQ693186
Triplaris longifolia **Huber**	*A. Sanchez 188 (WFU)	Peru	HQ693114	HQ693209	HQ693224	-	*HQ693187
Triplaris melaenodendron **(Bertol.) Standl. & Steyerm.**	A. Sanchez 405 (WFU)	Costa Rica	HQ693115	HQ693210	HQ693225	HQ693157	HQ693188
Triplaris peruviana **Fisch. & Meyer ex C.A. Meyer**	A. Sanchez 171 (WFU)	Peru	HQ693116	HQ693211	HQ693226	HQ693158	HQ693189
Triplaris poeppigiana **Wedd.**	*A. Sanchez 89 (WFU)	Peru	FJ154487	FJ154497	FJ154509	*HQ693159	*HQ693190
Triplaris purdiei **Meisn.**	*A. Sanchez 100 (WFU)	Peru	HQ693117	HQ693212	HQ693227	HQ693160	HQ693191
Triplaris setosa **Rusby**	*A.F. Fuentes et al. 5351 (MO)	Bolivia	FJ154488	FJ154498	FJ154510	*HQ693161	*HQ693192
Triplaris weigeltiana **(Rchb.) Kuntze**	*Unvouchered cultivated material	Montgomery Botanical Center (natural range: Colombia, Venezuela, Guiana shield)	*HM137446	*HM137405	*HM137426	*HQ693162	*HQ693193
Triplaris peruviana **Fisch. & Meyer ex C.A. Meyer**	A. Sanchez 171 (WFU)	Peru	KP271190	-	-	-	KP236780
Triplaris punctata **Standl.**	A. Sanchez 205 (WFU)	Peru	KP271191	-	-	-	KP236785
Triplaris dugandii **Brandbyge**	A. Sanchez 58 (WFU)	Peru	KP271194	-	-	-	KP236772

Table S6. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Species	Voucher	Location	ITS	trnL	matK	psbA-trnH
***Tachigali alba* Ducke**	M. Silva & R. Souza 2257, 14 Aug. 1969 (M)	Pará, Brazil	KR492614	KR872689	KR872709	KR872664
***Tachigali aurea* Tul.**	G. Hatschbach 23815, 19 Feb 1970 (M)	Brazil	-	-	KR872710	-
***Tachigali barnebyi* van der Werff**	M.G. Vieira *et al.* 910 (MO)	Rodonia, Brazil	KR492615	KR872690	KR872711	KR872665
***Tachigali bracteosa* (Harms) Zarucchi & Pipoly**	G.T. Prance *et al.* 59239, 02 Oct 1964 (M)	Serra do Roncador, Brazil	-	-	KR872733	KR872685
***Tachigali cavipes* (Spruce ex Benth.) J.F. Macbr.**	B. Maguire *et al.* 41769, 07 Oct. 1957 (M)	Rio Guainia, Temtono. Amazonas (Colombia-Venezuela)	-	-	KR872712	-
***Tachigali* cf. *paniculata* Aubl.**	F. Ayala 8075, 01 Apr 1997 (M), det. Merello (MOBG)	Perú	KR492623	KR872701	KR872726	KR872678
***Tachigali* cf. *paniculata* Aubl.**	G.T. Prance *et al.* 14257 (M), det. H.C. Lima (RB)	Brazil Río Purus, Río Itaxi	KR492622	KR872700	KR872725	KR872677
***Tachigali chrysaloides* van der Werff**	J. Jaramillo 27007 (QCA)	Ecuador, Pastaza-Tarangaro	-	KR872691	KR872713	KR872666
***Tachigali chrysophylla* (Poepp) Zarucchi & Herend.**	E. Gudiño 1382 (QCA)	Ecuador, Pastaza-Moretocōcha	-	-	KR872714	KR872667
***Tachigali denudata* (Vogel) Oliveira-Filho**	A. Andrade *et al.* RB 384069, 12 Jun 2003 (M)	Floresta de Tijuca, Brazil	KR492616	KR872692	KR872715	KR872668
***Tachigali densiflora* (Benth.) L.F. Gomes da Silva & H.C. Lima**	Carvalho 4095	Brazil	-	AY904429	-	-
***Tachigali dwyeri* (R.S.Cowan) Zarucchi & Herend.**	B. Maguire *et al.* 41652 (MO)	Amazonas, Venezuela	KR492617	KR872693	KR872716	KR872669
***Tachigali formicarum* Harms**	J. Jaramillo 19645 (QCA)	Ecuador, Orellana	-	-	KR872717	KR872670
***Tachigali glauca* Tul.**	C.C. Berg *et al.* P19811, 20 Oct. 1973 (M)	State of Mato grosso, Brazil	KR492618	KR872694	KR872718	KR872671
***Tachigali guianensis* (Benth.) Zarucchi & Herend.**	K. Kubitzki *et al.* 85-50, 25 Mar 1985 (M)	Brazil	-	KR872695	KR872719	KR872672
***Tachigali hypoleuca* (Benth.) Zarucchi &**	G.T. Prance *et al.* 16264, 11 Nov 1971 (M)	Brazil	KR492619	-	KR872720	KR872673

Herend.						
***Tachigali inconspicua* van der Werff**	A.J. Perez 3803 (QCA)	Ecuador Pastaza-Villano	-	KR872696	KR872721	KR872674
***Tachigali macrostachya* Huber**	D.G. Campbell *et al.* P22471, 02 Jun 1974 (M)	Basin Río Trombetas, Brazil	KR492620	KR872697	KR872722	KR872675
***Tachigali myrmecophila* (Ducke) Ducke**	R. S. Cowan 38220	Brazil	-	AY899706	-	-
***Tachigali odoratissima* (Spruce ex Benth.) Zarucchi & Herend.**	J. Clark 8303 (QCA)	Venezuela, Terr. Federal Amazonas	KR492621	KR872698	KR872723	KR872676
***Tachigali panamensis* van der Werff & N. Zamora**	H. van der Werff *et al.* 24406 (MO)	Colón, Panama	-	KR872699	KR872724	-
***Tachigali paraensis* (Huber) Barneby**	R. Valencia 67740 (QCA)	Ecuador, Sucumbios-Cuyabeno	KR492624	KR872702	KR872727	KR872679
***Tachigali paratyensis* (Vell.) H.C. Lima**	Hy. Mosen 3370 (M)	Brazil	-	-	KR872728	KR872680
***Tachigali peruviana* (Dwyer) Zarucchi & Herend.**	G.T. Prance *et al.* 5447, 02 Jul 1968 (M)	Basin Río Madeira, Brazil	-	-	KR872729	-
***Tachigali physophora* (Huber) Zarucchi & Herend.**	E. Lleras *et al.* P17479, 24 Aug 1973 (M)	Estado de Amazonas, Rio Solimoes and Rio Javani	KR492625	KR872703	KR872730	KR872681
***Tachigali plumbea* Ducke**	B. Boom *et al.* 8742 (MO)	Amazonas, Brazil	KR492626	-	KR872731	KR872682
***Tachigali poeppigiana* Tul.**	M.G. Vieira *et al.* 914 (MO)	Rodonia, Brazil	KR492627	-	-	KR872683
***Tachigali ptychophysca* Spruce ex Benth.**	R.E. Schultes & I. Cabrera 12955 (MO)	Amazonas, Colombia	KR492628	-	-	-
***Tachigali rigida* Ducke**	J. Clark 7921 (QCA)	Venezuela, Terr. Federal Amazonas	KR492629	KR872704	KR872732	KR872684
***Tachigali paniculata* Aubl.**	R. Blatrix 429 (M leaf voucher)	French Guiana, Kourou	KR492630	KR872705	KR872734	KR872686
***Tachigali paniculata* Aubl.**	J.J. Strudwick 3352 (CAS)	Brazil	-	AF430790	-	-
***Tachigali subvelutina* (Benth.) Oliveira-Filho**	R.M. Harley *et al.* 19962, 24 Mar 1977 (M)	Rio de Contas, Brazil	KR492631	KR872706	KR872735	KR872687
***Tachigali tinctoria* (Benth.) Zarucchi & Herend.**	G.T. Prance *et al.* 8840, 25 Nov 1968 (M)	Basin Río Madeira, Brazil	-	-	KR872736	-
***Tachigali venusta* Dwyer**	J.R. Nascimento & C.F. da Silva 658 (MO)	Amazonas, Brazil	KR492632	KR872707	KR872737	-
***Tachigali vulgaris* L.F. Gomes da Silva & H.C. Lima**	A. Janssen 687, 20 Oct. 1981 (M)	Estado Amazonas, MunBrazil	KR492633	KR872708	KR872738	KR872688
***Tachigali richardiana* Tul.**	D. Clarke 7212 (US)	Guyana	-	AY232775 AF365113	EU362054	-
Tachigali sp.	B. Klitgaard 687 (K)	Ecuador	-	KF794198 AF365111	EU362040	-

Table S7. The 12 partitions and models identified by PartitionFinder and used in maximum likelihood and Bayesian analyses of the 10-gene Pseudomyrmecinae data matrix.

Partition	**Blocks**	**Model**
p1	AbdA pos1, EF1aF2 pos2	JC+I
p2	AbdA pos2, Ubx pos2	F81
p3	AbdA pos3, Ubx pos3	K80+G
p4	EF1aF2 pos3, Top1 pos3	K80+I+G
p5	ArgK pos1, Enolase pos1, EF1aF2 pos1, Top1 pos1, Ubx pos1	GTR+I+G
p6	ArgK pos3, LW Rh pos3, Wg pos3	K80+I+G
p7	28S, LW Rh pos1	SYM+I+G
p8	ArgK pos2, LW Rh pos2	SYM+I+G
p9	CAD pos2, Enolase pos2, Top1 pos2	HKY+I+G
p10	CAD pos3, Enolase pos3	HKY+I+G
p11	CAD pos1	K80+I+G
p12	Wg pos1, Wg pos2	K80+I+G

Table S9. BioGeoBEARS statistics for biogeographic model testing applied to ant and plant clades. Details see *Materials and Methods*.

(a) Pseudomyrmecinae ants

Model	LnL	d	e	j	ΔAICc model1	ΔAICc model2
DEC	-608.90	0.011452	1E-12	0		
DEC + J	-608.91	0.011453	1E-12	0.00001	0.37	2.73
DIVALIKE	-647.46	0.013132	1E-12	0		
DIVALIKE + J	-646.90	0.012867	1E-12	0.00186	0.64	1.55
BAYAREALIKE	-463.52	0.001639	3.51E-2	0		
BAYAREALIKE + J	**-451.87**	**0.002216**	**2.55E-2**	**0.0047**	42289	2.4E-05

(b) *Tachigali* (Fabaceae: Caesalpinioideae)

Model	LnL	d	e	j	ΔAICc model1	ΔAICc model2
DEC	-78.06	0.032838	1.97E-2	0		
DEC + J	**-72.46**	**0.015679**	**5.23E-3**	**0.0512**	99.67	0.01
DIVALIKE	-76.09	0.035186	1.60E-2	0		
DIVALIKE + J	-73.31	0.016503	1E-12	0.0409	5.94	0.17
BAYAREALIKE	-97.46	0.019197	1.07E-1	0		
BAYAREALIKE + J	-77.88	0.013375	1.76E-2	0.0628	1.17E+8	8.6E-9

(c) *Triplaris/Ruprechtia* (Polygonaceae)

Model	LnL	d	e	j	ΔAICc model1	ΔAICc model2
DEC	-77.66	0.007798	4.12E-3	0		
DEC + J	**-76.51**	**0.005968**	**1E-12**	**0.0158**	1.16	0.86
DIVALIKE	-79.94	0.008199	2E-9	0		
DIVALIKE + J	-79.30	0.007084	1E-12	0.0122	0.70	1.43
BAYAREALIKE	-86.99	0.008726	0.03	0		
BAYAREALIKE + J	-82.18	0.005773	0.01	0.0272	45.21	0.02

(d) ***Vachellia*** **(**Fabaceae: Mimosoideae)

Model	**LnL**	**d**	**e**	**j**	**ΔAICc model1**	**ΔAICc model2**
DEC	**-37.550**	**0.020773**	**5.88-9**	**0**		
DEC + J	-37.551	0.020770	4.15E-9	1E-5	0.37	2.72
DIVALIKE	-39.795	0.022501	3.43E-9	0		
DIVALIKE + J	-39.796	0.022501	1E-12	1E-5	0.36	2.71
BAYAREALIKE	-39.58	0.017692	0.02	0		
BAYAREALIKE + J	-39.41	0.016119	0.01	0.004	0.44	2.28

(e) ***Platymiscium*** **(**Fabaceae: Faboideae)

Model	**LnL**	**d**	**e**	**j**	**ΔAICc model1**	**ΔAICc model2**
DEC	**-39.675**	**0.013688**	**1E-12**	**0**		
DEC + J	-39.676	0.013690	1E-12	1E-5	0.37	2.72
DIVALIKE	-42.460	0.016942	1E-12	0		
DIVALIKE + J	-42.461	0.016939	1E-12	1E-5	0.37	2.72
BAYAREALIKE	-48.50	0.013117	0.03	0		
BAYAREALIKE + J	-41.65	0.004849	1E-7	0.04	346.1	0.003

| Chapter 3 |

Evolutionary relationships and history of the ant-epiphytic genus *Squamalleria* (Rubiaceae: Psychotrieae) and their taxonomic implications

Guillaume Chomicki and Susanne S. Renner

PLoS ONE 11: e0151317 (2016).

.

PLOS | ONE

RESEARCH ARTICLE

Evolutionary Relationships and Biogeography of the Ant-Epiphytic Genus *Squamellaria* (Rubiaceae: Psychotrieae) and Their Taxonomic Implications

Guillaume Chomicki*, Susanne S. Renner

Systematic Botany and Mycology, University of Munich (LMU), Menzinger Str. 67, 80638, Munich, Germany

* guillaume.chomicki@gmail.com

Citation: Chomicki G, Renner SS (2016) Evolutionary Relationships and Biogeography of the Ant-Epiphytic Genus *Squamellaria* (Rubiaceae: Psychotrieae) and Their Taxonomic Implications. PLoS ONE 11(3): e0151317. doi:10.1371/journal.pone.0151317

Editor: William Oki Wong, Institute of Botany, CHINA

Received: December 2, 2015

Accepted: February 25, 2016

Published: March 30, 2016

Data Availability Statement: All relevant data have been submitted to GenBank and the accession numbers are provided in the Supporting Information file S1 Table.

Funding: This work was supported by a grant from the German Research Foundation (DFG), RE 603/20, and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to GC. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Ecological research on ant/plant symbioses in Fiji, combined with molecular phylogenetics, has brought to light four new species of *Squamellaria* in the subtribe Hydnophytinae of the Rubiaceae tribe Psychotrieae and revealed that four other species, previously in *Hydnophytum*, need to be transferred to *Squamellaria*. The diagnoses of the new species are based on morphological and DNA traits, with further insights from microCT scanning of flowers and leaf $\delta^{13}C$ ratios (associated with Crassulacean acid metabolism). Our field and phylogenetic work results in a new circumscription of the genus *Squamellaria*, which now contains 12 species (to which we also provide a taxonomic key), not 3 as in the last revision. A clock-dated phylogeny and a model-testing biogeographic framework were used to infer the broader geographic history of rubiaceous ant plants in the Pacific, specifically the successive expansion of *Squamellaria* to Vanuatu, the Solomon Islands, and Fiji. The colonization of Vanuatu may have occurred from Fiji, when these islands were still in the same insular arc, while the colonization of the Solomon islands may have occurred after the separation of this island from the Fiji/Vanuatu arc. Some of these ant-housing epiphytes must have dispersed with their specialized ants, for instance attached to floating timber. Others acquired new ant symbionts on different islands.

Introduction

The angiosperm family with the highest diversity of ant-plant is the Rubiaceae [1]. In Southeast Asia, it is the tribe Psychotrieae that is especially rich in epiphytic species occupied by ants living in specialized domatia. During ecological research on ant/plant symbioses in the Psychotrieae of Fiji, we discovered several new species that we are here placing in a phylogenetic and biogeographic context. Based on molecular-phylogenetic data, the four new species belong in the genus *Squamellaria* in the Hydnophytinae, a subtribe erected by Huxley and Jebb [2] to set apart a group of epiphytic ant plants from the rest of the Psychotrieae, which contain over 2000

Competing Interests: The authors have declared that no competing interests exist.

species. The Hydnophytinae include about 100 species in five genera *Hydnophytum* (55 species; Jebb and Huxley, unpublished revision), *Myrmecodia* (26 species; [3]), *Myrmephytum* (5 species; [4]), *Anthorrhiza* (9 species; [5]), and *Squamellaria* (3 species; [6]). These five genera share a unique synapomorphy consisting of a hypocotyl-derived tuber (domatium) that contains a network of galleries, connected to the exterior by entrance holes. The galleries and entrance holes form regardless of the presence of ants [7, 8]. The tubers of most Hydnophytinae are inhabited by ants, usually belonging to the dolichoderine genera *Philidris* and *Anonychomyrma* [9]. In most species, the walls of the galleries inside the domatium are of two types: smooth walls (where the ants nest) and warted walls with small root-like protuberances [7,8,10]. Heim [11] suggested that the warts might be absorptive, a suggestion supported by Janzen [12], who on Borneo observed workers of *Philidris myrmecodiae* placing dead insects inside warted chambers, indicating to a trophic mutualism. The demonstration of such a mutualism came from a seminal paper by Huxley [9] who used radiolabelled sugar solutions to prove that molecules taken up by the ants moved from their feces in the warted cavities into the plants. Huxley [9] also provided evidence for an additional anti-herbivore defence role of the symbiotic ants.

Systematic work on the ant plant species in the Hydnophytinae began with the research of the Italian botanist Odoardo Beccari (1843–1920) who spent 13 years in Sarawak (1865–1878) and undertook two expeditions to West Papua, one in 1872, the other in 1875 [13]. He described numerous ant-housing species, notably in *Myrmecodia* W.Jack and *Hydnophytum* W.Jack [14]. Beccari also studied relevant herbarium material, including the first ant-plant ever collected on Fiji, a specimen prepared by members of the Wilkes United States Exploring Expedition in 1840 and described by Asa Gray as *M. imberbis* (Wilkes Expl. Exped. s.n.; US Catalog No.: 62266, barcode: 00129869). John Horne (1848–1928), a British forester living in Fiji in 1876/1877, collected a second ant-housing species, *H. wilsonii* [15], a name validated by Baker [16]. Based on the Wilkes and Horne specimens, Beccari thought that these Fijian ant plants differed sufficiently from *Hydnophytum* and *Myrmecodia* species to deserve a separate genus (a decision supported by DNA sequences; *Results*). He diagnosed the new genus by the presence of fringed scales (squamellae) at the inner base of the flower petals (Beccari [14], p. 228: “tubo intus ad basin squamulis 4 barbatis aucto”), and accordingly named it *Squamellaria*, with the new combinations, *S. imberbis* (A.Gray) Beccari and *S. wilsonii* (Horne ex Baker) Beccari. Beccari could not know whether the two species of *Squamellaria* formed the inflated hypocotyl tubers found in all species of this group (Hydnophytinae), writing “Gli esemplari di *M. imberbis* che conosco, constant soltanto di rami e mancano di radici o di tubero. Non trovo nemmeno alcuna citazione che mi faccia credere che queste due piante producano alla base un rigonfiamento abitato da formiche come gli *Hydnophytum*” (Beccari [14], p. 228), meaning “The specimens of *M. imberbis* that I have seen consist only of branches and lack roots or tubers. I also cannot find any observations that make me believe that these two plants [*M. imberbis* and *S. wilsonii*] produce a swelling at the base inhabited by ants, as do *Hydnophytum*.

On Fiji, cars came into use in the early 20th century, and when A.C. Smith began collecting in the Fiji Archipelago in 1933–1934 [17], the road network was still limited, especially in the eastern part of Taveuni Island (Bouma), where roads were first built in the 1970’s [18]. With the increasingly easy access, a third species of *Squamellaria*, *S. major*, was discovered in 1953 by Smith [19] on the slopes of Mt. Manuka near Waikiri (*Smith 8323*; US Catalog No.: 2191043, barcode: 00129863), and a fourth, *S. thekii*, in 1983 by Jebb [6] from Taveuni at DesVoeux Peak near Somosomo (*Jebb 477*; BISH, K image barcode K000761985, SUVA). Benefitting from these known locations and easier road access, we conducted fieldwork in Viti Levu, Vanua Levu, and Taveuni, in 2014 and 2015, studied relevant herbarium material (see *Acknowledgments*), and used molecular phylogenetics to answer the following questions: (*i*) What are the species relationships in the Pacific Hydnophytinae and (*ii*) Are are the Pacific Hydnophytinae part of a

Table 1. δ ^{13}C value in Fijian *Squamellaria*. A value below 20‰ is suggestive of CAM metabolism, around 20‰ implies an intermediate CAM/C3 metabolism and above 20‰ indicates C3 metabolism. In all cases, Isotope-Ratio Mass Spectrometry (IR-MS) measurements were measured on leaves.

Species	Voucher	δ ^{13}C (‰)	Metabolism
***Squamellaria grayi* sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (M)	-24.35	C3
***Squamellaria huxleyana* sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 48 (M)	-18.89	CAM
Squamellaria imberbis	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	-28.01	C3
***Squamellaria jebbiana* sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	-29.79	C3
Squamellaria major	M.P.H. Jebb 475 (FHO)	-15.08	CAM
Squamellaria major	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	-16.23	CAM
***Squamellaria tenuiflora* comb. Nov.**	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	-34.72	C3
***Squamellaria tenuiflora* comb. Nov.**	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	-34.01	C3
Squamellaria thekii	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	-20.13	Intermediate
***Squamellaria wilkinsonii* comb. Nov.**	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	-30.44	C3
***Squamellaria wilkinsonii* comb. Nov.**	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	-30.18	C3
Squamellaria wilsonii	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	-30.19	C3

doi:10.1371/journal.pone.0151317.t001

single lineage that dispersed throughout the Pacific? We enhance our species descriptions with CT scanning of flowers and δ ^{13}C to account for photosynthetic types (Table 1).

Materials and Methods

Ethics statement

Some of the new species reported in this work were collected in non-protected forests (*S. huxleyana*, along the cross-island road on Vanua Levu), others in protected forests (*S. grayi* in the Bouma heritage reserve on Taveuni and Waisali Forest Park on Vanua Levu; *S. jebbiana* Des-Voeux peak reserve on Taveuni). All fieldwork was conducted jointly with members of the University of the South Pacific, Suva, Fiji (see *Acknowledgments*), thus no permits were required for the described study, which complied with all relevant regulations. The research did not endanger any protected species. Holotypes of our new species and duplicates of other collections are deposited in the SUVA herbarium in Fiji.

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: PubMed Central and LOCKSS.

DNA extraction, amplification, sequence alignment and phylogenetic inference

The isolation of DNA, amplification, and sequencing followed standard procedures, described in Chomicki and Renner [1, 20]. We sequenced and combined six plastid regions (*trnL* intron, *trnL-trnF* spacer, *ndhF*, *rps12-rpl20*, *trnS*-trnG, and *rps16*) and three nuclear regions (18S, ITS, and ETS) from 17 *Squamellaria* specimens. We sampled all species of Pacific Hydnophytinae (Fiji, Vanuatu, the Solomons). Vouchers, with their geographic origin and herbarium deposition, as well as the GenBank accession numbers for new sequences linked to this paper are shown in S1 Table. Dense species sampling of Hydnophytineae assured that the monophyly of *Squamellaria* could be rigidly tested. Sequence alignments were performed in MAFFT vs. 7 [21], under standard settings except for the ITS region aligned using the Q-INS-S option, which takes into consideration RNA secondary structure, as recommended for this marker. In the absence of statistically supported incongruence (defined as maximum likelihood bootstrap support >70%), we concatenated the datasets manually in Mesquite v. 2.75 [22]. Maximum likelihood tree inference relied on RAxML v. 8.1 [23], with 100 ML bootstrap replicates, using the GTR + G substitution model with six rate categories. We also conducted Bayesian analyses in MRBAYES v. 3.2 [24], using the best-fitting models identified by jModelTest2 [25] in a two-partition (chloroplast-nuclear) scheme. We used the default four chains (one cold and three heated), with uniform priors on most parameters. Substitution models for plastid (HYK+G) and nuclear (JC+G) were unlinked. The Markov chain Monte Carlo (MCMC) was run for 1 million generations, with parameters and trees sampled every 1,000 generations.

Molecular clock dating

Molecular dating analyses relied on BEAST v. 2 [26] and uncorrelated lognormal relaxed clock models. We used the GTR + G substitution model with four rate categories and a Yule tree prior. The MCMCs were run for 20 million generations, with parameters and trees sampled every 10,000 generations. We used Tracer v. 1.6 [27] to check that the effective sample size (ESS) of all parameters was >200, indicating that runs had converged. After discarding 20% as burn-in, trees were summarized in TreeAnnotator v. 1.8 (part of the BEAST package) using the options 'maximum clade credibility tree', which is the tree with the highest product of the posterior probability of all its nodes, 'mean node height,' and a posterior probability limit of 0.98. The final tree was visualized in FigTree v. 1.4 [28]. To calibrate our tree, we constrained the age of the root, i.e., the split between the Pacific clade and the so-called *Psychotria* clade IV of Barrabé et al. [29], to 22 ± 7 Ma, based on the age of this node estimated by these authors, using a normal prior and a standard deviation of 4 corresponding to the 95% confidence interval of Barrabé et al. [29].

Historical biogeography

We coded the geographic ranges of the Hydnophytinae and outgroup species as A = Fiji, B = Solomons, C = Vanuatu, D = Papua New Guinea, E = Australia, F = Wallis and Futuna, and G = Malesian region, H = Philippines, I = New Caledonia, J = Hawaii, K = French Polynesia. To infer ancestral areas, we used the multimodel approach implemented in the R package BioGeoBEARS [30, 31] and the chronogram obtained from the dating analysis in BEAST. BioGeoBEARS permits comparison of three biogeographic models, called dispersal-extinction-cladogenesis (DEC), dispersal-vicariance (DIVALIKE), and BAYAREA (BAYAREALIKE) [30, 31]. Founder-event speciation is modeled via a speciation parameter j that can be added to each of the models. We selected the best-fit model based on LogLikelihood values as well as the Akaike Information Criterion (ΔAICc). Statistics for these six models are shown in Table 2.

Measurement of δ^{13}C values

We performed Isotope-Ratio Mass Spectometry (IR-MS) to detect possible $\delta^{13}C$ differences among the species that might be associated with CAM versus C3 photosynthesis. We suspected

Table 2. Model-testing statistics from the BioGeoBEARS analysis. *d* refers to the rate of dispersal/range addition; *e*, to the extinction rate/range contraction; *j*, to the rate of founder-events. The best model (DEC+J) is highlighted in bold.

Models	LnL	Number of parameters	*d*	*e*	*j*
DEC	-117.86	2	0.0076	0.0284	0
DEC+J	**-86.44**	**3**	**1.00E-12**	**1.00E-12**	**0.0309**
DIVALIKE	-111.09	2	0.0073	0.0042	0
DIVALIKE+J	-86.95	3	1.00E-12	1.00E-12	0.0320
BAYAREALIKE	-133.49	2	0.0115	0.1052	0
BAYAREALIKE+J	-88.22	3	1.00E-07	1.00E-07	0.0309

doi:10.1371/journal.pone.0151317.t002

such differences because *Squamellaria* plants are epiphytes growing in drought-stressed tree canopies. We collected and ground 1–2 mg samples of silica-dried leaves or stems of 12 specimens, representing all nine Fijian ant-plant species. Dried samples were analysed with a mass spectrometer at the geoscience institute of the University of Mainz, Germany. Results are reported in Table 1.

Results

Phylogenetic position of the four new species

Our maximum likelihood and Bayesian tree searches based on up to 9300 aligned nucleotides from the combined plastid and nuclear markers (S1 Table) support the monophyly of a group of species close to *S. imberbis*, the type species of *Squamellaria*, while the type species of *Hydnophytum*, *H. formicarum* Jack, is embedded in an Australasian clade of Hydnophytinae (Fig 1), supporting Beccari's [14] gut feeling that the Fijian ant plant species are only distantly related to core-*Hydnophytum*. Four species of *Hydnophytum*, however, are more closely related to the type species of *Squamellaria* than that of *Hydnophytum* (marked with an asterisk in Fig 2M) and here transferred into *Squamellaria*. One of our new species, *S. grayi*, is placed as sister to *S. major*, the other, *S. huxleyana*, as sister to *S. thekii*, the third *S. jebbiana*, as sister to the remaining nine Fijian *Squamellaria* species, and the fourth, *S. vanuatuensis*, as sister to all other species in the genus (Figs 1 and 2M).

Dated phylogeny and historical biogeography of Pacific ant-plants

The BioGeoBEARS analysis selected the model 'Dispersal-Extinction-Cladogenesis + founder event speciation' (DEC + J) as best explaining our data (Table 2), and our phylogenetic analysis revealed two main clades of Hydnophytinae (Fig 1): A Pacific clade consisting of *Squamellaria* and the four species previously in *Hydnophytum* and an Australasian clade consisting of *Anthorrhiza*, *Hydnophytum* (as to its type species), *Myrmecodia* and *Myrmephytum*. The Solomon Islands (color-coded orange) were colonized at least twice by epiphytic ant-plants, while Fiji and Vanuatu each were each colonized only once. The Pacific ant-plant clade apparently dates to 10.7 ± 5 Ma, when *S. vanuatuensis* diverged from the ancestor of the remaining species. We reconstructed the most recent common ancestor of *Squamellaria* as living in Fiji and Vanuatu (Fig 1), at a time when these two archipelagos were part of the same volcanic arc [32]. Colonization of the Solomons at 9.1 ± 4 Ma, led to the pair of Solomon endemics *S. kajewskii* and *S. guppyana*, which diverged from each other at 1.3 ± 1 Ma. The most recent common ancestor of the Fijian *Squamellaria* species (*S. imberbis*, *S. wilsonii*, *S. huxleyana*, *S. grayi* and *S. thekii*) inhabited by the ant species *Philidris nagasau* Mann (1921) is dated to 1.8 ± 1 Ma (Fig 1).

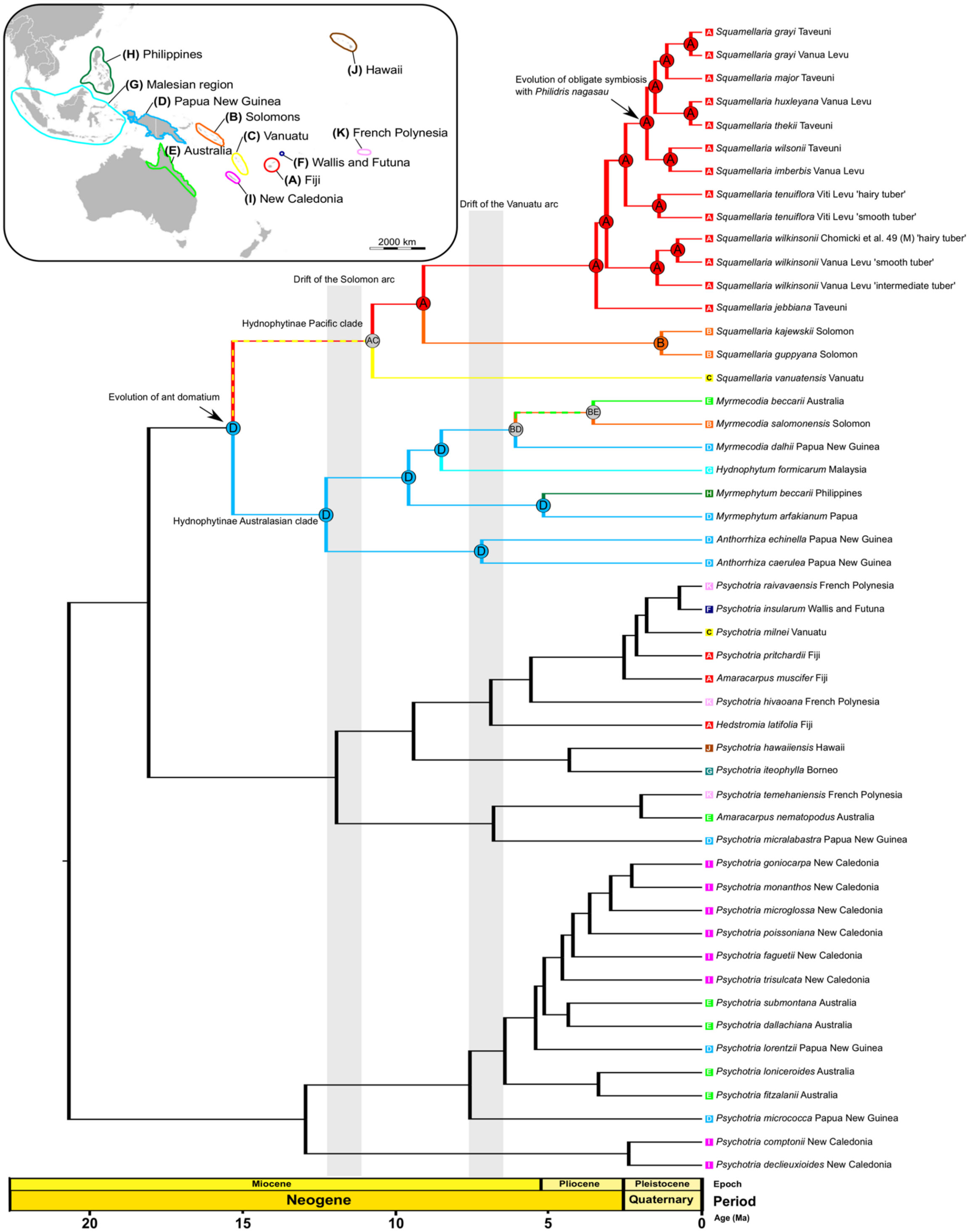


Fig 1. Phylogeny and biogeographic history of the Pacific Hydnophytinae, inferred under the DEC+J model (see Table 2) on the BEAST chronogram.

doi:10.1371/journal.pone.0151317.g001

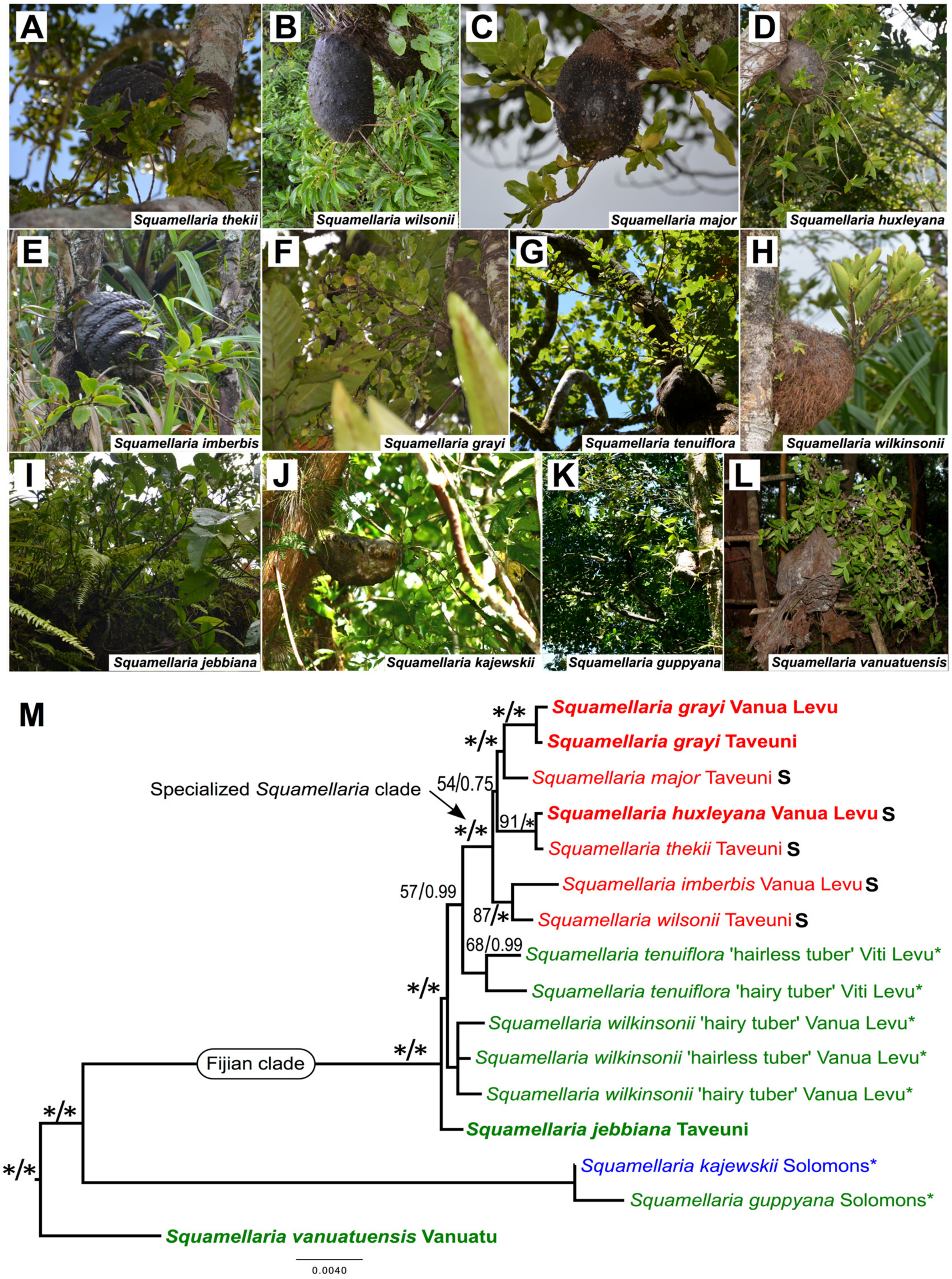


Fig 2. Photos of the 12 *Squamellaria* species and phylogenetic relationships among them. (**A**) *Squamellaria thekii*. (**B**) *S*. *wilsonii*. (**C**) *S*. *major*. (**D**) *S*. *huxleyana* Chomicki, sp. nov. (**E**) *S*. *imberbis*. (**F**) *S*. *grayi*, Chomicki & Wistuba, sp. nov. (**G**) *S*. *tenuiflora* comb. nov. (**H**) *S*. *wilkinsonii* comb. nov. (**I**) *S*. *jebbiana* Chomicki, sp. nov. (**J**) *S*. *kajewskii* comb. nov. (**K**) *S*. *guppyana* comb. nov. (**L**) *S*. *vanuatuensis*, sp. nov. (**M**) Maximum likelihood phylogenetic tree of the genus *Squamellaria* based on up to combined plastid and nuclear DNA regions (outgroups not shown). Numbers above branches are the maximum likelihood bootstrap support values, followed by the posterior probabilities from a Bayesian analysis of the same dataset. Asterisks above branches (*) indicate a maximal support (100 and 1 for ML and Bayesian analyses, respectively). Color-coding of the species names refers to obligate symbiosis with *Philidris nagasau* ants (red), facultative symbiosis with various ant species (green), or no symbiosis with ants (blue). Asterisks after species names refer to names that have been transferred to *Squamellaria*. An 'S' after species name refers to the presence of squamellae. Photographic credits: G. Chomicki except (J-K): Derrick Rowe and (L): Bruno Corbara.

doi:10.1371/journal.pone.0151317.g002

Taxonomic treatment

Squamellaria grayi Chomicki & Wistuba spec. nov. [urn:lsid:ipni.org:names:77153474–1] (Figs 2F, 3A–3D, 4 and 5).

Type. FIJI. Taveuni: Lavena, at the end of Lavena coastal walk, ~4 km (by walk) NW of Lavena village, 16°49'58.98"S, 179°58'36.9"E, 19 m alt., 21 March 2015, *G. Chomicki, J. Aroles & A. Naikatini 53* (SUVA holotype; GH, K, L, M barcode: M-0274839, Fig 5, MO, NOU, NSW, P, S isotypes).

Diagnosis. *Squamellaria grayi* differs from all other species in the genus in its calyx length (2–3 mm vs. 5–7 mm in the other species), corolla tube width (2.5–3.5 mm vs. 5–8 mm), and three carpels with straight pyrenes (vs. four carpels and more or less curved pyrenes). It also differs in one substitution at position 372 (GenBank # KU586339) in the nuclear ribosomal intergenic spacer region ITS (C vs. A in all other *Squamellaria* species).

Description. Tuber (hypocotyle-derived ant-housing structure) attached to tree trunks (see Figs 2F and 3A and 3B), 15–25 to 25–40 cm, cylindrical, tuber apex flattened, surface with small, dark spiny protuberances (1–3 mm long), tuber surface grey, domatium entrance holes 1.5 to 3 mm wide, except the first one, which is 5–8 mm wide. Stems several, branched, of sympodial structure, alternating with entrance hole rings, often imperfect. Internodes 1–5 cm long and 0.2–1 cm in diameter, nodes slightly swollen (Fig 3A), 0.3–1 cm in diameter. Leaves arranged in a decussate phyllotaxis, slightly succulent but performing C3 photosynthesis, curved on the apical-basal axis (Fig 3A), slightly anisophyllous (Fig 4G), lamina ovate (Figs 3A and 4A and 4G), at each mature leaf pair, larger leaf 4–5 cm long, 2.5–3.5 wide and smaller leaf 2.5–4 cm long, 2–3 cm wide, leaves 2–3.5 mm thick, apex acute, base rounded, pale green on both the adaxial (upper) and abaxial (lower) sides, the petiole 2–5 mm long and 2–3.5 mm wide. Primary leaf vein monopodial, secondary veins brochidromous (i.e., secondary veins connect to the connects directly to the next secondary vein via a loop; Fig 4G). Inflorescences (and infructescences) on lateral short shoots, axillary and terminal (Figs 3A and 4G). Flowers functionally unisexual, plants monoecious, female flowers with four sterile stamens, male flowers with a sterile gynoecium (Figs 3D and 4B, 4E and 4F), actinomorphic, 3–5 cm long, opening mostly at night, strongly fragrant, opening only once. Calyx light green, with 4 fused sepals, to 3 mm long and 2 mm wide; corolla with 4 petals, white, glabrous, each petal distal lobe 0.8–1.3 cm long and 0.5–0.8 cm wide, whorled, in a valvate aestivation with revolute margins, corolla tube 2.5–4 cm long. Squamellae absent (Fig 4E). Anthers basifixed, adnate to corolla, valvate, and with introrse dehiscence. Ovary inferior, with three congenitally fused carpels. Stigma flattened, four-parted, square in section, slightly hairy (Fig 4F). Pyrenes 3, straight (Fig 3M). Fruit oblong, round in section, to 1 cm long and 7 mm wide (Fig 3M).

Floral formulae. Male: $^{*}K(4)[C(4)A(4)]\underline{G}(3)$; Female: $^{*}K(4)[C(4)A^{0}(4)]\underline{G}(3)$

Distribution and ecology. *Squamellaria grayi* is known from the vicinity of Lavena on Taveuni (Fig 6), where it grows in forest at sea level, and from Waisali forest reserve in Central Vanua Levu, where it grows at low elevations. The species is inhabited by the Dolichoderinae ant species *Philidris nagasau*. At night, its flowers emit a strong sweet perfume while the other *Squamellaria* species from the clade obligately inhabited by *Philidris nagasau* (see Figs 1 and 2) flower during the day and lack any obvious sent. Its lack of scales (squamellae) at the inner petals bases (Fig 4E) appears to be a secondary loss (Fig 2M).

Etymology and common name. Named in honour of the American botanist Asa Gray who described the first *Squamellaria* (Gray [33]; cf. *Introduction*). Because the epithet *grayana* is occupied by *Psychotria grayana* K.Schum., we opted for *grayi*, so as avoid homonymy should *Squamellaria* be sunk into *Psychotria*. Like the other Fijian *Squamellaria*, *S. grayi* is locally called 'theke theke nkau' meaning testicles of the trees in Fijian, 'theketuwawa' meaning giant

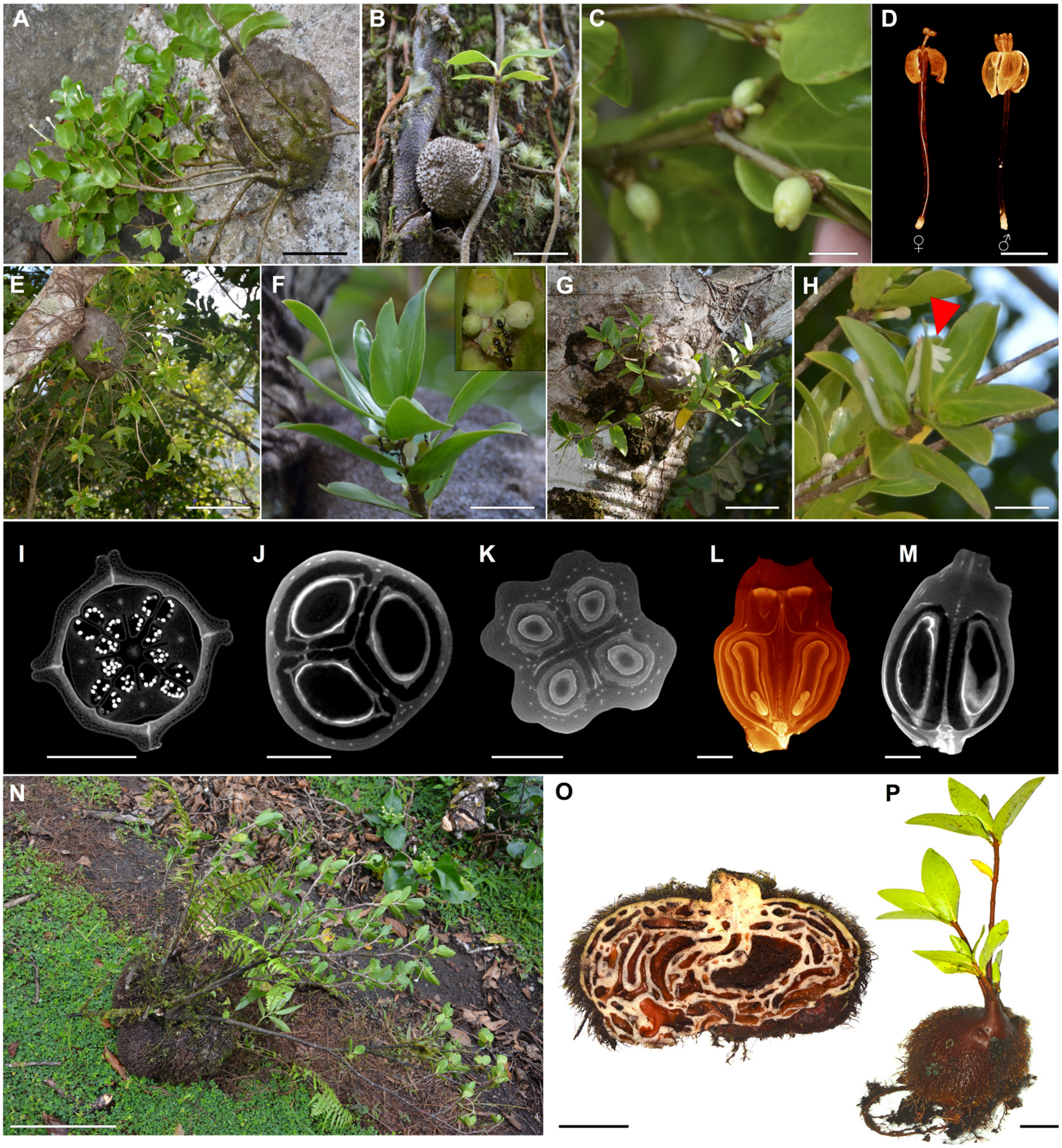


Fig 3. Photos of the three Fijian new species, *Squamellaria grayi*, Chomicki & Wistuba, sp. nov. (Taveuni), *S. huxleyana* Chomicki, sp. nov. (Vanua Levu) and *S. jebbiana* Chomicki, sp. nov. (A-D) *Squamellaria grayi*. (**A**) Mature adult with flowers closed during the day. (**B**) Seedling. (**C**) Fruits. (**D**) CT scanning image of the functionally unisexual flowers of *S. grayi* lacking the squamellae at the inner base of the flower tube (see also Fig 4B, 4E and 4F). (**E-H**) *Squamellaria huxleyana*. (**E**) Habit of a mature adult. (**F**) Shoot with calyx nectaries visited by *Philidris nagasau* workers. Inset shows details of nectary and fruits (see also Fig 6E). (**G**) Habit of two young individuals growing adjacently. (**H**) Flowering shoot including one flower whose corolla has split and which is therefore secondarily zygomorphic. (**I**) CT-scanning optical cross-section of *S. grayi* bud, with reduplicate petal margins (see also Fig 4C). (**J**) CT-scanning optical cross-section of *S. grayi* fruit, with three carpels. (**K**) CT-scanning cross-section of *S. huxleyana* bud, showing the four carpels. (**L**) CT-scanning longitudinal 3D reconstruction of an *S. huxleyana* fruit showing the curved pyrenes. (**M**) CT-scanning longitudinal section of *S. grayi* fruit showing the straight pyrenes. (**N-P**) *S. jebbiana*. (**N**) Habit of a mature adult (fall on the ground). (**M**) Domatium cross-section. (**O**) Juvenile individual. Photographic credit: G. Chomicki except D, I-M: Y. Staedler. Scale bars: A: 10 cm; B: 1.5 cm; C-D: 1 cm; E: 20 cm; F: 2 cm; G: 7 cm; H: 2 cm; I,J: 1.5 mm; K: 3 mm; L-M: 2 mm; N: 20 cm; O: 6 cm; P: 2.5 cm.

doi:10.1371/journal.pone.0151317.g003

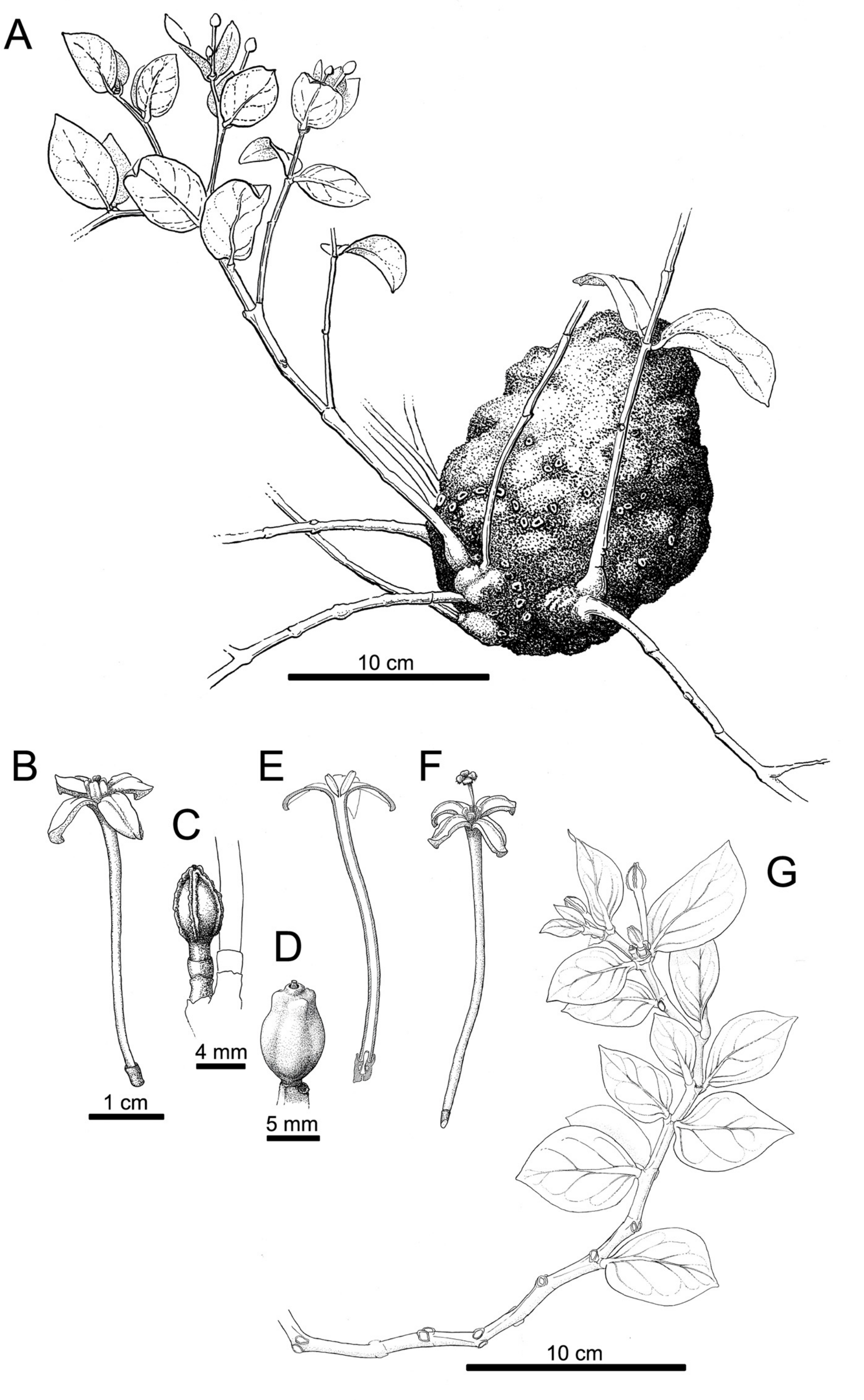


Fig 4. *Squamellaria grayi* Chomicki & Wistuba, spec. nov. (**A**) Habit showing the domatium. (**B**) Male flower. (**C**) Bud with reduplicate petal margins. (**D**) Fruit. (**E**) Male flower in longitudinal section. (**F**) Female flower. (**G**) Flowering shoot with anisophyllous paired leaves.

doi:10.1371/journal.pone.0151317.g004

Fig 5. Isotype of *Squamellaria grayi*, G. Chomicki, J. Aroles, N. Naikatini 53 (M, barcode: M-0274839).

doi:10.1371/journal.pone.0151317.g005

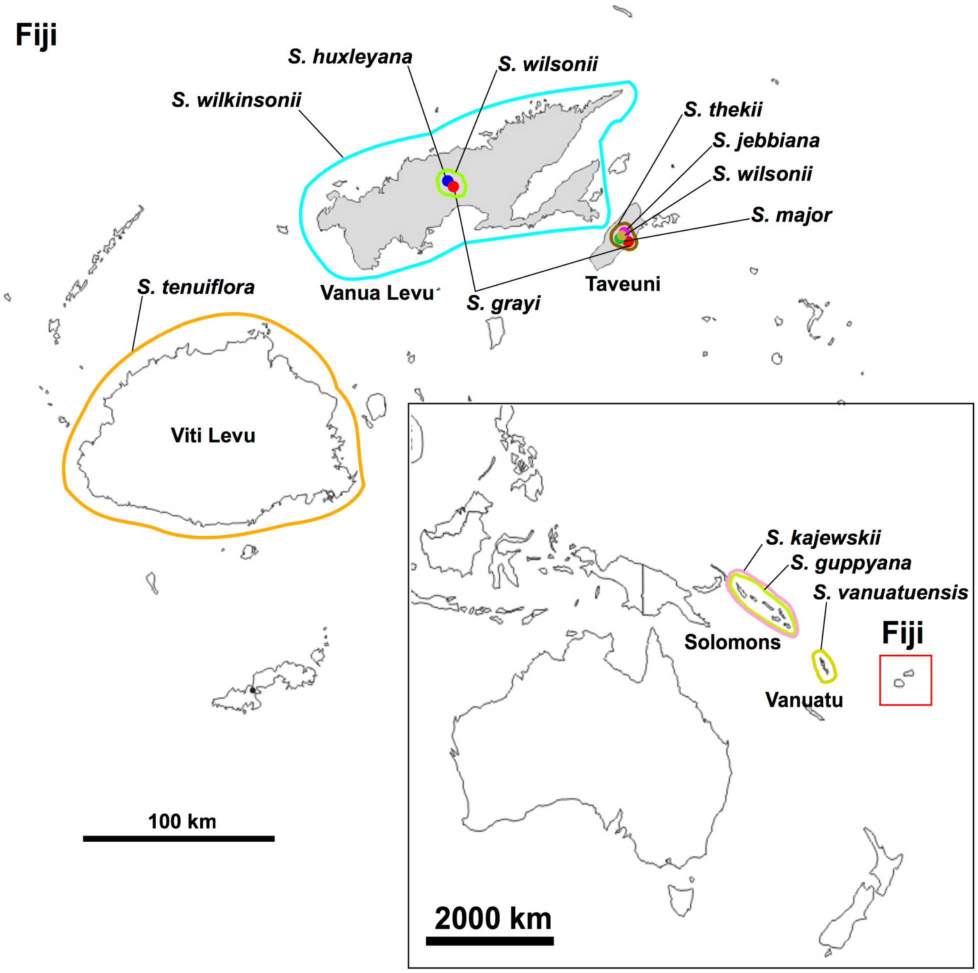


Fig 6. Geographic distribution of the 12 *Squamellaria* species.

doi:10.1371/journal.pone.0151317.g006

scrotum, or 'theketheke' meaning scrotum. Other names exist, but their meaning is uncertain 'mokamoka', 'ndatokaikai' (Alivereti Naikatini, pers. comm. to GC in June 2015).

Conservation status. The species is known from Lavena, where its range may be <10 km^2, and from Waisali forest reserve on Vanua Levu. Fijian law protects plants growing in Bouma National Heritage Park and Waisali forest reserve. Although the lack of data prevents us from assigning an IUCN status to this species, we suspect that it is at least endangered based on criteria B (extent of occurrence) and C (population size and decline) [34].

Specimens examined. FIJI. Vanua Levu: Waisali forest park reserve, 16°38'19.8"S, 179°13'19.7"E, 219 m lat., 20 March 2015, *G. Chomicki, J. Aroles & A. Naikatini 47* (SUVA, GH, K, L, M, MO, NOU, NSW, P, S).

Squamellaria huxleyana Chomicki **spec. nov.** [urn:lsid:ipni.org:names:77153475–1] (Figs 2D, 3E–3H, 3K and 3L, 7 and 8)

Type. FIJI. Vanua Levu: 45 km North of Savusavu towards Labasa, 16°37'16.1364"S, 179° 09'45.864"E, 271 m alt., 18 March 2015, *G. Chomicki, J. Aroles & A. Naikatini 48* (SUVA holotype; GH, K, L, M barcode: M-0274838, Fig 7, MO, NOU, NSW, P, S isotypes).

Diagnosis. *Squamellaria huxleyana* differs from all other species in the genus by the following combination of characters: oblong leaves with a rounded base, 4–5 cm long, 1.5–2 wide,

Isotype of *Squamellaria huxleyana* Chomicki
in G. Chomick & S. S. Renner, PLOS ONE, 2015 in review

Det. Guillaume Chomicki, 28 Nov. 2015
Botanische Staatssammlung München (M), Germany

FLORA OF FIJI
Rubiaceae

Squamellaria huxleyana Chomicki

Locality: Along main Savusavu-Labasa Highway, about 3km from Lomaloma village, Sasa District, Macuata Province, Vanua Levu, Fiji. Coordinates: S 16°37’16.1364”, E 179°09’45.8640”.
Notes: Epiphyte with domatium inhabited by the ant *Philidris nagasau*, growing on *Macaranga sp.* tree, ca. 7m above ground. DNA sequences deposited in GenBank.

Leg.: G. Chomicki, J. Aroles & A. Naikatini 48   18 March 2015

Fig 7. Isotype of *Squamellaria huxleyana*, G. Chomicki, J. Aroles, N. Naikatini 48 (M, barcode: M-0274838).

doi:10.1371/journal.pone.0151317.g007

ant houses (domatia) regularly globose and grey with dark protuberances, and fruits bilobed-quadrangular in section (versus round or quandrangular, but not bilobed fruits in the remaining species).

Description. Tuber attached to tree trunks (see Figs 2D and 3E) 15–25 x 25–30 cm, globose (in juveniles, Fig 3G) to ovoid (in mature plants, Figs 1G and 4A), its apex convex, surface grey with small dark protuberances (1–3 mm long) (Figs 2D and 3E), ant entrance holes to 3 mm in diameter, except the first (oldest) one, which is 5–8 mm in diameter. Stems several, in clusters, alternate with entrance hole rings around the tuber (Figs 3E and 8A), rarely branched, of sympodial structure. Stem diameter larger at distal ends (~0.8 cm), which are fleshy, than at the base (~0.3–0.4 cm). Internodes 0.5–4 cm long, 0.3–0.8 cm in diameter, nodes slightly swollen (Figs 3F and 8A), 0.4–1 cm in diameter. Internode length decreasing towards the distal end of each shoot. Leaves decussate (Figs 3E–3F and 8A), lamina oblong, 4–5 cm long, 1.5–2 cm wide, 2–3.5 mm thick, performing CAM or intermediate C3/CAM photosynthesis, pale green with translucent, entire margins, apex acute, base rounded, petiole 2–5 mm long and 2–3.5 mm wide (Figs 3F and 8F). Primary leaf vein monopodial, secondary veins festooned brochidromous (connecting to other secondary veins via multiple loops and not reaching the margin; Fig 8F). Inflorescences consisting of lateral short shoots in terminal and axillary position. Flowers bisexual, homostylous, 3–4 cm long, after the first days of anthesis, initially actinomorphic but the corolla often splitting at full anthesis resulting in a secondary zygomorphy (monosymmetry) (Fig 3H). Calyx light green, cup-shaped to 5 mm long and 5 mm wide, with a large interior nectary gland (Fig 3L); corolla white, glabrous, 3–5 cm long, 4 petals, hairy on the outside, whorled, in a valvate aestivation, tube 2.5–3.5 cm, lobes ca. 3 x 5 mm. Squamellae (scales inside petals) present, 4, one at the base of each petal (Fig 8D). Anthers basifixed, valvate, with introrse deshiscence (Fig 8D). Ovary inferior, with four carpels congenitally fused. Stigma flattened, four-parted, square in section. Fruit turbinate at the base, bilobed-quandrangular in section ca. 1 cm long and 7 mm large (Figs 3K–3L and 8E). Pyrenes, 4, curved (Fig 3K–3L).

Floral formula. $^*(\downarrow)K(4)[C(4)A(4)]\underline{G}(4)$

Distribution and ecology. *Squamellaria huxleyana* is only known from Vanua Levu, about 45 km northwest of Savusavu, along the road towards Labasa (Fig 6). It has been observed at elevations of 400–500 m in open areas and on trees. The species lives in obligate symbiosis with *Philidris nagasau* and is morphologically close to its sister species *S. thekii*, which occurs on Taveuni, but differs by the trait combination mentioned in the diagnosis. These two species appear to have evolved by allopatric speciation following dispersal between Taveuni and Vanua Levu, two islands only 6.5 km apart.

Etymology and common name. Named in honour of Camilla R. Huxley-Lambrick, née Huxley, for her key contributions to the biology and taxonomy of ant plants in the Rubiaceae [2–5, 9]. Its common names are the same as those of *S. grayi*.

Conservation status. The species is known from two localities separated by about one kilometre, and its range is likely <10 km^2. We have not found *S. huxleyana* in Waisali forest reserve, and the two locations where we found it are not protected sites. Although the lack of data prevents us from assigning an IUCN status to this species, we suspect that it is at least endangered based on criteria B (extent of occurrence) and C (population size and decline) [34].

Specimens examined. Only known from the type collection.

Squamellaria jebbiana Chomicki **spec. nov.** [urn:lsid:ipni.org:names:77153477–1] (Figs 2I, 3N–3P and 9)

Type. FIJI. Taveuni: Des Voeux peak, 16°48'25. 8133"S, 179°56'36.6843"E, 450 m alt., 22 March 2015, *G. Chomicki*, *J. Aroles & A. Naikatini 74* (SUVA holotype; GH, K, L, M isotype barcode: M-0274837, MO, NOU, NSW, P, S isotypes).

Diagnosis. *Squamellaria jebbiana* differs from the remaining species by consistently elliptic leaves. Two substitutions can be used to diagnose this species: a C in position 354 of ITS (GenBank # KU586342) instead of an A or T, a C at position 278 of rps16 (GenBank # KU586438) instead of an A in all other Fijian *Squamellaria*.

Description. Tuber attached to tree trunks, 25–40 to 25–40 cm, conical, tuber apex prominent, its surface dark brown, domatium entrance holes of two kinds, lipped to 1.5 cm in

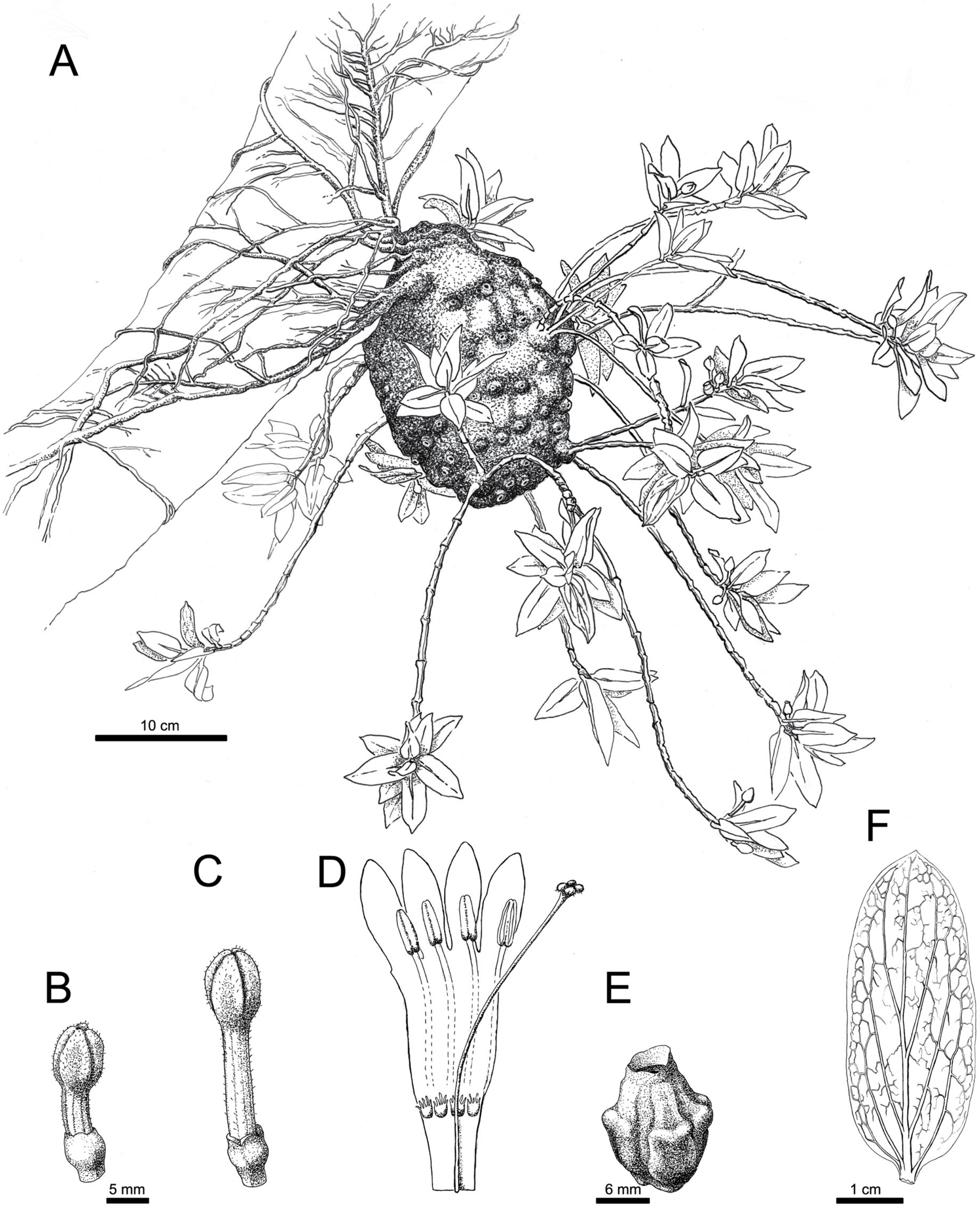


Fig 8. *Squamellaria huxleyana* Chomicki, spec. nov. (**A**) Habit showing the domatium. (**B**, **C**) Successive stages of flower bud opening, showing the corolla tube elongation. (**D**) Open corolla tube with the four squamellae. (**E**) Fruit with a bilobed-quadrangular shape in cross section. (**F**) Leaf, showing the festooned brochidromous venation (connecting to other secondary veins via multiple loops and not reaching the margin).

doi:10.1371/journal.pone.0151317.g008

Fig 9. Isotype of *Squamellaria jebbiana*, G. Chomicki, J. Aroles, N. Naikatini 74 (M, barcode: M-0274837).

doi:10.1371/journal.pone.0151317.g009

diameter, and funnel-like, often ovate, to 6 cm in diameter. Stems several, branched, of sympodial structure (Fig 3N), all emerging from the prominent tuber apex (Fig 3N and 3O). Internodes 1–8 cm long and 0.3–1.5 cm in diameter, nodes slightly swollen (Fig 3N). Leaves decussate, lamina elliptic (Figs 3N and 9) but acuminate apex in juveniles (Fig 3P), leaf 3–5 cm long, 2.5–3.5 wide, 2–3.5 mm thick, slightly succulent but performing C3 photosynthesis, apex rounded to slightly convex, base rounded, darker green on the adaxial (upper) than on the abaxial (lower) sides, the petiole 2–5 mm long and 3–3.5 mm wide. Primary leaf vein monopodial, secondary veins brochidromous (i.e., secondary veins connect to the connects directly to the next secondary vein via a loop; Fig 9). Inflorescences (and infructescences) on lateral short shoots, axillary and terminal (Figs 3N and 9). Flowers functionally unisexual, plants monoecious female flowers with four sterile stamens, male flowers with a sterile gynoecium (Figs 3N and 9), actinomorphic, 3–5 cm long. Calyx light green, made of 4 fused sepals, to 2 mm long and 2 mm wide; corolla with 4 petals, white, glabrous, whorled, in a valvate aestivation with revolute margins, corolla tube 2.5–4 cm long. Anthers basifixed, adnate to corolla, valvate, and with introrse dehiscence. Ovary inferior, flattened, with two congenitally fused carpels. Pyrenes 2, straight. Fruit oblong, ovate in section, to 1 cm long and 7 mm wide.

Floral formulae. Male: $^{*}K(4)[C(4)A(4)]\underline{G}(2)$; Female: $^{*}K(4)[C(4)A^{0}(4)]\underline{G}(2)$

Distribution and ecology. *Squamellaria jebbiana* is known from the path going from Somosomo to DesVoeux peak and Mt. Manuca, where it grows from 400 m to 600 m. It is usually inhabited by *Camponotus* ants (*C. chloroticus*) and several species of *Pheidole*, but not the Dolichoderinae *Philidris nagasau*.

Etymology and common name. Named in honour of the Irish botanist Matthew P. H. Jebb for his contributions to the taxonomy and biology of the Hydnophytinae [2–6, 35]. Its common names are the same as those of *S. grayi* and *S. huxleyana*.

Conservation status. The species is known from the DesVoeux peak reserve, where its range may be <10 km^2. Fijian law protects plants growing in DesVoeux peak reserve. Although the lack of data prevents us from assigning an IUCN status to this species, we suspect that it is at least endangered based on criteria B (extent of occurrence) and C (population size and decline) [34].

Specimens examined. Only known from the type collection.

Squamellaria vanuatuensis Jebb & C.R.Huxley in Chomicki & S.S. Renner **spec. nov.** [urn:lsid:ipni.org:names: 77153598–1] (Figs 2L and 10)

Type. Vanuatu, Espiritu Santo Island: 14°57’50”S, 166°38’52”E, 600 m alt., 17 Nov. 2006, G. *McPherson, M. Tuiwawa, and R. Rigault 19437* (PVNH holotype; MO, barcode 2530839; P, barcode: P04534466, NOU barcode NOU074252, SUVA, isotypes).

Diagnosis. *Squamellaria vanuatuensis* differs from the other species in the genus by the presence of dense triangular papery bracts around inflorescences with minute flowers (0.5 cm vs. >1.5 cm for all other *Squamellaria* species; Fig 10). It also differs in the following substitution in the nuclear ribosomal intergenic spacer region ITS (GenBank # JX155078): (*i*) a G at position 149 instead of a C for all Fijian *Squamellaria* (G shared with *S. kajewskii* and *S. guppyanum*); (*ii*) a A at position 171 instead of G or C in all other *Squamellaria*; and (*iii*) a T in position 186 instead of a A or C in all other *Squamellaria*.

Description. Tuber attached to tree trunks, to 40 cm across, globose to flattened (Figs 2L and 10A), tuber surface red-brown, its cavities large, entrances holes to 1 cm in diameter. Stems several, to 60 cm long and 0.2–0.5 cm of diameter, branched, of sympodial structure, all emerging from the flattened tuber apex. Internodes 1.5 to 5 cm. Leaves arranged in a decussate phyllotaxis, lamina ovate; 4 x 2.2 to 5.5 x 3.3 cm; apex acute; base rounded; succulent; pale green. Venation dark; petiole 2 cm. Stipules to 0.15 cm, rounded, papery, caducous. Inflorescence 1–3 per node, sessile, covered by papery, triangular bracts to 1 cm in length, forming a mass 1.5 cm across. Flowers minute, bisexual, homostylous, calyx dentate, 3 mm long with teeth to 1 mm (Fig 10C), corolla 2.5 mm overall, with dense hairs at the opening (Fig 10B). Anthers ca. 1.2 mm long, basifixed, adnate to corolla, valvate, and with introrse dehiscence. Pollen 3-colpate, 57.5 μm across; reticulation medium, 1–2 μm. Ovary inferior, stigma bifid. Fruit and pyrenes unknown.

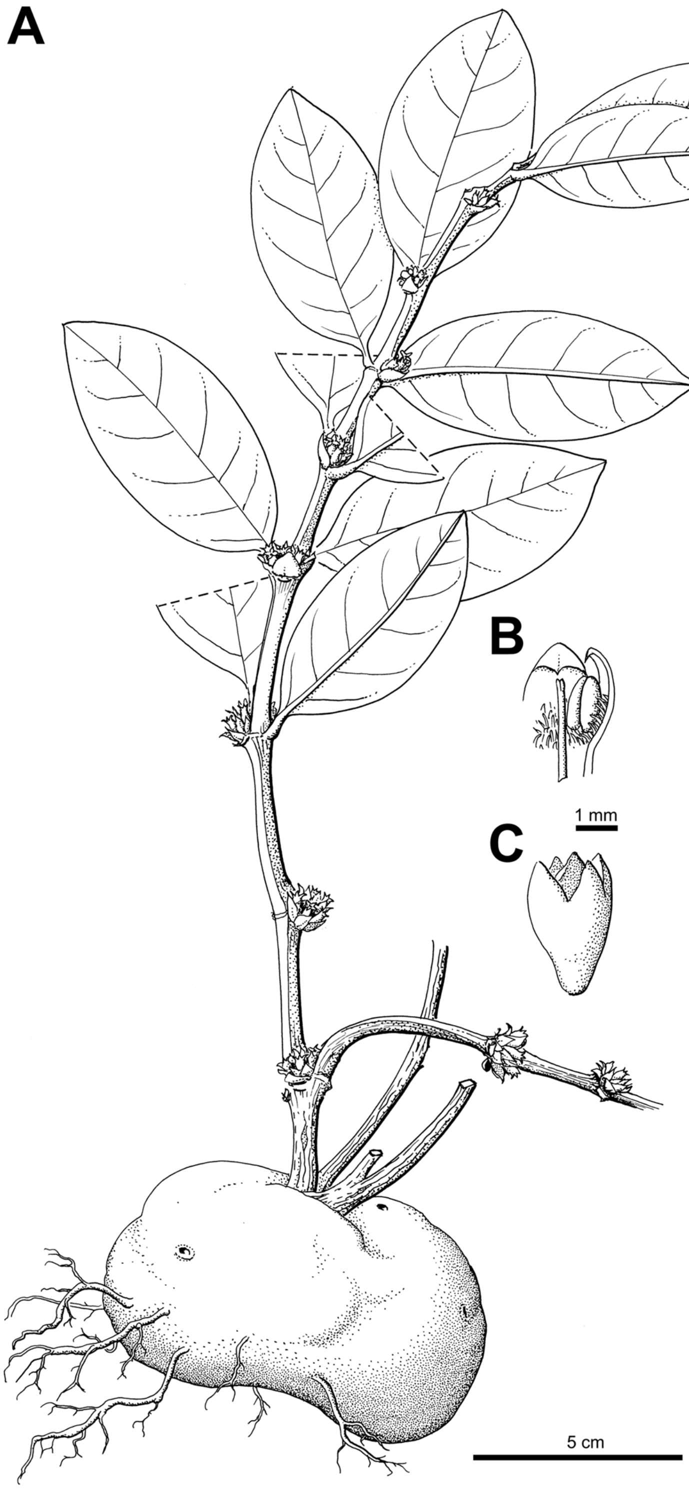


Fig 10. *Squamellaria vanuatuensis* Jebb & C.R.Huxley, spec. nov. (**A**). Habit showing the domatium; (**B**). Inner view of flower throat; (**C**). Hypanthium and calyx. Scale bar 5 cm for **A**; 5 mm for **B** and **C**. Drawn by Rosemary Wise from herbarium specimens: **A** = *L. Bernardi 13238* (G, barcode G-62446); **B, C** = Green 1274 (K, barcode K000772005).

doi:10.1371/journal.pone.0151317.g010

Distribution and ecology. *Squamellaria vanuatuensis* is known from rainforests on five islands of the Vanuatu archipelago: Erromango island, close to Nouankao camp, Efate island, Summit of Mt. Macdonald (647 m alt.), Undine Bay, and Maewo island, Saritamita. Pentecost island, close to Enkul village, Espiritu Santo Island, West coast of Cumberland peninsula, above the village of Penarou. Tuber inhabited by unspecialized ants of various genera (M.P.H. Jebb, pers. comm. to G.C., Feb. 2015).

Etymology and common name. Named for its geographic distribution on Vanuatu. The epithet was first used by M. Jebb and C. R. Huxley in December 1991 on annotation labels attached to *P.S. Green 1274* (K, P).

Conservation status. The species is known from five islands in the Vanuatu island group and thus have a small range. Although the lack of data prevents us from assigning an IUCN status to this species, we suspect, based on criteria B (extent of occurrence) and C (population size and decline) [34], that it may be endangered.

Specimens examined. VANUATU. **Erromango Island**: *L. Bernardi 13238* (G barcode G00405545); 18°54'0"S, 169°10'60"E, Erromanga, 5 Aug. 1971, *P. S. Green 1274* (K, P). **Efate Island**: Summit of Mt. Macdonald, Undine Bay, *Morrison s.n.* (K). **Maewo Island**: Saritamita, 23 April 1986, *Bourdy 532* (K, P). **Pentecost Island**: Near Enkul village, 500 m alt., 27 Sep. 1984, *P. Cabalion 2528* (K, P).

Key to the species of *Squamellaria*

1. Species not occurring in Fiji 2

2. Endemic to Vanuatu; inflorescence covered by triangular papery bracts (Figs 2L and 10A) . . . . *S. vanuatuensis*

2. Endemic to the Solomon Islands, inflorescence not covered by papery bracts 3

3. Lamina 5–20 cm long; peduncle 3-branched, with 4 or more fertile branch ends, corolla tube slender, at least 3 times as long as broad, tuber round to ovate (Fig 2K) . . . . *S. guppyana*

3. Lamina 2–4 cm long; peduncle 2-branched, with 2 or rarely 3 fertile branch ends; corolla tube scarcely longer than broad, tuber boat-shaped (Fig 2J). *S. kajewskii*

1. Species occurring in Fiji 4

4. Tuber entrance holes of 0.5–5 cm, arranged irregularly and concentrated at the basal part of the tuber, leaves 2–12 cm long, flowers with thin corolla tubes (2–3 mm), inhabited by various ant species, but not *Philidris nagasau*. 5

5. Herbarium and living material of the following three species cannot be securely distinguished morphologically; endemic to the South East Fiji islands Viti Levu, Ovalau. . . . . . . *S. tenuiflora*

5. Endemic to the North West Fiji islands Vanua Levu and Taveuni 6

6. Vanua Levu. . . . . *S. wilkinsonii*

6. Taveuni . . . . *S. jebbiana*

4. Tuber entrance holes <3 mm, in circles around the tuber, leaves 3–8.5 cm, flowers with large (4–6 mm) or thin (2–3 mm) corolla tubes, always inhabited by the ant species *Philidris nagasau* 7

7. Flower calyx 3 mm wide, corolla tubes 2.5–3.5 mm wide (Figs 3D and 4B, 4E and 4F); squamellae absent, carpels three with straight pyrenes (Fig 3J and 3M). . . . . *S. grayi*

7. Flower calyx 5–7 mm wide, corolla tube 4–10 mm wide, squamellae present, carpels four with curved pyrenes 8

8. Tuber lacking hairs but with dark brown protuberances (Figs 2D and 3E); leaves oblong (Figs 2F and 4F) . . . . *S. huxleyana*

8. Tuber with hairs (*S. imberbis*, *S. wilsonii*) and/or pale protuberances (*S. major*, *S. thekii*); leaves not oblong except in *S. major* 9

9. Leaves not succulent, lanceolate to rhomboid, 1–2 mm thick 10

10. Domatium globose, with hairs; species endemic to Vanua Levu (Fig 2E) . . . . *S. imberbis*

10. Domatium flattened, with pronounced bilateral symmetry (Fig 2B), with hairs, species endemic to Taveuni. . . . . *S. wilsonii*

9. Leaves succulent, cordate to ovate-rhomboid or ovate to oblong-elliptic, 3–4 mm thick. . . 11

11. Leaves ovate to oblong-elliptic, 8–16 cm long, stems solitary around the domatium (Fig 2C) . . . . *S. major*

11. Leaves cordate to ovate-rhomboid, 4–6 cm long, stems in clusters around the tuber (Fig 2A) . . . . *S. thekii*

New combinations

Squamellaria guppyana (Becc.) Chomicki, **comb. nov.** [urn:lsid:ipni.org:names:77153477–1] Basionym: *Hydnophytum guppyanum* Becc., Malesia 2: 133, pl. 40. 1885. **Type**: Solomon Islands, Shortland islands, May 1884, *H.B. Guppy s.n.* (FI holotype, barcode: FI008898).

Note: Kew has no duplicate of this collection, but instead *H.B. Guppy 140* (image barcode K000772006), which appears to represent the same collection.

Squamellaria kajewskii (Merr. & L.M.Perry) Chomicki, **comb. nov.** [urn:lsid:ipni.org:names: 77153478–1] Basionym: *Hydnophytum kajewskii* Merr. & L.M.Perry, J. Arnold Arbor. 26: 25. 1945. **Type**: Solomon Islands, Bougainville, April 1930, *S.F. Kajewski 1716* (A holotype, barcode: A00096843; BM, barcode: BM001040409, BO, BRI, barcode BRI-AQ0570119, G, barcode G00436269, P, barcode P04957009 isotypes).

Squamellaria tenuiflora (Becc.) Chomicki, **comb. nov.** [urn:lsid:ipni.org:names:77153479–1] Basionym: *Hydnophytum tenuiflorum* Becc., Malesia 2: 169, pl. 43, 1–14. 1885. **Syntypes**: Fiji, Viti Levu, Dec. 1864, *E.O. Graeffe 1573* (K, barcode: K000761993, lectotype designated by A.C.Smith, Fl. Vitiensis Nova 4: 244. 1988), Ovalau, *E.O. Graeffe 1555* (K, paratype, barcode: K000761992).

Gray and Beccari described three further species that may be synonyms of *S. tenuiflora*, but without DNA sequences from their type specimens it is not currently possible to decide the matter. They are *H. longiflorum* A.Gray (Proc. Am. Acad. 4: 42. 1858) based on US Expl. Exp. 62267 (US Catalog No. 62267, barcode: 00036508) from Ovalau; *H. grandiflorum* Becc. (Malesia 2: 126. 1884, Malesia 2: 171, pl. 44: 13–25. 1885) based on *E.O. Graeffe s.n.* (K barcode 000762000) from 'Fiji, Ovalau and Viti Levu, Dec. 1864'; and *H. horneanum* Becc. (Malesia 2: 125. 1884, Malesia 2: 168, pl. 43:15–25, 1885) based on *J. Horne 282* (K barcode: K000761999) from Fiji.

Squamellaria wilkinsonii (Horne ex Baker) Chomicki, **comb. nov.** [urn:lsid:ipni.org:names: 77153480–1] Basionym: *Hydnophytum wilkinsonii* Horne ex Baker, J. Linn. Soc., Bot. 20: 365. 1883 [1884 publ. 1883]. **Type**: Fiji, Vanua Levu, 1877–78, *J. Horne 1077* (K holotype, barcode: K000761990; K000761991, isotype).

Squamellaria wilkinsonii from Vanua Levu and the preceding species, S. *tenuiflora* from Viti Levu, are morphologically extremely similar.

Discussion

Traits and taxonomy of the Fijian Hydnophytinae (Psychotrieae, Rubiaceae)

The genus *Squamellaria* now comprises 12 species, four of them described here, four transferred from *Hydnophytum*, and one resurrected. The resurrected species is *S. wilsonii*, which was synonymized under *S. imberbis* by Jebb [6] but is distinct in tuber shape and DNA sequences (Fig 2B and 2M). The entire subtribe Hydnophytinae (*Anthorrhiza*, *Hydnophytum*, *Myrmecodia*, *Myrmephytum*, *Squamellaria*) is embedded in *Psychotria* (Fig 1), and Razafimandimbison et al. [36] have therefore transferred the type species of each of these genera into *Psychotria*, but without transferring the remaining names. For three reasons, we decided to describe our new species in *Squamellaria* instead of in *Psychotria*. Firstly, some 4000 names are available in *Psychotria*, and any transfers into that genus are therefore best left to *Psychotria* specialists. Secondly, Matthew Jebb (National Botanic Gardens, Glasnevin, Dublin) is working on a taxonomic revision of *Hydnophytum*. Thirdly, species and gene sampling densities in the Pacific clade of *Psychotria* are still sparse [29, 36], and two more broadly defined genera could be maintained separate from *Psychotria*, namely our expanded *Squamellaria* and the Australasian clade as an expanded *Hydnophytum* (Fig 1). Nevertheless, we selected the epithets of our four new species so as not to require replacement names should these species be transferred into *Psychotria* in the future. The genus *Squamellaria* can be defined by the combination of 4-merous sepal and petal whorls together with solitary inflorescences (except in *S. vanuatuensis* where this trait is variable) and distribution in the Pacific (Fiji, Vanuatu, Solomons).

The scales at the inner base of the flower tube used by Beccari [14] to set apart *Squamellaria* from *Hydnophytum* can no longer be used as the defining morphological trait of the genus, since they only arose within the genus, for example in *S. grayi* (species followed with a "S" in Fig 2). *Squamellaria grayi* is distinctive by the slight succulence and curved shape of its leaves (Figs 3A and 4A). The isotope $\delta^{13}C$ ratio for CAM versus C3 photosynthesis revealed that these leaves carry out standard C3 photosynthesis, as do *S. jebbiana*, *S. imberbis*, and *S. wilsonii*, while *S. huxleyana*, *S. major*, and *S. thekii* have CAM photosynthesis or intermediate C3/CAM photosynthesis.

Biogeographic history of *Squamellaria*

During the Oligocene, some 30 million years ago, Fiji, Vanuatu, the Solomon Islands, and the Bismarck Archipelago were part of a volcanic arc, the Vitiaz arc, with active volcanism that continued to build land [37]. By about 12 Ma, the Solomons had rifted from the Vanuatu-Fijian arc, and the latter two regions then became separated at about 7 Ma [32, 38]. The inferred ancestral area for *Squamellaria* in Fiji and Vanuatu is thus consistent with the Vitiaz arc (Fig 1). The colonization of Vanuatu by the ancestor of *S. vanuatuensis* could have occurred from Fiji, and the common ancestor of *S. kajewskii* and *S. guppyana* later reached the Solomon Islands (Fig 1). The six *Squamellaria* species (marked in red in Fig 2) that live in an obligate symbiosis with a single ant species (*Philidris nagasau*) are restricted to Taveuni and Vanua Levu together with their symbiont, two islands separated by only 6.5 km, implying that they arrived as epiphytes on floating tree trunks, with their domatia occupied by their own coevolved ants. By contrast, the *Squamellaria* species occupied by facultative ant symbionts are widespread on the archipelago. A biogeographic analysis of Neotropical *Pseudomyrmex* ants and their plant hosts showed that interacting ant and plant clades shared the same ancestral areas and that dispersal events outside of the partner ranges were rare [39].

One of our new species, *S. jebbiana* from Taveuni, is sister to the other Fijian *Squamellaria* (Figs 1 and 9) from which it appears to have diverged 3.7 ± 1.5 Ma (Fig 1). This is puzzling because Taveuni is supposed to have emerged only some 0.8 Ma ago [40, 41]. Perhaps the species arrived from an older island, such as nearby Vanua Levu (~4 Ma old; [41]) or Viti Levu (~28 Ma; [41]), followed by local extinction on these islands. Alternatively, the uncertainty of molecular clock dating, especially of young nodes for which error ranges cannot be calculated because of too few substitutions, may explain the age discrepancy between the island age and the inferred species divergence time.

Conclusion

Our four new species, four new combinations, and the resurrected *S. wilsonii* bring the number of ant-plant species worldwide to 685 [1]. The discovery of five new myrmecophytes on tiny islands of the Fiji Archipelago suggests that a modelling-based estimate of probably over 1,100 myrmecophyte species worldwide may well be realistic [1]. That new species were discovered on a tourist walk also illustrates how much botanical collecting remains to be done on Fiji.

Supporting Information

S1 Table. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).
(DOCX)

Acknowledgments

We thank Jeremy Aroles for help in the field; Two anonymous reviewers for comments on the manuscript; Bruno Corbara and Derrick Rowe for photographs; Alivereti Naikatini and Marika Tuiwawa from the University of South Pacific and the herbarium SUVA for technical support and advice; Gudrun Kadereit, University of Mainz, for the isotope-ratio mass spectrometry analyses carried out at the University of Mainz; Matthew Jebb and Sylvain Razafimandimbison for taxonomic advice, and Yannick Staedler for providing the microCT scanning images in Fig 3. Yasumin S. Lermer from the Botanical Institute in Munich drew two plates (*S. huxleyana* and *S. grayi*), and Rosemary Wise from Oxford drew the *S. vanuatensis* plate. Curators at Dublin (DUB), Kew (K), Leiden (L), Munich (M and MSB), Oxford (FHO), Paris (P), Sydney (NSW), and SUVA provided access to relevant collections. Material from Vanuatu was collected during “SANTO 2006”, an international expedition organized by the Paris Natural History Museum, Pro-Natura International, and the Institut de Recherche pour le Développement, and sponsored by the Stavros Niarchos Foundation, the French Fonds Pacifique and the Total Foundation. The expedition operated under a permit granted to Prof. P. Bouchet by the Environment Unit of the Government of Vanuatu. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20, and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to GC.

Author Contributions

Conceived and designed the experiments: GC. Performed the experiments: GC. Analyzed the data: GC. Contributed reagents/materials/analysis tools: SSR GC. Wrote the paper: GC SSR.

References

1. Chomicki G, Renner SS. Phylogenetics and molecular-clock dating reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. New Phytol. 2015; 207: 411–424. doi: 10.1111/nph.13271 PMID: 25616013
2. Huxley CR, Jebb MHP. The tuberous epiphytes of the Rubiaceae 1: A new subtribe- the Hydnophytinae. Blumea. 1991; 36: 1–20.
3. Huxley CR, Jebb MHP. The tuberous epiphytes of the Rubiaceae 5: A revision of *Myrmecodia*. Blumea 1993; 37: 271–334.
4. Huxley CR, Jebb MHP. The tuberous epiphytes of the Rubiaceae: 3. A revision of *Myrmephytum* to include *Myrmedoma*. Blumea 1991; 36: 43–52.
5. Huxley CR, Jebb MHP The tuberous epiphytes of the Rubiaceae 2: the new genus *Anthorrhiza*. Blumea. 1991; 36: 21–41.
6. Jebb MHP. The tuberous epiphytes of the Rubiaceae: 4. A revision of *Squamellaria*. Blumea 1991; 36: 53–61.
7. Treub MM. Sur le *Myrmecodia echinata* Gaudich. Ann Jard Bot Buitenz. 1883; 3: 129–59.
8. Treub MM. Nouvelles recherches sur le *Myrmecodia* de Java. Ann Jard Bot Buitenz. 1888; 7: 191.
9. Huxley CR. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. New Phytol. 1978; 80: 231–268.
10. Miehe H. Ueber die javanische *Myrmecodia* und die Beziehung zu ihren Ameisen. Biol Zbl. 1991; 31: 73
11. Heim DR. The biologic relations between plants and ants. Smithsonian Report for 1896. 1988; 411–455. Government Printing Office, Washington.
12. Janzen DH. Epiphytic myrmecophytes in Sarawak: mutualism through the feeding of plants by ants. *Biotropica*. 1974; 6: 237–259.
13. Barosi F. Odoardo Beccari. I viaggi e il contributo scientifico. Geostorie. 2010; 18: 7–85.
14. Beccari O. Piante ospitatrici, ossia piante formicarie della Malesia e della Papuasia. 1884–1886; *Malesia (Genoa)* vol. II, fasc. 1–2 (1884), fasc. 3 (1885), fasc. 2 (1886).
15. Horne J. A year in Fiji, or an Inquiry Into the Botanical, Agricultural, and Economical Resources of the Colony. 1881; London: Eyre GE & Spottiswoode W.
16. Baker JG. Recent additions to our knowledge of the flora of Fiji. J Linn Soc, Bot. 1884 [published in 1883] 20: 358–373.
17. Wagner WL, Lorence DH. Albert Charles Smith (1906–1999): a monumental botanist. Allertonia 2001; 8: 329–339.
18. Lin H-L. Colonial uneven development, Fijian Vanua, and modern ecotourism in Taveuni, Fiji. Pacific Asia Inquiry. 2012; 3: 41–57.
19. Smith AC. Studies of the Pacific Islands plants XVIII. New and noteworthy plants from Fiji. Contr US Natl Herb. 1967; 37: 67–107.
20. Chomicki G, Renner SS. Watermelon origin solved with molecular phylogenetics including Linnaean material: Another example of museomics. New Phytol. 2015; 205: 526–532. doi: 10.1111/nph.13163 PMID: 25358433
21. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013, 30: 772–780. doi: 10.1093/molbev/mst010 PMID: 23329690
22. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 2.75. 2011. *URL* http://mesquiteproject *org*.
23. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014; 30: 1312–1313. doi: 10.1093/bioinformatics/btu033 PMID: 24451623
24. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012; 61: 539–542. doi: 10.1093/sysbio/sys029 PMID: 22357727
25. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012; 9: 772.
26. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D et al. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comp Biol. 2014; 104, e1003537.
27. Rambaut A, Drummond AJ. Tracer—MCMC trace analysis tool version v1.5. 2007; URL http://beast.bio.ed.ac.uk.

28. Rambaut A. FigTree v. 1.4.0. 2012. http://tree.bio.ed.ac.uk/software/figtre.

29. Barrabé L, Maggia L, Pillon Y, Rigault F, Mouly A, Davis AP, et al. New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. Molecular Phylogenet Evol. 2014; 71: 15–35.

30. Matzke NJ. Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in dispersal–extinction–cladogenesis DEC analyses. Front Biogeog. 2012; 4: 210.

31. Matzke NJ. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. Syst Biol. 2014; 63: 951–970. doi: 10.1093/sysbio/syu056 PMID: 25123369

32. Hall R. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, models, and animations. J Asian Earth Sci. 2002; 20: 353–431.

33. Gray A. Myrmecodia imberbis. Proc Am Acad Arts Sci. 1858; 4: 31–78.

34. IUCN Species Survival Commission. *IUCN Red List Categories and Criteria*. 2001. IUCN.

35. Jebb M. Cavity structure and function in the tuberous Rubiaceae. 1991; Huxley CR, Cutler D, F ed (s). Ant-plant interactions. Oxford Univ. Press: Oxford, p.p. 374–89.

36. Razafimandimbison SG, Taylor CM, Wikström N, Pailler T, Khodabandeh A, Bremer B. Phylogeny and generic limits in the sister tribes Psychotrieae and Palicoureeae (Rubiaceae): Evolution of schizocarps in *Psychotria* and origins of bacterial leaf nodules of the Malagasy species. Am J Bot. 2014; 101: 1102–1126. PMID: 25049266

37. Ewart FT. Geological history of the Fiji–Tonga–Samoan Region of the S.W. Pacific, and some palaeogeographic and biogeographic implications. 1988; In: Lyneborg L. (Ed.). The Cicadas of the Fiji, Samoa and Tonga Islands, Their Taxonomy and Biogeography. EJ Brill/Scandinavian Science Press, Leiden, Netherlands.

38. Taylor GK, Gascoyne J, Colley H. Rapid rotation of Fiji; paleomagnetic evidence and tectonic implications. J Geophys Res, B, Solid Earth Planets. 2000; 105: 5771–5781.

39. Chomicki G, Ward PS, Renner SS. Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. Proc R Soc B. 2015; 282: 20152200. doi: 10.1098/rspb.2015.2200 PMID: 26582029

40. Rodda P, Kroenke L. Fiji: a fragmented arc, p. 87–110. In: Cenozoic Tectonic Development of the Southwest Pacific. Vol. Technical Bulletin. 1984; No. 6. Kroenke L. (ed.). U.N. ESCAP, CCOP/SOPAC.

41. Rodda P. Geology of Fiji. South Pacific Applied Geoscience Commission (SOPAC). Technical Bulletin. 1994; 8: 131–151.

S1 Table. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	Voucher	Geographic origin	ITS	ETS	18S	trnL-trnF	ndhF	trnS-trnG	rps16	rps12-rpl20
***Amaracarpus muscifer* A.C.Sm.**	L. Barrabe & M. Tuiwawa 1109 (NOU)	Fiji	KF675907	KF675790	-	-	KF675995	-	KF676083	-
***Amaracarpus nematopodus* (F.Muell.) P.I.Forst.**	L. Barrabe et al. 1030 (NOU)	Australia	JX155060	KF675791	-	-	JX155105	-	JX155152	-
***Anthorrhiza caerulea* Huxley & Jebb**	M.P.H. Jebb 358 (FHO)	Papua New Guinea	KU586349	KU586368	-	-	-	-	-	-
Anthorrhiza echinella	G. Chomicki 83 (M)	Cultivated Oxf. Bot Gard., origin New Guinea	KU586350	KU586369	-	-	-	-	-	-
***Hedstromia latifolia* A.C.Sm.**	L. Barrabe et al 1090 (NOU)	Fiji	KF675911	KF675795	-	-	KF675999	-	KF676087	-
***Hydnophytum formicarum* Jack**	G. Chomicki 87 (M)	Cultivated, origin Malaysian region	KU586346	KU586365	-	-	KU586397	-	-	-
***Myrmecodia beccarii* Hook f.**	G. Chomicki 99 (M)	Cultivated, origin Australia	KU586347	KU586366	-	-	KU586398	-	-	-
***Myrmecodia salomonensis* Becc.**	C. R. Huxley and L. M. Turton 3442 (FHO)	Solomons	KU586351	KU586370	-	-	-	-	-	-
***Myrmecodia dahlii* K.Schum.**	J.I. Menzies 5947 (FHO)	Papua New Guinea	KU586348	KU586367	-	-	KU586399	-	-	-

***Myrmephytum arfakianum* (Becc.) Huxley & Jebb**	G. Chomicki 116 (M)	Cultivated, origin Papua	KU586352	KU586371	-	-	KU586400	-	-	-
***Myrmephytum beccarii* Elmer**	G. Chomicki 118 (M)	Cultivated, origin Philippines	KU586353	KU586354	-	-	KU586401	-	-	-
***Psychotria comptonii* S.Moore**	L. Barrabe & Rigault 1014 (NOU)	New Caledonia	KF675927	KF675823	-	-	KF676015	-	KF676104	-
***Psychotria dallachiana* Benth.**	L. Barrabe & Rigault 1048 (NOU)	Australia	KF675928	KF675824	-	-	KF676016	-	KF676169	-
***Psychotria declieuxioides* S.Moore**	L. Barrabe & Nigote 937 (NOU)	New Caledonia	KF675932	KF675828	-	-	KF676020	-	KF676107	-
***Psychotria faguetii* (Baill.) Schltr.**	L. Barrabe et al. 820 (NOU)	New Caledonia	KF675934	KF675831	-	-	KF676023	-	-	-
***Psychotria fitzalanii* Benth.**	L. Barrabe & Rigault 1057 (NOU)	Australia	KF675935	KF675832	-	-	KF676024	-	KF676110	-
***Psychotria goniocarpa* (Baill.) Guillaumin**	L. Barrabe 586 (NOU)	New Caledonia	KF675940	KF675838	-	-	KF676029	-	KF676115	-
***Psychotria hawaiiensis* (A.Gray) Fosberg**	Y. Pillon 1425 (NOU)	Hawaii	KF675941	KF675840	-	-	KF676030	-	KF676116	-
***Psychotria hivaoana* Fosberg**	Meyer 3071 (PAP)	French Polynesia	KF675942	KF675841	-	-	KF676031	-	KF676117	-
***Psychotria insularum* A.Gray**	Y. Pillon 909 (NOU)	Wallis & Futuna	KF675943	KF675842	-	-	KF676032	-	KF676118	-

***Psychotria iteophylla* Stapf**	Axelius 303 (S)	Borneo	-	-	-	-	-	-	AF410726	-
***Psychotria loniceroides* Sieber ex DC.**	L. Barrabe & Rigault 1042 (NOU)	Australia	KF675945	KF675846	-	-	KF676033	-	KF676120	-
***Psychotria lorentzii* Valeton**	Puradyatmika 10460 (K)	Papua New Guinea	KF675946	KF675847	-	-	KF676034	-	KF676121	-
***Psychotria micralabastra* (Lauterb. & K.Schum.) Valeton**	Takeuchi 16163 (K)	Papua New Guinea	KF675949	KF675851	-	-	KF676036	-	KF676124	-
***Psychotria micrococca* (Lauterb. & K.Schum.) Valeton**	Drozd & Molem s.n. (PSF)	Papua New Guinea	KF675951	KF675853	-	-	KF676038	-	KF676126	-
***Psychotria microglossa* (Baill.) Baill. ex Guillaumin**	L. Barrabe 585 (NOU)	New Caledonia	KF675950	KF675852	-	-	KF676037	-	KF676125	-
***Psychotria monanthos* (Baill.) Schltr.**	Y. Pillon 1370 (NOU)	New Caledonia	KF675953	KF675855	-	-	KF676040	-	KF676128	-
***Psychotria poissoniana* (Baill.) Guillaumin**	J. Munzinger 5156 (NOU)	New Caledonia	KF675958	KF675861	-	-	KF676045	-	KF676133	-
***Psychotria pritchardii* Seem.**	L. Barrabe et al 1124 (NOU)	Fiji	KF675992	KF675903	-	-	KF676078	-	KF676165	-
***Psychotria raivavaensis* Fosberg**	Meyer 3088 (PAP)	French Polynesia	KF675960	-	-	-	KF676047	-	KF676135	-
***Psychotria submontana* Domin**	L. Barrabe et al. 1044 (NOU)	Australia	KF675988	KF675899	-	-	-	-	KF676168	-

***Psychotria temehaniensis* J.W.Moore**	Mouly 403 (P)	French Polynesia	KF675989	KF675900	-	-	KF676075	-	KF676162	-
***Psychotria trisulcata* (Baill.) Guillaumin**	L. Barrabe et al. 902 (NOU)	New Caledonia	KF675990	KF675901	-	-	KF676076	-	KF676163	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (SUVA) HOLOTYPE	Taveuni, Bouma falls, Lavena	KU586339	KU586358	KU586427	KU586376	KU586388	KU586406	KU586436	KU586417
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 47 (M)	Vanua Levu, Waisali forest park	-	-	-	KU586372	-	KU586402	KU586432	KU586413
***Squamellaria guppyana* (Becc.) Chomicki, comb. nov.**	G. Chomicki 123 (M)	Cultivated, origin Solomons	KU586345	-	-	-	KU586396	-	-	-
***Squamellaria huxleyana* Chomicki sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 48 (SUVA) HOLOTYPE	Fiji, Vanua Levu, road between Savusavu to Labasa.	KU586336	KU586355	KU586425	KU586373	KU586385	KU586403	KU586433	KU586414
***Squamellaria imberbis* (A. Gray) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	Fiji, Vanua Levu, track to vodaphone tower.	KU586337	KU586356	-	KU586374	KU586386	KU586404	KU586434	KU586415
***Squamellaria jebbiana* Chomicki, sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	Fiji, Taveuni, Mt Manuca area.	KU586342	KU586361	-	KU586379	KU586391	KU586408	KU586438	KU586419
***Squamellaria kajewskii* (Merr. & L.M.Perry) Chomicki, comb. nov.**	G. Chomicki 122 (M)	Cultivated, origin Solomons	KU586335	-	-	-	KU586384	-	-	-
***Squamellaria major* A.C. Sm.**	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586338	KU586357	KU586426	KU586375	KU586387	KU586405	KU586435	KU586416
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	-	-	KU586430	KU586381	KU586393	KU586410	KU586440	KU586421
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	KU586343	KU586362	KU586431	KU586382	KU586394	KU586411	-	KU586422

***Squamellaria thekii* Jebb**	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586340	KU586359	KU586428	KU586377	KU586389	KU586407	KU586437	KU586418
***Squamellaria vanuatuensis* (Jebb & Huxley) Chomicki, comb. nov.**	McPherson 19437 (P)	Vanuatu	JX155078	-	-	-	-	-	JX155170	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	Fiji, Vanua Levu, Waisali forest park.	-	-	KU586429	KU586380	KU586392	KU586409	KU586439	KU586420
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 49 (M)	Fiji, Vanua Levu, Waisali forest park.	-	KU586364	-	-	-	-	-	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	Fiji, Vanua Levu, Waisali forest park.	KU586344	KU586363	-	KU586383	KU586395	KU586412	KU586441	KU586423
***Squamellaria wilsonii* (Horne ex Baker) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586341	KU586360	-	KU586378	KU586390	-	-	KU586424

| Chapter 4 |

Partner choice through concealed floral sugar rewards evolved with the specialization of ant/plant mutualisms

Guillaume Chomicki, Yannick Staedler, Jürg Schönenberger, and Susanne S. Renner

New Phytologist (2016).

.

New Phytologist

Research

Partner choice through concealed floral sugar rewards evolved with the specialization of ant–plant mutualisms

Guillaume Chomicki[1], Yannick M. Staedler[2], Jürg Schönenberger[2] and Susanne S. Renner[1]

[1]Systematic Botany and Mycology, Department of Biology, University of Munich, 67 Menzinger Str., 80638 Munich, Germany; [2]Department of Botany and Biodiversity Research, Faculty of Life Sciences, University of Vienna, Rennweg 14, 1030 Vienna, Austria

Author for correspondence:
Guillaume Chomicki
Tel: +49 89 17861 285
Email: guillaume.chomicki@gmail.com

Received: *12 February 2016*
Accepted: *25 March 2016*

New Phytologist (2016)
doi: 10.1111/nph.13990

Key words: animal–plant interactions, ant–plant mutualisms, mutualism maintenance, partner choice, symbioses.

Summary

- Obligate mutualisms require filtering mechanisms to prevent their exploitation by opportunists, but ecological contexts and traits facilitating the evolution of such mechanisms are largely unknown.
- We investigated the evolution of filtering mechanisms in an epiphytic ant–plant symbiotic system in Fiji involving Rubiaceae and dolichoderine ants, using field experiments, metabolomics, X-ray micro-computed tomography (micro-CT) scanning and phylogenetics.
- We discovered a novel plant reward consisting of sugary sap concealed in post-anthetic flowers only accessible to *Philidris nagasau* workers that bite through the thick epidermis. In five of the six species of Rubiaceae obligately inhabited by this ant, the nectar glands functioned for 10 d after a flower's sexual function was over. Sugar metabolomics and field experiments showed that ant foraging tracks sucrose levels, which only drop at the onset of fruit development. Ontogenetic analyses of our focal species and their relatives revealed a 25-fold increase in nectary size and delayed fruit development in the ant-rewarding species, and Bayesian analyses of several traits showed the correlated evolution of sugar rewards and symbiosis specialization.
- Concealed floral nectar forestalls exploitation by opportunists (generalist ants) and stabilizes these obligate mutualisms. Our study pinpoints the importance of partner choice mechanisms in transitions from facultative to obligate mutualisms.

Introduction

How does cooperation among species remain stable over time and escape exploitation by non-reciprocators that do not pay back for what they gain? Understanding this puzzling question is a fundamental research goal in ecology and evolutionary biology (Axelrod & Hamilton, 1981; Sachs *et al.*, 2004; Sachs & Simms, 2006; Frederickson, 2013). Exploiters can have higher fitness than mutualists as they gain the benefits of a mutualistic interaction without incurring the associated costs (Yu, 2001), which can ultimately lead to mutualism breakdown (Sachs & Simms, 2006). Two types of exploitation are distinguished. Cheaters or 'cheater mutants' are exploiters that evolved from mutualistic ancestors (Emery, 1909; Bronstein, 2001; Bull & Rice, 1991; Sachs *et al.*, 2004). Despite being predicted by theory (Trivers, 1971; Axelrod & Hamilton, 1981), there are only a few well-documented examples (Sachs & Simms, 2006), for example in bees (Schaefer & Renner, 2008; Litman *et al.*, 2013), but a majority of parasites are nested within non-mutualistic clades (Sachs & Simms, 2006; Chomicki *et al.*, 2015). The other class of exploiters is referred to as 'parasites of mutualisms' *sensu* Yu (2001), and comprises unrelated opportunistic species that invade mutualisms; some invaders are specialized parasites, such as the ant *Cautalacus*, which exploits the mutualism between *Leonardoxa africana* and *Petalomyrmex phylax* (Gaume & McKey, 1999). Three types of mechanism are generally considered in mutualism stabilization, namely by-product mutualism, partner fidelity feedback and partner choice (Sachs *et al.*, 2004). By-product mutualism occurs when the mutualistic behaviour is cost-free (i.e. involving by-products of other traits), and selection for cheating is thus unlikely to arise (Sachs *et al.*, 2004; Foster & Wenseleers, 2006). Partner fidelity feedback posits that the positive feedback between host and symbiont finesses is sufficient to prevent exploitation, a mechanism that has gained recent theoretical support from economic contract theory (Weyl *et al.*, 2010; Archetti *et al.*, 2011). Finally, partner choice consists in excluding non-cooperative partners by preferentially, or only, rewarding cooperative ones (Bull & Rice, 1991). Individuals choosing cooperative partners enhance their own fitness, and the filtering (choice), in turn, promotes the maintenance of cooperation in the cooperative partner (Sachs *et al.*, 2004). The ecological contexts and traits facilitating the evolution of partner choice mechanisms, however, remain poorly understood (Sachs *et al.*, 2004; Frederickson, 2013).

Ant–plant symbioses involve plants with specialized structures (domatia) in which ants nest, sometimes with the same plant also offering food rewards (e.g. extrafloral nectar (EFN), food bodies), in return for defence against herbivores, extra nutrients and occasionally the physical or chemical removal of competing plant species (Huxley, 1978; Davidson & McKey, 1993; Renner & Ricklefs, 1998; Frederickson *et al.*, 2005). The evolutionary specialization of such mutualisms could involve an increased investment in rewards, so as to maintain the desired symbiont, for example, by increasing the amount of EFN offered. However, increasing reward levels also increase the interest of opportunists, and partner choice mechanisms should thus evolve to exclude the less desired partners. Three such mechanisms, all involving food rewards, have been documented in myrmecophytic Mesoamerican *Vachellia* (Fabaceae) that host *Pseudomyrmex* (Pseudomyrmecinae) ants (Heil *et al.*, 2005, 2014; Orona-Tamayo *et al.*, 2013), illustrating the importance of rewards as a substrate for the evolution of partner choice in ant–plant symbioses.

The family richest in ant-housing species is the Rubiaceae, which includes over 160 species that develop domatia regardless of the presence of ants. Surprisingly, no ant-plant species in this family has extrafloral nectaries (Weber & Keeler, 2013). Within Rubiaceae, a clade of *c.* 100 epiphytic species from the Australasian region (Psychotriae subtribe Hydnophytinae) is characterized by large hypocotyl domatia with networks of galleries (Fig. 1). The domatia are inhabited by ants, frequently of the dolichoderine genera *Philidris* and *Anonychomyrma*, that feed the plants by defecating inside the cavities, and, in some instances, also provide antiherbivore defence (Huxley, 1978). During fieldwork on rubiaceous ant-plants in Fiji, however, we discovered a novel type of exclusive food reward, when we noticed the more than week-long persistence of old (post-anthetic) flowers visited by the ant mutualist. Our system consists of a clade of nine species from the genus *Squamellaria* (Rubiaceae, Psychotriae, Hydnophytinae), three of which form facultative symbioses with a wide range of ants, and six of which are obligately associated with the dolichoderine ant *Philidris nagasau*. In addition to this Fijian study system, we produce here a phylogeny for the whole subtribe Hydnophytinae and reconstruct the evolutionary histories of symbiosis specialization and partner choice mechanisms. Based on behavioural experiments, three-dimensional reconstructions of nectar gland ontogeny, sugar metabolomics and phylogenetics, we describe the new type of food reward and then address the following questions: By which developmental steps did the new partner choice mechanism evolve? And did the partner choice mechanisms evolve concurrently with increasing symbiosis specialization?

Materials and Methods

Collection of material on Fiji and study sites

In September 2014 and March 2015, we conducted fieldwork on the islands of Viti Levu, Vanua Levu and Taveuni, and collected all nine species of the genus *Squamellaria* that occur on these islands (Chomicki & Renner, 2016). The study sites in Viti Levu were Colo-i-Suva Forest Reserve in the south of the island (18°1′46.808″S, 178°24′0.4175″E) and forest around Navai in the centre of the island (17°37′ 49.5979″S, 177°58′34.9315″E). In Vanua Levu, the collection sites were Waisali Forest Reserve (16°38′19.8″S, 179°13′19.7″E) and along the Cross Island road before the bifurcation to Nabouwalu and Labasa. In Taveuni, the collections were made along the trail to DesVoeux peak and Mt. Manuca on the western side of the island (16°48′ 25.8133″S, 179°56′36.6843″E) and at the end of Lavena coastal walk, Bouma heritage park, on the eastern side of the island (16°51′45.4433″S, 179°54′ 6.5149″E). All collections were made in collaboration with Alivereti Naikatini and Marika Tuiwawa from the University of South Pacific, Suva, and vouchers have been deposited in the herbaria of Suva (SUVA) and Munich (M). For DNA extraction, we collected young leaves and dried them in silica gel. Except for a few cases, *Squamellaria* plants were accessed by tree climbing, using a rope secured by a partner on the ground. This technique allowed long stays in the canopy with minimal disturbance of the ant colony.

Cafeteria experiments addressing the attraction of *Philidris nagasau* to different sugars

To determine whether a decrease in the concentration of sucrose, glucose or fructose affected *P. nagasau* attendance, we conducted ‘cafeteria’-style experiments. During these experiments, we synchronously offered different sugar solutions to ants. All experiments were performed without displacements of ants and without artificial platforms, as a pilot had shown that transport and platforms affected ant behaviour. Distilled water was used as a negative control. For each cafeteria, 10 replicates were performed, and five independent ant colonies were used. At each single site, three droplets (10 μl) of each sugar solution were placed on the host tree bark (close to the epiphytic plants), and the order of each solution was randomized, with all drops present at *c.* 10 cm from each other. Ants feeding on each solution were counted twice, at 4 and 6 min following droplet placement, as droplets generally dried out in *c.* 15 min. Droplets were replaced three times and the same procedure was repeated (so that each individual cafeteria consisted of a triplicate, itself performed 10 times on different ant colonies, days and time of the day). Their numbers were summed to calculate the relative numbers of ants that had been attracted to the respective sugar solution. Statistical evaluation was performed by summing the ant numbers attracted to one particular sugar solution for each replicate, and subjected to one-way ANOVA and Tukey’s *post-hoc* test, all performed in R v3.2.0 (R Core Team, 2015).

Experiments addressing ant behaviour on young and old *Squamellaria* flowers

To determine whether post-anthetic *Squamellaria* flowers (i.e. floral cups without the petals) were attractive to opportunistic ants, we offered *S. imberbis* (in Vanua Levu) inflorescences to the opportunistic ant species *Camponotus chloroticus*, *Colobopsis polynesica* (*Camponotus polynesicus*; Ward *et al.*, 2016), *Pheidole* sp. 1 and *Pheidole* sp. 2, which live in non-specialized species of *Squamellaria*. As these ants showed no interest in the post-anthetic flowers (different from *P. nagasau* workers, below), we decided to test whether this was caused by the absence of any

Fig. 1 Facultative and obligate symbioses in Fijian epiphytic ant-plants. (a) *Squamellaria imberbis*, Taveuni, an obligate ant-epiphyte species. (b) *Squamellaria wilkinsonii*, Vanua Levu, a facultative ant-epiphyte. (c) Foraging of the (single) ant symbiont of *Squamellaria* (*Philidris nagasau*) inside post-anthetic nectaries. Inset: scars of *P. nagasau* bites after a few days. (d) *Philidris nagasau* exploiting the concealed nectar of *S. wilsonii* by biting into the nectary disc. Bars: (a) 20 cm; (b) 12 cm; (c) 2 cm; (d) 6 mm.

secretion in post-anthetic flowers. We therefore offered the same four opportunistic ant species as well as workers of *P. nagasau* the following: (1) intact floral cups of post-anthetic flowers; (2) floral cups of post-anthetic flowers in which the epidermis had been scratched to expose the accumulated nectar; and (3) intact floral cups bearing a drop of aqueous solution with a similar concentration in sucrose, glucose and fructose (~2400 ng μg^{-1} dry mass), mimicking secreted nectar. Each experiment was replicated five times, each time on a different ant colony. Ants were counted at 5, 7 and 10 min. The numbers of ants attracted to any particular sugar solution were summed and subjected to one-way ANOVA and Tukey's *post-hoc* test, all performed in R.

Philidris nagasau monitoring

We also monitored *P. nagasau* foraging on anthetic and post-anthetic *Squamellaria* flowers throughout the day, focusing on five ant colonies (each living on a different tree) over a 3-d rolling basis. For each colony, behaviour on the floral cups was recorded once an hour for 10 min during daylight. To relate foraging to flower age, we marked and observed 53 flowers of *S. imberbis* (on Taveuni) from the time at which they had just opened to 20 d after anthesis, returning to each flower for 10-min periods between 13:00 and 15:00 h. We also monitored the location of *P. nagasau* workers on *Squamellaria* plants, by counting all ants on the domatia, stems, leaves, post-anthetic nectaries and fresh flowers. We monitored the worker distribution from 20 *Squamellaria wilsonii* (Taveuni) and *S. imberbis* (Vanua Levu) plants, by counting every worker present on all plant parts, for a total of 534 different workers.

Fruit phenology

To test whether *Squamellaria* species characterized by the production of ant-addressed post-anthetic rewards show delayed fruit development compared with closely related species without such rewards, we measured ovary diameter (in the middle) daily from anthesis to 20 d after anthesis in at least 10 flowers of each of the nine species of this genus that occur on Viti Levu, Vanua Levu and Taveuni.

Metabolomics and absolute sugar measurements

Metabolites for gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) were extracted and derivatized using a modified version of the method described in Roessner *et al.* (2001), Lisec *et al.* (2006) and Erban *et al.* (2007). We determined the metabolomic composition of post-anthetic floral rewards in all five rewarding species (*Squamellaria huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, *S. wilsonii*). For each species, we selected a healthy specimen, with similar sun exposure, and collected its ant-rewarding nectaries (i.e. 2–6 d post-anthesis). To measure the absolute concentration of sugars in each of the key phases of nectary development, we collected *S. imberbis* (Vanua Levu) nectaries from three stages: (1) at anthesis; (2) 2–6 d post-anthesis (i.e. in the phase in which they were actively rewarding ants); and (3) after the onset of fruit development, when the ovary had just started to bulge (i.e. non-ant-rewarding, 14–16 d post-anthesis). In all cases, nectaries were immediately dissected and microwave dried, a method that preserves metabolites (Popp *et al.*, 1996) and is ideal under field conditions. For the extraction, ~5 mg of plant material (dry weight) was ground in 300 μl of cold (–20°C) methanol (80%) containing 15 μl of ribitol (0.1 mg ml^{-1} in water) and 15 μl of ^{13}C-sorbitol (0.1 mg ml^{-1} in water), which were added as internal standards for the quantification of metabolite abundances. After incubation at 70°C for 15 min, 30 μl of the extract was dried *in vacuo*. The pellet was resuspended in 10 μl of methoxyaminohydrochloride (20 mg ml^{-1} in pyridine) and derivatized for 90 min at 37°C. After the addition of 20 μl of BSTFA (*N,O*-bis[trimethylsilyl] trifluoroacetamide) containing 5 μl of retention time standard mixture of linear alkanes (n-decane, n-dodecane, n-pentadecane, n-nonadecane, n-docosane, n-octacosane, n-dotriacontane), the mix was incubated at 37°C for a further 45 min. A volume of 1 μl of each sample was injected into a GC-TOF-MS system (Pegasus HT, Leco, St Joseph, MI, USA). Samples were derivatized and injected by an autosampler system (Combi PAL, CTC Analytics AG, Zwingen, Switzerland). We used helium as carrier gas at a constant flow rate of 1 ml min^{-1}. We performed GC on an Agilent GC system (7890A, Agilent, Santa Clara, CA, USA) using a 30-m VF-5 ms column with a 10-m EZ-Guard column. The injection temperature of the CIS injector (CIS4, Gerstel, Mühlheim, Germany) increased with a rate of 12°C s^{-1} from an initial temperature of 70°C to 275°C. Transfer line and ion source temperatures were set to 250°C, with an initial oven temperature of 70°C gradually increased by 9°C min^{-1} to a final temperature of 320°C. To avoid solvent contamination, the solvent delay was set to 340 s. Metabolites that passed the column were released into the TOF-MS. The transfer line connecting the GC and the TOF-MS was set to 250°C. The ion source at which the in-streaming metabolites were ionized and fractionated by an ion pulse of 70 eV was also set to 250°C. Mass spectra were recorded at 20 scans s^{-1} with an *m/z* 35–800 scanning range. Chromatograms and mass spectra were evaluated using CHROMATOF 4.5 and TAGFINDER 4.1 software (Luedemann *et al.*, 2008). Absolute quantitative estimation was performed using external standards of each compound. Relative values are the specific ratios of the metabolite intensity multiplied by the intensity of the internal standard compound, normalized by the amount of dry weight. The full list of metabolites is given in Supporting Information Table S1.

DNA extraction, phylogenetic analyses and molecular clock dating

We generated two phylogenies for this study. First, a nine-marker phylogeny for the nine Fijian *Squamellaria* species using six plastid regions (*trnL* intron, *trnL-trnF* spacer, *ndhF*, *rps12-rpl20*, *trnS*-trnG and *rps16*) and three nuclear regions (18S, ITS and ETS), which have been proven to be useful in Rubiaceae phylogenetics (e.g. Barrabé *et al.*, 2014). The primers used are reported in Table S2. All accessions of Fijian *Squamellaria* were extracted from silica-dried leaves collected by GC and are all linked to herbarium specimens deposited in the herbaria SUVA and M (Table S3). Outgroups (in the tribe Psychotrieae) were selected based on Barrabé *et al.* (2014). Second, a six-marker phylogeny for the whole subtribe Hydnophytinae, sampling 50% of their *c.* 100 species (55 ingroup plus 22 outgroup) for two nuclear markers (ITS and ETS) and three plastid markers (*ndhF*, *trnL* intron and *trnL-trnF* spacer), obtained from a combination of herbarium material, material collected in Fiji by the first author and vouchered cultivated material. The outgroup sequences were downloaded from GenBank and came from Barrabé *et al.* (2014). Vouchers, geographical origin and GenBank accession numbers are reported in Table S3.

Total genomic DNA was extracted from *c.* 20 mg of leaf tissues using a commercial plant DNA extraction kit (NucleoSpin; Macherey-Nagel, Düren, Germany) according to the manufacturer's protocols. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England Biolabs, Cambridge, MA, USA) and a standard protocol (39 cycles, annealing temperature of 56°C). PCR products were purified using the ExoSap clean-up kit (Fermentas, St Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in SEQUENCHER 5.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were BLAST searched in GenBank. Sequence alignment was performed in MAFFT v.7 in the online server (http://mafft.cbrc.jp/alignment/server; Katoh & Standley, 2013) under standard parameters, except for the ITS region which was aligned under Q-INS-i optimization, which takes rRNA secondary structure into consideration. Minor alignment errors were corrected manually in MESQUITE v.2.75 (Maddison & Maddison, 2011).

In the absence of statistically supported incongruence (i.e. maximum likelihood (ML) bootstrap support > 75) between the plastid and nuclear data partitions, we concatenated all DNA matrices, yielding an alignment of 9346 bp for the *Squamellaria* matrix and 5895 bp for the Hydnophytinae matrix. ML inference relied on RAxML v.8.0 (Stamatakis *et al.*, 2008) with 100 ML bootstrap replicates and the analysis partitioned by gene region, all under the GTR + Γ substitution model, with empirical nucleotide frequencies and 25 gamma rate categories. We also conduced Bayesian inference in MRBAYES v.3.2 (Ronquist *et al.*, 2012), using the default two runs and four chains (one cold and three heated), with the uniform default priors. Model parameters were unlinked, and posterior probabilities of the tree topologies were estimated from all 10 partitions, each running under its best-fitting model according to the Akaike information criterion (AIC) as determined in JMODELTEST2 (Darriba *et al.*, 2012). We set a 10 × 10^6 Markov chain Monte Carlo (MCMC) chain, sampling trees every 1000th generation. Split frequencies approaching zero indicated convergence. We used the 50% consensus tree to assess the posterior probabilities for the nodes of interest. Molecular clock dating was performed in BEAST 2 (Bouckaert *et al.*, 2014) and used Yule tree priors, with an MCMC chain length of 20 million, sampling every 10 000th generation, with the chain length depending on convergence, as determined by examining the log files in TRACER v.1.5 (Rambaut & Drummond, 2007) after removal of an initial burn-in proportion of 10% of the trees. The tree was calibrated using a secondary constraint from Barrabé *et al.* (2014) for the clade (*Psychotria* clade IV + *Psychotria* Pacific clade (including Hydnophytinae)) of 22 ± 7 million yr ago (Ma), with a normal prior and a standard deviation corresponding to the 95% confidence interval (CI).

Ancestral state reconstructions of nectary types

The floral nectary types of 55 ingroup (including the nine Fiji species) and 22 outgroup taxa were coded '0' for non-ant-rewarding and '1' for ant-rewarding based on published and unpublished observations (Huxley, 1981; M. P. H. Jebb and C. Huxley-Lambrick, pers. comm. to G.C., February 2015 and November 2015; G.C. own observations on Fiji). We used stochastic character mapping to infer possible histories of floral nectary types, using the function 'MAKE.SIMMAP' in the PHYTOOLS package v.04-60 (Revell, 2012), which implements the stochastic character mapping approach developed by Bollback (2006). We estimated ancestral states using a symmetric rate model, and then simulated 1000 character histories on the maximum clade credibility trees from BEAST. We summarized the 1000 simulated character histories using the function DENSITYMAP (also in PHYTOOLS).

Correlated evolution of concealed sugar rewards and symbiosis specialization

To test whether concealed sugar rewards evolved with symbiosis specialization, we used BAYESTRAITS v.2 (Pagel & Meade, 2014), which allows the detection of correlated evolution between pairs of discrete binary traits. Absence of concealed sugar reward was coded as '0' and presence as '1'. Based on observations by C. R. Huxley, M. P. H. Jebb and M. Janda, gathered over the last 35 yr in Papua New Guinea, and by G. Chomicki in Fiji in September–October 2014 and March–April 2015, we distinguished two main mutualism types: facultative, when species were inhabited by several (often unrelated) generalist ant species, and specialized, when species were either obligately inhabited by *P. nagasau* (*Squamellaria grayi*, *S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, *S. wilsonii*) or inhabited by one or two specialized plant-ants (from the genera *Philidris* or *Anonychomyrma*, all *Myrmecodia* species, and a few *Hydnophytum*). For all nine Fijian species, we quantified occupancy rates and ant partner types by examining the ants present in at least 20 specimens per species. *Squamellaria jebbiana*, *S. tenuiflora* and *S. wilkinsonii* were inhabited by various generalist ant species (several species of *Pheidole*, *Camponotus chloroticus*, *Colobopsis polynesica* (*Camponotus polynesicus*)). Furthermore, 30–45% of the individuals of this species were not inhabited by ants. Sarnat (2009) reported further ant species inhabiting *S. tenuiflorum*. Altogether, this indicates that *S. jebbiana*, *S. tenuiflora* and *S. wilkinsonii* form only facultative symbioses with ants. By contrast, the six other Fijian *Squamellaria* species (*S. grayi*, *S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, *S. wilsonii*) were all inhabited by *P. nagasau* (> 300 mature individuals observed, all were inhabited), indicating an obligate symbiosis with *P. nagasau*. Moreover, *P. nagasau* has never been found outside of *Squamellaria* (Sarnat & Economo, 2012; this study), suggesting that the symbiosis is obligate for both partners. We used two proxies for symbiosis specialization: the number of ant partners per plant species, with species scored as '0' if occupied by ants from two or more genera and as '1' if occupied by ≤ 2 ant species from the same genus; and the level of domatium specialization, with species scored as '0' if their domatia have entrance holes > 0.5 cm in diameter and reticulated, unlinked cavities (indicative of facultative symbioses) or if their domatia have entrance holes > 1 cm and bulbous cavities (forming no symbioses with ants) and as '1' if their domatia have entrance holes < 0.5 cm in diameter and highly reticulated, linked cavities. Although the number of ant partners might directly reflect the presence or absence of concealed sugar rewards, tuber traits are an independent measure of symbiosis specialization. We used the maximum clade credibility (MCC) tree from BEAST, but pruned the 22 outgroups and first ran a model of independent trait evolution estimating the four transition rate parameters $\alpha1$, $\alpha2$, $\beta1$, $\beta2$, wherein double transitions from state 0,0 to 1,1 or from 0,1 to 1,0 are set to zero. We then ran a model of dependent trait evolution with eight parameters (q12, q13, q21, q24, q31, q34, q42, q43). To compare these non-nested models, we calculated the Bayes Factor score.

X-ray micro-computed tomography (micro-CT)

Flowers were fixed in formalin–acetic acid–alcohol (FAA) in the field. For X-ray micro-CT, all samples were treated with a solution of 1% (w/v) phosphotungstic acid in FAA for at least 1 wk, changing the solution every other day following the protocol of Staedler *et al.* (2013). The flowers were imaged at 2–33.7 μm voxel size with a microXCT-200 X-ray tomography system from Zeiss Microscopy (Jena, Germany). This system uses a 90-kV microfocus X-ray source (L9421-02 from Hamamatsu, Hamamatsu City, Japan), a cooled 2k 2k CCD camera, and switchable scintillator objective lens units. The scanning settings are summarized in Table S4. XMRECONSTRUCTOR 8.1.6599 software (Zeiss Microscopy) was used to perform the three-dimensional reconstruction from the scanning data. For samples that were scanned in several steps, XMECONTROLLER 8.1.6599 software was used to stitch together the resulting scan data. TXM3D VIEWER software (Xradia Inc., Concord, CA, USA) was used to acquire images of the samples.

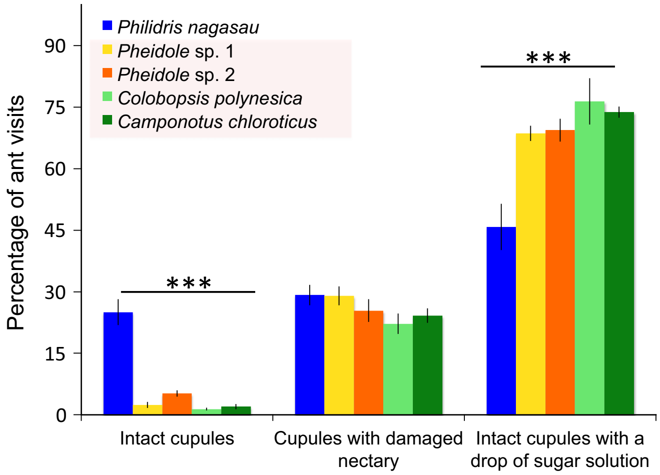


Fig. 2 *Squamellaria* conceals nectar as an exclusive reward. The transparent red square highlights opportunistic ant species (*Camponotus chloroticus*, *Colobopsis polynesica* (*Camponotus polynesicus*), *Pheidole* sp. 1, *Pheidole* sp. 2), which are compared with the specialist plant-ant *Philidris nagasau*. Error bars, ± SE. ***, *P* values significant at the $P < 0.001$ level of a *post-hoc* Tukey's test.

Results

Only a specialized symbiont exploits the concealed sugar reward produced for *c.* 10 d in its hosts' post-anthetic flowers

Two types of symbioses are found in the nine Fijian ant-plant species in the genus *Squamellaria*: facultative symbioses with several generalist ants in *S. jebbiana*, *S. wilkinsonii* and *S. tenuiflora*, and obligate symbioses with a single ant mutualist, the dolichoderine ant *P. nagasau*, in the six remaining species (*S. grayi*, *S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, *S. wilsonii*) (Fig. 1a,b). In five of the latter species, old flowers in which the corolla has already been lost stay on the plants unchanged, instead of falling off or beginning to develop into fruits. Each of these old flowers has a conspicuous nectary disc that is not exposed whilst the flowers still have their petals (Fig. 1c). Only *P. nagasau* actively forage on these cup-shaped post-anthetic nectaries by biting into the epidermis with their mandibles (Fig. 1c,d; Movie S1). The absence of any nectar as liquid or as crystals on the nectary disc made post-anthetic flowers

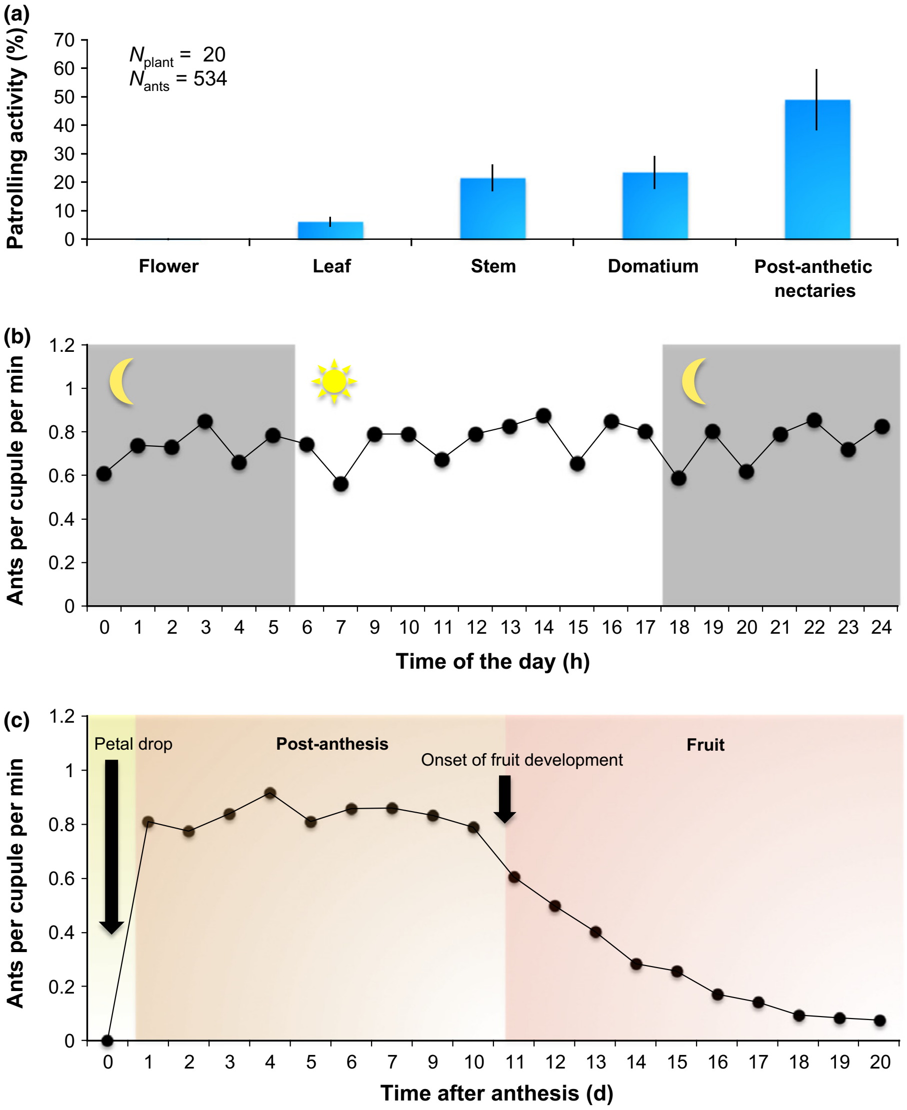


Fig. 3 *Philidris nagasau* foraging behaviour on *Squamellaria* nectaries. (a) *Philidris nagasau* patrolling activity on different *Squamellaria* organs. (b) Ant foraging activity throughout the day. (c) Ant foraging activity on 53 individual nectaries followed after anthesis. Error bars, ± SE.

unattractive to opportunists who appeared unable to perceive or exploit the concealed sugar reward as confirmed by cafeteria experiments (Fig. 2; ANOVA, $P<0.001$; *post-hoc* Tukey's test, $P<0.001$). Monitoring of ants also showed that *P. nagasau* hardly patrolled anthetic flowers (Fig. 3a), but visited post-anthetic flowers with their concealed sugar reward more or less constantly during the day and night (Fig. 3b) for *c.* 10 d, after which visitation dropped as fruit development started (Fig. 3c).

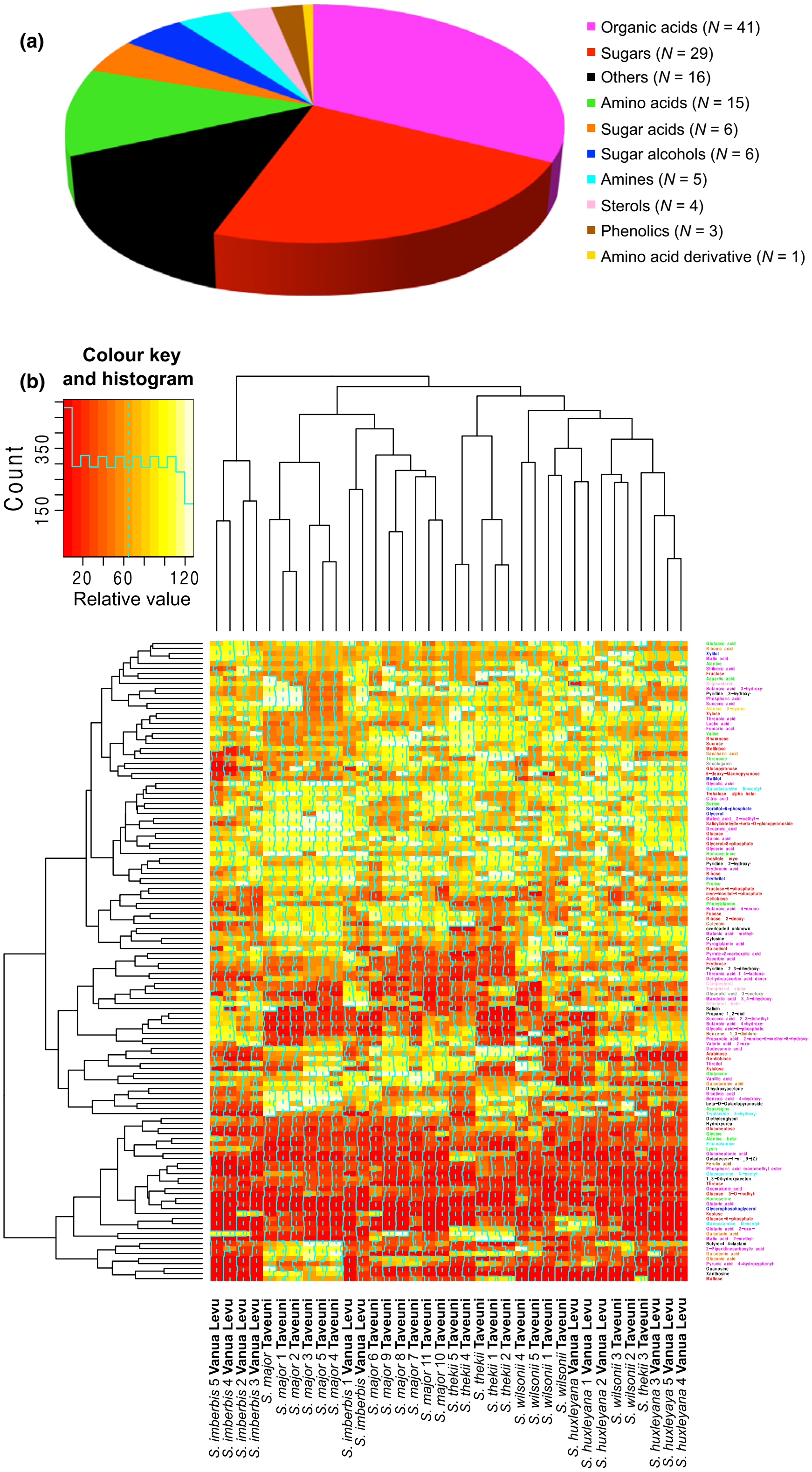


Fig. 4 Metabolomics of *Squamellaria* exclusive post-anthetic sugar rewards. (a) Pie chart showing the main categories for the 128 metabolites common to all five rewarding *Squamellaria* species (*S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, *S. wilsonii*). (b) Heatmap showing the relative quantities of all 128 metabolites across all five species and samples. Metabolite names on the right are colour coded as in (a).

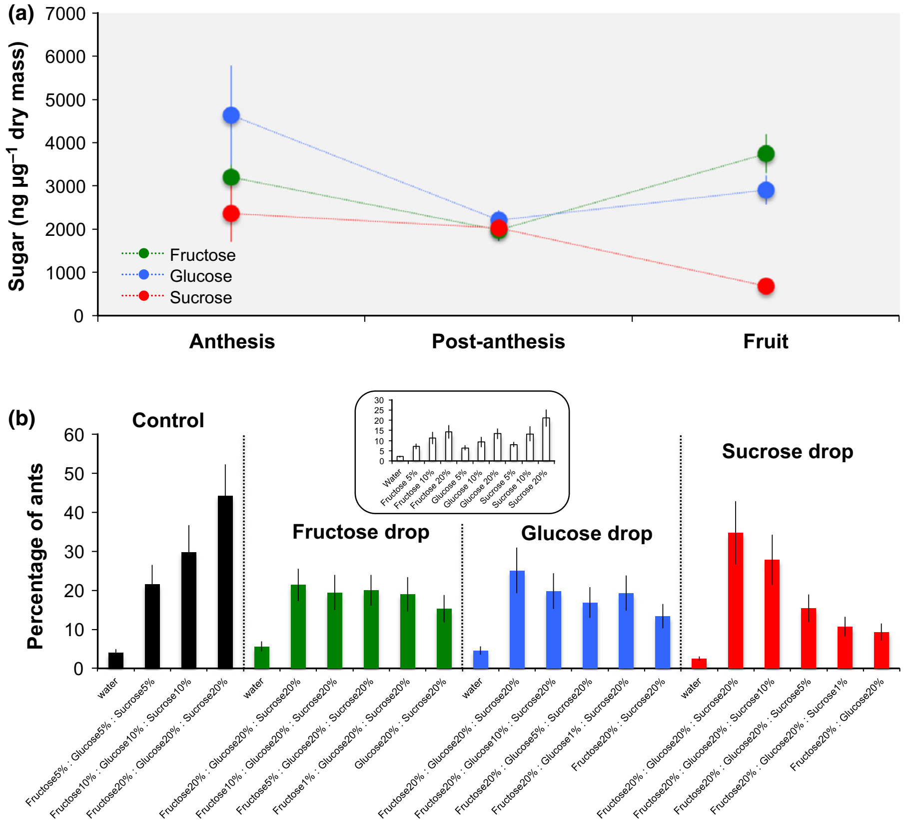


Fig. 5 Sucrose drop during early fruit development regulates *Philidris nagasau* foraging. (a) Sugar concentration in *Squamellaria wilsonii* nectaries at different stages. Post-anthesis nectaries collected on days 2–6; fruit nectaries sampled on immature fruits on days 14–16. (b) *Philidris nagasau* sugar preferences evaluated from cafeteria experiments (see the Materials and Methods section). Error bars, ± SE.

Metabolomic composition of *Squamellaria* post-anthetic concealed sugar reward

Metabolomic analysis of nectary tissue at anthesis, post-anthesis and during early fruit development (see the Materials and Methods section) revealed 128 metabolites, most notably 29 sugars and 15 amino acids (Fig. 4a,b; Table S1), indicating that post-anthetic rewards are very nutritious. At anthesis, the sugary sap is richer in glucose and fructose than in sucrose, but, after anthesis, the concentration of the first two sugars drops to reach *c.* 2400 ng µg^{-1} dry mass, whereas the sucrose level is maintained (Fig. 5a). After the onset of fruit development (i.e. ovary bulging), the sucrose level drops, resulting in correspondingly higher glucose and fructose levels (Fig. 5a).

The association of a drop in sucrose concentration and lower ant visitation suggests that the sucrose level controls *P. nagasau* foraging. To test this, we carried out a second series of cafeteria experiments in which we fed ants with different sugar solutions to test whether *P. nagasau* was sensitive to changes in one of the three sugars (Fig. 5b). This turned out to be true for all three, either separately or together at the same stoichiometry (Fig. 5b and inset). To specifically test how a lower level of one of the three sugars affects *P. nagasau* preferences, we performed three more series of cafeteria experiments in which only one of the three sugars was presented at different concentrations, whilst the two others were kept constant (see the Materials and Methods section). Varying glucose or fructose levels (whilst keeping sucrose constant) resulted in only small decreases in ant attendance (Fig. 5b). By contrast, when sucrose was offered in different concentrations (whilst the levels of glucose and fructose remained unchanged), ant attendance decreased dramatically, tracking the sucrose decrease (Fig. 5b). This confirmed that sucrose levels control *P. nagasau* foraging behaviour.

Evolution of the concealed sugar reward: increase in nectary volume and delayed onset of fruit development

Sugar rewards form early (Fig. 6a), and ant-addressed nectaries have a volume *c.* 25-fold larger than non-ant-addressed nectaries in unspecialized *Squamellaria* or the secondarily reduced glands of the rewardless *S. grayi* (8–10 mm^3 vs 0.3–0.4 mm^3; Fig. S1). In the ant-addressed nectaries, the vascular network is prominent with a high bundle density (Fig. S2).

In addition to the conspicuous difference in gland size, the onset of fruit development in the species of *Squamellaria* with ant-addressed nectaries is phenologically delayed compared with that in related species that do not offer post-anthetic sugar rewards to their symbiotic ants. This delay causes the accumulation of old (post-anthetic) flowers (Figs 1c,d, 6a). Assessment of the timing of fruit development (ovary bulging) in all nine species of Fijian *Squamellaria* by measuring the calyx diameter for 20 d after anthesis revealed that, in the five species with concealed sugar rewards, fruit development started *c.* 10 d after anthesis, whereas in the other (non-ant-rewarding) species, ovary enlargement was noticeable after 48–72 h (Fig. 6b). This delay retards sucrose hydrolysis (previous section), which begins during

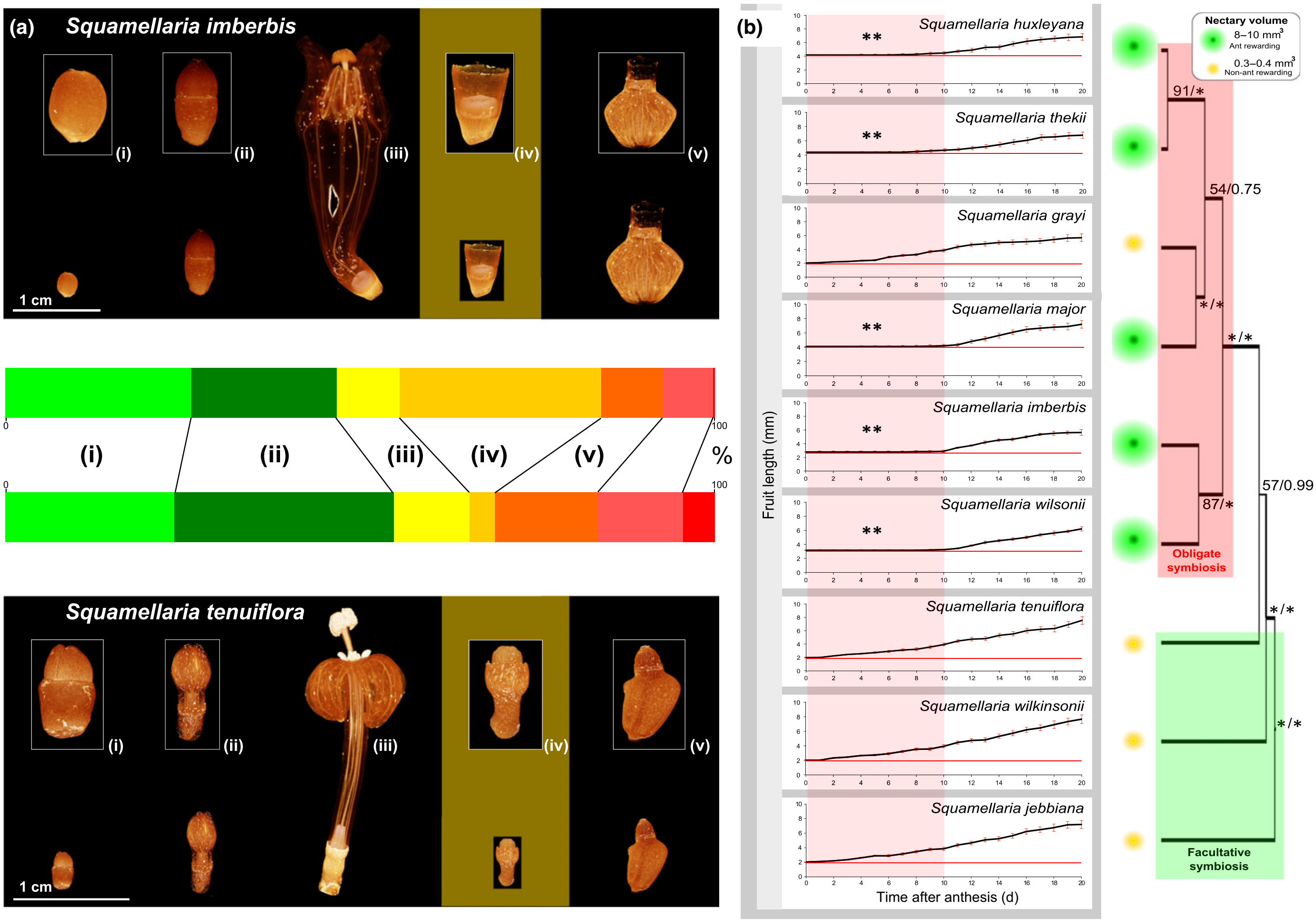


Fig. 6 *Squamellaria* post-anthetic sugar rewards evolved via heterochronic fruit development and nectary enlargement. (a) Micro-computed tomography (µCT) scanning images showing floral developmental stages of the ant-addressed nectaries of *Squamellaria imberbis* and the non-ant-addressed nectaries of *S. tenuiflora*. The middle chart shows the proportion of each of the developmental stages, recorded as a percentage, in 20 inflorescences for each species. (b) Fruit developmental timing in rewarding and non-rewarding Fijian ant-epiphytes. Fruit length (ovary length) is over the time shown, and linked to phylogenetic relationships. Numbers at the branches show the maximum likelihood (ML) bootstrap support and the posterior probabilities. * indicates maximal support. Error bars, ± SE. **, *P* values of *t*-tests significant at the $P < 0.01$ level.

fruit development and results in the accumulation of ant-rewarding post-anthetic flowers. During the months of September/October and March/April when we studied *Squamellaria*, the symbiotic ant colonies were constantly provided with sugar rewards, and observations of *Squamellaria* herbarium specimens (K, FHO, SUVA, L, NSW, US) confirmed that flowers are produced year-round. *Squamellaria* flowering phenology thus ensures that rewards are produced year-round.

Concealed sugar rewards evolved with mutualism specialization

To understand the evolution of concealed sugar rewards produced after anthesis and accessible only to visitors capable of chewing (not pollinators), we investigated nectary ontogeny in all Fijian ant-plant Rubiaceae species. All nine species have floral nectary discs, but *P. nagasau* ants forage only on five of the six *Squamellaria* species it inhabits (*S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, *S. wilsonii*, *S. grayi*). To study the evolution of gland structure and volume, we inferred a molecular clock-dated phylogeny based on up to 10 nuclear and plastid DNA markers obtained for 55 species of Hydnophytinae (*c.* 50% of all species in the clade; Chomicki & Renner, 2016). Large ant-addressed nectaries that are sugar-rich post-anthesis evolved in the most recent common ancestor (MRCA) of *Squamellaria*, *c.* 2.1 ± 1 Ma (Fig. S3), and were secondarily lost in *S. grayi*, which has small glands similar to those of unspecialized *Squamellaria* (Figs 6b, 7, S2).

Mapping the evolution of concealed sugar rewards on a large Hydnophytinae phylogeny revealed an apparent correlation with specialized symbiosis (Fig. 7). The BAYESTRAIT test (see the Materials and Methods section) for correlated evolution of ant symbiont specialization and domatium specialization showed that models of correlated trait evolution were strongly favoured over models that assumed independent trait change (Bayes Factor = 55.8 and 43.1, respectively), confirming the concurrent evolution of the cheater exclusion mechanism 'concealed sugar rewards' jointly with increasing symbiosis specialization

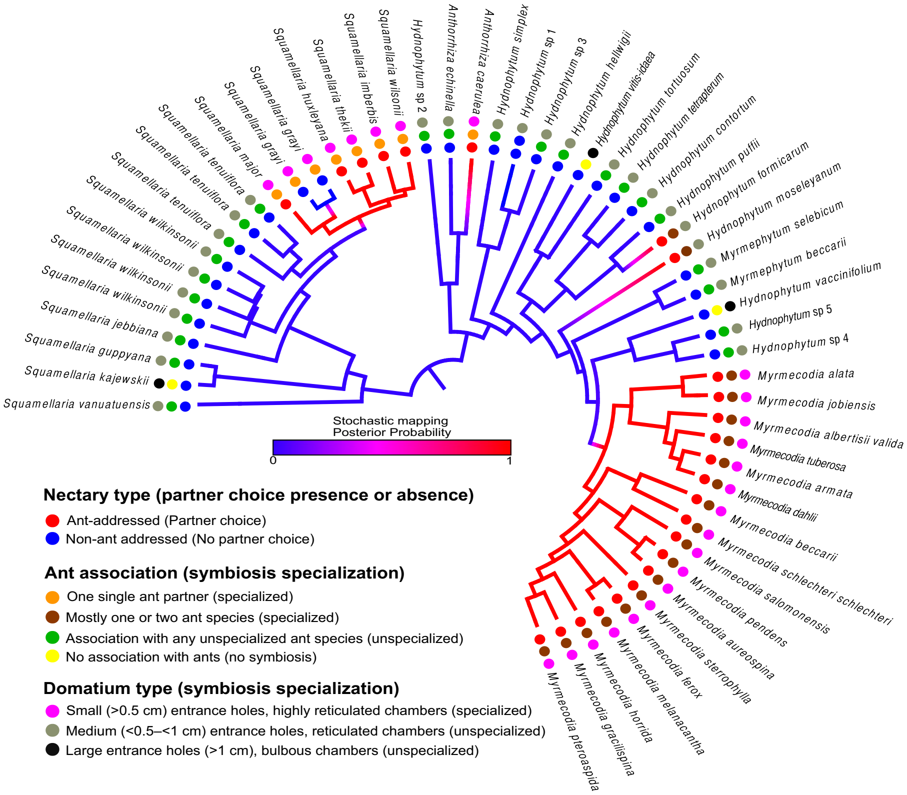


Fig. 7 Evolution of exclusive rewards in the Hydnophytinae and correlated evolution with mutualism specialization. Stochastic mapping reconstruction of nectary type performed on the BEAST maximum credibility tree, and correlated evolution of concealed rewards and symbiosis specialization, as evaluated via two proxies (ant inhabitants and domatium type).

(measured by proxies, namely ant partner number and the domatium traits 'entrance hole diameter' and 'type of cavity').

Discussion

Concealed nectary rewards compared with other partner choice mechanisms in ant–plant symbioses

The concealed sugar reward in these rubiaceous ant-plants (genus *Squamellaria*) filters out opportunistic nectar foragers (Fig. 2). However, the post-anthetic nectar rewards are unlikely to be the main asset that ties *P. nagasau* to *Squamellaria*, given that one species, *S. grayi*, secondarily lacks the sugary rewards (and hence the partner choice mechanism) and still retains its obligate symbiosis with *P. nagasau*. Selective access to food rewards has evolved as a partner choice mechanism in several other ant–plant systems. In Central American *Vachellia*, post-secretory hydrolysis of sucrose by invertase renders the EFN unattractive to opportunistic ants (Heil *et al.*, 2005; Kautz *et al.*, 2009), whereas the mutualist species *Pseudomyrmex ferrugineus* is manipulated by its host (*Vachellia*), which inhibits the digestive ability via chitinase that blocks invertase activity (Heil *et al.*, 2014). *Vachellia* thus filters out opportunistic foragers, but also manipulates its partner to restrict it from exploiting other food sources. Such partner restriction can theoretically stabilize mutualisms (Wyatt *et al.*, 2016). In this *Vachellia–Pseudomyrmex* system, the plant hosts produce food bodies (Beltian bodies) that are protein- and lipid-rich, and that are protected from exploitation by a protease inhibitor that prevents leaf beetles and opportunistic ants from digesting them (Orona-Tamayo *et al.*, 2013). *Squamellaria* concealed sugar rewards differ from these systems in that filtering is physical, not chemical. In South-East Asian domatium-bearing *Macaranga*, about half of the species have slippery waxy stems that limit stem exploitation by opportunists, whereas mutualists possess biomechanical adaptations enabling them to adhere to these waxy surfaces (Federle *et al.*, 1997, 2000). Domatium-bearing *Macaranga* species without waxy surfaces have Beltian bodies hidden under stipules and almost no EFN, whereas waxy *Macaranga* secrete abundant EFN (Federle & Rheindt, 2005), showing that wax-covered stems are also a partner choice mechanism. Yet another type of physical partner choice occurs in one species of the African Fabaceae *Leonardoxa*, in which ant and plant have coevolved to produce a prostoma matching the ant mutualist's size and shape (Brouat *et al.*, 2001).

When is partner choice needed in ant–plant symbioses?

It is currently debated whether partner fidelity feedback alone can maintain mutualism (West *et al.*, 2002; Kiers *et al.*, 2003; Weyl *et al.*, 2010; Kiers *et al.*, 2011; Frederickson, 2013). Frederickson (2013) argued that 'sanction' mechanisms in fig–wasp, yucca–moth and legume–rhizobia mutualisms are a misinterpretation of host pre-adaptations and are instead best understood as partner fidelity feedbacks (Weyl *et al.*, 2010). In *Cordia nodosa*, young shoots that suffer heavy herbivory are shed, which has been interpreted as a 'host sanction' that evolved in response to selection from cheaters (Edwards *et al.*, 2006). This seems unlikely as organ abscission following biotic or abiotic damage is frequent in plants (e.g. Addicott, 1982), and thus this is likely to be a pre-adaptation, best understood within the partner fidelity feedback framework (Weyl *et al.*, 2010). A potential example of a sanction induced by cheater selection is found in *Hirtella myrmecophila* (Chrysobalanaceae), the leaf pouch domatia of which are inhabited by *Allomerus octoarticulatus*, an ant that protects *Hirtella* against herbivores, but castrates its host. *Hirtella* shed the domatia in older leaves (on shoots that will flower), which mitigates the effect of castration (Izzo & Vasconcelos, 2002). If *Allomerus* is the principal partner of *Hirtella*, this would be a case in which partner fidelity feedback alone cannot efficiently maintain mutualism.

More generally, where ant–plant symbioses involve specialized food rewards, there seems to be selection for reducing the attraction of opportunists (parasites of mutualisms), whereas cheating by the plant's own symbionts appears to be too rare to have induced the evolution of sanctions (Frederickson, 2013). EFNs provide a good example. In over 457 plant lineages and > 3900 species (Weber & Keeler, 2013), EFNs attract a wide range of ants and parasitoid wasp species that forage for nectar and deter herbivores (Heil & McKey, 2003). Of the 158 lineages of vascular plants (685 species) with ant domatia, only 14 have EFNs (Chomicki & Renner, 2015), and almost all of these form facultative symbioses because their nectaries can be exploited by numerous ant species without partner filtering (e.g. *Barteria nigritana* (Passifloraceae), Djiéto-Lordon *et al.*, 2004; *Humboldtia brunonis* (Fabaceae), Gaume *et al.*, 2005). Specialized ant–plant symbioses involving EFN rewards, however, limit opportunistic foraging through partner choice (Heil *et al.*, 2005; Federle & Rheindt, 2005; D. McKey, pers. comm. to G.C., May 2015).

Partner choice evolved with mutualism specialization

Our finding of the striking correlation between partner choice (concealed sugary rewards) and symbiosis specialization provides a strong argument of when partner choice is needed to stabilize a mutualism. It suggests that partner choice is necessary in specialized, coevolved mutualisms when costly trophic rewards are offered, and indirectly shows the strength of food competition from opportunists. In ant–plant symbioses, partner choice mechanisms (reviewed above) are always present in highly specialized mutualisms, all involving costly food rewards. The abundance and ubiquity of opportunists are thus unlikely to be balanced by mere partner fidelity feedback, requiring the evolution of a partner choice mechanism during the transition from facultative to obligate mutualisms.

Conclusion

Our study illustrates a novel partner choice mechanism that consists of post-anthetic sugar rewards and that evolved via a developmental shift in fruit development and nectary enlargement. The concealed sugar rewards appear to have played a central role in the transition from facultative to obligate mutualisms by increasing benefit trading whilst preventing partner exploitation. Both our experimental and comparative data for the nine Fijian species of *Squamellaria*, and our larger scale phylogenetic analysis of the Hydnophytinae, imply the correlated evolution of partner choice and mutualism specialization. Our study highlights that partner choice may be necessary to maintain mutualisms from exploitation by opportunists when mutualisms involve the trading of highly valuable 'goods' between the partners. This suggests that, in such specialized (coevolved) mutualisms, the selection pressure exerted by opportunists exceeds that exerted by cheaters.

Acknowledgements

We thank Jeremy Aroles for help with fieldwork and for proofreading of the manuscript, Alivereti Naikatini and Marika Tuiwawa (University of South Pacific, Suva) with whom all collections were made, Benoît Chomicki for help with the editing of Movie S1, Evans Economo for help with ant identification, Andreas Wistuba for cultivated Hydnophytinae samples, Martin Heil and three anonymous reviewers for their comments on the manuscript, and the German Research Foundation (RE 603/20), the Society of Systematic Biologists and the American Association of Plant Taxonomy for funding.

Author contributions

G.C. designed and planned the research; G.C. and Y.S. performed the research and analysed the data; G.C. and S.S.R. wrote the manuscript; all authors read and agreed the manuscript. S.S.R., G.C. and J.S. provided reagents.

References

Addicott FT. 1982. *Abscission.* Berkeley, CA, USA: University of California Press.

Archetti M, Scheuring I, Hoffman M, Frederickson ME, Pierce NE, Yu DW. 2011. Economic game theory for mutualism and cooperation. *Ecology Letters* 14: 1300–1312.

Axelrod R, Hamilton WD. 1981. The evolution of cooperation. *Science* 211: 1390–1396.

Barrabé L, Maggia L, Pillon Y, Rigault F, Mouly A, Davis AP, Buerki S. 2014. New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Molecular Phylogenetics and Evolution* 71: 15–35.

Bollback JP. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* 7: 88.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 104: e1003537.

Bronstein JL. 2001. The exploitation of mutualisms. *Ecology Letters* 4: 277–287.

Brouat C, Garcia N, Andary C, McKey D. 2001. Plant lock and ant key: pairwise coevolution of an exclusion filter in an ant–plant mutualism. *Proceedings of the Royal Society of London B: Biological Sciences* 268: 2131–2141.

Bull JJ, Rice WR. 1991. Distinguishing mechanisms for the evolution of co-operation. *Journal of Theoretical Biology* 149: 63–74.

Chomicki G, Renner SS. 2015. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist* 207: 411–424.

Chomicki G, Renner SS. 2016. Evolutionary relationships and history of the ant-epiphytic genus *Squamalleria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *PLoS ONE* 11: e0151317.

Chomicki G, Ward PS, Renner SS. 2015. Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. *Proceedings of the Royal Society of London B: Biological Sciences* **282**: 1819.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

Davidson DW, McKey D. 1993. The evolutionary ecology of symbiotic ant/plant relationships. *Journal of Hymenopteran Research* **2**: 13–83.

Djiéto-Lordon C, Dejean A, Gibernau M, Hossaert-McKey M, McKey D. 2004. Symbiotic mutualism with a community of opportunistic ants: protection, competition, and ant occupancy of the myrmecophyte *Barteria nigritana* (Passifloraceae). *Acta Oecologia* **26**: 109–116.

Edwards DP, Hassall M, Sutherland WJ, Douglas WY. 2006. Selection for protection in an ant–plant mutualism: host sanctions, host modularity, and the principal–agent game. *Proceedings of the Royal Society of London B: Biological Sciences* **273**: 595–602.

Emery C. 1909. Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. *Biologisches Zentralblatt* **29**: 352–362.

Erban A, Schauer N, Fernie AR, Kopka J. 2007. Nonsupervised construction and application of mass spectral and retention time index libraries from time-of-flight gas chromatography-mass spectrometry metabolite profiles. In: Weckwerth W, ed. *Methods in molecular biology vol. 358: Metabolomics-methods and protocols*. New York, NY, USA: Humana Press, 19–38.

Federle W, Maschwitz U, Fiala B, Riederer M, Hölldobler B. 1997. Slippery ant-plants and skilful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). *Oecologia* **112**: 217–224.

Federle W, Rheindt FE. 2005. *Macaranga* ant-plants hide food from intruders: correlation of food presentation and presence of wax barriers analysed using phylogenetically independent contrasts. *Biological Journal of the Linnean Society* **84**: 177–193.

Federle W, Rohrseitz K, Hölldobler B. 2000. Attachment forces of ants measured with a centrifuge: better 'wax-runners' have a poorer attachment to a smooth surface. *Journal of Experimental Biology* **203**: 505–512.

Foster KR, Wenseleers T. 2006. A general model for the evolution of mutualisms. *Journal of Evolutionary Biology* **19**: 1283–1293.

Frederickson ME. 2013. Rethinking mutualism stability: cheaters and the evolution of sanctions. *Quarterly Review of Biology* **88**: 269–295.

Frederickson ME, Greene MJ, Gordon DM. 2005. Ecology: 'Devil's gardens' bedevilled by ants. *Nature* **437**: 495–496.

Gaume L, McKey D. 1999. An ant–plant mutualism and its host-specific parasite: activity rhythms, young leaf patrolling, and effects on herbivores of two specialist plant-ants inhabiting the same myrmecophyte. *Oikos* **84**: 130–144.

Gaume L, Zacharias M, Grosbois V, Borges RM. 2005. The fitness consequences of bearing domatia and having the right ant partner: experiments with protective and non-protective ants in a semi-myrmecophyte. *Oecologia* **145**: 76–86.

Heil M, Barajas-Barron A, Orona-Tamayo D, Wielsch N, Svatos A. 2014. Partner manipulation stabilises a horizontally transmitted mutualism. *Ecology Letters* **17**: 185–192.

Heil M, McKey D. 2003. Protective ant–plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution and Systematics* **34**: 425–453.

Heil M, Rattke J, Boland W. 2005. Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* **308**: 560–563.

Huxley CR. 1978. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* **80**: 231–268.

Huxley CR. 1981. *Evolution and taxonomy of myrmecophytes with particular reference to* Myrmecodia *and* Hydnophytum *(Rubiaceae)*. DPhil thesis, University of Oxford, UK.

Izzo TJ, Vasconcelos HL. 2002. Cheating the cheater: domatia loss minimizes the effects of ant castration in an Amazonian ant-plant. *Oecologia* **133**: 200–205.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kautz S, Lumbsch HT, Ward PS, Heil M. 2009. How to prevent cheating: a digestive specialization ties mutualistic plant-ants to their ant-plant partners. *Evolution* **63**: 839–853.

Kiers ET, Rousseau RA, West SA, Denison RF. 2003. Host sanctions and the legume–rhizobium mutualism. *Nature* **425**: 78–81.

Kiers ET, Denison RF, Kawakita A, Herre EA. 2011. The biological reality of host sanctions and partner fidelity. *Proceedings of the National Academy of Sciences, USA* **108**: E7.

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR. 2006. Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nature Protocols* **1**: 387–396.

Litman JR, Praz CJ, Danforth BN, Griswold TL, Cardinal S. 2013. Origins, evolution, and diversification of cleptoparasitic lineages in long-tongued bees. *Evolution* **67**: 2982–2998.

Luedemann A, Strassburg K, Erban A, Kopka J. 2008. TagFinder for the quantitative analysis of gas chromatography-mass spectrometry (GC-MS)-based metabolite profiling experiments. *Bioinformatics* **24**: 732–737.

Maddison WP, Maddison DR. 2011. *Mesquite: a modular system for evolutionary analysis, version 2.75*. URL [WWW document] http://mesquiteproject.org [accessed 1 October 2015].

Orona-Tamayo D, Wielsch N, Blanco-Labra A, Svatos A, Farías-Rodríguez R, Heil M. 2013. Exclusive rewards in mutualisms: ant proteases and plant protease inhibitors create a lock–key system to protect *Acacia* food bodies from exploitation. *Molecular Ecology* **22**: 4087–4100.

Pagel M, Meade A. 2014. *BayesTraits, version 2*. Berkshire, UK: University of Reading. http://www.evolution.rdg.ac.uk.

Popp M, Lied W, Meyer AJ, Richter A, Schiller P, Schwitte H. 1996. Sample preservation for determination of organic compounds: microwave versus freeze-drying. *Journal of Experimental Botany* **47**: 1469–1473.

R Core Team 2015. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rambaut A, Drummond AJ. 2007. *Tracer – MCMC trace analysis tool version v1.5*. URL [WWW document] http://beast.bio.ed.ac.uk. [accessed 1 October 2015].

Renner SS, Ricklefs RE. 1998. Herbicidal activity of domatia-inhabiting ants in patches of *Tococa guianensis* and *Clidemia heterophylla*. *Biotropica* **30**: 324–327.

Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Roessner U, Luedemann A, Brust D, Fiehn O, Linke T, Willmitzer L, Fernie AR. 2001. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* **13**: 11–29.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Sachs JL, Mueller UG, Wilcox TP, Bull JJ. 2004. The evolution of cooperation. *Quarterly Review of Biology* **79**: 135–160.

Sachs JL, Simms EL. 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution* **21**: 585–592.

Sarnat EM. 2009. *The ants [Hymenoptera: Formicidae] of Fiji: systematics, biogeography and conservation of an island arc fauna*. PhD dissertation, University of California, Davis, CA, USA.

Sarnat EM, Economo EP. 2012. *The ants of Fiji (vol 132)*. Berkeley, CA, USA: University of California Press.

Schaefer H, Renner SS. 2008. A phylogeny of the oil bee tribe Ctenoplectrini (Hymenoptera: Anthophila) based on mitochondrial and nuclear data: evidence for Early Eocene divergence and repeated out-of-Africa dispersal. *Molecular Phylogenetics and Evolution* **47**: 799–811.

Staedler YM, Masson D, Schönenberger J. 2013. Plant tissues in 3D via X-ray tomography: simple contrasting methods allow high resolution imaging. *PLoS ONE* **8**: e75295.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**: 758–771.

Trivers RL. 1971. The evolution of reciprocal altruism. *Quarterly Review of Biology* **46**: 35–57.

Ward PS, Blaimer BB, Fisher BL. 2016. A revised phylogenetic classification of the ant subfamily Formicinae (Hymenoptera: Formicidae), with resurrection of the genera *Colobopsis* and *Dinomyrmex*. *Zootaxa* **4072**: 343–357.

Weber MG, Keeler KH. 2013. The phylogenetic distribution of extrafloral nectaries in plants. *Annals of Botany* **111**: 1251–1261.

West SA, Kiers ET, Pen I, Denison RF. 2002. Sanctions and mutualism stability: when should less beneficial mutualists be tolerated? *Journal of Evolutionary Biology* **15**: 830–837.

Weyl EG, Frederickson ME, Douglas WY, Pierce NE. 2010. Economic contract theory tests models of mutualism. *Proceedings of the National Academy of Sciences, USA* **107**: 15712–15716.

Wyatt GA, Kiers ET, Gardner A, West SA. 2016. Restricting mutualistic partners to enforce trade reliance. *Nature Communications* **7**: 10332.

Yu DW. 2001. Parasites of mutualisms. *Biological Journal of the Linnean Society* **72**: 529–546.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1 Computed tomography (CT) scanning images showing the developmental stages of *Squamellaria grayi* nectary.

Fig. S2 Vascular system of nectaries visualized with computed tomography (CT) scanning data.

Fig. S3 Dated phylogeny of the Hydnophytinae.

Table S1 Full list of metabolites of *Squamellaria* nectaries from metabolomic analyses (see the Materials and Methods section)

Table S2 Primers used in this study

Table S3 Plant material included in this study with authors of species names, vouchers and their geographical origin and GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp)

Table S4 Scanning conditions for micro-computed tomography

Movie S1 *Philidris nagasau* foraging on post-anthetic nectaries of *Squamellaria wilsonii*, Taveuni, DesVoeux peak track.

New Phytologist Supporting Information

Article title: Partner choice through concealed floral sugar rewards evolved with the specialization of ant/plant mutualisms

Authors: Guillaume Chomicki, Yannick Staedler, Jürg Schönenberger, Susanne S. Renner

Article acceptance date: 25 March 2016

The following Supporting Information is available for this article:

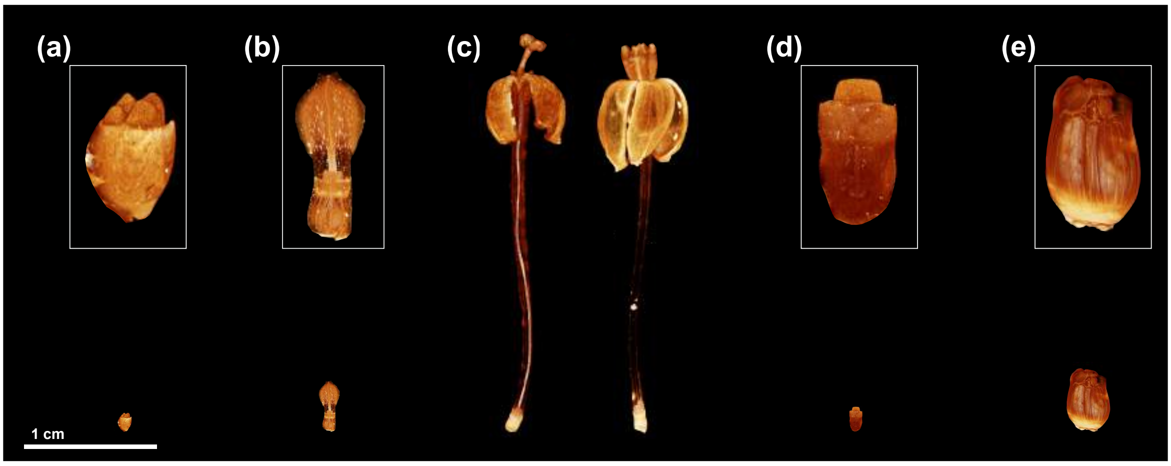


Figure S1. Developmental stages in *Squamellaria grayi* nectary. (a) Early bud. (b) Late bud. (c) Male and female flowers at anthesis. (d) Post-anthesis nectary. (e) fruit.

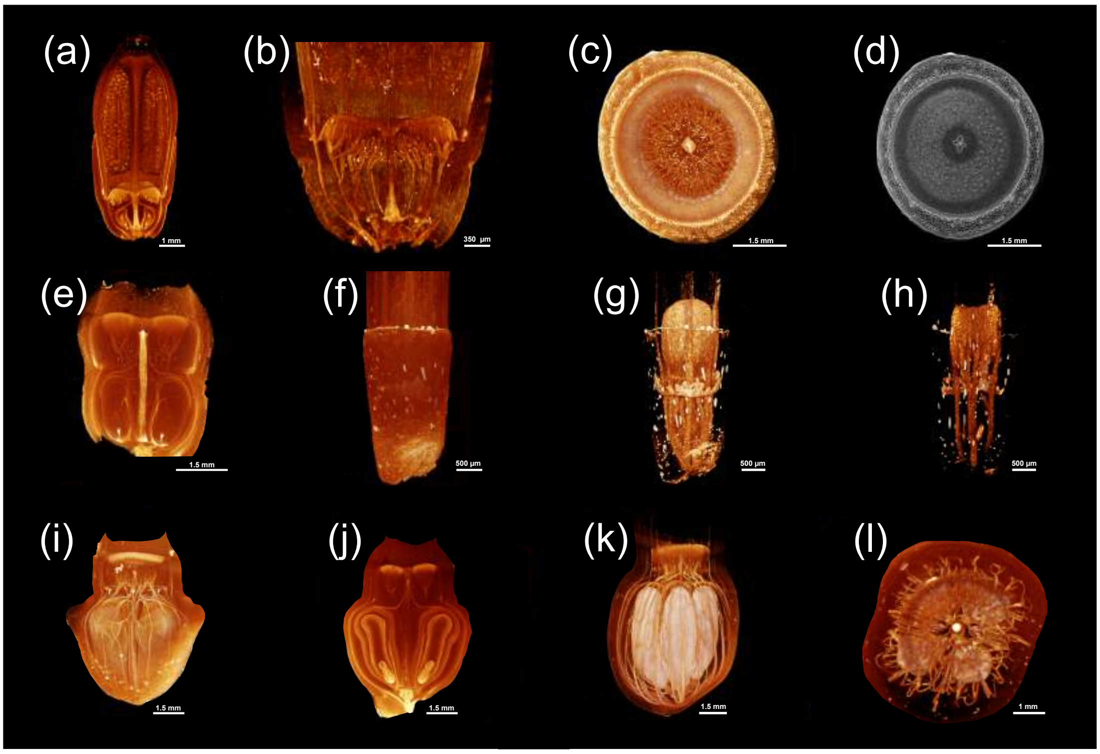


Figure S2. Vascular system of nectaries. (**a-b**). Well-established vascular system in nectaries of *Squamellaria imberbis* buds, 3D longitudinal section. (**c-e**) *S. major*. (**c**) 3D cross-section through a mature nectary reveals an intense vascular system. (**d**) 2D cross-section of the same specimen. (**e**) 3D longitudinal section of a mature nectary reveals intense vascular branching towards the exposed side of the nectary. (**f-h**) *Hydnophytum tenuiflorum* calyx (**f**), nectary (**g**) and nectary vascular system (**h**) showing the limited vascular system as compared to *Squamellaria*. (**i-l**) Maintenance of the nectary structure during fruit development. (**i**) *S. huxleyi*. (**j**) *S. thekii*. (**k-l**) *S. major*. (**k**) side view. (**l**) Top view showing the nectary vasculature.

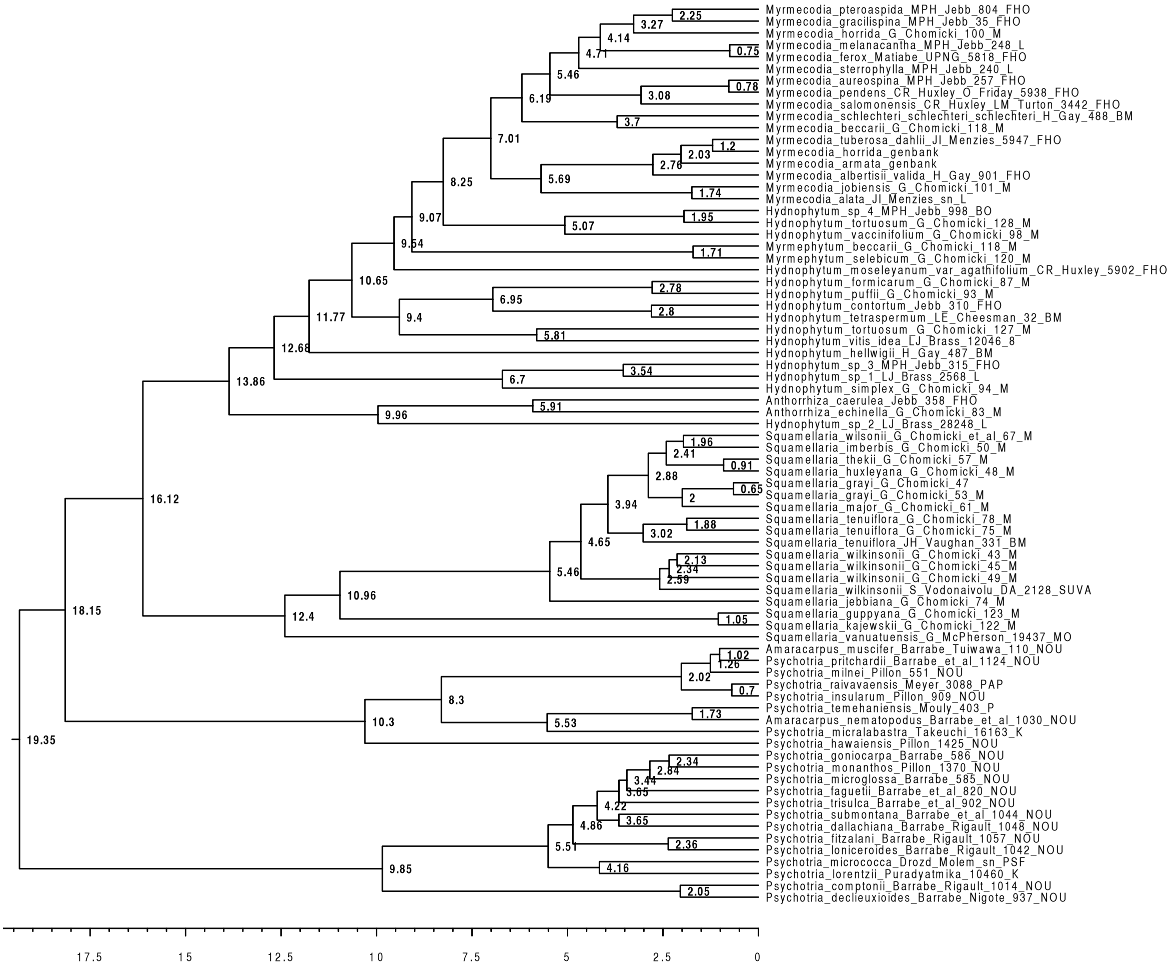


Figure S3. Dated phylogeny for the Hydnophytinae.

Table S2. Primers used in this study.

Marker	Forward	Reverse	References
ITS region	ITS1: TCCGTAGGTGAACCTGCGG	ITS4: TCCTCCGCTTATTGATATGC	White et al. (1990)
trnL-trnF region	C (Fw): CGAAATCGGTAGACGCTACG E (Fw): GGTTCAAGTCCCTCTATCCC	D (Rev): GGGGATAGAGGGACTTGAAC F (Rev): ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)
ETS	Psy1: GTGTGAGTGGTAAATGGATAGC[1]	18S-ETS: ACTTACACATGCATGGCTTAATCT[2]	Nepokroeff et al. (2003); Baldwin et al. (1998)
ndhF	ndhF 1F: ATGGAACAKACATATSAATATGCTGG	ndhF 972R: CATAATATAACCCAATTGAGAC	Olmstead et al. (1994)
Rps16	rpsF: GTGGTAGAAAGCAACGTGCGACTT	rpsR2: TCGGGATCGAACATCAATTGCAAC	Oxelman et al. (1997)
trnS-trnG	trnG(UUC): GAATCGAACCCGCATCGTTAG	trnS(GCU): AACTCGTACAACGGATTAGCAATC	Shaw et al. (2007)
rps12-rpl20	5'rpS12: ATTAGAAANRCAAGACAGCCAAT	rpL20: CGY YAY CGA GCT ATA TAT CC	Shaw et al. (2005)
18S	NS1: GTAGTCATATGCTTGTCTC	NS2: GGCTGCTGGCACCAGACTTGC	White et al. (1990)

Supplementary references

Baldwin BG, Markos S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of Calycadenia (Compositae). *Molecular Phylogenetics and Evolution* **10**: 449–463.

Nepokroeff M, Systma KJ, Wagner WL, Zimmer EA. 2003. Reconstructing ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. *Systematic Biology* **52**: 820–838.

Olmstead RG, Sweere JA. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Systematic Biology **43**: 467–481.

Oxelman B, Lidén M, Berglund D. 1997. Chloroplast rps16 intron phylogeny of the Sileneae (Caryophyllaceae). Pl Syst Evol 206: 393–410.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142-166.

Shaw J, Lickey EB, Schilling EE, Small RL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* **94**: 275-288.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105-1109.

White T, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR-protocols a guide to methods and applications Edited by: Innis MA, Gelfand DH, Sninski JJ, White TJ. San Diego: Academic press; pp. 315-322.

Table S3. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	Voucher	Geographic origin	ITS	ETS	18S	trnL intron	trnL-trnF spacer	ndhF	trnS-trnG	rps16	rps12-rpl20
***Amaracarpus muscifer* A.C.Sm.**	L. Barrabé & M. Tuiwawa 1109 (NOU)	Fiji	KF675907	KF675790	-	-	-	KF675995	-	KF676083	-
***Amaracarpus nematopodus* (F.Muell.) P.I.Forst.**	L. Barrabé et al. 1030 (NOU)	Australia	JX155060	KF675791	-	-	-	JX155105	-	JX155152	-
***Anthorrhiza caerulea* Huxley & Jebb**	M.P.H. Jebb 358 (FHO)	Papua New Guinea	KU586349	KU586368	-	-	-	-	-	-	-
Anthorrhiza echinella	G. Chomicki 83 (M)	Cultivated Oxf. Bot Gard., origin New Guinea	KU586350	KU586369	-	-	-	-	-	-	-
***Hedstromia latifolia* A.C.Sm.**	L. Barrabé et al 1090 (NOU)	Fiji	KF675911	KF675795	-	-	-	KF675999	-	KF676087	-
***Hydnophytum formicarum* Jack**	G. Chomicki 87 (M)	Cultivated, origin Malaysian region	KU586346	KU586365	-	-	-	KU586397	-	-	-
***Hydnophytum simplex* Becc.**	G. Chomicki 94 (M)	Cultivated, origin Aru Island, Papua New Guinea	KU963311	KU963332	-	KU963350	KU963362	KU963377	-	-	-
Hydnophytum sp. 1 (=*H. dentrecastense* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)	L.J. Brass 2568 (L)	Papua New Guinea	KU963312	-	-	-	-	-	-	-	-
Hydnophytum sp. 2 (=*H. orichalcum* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)	L.J. Brass 28248 (L)	Papua New Guinea	KU963313	-	-	-	-	-	-	-	-
Hydnophytum sp. 3 (=*H. terrestris* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)	M.P.H. Jebb 315 (FHO)	Papua New Guinea	KU963314	-	-	-	KU963376	-	-	-	-

Hydnophytum hellwigii **Warb.**	H. Gay 487 (BM)	Papua New Guinea	KU963315	KU963336	-	-	KU963374	-	-	-	-
Hydnophytum vitis-idaea **Merr. & L.M.Perry**	L.J. Brass 12046 (BM)	Papua New Guinea	KU963316	KU963337	-	KU963351	KU963363	-	-	-	-
Hydnophytum tortuosum **Becc.**	G. Chomicki 128 (M)	Cultivated, origin Papua	KU963317	-	-	KU963353	KU963365	KU963380	-	-	-
Hydnophytum **sp. 5**	G. Chomicki 127 (M)	Cultivated, origin Papua	KU963318	KU963339	-	KU963352	KU963364	KU963379	-	-	-
Hydnophytum tetrapterum **Becc.**	L.E. Cheesman 32 (BM)	Papua New Guinea	KU963319	KU963340	-	-	-	-	-	-	-
Hydnophytum contortum **Merr. & L. M. Perry**	M.P.H Jebb 310 (FHO)	Papua New Guinea	KU963321	KU963342	-	-	KU963375	-	-	-	-
Hydnophytum puffii **Low, Sugau & Wong**	G. Chomicki 93 (M)	Cultivated, origin Borneo	KU963322	KU963343	-	KU963354	KU963366	KU963381	-	-	-
Hydnophytum moseleyanum **Becc. *'agathifolium'***	C.R. Huxley 5902 (FHO)	Papua New Guinea	KU963323	KU963344	-	KU963355	KU963367	-	-	-	-
Hydnophytum vaccinifolium **P.Royen**	G. Chomicki 98 (M)	Cultivated, origin Papua	KU963324	KU963345	-	KU963356	KU963368	KU963382	-	-	-
Myrmecodia beccarii **Hook f.**	G. Chomicki 99 (M)	Cultivated, origin Australia	KU586347	KU586366	-	-	-	KU586398	-	-	-
Myrmecodia salomonensis **Becc.**	C. R. Huxley and L. M. Turton 3442 (FHO)	Solomons	KU586351	KU586370	-	-	-	-	-	-	-
Myrmecodia dahlii **K.Schum.**	J.I. Menzies 5947 (FHO)	Papua New Guinea	KU586348	KU586367	-	KU963357	KU963369	KU586399	-	-	-
Myrmecodia alata **Becc.**	J.I. Menzies s.n. (L)	Papua	-	-	-	-	-	-	-	-	-
Myrmecodia jobiensis **Becc.**	G. Chomicki 101 (M)	Cultivated, origin Papua	KU963326	KU963347	-	KU963358	KU963370	KU963384	-	-	-
Myrmecodia albertisii **Becc. subsp. *valida* C.R.Huxley & Jebb**	H.J. Gay 901 (FHO)	Papua New Guinea	-	KU963327	KU963348	-	-	-	-	-	-
Myrmecodia tuberosa **Jack *'armata'* C.R. Huxley & Jebb**	unvouchered	Papua New Guinea	AF034917	-	-	-	-	-	-	-	-
Myrmecodia schlechteri **subsp. *schlechteri*** **var. *schlechteri*** **C.R.Huxley & Jebb**	H. J. Gay 488 (FHO)	Papua New Guinea	AF071988	-	-	JN643394	JN643394	-	-	-	-

***Myrmecodia pendens* Merr. & L.M.Perr.**	C.R. Huxley and J. Friday 5938 (FHO)	Papua New Guinea	-	KU963328	KU963349	-	-	-	-	-	-
***Myrmecodia aureospina* C.R.Huxley & Jebb**	M.P.H. Jebb 257 (FHO)	Papua New Guinea	-	KU963335	-	-	-	-	-	-	-
***Myrmecodia sterrophylla* Merr. & L.M. Perry**	M.P.H. Jebb 240 (L)	Papua New Guinea	KU963330	-	-	-	-	-	-	-	-
***Myrmecodia ferox* C.R.Huxley & Jebb**	C.R. Huxley & Matiabe UPNG 5818 (FHO)	Papua New Guinea	-	KU963334	-	-	-	-	-	-	-
***Myrmecodia melanacantha* C.R.Huxley & Jebb**	M.P.H. Jebb 240 (L)	Papua New Guinea	KU963331	-	-	-	-	-	-	-	-
***Myrmecodia horrida* C.R.Huxley & Jebb**	G. Chomicki 100 (M)	Cultivated, origin Papua New Guinea	KU963329	KU963338	-	KU963359	KU963371	KU963385	-	-	-
***Myrmecodia gracilispina* C.R.Huxley & Jebb**	M.P.H. Jebb 35 (FHO)	Papua New Guinea	-	KU963333	-	-	-	-	-	-	-
***Myrmecodia pteroaspida* C.R.Huxley & Jebb**	M.P.H. Jebb 804 (FHO)	Papua New Guinea	KU963325	KU963346	-	-	-	-	-	-	-
***Myrmephytum selebicum* (Becc.) Becc.**	G. Chomicki 120 (M)	Cultivated, origin Papua	KU963320	KU963341	-	KU963360	KU963372	KU963386	-	-	-
***Myrmephytum beccarii* Elmer**	G. Chomicki 118 (M)	Cultivated, origin Philippines	KU586353	KU586354	-	KU963361	KU963373	KU586401	-	-	-
***Psychotria comptonii* S.Moore**	L. Barrabé & Rigault 1014 (NOU)	New Caledonia	KF675927	KF675823	-	-	-	KF676015	-	KF676104	-
***Psychotria dallachiana* Benth.**	L. Barrabé & Rigault 1048 (NOU)	Australia	KF675928	KF675824	-	-	-	KF676016	-	KF676169	-
***Psychotria declieuxioides* S.Moore**	L. Barrabé & Nigote 937 (NOU)	New Caledonia	KF675932	KF675828	-	-	-	KF676020	-	KF676107	-
***Psychotria faguetii* (Baill.) Schltr.**	L. Barrabé et al. 820 (NOU)	New Caledonia	KF675934	KF675831	-	-	-	KF676023	-	-	-
***Psychotria fitzalanii* Benth.**	L. Barrabé & Rigault 1057 (NOU)	Australia	KF675935	KF675832	-	-	-	KF676024	-	KF676110	-
***Psychotria goniocarpa* (Baill.) Guillaumin**	L. Barrabé 586 (NOU)	New Caledonia	KF675940	KF675838	-	-	-	KF676029	-	KF676115	-
***Psychotria hawaiiensis* (A.Gray) Fosberg**	Y. Pillon 1425 (NOU)	Hawaii	KF675941	KF675840	-	-	-	KF676030	-	KF676116	-

***Psychotria hivaoana* Fosberg**	Meyer 3071 (PAP)	French Polynesia	KF675942	KF675841	-	-	-	KF676031	-	KF676117	-
***Psychotria insularum* A.Gray**	Y. Pillon 909 (NOU)	Wallis & Futuna	KF675943	KF675842	-	-	-	KF676032	-	KF676118	-
***Psychotria iteophylla* Stapf**	Axelius 303 (S)	Borneo	-	-	-	-	-	-	-	AF410726	-
***Psychotria loniceroides* Sieber ex DC.**	L. Barrabé & Rigault 1042 (NOU)	Australia	KF675945	KF675846	-	-	-	KF676033	-	KF676120	-
***Psychotria lorentzii* Valeton**	Puradyatmika 10460 (K)	Papua New Guinea	KF675946	KF675847	-	-	-	KF676034	-	KF676121	-
***Psychotria micralabastra* (Lauterb. & K.Schum.) Valeton**	Takeuchi 16163 (K)	Papua New Guinea	KF675949	KF675851	-	-	-	KF676036	-	KF676124	-
***Psychotria micrococca* (Lauterb. & K.Schum.) Valeton**	Drozd & Molem s.n. (PSF)	Papua New Guinea	KF675951	KF675853	-	-	-	KF676038	-	KF676126	-
***Psychotria microglossa* (Baill.) Baill. ex Guillaumin**	L. Barrabé 585 (NOU)	New Guinea	KF675950	KF675852	-	-	-	KF676037	-	KF676125	-
***Psychotria monanthos* (Baill.) Schltr.**	Y. Pillon 1370 (NOU)	New Caledonia	KF675953	KF675855	-	-	-	KF676040	-	KF676128	-
***Psychotria poissoniana* (Baill.) Guillaumin**	J. Munzinger 5156 (NOU)	New Caledonia	KF675958	KF675861	-	-	-	KF676045	-	KF676133	-
***Psychotria pritchardii* Seem.**	L. Barrabé et al 1124 (NOU)	Fiji	KF675992	KF675903	-	-	-	KF676078	-	KF676165	-
***Psychotria raivavaensis* Fosberg**	Meyer 3088 (PAP)	French Polynesia	KF675960	-	-	-	-	KF676047	-	KF676135	-
***Psychotria submontana* Domin**	L. Barrabé et al. 1044 (NOU)	Australia	KF675988	KF675899	-	-	-	-	-	KF676168	-
***Psychotria temehaniensis* J.W.Moore**	Mouly 403 (P)	French Polynesia	KF675989	KF675900	-	-	-	KF676075	-	KF676162	-
***Psychotria trisulcata* (Baill.) Guillaumin**	L. Barrabé et al. 902 (NOU)	New Caledonia	KF675990	KF675901	-	-	-	KF676076	-	KF676163	-
***Squamellaria grandiflora* (Becc.) Chomicki, comb. nov.**	S. Vodonaivolu DA2128 (SUVA)	Fiji, Kadavu Island	-	KU963388	-	-	-	-	-	-	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (SUVA) HOLOTYPE	Taveuni, Bouma falls, Lavena	KU586339	KU586358	KU586427	KU586376	KU586376	KU586388	KU586406	KU586436	KU586417

Squamellaria grayi Chomicki & Wistuba sp. nov.	G. Chomicki, J. Aroles, A. Naikatini 47 (M)	Vanua Levu, Waisali forest park	-	-	-	KU586372	KU586372	-	KU586402	KU586432	KU586413
Squamellaria guppyana (Becc.) Chomicki, comb. nov.	G. Chomicki 123 (M)	Cultivated, origin Solomons	KU586345	-	-	-	-	KU586396	-	-	-
Squamellaria huxleyana Chomicki sp. nov.	G. Chomicki, J. Aroles, A. Naikatini 48 (SUVA)	Fiji, Vanua Levu, road between Savusavu to Labasa.	KU586336	KU586355	KU586425	KU586373	KU586373	KU586385	KU586403	KU586433	KU586414
Squamellaria imberbis (A. Gray) Becc.	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	Fiji, Vanua Levu, track to vodaphone tower.	KU586337	KU586356	-	KU586374	KU586374	KU586386	KU586404	KU586434	KU586415
Squamellaria jebbiana Chomicki, sp. nov.	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	Fiji, Taveuni, Mt Manuca area.	KU586342	KU586361	-	KU586379	KU586379	KU586391	KU586408	KU586438	KU586419
Squamellaria kajewskii (Merr. & L.M.Perry) Chomicki, comb. nov.	G. Chomicki 122 (M)	Cultivated, origin Solomons	KU586335	-	-	-	-	KU586384	-	-	-
Squamellaria major A.C. Sm.	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586338	KU586357	KU586426	KU586375	KU586375	KU586387	KU586405	KU586435	KU586416
Squamellaria tenuiflora (Becc.) Chomicki, comb. nov.	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	-	-	KU586430	KU586381	KU586381	KU586393	KU586410	KU586440	KU586421
Squamellaria tenuiflora (Becc.) Chomicki, comb. nov.	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	KU586343	KU586362	KU586431	KU586382	KU586382	KU586394	KU586411	-	KU586422
Squamellaria thekii Jebb	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586340	KU586359	KU586428	KU586377	KU586377	KU586389	KU586407	KU586437	KU586418
Squamellaria vanuatuensis (Jebb & Huxley) Chomicki, comb. nov.	McPherson 19437 (P)	Vanuatu	JX155078	-	-	-	-	-	-	JX155170	-
Squamellaria wilkinsonii (Horne ex Baker) Chomicki, comb. nov.	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	Fiji, Vanua Levu, Waisali forest park.	-	-	KU586429	KU586380	KU586380	KU586392	KU586409	KU586439	KU586420
Squamellaria wilkinsonii (Horne ex Baker) Chomicki, comb. nov.	G. Chomicki, J. Aroles, A. Naikatini 49 (M)	Fiji, Vanua Levu, Waisali forest park.	-	KU586364	-	-		-	-	-	-
Squamellaria wilkinsonii (Horne ex Baker) Chomicki, comb. nov.	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	Fiji, Vanua Levu, Waisali forest park.	KU586344	KU586363	-	KU586383		KU586395	KU586412	KU586441	KU586423
Squamellaria wilsonii (Horne ex Baker) Becc.	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586341	KU586360	-	KU586378		KU586390	-	-	KU586424

Table S4. Scanning conditions for micro-computed tomography.

Illustration	Species	Link to herbarium voucher	Stage	Acceleration voltage [kV]	Source current [µA]	Exposure time [s]	Pictures per sample	Camera binning	Optical magnification	Pixel size
Fig. 6a (i, upper panel)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	early bud	30	200	4	728	2	10	1,9
Fig. 6a (ii, upper panel)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	late bud	30	200	1	728	2	4	4
Fig. 6a (iii, upper panel)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	anthetic flower male	30	200	8,5	728	2	1	14,7
Fig. 6a (iv, upper panel)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	Post-anthetic flower	25	200	6	728	2	10	2
Fig. 6a (v, upper panel)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	late fruit	25	200	4	728	2	4	4,65
Fig. 6a (i, lower panel)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	early bud	30	200	4	728	2	4	3,2
Fig. 6a (ii, lower panel)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	late bud	33	181	2	728	2	4	5,1
Fig. 6a (iii, lower panel)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	anthetic flower (bisexual)	30	200	39	728	2	1	17,3
Fig. 6a (iv, lower panel)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	Post-anthethic flower	30	200	20	728	2	1	10,3
Fig. 6a (v, lower panel)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	late fruit	30	200	9,5	728	2	1	14,9
Fig. S1 (a)	*Squamellaria grayi* Chomicki & Wistuba	G. Chomicki, J. Aroles and A. Naikatini 53 (M)	early bud	30	20	2	728	2	4	3
Fig. S1 (b)	*Squamellaria grayi* Chomicki & Wistuba	G. Chomicki, J. Aroles and A. Naikatini 53 (M)	late bud	25	200	3	728	2	4	4,8
Fig. S1 (c, right)	*Squamellaria grayi* Chomicki & Wistuba	G. Chomicki, J. Aroles and A. Naikatini 53 (M)	anthetic flower male	27	196	10	728	4	1	33,7
Fig. S1 (c, left)	*Squamellaria grayi* Chomicki & Wistuba	G. Chomicki, J. Aroles and A. Naikatini 53 (M)	anthetic flower female	27	200	10	728	4	1	33,4
Fig. S1 (d)	*Squamellaria grayi* Chomicki & Wistuba	G. Chomicki, J. Aroles and A. Naikatini 53 (M)	Post-anthetic flower	30	20	2	728	2	4	3
Fig. S1 (e)	*Squamellaria grayi* Chomicki & Wistuba	G. Chomicki, J. Aroles and A. Naikatini 53 (M)	late fruit	40	200	4	728	2	1	18,1
Fig. S2 (a)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	Late bud	33	181	2	728	2	4	5,1
Fig. S2 (b)	*Squamellaria imberbis* (A. Gray) Becc.	G. Chomicki, J. Aroles and A. Naikatini 50 (M)	Late bud	33	181	2	728	2	4	5,1
Fig. S2 (c)	*Squamellaria major* A.C. Sm.	G. Chomicki, J. Aroles and A. Naikatini 61 (M)	Post-anthetic flower	40	200	1	728	2	4	5
Fig. S2 (d)	*Squamellaria major* A.C. Sm.	G. Chomicki, J. Aroles and A. Naikatini 61 (M)	Post-anthetic flower	40	200	1	728	2	4	5
Fig. S2 (e)	*Squamellaria major* A.C. Sm.	G. Chomicki, J. Aroles and A. Naikatini 61 (M)	Post-anthetic flower	40	200	1	728	2	4	5
Fig. S2 (f)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	Anthetic flower	35	200	2	728	2	4	5,1
Fig. S2 (g)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	Anthetic flower	35	200	2	728	2	4	5,1
Fig. S2 (h)	*Squamellaria tenuiflora* (Becc.) Chomicki	G. Chomicki, J. Aroles and A. Naikatini 75 (M)	Anthetic flower	35	200	2	728	2	4	5,1
Fig. S2 (i)	*Squamellaria huxleyi* Chomicki	G. Chomicki, J. Aroles and A. Naikatini 48 (M)	Fruit	50	160	2	728	2	1	17,8
Fig. S2 (j)	*Squamellaria thekii* Jebb	G. Chomicki, J. Aroles and A. Naikatini 57 (M)	Fruit	50	160	2	728	2	1	17,8
Fig. S2 (k)	*Squamellaria major* A.C. Sm.	G. Chomicki, J. Aroles and A. Naikatini 61 (M)	Fruit	50	160	4	728	2	1	19,6

Video/Movie S1 *Philidris nagasau* foraging on post-anthetic nectaries of *Squamellaria wilsonii*, Taveuni, DesVoeux peak track.

| Chapter 5 |

Active and exclusive planting and fertilization by ants of their hosts' offspring

Guillaume Chomicki and Susanne S. Renner

Nature Plants
Accepted pending revisions

Manuscript submitted to *Nature Plants* – Brief communication

Title: **Active and exclusive planting and fertilization by ants of their plant hosts' offspring**

Authors: Guillaume Chomicki[*]and Susanne S. Renner

Affiliation:
Department of Biology, Systematic Botany and Mycology, Menzinger Str. 67,
University of Munich (LMU), Munich 80638

*Correspondence to: guillaume.chomicki@gmail.com

Abstract: **[67 words]**
Associating with ants has been a convergent strategy to gain nutrients in epiphytic plants. Here, we report a novel type of ant/plant symbiosis in Fiji where one ant species actively and exclusively plants the seeds and fertilizes the seedlings of six species of *Squamellaria* (Rubiaceae). Comparison with related facultative ant-plants suggests that such farming plays a key role in mutualism stability by mitigating the critical re-establishment step.

Main text [1304 words].

Ant/plant symbioses involve plants with preformed cavities (domatia) that house ants in return for protection or extra nutrients[1]. Typically, they need to be re-established at each generation. Longer living symbioses are found in ant gardens, associations of epiphytes planted by ant workers inside carton nests[2]. Different from terrestrial ant/plant symbioses that are mostly defense mutualisms, epiphytic ant-plants usually are nutritional mutualisms[1,3]. Such trophic mutualisms, which involve ants that provide detritus or feces to their host plants' domatia, have evolved many times[4-6], probably because epiphytes are usually more nutrient-stressed than ground-living plants, especially during seedling establishment[7].

We focused on a clade of domatium-bearing epiphytes in the genus *Squamellaria* (Rubiaceae), involving nine epiphytic species in Fiji[8]. All inspected domatia of a clade of six species (*S. grayi, S. huxleyana*, *S. imberbis*, *S. major*, *S. thekii*, and *S. wilsonii*; in total 337 individuals) were occupied by workers or colonies of *Philidris nagasau* (Dolichoderinae)*,* while only 70-80% of the domatia of *S. jebbiana*, *S. tenuiflora*, and *S. wilkinsonii* were ant-occupied, all by generalist ant species that can also nest outside plants (Fig. 1a). Specialized *Squamellaria* offer housing to *P. nagasau* and five of them also offer nutritious sugar rewards[9]. A phylogenetic framework indicates that facultative mutualism with opportunistic ants is the ancestral condition, while obligate symbioses with *P. nagasau* arose in the common ancestor of the six species (Fig. 1a).

To test whether any *Squamellaria* species are ant-dispersed, we first recorded the spatial distribution of *P. nagasau*-inhabited *Squamellaria*, which was >3 times more clustered than that of the non-*P. nagasau*-inhabited species (Fig. 1b, Fig. S1; t-tests, all $P<0.001$), pointing to a difference in seed dispersal or establishment. *Philidris nagasau*-inhabited plant clusters sometimes comprised over 25 individuals (Fig. 1b), all linked by ant trails (i.e., the ant species is polydomous, with the offspring of one queen living in several domatia), sometimes spanning across several trees with touching branches. In contrast, all the generalist ant species nesting in *S. jebbiana*, *S. tenuiflora*, and *S. wilkinsonii* are monodomous (the queen and all her offspring live in a single nest). The pattern of trails linking was centralized towards the queen-bearing domatium and distance appeared important in determining network structure (Fig. S2; *Materials and Methods*).

We observed that *P. nagasau* inserted the seeds of its plant hosts in cracks in tree bark (Fig. 1f, g) and that workers constantly patrol the planting sites. The seedlings have a unique morphology that involves an elongation of the hypocotyl (hypocotyl foot) prior to domatium formation (Fig. 1c-e). This type of seedling growth evolved in the *P. nagasau*-inhabited clade (Fig. 1a), but is absent in 'facultative' (i.e., occupied by generalist ants) *Squamellaria* species (Fig. 1a,e) and is, as far as we know, unique in Hydnophytinae. This strongly suggests trait coevolution whereby the seedlings first escape their sunken germination sites thanks to the elongated hypocotyl foot (Fig. 1c,d) and then develop the globose domatia outside tree bark.

To test whether *P. nagasau* can recognize its hosts' seeds and prefers them over seeds of the closely related facultative *Squamellaria* species, we conducted a cafeteria experiment, which showed that when ants face a choice between the seeds of an obligate and of a facultative species of *Squamellaria* (here, *S. huxleyana* vs. *S. wilkinsonii*; Fig. 1h), they only collect the seeds of the specialized species (GLM, $X^2_{1,13}= 6.69$, $P < 0.01$). In Neotropical ant gardens, the seeds of *Peperomia* *macrostachya* are recognized by *Camponotus femoratus* via chemical cues[10], but this ant species also plants other unrelated epiphytes[3]. The absence of fatty rewards on the seeds (elaiosomes) in *Squamellaria* combined with the results of our seed removal assays points to some chemical cue with stringent specificity (limited to six *Squamellaria* species).

We tested for ant versus bird dispersal in *Squamellaria* by exclusion experiments that prevented either birds (bag only) or ants and birds (Vaseline and bag) from accessing fruits and seeds (*Material and Methods*). Bird exclusion (bag only) did not affect fruit removal of *P. nagasau*-inhabited *Squamellaria* (*S. huxleyana*), while the treatment that excluded ants and birds (Vaseline) decreased it significantly (Fig. 1i; GLM, $X^2_{1,17} = 13.32$, $P < 0.001$). Exclusion experiments in one of the non-*P. nagasau*-inhabited species (*S. wilkinsonii*) yielded no differences between the two treatments (control vs. Vaseline or bag: GLM, $X^2_{1,17}=7.23$, $P < 0.01$; Vaseline vs. bag: n.s. (color-changing fruits) and n.s. (mature fruits); Fig. 1j). These data confirm that *P. nagasau*-inhabited (i.e., specialized) *Squamellaria* species are dispersed by their ant symbionts, while *Squamellaria* species occupied by unspecialized (only facultatively plant nesting) ant species are bird-dispersed.

Typically, ant-garden ants plant seeds inside carton nest[2,11]. The puzzling lack of carton nest building in the *P. nagasau*/*Squamellaria* symbioses led us to investigate the life history of *Philidris* ants. We generated a clock-dated phylogeny for this group and reconstructed ancestral states for carton nesting, other nesting habits, and seed dispersal behaviour (Fig. 2, Figs. S5-S7). Our phylogeny and state reconstructions show that the carton nest making has been lost in *P. nagasau*, while the remaining *Philidris* species all make carton nests. Although many ground-dwelling and arboreal dolichoderines disperse seeds, it appears that only *P. nagasau* plants just *Squamellaria* seeds (Fig. 2, Fig. S7).

We observed that each *Squamellaria* seedling was transiently entered by *P. nagasau* workers as soon as it had formed its first cavity, which occurs in ~2 cm-tall seedlings (Fig. 1f, upper inset); typically, 3-10 workers coming from the queen-bearing domatium were constantly shuttling in and out of the tiny seedling domatium. Our ^{15}N labelling experiment (*Materials and Methods*) revealed a ca. 300-fold increase in $\delta\,^{15}$N as compared to the control (285.93 ± 133.32 vs. 0.32 ± 0.59). Taken together, these findings demonstrate that the workers fertilize the seedlings by actively defecating into their tiny domatia.

The difference in seed dispersal mechanisms between facultative and obligate *Squamellaria* species raises the question whether they vary in the range of host trees on which they grow. We addressed this with a forest transect where *S. imberbis* (only and always inhabited by *P. nagasau*) and *S. wilkinsonii* (inhabited by generalist ants) grow sympatrically (Table S1). *Squamellaria imberbis* occurred on only 34% of the 35 tree species, while *S. wilkinsonii* occurred on 69%. Four tree species (*Ficus vitiense*, *Erythrina spec.*, *Macaranga spec.1*, *Macaranga spec. 2*) were the most frequent hosts of *S. imberbis* (Fig. S3; Kolmogorov-Smirnov test, P<0.001), indicating a preference of the seed-planting workers for these species. *Squamellaria wilkinsonii* were not found on these tree species. Of the four species, *Erythrina* and *Macaranga* have extrafloral nectaries (EFNs) on the petiole and rachis or on the leaf blade (Fig. S4), and we observed *P. nagasau* workers foraging on these nectaries. *Ficus vitiense* does not offer nectar but has sugary fruits as well as soft, easily hollowed piths (Fig. S4G-K) transiently occupied by *P. nagasau* workers (Fig. S4J). GC–TOF–MS analyses (*Materials and Methods*) showed that the chemical composition of the rewards of *Macaranga* and *Erythrina* largely overlaps that of *P.*

nagasau-inhabited *Squamellaria* nectar in sugars, amino acids and organic acids (Fig. S4M-N). Host tree selection by workers that choose the most suitable trees to establish their 'farms' maximises colony fitness and has also been reported in Neotropical and South-East Asian ant gardens, typically involving trees with food rewards or chemical defences[2,3].

The planting of seeds inside cracks in the bark of tall trees reported here is unique. In all ant gardens known previously, ants place seeds in carton nests or runways, both in the Neotropics[2] and in South East Asia[11], where seeds germinate rarely and are never actively fertilized by the ants. *Philidris nagasau* never builds carton nests, and its workers *actively* fertilize *Squamellaria* seedlings via defecation inside the seedling's minute domatium (too small to house a colony), which will only becomes inhabitable several years later. The mutualisms between *Squamellaria* and *P. nagasau* thus is extremely specialized, in spite of only being about 3 million years old (Fig. 1A).

Materials and Methods summary

All material and methods are detailed in the online supplementary material and methods.

Collection of material on Fiji and study sites

In September 2014 and March 2015, the first author conducted fieldwork on Viti Levu, Vanua Levu, and Taveuni and collected all species of Fijian *Squamellaria*, including three new species (*S. jebbiana*, *S. grayi*, and S. *huxleyana;* Chomicki and Renner, 2016). Except for a few cases, *Squamellaria* plants were accessed by tree climbing, using a rope secured by a partner on the ground. This technique allowed long stays in the canopy with minimal disturbance of the ant colony. See the OSM for details on the field sites and *Squamellaria* host tree association.

Experiments on seed dispersal by ant versus birds and seed cafeteria experiment

To find out the seed-dispersing vectors of specialized and unspecialized *Squamellaria*, we excluded either birds only (bags) or birds and ants (Vaseline) on specialized and generalist Squamellaria species. Samples sizes and statistical analyses are described in the OSM. Seed cafeteria was performed by providing seeds from specialized vs. generalist (plus control) to *P. nagasau*, followed by 6-hour monitoring.

^{15}N sugar feeding experiments and $\delta\,^{15}N$ isotope analyses

We tested whether *P. nagasau* workers fertilizes seedling by providing sugar solutions with 15N glycine for 10 days, and subsequently analysing samples (with appropriate control) via Isotope-Ratio Mass Spectrometry. See the OSM for details.

DNA extraction, phylogenetic analyses, molecular clock dating and ancestral state reconstructions

We used our recent nine-gene matrix for *Squamellaria* (Chomicki and Renner, 2016), and generated a 3-gene matrix for *Philidris*. All procedures are described in details in the OSM.

GC-TOF-MS determination of metabolic profiles

All procedures are described in details in the OSM.

References

1. Chomicki, G. & Renner, S. S. Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytol* 207, 411-424 (2015).
2. Davidson, D.W. Ecological studies of neotropical ant gardens. *Ecology* 69, 1138-1152 (1988).
3. Davidson, D.W. & Epstein, W.W. Epiphytic associations with ants. In *Vascular plants as epiphytes* (pp. 200-233). Springer Berlin Heidelberg (1989).
4. Huxley, C. R. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytol* 80, 231-268 (1978).
5. Rico-Gray, V., Barber, J. T., Thien, L. B., Ellgaard, E. G. & Toney, J. J. An unusual animal-plant interaction: feeding of *Schomburgkia tibicinis* (Orchidaceae) by ants. *Amer J Botany* 76, 603-608 (1989).
6. Treseder, K. K., Davidson, D. W. & Ehleringer, J. R. Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* 375:137-139 (1995).
7. Laube, S. & Zotz, G. Which abiotic factors limit vegetative growth in a vascular epiphyte?. *Funct Ecol* 17, 598-604 (2003).
8. Chomicki, G. & Renner, S. S. Evolutionary relationships and biogeography of the ant-epiphytic genus *Squamellaria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *Plos One* 11(3), e0151317 (2016).
9. Chomicki, G., M Staedler, Y., Schönenberger, J. & Renner, S.S. Partner choice through concealed floral sugar rewards evolved with the specialization of ant–plant mutualisms. *New Phytologist*. In press. (2016) DOI: 10.1111/nph.1399
10. Youngsteadt, E., Nojima, S., Häberlein, C., Schulz, S. & Schal, C. Seed odor mediates an obligate ant–plant mutualism in Amazonian rainforests. *Proc Nat Acad Sci USA* 105:4571-4575 (2008).
11. Kaufmann, E. & Maschwitz, U. Ant-gardens of tropical Asian rainforests. *Naturwissenschaften* 93: 216-227 (2006).

Acknowledgments: We thank J. Aroles for essential help in the field and critical reading of the manuscript; A. Naikatini and M. Tuiwava for help with permissions and voucher collection; V. Mayer, University of Vienna, for instructions and material for the nitrogen isotope experiments; C. Mayr, University of Munich, for the analyses of nitrogen isotope ratios; M. Lehman and P. Geigenberger, University of Munich, for metabolomic analyses; E. Kaufmann and M. Janda for *Philidris* samples and for discussion; E. Economo for help with ant identification, P. S. Ward, C.S. Moreau, M. Jebb, A. Wistuba, and D. McKey for discussion; and M. Frederickson, N. Pierce and R. Ricklefs for comments on the manuscript. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20, and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to GC.

Fig. 1. *Philidris nagasau* exclusively plants specialized *Squamellaria* seeds under tree bark. (**a**) Phylogenetic distribution of occupancy rates and the nature of ant symbionts in Fijian ant plant symbioses. (**b**) *Macaranga* branch harboring 28 *Squamellaria* species from three species, DesVoeux peak, Taveuni. (**c-d**) Young seedlings of *S. thekii* emerging from under the bark. (**e**) Morphology of seedling from obligate (left) and facultative (right) *Squamellaria*, showing the hypocotyl foot of P. nagasau-inhabited species. (**f-h**) Seed planting by *P. nagasau*. (**f**) *S. huxleyana* seed inserted in a bark crack with other seedlings (see lower inset too). Upper inset shows P. nagasau worker inside the tiny domatium of 2 cm-long seedlings. (**g**) *P. nagasau* transporting a *S. huxleyana* seed. (**h**) Seed removal assay in which seeds from specialized and unspecialized *Squamellaria* species were placed on the surface of a host tree, with rice grains as controls. (**i**) Dispersal experiment in *S. huxleyana* (see *Material and Method*). (**j**) Dispersal experiment in *S. wilkinsonii*. (**i**) Correlated evolution of seed dispersal by ants and symbiosis specialization in epiphytic ant/plants (Rubiaceae, Hydnophytinae).

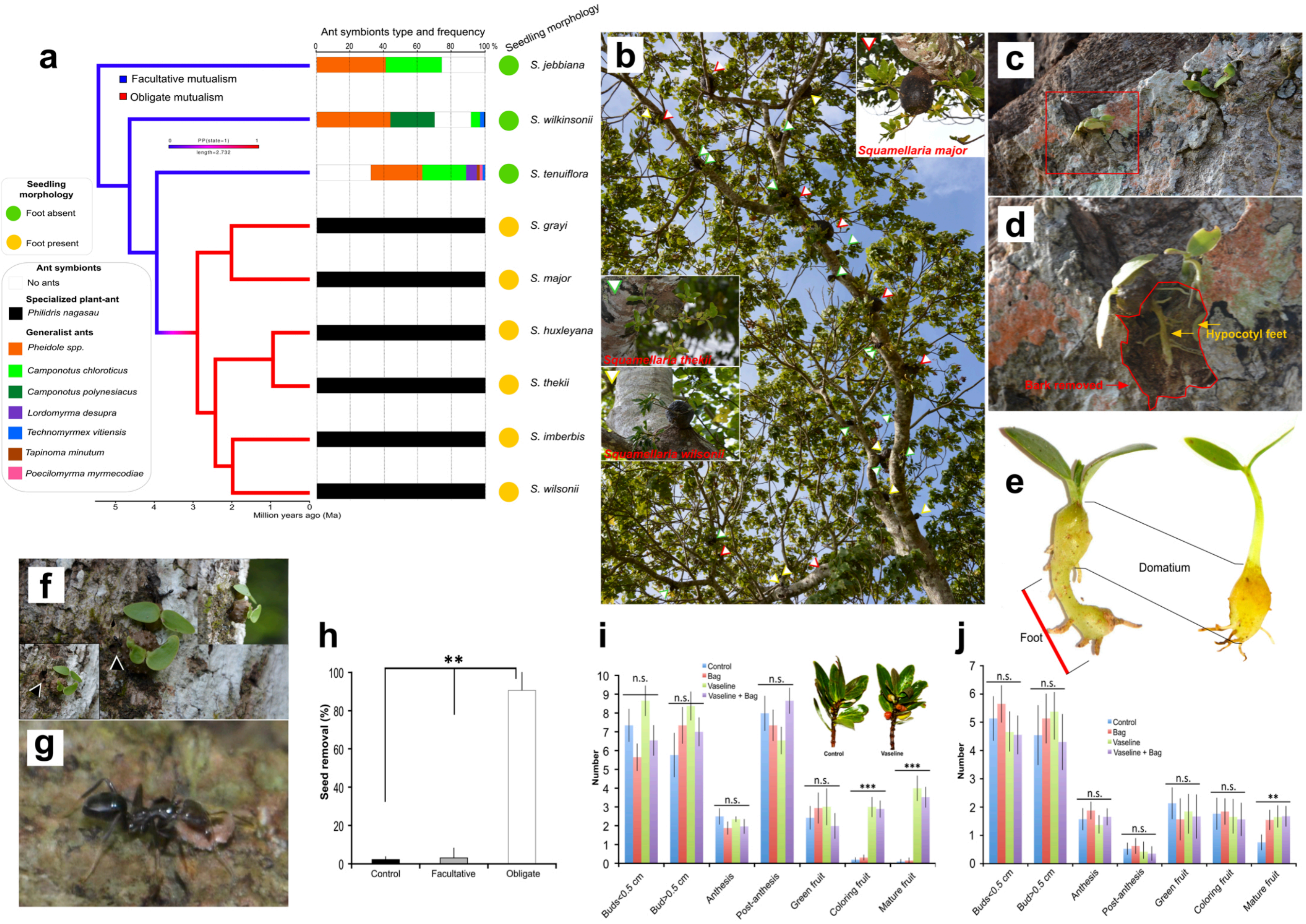
Fig. 2. Evolution of *Squamellaria* and *Philidris* life histories reconstructed on the BEAST chronograms for the Hydnophytinae and *Philidris*. Stochastic character mapping using phytools shows the evolutionary history of seed dispersal strategies on the Hydnophytinae tree and the evolution of carton nest building on the *Philidris* tree. The loss of carton building in *P. nagasau* is to compare to the evolution of the hypocotyl foot in specialized *Squamellaria* (Fig. 1a)

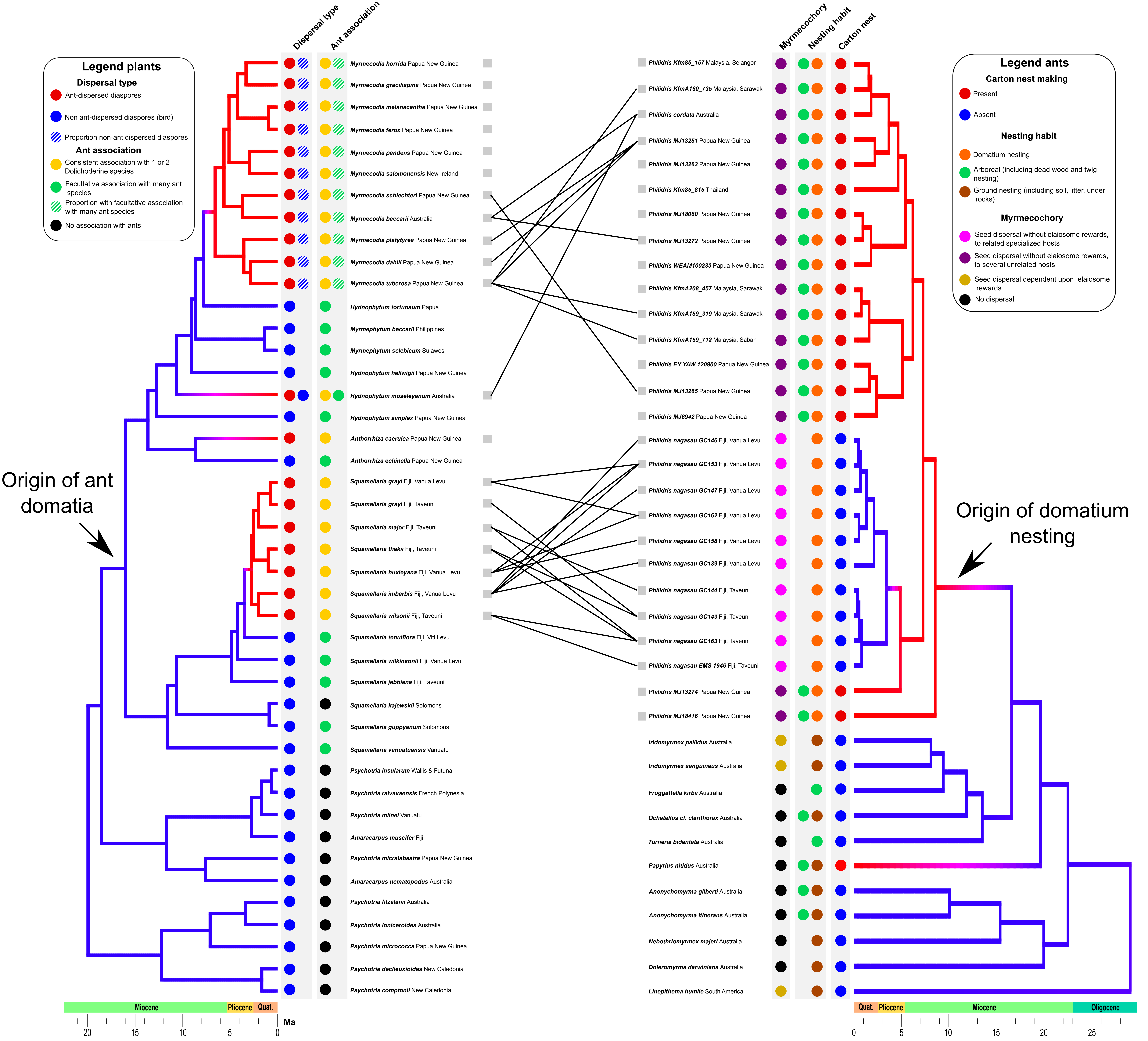
Online Supplementary Materials

Supplementary Materials and Methods

Figs. S1-S7

Tables S1-S5


a
Facultative mutualism
Obligate mutualism
0 PP(state=1) 1
length=2.732
Ant symbionts type and frequency
0 20 40 60 80 100 %
Seedling morphology
S. jebbiana
S. wilkinsonii
S. tenuiflora
S. grayi
S. major
S. huxleyana
S. thekii
S. imberbis
S. wilsonii
Seedling morphology
Foot absent
Foot present
Ant symbionts
No ants
Specialized plant-ant
Philidris nagasau
Generalist ants
Pheidole spp.
Camponotus chloroticus
Camponotus polynesiacus
Lordomyrma desupra
Technomyrmex vitiensis
Tapinoma minutum
Poecilomyrma myrmecodiae
5 4 3 2 1 0
Million years ago (Ma)
b
Squamellaria major
Squamellaria thekii
Squamellaria wilsonii
c
d
Hypocotyl feet
Bark removed
e
Domatium
Foot
f
g
h
**
Seed removal (%)
0 20 40 60 80 100
Control
Facultative
Obligate
i
Control
Bag
Vaseline
Vaseline + Bag
Control
Vaseline
Number
0 1 2 3 4 5 6 7 8 9 10
n.s.
n.s.
n.s.
n.s.
n.s.
***
***
Buds<0.5 cm
Bud>0.5 cm
Anthesis
Post-anthesis
Green fruit
Coloring fruit
Mature fruit
j
Control
Bag
Vaseline
Vaseline + Bag
Number
0 1 2 3 4 5 6 7
n.s.
n.s.
n.s.
n.s.
n.s.
n.s.
**
Buds<0.5 cm
Bud>0.5 cm
Anthesis
Post-anthesis
Green fruit
Coloring fruit
Mature fruit


Dispersal type
Ant association
Myrmecochory
Nesting habit
Carton nest
Legend plants
Dispersal type
Ant-dispersed diaspores
Non ant-dispersed diaspores (bird)
Proportion non-ant dispersed diaspores
Ant association
Consistent association with 1 or 2 Dolichoderine species
Facultative association with many ant species
Proportion with facultative association with many ant species
No association with ants
Legend ants
Carton nest making
Present
Absent
Nesting habit
Domatium nesting
Arboreal (including dead wood and twig nesting)
Ground nesting (including soil, litter, under rocks)
Myrmecochory
Seed dispersal without elaiosome rewards, to related specialized hosts
Seed dispersal without elaiosome rewards, to several unrelated hosts
Seed dispersal dependent upon elaiosome rewards
No dispersal
Myrmecodia horrida Papua New Guinea
Myrmecodia gracilispina Papua New Guinea
Myrmecodia melanacantha Papua New Guinea
Myrmecodia ferox Papua New Guinea
Myrmecodia pendens Papua New Guinea
Myrmecodia salomonensis New Ireland
Myrmecodia schlechteri Papua New Guinea
Myrmecodia beccarii Australia
Myrmecodia platytyrea Papua New Guinea
Myrmecodia dahlii Papua New Guinea
Myrmecodia tuberosa Papua New Guinea
Hydnophytum tortuosum Papua
Myrmephytum beccarii Philippines
Myrmephytum selebicum Sulawesi
Hydnophytum hellwigii Papua New Guinea
Hydnophytum moseleyanum Australia
Hydnophytum simplex Papua New Guinea
Anthorrhiza caerulea Papua New Guinea
Anthorrhiza echinella Papua New Guinea
Squamellaria grayi Fiji, Vanua Levu
Squamellaria grayi Fiji, Taveuni
Squamellaria major Fiji, Taveuni
Squamellaria thekii Fiji, Taveuni
Squamellaria huxleyana Fiji, Vanua Levu
Squamellaria imberbis Fiji, Vanua Levu
Squamellaria wilsonii Fiji, Taveuni
Squamellaria tenuiflora Fiji, Viti Levu
Squamellaria wilkinsonii Fiji, Vanua Levu
Squamellaria jebbiana Fiji, Taveuni
Squamellaria kajewskii Solomons
Squamellaria guppyanum Solomons
Squamellaria vanuatuensis Vanuatu
Psychotria insularum Wallis & Futuna
Psychotria raivavaensis French Polynesia
Psychotria milnei Vanuatu
Amaracarpus muscifer Fiji
Psychotria micralabastra Papua New Guinea
Amaracarpus nematopodus Australia
Psychotria fitzalanii Australia
Psychotria loniceroides Australia
Psychotria micrococca Papua New Guinea
Psychotria declieuxioides New Caledonia
Psychotria comptonii New Caledonia
Philidris Kfm85_157 Malaysia, Selangor
Philidris KfmA160_735 Malaysia, Sarawak
Philidris cordata Australia
Philidris MJ13251 Papua New Guinea
Philidris MJ13263 Papua New Guinea
Philidris Kfm85_815 Thailand
Philidris MJ18060 Papua New Guinea
Philidris MJ13272 Papua New Guinea
Philidris WEAM100233 Papua New Guinea
Philidris KfmA208_457 Malaysia, Sarawak
Philidris KfmA159_319 Malaysia, Sarawak
Philidris KfmA159_712 Malaysia, Sabah
Philidris EY YAW 120900 Papua New Guinea
Philidris MJ13265 Papua New Guinea
Philidris MJ6942 Papua New Guinea
Philidris nagasau GC146 Fiji, Vanua Levu
Philidris nagasau GC153 Fiji, Vanua Levu
Philidris nagasau GC147 Fiji, Vanua Levu
Philidris nagasau GC162 Fiji, Vanua Levu
Philidris nagasau GC158 Fiji, Vanua Levu
Philidris nagasau GC139 Fiji, Vanua Levu
Philidris nagasau GC144 Fiji, Taveuni
Philidris nagasau GC143 Fiji, Taveuni
Philidris nagasau GC163 Fiji, Taveuni
Philidris nagasau EMS 1946 Fiji, Taveuni
Philidris MJ13274 Papua New Guinea
Philidris MJ18416 Papua New Guinea
Iridomyrmex pallidus Australia
Iridomyrmex sanguineus Australia
Froggattella kirbii Australia
Ochetellus cf. clarithorax Australia
Turneria bidentata Australia
Papyrius nitidus Australia
Anonychomyrma gilberti Australia
Anonychomyrma itinerans Australia
Nebothriomyrmex majeri Australia
Doleromyrma darwiniana Australia
Linepithema humile South America
Origin of ant domatia
Origin of domatium nesting
Miocene
Pliocene
Quat.
Oligocene
20
15
10
5
0
Ma
25

Supplementary Materials and Methods

Supplementary information on collection of material on Fiji and study sites

The study sites in Viti Levu were Colo-i-Suva forest reserve in the south of the island (S 18° 1' 46.808", E 178° 24' 0.4175") and forest around Navai in the center of the island (S 17° 37' 49.5979", E 177° 58' 34.9315"); in Vanua Levu, the collection sites were in Waisali forest reserve (S 16 38'19.8", E 179 13'19.7"), and along the Cross Island road before the bifurcation to Nabouwalu and Labasa; in Taveuni, the collections were made along the trail to DesVoeux peak and Mt. Manuca on the western side of the island (S 16° 48' 25.8133", E 179° 56' 36.6843"), and at the end of Lavena coastal walk, Bouma heritage park, on the eastern side of the island (S 16° 51' 45.4433", E 179° 54' 6.5149"). All collections were made in collaboration with colleagues from the University of South Pacific (Acknowledgements), and vouchers have been deposited in the herbaria of Suva (SUVA) and Munich (M). For DNA extraction, we collected young leaves and dried them in silica gel. *Squamellaria* taxonomy follows Chomicki and Renner (2016).

Host specificity, occupancy rates, and Philidris nagasau colony structure

Philidris nagasau form large colonies expanding often into several dozen of *Squamellaria* (Fig. 1a), and with several thousands of workers (one large 80 cm-long, queen-bearing domatium had 10,000 workers). We assessed host specificity and host occupancy rates for each species using at least 20 specimens. 'Host' here refers to the Hydnophytinae species with domatia suitable as nesting sites for ants. Observations were designed to be as non-invasive as possible. For each plant we recorded whether it was inhabited by ants or not and determined all domatium-inhabiting species. We analysed Statistical difference of each species from another using Welch t-tests, performed in R. Since we found that six species of *Squamellaria* were exclusively inhabited by *P. nagasau*, we investigated ant colony size and structure using the two most common *Squamellaria* species, *S. imberbis* (Vanua Levu) and *S. wilsonii* (Taveuni). We dissected a *S. wilsonii* colony of 23 individuals to determine presence of one or more queens, which revealed a single queen in the largest domatium (monogynous), but many alates, with the number of alates correlated with domatium size (Fig. S2B), suggesting dispersal from the queen-bearing domatium. Monitoring of ant traits on a colony of 15 individuals where all specimens where classified as seedlings, small, medium, large and queen housing (see Fig. S2). We recorded all trails connecting pairs of *Squamellaria* within the system. Monitoring was carried out three times a day for two days. The 25 links observed (realised) have to be opposed to the 105 links possible (number of links = 15!/2!(15-2)!). To test whether distance, beyond connectivity to the queen-bearing domatium, was an important factor determining the polydomous network, we recorded the distance (either by direct measurement when possible, or by estimation when domatium were out of reach) between each domatium pairs and noted whether the connection was realised or not. We performed a one-way ANOVA on these data with a Tukey's post-hoc test. Ant monitoring showed that the trails on any tree are hierarchically organized towards the queen-housing plant (Fig. S2) and that distance is an important mediator of link realization (Fig. S2A; Tukey's test; P<0.001), indicating that continuous visit of all-sized plants is centrally controlled.

To evaluate whether the ant species inhabiting unspecialized *Squamellaria* were polydomous or monodomous, we searched for trails connecting their colonies, but found none. Instead, we discovered that different ant species inhabited them, proving monodomy.

Squamellaria seedling morphology

To ensure that the unique morphology of *Squamellaria* seedlings was not the result of etiolation, seeds of the specialized *S. thekii* and *S. imberbis* and the unspecialized *S. tenuiflora* and *S. wilkinsonii* were germinated under high light levels, which confirmed that delayed domatium formation is an inherited trait. All nine species were coded hypocotyl foot absent (0) or hypocotyl foot present based on seedlings observed in the field. Species from Hydnophytinae genera *Myrmecodia* (*M. tuberosa*) and *Hydnophytum* (*H. formicarum*) have been germinated under the same conditions to and none showed a hypocotyl foot. Moreover, Hydnophytinae taxonomists Camilla R. Huxley and Matthew P. Jebb were consulted and from their extensive field experience no other Hydnophytinae species has hypocotyl feet.

Experiments on seed dispersal by ant versus birds

To find out the seed-dispersing vectors of specialized and unspecialized *Squamellaria*, we selected five large flowering and fruiting plants of *S. imberbis* (specialized) and *S. wilkinsonii* (unspecialized) along the cross-island road in Vanua Levu. In each plant, three branches were marked as controls, three others were enclosed in a bag with holes, in three other, all reproductive structures and developing buds were enclosed in Vaseline, and three others were enclosed in Vaseline and additionally a bag with holes (as a treatment control). A bag with holes should prevent fruit collection by birds but not by ants (confirmed by observation), and Vaseline should prevent ants from removing the fruits. All treatments were applied during ten days in March 2015. We expected that if ants are the main dispersers, the presence of a bag preventing bird access would not significantly affect fruit removal, while conversely, Vaseline treatment preventing ant access would significantly decrease fruit removal. Each stage (buds <0.5 cm in length, buds >0.5 cm, flowers at anthesis, post-anthesis, green fruits, fruits turning red, and mature fruits) was recorded for each shoot at the end of the 10 days, and the results were normalized by the number of shoot metamers (segments between leaf nodes) to ensure comparability across shoots. All corresponding data (N = 3 x 5 replicates) were summarized, and we used R (R core team 2013) to test the difference in means for each treatment relative to the control using generalized linear models (GLM) with a Poisson distribution, followed by an ANOVA of the deviance table. Significance of the p-value are reported as * for P<0.05, ** for P<0.01 and *** for P<0.001. Pairwise analyses using Welch t-tests were similarly significant.

Seed cafeteria experiment

To test whether *P. nagasau* can recognize seeds of specialized *Squamellaria* and differentiate them from those of unspecialized *Squamellaria* (often growing nearby) we placed 20 *S. wilkinsonii* seeds, 20 *S. huxleyana* seeds, and 20 rice grains (as controls) on a large branch, and subsequently monitored seed removal for 6 hours. Replications consisted of blocking by 4 seeds, hence with 5 replicates. We analysed statistical difference of each category from the control using a Generalized Linear Model (GLM) with a Poisson distribution followed by an ANOVA of the deviance table. Pairwise analyses of control/obligate or facultative/obligate using Welch t-tests were similarly significant.

^{15}N sugar feeding experiments and $\delta\ ^{15}$N isotope analyses

Related mature *Myrmecodia* plants are fed by their *Philidris* ants by defecation in specific 'warted' chambers (Huxley, 1978), and we therefore tested whether the seedlings of ant-dispersed *Squamellaria* species that are developing their first cavity (domatium), usually at a seedling diameter ~ 2 cm (Fig. 1), were already being fed by ants. To do so, we selected a *Macaranga* tree along the cross-island road in Vanua Levu with a colony of *S. huxleyana* with both mature plants and seedlings. We placed a solution of 20 mM ^{15}N glycine (enriched at 98% at, Isotec) with 40% (w/v) 1:1:1 mix of sucrose, glucose and fructose in a flacon tube close to the mature plants, and at about 2 meters of the seedlings. A paper wick allowed the ants to reach the solution without drowning in it. We added two millilitres of solution to the falcon tube twice a day during the 10 days of the experiment. On the 11th day, we collected six seedlings of ~2 cm (see Fig. 1f) and microwave-dried them. Five *S. huxleyana* seedling controls of the same stage were collected from a neighboring tree (at about 500 m) and prepared in the same way. Samples were homogenized with a ball mill and ca. 1-3 mg of dry powder was weighted in tin capsules. Isotope-ratio mass spectrometry (IR-MS) analyses were performed at the GeoBiocenter, University of Munich (LMU). Capsules were combusted in an elemental analyser (NC2500, Carlo Erba) in a continuous helium flow at 1080^{0}C. The combustion gases passed through a reaction tube filled with chromium and silvered cobaltous oxides, a subsequent reduction tube (560^{0}C) filled with copper wires, a water trap filled with magnesium perchlorate, and a gas-chromatography column. The isolated gases N_2 and CO_2 were then analysed in an isotope-ratio mass spectrometer (DeltaPlus, Thermo-Finnigan) to determine the isotope ratios of organic carbon (δ^{13}Corg) and nitrogen (δ^{15}N). The total organic carbon (TOC) and total nitrogen (TN) mass percentages were calculated from sample peak areas using the elemental standards atropine, cyclohexanone-2,4-dinitrophenylhydrazone, and peptone for calibration.

Squamellaria host tree association

To evaluate the host tree range of specialized *Squamellaria* species versus that of the unspecialized species and to test for a possible selection of particular tree species by *P. nagasau* workers, we evaluated tree occupancy rate along transects. Tree height and *Squamellaria* distance from the canopy were evaluated using a compass and a simple trigonometric method, for a subset of 20 host trees where possible. By comparing research sites on the three islands, we determined that the best study site to compare host tree association of specialized and unspecialized *Squamellaria* species was on Vanua Levu, where *S. imberbis* and *H. wilkinsonii* are abundant and grow in close proximity to each other. The transect started at the track to the Vodafone Tower, near the Cakaudrove-Macuata Provincial boundary line, and extended along the cross-island road until Waisali forest reserve, and inside Waisali forest reserve. Each tree along the transect with a diameter at breast height (DBH) >5 cm was recorded, and its *Squamellaria* epiphytes (*S. imberbis* and *S. wilkinsonii*) were counted. Data for the 35 host tree species and 253 tree individuals are reported in Table S1. We found high correlations between tree size (log(DBH)) and number of epiphytes per tree (log(plant number)) for the specialized *S. imberbis* (Pearson's correlation coefficient R=0.58) and the unspecialized *S. wilkinsonii* (R=0.55). Specialized *Squamellaria* further appeared to be concentrated on four tree species (*Macaranga spec. 1*, *Macaranga spec. 2*, *Ficus vitiense* and *Erythrina spec.*), all of which reward the *Squamellaria*-inhabing ants *Philidris nagasau*. To test for a potential significance of this observation, we determined the occupancy frequencies of rewarding trees versus non-rewarding trees by specialized versus non-specialized *Squamellaria*. Because the occupancy frequencies were too far from a normal distribution and homoscedascity was not verified, we used a non-parametric test. The Kolmogorov-Smirnov test ('ks.test' function in R, null hypothesis (H0): the samples have the same distribution) confirmed that the difference was statistically significant.

DNA extraction and phylogenetic analyses

For *Squamellaria*, we used our recently generated a matrix of nine gene regions based on plastid (trnL-trnF region (trnL intron and trnL-trnF spacer), ndhF, rps16, rpl20-rps12, trnG-trnS spacer), nuclear (ITS region (ITS1, 5.8S, ITS2), ETS, 18S) (Chomicki and Renner, 2016). All accessions of Fijian *Squamellaria* were extracted from silica-dried leaves collected by GC and are all linked to herbarium specimens deposited in the herbaria SUVA and M. A sampling of outgroups (in the tribe Psychotrieae) was selected based on Barrabé et al. (2014). We also generated another matrix using six markers (nuclear ITS and ETS and plastid ndhF, psbA-trnH, trnL intron and trnL-trnF spacer) using a wider sampling of Hydnophytinae, in order to infer the evolution of dispersal type and ant inhabitation, sampling 31 species out of ca. 100, selected based on the current knowledge of their ant occupants. For the ants, we generated a matrix of three nuclear markers (CAD, EFαF1 and EFαF2) sampled for 27 ingroup taxa and an additional 11 taxa as outgroups, based on Ward et al. (2010). All primers are shown in Table S2. Because the taxonomy of *Philidris* is poorly understood, and species delimination is problematic (M. Janda pers. comm. to G.C. and S.S.R. Nov. 2014), we selected samples representing the broadest range of hosts plants and geography as possible. Voucher information is reported in Table S3 for plants, and Table S4 for ants. Total genomic DNA was extracted from c. 20 mg of leaf tissues, using a commercial plant DNA extraction kit (NucleoSpin; Macherey–Nagel, Düren, Germany) according to manufacturer protocols. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England Biolabs, Cambridge, MA, USA) and a standard protocol (39 cycles, annealing temperature 56^0C). PCR products were purified using the ExoSap clean-up kit (Fermentas, St Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were BLAST-searched in GenBank. Sequence alignment was performed in MAFFT v. 7 in the online server (http://mafft.cbrc.jp/alignment/ server;) (Katoh and Standley, 2013) under standard parameters except for the ITS region, which was aligned under Q-INS-i optimization, which takes rRNA secondary structure into consideration. Minor alignment errors were corrected manually in Mesquite v. 2.75 (Maddison and Maddison, 2011). In the absence of statistically supported incongruence (i.e., maximum likelihood bootstrap (BS) support >75) between the plastid and nuclear data partitions), we concatenated all markers, yielding an alignment of 9346 bp for the Squamellaria matrix, 3055 bp for the Hydnophytinae matrix and 1592 for *Philidris*. Maximum-likelihood (ML) inference relied on RAxML v8.0 (Stamatakis, 2014) and the GTR + Γ substitution model, with empirical nucleotide frequencies and 25 gamma rate categories; bootstrap support was assessed from 100 replicates under the same model. We also conduced Bayesian inference in MrBayes v. 3.2 (Ronquist et al., 2012) under the same substitution model (but with 4 rate categories) and using the program's default two runs and four chains (one cold and three heated), with the uniform default priors. We set a $10X10^6$ MCMC chain, sampling trees every 1000^{th} generation. Split frequencies approaching zero indicated convergence. We used the 50% consensus tree to assess posterior probabilities for nodes of interest.

Molecular clock dating

Molecular dating analyses relied on BEAST v. 2 (Bouckaert et al., 2014) and uncorrelated lognormal relaxed clock models. We used the GTR + G substitution model with four rate categories and a Yule tree prior. For both our plant and ant trees, MCMCs were run for 20 million generations, with parameters and trees sampled every 10,000 generations. We used Tracer v. 1.6 (Rambaut and Drummond, 2007) to check that the effective sample size (ESS) of all parameters was >200, indicating that runs had converged. After discarding 10% as burn-in, trees were summarized in TreeAnnotator v. 1.8 (part of the BEAST package) using the options 'maximum clade credibility tree', which is the tree with the highest product of the posterior probability of all its nodes, 'mean node height,' and a posterior probability limit of 0.98. The final tree was visualized in FigTree v. 1.4 (Rambaut, 2012). To calibrate our tree, we constrained the age of the root, i.e., the split between the Pacific clade and the so-called *Psychotria* clade IV of Barrabé et al. (2014), to 22 ± 7 Ma, based on the age of this node estimated by these authors, using a normal prior and a standard deviation of 4 corresponding to the 95% confidence interval of Barrabé et al. (2014). The Hydnophytinae dated tree is shown in Fig. S5 with support values. To calibrate our ant tree, we used a secondary constraint from Ward et al. (2010), specifically the split of *Linepithema humile* from all other taxa to 33±8 Ma, using a normal prior with a standard deviation of 4. Because *Philidris nagasau* contains a clade endemic from the island of Taveuni, which has been dated to 0.8 Ma (Rodda and Kroenke, 1984; Rodda, 1994), we used this age as a geological maximal constraint for the age of this clade, using a uniform prior with a 0.8-0 Ma bound. The *Philidris* dated tree is shown in Fig. S6 with support values.

Ancestral state reconstructions of ant and plant life histories

We inferred the evolutionary history of dispersal type and ant association in the Hydnophytinae and that seed planting, nesting habit and carton nest making in *Philidris* ants (Fig. 2).

The seed dispersal type of the 31 ingroup species plus outgroups (all "0") was coded "0" for dispersal by frugivorous animals (endozoochory), "1" for dispersal only by ants (myrmecochory), based on published and unpublished data, especially (*i*) the clustering of individual epiphytes, with the clustered distribution allusive of ant dispersal and dispersed distribution suggestive of bird dispersal, (*ii*) polydomy versus monodomy of ant colonies (indicated by trails linking distinct specimens), with polydomy indicative of ant dispersal of the seeds, while monodomy points to bird dispersal (Huxley, 1978; personal observations by M.P.H. Jebb and C.R. Huxley-Lambrick, during fieldwork in the 1975-1990 in Papua New Guinea) and observations and experiments made for this study (for the nine species of Fijian *Squamellaria*). The above-described experiments were conducted on *S. wilkinsonii* and *S. huxleyana*. For the seven other Fijian *Squamellaria* species, we use the described traits (clustering and polydomy vs. monodomy) to assign bird versus ant dispersal. Clustering was measured by counting the number of epiphytes per tree and in at least 10 epiphyte-bearing trees for each of the nine species, normalizing the data by the tree diameter at breast height (DBH; see Fig. S1).

Ant association was determined based on Huxley and Jebb (1991a, b), Huxley (1993), and an unpublished revision of *Hydnophytum* from M. Jebb and C.R. Huxley, personal communications to G.C. from M. Janda and observations made by G.C. for this study for the nine Fijian *Squamellaria*.

For the ants, presence of carton nest building, nesting habit and ant dispersal was search for all outgroup species, further information came from consultation with ant taxonomist P.S. Ward, and for all *Philidris* samples, the information came from G.C., E. Kaufmann and M. Janda who collected the specimens.

To infer ancestral dispersal types, we used the Maximum Clade Credibility (MCC) tree from BEAST, and (*i*) the stochastic mapping approach implemented in the phytools package (Revell, 2012) and (*ii*) the ML approach implemented in ape (Paradis et al., 2014). We used the function make.simmap in the phytools package (v. 04-60) (Revell, 2012), which implements the stochastic character mapping approach developed by Bollback (2006). We estimated ancestral states using three models (see thereafter), and then simulated 1,000 character histories on the MCC tree. We summarized the 1,000 simulated character histories using the function densityMap (also in phytools).

We performed the stochastic mapping analyses using three different models: (*i*) Equal rates (ER) model, wherein all rates of transitions among character states are equal; (*ii*) Symmetrical rate model (SYM), wherein the backward and forward character state transition rates are equal for each combination of character states, but each distinct state combination can have a distinct rate; (*iii*) All rates different (ARD), wherein all rates are allowed to vary. The likelihood of each model was compared to select the one fitting our data best, in this case the ARD model. ML ancestral state reconstructions for dispersal by ants are shown in Fig. S7.

GC-TOF-MS determination of metabolic profiles

All samples were field-collected and immediately microwave-dried, a method that preserves metabolites (Popp et al., 1992). Metabolites for gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) were extracted and derivatized using a method based on Roessner et al. (2001), Lisec et al. (2006) and Erban et al. (2007). For the extraction, ~ 5 mg plant material (dry weight) was ground in 300 µl cold (-20°C) methanol (80 %) containing 15 µl ribitol (0.1 mg ml-1 in water) and 15 µl ^{13}C-sorbitol (0.1 mg ml-1 in water), which were added as internal standards. After incubation at 70°C for 15 min, 30 µl of the extract was dried *in vacuo*. The pellet was resuspend in 10 µl of methoxyaminhydrochloride (20 mg ml-1 in pyridine) and derivatized for 90 min at 37°C. After the addition of 20 µl of BSTFA (*N*,*O*-Bis[trimethylsilyl]trifluoroacetamide) containing 5 µl retention time standard mixture of linear alkanes (n-decane, n-dodecane, n-pentadecane, n-nonadecane, n-docosane, n-octacosane, n-dotriacontane), the mix was incubated at 37°C for further 45 min. A volume of 1 µl of each sample was injected into a GC–TOF–MS system (Pegasus HT, Leco, St Joseph, USA). Samples were derivatized and injected by an autosampler system (Combi PAL, CTC Analytics AG, Zwingen, Switzerland). Helium acted as carrier gas at a constant flow rate of 1 ml/min. Gas chromatography was performed on an Agilent GC (7890A, Agilent, Santa Clara, USA) using a 30 m VF-5ms column with 10 m EZ-Guard column. The injection temperature of the CIS injector (CIS4, Gerstel, Mühlheim, Germany) increased with a rate of 12°C s-1 from initially 70°C to finally 275°C. Transfer line and ion source were set to 250°C. The initial oven temperature (70°C) was permanently increased to a final temperature of 320°C by 9°C per minute. To avoid solvent contaminations, the solvent delay was set to 340 s. Because of the chemical and physical properties of the different metabolites the mixture was separated on the column over time. Metabolites that passed the column were released into the TOF-MS. The transfer line, connecting the GC and the TOF-MS, was set to 250°C, as was the ion source where the in-coming metabolites got ionized and fractionated by an ion pulse of 70 eV. Mass spectra were recorded at 20 scans per second with an *m/z* 35– 800 scanning range. Chromatograms and mass spectra were evaluated using ChromaTOF 4.5 and TagFinder 4.1 software (Luedemann et al., 2008). All metabolite profiles are shown in Table S5.

Supplementary references

Barrabé, L., Maggia, L., Pillon, Y., Rigault, F., Mouly, A., Davis, A. P. & Buerki S. New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Mol. Phylogenet. Evol.* 71, 15-35 (2014).

Bollback, J. P. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC bioinformatics* 7, 88 (2006).

Chomicki, G. & Renner, S. S. Evolutionary relationships and biogeography of the ant-epiphytic genus *Squamellaria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *Plos One* 11, e0151317 (2016).

Erban A, Schauer N, Fernie AR, Kopka J. Nonsupervised construction and application of mass spectral and retention time index libraries from time-of-flight gas chromatography-mass spectrometry metabolite profiles. *Methods Mol Biol* 358. Metabolomics-Methods and Protocols. Humana Press, 19-38 (2007).

Huxley, C. R. The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytol* 80, 231-268 (1978).

Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772-780 (2013).

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L. & Fernie AR. Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nat Protoc* 1, 387-396 (2006).

Luedemann, A., Strassburg, K., Erban, A. & Kopka, J. TagFinder for the quantitative analysis of gas chromatography - mass spectrometry (GC-MS)-based metabolite profiling experiments. *Bioinformatics* 24, 732-737 (2008).

Maddison, W. P & Maddison, D. R. Mesquite: a modular system for evolutionary analysis. Version 2.75. *Available at: mesquiteproject.org/mesquite/download/download.* (2011).

Pagel, M. & Meade, A. BayesTraits, version 2. *Univ. of Reading, Berkshire, UK Available at http://www.evolution.rdg.ac.uk.* (2013)

Paradis, E, Claude, J., Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289-290 (2004).

Popp, M., Lied, W., Meyer, A. J., Richter, A., Schiller, P. & Schwitte, H. Sample preservation for determination of organic compounds, microwave versus freeze-drying. *J Exp Bot* 47, 1469-1473 (1996).

Rambaut A. FigTree v. 1.4.0. http://tree.bio.ed.ac.uk/software/figtre. (2012)

Rambaut A, Drummond AJ. Tracer – MCMC trace analysis tool version v1.5. URL http://beast.bio.ed.ac.uk. (2007).

Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3:217-223 (2012)

Ripley, B., Venables, B., Bates, D. M., Hornik, K., Gebhardt, A. R package 'MASS'. https://cran.r-project.org/web/packages/MASS/MASS.pdf. (2015).

Roessner, U., Luedemann, A., Brust, D., Fiehn, O., Linke, T., Willmitzer, L., Fernie, A. R. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* 13, 11-29 (2001).

Rodda, P. Geology of Fiji. South Pacific Applied Geoscience Commission (SOPAC) *Technical Bulletin* 8:131-151 (1994).

Rodda, P. & Kroenke, L. Fiji: a fragmented arc. In: Cenozoic Tectonic Development of the Southwest Pacific (ed. L. Kroenke), pp. 87-110. U.N. ESCAP,CCOP/SOPAC (1984).

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. MrBayes 3.2, efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61, 539-542 (2012).

Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312-1313 (2014).

Ward, P. S., Brady, S. G., Fisher, B. L & Schultz, T. R. Phylogeny and biogeography of dolichoderine ants: effects of data partitioning and relict taxa on historical inference. *Syst Biol* 59, 1-21 (2010).

Figure S1. Clustering value (mean number of plants per tree normalized by DBH) in all species of the Fijian ant-plant clade. Star refer to the significance of the t-tests (*Materials and Methods*).

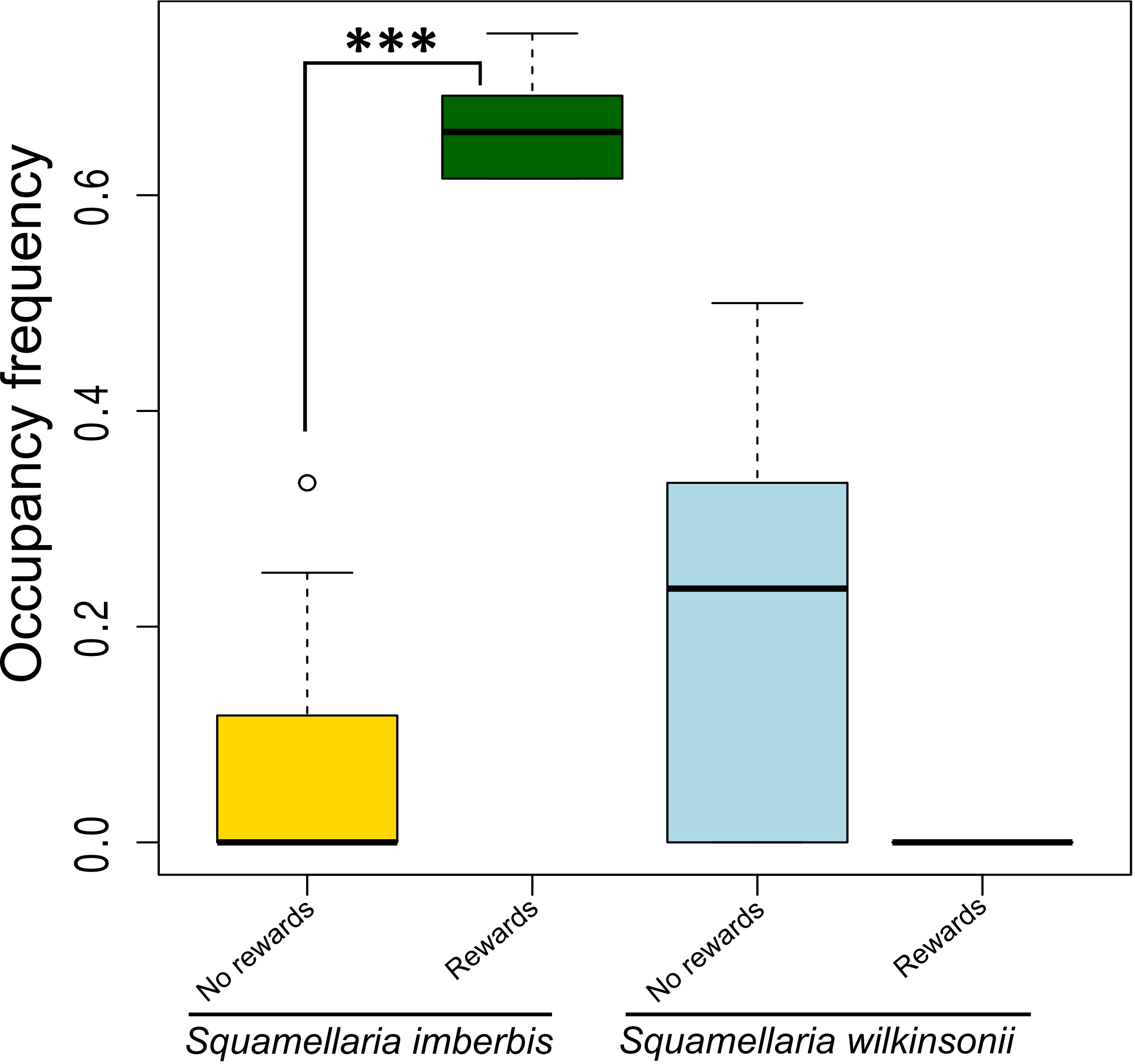


Figure S2. The distribution of specialized *Squamellaria* epiphytes on host trees is biased. Occupancy frequencies by *Squamellaria imberbis* or *S. wilkinsonii* in trees with or without rewards (including extrafloral nectar, floral nectar, and cavities suitable for ant nests). Stars refer to the significance of the Kolmogorov-Smirnov test (*Materials and Methods*).

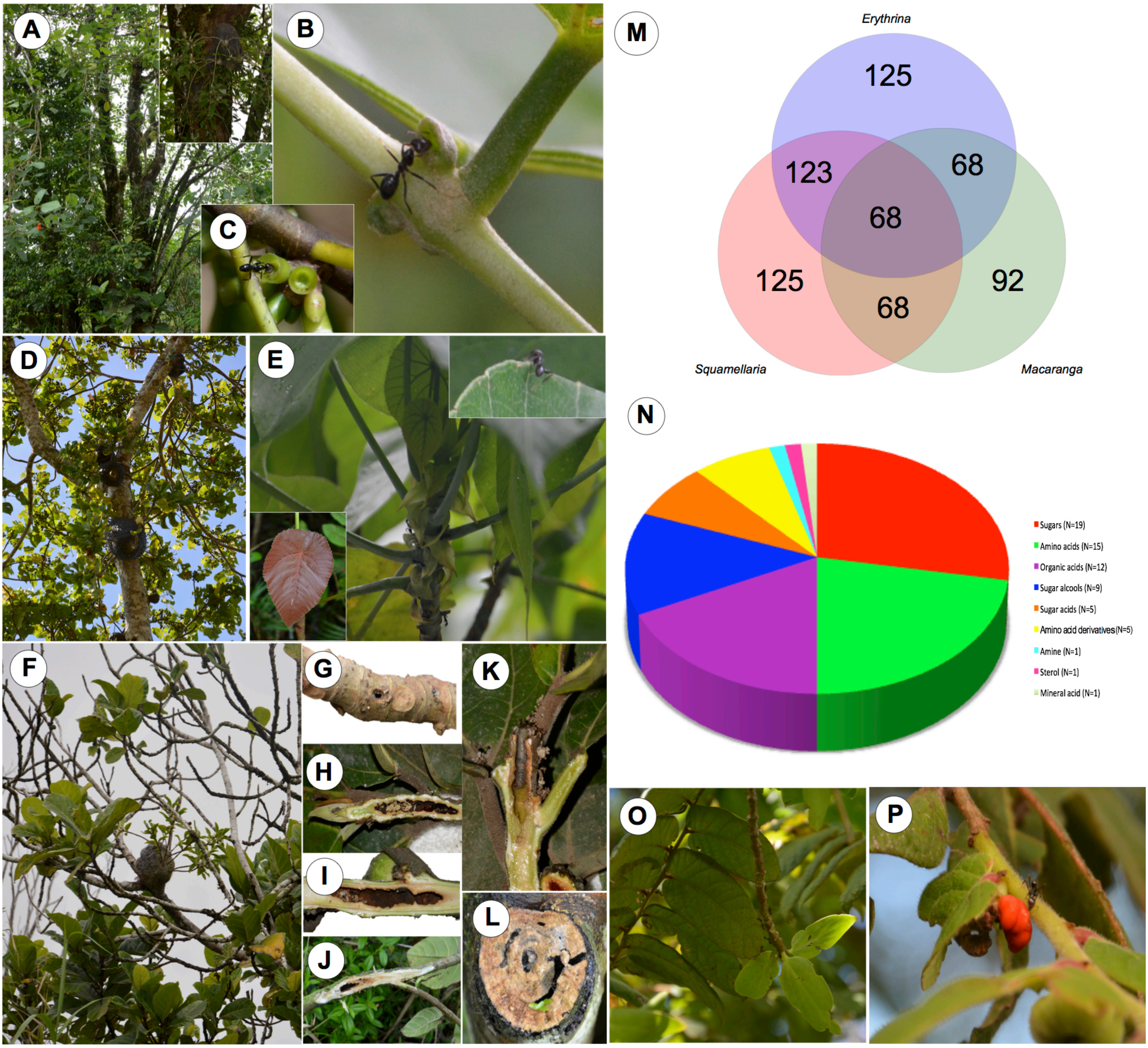


Figure S3. *P. nagasau* exploits facultative ant-plant mutualisms. (A-B) *Erythrina*. (A) *Erythrina* host *Squamellaria imberbis*. (b) *Philidris nagasau* feeds on *Erythrina* EFNs. (C) *Philidris nagasau* feeds on *S. imberbis* post-floral nectaries. (D-E) *Maracanga*. (D) *S. major* on *Macaranga spec. 1*. (E) *P. nagasau* feeds on the sugary secretions of *Macaranga* stipules and EFNs. (F-L) *Ficus vitense*. (F) *S. imberbis* on *Ficus vitense*. (G) Entrance hole of *F. vitense* domatium. (H) Nest of *Camponotus spec*. in a *F. vitense* lacking *Squamellaria*. (I) Nest of *Pheidole spec*. in a *F. vitense* lacking *Squamellaria*. (J) *P. nagasau* on *F. vitense*, transiently occupying domatium. (K) Wood borer eating the soft pith of *F. vitense* stem, resulting in domatium formation. (L) *S. imberbis* seedlings planted and guarded by *P. nagasau* in a broken hollow branch of *F. vitense*. (M) Venn diagram showing the overlap of *S. imberbis* post-floral nectaries, *Erythrina* EFNs and *Macaranga spec. 1* EFNs metabolomes. (N) Nature of the 68 metabolites shared by *Squamellaria*, *Macaranga* and *Erythrina*. (O) *P. nagasau* moving from their *Squamellaria huxleyana* to another tree (*Aglaia spec.* without *Squamellaria*) through the canopy. (P) The same *P. nagasau* feeding on *Aglaia* fruits.

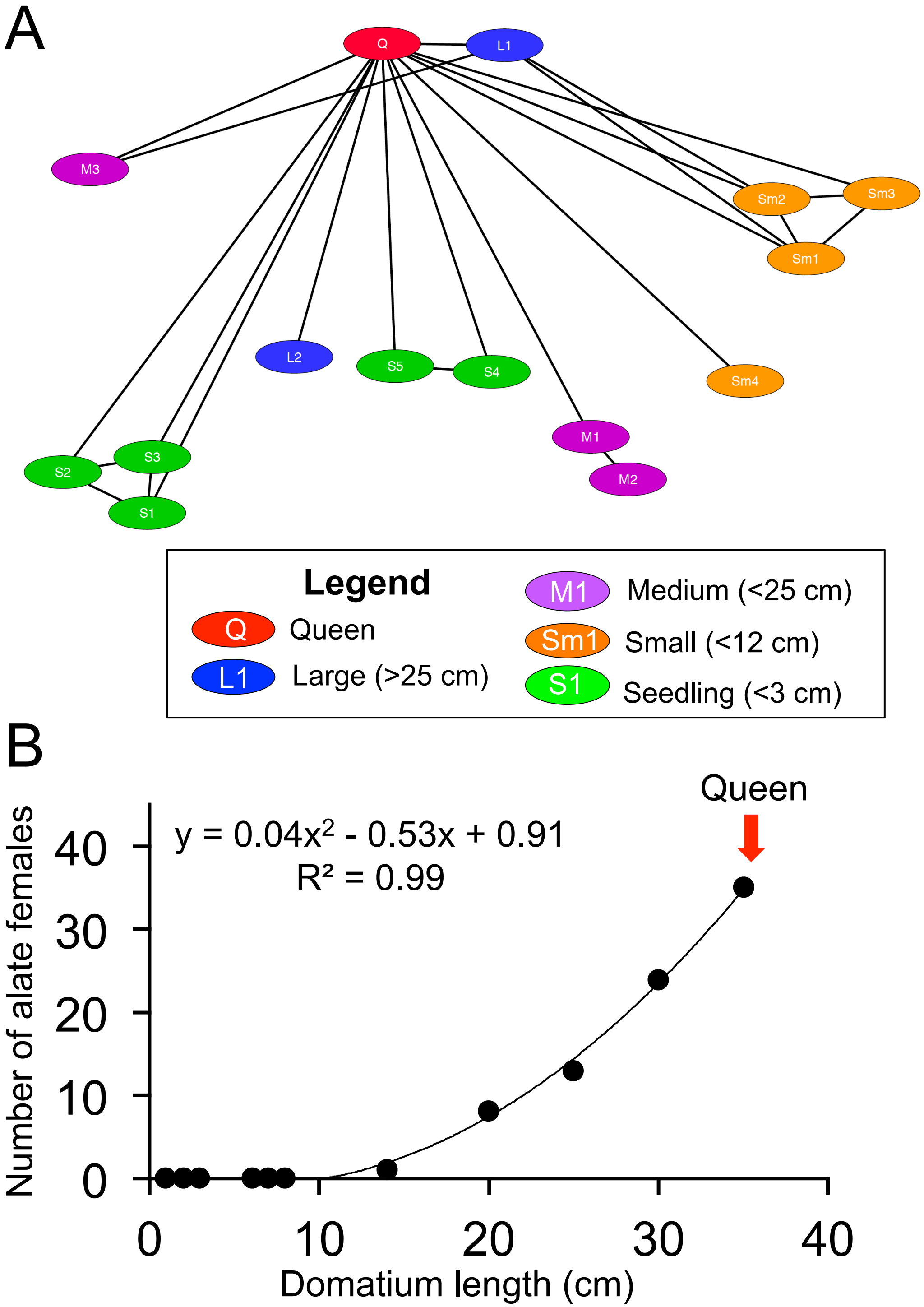


Figure S4. Polydomy of *Philidris nagasau* colonies. (A) detail of a network present on a single *Macaranga spec. 1* tree in Taveuni, all *Squamellaria* specimen are *S. wilsonii*. (B) Relationship between domatium size and alate number.

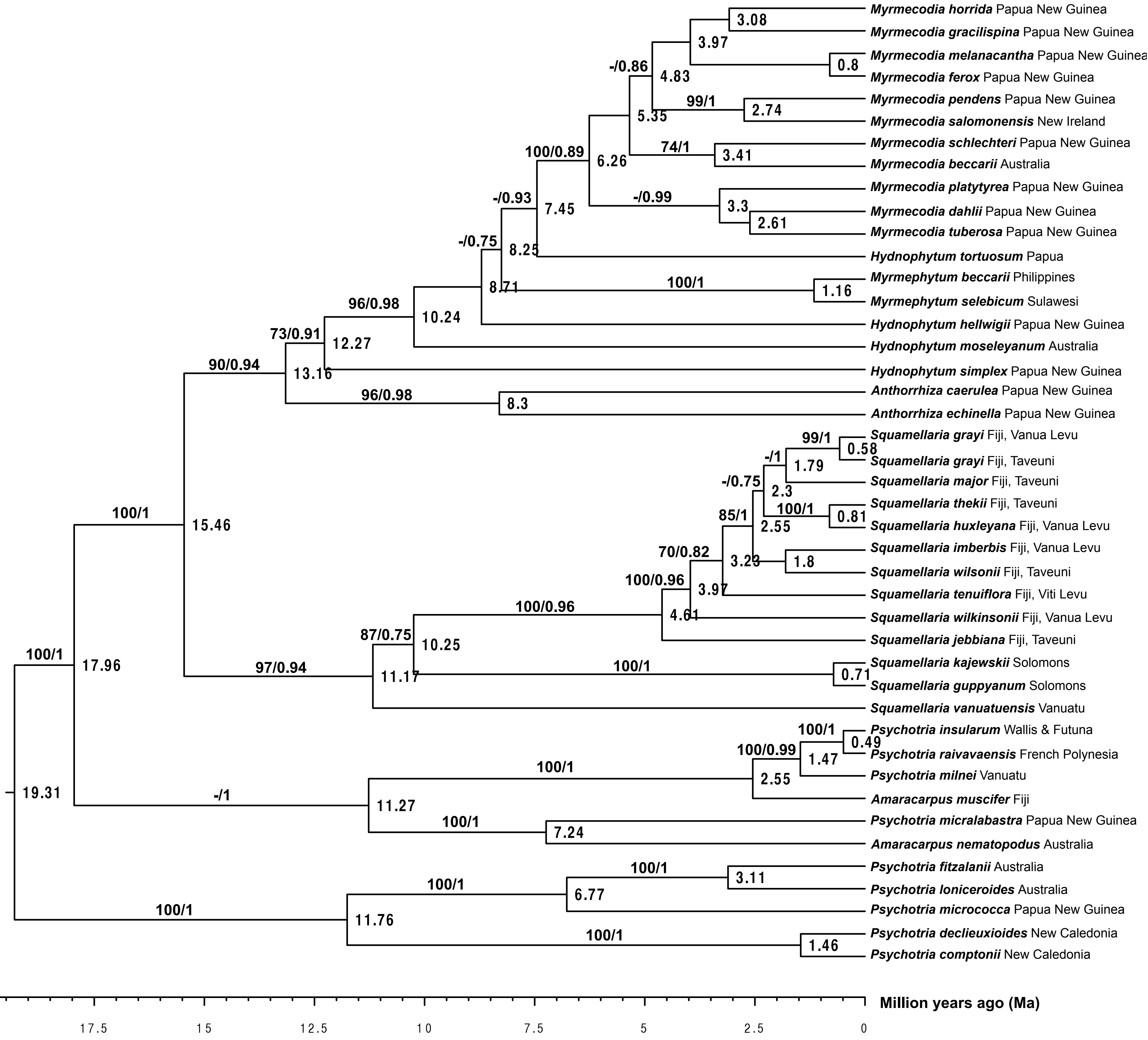


Figure S5. Dated phylogeny for the Hydnophytinae. Dates are shown at nodes. Maximum likelihood bootstrap support from RAxML and posterior probability from BEAST are shown above branches.

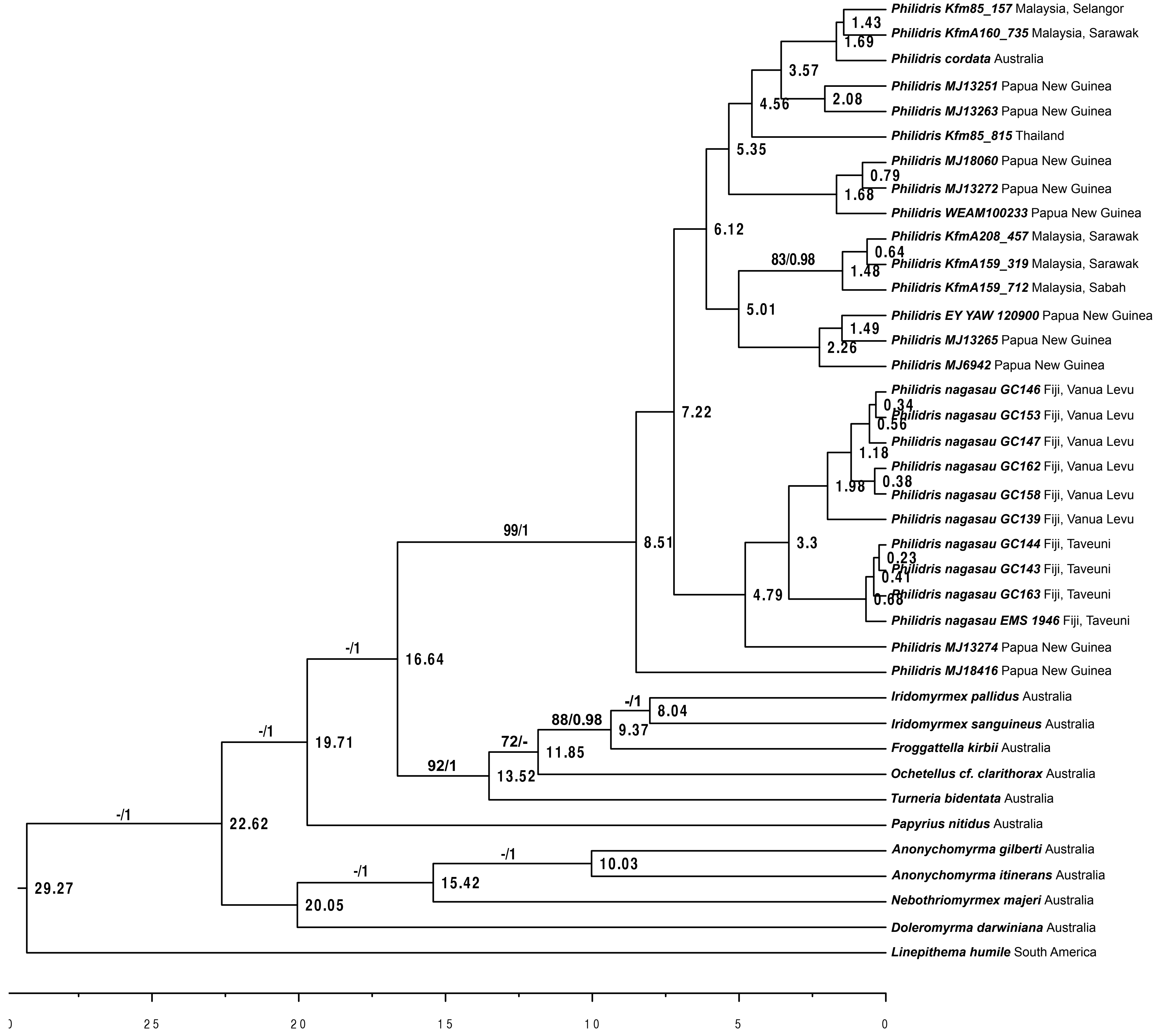


Figure S6. Dated phylogeny for *Philidris*. Ages are shown at nodes. Number above branches show the bootstrap support values from RAxML and the posterior probabilities from the BEAST dating.

Figure S7. Ancestral state reconstruction for seed dispersal in *Philidris*, performed using the R package 'Ape'.

Table S1. Occupancy, abundance and DBH of host trees for specialized (*S. imberbis*) versus facultative Squamellaria (*S. wilkinsonii*) along the transect.

Host tree species	**Family**	**mean DBH**	**Abundance**	**Occupancy Specialized S**	**Occupancy Facultative S**
Agathis macrophylla	Araucariaceae	37.5	0.0825	0.098	0.04
Aglaia sp.	Meliaceae	22	0.01	0	0
Alstonia pacifica	Apocynaceae	35	0.015	0	0
Araucariaceae sp.	Araucariaceae	25.6	0.0125	0	0.02
Atuna racemosa	Chrysobalanaceae	25	0.005	0	0
Barringtonia edulis	Lecythidaceae	35	0.0125	0	0.02
Callitris sp.	Cupressaceae	32.5	0.005	0	0
Clusia sp.	Clusiaceae	19.3	0.0325	0.0193	0.02
Cyathea lunulata	Cyatheaceae	7.3	0.08	0	0.04
Dacrydium nidulum	Podocarpaceae	20	0.0375	0.039	0.02
Decaspermum vitiense	Myrtaceae	23.1	0.0375	0	0.05
Dillenia biflora	Dilleniaceae	28	0.02	0	0.04
Dysoxylum sp.	Meliaceae	24.8	0.065	0.029	0.1
Endiandra sp.	Lauraceae	22	0.01	0	0.02
Endospermum macrophyllum	Euphorbiaceae	23	0.03	0.087	0.02
Erythrina sp.	Fabaceae	22.1	0.015	0.156	0
Fabaceae sp.	Fabaceae	27.6	0.05	0	0.06
Fabaceae sp. 2	Fabaceae	27.6	0.0175	0	0
Fagraea sp.	Gentianaceae	15.3	0.02	0	0
Ficus vitiense	Moraceae	13.7	0.0175	0	0.02
Geissois ternata	Cunoniaceae	24	0.0075	0	0.02
Homalium sp	Salicaceae	30.2	0.03	0.039	0.04

Ixora sp.	Rubiaceae	18	0.06	0.058	0.06
Loganiaceae sp	Loganiaceae	21.3	0.0675	0	0.06
Macaranga sp. 1	Euphorbiaceae	29.5	0.035	0.235	0
Macaranga sp. 2	Euphorbiaceae	24	0.025	0.176	0
Myristica sp	Myristicaceae	24.4	0.0225	0	0
Myrtaceae sp1.	Myrtaceae	14.7	0.0125	0	0
Palaquium sp.	Sapotaceae	19.8	0.0375	0	0.02
Pandanus sp.	Pandanaceae	17.4	0.02	0	0.04
Parasponia andersonii	Cannabaceae	20.7	0.035	0.156	0.05
Pinus sp.	Pinaceae	27.6	0.0475	0	0
Planchonella sp.1	Sapotaceae	30.5	0.035	0	0.05
Planchonella sp.2	Sapotaceae	24.1	0.0015	0	0.04
Vavaea sp	Lauraceae	18	0.0025	0	0

Table S2. Primers used in this study.

Plant marker	Forward	Reverse	References
ITS region	ITS1: TCCGTAGGTGAACCTGCGG	ITS4: TCCTCCGCTTATTGATATGC	White et al. (1990)
trnL-trnF	C (Fw): CGAAATCGGTAGACGCTACG E (Fw): GGTTCAAGTCCCTCTATCCC	D (Rev): GGGGATAGAGGGACTTGAAC F (Rev): ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)
ETS	Psy1: GTGTGAGTGGTAAATGGATAGC[1]	18S-ETS: ACTTACACATGCATGGCTTAATCT[2]	[1]Nepokroeff et al. (2003) [2]Baldwin and Marcos (1998)
ndhF	ndhF 1F: ATGGAACAKACATATSAATATGCTGG	ndhF 972R: CATAATATAACCCAATTGAGAC	Olmstead and Sweere (1994)
Rps16	rpsF: GTGGTAGAAAGCAACGTGCGACTT	rpsR2: TCGGGATCGAACATCAATTGCAAC	Oxelman et al. (1997)
trnS-trnG	trnG(UUC): GAATCGAACCCGCATCGTTAG	trnS(GCU): AACTCGTACAACGGATTAGCAATC	Shaw et al. (2007)
18S	NS1: GTAGTCATATGCTTGTCTC	NS2: GGCTGCTGGCACCAGACTTGC	White et al. (1990)
rps12-rpl20	5'rpS12: ATTAGAAANRCAAGACAGCCAAT	rpL20: CGYYAYCGAGCTATATATCC	Shaw et al. (2005)
Ant marker	**Forward**	**Reverse**	**References**
EFαF1	F1-1424F: GCGCCKGCGGCTCTCACCACCGAGG	F1-1829R: GGAAGGCCTCGACGCACATMGG	Brady et al. (2006)
EFαF2	F2-557F: GAACGTGAACGTGGTATYACSAT	F2-1118R TTACCTGAAGGGGAAGACGRAG	Brady et al. (2006)
CAD	CD847F: ATGAATTACGGYAATCGCGGYCAYAAYCARCC	CD1459R: GCARTTDAGAGCGGTYTGYCCRCCRAAYGT	Schmidt (2013)

Supplementary references

Baldwin BG, Markos S (1998) Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of Calycadenia (Compositae). *Mol Phylogenet Evol* 10: 449–463.

Brady SG, Schultz TR, Fisher BL, Ward PS (2006) Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences* 103: 18172-18177.

Schmidt C (2013) Molecular phylogenetics of ponerine ants (Hymenoptera: Formicidae: Ponerinae). *Zootaxa* 3647: 201-250.

Nepokroeff M, Systma KJ, Wagner WL, Zimmer EA (2003) Reconstructing ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. *Syst Biol* 52: 820–838.

Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Syst Biol*

43: 467–481.

Oxelman B, Lidén M, Berglund D (1997) Chloroplast rps16 intron phylogeny of the Sileneae (Caryophyllaceae). *Pl Syst Evol* 206: 393–410.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Am J bot* 92: 142-166.

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *Am J bot* 94: 275-288.

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol Biol* 17: 1105-1109.

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR-protocols a guide to methods and applications Edited by: Innis MA, Gelfand DH, Sninski JJ, White TJ. San Diego: Academic press; pp. 315-322.

Table S3. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	Voucher	Geographic origin	ITS	ETS	18S	trnL-trnF	ndhF	trnS-trnG	rps16	rps12-rpl20
***Amaracarpus muscifer* A.C.Sm.**	L. Barrabe & M. Tuiwawa 1109 (NOU)	Fiji	KF675907	KF675790	-	-	KF675995	-	KF676083	-
***Amaracarpus nematopodus* (F.Muell.) P.I.Forst.**	L. Barrabe et al. 1030 (NOU)	Australia	JX155060	KF675791	-	-	JX155105	-	JX155152	-
***Anthorrhiza caerulea* Huxley & Jebb**	M.P.H. Jebb 180 (FHO)	Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Anthorrhiza echinella	G. Chomicki 83 (M)	Cultivated Oxf. Bot Gard., origin New Guinea	Submitted	Submitted	-	Submitted	-	-	-	-
***Hedstromia latifolia* A.C.Sm.**	L. Barrabe et al 1090 (NOU)	Fiji	KF675911	KF675795	-	-	KF675999	-	KF676087	-
***Hydnophytum simplex* Becc.**	G. Chomicki 94 (M)	Cultivated, origin Aru Island, Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
***Hydnophytum hellwigii* Warb.**	H. Gay 487 (BM)	Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
***Hydnophytum tortuosum* Becc.**	G. Chomicki 128 (M)	Cultivated, origin Papua	Submitted	Submitted	-	Submitted	Submitted	-	-	-
***Hydnophytum moseleyanum* Becc.**	Barrabe & Rigault 1041 (NOU)	Australia	KF675912	KF675798	-	KF676176	KF676000	-	KF676088	-

Myrmecodia beccarii **Hook f.**	G. Chomicki 99 (M)	Cultivated, origin Australia	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Myrmecodia salomonensis **Becc.**	C. R. Huxley and L. M. Turton 3442 (FHO)	Solomons	Submitted	Submitted	-	Submitted	-	-	-	-
Myrmecodia dahlii **K.Schum.**	J.I. Menzies 5947 (FHO)	Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Myrmecodia schlechteri **subsp.** ***schlechteri*** **var.** ***schlechteri*** **C.R.Huxley & Jebb**	H. J. Gay 488 (FHO)	Papua New Guinea	AF071988	-	-	JN643394	-	-	-	-
Myrmecodia pendens **Merr. & L.M.Perr.**	C.R. Huxley and J. Friday 5938 (FHO)	Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Myrmecodia ferox **C.R.Huxley & Jebb**	C.R. Huxley & Matiabe UPNG 5818 FHO	Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Myrmecodia melanacantha **C.R.Huxley & Jebb**	M.P.H. Jebb 240 (L)	Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Myrmecodia horrida **C.R.Huxley & Jebb**	G. Chomicki 100 (M)	Cultivated, origin Papua New Guinea	Submitted	Submitted	-	Submitted	Submitted	-	-	-
Myrmephytum selebicum **(Becc.) Becc.**	G. Chomicki 120 (M)	Cultivated, origin Papua	Submitted	Submitted	-	Submitted	-	-	-	-
Myrmephytum beccarii **Elmer**	G. Chomicki 118 (M)	Cultivated, origin Philippines	Submitted	Submitted	-	Submitted	-	-	-	-
Psychotria comptonii **S.Moore**	L. Barrabe & Rigault 1014 (NOU)	New Caledonia	KF675927	KF675823	-	-	KF676015	-	KF676104	-

***Psychotria dallachiana* Benth.**	L. Barrabe & Rigault 1048 (NOU)	Australia	KF675928	KF675824	-	-	KF676016	-	KF676169	-
***Psychotria declieuxioides* S.Moore**	L. Barrabe & Nigote 937 (NOU)	New Caledonia	KF675932	KF675828	-	-	KF676020	-	KF676107	-
***Psychotria faguetii* (Baill.) Schltr.**	L. Barrabe et al. 820 (NOU)	New Caledonia	KF675934	KF675831	-	-	KF676023	-	-	-
***Psychotria fitzalanii* Benth.**	L. Barrabe & Rigault 1057 (NOU)	Australia	KF675935	KF675832	-	-	KF676024	-	KF676110	-
***Psychotria goniocarpa* (Baill.) Guillaumin**	L. Barrabe 586 (NOU)	New Caledonia	KF675940	KF675838	-	-	KF676029	-	KF676115	-
***Psychotria hawaiiensis* (A.Gray) Fosberg**	Y. Pillon 1425 (NOU)	Hawaii	KF675941	KF675840	-	-	KF676030	-	KF676116	-
***Psychotria hivaoana* Fosberg**	Meyer 3071 (PAP)	French Polynesia	KF675942	KF675841	-	-	KF676031	-	KF676117	-
***Psychotria insularum* A.Gray**	Y. Pillon 909 (NOU)	Wallis & Futuna	KF675943	KF675842	-	-	KF676032	-	KF676118	-
***Psychotria iteophylla* Stapf**	Axelius 303 (S)	Borneo	-	-	-	-	-	-	AF410726	-
***Psychotria loniceroides* Sieber ex DC.**	L. Barrabe & Rigault 1042 (NOU)	Australia	KF675945	KF675846	-	-	KF676033	-	KF676120	-
***Psychotria lorentzii* Valeton**	Puradyatmika 10460 (K)	Papua New Guinea	KF675946	KF675847	-	-	KF676034	-	KF676121	-

Psychotria micralabastra **(Lauterb. & K.Schum.) Valeton**	Takeuchi 16163 (K)	Papua New Guinea	KF675949	KF675851	-	-	KF676036	-	KF676124	-
Psychotria micrococca **(Lauterb. & K.Schum.) Valeton**	Drozd & Molem s.n. (PSF)	Papua New Guinea	KF675951	KF675853	-	-	KF676038	-	KF676126	-
Psychotria microglossa **(Baill.) Baill. ex Guillaumin**	L. Barrabe 585 (NOU)	New Guinea	KF675950	KF675852	-	-	KF676037	-	KF676125	-
Psychotria monanthos **(Baill.) Schltr.**	Y. Pillon 1370 (NOU)	New Caledonia	KF675953	KF675855	-	-	KF676040	-	KF676128	-
Psychotria poissoniana **(Baill.) Guillaumin**	J. Munzinger 5156 (NOU)	New Caledonia	KF675958	KF675861	-	-	KF676045	-	KF676133	-
Psychotria pritchardii **Seem.**	L. Barrabe et al 1124 (NOU)	Fiji	KF675992	KF675903	-	-	KF676078	-	KF676165	-
Psychotria raivavaensis **Fosberg**	Meyer 3088 (PAP)	French Polynesia	KF675960	-	-	-	KF676047	-	KF676135	-
Psychotria submontana **Domin**	L. Barrabe et al. 1044 (NOU)	Australia	KF675988	KF675899	-	-	-	-	KF676168	-
Psychotria temehaniensis **J.W.Moore**	Mouly 403 (P)	French Polynesia	KF675989	KF675900	-	-	KF676075	-	KF676162	-
Psychotria trisulcata **(Baill.) Guillaumin**	L. Barrabe et al. 902 (NOU)	New Caledonia	KF675990	KF675901	-	-	KF676076	-	KF676163	-
Squamellaria grandiflora **(Becc.) Chomicki, comb. nov.**	S. Vodonaivolu DA2128 (SUVA)	Fiji, Kadavu Island	-	Submitted	-	-	-	-	-	-

***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (SUVA) HOLOTYPE	Taveuni, Bouma falls, Lavena	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 47 (M)	Vanua Levu, Waisali forest park	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria guppyana* (Becc.) Chomicki, comb. nov.**	G. Chomicki 123 (M)	Cultivated, origin Solomons	Submitted	Submitted	-	Submitted	Submitted	-	-	-
***Squamellaria huxleyana* Chomicki sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 48 (SUVA) HOLOTYPE	Fiji, Vanua Levu, road between Savusavu to Labasa.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria imberbis* (A. Gray) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	Fiji, Vanua Levu, track to vodaphone tower.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria jebbiana* Chomicki, sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	Fiji, Taveuni, Mt Manuca area.	Submitted	Submitted	Submitted	EF013453	Submitted	Submitted	Submitted	Submitted
***Squamellaria kajewskii* (Merr. & L.M.Perry) Chomicki, comb. nov.**	G. Chomicki 122 (M)	Cultivated, origin Solomons	Submitted	Submitted	-	Submitted	Submitted	-	-	-
***Squamellaria major* A.C. Sm.**	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	Fiji, Taveuni, road to DesVoeux peak.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	-	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria thekii* Jebb**	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	Fiji, Taveuni, road to DesVoeux peak.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria vanuatuensis* (Jebb & Huxley) Chomicki, comb. nov.**	McPherson 19437 (P)	Vanuatu	JX155078	-	-	-	-	-	JX155170	-

***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	Fiji, Vanua Levu, Waisali forest park.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 49 (M)	Fiji, Vanua Levu, Waisali forest park.	Submitted	Submitted	-	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	Fiji, Vanua Levu, Waisali forest park.	Submitted	Submitted	-	Submitted	Submitted	Submitted	Submitted	Submitted
***Squamellaria wilsonii* (Horne ex Baker) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	Fiji, Taveuni, road to DesVoeux peak.	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted

Table S4. Ant material included in this study. Information for CASENT vouchers can be found in ant web: https://www.antweb.org.

Taxon	**Voucher**	**Geographic origin**	**CAD**	**EFαF1**	**EFαF2**
Philidris sp.	Kfm85_157	Malaysia, Selangor	-	Submitted	-
Philidris sp.	KfmA160_735	Malaysia, Sarawak	-	Submitted	Submitted
Philidris cordata	CASENT0106011	Australia, Queensland	FJ939937	EF013320	EF013482
Philidris sp.	MJ13251	Papua New Guinea	Submitted	Submitted	Submitted
Philidris sp.	MJ13263	Papua New Guinea	Submitted	Submitted	Submitted
Philidris sp.	Kfm85_815	Thailand	-	Submitted	-
Philidris sp.	MJ18060	Papua New Guinea	Submitted	Submitted	Submitted
Philidris sp.	MJ13272	Papua New Guinea	Submitted	Submitted	Submitted
Philidris sp.	WEAM100233	Papua New Guinea	Submitted	Submitted	Submitted

Philidris sp.	KfmA208_457	Malaysia, Sarawak	-	Submitted	-
Philidris sp.	KfmA159_319	Malaysia, Sarawak	-	Submitted	-
Philidris sp.	KfmA159_712	Malaysia, Sabah	-	Submitted	-
Philidris sp.	EY YAW 120900	Papua New Guinea	AF071988	Submitted	Submitted
Philidris sp.	MJ13265	Papua New Guinea	Submitted	Submitted	Submitted
Philidris sp.	MJ6942	Papua New Guinea	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC146	Fiji, Vanua Levu	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC153	Fiji, Vanua Levu	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC147	Fiji, Vanua Levu	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC162	Fiji, Vanua Levu	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC158	Fiji, Vanua Levu	Submitted	Submitted	Submitted

Philidris nagasau Mann (1921)	GC144	Fiji, Taveuni	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC163	Fiji, Taveuni	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	EMS 1946	Fiji, Taveuni	Submitted	Submitted	Submitted
Philidris sp.	MJ13274	Papua New Guinea	Submitted	Submitted	Submitted
Philidris sp.	MJ18416	Papua New Guinea	Submitted	Submitted	Submitted
Iridomyrmex pallidus	CASENT0106152	Australia, Queensland	FJ939922	FJ940066	FJ939969
Iridomyrmex sanguineus	AIMI	Australia	FJ939923	FJ940067	FJ939970
Froggattella kirbii	CASENT0009944	Australia, Queensland	FJ939919	FJ940063	FJ939966
Ochetellus cf. clarithorax	CASENT0106166	Australia, Queensland	FJ939935	FJ940071	FJ939974
Turneria bidentata	CASENT0106019	Australia, Queensland	FJ939946	EF013365	EF013527
Papyrius nitidus	CASENT0106012	Australia, Queensland	FJ939936	EF013314	EF013476

Anonychomyrma gilberti	CASENT0106003	Australia, Queensland	FJ939895	EF013222	EF013384
Anonychomyrma itinerans	CASENT0009959	Australia, Western Australia	FJ939896	FJ940044	FJ939947
Nebothriomyrmex majeri	CASENT0106174	Australia, Western Australia	FJ939933	FJ940070	FJ939973
Doleromyrma darwiniana	CASENT0009949	Australia, AUST ACT	FJ939908	FJ940055	FJ939958
Linepithema humile	CASENT0106119	Argentina, Santa Fe	-	EF013277	EF013439

| Chapter 6 |

Altitude drives mutualism breakdown, leading to accelerated morphological evolution

Guillaume Chomicki and Susanne S. Renner

Unpublished manuscript

.

Title: **Altitude drives mutualism breakdown, leading to accelerated morphological evolution**

Authors: Guillaume Chomicki* and Susanne S. Renner

Affiliation:
[1]Department of Biology, Systematic Botany and Mycology, Menzinger Str. 67,
University of Munich (LMU), Munich 80638

Author for correspondence:

Guillaume Chomicki

Email: guillaume.chomicki@gmail.com

Abstract [185]

Mutualisms that involve symbioses among specialized partners may be more stable than facultative ones, and theoretical models predict that mutualists exert reciprocal stabilizing selection on traits directly involved in the interaction. A corollary is that mutualism breakdown should increase morphological rates of evolution. We here use the largest ant-plant clade (Hydnophytinae), with different levels of dependency on mutualistic ant symbionts, to study the ecological context of mutualism breakdown and the response of a key symbiosis-related trait, domatium entrance hole size (filtering 'allowed in' symbionts). Our analyses support three predictions from mutualism theory. First, all ten losses of mutualism occurred from a facultative symbiotic state. Second, breakdowns occur where symbionts are scarce, here at high altitudes. Thirdly, domatium entrance hole size barely changes in obligate symbiotic species, but evolves rapidly once symbiosis with specialized ants has broken down, with a 'morphorate map' revealing that hotspots of entrance hole evolution are clustered in high altitude areas. This suggests that partners' relative abundances is an important component controlling the evolutionary direction in which a facultative symbiosis will evolve (either dissolving or becoming obligate), here with entrance diameter evolution mediating symbiotic strategy.

mutualism | symbiosis | phylogeny | plants | ants

Significance [119]

Some epiphytes gain most of their nutrients from ants that nest in plant-provided cavities, accessible *only* through plant-formed entrance holes. We use a large clade of ant-epiphytes to study when mutualisms break down and how this affects the symbiont filtering system. Results support three theoretical predictions: (i) evolutionary returns to an ant-free state occur where partners are rare, in our system at high altitudes; (ii) only facultative symbioses successfully returned to a non-ant-associated state; and (iii) the rate of hole diameter evolution increases drastically after release from stabilizing selection exerted by mutualistic ants. This suggests that partners' relative abundances determine mutualistic strategy and explains the convergent evolution of ant/plant symbioses in tropical lowlands where plant-nesting ants are abundant.

Introduction

Understanding how mutualisms arise, persist, or break down is a major focus in ecology and evolutionary biology (Sachs et al., 2004; Sachs and Simms, 2006; Frederickson, 2013). Symbiotic mutualisms can revert to the free-living state if the benefit-cost ratio shifts so that costs outweigh benefits. There are three main pathways through which mutualism can break down, namely extinction of the partner, reversion to autonomy, or shift to parasitism (Sachs and Simms, 2006). In obligate mutualisms, scarcity of a partner can result in extinction, but in facultative mutualisms, it can lead to a reversion to autonomy (Vandermeer and Boucher, 1978; Keeler, 1985; Schemske and Lande, 1985; Holland et al., 2004; Fosters and Wenseleers, 2006). Partner abundances may be especially important in laterally transferred mutualisms, where partners have to first find each other to re-establish their interaction at each generation, involving vulnerable un-associated stages for both. Mutualisms can also break down by shifting to parasitism. Such shifts are predicted by theory (Trivers, 1971; Axelrod and Hamilton, 1981) because reducing reciprocation can increase the fitness of the cheating partner, and there are several well-documented examples (e.g., Pellmyr et al., 1996; Bidartondo and Bruns, 2001; Machado et al., 2001; Als et al., 2004). While shifts to parasitism may be rare (Sachs and Simms, 2006), unrelated exploiters may also disrupt a mutualism by parasitizing it (Bronstein et al., 2003; Wilson et al., 2003). Finally, mutualism can also breakdown if benefits can be obtained cheaply or freely from the environment, for example, when plants involved in mycorrhizal or rhizobia symbioses grow in nutrient-rich soils (Allen, 1991; Sprent, 2001), or when defence by mutualists is no longer required (Palmer et al., 2010). Here we use a species-rich and diverse ant/plant interaction system to study the ecological context under which breakdown of symbiotic mutualisms occurs on the geologic timescale.

Ant/plant mutualisms are ubiquitous in tropical ecosystems and encompass a wide range of strategies (Davidson and Epstein, 1989; Davidson and McKey, 1993; Chomicki and Renner, 2015). In Australasia, the majority of ant-plants are epiphytes and appear to be primarily involved in trophic mutualisms, rather than defence mutualisms (Davidson and Epstein, 1989; Chomicki and Renner, 2015). An epiphytic habit means uneven water and nutrient supplies (Laube and Zotz, 2003), and mutualisms with plant-nesting ants that provide detritus and faeces for their host (Benzing, 1970; Huxley, 1978; Rickson, 1979; Rico-Gray et al., 1989; Gay, 1993; Tredeser et al., 1995; Gegenbauer et al., 2012) are therefore overrepresented among epiphytes (Chomicki and Renner, 2015). These mutualistic symbioses range from facultative interactions involving many arboreal ant species to obligate interactions that can be species-specific (Huxley, 1978; Gay, 1993; Treseder et al., 1995; Chomicki and Renner, 2016).

We here use the World's most species-rich epiphytic ant-plant clade, the Hydnophytinae subtribe of the Rubiaceae (Chomicki and Renner, 2015), to study the occurrence and breakdown of mutualistic strategies and how this affects rates of morphological evolution. The Hydnophytinae comprise ca. 100 epiphytic species in Australasia. They produce large characteristic ant-housing structures (domatia) that result from a modified hypocotyl with a network of galleries (Fig. 1B-F). Three strategies are present: obligate or specialized ant-plants, where species associate consistently with one or few species of ants; facultative ant-plants, where plants sometimes, but not always, associate with generalist ants; and finally species that form no associations with ants. Theoretical models predict that mutualists exert stabilizing selection on each other, notably to maintain trait-matching phenotypes (Thompson, 2005; Kopp and Gavrilets, 2006; Yoder and Nuismer, 2010; Raimundo et al., 2014). A corollary is that loss of mutualistic interactions, especially obligate ones among specific partners, will relax selection on traits previously involved in the interaction. To probe this expectation, we investigated the rate of morphological evolution of a pivotal mutualism-related trait: the diameter of domatium entrance holes. These holes filter the type of animal that will inhabit a domatium. When they are large, the domatium can contain a range of invertebrates, such as spiders or cockroaches, and even small vertebrates, such as gecko that lays eggs inside the domatium (Fig. 2) or frogs whose tadpoles develop in rainwater-filled domatia (Fig.1F, 2F).

The size of the Hydnophytinae clade and array of domatium types and symbioses suit it for investigating shifts between strategies. Specifically, we address three questions: (*i*) Are mutualism losses associated with particular ancestral states (such as facultative or obligate symbiosis)? (*ii*) Are losses associated with a particular ecological context, for example shifts to habitats where partners are scarcer or where nutritional resources are freely available? (*iii*) Given the role of the domatium entrance holes as a filter for ‘permitted’ mutualists, how do shifts in strategies affect the rate of change in the size of these holes?

Results and discussion

Recurrent losses of symbioses in facultative symbioses

Our matrix of six plastid and nuclear markers (*ndhF, trnH-psbA, trnL intron, trnL-trnF spacer, ITS, ETS*) includes 75% of the 104 species of Hydnophytinae and yields a statistically strongly supported tree in both ML and Bayesian analyses (Fig. S1). Consistent with our previous analysis (Chomicki and Renner, 2016), we found a sister relationship between two clades: a Pacific clade comprising all 12 species of *Squamellaria* and an Australasian clade of species in the genera *Anthorrhiza*, *Myrmephytum* and *Myrmecodia*, together nested within the paraphyletic genus *Hydnophytum*. Stochastic mapping and Bayesian ancestral state reconstructions (*Materials and Methods*) yielded facultative symbiosis as the ancestral state in the clade. We inferred 4-5 subsequent specializations of the symbioses in *Squamellaria*, *Anthorrhiza* (1 or 2), and *Myrmephytum* and *Myrmecodia*, involving preferential interactions with the dolichoderine genera *Philidris* and *Anonychomyrma* (Huxley, 1978; Jebb, 1985; Huxley and Jebb, 1991a; 1991b; 1993; Chomicki and Renner, 2016).

We inferred at least ten losses of symbiosis with ants (at nodes in the phylogeny including 17 of the 23 species that lack association with ants), always from facultative ant-plant ancestors (Fig. 1A, blue rectangles). Computed-tomography scanning revealed distinct domatium structures for the three strategies, with apically growing domatia in the specialized ant-plant *Myrmecodia* and diffusely growing domatia in the facultative and non-ant associated species, the latter typically having larger bulbous cavities and entrance holes (Fig. 1F-I; Movies S1-S4).

Relaxed selective constraints on entrance diameter after loss of mutualism

Ancestral state reconstruction for entrance hole diameter showed that the loss of obligate mutualism with ants is accompanied by increases in entrance hole size (Fig. S2A). Most species that have lost symbiosis have large entrance holes (Fig. 2D-F) and are inhabited by a range of invertebrates (cockroaches, millipedes, *Peripatus* worms, spiders, slugs, leeches) and small vertebrates (frogs, skinks, geckos; Figs. 1F, 2E). We used BAMM (Rabosky, 2014) to investigate the rates of morphological evolution (morphorate). We extracted the tip morphorates for each species (*Materials and Methods*) based on the best shift configuration (Fig. S2B, S7) and then fitted a logistic model to obtain a probabilistic framework linking morphorate to mutualistic strategy (Fig. 2A; t=1.203, AIC=282.29). This revealed that species that have lost symbiosis with ants have the highest morphorates, while obligate species have the lowest morporates, with facultative ones in-between (Fig. 2G, left inset, Fig. S3). We confirmed these results with a hierarchical Bayesian analysis accounting for phylogenetic autocorrelation (Fig. 3G, right inset). Morphorate-through-time analysis showed that specialized clades (*Myrmecodia* and obligate *Squamellaria* clade) have low morphological change rates as compared to the rest of the Hydnophytinae (Fig. 2G). This provides support for the prediction that in specialized mutualisms, traits involved in the interaction are under stabilizing selection (Thompson, 2005; Kopp and Gavrilets, 2006; Yoder and Nuismer, 2010; Raimundo et al., 2014).

Since seven of the ten losses of mutualism coincide with occurrence at high altitudes (Fig. S4), we investigated the geography of evolutionary rate of entrance holes, using a novel method that couples species-based morphorates inferred from BAMM and georeferenced specimen-based species' distribution (*Materials and Methods*). The resulting 'morphorate map' reveals that hotspots of entrance hole evolution are strongly clustered in the high altitude areas of Papua New Guinea (Fig. 3). Morphological evolution can accelerate when selection on a trait is removed and the body plan is free to change if developmental constraints are limited (Barkman et al., 2008; Davis et al., 2014) or it can slow down when ecological opportunity diminishes simultaneously (Mahler et al., 2010). Further field data are needed to resolve whether large domatium entrance holes, such as present in the minimally three species housing the frog *Cophixalus riparius*, are the result of directional selection or a by-product of simple tuber growth, leading to larger holes.

Loss of mutualism with ants coincides with shifts to montane habitats

The inferred minimally ten losses of ant symbiosis –involving single species or the ancestors of clades comprising 2 to 4 species– prompted us to study their ecological context. Of the 23 species, 17 are highland species from Papua New Guinea living between 1500 and over 3500 meters elevation. Three of these species have domatia that fill with rainwater and then harbor *C. riparius* at 1900-2600 meter (Jebb, 1985; 1991; Fig. 1F-G). This frog is endemic from Papua New Guinea, where it occurs above 1,900 m (IUCN, 2015). Six of the 23 species that have lost mutualistic interactions may obtain nutrients more cheaply from soil, but two specialized ant-plant species (*Myrmecodia lamii* and *M. brassii*) also often grow terrestrially and still have obligately occupied domatia (Huxley, 1978; Huxley and Jebb, 1993), suggesting that mutualism loss in these six species was not driven by return to the terrestrial habit.

To evaluate whether mutualism breakdown coincides with occurrence at higher altitude, we recorded the mean and the maximum altitude for each species and asked whether there were significant differences between the three-mutualism categories (facultative, specialized, and loss) encountered in the group. We found significant differences between the groups (one-way ANOVA, F=17.38, $P > 0.01$), with species that have lost symbiosis with ants growing at significantly higher elevation (Tukey's post-hoc test, $P = 1E-07$). A multinomial logistic regression ($t = 4.42$, $AIC = 233.1$) identified a positive exponential relationship between increasing altitude and the probability of having a 'loss' type of tuber, as opposed to facultative symbiosis, which showed the reverse pattern (Fig. 4A). We found similar relationships using mean altitude calculated for all taxa from over 1,000 herbarium records (Fig. S7; Table S1). We confirmed these results with a hierarchical Bayesian analysis that accounted for phylogenetic autocorrelation (Fig. 4A, inset; *Materials and Methods*).

To further investigate the apparent loss of ant symbioses with altitude, we performed ML ancestral state reconstruction of species' altitudinal niches, coding both maximum and mean altitude based on the same species' distribution data as before (*Materials and Methods*). Results confirmed that mutualism loss was associated with occurrence at higher elevations (Fig. S5). We further performed ancestral biome reconstruction (with the states tropical rainforest <1500 m alt., montane habitat $\geq$1500 m alt., savanna, and mangrove), using stochastic mapping (*Materials and Method*s), which revealed that seven of the ten losses of mutualisms with ants coincide with a shift to the montane biome (Fig. S4). Altogether, these approaches show that loss of mutualism with ants was associated with shifts to higher elevations.

To confirm that facultative ant plant species and non-ant-associated species have different niches, we evaluated the niche space occupied by each strategy using the same herbarium records and non-dimensional metric scaling (NDMS). We first performed an analysis using all Hydnophytinae species, which revealed that the 95% confidence clusters of facultative versus non-ant-associated species did not overlap and that both are nested within the larger cluster of obligate ant-plants, as expected (Fig. S7). We next repeated the analysis using the species sampled in our tree (~75%), plotted in a phylomorphospace (*Materials and Methods*), which showed the same pattern (Fig. 4B). The larger climatic niche space for obligate species results from their principal ant partners occupying different niches. *Philidris* ants occupy mostly plants in lowland to middle elevation savannah or disturbed forest while *Anonychomyrma* species occupy mostly lowland to mid-elevation rainforest or montane habitats (Huxley, 1978; Jebb, 1985; Huxley and Jebb, 1993).

The more restricted niche space of facultative ant-plant species compared to obligate ones is at first puzzling because their partner can potentially be any arboreal ant-plant species (from 17 ant genera; Jebb, 1985). However, ant species richness and abundance both decrease with increasing elevation (Longino et al., 2014; Gillette et al., 2015), likely because of the decreasing temperature (Sanders et al., 2007). Partner rarity is thought to lead to the local extinction of obligate mutualistic partnerships and the reversion to the free-living state in facultative ones (Vandermeer and Boucher, 1978; Keeler, 1985; Holland et al., 2004; Fosters and Wenseleers, 2006). In facultative ant/plant symbioses, nesting space limitation may determine the extent to which generalist ants nest inside domatia. Such nesting limitation has been shown in lowland tropical forests (Wilson, 1959; Philpott and Fosters, 2005) and has long been thought to be a driver for the evolution of ant plants in lowland tropics (Davidson and McKey, 1993; Chomicki and Renner, 2015).

Conclusion

In symbiotic mutualisms, symbiont abundance is known to increase host fitness as well as symbiont fitness, involving partner fidelity feedbacks (Agrawal and Karban, 1997; Strack et al., 2003; Backhed et al., 2005; Fosters and Wenseleers, 2006). Hydnophytinae provide an example for how a decrease in partners' abundances at higher altitudes, over evolutionary time scales has driven the loss of ant/plant symbiosis, by releasing a key mutualistic trait previously under selection by ant body size, namely domatium entrance holes. Our study suggests that shifts in mutualism strategies control the pace of morphological changes in partners and therefore also affect mutualistic strategy itself.

Acknowledgements

We thank J. Aroles for proofreading the manuscript, M. Jebb, C. Huxley-Lambrick for discussion, and A. Wistuba for plant material, O. Pérez for help with the map, and C. Zohner for help with the Bayesian hierarchical model. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20, and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to GC.

References

Allen MF (1991) *The Ecology of Mycorrhizae*. Cambridge University Press, Cambridge, UK.

Als TD, Vila R, Kandul NP, Nash DR, Yen SH, Hsu YF, Mignault AA, Boomsma JJ, Pierce NE (2004) The evolution of alternative parasitic life histories in large blue butterflies. *Nature* 432(7015):386-390.

Axelrod R, Hamilton WD (1981) The evolution of cooperation. *Science* 211(4489):1390-1396.

Barrabé L, Maggia L, Pillon Y, Rigault F, Mouly A, Davis AP, Buerki S (2014) New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Mol Phylogenet Evol* 71:15-35.

Barkman TJ, Bendiksby M, Lim SH, Salleh KM, Nais J, Madulid D, Schumacher T, (2008) Accelerated rates of floral evolution at the upper size limit for flowers. *Curr Biol 18*(19):1508-1513.

Benzing DH, Burt KM (1970) Foliar permeability among twenty species of the Bromeliaceae. *Bull Torrey Bot Club* 97(5):269-279.

Bidartondo MI, Bruns TD (2001) Extreme specificity in the epiparasitic Monotropoideae (Ericaceae). *Mol Ecol* 10(9):2285–2295.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol 10*(4):e1003537.

Bronstein JL, Wilson WG, Morris WF (2003) Ecological dynamics of mutualist/antagonist communities. *Am Nat 162*(S4):S24-S39.

Chomicki G, Renner SS (2015) Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytol* 207(2):411-424.

Chomicki G, Renner SS (2016) Evolutionary relationships and biogeography of the ant-epiphytic genus *Squamellaria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *PloS one* 11(3):e0151317.

Chomicki G, Ward PS, Renner SS (2015) Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. *Proc. R. Soc. B* 282(1819):20152200).

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat methods 9*(8):772-772.

Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant/plant relationships. Journal of Hymenopteran Research 2(1):13–83.

Davidson DW, Epstein WW (1989) Epiphytic associations with ants. In *Vascular plants as epiphytes* (pp. 200-233). Springer Berlin Heidelberg.

Davis CC, Schaefer H, Xi Z, Baum DA, Donoghue MJ, Harmon LJ (2014) Long-term morphological stasis maintained by a plant–pollinator mutualism. *Proc Natl Acad Sci USA 111*(16):5914-5919.

de Villemereuil P, Wells JA, Edwards RD, Blomberg SP (2012) Bayesian models for comparative analysis integrating phylogenetic uncertainty. *BMC Evolut Biol* 12(1): 102.

ESRI E. ArcMap 9.3. ArcGIS 9, 1999-2008.

Foster KR, Wenseleers T (2006) A general model for the evolution of mutualisms. *J Evol Biol* 19:1283-1293.

Frederickson ME (2013) Rethinking mutualism stability: cheaters and the evolution of sanctions. *Q Rev Biol 88*(4):269-295.

Fridley JD, Craddock A (2015) Contrasting growth phenology of native and invasive forest shrubs mediated by genome size. *New Phytol 207*(3):659-668.

Gathmann A, Tscharntke T (2002) Foraging ranges of solitary bees. *J Anim Ecol* 71(5):757–764.

Gay H. (1993) Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw.(Polypodiaceae). *Biol J Linn Soc 50*(3):221-233.

Gegenbauer C, Mayer VE, Zotz G, Richter A (2012) Uptake of ant-derived nitrogen in the myrmecophytic orchid *Caularthron bilamellatum*. *Ann Bot 110*(4):757-766.

Gelman A, Hill J (2007) Data analysis using regression and multilevel/hierarchical models. Cambridge, UK: Cambridge University Press.

Gillette PN, Ennis KK, Domínguez Martínez G, Philpott SM (2015) Changes in species richness, abundance, and composition of arboreal twig-nesting ants along an elevational gradient in coffee landscapes. *Biotropica 47*(6):712-722.

Hijmans RJ, Van Etten J (2013) Raster: geographic data analysis and modeling. R package version 2.1-49.

Holland JN, DeAngelis DL, Schultz ST (2004) Evolutionary stability of mutualism: interspecific population regulation as an evolutionary stable strategy. *Proc R Soc B* 271(1550):1807–1814.

Huxley CR, Jebb MHP (1991) The tuberous epiphytes of the Rubiaceae 3: A revision of *Myrmephytum* to include *Myrmedoma*. *Blumea* 36(1):43-52.

Huxley CR, Jebb MHP (1991) The tuberous epiphytes of the Rubiaceae 2: the new genus *Anthorrhiza*. *Blumea* 36(1):21-41.

Huxley CR, Jebb MHP (1993) The tuberous epiphytes of the Rubiaceae 5. A revision of *Myrmecodia*. *Blumea* 37(2):271-334.

Huxley CR (1978) The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* 80(1):231-268.

I Marín XF (2013) ggmcmc: Graphical tools for analyzing Markov Chain Monte Carlo simulations from Bayesian inference. *URL: http://xavier-fim. net/packages/ggmcmc*

IUCN (2015) http://www.iucnredlist.org/details/57785/0

Janzen DH (1974) Epiphytic myrmecophytes in Sarawak: mutualism through the feeding of plants by ants. *Biotropica* 6(4):237-259.

Jebb MHP (1985) *Taxonomy and tuber morphology of the rubiaceous Ant-Plants*. Doctoral dissertation, University of Oxford.

Jebb MHP (1991) Cavity structure and function in the tuberous Rubiaceae. pp. 374-390. In Huxley CR and Cutler DF eds. *Ant-plant interactions*. Oxford: Oxford University Press.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol 30*(4):772-780.

Keeler KH (1985) Cost:benefit models of mutualism. In The Biology of Mutualism, Ecology and Evolution (Boucher, D.H., ed.), pp. 100–127, Oxford University Press.

Kopp M, Gavrilets S (2006) Multilocus genetics and the coevolution of quantitative traits. *Evolution 60*(7):1321-1336.

Laube S, Zotz G (2003) Which abiotic factors limit vegetative growth in a vascular epiphyte? *Funct Ecol 17*(5):598-604.

Longino JT, Branstetter MG, Colwell RK (2014) How ants drop out: ant abundance on tropical mountains. *PloS one* 9(8):e104030.

Machado CA, Jousselin E, Kjellberg F, Compton SG, Herre EA (2001) Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proc R Soc B* 268(1468):685-694.

Maddison WP, Maddison DR (2011) Mesquite: a modular system for evolutionary analysis. Version 2.75. Available at: *mesquiteproject.org/mesquite/download/download.html*.

Mahler DL, Revell LJ, Glor RE, Losos JB (2010) Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution 64*(9):2731-2745.

Margulis L, Fester R (1991) *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. MIT Press, Cambridge, MA, USA.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H, Oksanen MJ (2013) Package 'vegan'. *Community ecology package, version*, *2*(9).

Pagel M, Meade A (2013) BayesTraits, version 2. *Univ. of Reading, Berkshire, UK Available at http://www.evolution.rdg.ac.uk*

Palmer TM, Stanton ML, Young TP, Goheen JR, Pringle RM, Karban R (2008) Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. *Science* 319(5860):192-195.

Pellmyr O, Leebens-Mack J, Huth CJ (1996) Non-mutualistic yucca moths and their evolutionary consequences. *Nature* 380:155-156.

Philpott SM, Foster PF (2005) Nest-site limitation in coffee agroecosystems: artificial nests maintain diversity of arboreal ants. *Ecol Appl* 15(4): 1478–1485.

Plummer M (2003) JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. In: Hornik K, Leisch F, Zeileis A, eds. Proceedings of the 3rd International Workshop on Distributed Statistical Computing (DSC 2003). Vienna, Austria: Achim Zeileis.

Rabosky DL (2014) Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PloS one 9*(2):e89543.

Rabosky DL, Grundler M, Anderson C, Shi JJ, Brown JW, Huang H, Larson JG (2014) BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods Ecol Evol* 5(7): 701-707.

Rabosky DL, Santini F, Eastman J, Smith SA, Sidlauskas B, Chang J, Alfaro ME (2013) Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat Commun* 4:1958.

Raimundo RL, Gibert JP, Hembry DH, Guimaraes Jr PR (2014) Conflicting selection in the course of adaptive diversification: the interplay between mutualism and intraspecific competition. *Am Nat* 183:363-375.

Rambaut, A., Suchard, M., Xie, W. and Drummond, A., 2014. Tracer v. 1.6. Institute of Evolutionary Biology, University of Edinburgh.

Revell LJ (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3:217-223.

Rickson FR (1979) Absorption of animal tissue breakdown products into a plant stem-the feeding of a plant by ants. *Am J Bot* 66: 87-90.

Rico-Gray V, Barber JT, Thien LB, Ellgaard EG, Toney JJ (1989) An unusual animal-plant interaction: feeding of *Schomburgkia tibicinis* (Orchidaceae) by ants. *Am J Bot* 76(4):603-608.

Sachs JL, Simms EL (2006) Pathways to mutualism breakdown. *Trends Ecol Evol 21*(10):585-592.

Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. *Q Rev Biol 79*(2):135-160.

Sanders NJ, Lessard JP, Fitzpatrick MC, Dunn RR (2007) Temperature, but not productivity or geometry, predicts elevational diversity gradients in ants across spatial grains. *Global Ecol Biogeo* 16(5):640-649.

Schemske DW, Lande R (1985) The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39(1): 41-52.

Schluter D (2000) *The ecology of adaptive radiation*. Oxford Univ Press, New York, 288 pp.

Sprent JI (2001) *Nodulation in Legumes*. Royal Botanic Gardens.

Su Y-S, Yajima M (2014) R2jags: a package for running JAGS from R. R package version 0.04-03. [WWW document] URL http://CRAN.R-project.org/ package=R2jags.

Thompson,JN (2005) *The geographic mosaic of coevolution*. University of Chicago Press.

Treseder KK, Davidson DW, Ehleringer JR (1995) Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature 375*(6527):137-139.

Trivers RL (1971) The evolution of reciprocal altruism. *Q Rev* Biol 46(1):35-57.

Vandermeer JH, Boucher DH (1978) Varieties of mutualistic interaction in population models. *J Theor Biol* 74(4):549–558.

Wickham H (2009) *ggplot2: elegant graphics for data analysis*. Springer Science & Business Media.

Wilson EO (1959) Some ecological characteristics of ants in New Guinea rainforests. *Ecology* 40(3):437-447.

Wilson WG, Morris WF, Bronstein JL (2003) Coexistence of mutualists and exploiters on spatial landscapes. *Eco Monogr* 73(3):397-413.

Yoder JB, Nuismer SL (2010) When does coevolution promote diversification? *Am Nat* 176(6):802-817.

Materials and Methods

Taxon sampling, DNA extraction and phylogenetic analyses

We generated a matrix of six markers (nuclear ITS and ETS and plastid *ndhF*, *psbA-trnH*, *trnL* intron and *trnL-trnF* spacer), sampling 76 species out of *ca.*106 Hydnophytinae species. We sampled all 12 *Squamellaria* species recognized by Chomicki and Renner (2016), 5 of the 8 species of *Anthorrhiza* recognized by Huxley and Jebb (1991), 32 of the 51 *Hydnophytum* species recognized by Jebb and Huxley in an unpublished revision, 4 of the 5 described species of *Myrmephytum* (Huxley and Jebb, 1991b) and 19 of the 26 *Myrmecodia* species recognized by Huxley and Jebb (1993). In addition to these 72 species, we also sequenced 4 species that were previously synonymized but stand as good species in light of molecular data. The new *Hydnophytum* species have been long described by Jebb, distributed into many herbaria, but never formally published. We refer to them as *Hydnophytum* followed by the voucher name, and provide the name (nomen nudum) in the supplementary table S2. A sampling of outgroups (in the tribe Psychotrieae) was selected based on Barrabé et al. (2014). Voucher information is reported in Table S2.

Total genomic DNA was extracted from c. 20 mg of leaf tissues, using a commercial plant DNA extraction kit (NucleoSpin; Macherey–Nagel, Düren, Germany) according to manufacturer protocols. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England Biolabs, Cambridge, MA, USA) and a standard protocol (39 cycles, annealing temperature 56^0C). PCR products were purified using the ExoSap clean-up kit (Fermentas, St Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were BLAST-searched in GenBank. Sequence alignment was performed in MAFFT v. 7 in the online server (http://mafft.cbrc.jp/alignment/server) (Katoh and Standley, 2013) under standard parameters except for the ITS region, which was aligned under Q-INS-i optimization, which takes rRNA secondary structure into consideration. Minor alignment errors were corrected manually in Mesquite v. 2.75 (Maddison and Maddison, 2011). In the absence of statistically supported incongruence (i.e., maximum likelihood bootstrap (BS) support >75) between the plastid and nuclear data partitions), we concatenated all markers, yielding an alignment of 3055 bp. Maximum-likelihood (ML) inference relied on RAxML v8.0 (Stamatakis, 2014) and the GTR + Γ substitution model, with empirical nucleotide frequencies and 25 gamma rate categories; bootstrap support was assessed from 100 replicates under the same model. We also conduced Bayesian inference in MrBayes v. 3.2 (Ronquist et al., 2012) under the substitution model selected by jmodeltest2 (Darriba et al., 2012), and using the program's default two runs and four chains (one cold and three heated), with the uniform default priors. We set a $10X10^6$ MCMC chain, sampling trees every 1000th generation. Split frequencies approaching zero indicated convergence. We used the 50% consensus tree to assess posterior probabilities for nodes of interest.

Molecular clock dating

Molecular dating analyses relied on BEAST v. 2 (Bouckaert et al., 2014) and uncorrelated lognormal relaxed clock models. We used the GTR + G substitution model with four rate categories and a Yule tree prior. For both our plant and ant trees, MCMCs were run for 20 million generations, with parameters and trees sampled every 10,000 generations. We used Tracer v. 1.6 (Rambaut et al., 2014) to check that the effective sample size (ESS) of all parameters was >200, indicating that runs had converged. After discarding 10% as burn-in, trees were summarized in TreeAnnotator

v. 1.8 (part of the BEAST package) using the options ‘maximum clade credibility tree’, which is the tree with the highest product of the posterior probability of all its nodes, ‘mean node height,’ and a posterior probability limit of 0.98. The final tree was visualized in FigTree v. 1.4 (Rambaut, 2012). To calibrate our tree, we constrained the age of the root, i.e., the split between the Pacific clade and the so-called *Psychotria* clade IV of Barrabé et al. (2014), to 22 ± 7 Million years, based on the age of this node estimated by these authors, using a normal prior and a standard deviation of 4 corresponding to the 95% confidence interval of Barrabé et al. (2014).

Ancestral state reconstructions of mutualistic strategies, entrance hole diameter, elevation and biome

We inferred the evolutionary history of mutualism strategy in the Hydnophytinae. All outgroups were coded ‘0’ for non-ant associated, with no tuber. We coded the 76 ingroup species as ‘1’, facultative ant-plants, for species that can be inhabited by a range of generalist ant species, ‘2’ for obligate or specialized species that are inhabited typically by one (or two) species of the dolichoderine genera *Philidris* and *Anonychomyrma* or ‘3’ for species that are not ant-inhabited but have a tuber. Each species was coded based on Jebb (1985, 1991), Huxley and Jebb (1991a, 1991b, 1992, 1993), Chomicki and Renner (2016) and an unpublished revision of *Hydnophytum* from M. Jebb and C.R. Huxley.

To infer ancestral mutualistic strategies, we used the Maximum Clade Credibility (MCC) tree from BEAST, and (*i*) the stochastic mapping approach implemented in the phytools package (Revell, 2012) and (*ii*) the reverse MCMC approach implemented in BayesTraits v. 2 (Pagel and Meade, 2013). For the stochastic mapping, we used the function ‘make.simmap’ in the phytools package (v. 04-60) (Revell, 2012), which implements the stochastic character mapping approach developed by Bollback (2006). We estimated ancestral states using under the ER model, and then simulated 1,000 character histories on the MCC tree. We summarized the 1,000 simulated character histories using the function ‘describe.simmap’. For the Bayesian reversible MCMC approach, we used a chain of 50 million generations, and rate coefficients and ancestral states were sampled every 1,000th generation. We ensured that the acceptance rate was between 20 and 40%, as recommended in the manual, and reconstructed the nodes of interest using the command ‘addnode’. We reconstructed all key nodes and reported the probabilities above nodes in Fig. 1.

We estimated ancestral state for maximum and mean elevation using the continuous character ML approach implemented in phytools (Revell, 2012). We relied on the function ‘fastAnc’ and plotted it using the function ‘contMap’ in phytools.

We reconstructed ancestral entrance hole size using the same method. For each species we recorded the maximal entrance hole diameter based on Huxley and Jebb (1991a, 1991b, 1992, 1993), Chomicki and Renner (2016) and an unpublished revision of *Hydnophytum* from M. Jebb and C.R. Huxley. Additional measures from herbarium specimens from Leiden (L), Oxford (FHO) and British Museum (BM) were performed when required for verification.

We also reconstructed ancestral biomes using the same stochastic mapping approach in phytools. Species were coded ‘0’ as belonging to the ‘tropical forest’ biome when they were native to tropical forest below 1,500 meters in altitude, ‘1’ as belonging to the montane environment above 1,500 meters, ‘2’ from species native to savanna or grassland, and ‘3’ from species growing mostly in mangroves (a few species are sometimes found in mangal habitats, but these ML approaches do not allow for multistate characters). Again, each species was coded based on Jebb (1985, 1991), Huxley and Jebb (1991a, 1991b, 1992, 1993), Chomicki and Renner (2016) and an unpublished revision of *Hydnophytum* from M. Jebb and C.R. Huxley.

Computed-Tomography 3D reconstructions

Tubers (either pickled or dried) were scanned in a Nanotom m (Phoenix) X-ray tomography scanner at the Zoologische Staatssammlung in Munich. 3D processing was performed with the software Amira (version 6.0.1, TGS Europe, S.A., Merignac Cedex, France; Mercury Computer Systems Inc., Chelmsford, MA). For slice alignment, the section edges representing the bottom of the block (mold) were used as reference in addition to bringing the specimen structures of neighboring slices to a maximum congruence. Labeling of structures (Amira: segmentation) was done by hand, with the brush (internal structures) and lasso (external surfaces) tools. Initially, every third slice was labeled, with subsequent interpolation of structures on intervening slices, followed by a check of each interpolation and correction if necessary. Before surface rendering, structures were separated from the ‘master’ LabelField ‘am’ file into several LabelFields, each containing one specimen component. Specifically, we separated each independent cavity, as well as the surface outline of each tuber (obtained by merging all elements to 'tuber'). This allowed visualizing easily the tuber 3D structure. In addition, most new LabelFields were reduced in resolution by applying the Resample module to enable (fast) surface rendering, mostly using the default settings (binning x and y values by 2). Surface rendering was performed with the SurfaceGen module, leaving all settings at default. This was followed by the smoothening of the reduced surfaces using the (SmoothSurface module, mostly 40 iterations). To facilitate visualization of domatium structure, we applied a transparency of 70% for each individual cavity and 90% for the tuber surface. Movies were created using the MovieMaker module.

Comparing niche space across mutualistic strategies

To test the hypothesis that 'facultative' and 'loss' strategies have separate smaller niches embedded into the larger niche of specialised/obligate ant-plants, we generated a list of over 1,000 occurrence data for all species, subspecies, varieties and forms of Hydnophytinae. We first downloaded all information from the Global Biodiversity Information Facility (gbif, http://www.gbif.org) and cleaned each of data point, checking that the geographic and altitudinal range matched the protologue or description from the revisions from Huxley and Jebb (1991a, 1991b, 1992, 1993), Chomicki and Renner (2016) and an unpublished revision of *Hydnophytum* from M. Jebb and C.R. Huxley. The majority of the data (>70%) comes from herbarium specimens that were either cited within the aforementioned revisions, of viewed by the first author at Leiden (L), Kew (K) or Oxford (FHO). For some important specimens were GPS data was missing, we inferred it when sufficient geographic data was provided (e.g. 3 km NE of Sorong, Papua, Indonesia) on google maps (https://maps.google.com/).

We next downloaded all 19 bioclim variables (plus altitude) at 30 second resolution on WordClim (http://www.worldclim.org). For each data point, we extracted the 19 bioclim variable values plus altitude using the function 'extract' from the R package 'raster' (Hijmans and Van Etten, 2013). We next generated a file with a single average value per bioclim variable for each species and subspecific form. Since using correlated variables can result in spurious results, we first determined the Pearson's correlation coefficients between all 19 bioclim variables plus altitude. We then selected variables for analysis with a Pearson's correlation coefficient < 0.5, taking a single variable in correlated clusters. The analyses were thus performed with bio_2, bio_3, bio_13, bio_15, bio_18 and altitude. We used the R package vegan (Oksanen et al., 2013) to perform non-dimensional metric scaling analyses (NMDS) and used (*i*) the whole dataset and (*ii*) only the 76 species sampled in our phylogeny. We plotted the first analysis using the package ggplot2 (Wickham, 2009). For the second analysis, we extracted the NMDS1 and NMDS2 values for each species, and used this data matched to the phylogeny tips as input data for a phylomorphospace that showed the ordination, phylogeny and mutualism strategies at once, using the function 'phylomorphospace' of the R package phytools (Revell, 2012).

Inferring rates of entrance hole diameter evolution

To assess whether the morphological rate of entrance hole evolution in the Hydnophytinae, we first used the time-dependent model implemented in BAMM v.2.5.0 (Rabosky, 2014), accounting for incomplete taxon sampling. We performed three runs with 1 million MCMC generations, sampling parameters every 10,000 generations. Morphorate and rate shifts configurations were plotted using the R package BAMMtools (Rabosky et al., 2014). After checking for convergence of the chains, we relied on Bayes Factor to evaluate the best model. Using this approach, a model with nine morphorate shifts fitted best our data (Fig. S2)

Hierarchical Bayesian Modeling

To take into account phylogenetic distance, we used a hierarchical Bayesian (HB) approach for testing to test (*i*) whether mutualistic strategies significantly varied in altitude and (*ii*) whether mutualistic strategies significantly varied in entrance hole morphorate, while simultaneously correcting for phylogenic signal. Our approach followed Fridley and Craddock (2015).

Regression components of the model are the following:

$\mu_{Altitude\ i} = \alpha 1 + \beta 1 \times S_{Facultative\ i}$

$\mu_{Altitude\ i} = \alpha 2 + \beta 2 \times S_{Obligate\ i}$

$\mu_{Altitude\ i} = \alpha 3 + \beta 3 \times S_{Loss\ i}$

$\mu_{Hole\ i} = \alpha 4 + \beta 4 \times S_{Facultative\ i}$

$\mu_{Hole\ i} = \alpha 5 + \beta 5 \times S_{Obligate\ i}$

$\mu_{Hole\ i} = \alpha 6 + \beta 6 \times S_{Loss\ i}$

$\mu_{Hole\ i} = \alpha 7 + \beta 7 \times \mu_{Altitude\ i}$

$\mu_{Rate\ i} = \alpha 8 + \beta 8 \times S_{Facultative\ i}$

$\mu_{Rate\ i} = \alpha 9 + \beta 9 \times S_{Obligate\ i}$

$\mu_{Rate\ i} = \alpha 10 + \beta 10 \times S_{Loss\ i}$

$\mu_{Rate\ i} = \alpha 11 + \beta 11 \times \mu_{Altitude\ i}$

Where the terms α refer to the intercept and β for the slope. We accounted for each mutualistic strategy by three dummy (binary) variables $S_{Facultative\ i}$, $S_{Obligate\ I}$, and $S_{Loss\ I}$, where each taxa is coded absent "0" or present "1". We used mean altitude ($\mu_{Altitude\ i}$), entrance hole maximum diameter per species ($\mu_{Hole\ i}$), and entrance hole morphorate (inferred using BAMM, see above, $\mu_{Rate\ i}$), as dependent variables. To examine the relative effect sizes, all continuous variables were standardized by subtracting their mean and dividing by 2 SD before the analysis (Gelman & Hill, 2007). We integrated phylogenetic information into the model using the Bayesian phylogenetic regression method of de Villemereuil et al. (2012), by converting the 76 species-dated tree into a scaled (0-1) variance-covariance matrix, using the function 'vcv.phylo' of the ape package (Paradis et al., 2004). We adapted the script from Fridley and Craddock (2015), where the model is parameterized using 'JAGS' package (Plummer, 2003), into the $R2_{JAGS}$ package (Su & Yajima, 2014). We ran three parallel MCMC chains for 20,000 iterations followed by a 5000-iteration burn-in, and evaluated model convergence with the Gelman & Rubin (1992) statistic using the 'ggmcmc' R package (I Marín, 2013). Noninformative priors were specified for all parameter distributions, following Fridley and Craddock (2015).

Mapping morphological evolutionary rate in Papua New Guinea

To identify hotspots of morphological evolutionary rate, we developed a novel method to infer rates of morphological evolution using (*i*) a morphological rate analysis in BAMM (Rabosky, 2014) and (*ii*) a matrix of GPS coordinate data for each species sampled in the tree used in the BAMM analysis. To do so, we retrieved speciation morphorate from each tip from the BAMM analyses using the function 'GetTipsRates' in BAMMtools v.2.1 (Rabosky et al., 2014). Rates were interpolated to a polygon representing mainland Papua New Guinea, using the Inverse Distance Weight method implemented in the software ArcMap v.9.3 (ESRI).

Figure legends

Figure 1. The evolution of mutualistic strategies in the Hydnophytinae. (A) Ancestral state reconstruction of mutualistic strategies from 1,000 simulations of characters (phytools) states on a dated phylogeny and a reverse-jump MCMC approach on 1,000 trees (probability shown at key nodes; BayesTraits) with 72% of all Hydnophytinae. (B-G) Images of the mutualistic strategies and Computed-Tomography scanning-based 3D reconstruction of tuber structures. (B-C) *Squamellaria wilkinsonii* (G. Chomicki, J. Aroles, A. Naikatini 45 (M)), a facultative ant-plant from Fiji. (D) *Myrmecodia alata* and E *Myrmecodia tuberosa* specialized ant-plant from lowland Papua New Guinea. (F-G) *Hydnophytum myrtifolium* (M.H.P. Jebb 322) a species from the highlands of Papua New Guinea that is not associated with ants, but instead accumulates rainwater where the frog *Cophixalus riparius* breeds. (H) *Hydnophytum spec nov. magnirubrum*, from Papua New Guinea. The red-fleshed tubers of this species have large entrance holes and are inhabited by a range of arthropods, but not ants. (I) 3D model of *H. petiolatum* (MHP Jebb 377 (FHO)). This form is endemic from Normanby Island and is not associated with ants, instead trapping rainwater and holding cockroaches. The 3D reconstruction revealed 67 unlinked cavities.

Figure 2. Entrance hole evolution correlates with mutualistic strategy. (**A-C**) Diversity of entrance holes in specialized Hydnophytinae. (**A-B**) *Squamellaria wilsonii*, Taveuni, Fiji, with tiny entrance holes fitting the size of the ant partner *Philidris nagasau*. (**C**) *Myrmecodia tuberosa* (form *"versteegii*" sensu Huxley and Jebb, 1993), Papua New Guinea. (**D-F**) Diversity of entrance holes in non-ant associated Hydnophytinae**.** (**D**) *Hydnophytum* probably spec. nov., Papua New Guinea. (**E**) Eggs of *Lepidodactylus buleli*, a gecko endemic from Espiritu Santo island, Vanuatu, inside a *Squamellaria vanuatuensis* domatium. (**F**) Frog-inhabited *Hydnophytum myrtifolium*, Papua New Guinea. (**G**) Rate-through-time plot showing the rate of entrance hole size evolution. Left inset: Logistic regression showing probability of having a particular mutualistic strategy (facultative, obligate, or loss) in function of the morphorate. Right inset: The morphorate as a function of the strategies, taking into account phylogenetic distance (Bayesian hierarchical model).

The rate-through-time plot shows the morphorates for whole Hydnophytinae (see tree Fig. 1A), the specialists clades *Myrmecodia* and the obligate *Squamellaria* clade, and the Hydnophytinae minus these specialized clades. Photographic credits: A-B: G. Chomicki, C: M. Janda, D-E: U. Bauer, F: M. Jebb.

Figure 3. Map showing the morphological rate of entrance hole size in mainland Papua New Guinea. Black dots with red circles show Hydnophytinae occurrences.

Figure 4. Breakdown of mutualisms coincides with occurrence at high-altitudes. (A) Logistic regression showing the probability of having a particular mutualistic strategy (facultative, obligate, or loss) as a function of altitude (for all species of Hydnophytinae). Inset shows the results of a Bayesian hierarchical model that takes phylogenetic information into account (*Materials and Methods*). (B) Phylomorphospace showing the non-metric multidimensional scaling analysis of species' climatic niches (*Materials and Methods*). Note that 'facultative' and 'loss' strategies form discrete clusters (niche space) that are embedded in the larger 'obligate' cluster (compare with Fig. S7).

Online Supplementary Material

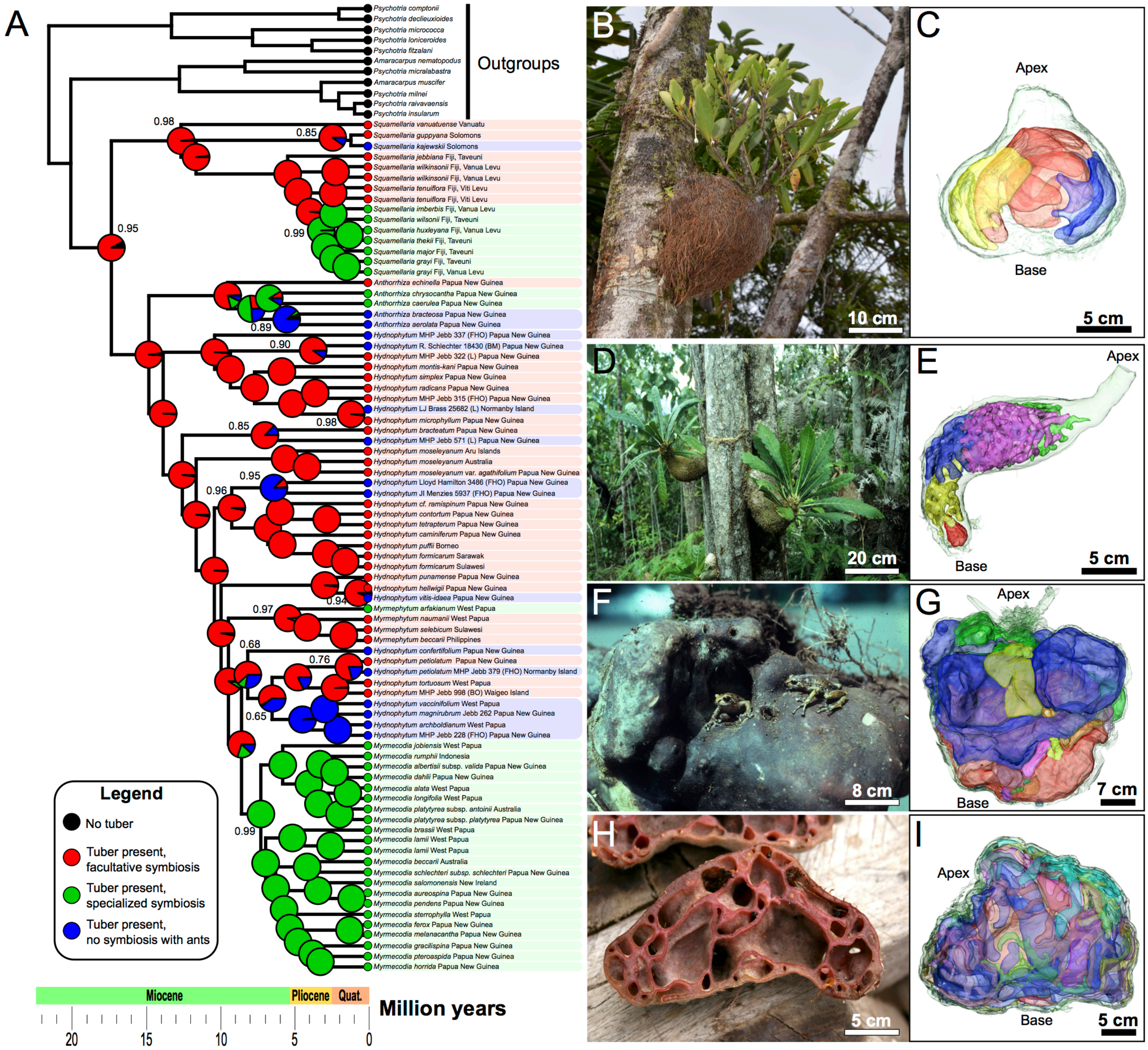
Supplementary Materials and methods
Figs. S1-S7
Tables S1-S2.
Movies S1-S4

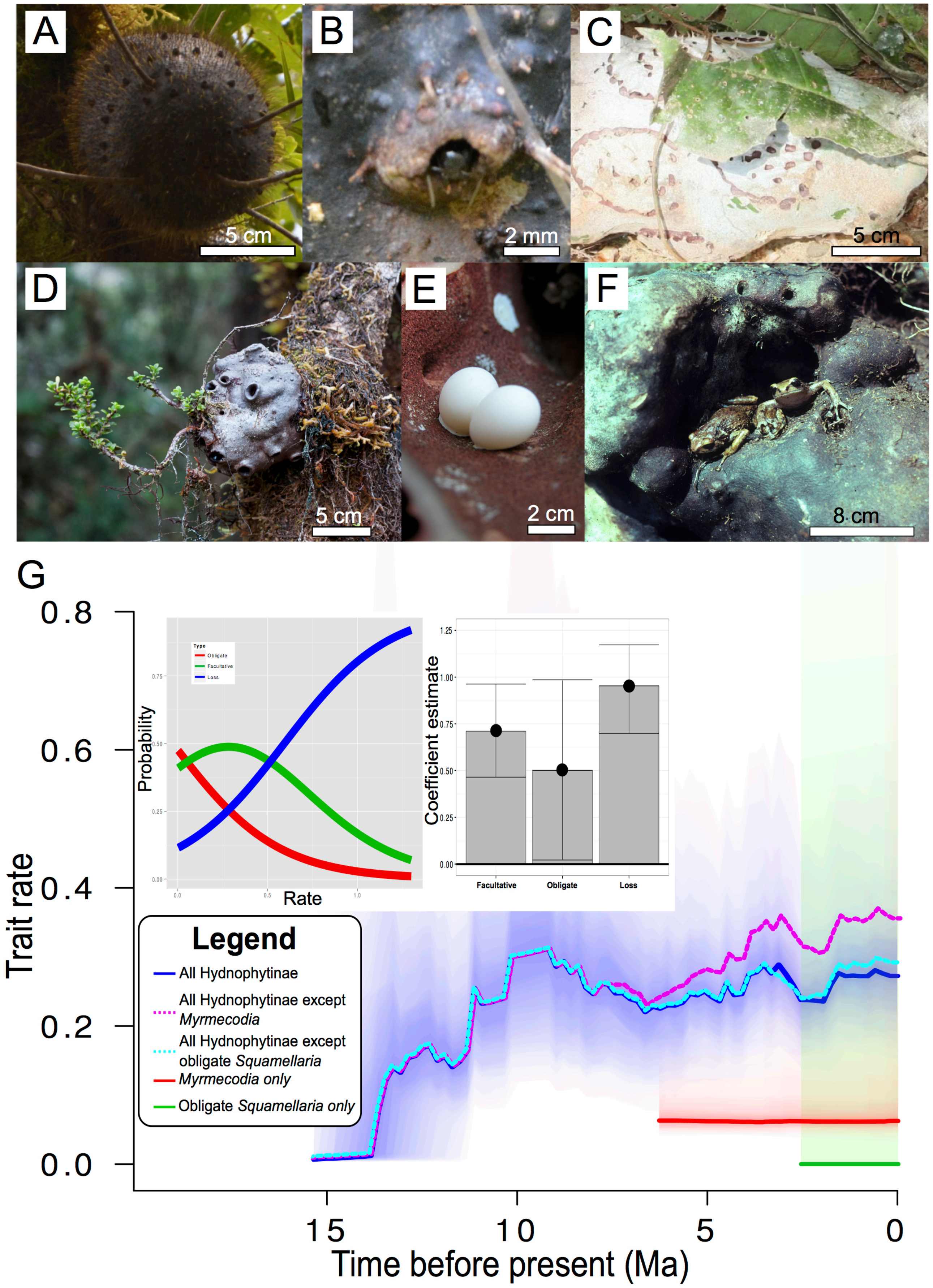
Movies S1. CT scanning 3D model for *Squamellaria wilkinsonii* (voucher G. Chomicki, J. Aroles, A. Naikatini 45 (M)), a facultative ant-plant from Fiji.

Movie S2. CT scanning 3D model for *Myrmecodia tuberosa*, a specialized ant-plant from lowland Papua New Guinea.

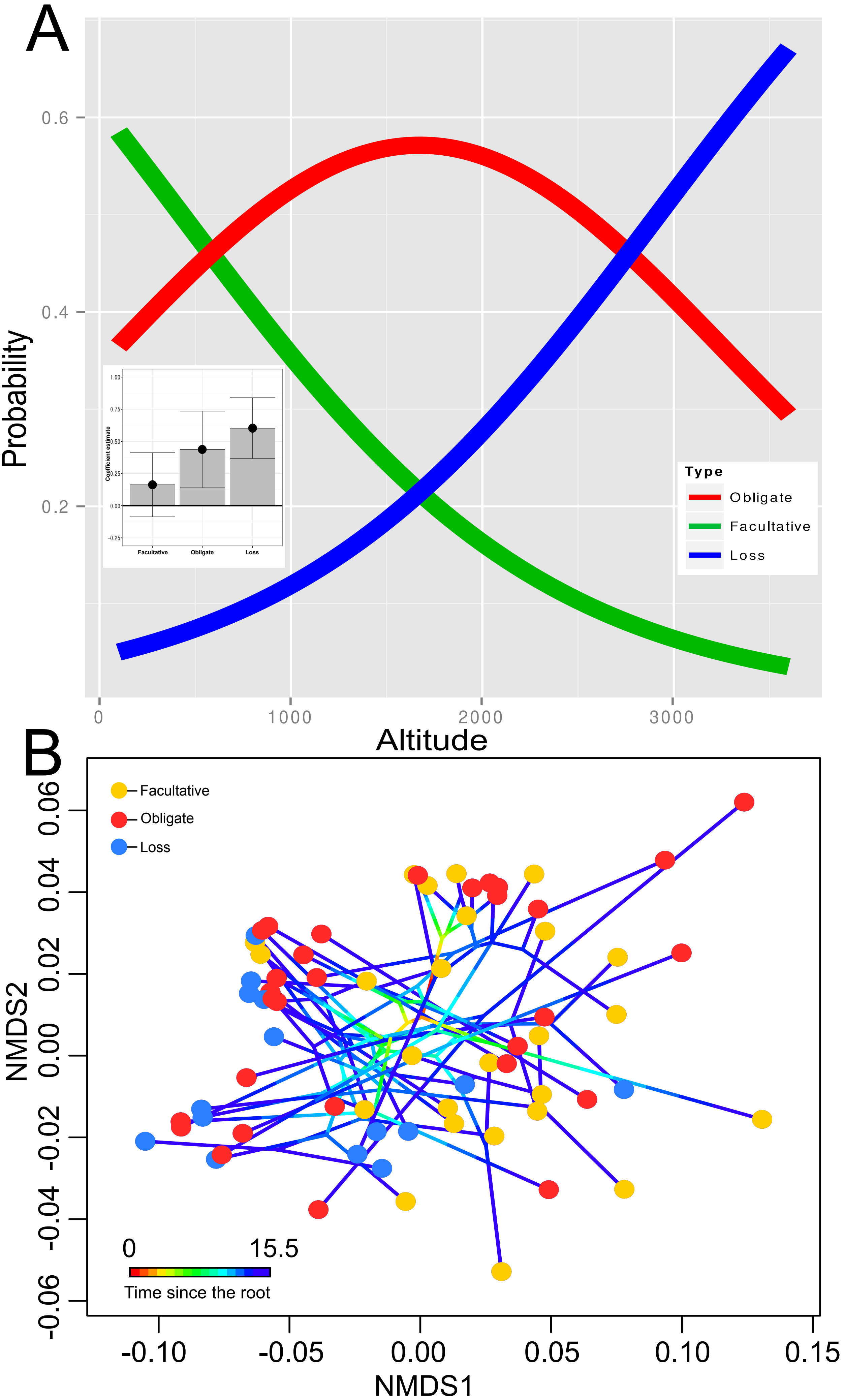
Movie S3. CT scanning 3D model for *Hydnophytum myrtifolium* (voucher M.H.P. Jebb 322), a species from the highlands of Papua New Guinea that is not associated with ants, but instead accumulates rainwater where the frog *Cophixalus riparius* breeds.

Movie S4. CT scanning 3D model for *Hydnophytum petiolatum* ("var. *argentatum*" from Normanby island, voucher MHP Jebb 377 (FHO)).


A
Psychotria comptonii
Psychotria declieuxioides
Psychotria micrococca
Psychotria loniceroides
Psychotria fitzalani
Amaracarpus nematopodus
Psychotria micralabastra
Amaracarpus muscifer
Psychotria milnei
Psychotria raivavaensis
Psychotria insularum
Outgroups
Squamellaria vanuatuense Vanuatu
Squamellaria guppyana Solomons
Squamellaria kajewskii Solomons
Squamellaria jebbiana Fiji, Taveuni
Squamellaria wilkinsonii Fiji, Vanua Levu
Squamellaria wilkinsonii Fiji, Vanua Levu
Squamellaria tenuiflora Fiji, Viti Levu
Squamellaria tenuiflora Fiji, Viti Levu
Squamellaria imberbis Fiji, Vanua Levu
Squamellaria wilsonii Fiji, Taveuni
Squamellaria huxleyana Fiji, Vanua Levu
Squamellaria thekii Fiji, Taveuni
Squamellaria major Fiji, Taveuni
Squamellaria grayi Fiji, Taveuni
Squamellaria grayi Fiji, Vanua Levu
Anthorrhiza echinella Papua New Guinea
Anthorrhiza chrysocantha Papua New Guinea
Anthorrhiza caerulea Papua New Guinea
Anthorrhiza bracteosa Papua New Guinea
Anthorrhiza aerolata Papua New Guinea
Hydnophytum MHP Jebb 337 (FHO) Papua New Guinea
Hydnophytum R. Schlechter 18430 (BM) Papua New Guinea
Hydnophytum MHP Jebb 322 (L) Papua New Guinea
Hydnophytum montis-kani Papua New Guinea
Hydnophytum simplex Papua New Guinea
Hydnophytum radicans Papua New Guinea
Hydnophytum MHP Jebb 315 (FHO) Papua New Guinea
Hydnophytum LJ Brass 25682 (L) Normanby Island
Hydnophytum microphyllum Papua New Guinea
Hydnophytum bracteatum Papua New Guinea
Hydnophytum MHP Jebb 571 (L) Papua New Guinea
Hydnophytum moseleyanum Aru Islands
Hydnophytum moseleyanum Australia
Hydnophytum moseleyanum var. agathifolium Papua New Guinea
Hydnophytum Lloyd Hamilton 3486 (FHO) Papua New Guinea
Hydnophytum JI Menzies 5937 (FHO) Papua New Guinea
Hydnophytum cf. ramispinum Papua New Guinea
Hydnophytum contortum Papua New Guinea
Hydnophytum tetrapterum Papua New Guinea
Hydnophytum caminiferum Papua New Guinea
Hydnophytum puffii Borneo
Hydnophytum formicarum Sarawak
Hydnophytum formicarum Sulawesi
Hydnophytum punamense Papua New Guinea
Hydnophytum hellwigii Papua New Guinea
Hydnophytum vitis-idaea Papua New Guinea
Myrmephytum arfakianum West Papua
Myrmephytum naumanii West Papua
Myrmephytum selebicum Sulawesi
Myrmephytum beccarii Philippines
Hydnophytum confertifolium Papua New Guinea
Hydnophytum petiolatum Papua New Guinea
Hydnophytum petiolatum MHP Jebb 379 (FHO) Normanby Island
Hydnophytum tortuosum West Papua
Hydnophytum MHP Jebb 998 (BO) Waigeo Island
Hydnophytum vaccinifolium West Papua
Hydnophytum magnirubrum Jebb 262 Papua New Guinea
Hydnophytum archboldianum West Papua
Hydnophytum MHP Jebb 228 (FHO) Papua New Guinea
Myrmecodia jobiensis West Papua
Myrmecodia rumphii Indonesia
Myrmecodia albertisii subsp. valida Papua New Guinea
Myrmecodia dahlii Papua New Guinea
Myrmecodia alata West Papua
Myrmecodia longifolia West Papua
Myrmecodia platytyrea subsp. antoinii Australia
Myrmecodia platytyrea subsp. platytyrea Papua New Guinea
Myrmecodia brassii West Papua
Myrmecodia lamii West Papua
Myrmecodia lamii West Papua
Myrmecodia beccarii Australia
Myrmecodia schlechteri subsp. schlechteri Papua New Guinea
Myrmecodia salomonensis New Ireland
Myrmecodia aureospina Papua New Guinea
Myrmecodia pendens Papua New Guinea
Myrmecodia sterrophylla West Papua
Myrmecodia ferox Papua New Guinea
Myrmecodia melanacantha Papua New Guinea
Myrmecodia gracilispina Papua New Guinea
Myrmecodia pteroaspida Papua New Guinea
Myrmecodia horrida Papua New Guinea
0.98
0.85
0.95
0.99
0.89
0.90
0.98
0.85
0.95
0.96
0.94
0.97
0.68
0.76
0.65
0.99
Legend
● No tuber
● Tuber present, facultative symbiosis
● Tuber present, specialized symbiosis
● Tuber present, no symbiosis with ants
Miocene
Pliocene
Quat.
Million years
20
15
10
5
0
B
10 cm
C
Apex
Base
5 cm
D
20 cm
E
Apex
Base
5 cm
F
8 cm
G
Apex
Base
7 cm
H
5 cm
I
Apex
Base
5 cm


A
5 cm
B
2 mm
C
5 cm
D
5 cm
E
2 cm
F
8 cm
G
Trait rate
0.8
0.6
0.4
0.2
0.0
Time before present (Ma)
15
10
5
0
Probability
Rate
Type
Obligate
Facultative
Loss
0.00
0.25
0.50
0.75
0.0
0.5
1.0
Coefficient estimate
1.25
1.00
0.75
0.50
0.25
0.00
Facultative
Obligate
Loss
Legend
All Hydnophytinae
All Hydnophytinae except *Myrmecodia*
All Hydnophytinae except obligate *Squamellaria*
Myrmecodia only
Obligate *Squamellaria only*


130°0'0"E
140°0'0"E
150°0'0"E
0°0'0"
10°0'0"S
Legend
● Hydnophytinae records
Entrance hole morphorate
0.0067 - 0.0599
0.0599 - 0.1131
0.1131 - 0.1662
0.1662 - 0.2194
0.2194 - 0.2726
0.2726 - 0.3257
0.3257 - 0.3789
0.3789 - 0.4321
0.4321 - 0.4852
0.4852 - 0.5384
0.5384 - 0.5916
0.5916 - 0.6447
0.6447 - 0.6979
0.6979 - 0.7511
0.7511 - 0.8043
0.8043 - 0.8574
0.8574 - 0.9106
0.9106 - 0.9638
0.9638 - 1.0169
1.0169 - 1.0701
1.0701 - 1.1233
1.1233 - 1.1764
1.1764 - 1.2296
1.2296 - 1.2828
1.2828 - 1.3359
1.3359 - 1.3891
1.3891 - 1.4423
1.4423 - 1.4954
1.4954 - 1.5486
1.5486 - 1.6018
1.6018 - 1.6549
1.6549 - 1.7081
Altitude (m)
0 - 38
38 - 85
85 - 144
144 - 213
213 - 288
288 - 367
367 - 449
449 - 534
534 - 623
623 - 716
716 - 812
812 - 910
910 - 1,011
1,011 - 1,116
1,116 - 1,226
1,226 - 1,342
1,342 - 1,463
1,463 - 1,589
1,589 - 1,721
1,721 - 1,859
1,859 - 2,005
2,005 - 2,159
2,159 - 2,323
2,323 - 2,495
2,495 - 2,676
2,676 - 2,863
2,863 - 3,064
3,064 - 3,289
3,289 - 3,543
3,543 - 3,853
3,853 - 4,650
4,650 - 4,884
0
62.5
125
250
375
500
Km


A
Probability
0.6
0.4
0.2
0
1000
2000
3000
Altitude
Type
Obligate
Facultative
Loss
Coefficient estimate
1.00
0.75
0.50
0.25
0.00
-0.25
Facultative
Obligate
Loss
B
Facultative
Obligate
Loss
NMDS2
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
NMDS1
-0.10
-0.05
0.00
0.05
0.10
0.15
0
15.5
Time since the root

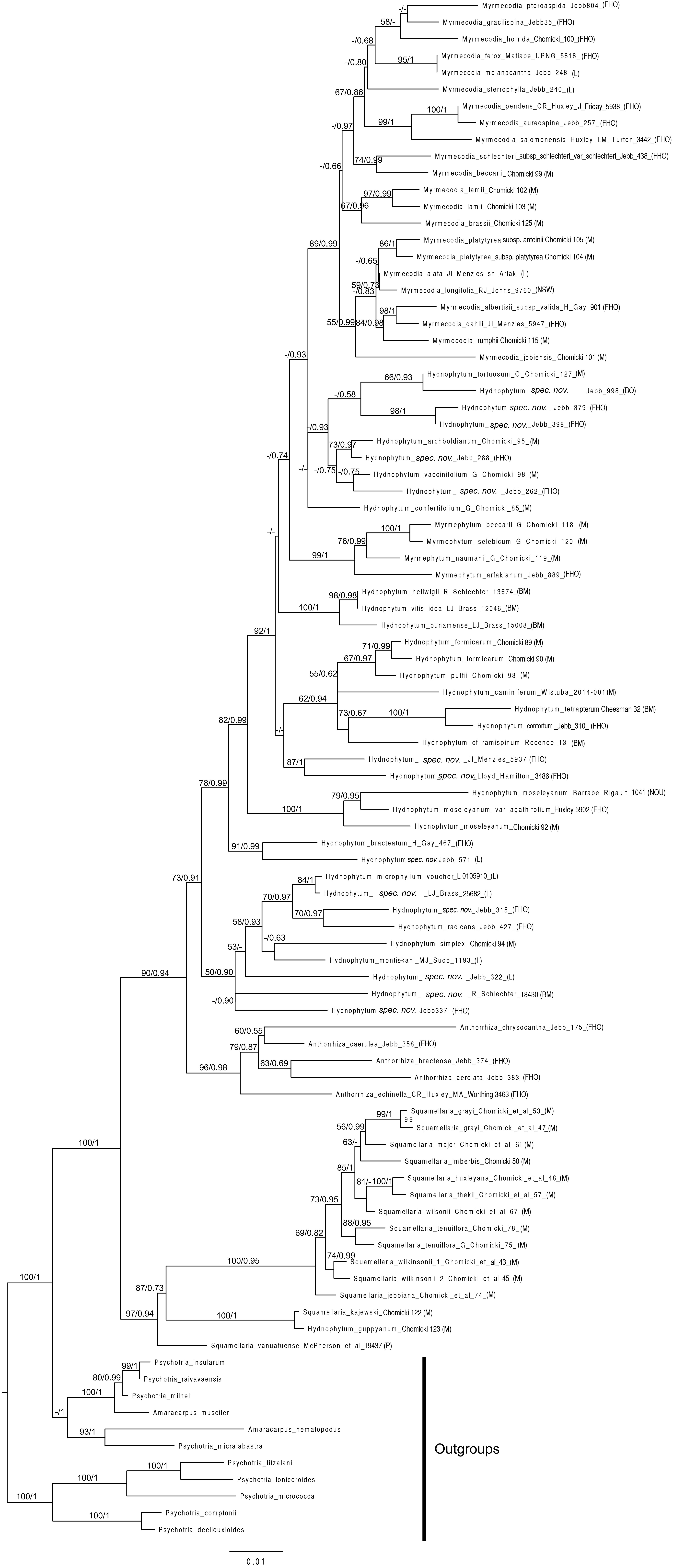


Figure S1. Maximum likelihood tree for the Hydnophytinae. Numbers above branches show the bootstrap support from 100 replicates, and the posterior probabilities from a Bayesian analysis.

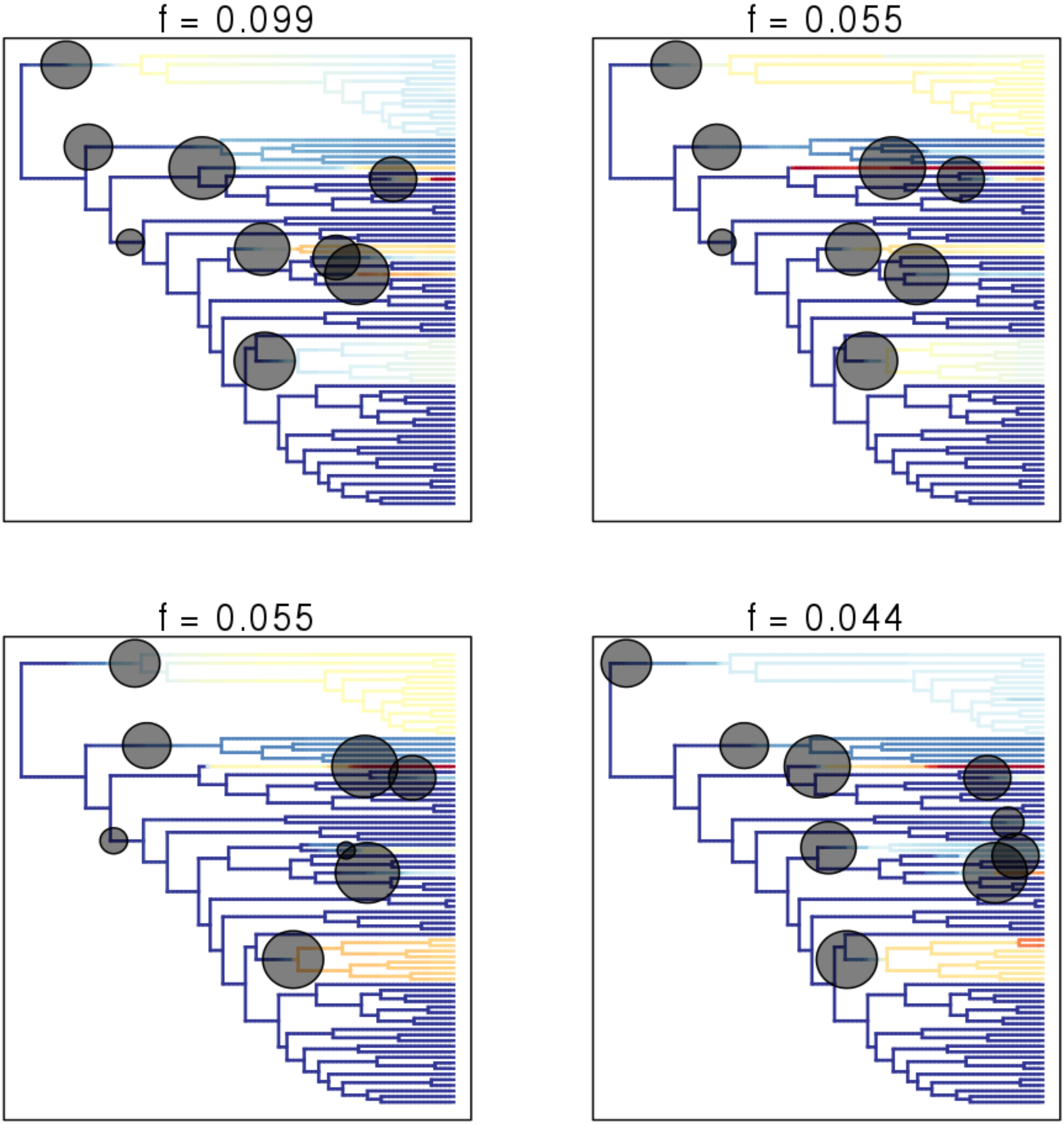


Figure S2. Credible shifts in domatium entrance hole size evolutionary rate inferred i
BAMM. Each black dot shows a shift in morphological rate with diameter proportional
to posterior probability.

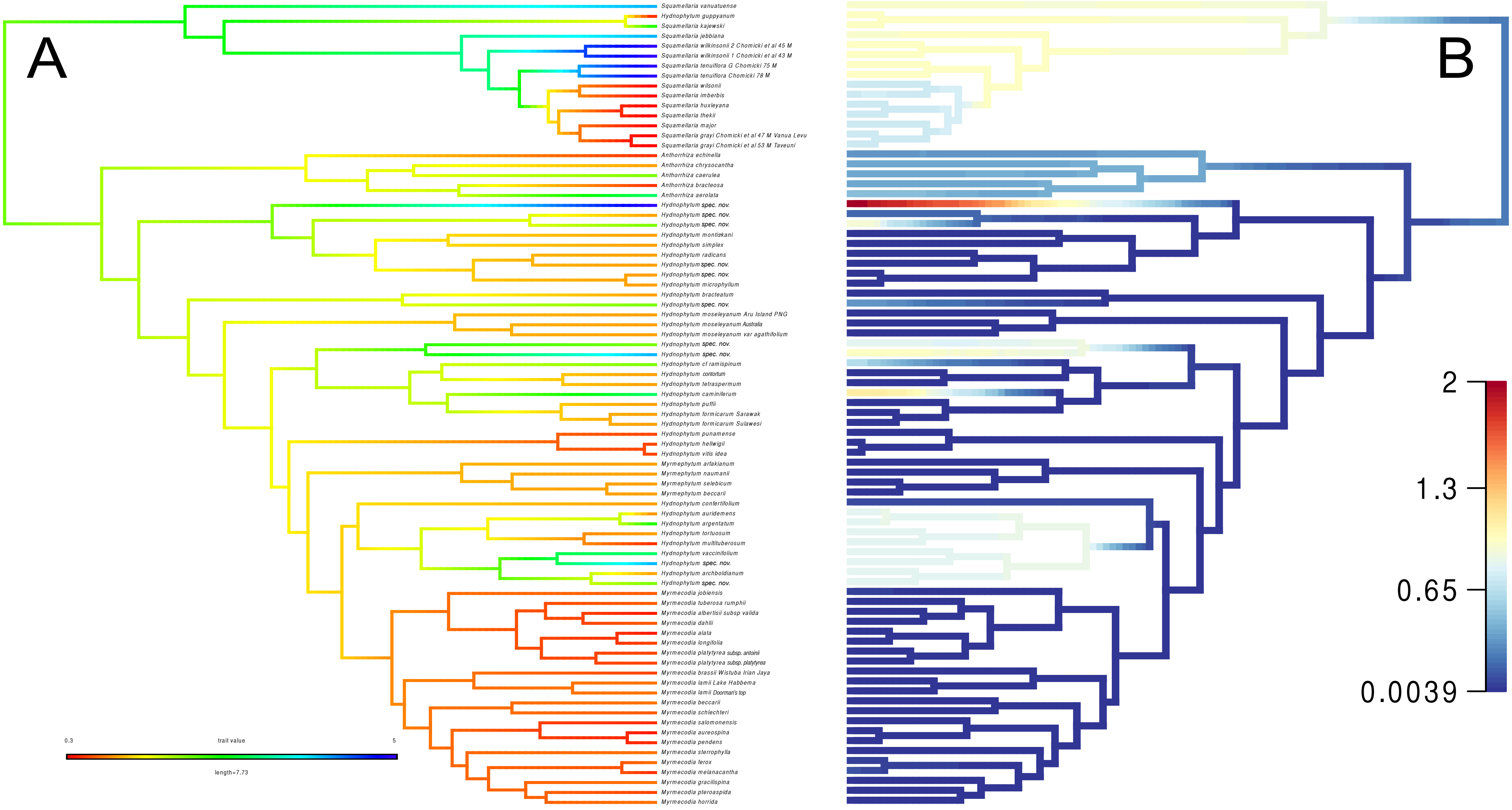


Fig. S3. The evolution of domatium entrance hole size in the Hydnophytinae. (A) Ancestral state reconstruction of entrance hole size using the functions 'fastAnc' and 'contMap' in the Phytools package. (B) Rate of entrance hole evolution inferred using 'Bayesian Analysis of Macroevolutionary Mixture (BAMM).

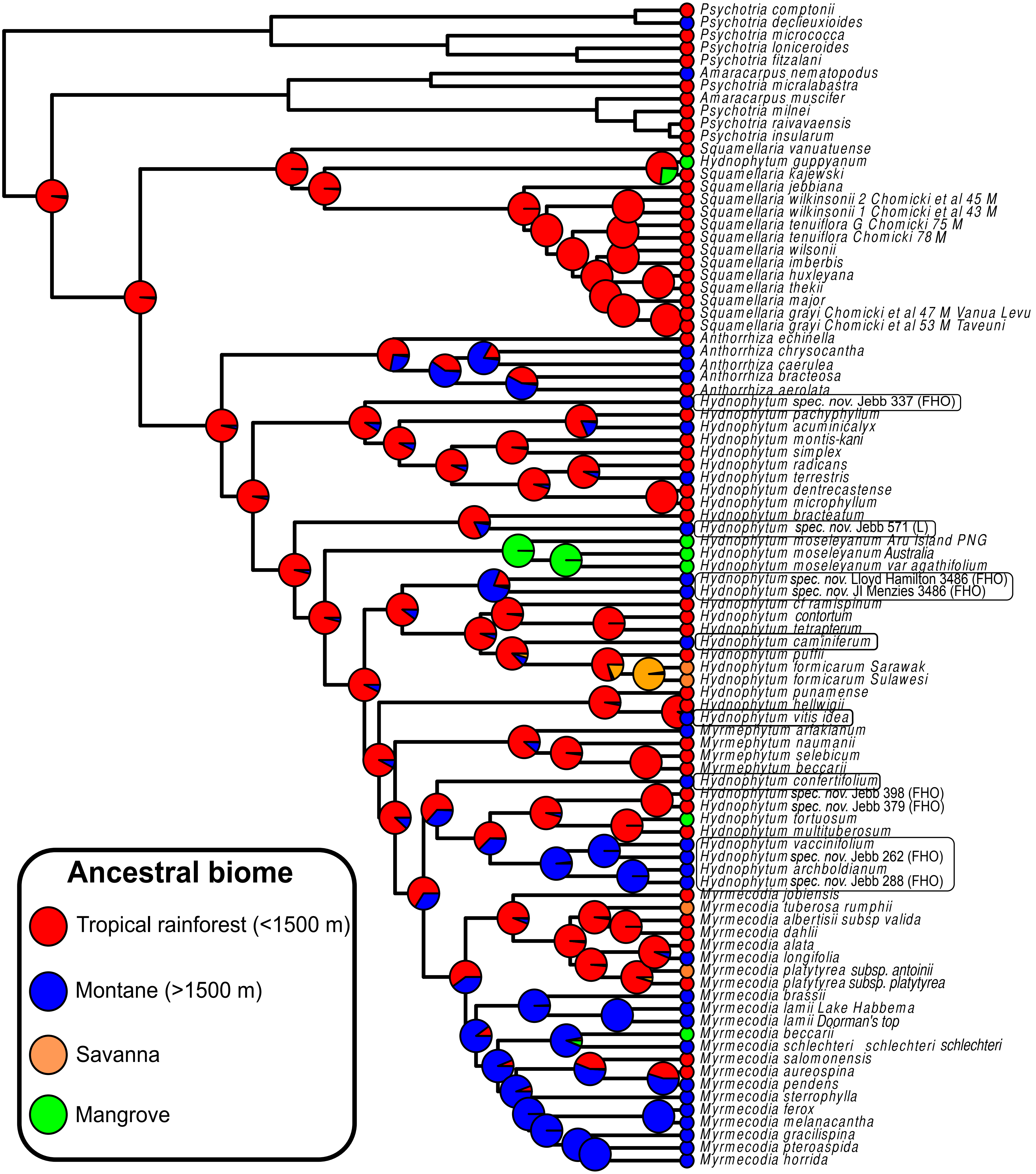


Fig. S4. Ancestral state reconstruction of species occurence using stochastic mapping in the Phytools package. Rectangles highlight the lineages where loss of mutualism with ants is correlated with shift to montane habitat. For coding, see Materials and Methods.

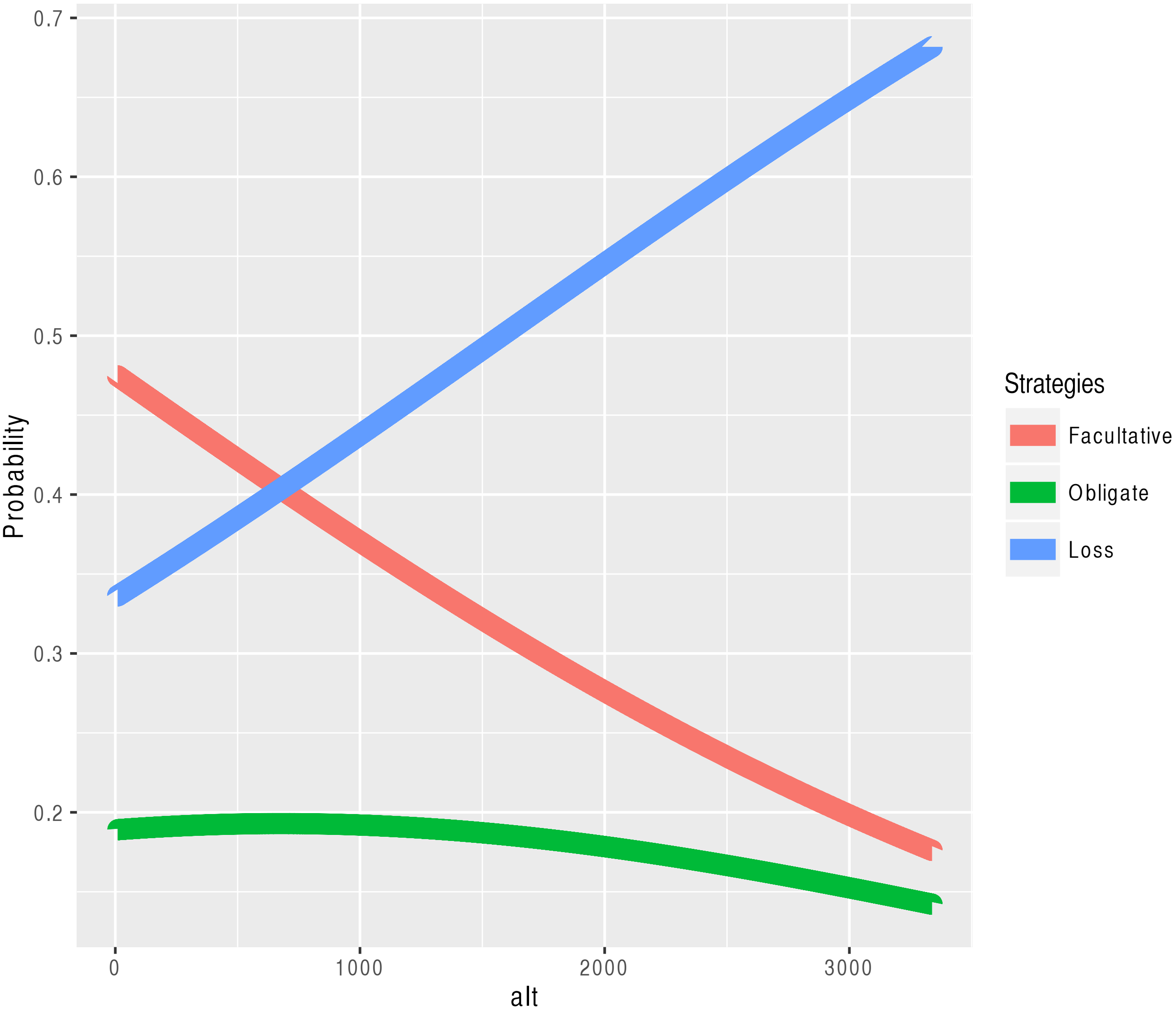


Figure S5. Logistic regression (t = 2.0, AIC = 270) of mean altitude on mutualistic strategies, calculated for each Hydnophytinae taxa (including subspecific forms, 130 taxa in total) from over 1,000 occurence data points.

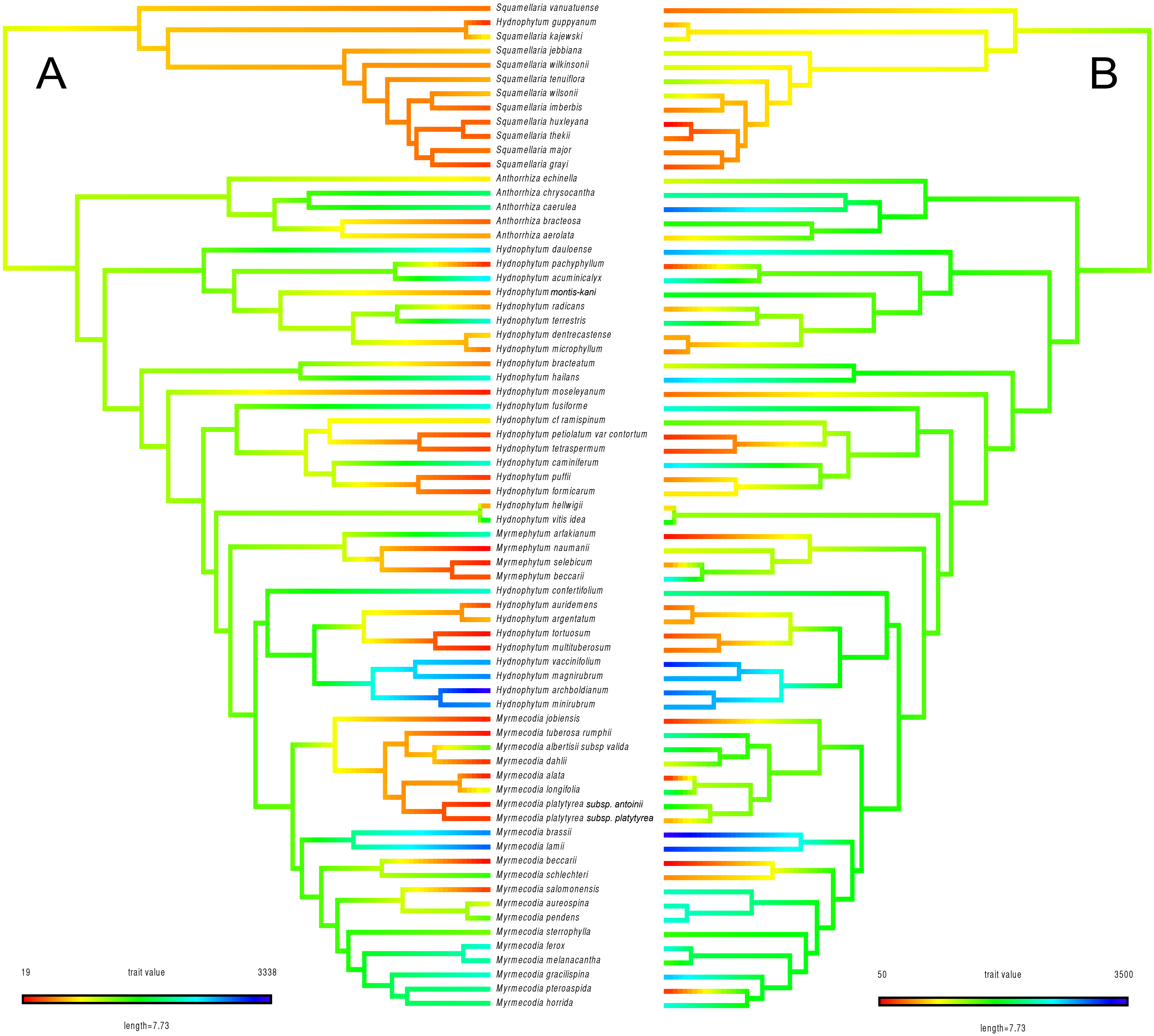


Fig. S6. Ancestral state reconstruction of altitude as a continuous character in the Phytools package. (A) Mean altitude calculated based on over 1,000 occurence data points. (B) Maximum altitudinal range taken from the literature (see Materials and Methods).

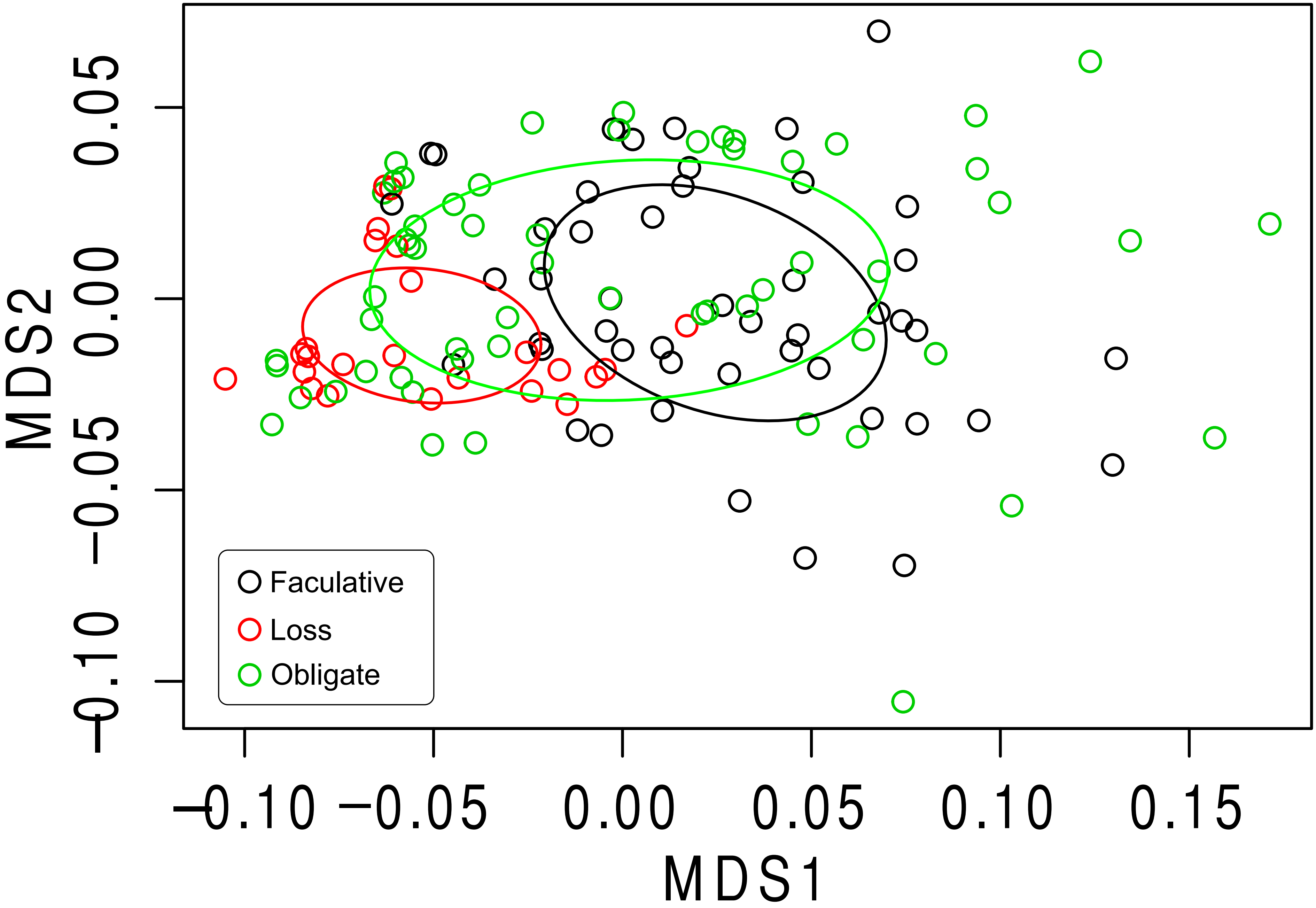


Fig. S7. Non-dimensional metric scaling analysis (NDMS) showing the climatic niche space of the three mutualistic strategies in the Hydnophytinae. Ellipses show the 95% confidence intervals for each strategy. This analysis is based on all species, subspecies or forms (130 taxa). A large dataset of over 1,000 occurences was compiled, and we then took the mean for Altitude or each Bioclim variable. We excluded all variables that were correlated among themselves (Pearson's coefficient > 0.5) and only kept one single variable per cluster. This analysis is based on bio_2, bio_3, bio_13, bio_18 and altitude.

Table S2. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	**Voucher**	**Geographic origin**	**ITS**	**ETS**	**trnL intron**	**trnL-trnF spacer**	**ndhF**	**trnH-psbA**
***Amaracarpus muscifer* A.C.Sm.**	L. Barrabé & M. Tuiwawa 1109 (NOU)	Fiji	KF675907	KF675790	-	-	KF675995	-
***Amaracarpus nematopodus* (F.Muell.) P.I.Forst.**	L. Barrabé et al. 1030 (NOU)	Australia	JX155060	KF675791	-	-	JX155105	-
***Anthorrhiza aerolata* Huxley & Jebb**	M.P.H. Jebb 383 (FHO)	Papua New Guinea, Normanby island	Submitted	Submitted	-	-	-	Submitted
***Anthorrhiza caerulea* Huxley & Jebb**	M.P.H. Jebb 358 (FHO)	Papua New Guinea	KU586349	KU586368	-	-	-	Submitted
***Anthorrhiza chrysocantha* Huxley & Jebb**	M.P.H. Jebb 175 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Anthorrhiza bracteosa* Huxley & Jebb**	M.P.H. Jebb 374 (FHO)	Papua New Guinea, Normanby island	Submitted	Submitted		-	-	Submitted
Anthorrhiza echinella	G. Chomicki 83 (M)	Cultivated Oxf. Bot Gard., origin New Guinea	KU586350	KU586369	-	-	-	Submitted
***Hedstromia latifolia* A.C.Sm.**	L. Barrabé et al 1090 (NOU)	Fiji	KF675911	KF675795	-	-	KF675999	-
***Hydnophytum formicarum* Jack**	G. Chomicki 87 (M)	Cultivated, origin Malaysian region	KU586346	KU586365	-	-	KU586397	Submitted
***Hydnophytum formicarum* Jack**	G. Chomicki 90 (M)	Cultivated, origin Sumatra	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum simplex* Becc.**	G. Chomicki 94 (M)	Cultivated, origin Aru Island, Papua New Guinea	KU963311	KU963332	KU963350	KU963362	KU963377	Submitted
***Hydnophytum montis-kani* Valeton**	M.J. Sudo 1193 (L)	Papua New Guinea	Submitted	Submitted	-	-	-	Submitted
***Hydnophytum sp. 1* (=*H. dentrecastense* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	L.J. Brass 2568 2 (L)	Papua New Guinea	KU963312	Submitted	-	-	-	Submitted
***Hydnophytum sp. 2* (=*H. orichalcum* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	L.J. Brass 28248 (L)	Papua New Guinea	KU963313	Submitted	-	-	-	Submitted

***Hydnophytum sp. 3* (=*H. terrestris* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	M.P.H. Jebb 315 (FHO)	Papua New Guinea	KU963314	-	-	KU963376	-	Submitted
***Hydnophytum hellwigii* Warb.**	R. Schlechter 13674 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum radicans* Becc.**	M.H.P. Jebb 427 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum punamense* Lauterb.**	L.J. Brass 15008 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum vitis-idaea* Merr. & L.M.Perry**	L.J. Brass 12046 (BM)	Papua New Guinea	KU963316	KU963337	KU963351	KU963363	-	Submitted
***Hydnophytum sp. (Hydnophytum petiolatum var. argentatum* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	M.H.P. Jebb 379 (FHO)	Normanby island, Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum petiolatum var. auridemens* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	M.H.P. Jebb 398 (FHO)	Missima island, Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum sp. (Hydnophytum dauloense* in Jebb and C.R. Huxley unpublished *Hydnophytum	M.H.P. Jebb 337 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum tortuosum* Becc.**	G. Chomicki 127 (M)	Cultivated, origin Indonesian Papua	KU963318	KU963339	KU963352	KU963364	KU963379	Submitted
***Hydnophytum archboldianum* Merr. & L.M.Perry**	G. Chomicki 95 (M)	Cultivated, origin Indonesian Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum minirubrum* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 288 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum magnirubrum* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 262 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp.* (*Hydnophytum multituberosum* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 998 (BO)	Waigeo Island, Indonesia	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum tetrapterum* Becc.**	L.E. Cheesman 32 (BM)	Papua New Guinea	KU963319	KU963340	-	-	-	Submitted
***Hydnophytum ramispinum* Merr. & L.M.Perry**	Recende 13 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted

***Hydnophytum confertifolium* Merr. & L.M.Perry**	G. Chomicki 85 (M)	Cultivated, origin West Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum contortum* Merr. & L. M. Perry**	M.P.H Jebb 310 (FHO)	Papua New Guinea	KU963321	KU963342	-	KU963375	-	Submitted
***Hydnophytum sp. (Hydnophytum fusiforme* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	Lloyd Hamilton 3486 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum magnirubrum* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	J. I. Menzies 5937 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum trichomanes* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	R. Schlechter 18430 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum puffii* Low, Sugau & Wong**	G. Chomicki 93 (M)	Cultivated, origin Borneo	KU963322	KU963343	KU963354	KU963366	KU963381	Submitted
***Hydnophytum sp. (Hydnophytum acuminicalyx* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 322 (L)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum caminiferum* Wistuba, U.Zimm., Gronem. & Marwinski**	A. Wistuba 2014-001 (M)	West Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum moseleyanum* Becc. *'agathifolium'	C.R. Huxley 5902 (FHO)	Papua New Guinea	KU963323	KU963344	KU963355	KU963367	-	Submitted
***Hydnophytum moseleyanum* Becc.**	L. Barrabeé & Rigault 1041 (NOU)	Australia	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum moseleyanum* Becc.**	G. Chomicki 92 (M)	Cultivated, origin Aru Island	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum bracteatum* Valeton**	H. Gay 467 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum hailans* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 571 (L)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum microphyllum* Becc.**	L0105910 (L)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum vaccinifolium* P.Royen**	G. Chomicki 98 (M)	Cultivated, origin Papua	KU963324	KU963345	KU963356	KU963368	KU963382	Submitted
***Myrmecodia beccarii* Hook f.**	G. Chomicki 99 (M)	Cultivated, origin Australia	KU586347	KU586366	-	-	KU586398	Submitted

***Myrmecodia salomonensis* Becc.**	C. R. Huxley and L. M. Turton 3442 (FHO)	Solomons	KU586351	KU586370	-	-	-	Submitted
***Myrmecodia dahlii* K.Schum.**	J.I. Menzies 5947 (FHO)	Papua New Guinea	KU586348	KU586367	KU963357	KU963369	KU586399	Submitted
***Myrmecodia rumphii* Becc.**	G. Chomicki 115 (M)	Cultivated	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia alata* Becc.**	J.I. Menzies s.n. (L)	Papua	Submitted	Submitted	-	-	-	Submitted
***Myrmecodia jobiensis* Becc.**	G. Chomicki 101 (M)	Cultivated, origin Papua	KU963326	KU963347	KU963358	KU963370	KU963384	Submitted
***Myrmecodia albertisii* Becc. subsp. *valida* C.R.Huxley & Jebb**	H.J. Gay 901 (FHO)	Papua New Guinea	-	KU963327	-	-	-	Submitted
***Myrmecodia schlechteri* subsp. *schlechteri* var. *schlechteri* C.R.Huxley & Jebb**	H. J. Gay 488 (FHO)	Papua New Guinea	AF071988	-	JN643394	JN643394	-	Submitted
***Myrmecodia pendens* Merr. & L.M.Perr.**	C.R. Huxley and J. Friday 5938 (FHO)	Papua New Guinea	-	KU963328	-	-	-	Submitted
***Myrmecodia aureospina* C.R.Huxley & Jebb**	M.P.H. Jebb 257 (FHO)	Papua New Guinea	-	KU963335	-	-	-	Submitted
***Myrmecodia sterrophylla* Merr. & L.M. Perry**	M.P.H. Jebb 240 (L)	Papua New Guinea	KU963330	-	-	-	-	Submitted
***Myrmecodia lamii* Merr. & L.M.Perry**	G. Chomicki 102 (M)	Cultivated, origin Indonesian Papua, Doorman’ top	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia lamii* Merr. & L.M.Perry**	G. Chomicki 103 (M)	Cultivated, origin Indonesian Papua, Lake Habbema	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia brassii* Merr. & L.M.Perry**	G. Chomicki 125 (M)	Cultivated, origin Indonesian Papua, Doorman’	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia platytyrea subsp. platytyrea* (Becc.) C.R.Huxley & Jebb**	G. Chomicki 104 (M)	Cultivated, origin Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia platytyrea subsp. antoinii* (Becc.) C.R.Huxley & Jebb**	G. Chomicki 105 (M)	Cultivated, origin Australia	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia ferox* C.R.Huxley & Jebb**	C.R. Huxley & Matiabe UPNG 5818 (FHO)	Papua New Guinea	-	KU963334	-	-	-	Submitted
***Myrmecodia longifolia* Valeton**	R.J. Johns 9760 (NSW)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted

Myrmecodia melanacantha **C.R.Huxley & Jebb**	M.P.H. Jebb 248 (L)	Papua New Guinea	KU963331	Submitted	-	-	-	Submitted
Myrmecodia horrida **C.R.Huxley & Jebb**	G. Chomicki 100 (M)	Cultivated, origin Papua New Guinea	KU963329	KU963338	KU963359	KU963371	KU963385	Submitted
Myrmecodia gracilispina **C.R.Huxley & Jebb**	M.P.H. Jebb 35 (FHO)	Papua New Guinea	-	KU963333	-	-	-	Submitted
Myrmecodia pteroaspida **C.R.Huxley & Jebb**	M.P.H. Jebb 804 (FHO)	Papua New Guinea	KU963325	KU963346	-	-	-	Submitted
Myrmephytum selebicum **(Becc.) Becc.**	G. Chomicki 120 (M)	Cultivated, origin Papua	KU963320	KU963341	KU963360	KU963372	KU963386	Submitted
Myrmephytum beccarii **Elmer**	G. Chomicki 118 (M)	Cultivated, origin Philippines	KU586353	KU586354	KU963361	KU963373	KU586401	Submitted
Myrmephytum naumanii **(Warb.) Huxley & Jebb**	G. Chomicki 119 (M)	Cultivated, origin West Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Myrmephytum arfakianum **(Becc.) Huxley & Jebb**	M.H.P. Jebb 889 (FHO)	West Papua, Arfak Mountains	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Psychotria comptonii **S.Moore**	L. Barrabé & Rigault 1014 (NOU)	New Caledonia	KF675927	KF675823	-	-	KF676015	-
Psychotria dallachiana **Benth.**	L. Barrabé & Rigault 1048 (NOU)	Australia	KF675928	KF675824	-	-	KF676016	-
Psychotria declieuxioides **S.Moore**	L. Barrabé & Nigote 937 (NOU)	New Caledonia	KF675932	KF675828	-	-	KF676020	-
Psychotria faguetii **(Baill.) Schltr.**	L. Barrabé et al. 820 (NOU)	New Caledonia	KF675934	KF675831	-	-	KF676023	-
Psychotria fitzalanii **Benth.**	L. Barrabé & Rigault 1057 (NOU)	Australia	KF675935	KF675832	-	-	KF676024	-
Psychotria goniocarpa **(Baill.) Guillaumin**	L. Barrabé 586 (NOU)	New Caledonia	KF675940	KF675838	-	-	KF676029	-
Psychotria hawaiiensis **(A.Gray) Fosberg**	Y. Pillon 1425 (NOU)	Hawaii	KF675941	KF675840	-	-	KF676030	-
Psychotria hivaoana **Fosberg**	Meyer 3071 (PAP)	French Polynesia	KF675942	KF675841	-	-	KF676031	-
Psychotria insularum **A.Gray**	Y. Pillon 909 (NOU)	Wallis & Futuna	KF675943	KF675842	-	-	KF676032	-
Psychotria iteophylla **Stapf**	Axelius 303 (S)	Borneo	-	-	-	-	-	-
Psychotria loniceroides **Sieber ex DC.**	L. Barrabé & Rigault 1042 (NOU)	Australia	KF675945	KF675846	-	-	KF676033	-

***Psychotria lorentzii* Valeton**	Puradyatmika 10460 (K)	Papua New Guinea	KF675946	KF675847	-	-	KF676034	-
***Psychotria micralabastra* (Lauterb. & K.Schum.) Valeton**	Takeuchi 16163 (K)	Papua New Guinea	KF675949	KF675851	-	-	KF676036	-
***Psychotria micrococca* (Lauterb. & K.Schum.) Valeton**	Drozd & Molem s.n. (PSF)	Papua New Guinea	KF675951	KF675853	-	-	KF676038	-
***Psychotria microglossa* (Baill.) Baill. ex Guillaumin**	L. Barrabé 585 (NOU)	New Guinea	KF675950	KF675852	-	-	KF676037	-
***Psychotria monanthos* (Baill.) Schltr.**	Y. Pillon 1370 (NOU)	New Caledonia	KF675953	KF675855	-	-	KF676040	-
***Psychotria poissoniana* (Baill.) Guillaumin**	J. Munzinger 5156 (NOU)	New Caledonia	KF675958	KF675861	-	-	KF676045	-
***Psychotria pritchardii* Seem.**	L. Barrabé et al 1124 (NOU)	Fiji	KF675992	KF675903	-	-	KF676078	-
***Psychotria raivavaensis* Fosberg**	Meyer 3088 (PAP)	French Polynesia	KF675960	-	-	-	KF676047	-
***Psychotria submontana* Domin**	L. Barrabé et al. 1044 (NOU)	Australia	KF675988	KF675899	-	-	-	-
***Psychotria temehaniensis* J.W.Moore**	Mouly 403 (P)	French Polynesia	KF675989	KF675900	-	-	KF676075	-
***Psychotria trisulcata* (Baill.) Guillaumin**	L. Barrabé et al. 902 (NOU)	New Caledonia	KF675990	KF675901	-	-	KF676076	-
***Squamellaria grandiflora* (Becc.) Chomicki, comb. nov.**	S. Vodonaivolu DA2128 (SUVA)	Fiji, Kadavu Island	-	KU963388	-	-	-	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (SUVA) HOLOTYPE	Taveuni, Bouma falls, Lavena	KU586339	KU586358	KU586376	KU586376	KU586388	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 47 (M)	Vanua Levu, Waisali forest park	-	-	KU586372	KU586372	-	-
***Squamellaria guppyana* (Becc.) Chomicki, comb. nov.**	G. Chomicki 123 (M)	Cultivated, origin Solomons	KU586345	-	-	-	KU586396	-
***Squamellaria huxleyana* Chomicki sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 48 (SUVA)	Fiji, Vanua Levu, road between Savusavu to	KU586336	KU586355	KU586373	KU586373	KU586385	-
***Squamellaria imberbis* (A. Gray) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	Fiji, Vanua Levu, track to vodaphone tower	KU586337	KU586356	KU586374	KU586374	KU586386	-
***Squamellaria jebbiana* Chomicki, sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	Fiji, Taveuni, Mt Manuca area.	KU586342	KU586361	KU586379	KU586379	KU586391	-
***Squamellaria kajewskii* (Merr. & L.M.Perry) Chomicki, comb. nov.**	G. Chomicki 122 (M)	Cultivated, origin Solomons	KU586335	-	-	-	KU586384	-
***Squamellaria major* A.C. Sm.**	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	Fiji, Taveuni, road to DesVoeux peak	KU586338	KU586357	KU586375	KU586375	KU586387	-

***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	-	-	KU586381	KU586381	KU586393	-
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	KU586343	KU586362	KU586382	KU586382	KU586394	-
***Squamellaria thekii* Jebb**	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586340	KU586359	KU586377	KU586377	KU586389	-
***Squamellaria vanuatuensis* (Jebb & Huxley) Chomicki, comb. nov.**	McPherson 19437 (P)	Vanuatu	JX155078	-	-	-	-	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	Fiji, Vanua Levu, Waisali forest park.	-	-	KU586380	KU586380	KU586392	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 49 (M)	Fiji, Vanua Levu, Waisali forest park.	-	KU586364	-		-	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	Fiji, Vanua Levu, Waisali forest park.	KU586344	KU586363	KU586383		KU586395	-
***Squamellaria wilsonii* (Horne ex Baker) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586341	KU586360	KU586378		KU586390	-

| Chapter 7 |

Domatium 3D structure maximizes benefits in facultative and obligate nutritional mutualisms

Guillaume Chomicki, Dirk Metzler and Susanne S. Renner

Unpublished manuscript

Title: Domatium 3D structure maximizes benefits in facultative and obligate nutritional mutualisms

Authors: Guillaume Chomicki[1]*, Dirk Metzler[2], Susanne S. Renner[1]

Affiliations: [1]Systematic Botany and Mycology, University of Munich (LMU), Menzinger Str. 67, 80638 Munich. [2]Evolutionary Biology, University of Munich (LMU), Grosshaderner Str. 2, 82152 Martinsried, Germany.

*Correspondence to: guillaume.chomicki@gmail.com

Abstract: Mutualisms vary from facultative associations between generalist species to obligate pairs of specialists. The mechanisms allowing maximizing benefits in different strategies are poorly known. In symbiotic ant/plant mutualisms in Fiji, we show that the 3D structure of plant-provided nesting sites (domatia) maximizes benefits in both cases. Specialized plant hosts have a single domatium cavity with highly absorptive 'warts' that serve as exclusive defecation sites, thus maximizing the plants' nitrogen gains. In contrast, the domatium of facultative hosts lacks absorptive warts and contains several separate, unlinked cavities, with separate entrance holes, permitting conflict-free occupation by different ant colonies. Our study suggests that minimizing competition to increase interactions with a guild of generalists may be a common strategy in facultative mutualisms.

One Sentence Summary: We show that plant-produced ant nesting sites have a 3D inner structure that differentially maximizes mutualism benefits in facultative vs. obligate ant-plant species.

Main Text [2169 words]: Theory predicts that partners involved in mutualisms should maximize benefits by reducing the costs associated with mutual services, leading to cheating and ultimately shift to parasitism (Trivers, 1971; Axelrod and Hamilton, 1981; Sachs and Simms, 2006). Nevertheless, the persistence of mutualisms over long periods either indicates that selection for cheating is not as strong as previously thought (Frederickson, 2013) or that benefits are maximized by increasing payoff rather than reducing costs. Increasing payoff can occur via filtering out the undesired partners (Federle et al., 1997; Federle and Rheindt, 2005; Chomicki et al., 2016). Increasing net benefits can also occur via a phenotypic loop wherein increased benefits to a partner yield an increased return (partner fidelity feedback), which can occur when partners' fitnesses are aligned, for example by vertical transmission (Sachs et al., 2004; Frederickson, 2013).

These mechanisms typically occur in specialized mutualisms, which often involve coevolution. Facultative or unspecialized mutualisms are thought to arise as ‘by-product’ mutualisms, where the service provided has no costs and thus selection for cheating is weak or non-existent (Sachs et al., 2004; Sachs and Simms, 2006). Facultative mutualisms should be variable in time and space because of changing local densities of the partners and other context-dependent parameters, for instance herbivore density in protection mutualisms (Rudgers and Strauss, 2004; Hoeksema and Bruna, 2015). Selection of facultative symbionts on host traits has been documented at the population level (Rudgers, 2004), but how facultative strategists maximize benefits gained from a fluctuating pool of symbionts is poorly understood. A potential solution for facultative hosts to maximize benefits would be to evolve traits that allow to accommodate the fluctuating pool of symbionts, for example by limiting competition between them, thereby maximizing the benefits received from (many different) facultative partners.

To test this hypothesis, we focused on a Fijian ant-plant symbiotic system with three basal species forming facultative symbioses with a range of ant species and a clade of six species forming obligate symbioses with the dolichoderine ant *Philidris nagasau* (Chomicki and Renner, 2016a, b; Chomicki and Renner, in revision; Fig. 1). Contrary to terrestrial ant/plant systems where plant-produced ant nesting sites (domatia) have a determinate development, *Squamellaria* species (like other genera of the Rubiaceae subtribe Hydnophytinae) have a domatium that grows continuously throughout a plant’s lifespan and becomes the major part of the plant (Fig. 1A, B). We thus first asked how domatium structure relates to mutualism strategy by generating 3D models based on CT scanning data (Online Supplementary Materials and Methods (OSM)). The 3D models revealed that the domatia of facultative *Squamellaria* species contain between several unlinked cavities that encase the first (oldest) cavity (shown in red) (Fig. 1C, D, Movie S1). This first cavity expand diffusely throughout the lifespan of facultative hosts, but not in obligate hosts (Fig. S1). The domatium of specialized *Squamellaria* species always contain a single cavity (in addition to a small embryonic cavity shown in red in Fig. 1C-E, G), which grows from the tuber apex (Fig. 1E, F, Movie S2). A phylogenetic framework and CT scanning 3D models for all nine species of *Squamellaria* shows the direction of domatium structural evolution and how it correlates with mutualistic strategy. The facultative strategy with unlinked galleries is ancestral while the obligate strategy with linked galleries is derived (Fig. 1G).

The differences in inner cavity structure suggested functional differences in mutualistic gains between strategies. To address this, we performed two series of experiments to determine the physiological capabilities of the domatium to uptake nitrogen and to probe the pattern of nutrient deposition by ants. Observations of the internal domatium structure revealed that obligate hosts have two cavity types, namely warty chambers, which occupy ~20% of the total inner cavity surface and in which small protuberances are evenly distributed, and smooth chambers lacking warts (similar to the inner cavity wall described in the Hydnophytinae genus *Myrmecodia*; Huxley, 1978). By contrast, facultative hosts have only one cavity type whose surface is poorly differentiated, with some areas 'warty-like', other 'smooth-like' (Fig. 1H, I).

To determine different cavity types' physiological uptake efficiency, we designed 'uptake experiments' by injecting solutions of different concentrations of mineral (NH_4^+) and organic (glycine) of ^{15}N stable isotopes solutions inside the domatium (OSM). In obligate ant plants (*S. huxleyana*), warty chambers were >3-fold more absorptive than smooth chambers for mineral nitrogen and 10-fold for organic nitrogen (ANOVA, post hoc test, both $p < 0.001$), and they were also 5-fold more absorptive than the unspecialized cavity surfaces of facultative species (*S. tenuiflora*) (Fig. 2A, B, Kolmogorov-Smirnov test, D=0.57, $p = 0.002$). In the facultative host chambers, the $\delta^{15}N$ value did not increase further at 500 µM (Fig. 2A, B), indicating that saturation occurred at 250 µM. This revealed the physiological limitation of the uptake function in the poorly differentiated cavities of facultative species and the physiological differentiation of chambers in obligate species, with the warty cavities being highly efficient in nutrient uptake and the smooth cavities being poorly absorptive.

The difference in nutrient uptake between mutualistic strategies raised the question of whether the deposition of nutrients by ants matched the plant physiology. We addressed this question with 'feeding experiments' where ^{15}N glycine in a sugary solution was fed to the ants during 10 days. Overall, fertilization by ants was significantly more efficient in obligate than facultative *Squamellaria* species (Fig. 2C; Kolmogorov-Smirnov test, D=0.41, $p = 0.001$). In facultative hosts, smooth-like cavities were significantly more enriched than warty-like cavities (ANOVA, post hoc test, $p < 0.001$), and both were significantly more enriched than leaves and stems, indicating that ants did not discriminate between cavity surface types. By contrast, in obligate *Squamellaria* species, we found large differences in $\delta^{15}N$ between the warty cavities and the smooth cavities (ANOVA, post hoc test, $p < 0.001$), although the latter were not significantly more enriched than leaves or stems (Fig. 2C, ANOVA, post hoc test, $p = 0.87$), indicating that ants defecate exclusively on the warts. Altogether, this suggests coevolution of plant physiology and ant behavior, resulting in the detection of the warts and exclusive defecation onto these highly absorptive structures, thus maximizing benefits to the plant (Fig. 2E, F).

The different structure and function of facultative vs. obligate domatia led us to investigate the relationship between colony size and domatium volume. We found a strong positive correlation between both variables in obligate but not in facultative symbioses, relating to the multi-cavity domatium structure that prevents colony expansion (Fig. 2D, specialists: $R^2 = 0.98$ vs. 0.37 in facultative; Pearson's correlation coefficient 0.99 vs. 0.63). The surface/volume ratio was >2-fold greater in obligate hosts as compared to facultative ones (Fig. 2D, inset, t-test, $p < 0.001$). This implies partner-fidelity feedback in obligate symbioses, where more domatium growth leads to more nesting space, thus more workers and more nutrient input, which feeds back on host growth, and thus participates in maximizing benefits to both partners. The uncoupling of domatium volume and colony size, however, was puzzling in terms of how this strategy maximizes benefits and how this can be maintained in natural populations.

To test if the unusual modular domatia of facultative hosts could be an adaptation to changing symbiont occupation, we monitored ant inhabitants in an obligate species (*S. imberbis*) and a facultative species (*S. wilkinsonii*) growing sympatrically at a six months interval. While 100% of the domatia in the obligate *S. imberbis* were inhabited by *Philidris nagasau*, the ants occupying the facultative *S. wilkinsonii* varied in species composition and number (Fig. 3A). Potentially following seasonal community dynamics, founding queens from distinct species continuously colonize facultative hosts, and we found 16 % of *S. wilkinsonii* individuals inhabited by two ant species living in distinct cavities (unlinked and with separate entrance holes). This suggests that the multi-cavity domatium may be an adaptation to reduce the competition between different mutualists by reducing the probability of encounters between founding queens and already established or establishing ant colonies.

To probe this hypothesis, we developed a probabilistic model to estimate the optimal cavity number as a function of ant turnover. We consider that founding ant queens in search of cavities arrive at a facultative plant with rate a. The queen will then randomly select one of the n cavities and try to found a nest there, meaning that for each cavity the time until the next queen arrives is exponentially distributed with rate a/n. We assume that it takes a time span g until the nest is large enough to defend the cavity against invaders. If other queens arrive at the same cavity while the nest is still small, we assume that the ants will fight, such that none of the nests can be established. We further assume that only large nests are beneficial for the plant. Thus there are three possible states for each of the cavities. It can be empty (E), it can harbor a small nest (S), or it can harbor a large nest (L). Let d be the average life span of nests after they became large. After a large nest dies, the cavity will be empty again. Hence, if a cavity is in state L, the next state will always be E, and if it is in state E, the next state will always be S. If a cavity is in state S, the probability that the next state will be L is $e^{-ga/n}$, as this is the probability that no competing ants will arrive for a time span g. Hence, the probability that the next state is E is $1 - e^{-ga/n}$.

If we consider a series of states of a cavity, disregarding how long the cavity is in each state at a time, we see (at least in the long run) E as often as we see S, because E is always followed by S. Thus, if p_E, p_S, and p_L are the equilibrium probabilities of this Markov chain (still disregarding the durations of each state) we obtain $p_E = p_S$. Furthermore, from the equilibrium conditions follows that $p_S\cdot e^{-g\cdot a/n} = p_L$, and together with $p_L+p_S+p_E = 1$ this implies $p_E = p_S = 1/(2+e^{-g\cdot a/n})$ and $p_L = 1/(2e^{g\cdot a/n}+1)$.

The expectations values for the time spent in a state E, S or L before leaving it again are $t_E = n/a$, $t_L = d$ and

$$t_S = e^{-g\cdot a/n}\cdot g + \int_0^g x\cdot\frac{a}{n}\cdot e^{-x\cdot a/n}\,dx = (n/a)\cdot\left(1-e^{-g\cdot a/n}\right)$$

By weighting p_E, p_S and p_L with the expected times spans t_E, t_S and t_L, we can calculate the fraction of time in which the cavity harbors a large nest, averaged over a long time span:

$$f_L = \frac{p_L\cdot t_L}{p_E\cdot t_E + p_S\cdot t_S + p_L\cdot t_L} = \frac{\dfrac{d}{2e^{g\cdot a/n}+1}}{\dfrac{n}{a\cdot\left(2+e^{-g\cdot a/n}\right)} + \dfrac{n\cdot\left(1-e^{-g\cdot a/n}\right)}{a\cdot\left(2+e^{-g\cdot a/n}\right)} + \dfrac{d}{2e^{g\cdot a/n}+1}} = \frac{a\cdot d}{a\cdot d + n\cdot\left(2e^{g\cdot a/n}-1\right)}. \qquad (1)$$

We can also consider f_L as the average fraction of cavities filled with large nests, again averaged over a long period of time. Thus, the optimal n for a plant to have most of its inner volume filled with large ant nests can be calculated by minimizing the term $n\cdot(2e^{g\cdot a/n}-1)$. The product $g\cdot a$ is the expected number of queens arriving at a plant during a time span that is needed to establish a nest in a cavity.

Solving equation (1) shows that with no or only mild turnover, a single cavity is the optimal solution, while a multiple cavity system is optimal when the probability of turnover ($g.a$) is greater than 0.65 (Fig. 3B). This confirms our prediction that the modular structure of facultative host domatia (discovered with CT scanning of entire domatia) reduces colony encounters and aggression, thereby forestalling symbiont turnover and maximizing the time a plant spends with large ant colonies.

Examination of facultative and obligate hosts at all developmental stages revealed that in facultative species, the first cavity in young plants is occupied by ants, but that it is taken over by other arthropods (or even small vertebrates) once it becomes large and old, with the change in occupants mediated by an increase in entrance hole diameter over ontogeny (Fig. S2). By contrast, the entrance hole diameter in the obligate species does not change with domatium age. This further increases the diversity of partner available to hosts, which is beneficial when partner abundance and quality fluctuate (Waser et al., 1996).

Competition for nesting space is thought to be a driving force behind the evolution of ant/plant symbioses (Davidson et al., 1989; Davidson and McKey, 1993; Stanton et al., 1999), with plant hosts being the limiting resource. Our study illuminated the converse situation of generalization occurring by limiting the competition between generalist ant symbionts.

The nestedness of mutualistic networks increases biodiversity by reducing competition (Bastolla et al., 2009). Here we showed how domatium modularity reduces competition among ant colonies and increases the overall time that cavities are occupied by ant partners. The specialization of mutualism may occur via a trait change that favors a particular partner within a competing guild (Palmer et al., 2003). Minimizing competition to increase interactions with a guild in facultative mutualisms may thus be a widespread mechanism of species coexistence.

References and Notes

1. R. L. Trivers, *Q. Rev. Biol.* **46**, 35-57 (1971).

2. R. Axelrod, W. D. Hamilton, *Science* **211**,1390-1396 (1981).
3. J. L. Sachs, E. L. Simms, *Trends Ecol. Evol.* **21**, 585-592 (2006).
4. M.E. Frederickson, *Q. Rev. Biol.* **88**, 269-295 (2013).
5. W. Federle, *et al. Oecologia* **112**: 217–224 (1997).
6. W. Federle, F. E. Rheindt, *Biol. J. Linn. Soc.* **84**, 177–193 (2005).
7. G. Chomicki, Y. Staedler, J. Schönenberger, S. S. Renner, *New Phytol.* (DOI: 10.1111/nph.13990) (2016).
8. J. L. Sachs, U. G. Mueller, T. P. Wilcox, J. J. Bull, *Q. Rev. Biol.* **79**, 135-160 (2004).
9. J. D. Hoeksema, E. M. Bruna, In *Mutualism. Ed. J.L. Bronstein. Oxford University Press, Oxford, UK*, pp.180-202 (2015).
10. J. A. Rudgers, S. Y. Strauss, Proc. R. Soc. B. **271**: 2481-2488 (2004).
11. J. A. Rudgers, *Ecology* **85**,192-205 (2004).
12. G. Chomicki, S. S. Renner, *PLoS ONE* **11**: e0151317 (2016).
13. G. Chomicki, S. S. Renner, *Nature Plants* (Submitted 21 May 2016, in revision).
14. C. R. Huxley, *New Phytologist* **80**, 231-268 (1978).
15. N. M. Waser, *et al.*, *Ecology* **77**, 1043-1060 (1996).
16. D. W. Davidson, R. R. Snelling, J. T. Longino,1989. *Biotropica* **21**, 64-73 (1989).
17. D. W. Davidson, D. McKey, *J Hymenopt Res* **2**,13–83 (1993).
18. M. L. Stanton *et al.*, *Nature* **401**, 578-581 (1999).
19. U. Bastolla *et al. Nature* **458**, 1018-1020 (2009).
20. T. M. Palmer, M. L. Stanton, T. P. Young, *Am. Nat.***162**, S63-S79 (2003).

Acknowledgements:

We thank Jeremy Aroles extensive help with fieldwork and for proofreading the manuscript, Veronika Mayer for suggestions on experimental design, Christoph Mayr for assistance of IR-MS analysis, Erol Akçay, Matthew Jebb, Camilla Huxley-Lambrick for discussion. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20, and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to GC.

Figure captions

Fig. 1. Structure of domatium in facultative and obligate Fijian *Squamellaria* hosts. (A) Habit of *Squamellaria tenuiflora*, a facultative ant-plant. (B) Habit of *S. wilsonii*, an obligate ant-plant. (C-D) CT scanning-based 3D models of *S. tenuilflora* (facultative) domatium. (E-F) CT scanning-based 3D models of *S. wilsonii*. (G) Domatium 3D models for all Fijian *Squamellaria* mapped on a 9-gene phylogeny. (H-I) Internal cavity structure for the facultative host *S. tenuiflorum* (H) and for the obligate host *S. wilsonii* (I).

Fig. 2. Physiological nitrogen uptake capacity for facultative and obligate hosts and patterns of nitrogen deposition by ants. (A-B) Uptake experiments to test the uptake capacity of facultative (*S. wilkinsonii*) and obligate hosts (*S. huxleyana*) internal cavities using mineral nitrogen (^{15}N NH4+) (A) and organic (1^{5}N glycine) (B). (C) Feeding experiments to determine the pattern of nitrogen deposition by ants inside domatium chambers of facultative (*S. wilkinsonii*) and obligate hosts (*S. huxleyana*). (D) Relationships between domatium volume and ant colony size, and surface: volume ratios in facultative and obligate hosts. (E-F) Schematic representation of domatium functional partitioning in facultative (E) and obligate (F) hosts.

Fig. 3. Modular colony structure in facultative ant-plants forestalls symbiont turnover. (A) Ant inhabitants in facultative (*S. wilkinsonii*) and obligate (*S. imberbis*) that grow in the same site, sampled 6 months apart. (B) Optimal cavity number to maximize the time spent with large (L) ant colony in function of ant turnover as determined by our probabilistic modeling.

Supplementary Materials:

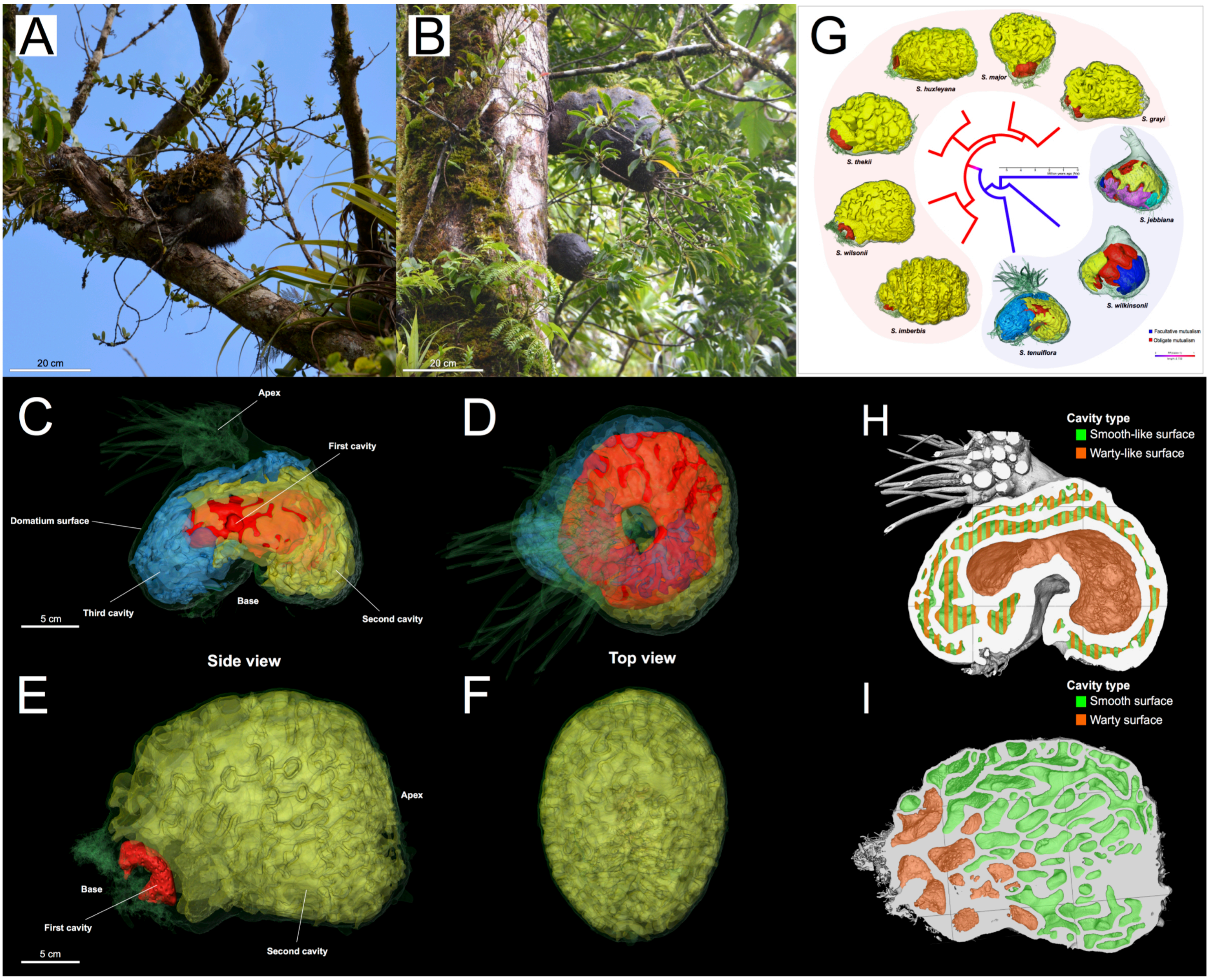
Materials and Methods

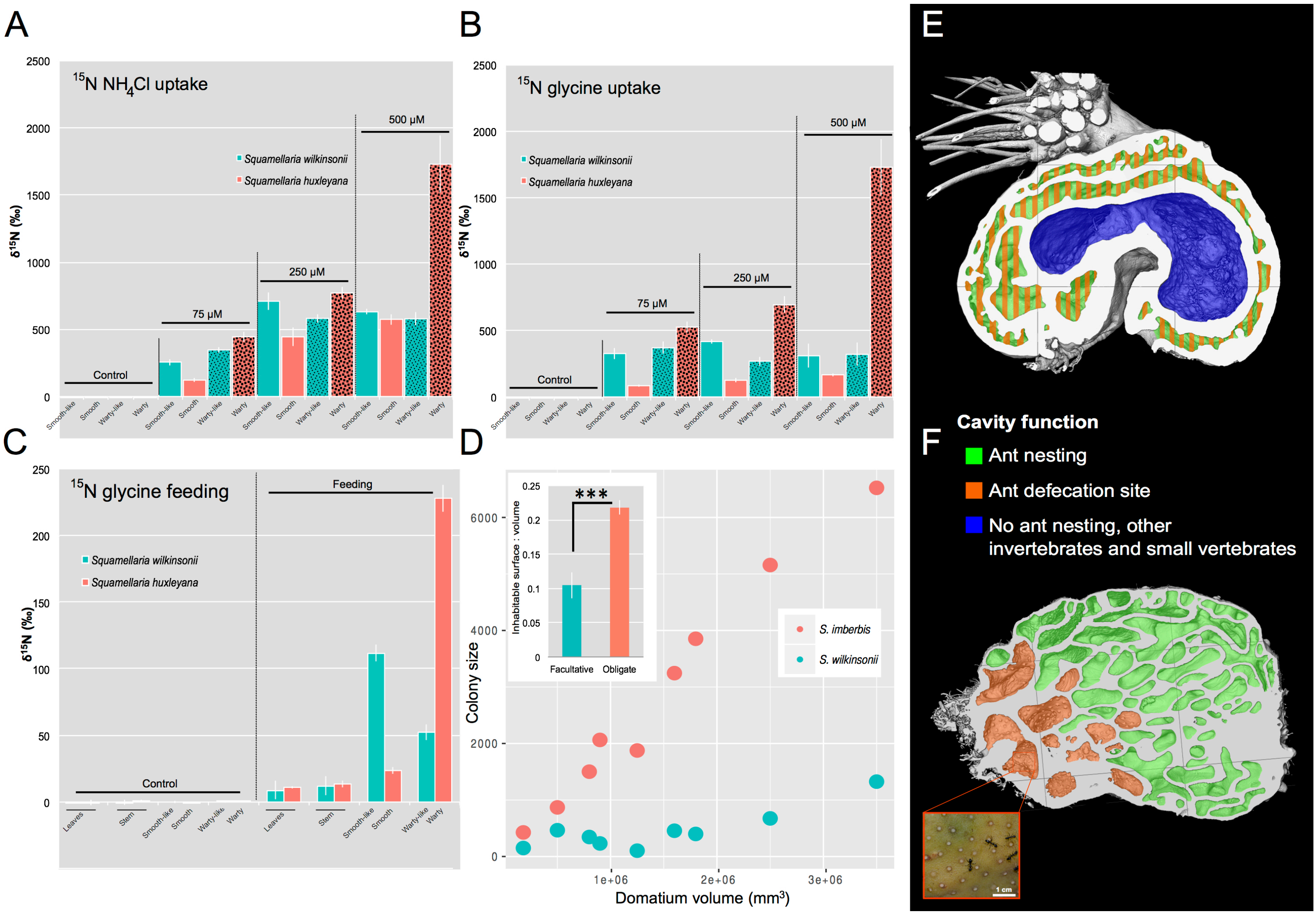
Figures S1-S2

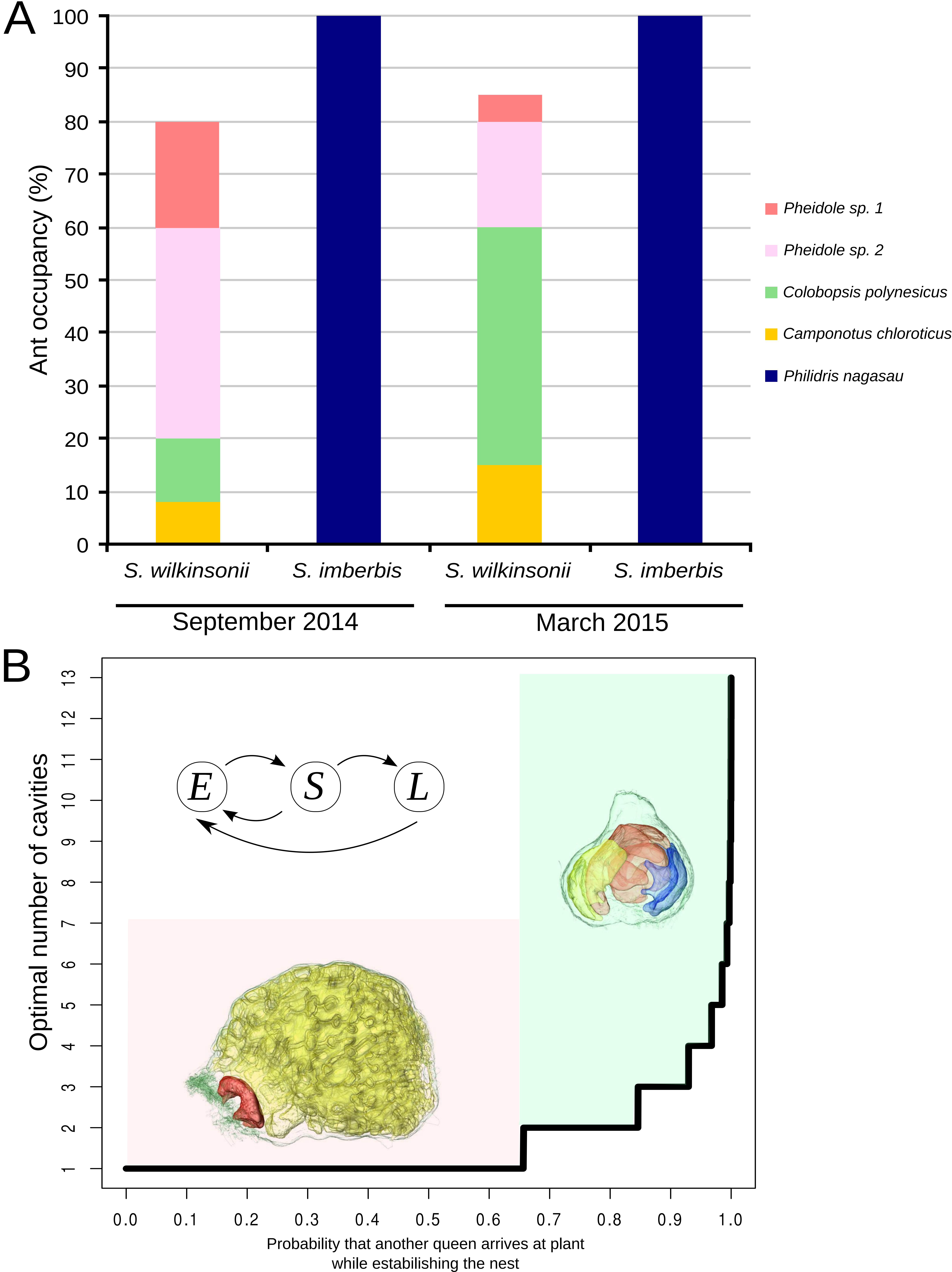
Table S1

Movies S1-S2

References (21-30)


A
20 cm
B
20 cm
G
S. huxleyana
S. major
S. grayi
S. thekii
S. wilsonii
S. imberbis
S. tenuiflora
S. jebbiana
S. wilkinsonii
Million years ago (Ma)
5 4 3 2 1 0
Facultative mutualism
Obligate mutualism
C
Apex
First cavity
Domatium surface
Third cavity
Base
Second cavity
5 cm
Side view
D
Top view
H
Cavity type
Smooth-like surface
Warty-like surface
E
Apex
Base
First cavity
Second cavity
5 cm
F
I
Cavity type
Smooth surface
Warty surface


A
15N NH4Cl uptake
Squamellaria wilkinsonii
Squamellaria huxleyana
δ15N (‰)
2500
2000
1500
1000
500
0
Control
75 µM
250 µM
500 µM
Smooth-like
Smooth
Warty-like
Warty
B
15N glycine uptake
Squamellaria wilkinsonii
Squamellaria huxleyana
δ15N (‰)
Control
75 µM
250 µM
500 µM
C
15N glycine feeding
Squamellaria wilkinsonii
Squamellaria huxleyana
δ15N (‰)
250
200
150
100
50
0
Control
Feeding
Leaves
Stem
Smooth-like
Smooth
Warty-like
Warty
D
Colony size
6000
4000
2000
0
Domatium volume (mm3)
1e+06
2e+06
3e+06
S. imberbis
S. wilkinsonii
Inhabitable surface : volume
0.25
0.2
0.15
0.1
0.05
0
***
Facultative
Obligate
E
F
Cavity function
🟩 Ant nesting
🟧 Ant defecation site
🟦 No ant nesting, other invertebrates and small vertebrates
1 cm


A
Ant occupancy (%)
0
10
20
30
40
50
60
70
80
90
100
S. wilkinsonii
S. imberbis
S. wilkinsonii
S. imberbis
September 2014
March 2015
Pheidole sp. 1
Pheidole sp. 2
Colobopsis polynesicus
Camponotus chloroticus
Philidris nagasau
B
Optimal number of cavities
1
2
3
4
5
6
7
8
9
10
11
12
13
E
S
L
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Probability that another queen arrives at plant
while estabilishing the nest

Supplementary Materials for

Domatium 3D structure maximize benefits in facultative and obligate nutritional mutualisms

Guillaume Chomicki, Dirk Metzler, Susanne S. Renner

correspondence to: guillaume.chomicki@gmail.com

This PDF file includes:

Materials and Methods

Collection of material on Fiji and study sites

In September 2014 and March 2015, the first author conducted fieldwork on Viti Levu, Vanua Levu, and Taveuni and collected all species of Fijian *Squamellaria*, including three new species (*S. jebbiana*, *S. grayi*, and S. *huxleyana;* Chomicki and Renner, 2016). The study sites in Viti Levu were Colo-i-Suva forest reserve in the south of the island (S 18° 1’ 46.808”, E 178° 24’ 0.4175”) and forest around Navai in the center of the island (S 17° 37’ 49.5979”, E 177° 58’ 34.9315”); in Vanua Levu, the collection sites were in Waisali forest reserve (S 16 38’19.8”, E 179 13’19.7”), and along the Cross Island road before the bifurcation to Nabouwalu and Labasa; in Taveuni, the collections were made along the trail to DesVoeux peak and Mt. Manuca on the western side of the island (S 16° 48’ 25.8133”, E 179° 56’ 36.6843”), and at the end of Lavena coastal walk, Bouma heritage park, on the eastern side of the island (S 16° 51’ 45.4433”, E 179° 54’ 6.5149”). All collections were made in collaboration with colleagues from the University of South Pacific (*Acknowledgements*), and vouchers have been deposited in the herbaria of Suva (SUVA) and Munich (M). For DNA extraction, we collected young leaves and dried them in silica gel. *Squamellaria* taxonomy follows Chomicki and Renner (2016). Except for a few cases, *Squamellaria* plants were accessed by tree climbing, using a rope secured by a partner on the ground. This technique allowed long stays in the canopy with minimal disturbance of ant colonies.

^{15}N uptake and feeding experiments and δ ^{15}N isotope analyses

We designed two types of experiments to determine the uptake capacity of the distinct cavity types by facultative versus obligate hosts (uptake experiments) and to determine the actual fertilization by ants (feeding experiments). For the **uptake experiments**, we selected two species: *Squamellaria wilkinsonii* (facultative host) and *S. huxleyana* (obligate host). We tested the uptake capacity of mineral and organic nitrogen using $^{15}NH_4Cl$ ($^{15}NH_4^+$; Isotec, 98% at) as mineral nitrogen source and ^{15}N glycine (Isotec, 98% at) as organic nitrogen source. To determine the potential for nutrient uptake in the distinct cavities of facultative versus obligate hosts, ^{15}N labelled mineral or organic nitrogen-containing molecules were injected in the domatium cavities by injection with a syringe for an incubation time of 1h. To prevent that the solution leaked from the many entrance holes, the whole domatium was wrapped up in parafilm M ®, with the plant being intact (shoots attached to domatium). We used $^{15}NH_4^+$ and ^{15}N glycine at concentrations of 75, 250 and 500 µM with three replicates per nitrogen form and concentration. Three controls were injected with distilled water only. To ensure replicability across replicates, we selected plants of similar size with domatia of ca. 12-15 cm in diameter. After the 1h incubation, apoplastically bound ions were removed by flushed the domatia twice with a 10 mM $CaCl_2$ solution (for one minute each). Domatia were subsequently cut in halves and further washed carefully with distilled water. Samples from warty and smooth chambers (for *S. huxleyana*) and from warty-like and smooth-like chambers (for *S. wilkinsonii*) and were then dissected with a scalpel, with a thickness of *ca*. 1 mm and subsequently microwave-dried.

For the **feeding experiments**, we used the same two species: *Squamellaria wilkinsonii* (facultative host) and *S. huxleyana* (obligate host). The aim of the experiment was to determine whether ants fertilized the plant, and if so, where and how efficiently defecation occurs. To do so, we selected five trees with distinct *P. nagasau* colonies (on five distinct trees), five trees with *Squamellaria wilkinsonii*. We then placed a solution of 20 mM ^{15}N glycine (enriched at 98% at, Isotec) with 40% (w/v) 1:1:1 mix of sucrose, glucose and fructose in a falcon tube close to a mature plants. A paper wick allowed the ants to reach the solution without drowning in it. We added two millilitres of solution to the falcon tube twice a day during the 10 days of the experiment. On the 11th day, we collected the mature plant closest to the nitrogen source. Each plant was cut in half, washed and dried as described above. For each species, five controls of the same stage were collected from a neighboring tree (at about 500 m) and prepared in the same way.

For both the uptake and feeding experiments, samples were homogenized with a ball mill and ca. 1-3 mg of dry powder was weighted in tin capsules. Isotope-ratio mass spectrometry (IR-MS) analyses were performed at the GeoBiocenter, University of Munich (LMU). Capsules were combusted in an elemental analyser (NC2500, Carlo Erba) in a continuous helium flow at 1080^0C. The combustion gases passed through a reaction tube filled with chromium and silvered cobaltous oxides, a subsequent reduction tube (560^{0}C) filled with copper wires, a water trap filled with magnesium perchlorate, and a gas-chromatography column. The isolated gases N_2 and CO_2 were then analysed in an isotope-ratio mass spectrometer (DeltaPlus, Thermo-Finnigan) to determine the isotope ratios of organic carbon (δ^{13}Corg) and nitrogen (δ^{15}N). The total organic carbon (TOC) and total nitrogen (TN) mass percentages were calculated from sample peak areas using the elemental standards atropine, cyclohexanone-2,4-dinitrophenylhydrazone, and peptone for calibration.

DNA extraction and phylogenetic analyses

We used our recently generated *Squamellaria* matrix of nine gene regions based on plastid (trnL-trnF region (trnL intron and trnL-trnF spacer), ndhF, rps16, rpl20-rps12, trnG-trnS spacer) and nuclear regions (ITS region (ITS1, 5.8S, ITS2), ETS, 18S) (Chomicki and Renner, 2016). All accessions of Fijian *Squamellaria* were extracted from silica-dried leaves collected by GC and are all linked to herbarium specimens deposited in SUVA, Suva, Fiji and M, Munich, Germany. A sampling of outgroups (in the tribe Psychotrieae) was selected based on Barrabé et al. (2014). Voucher information is reported in Table S1.Total genomic DNA was extracted from c. 20 mg of leaf tissues, using a commercial plant DNA extraction kit (NucleoSpin; Macherey–Nagel, Düren, Germany) according to manufacturer protocols. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England Biolabs, Cambridge, MA, USA) and a standard protocol (39 cycles, annealing temperature 56^{0}C). PCR products were purified using the ExoSap clean-up kit (Fermentas, St Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were BLAST-searched in GenBank. Sequence alignment was performed in MAFFT v. 7 in the online server (http://mafft.cbrc.jp/alignment/ server;) (Katoh and Standley, 2013) under standard parameters except for the ITS region, which was aligned under Q-INS-i optimization, which takes rRNA secondary structure into consideration. Minor alignment errors were corrected manually in Mesquite v. 2.75 (Maddison and

Maddison, 2011). In the absence of statistically supported incongruence (i.e., maximum likelihood bootstrap (BS) support >75) between the plastid and nuclear data partitions), we concatenated all markers, yielding an alignment of 9346 bp for the *Squamellaria* matrix. Maximum-likelihood (ML) inference relied on RAxML v8.0 (Stamatakis, 2014) and the GTR + Γ substitution model, with empirical nucleotide frequencies and 25 gamma rate categories; bootstrap support was assessed from 100 replicates under the same model. We also conduced Bayesian inference in MrBayes v. 3.2 (Ronquist et al., 2012) under the same substitution model (but with 4 rate categories) and using the program's default two runs and four chains (one cold and three heated), with the uniform default priors. We set a $10X10^6$ MCMC chain, sampling trees every 1000^{th} generation. Split frequencies approaching zero indicated convergence. We used the 50% consensus tree to assess posterior probabilities for nodes of interest.

Stochastic mapping ancestral state reconstruction of facultative vs. obligate mutualism

To trace the evolution of mutualistic strategies along the branches of the tree, we used stochastic character mapping in the function 'make.simmap' in the phytools R package (v. 04-60) (Revell, 2012), which implements the stochastic character mapping approach developed by Bollback (2006). We estimated ancestral states using under the ER model, and then simulated 1,000 character histories on the MCC tree. We summarized the 1,000 simulated character histories using the function 'densityMap'.

Molecular clock dating

Molecular dating analyses relied on BEAST v. 2 (Bouckaert et al., 2014) and uncorrelated lognormal relaxed clock models. We used the GTR + G substitution model with four rate categories and a Yule tree prior. For both our plant and ant trees, MCMCs were run for 20 million generations, with parameters and trees sampled every 10,000 generations. We used Tracer v. 1.6 (Rambaut et al., 2014) to check that the effective sample size (ESS) of all parameters was >200, indicating that runs had converged. After discarding 10% as burn-in, trees were summarized in TreeAnnotator v. 1.8 (part of the BEAST package) using the options 'maximum clade credibility tree', which is the tree with the highest product of the posterior probability of all its nodes, 'mean node height,' and a posterior probability limit of 0.98. The final tree was visualized in FigTree v. 1.4 (Rambaut, 2014). To calibrate our tree, we constrained the age of the root, i.e., the split between the Pacific clade and the so-called *Psychotria* clade IV of Barrabé et al. (2014), to 22 ± 7 Ma, based on the age of this node estimated by these authors, using a normal prior and a standard deviation of 4 corresponding to the 95% confidence interval of Barrabé et al. (2014).

Computed-Tomography 3D reconstructions

Domatia were collected in the field and immediately immersed in a 70% EtOH solution until scanned. Computed-tomography scanning was performed on a Nanotom m (Phoenix) X-ray tomography scanner at the Zoologische Staatssammlung in Munich. 3D processing was performed with the software Amira (version 6.0.1, TGS Europe, S.A., Merignac Cedex, France; Mercury Computer Systems Inc., Chelmsford, MA). For slice alignment, the section edges representing the bottom of the block (mold) were used as reference in addition to bringing the specimen structures of neighboring slices to a maximum congruence. Labeling of structures (Amira: segmentation) was done by hand, with the brush (internal structures) and lasso (external surfaces) tools. Initially, every third slice was labeled, with subsequent interpolation of structures on intervening slices, followed by a check of each interpolation and correction if necessary. Before surface rendering, structures were separated from the 'master' LabelField '.am' file into several LabelFields, each containing one specimen component. Specifically, we separated each independent cavity, as well as the surface outline of each tuber (obtained by merging all elements to 'tuber'). This allowed an easy visualization of the tuber 3D structures. In addition, most new LabelFields were reduced in resolution by applying the Resample module to enable (fast) surface rendering, mostly using the default settings (binning x and y values by 2). Surface rendering was performed with the SurfaceGen module, leaving all settings at default. This was followed by the smoothening of the reduced surfaces using the (SmoothSurface module, mostly 40 iterations). To facilitate visualization of domatium structure, we applied a transparency of 70% for each individual cavity and 90% for the tuber surface. Movies were created using the MovieMaker module.

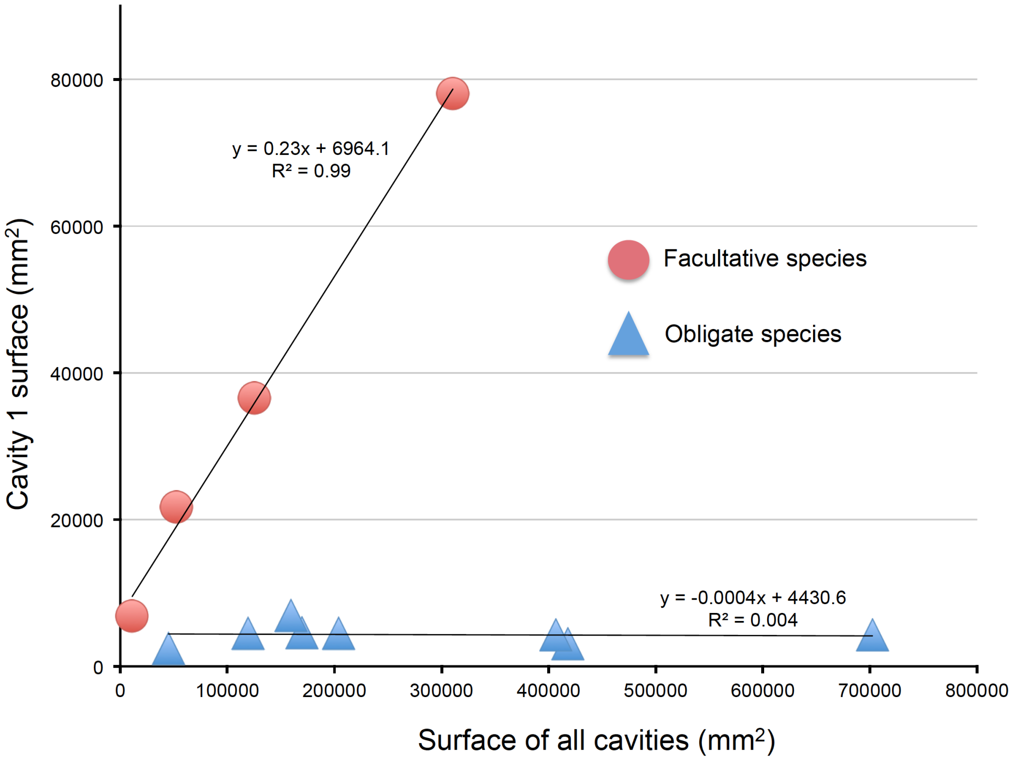


Fig. S1. Growth of the first cavity (shown in red in Figure 1) in facultative and obligate Fijian *Squamellaria*. In facultative species, cavity one growth together with other cavities, while in obligate species it stops expanding early in ontogeny, while the second cavity grows apically.

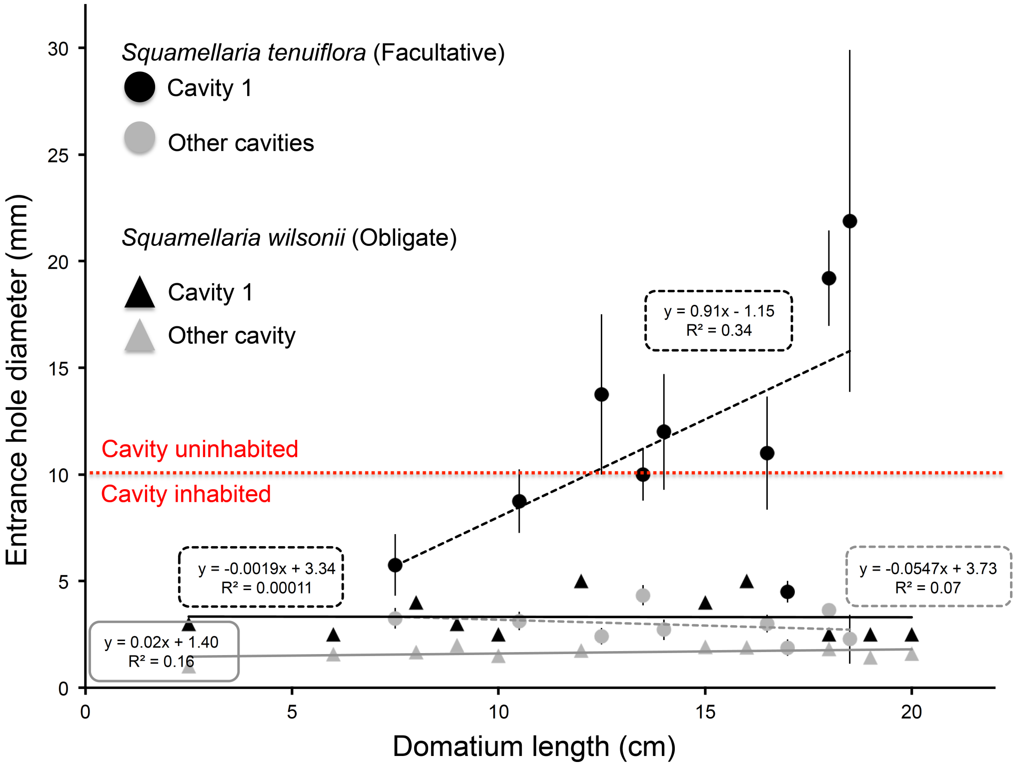


Fig. 2. Entrance hole diameter in function of domatium length in facultative (*Squamellaria tenuiflora*) and obligate (*S. wilsonii*) species. In facultative species, entrance holes connected to the cavity 1 expand as the cavity grows (see also Fig. S1). Because when entrance holes are over one centimeter, the filtering is no longer proficient, ants are replaced by other arthropods such as cockroaches, millipedes, spiders, and even small vertebrates such as gecko or skins that transiently use these sites to breed.

S1 Table. Plant material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	Voucher	Geographic origin	ITS	ETS	18S	trnL-trnF	ndhF	trnS-	rps16	rps12-rpl20
***Amaracarpus muscifer* A.C.Sm.**	L. Barrabe & M. Tuiwawa 1109 (NOU)	Fiji	KF675907	KF675790	-	-	KF675995	-	KF676083	-
***Amaracarpus nematopodus* (F.Muell.) P.I.Forst.**	L. Barrabe et al. 1030 (NOU)	Australia	JX155060	KF675791	-	-	JX155105	-	JX155152	-
***Anthorrhiza caerulea* Huxley & Jebb**	M.P.H. Jebb 358 (FHO)	Papua New Guinea	KU586349	KU586368	-	-	-	-	-	-
Anthorrhiza echinella	G. Chomicki 83 (M)	Cultivated Oxf. Bot Gard., origin New Guinea	KU586350	KU586369	-	-	-	-	-	-
***Hedstromia latifolia* A.C.Sm.**	L. Barrabe et al 1090 (NOU)	Fiji	KF675911	KF675795	-	-	KF675999	-	KF676087	-
***Hydnophytum formicarum* Jack**	G. Chomicki 87 (M)	Cultivated, origin Malaysian region	KU586346	KU586365	-	-	KU586397	-	-	-
***Myrmecodia beccarii* Hook f.**	G. Chomicki 99 (M)	Cultivated, origin Australia	KU586347	KU586366	-	-	KU586398	-	-	-
***Myrmecodia salomonensis* Becc.**	C. R. Huxley and L. M. Turton 3442 (FHO)	Solomons	KU586351	KU586370	-	-	-	-	-	-
***Myrmecodia dahlii* K.Schum.**	J.I. Menzies 5947 (FHO)	Papua New Guinea	KU586348	KU586367	-	-	KU586399	-	-	-
***Myrmephytum arfakianum* (Becc.) Huxley & Jebb**	G. Chomicki 116 (M)	Cultivated, origin Papua	KU586352	KU586371	-	-	KU586400	-	-	-
***Myrmephytum beccarii* Elmer**	G. Chomicki 118 (M)	Cultivated, origin Philippines	KU586353	KU586354	-	-	KU586401	-	-	-
***Psychotria comptonii* S.Moore**	L. Barrabe & Rigault 1014 (NOU)	New Caledonia	KF675927	KF675823	-	-	KF676015	-	KF676104	-
***Psychotria dallachiana* Benth.**	L. Barrabe & Rigault 1048 (NOU)	Australia	KF675928	KF675824	-	-	KF676016	-	KF676169	-
***Psychotria declieuxioides* S.Moore**	L. Barrabe & Nigote 937 (NOU)	New Caledonia	KF675932	KF675828	-	-	KF676020	-	KF676107	-

***Psychotria faguetii* (Baill.) Schltr.**	L. Barrabe et al. 820 (NOU)	New Caledonia	KF675934	KF675831	-	-	KF676023	-	-	-
***Psychotria fitzalanii* Benth.**	L. Barrabe & Rigault 1057 (NOU)	Australia	KF675935	KF675832	-	-	KF676024	-	KF676110	-
***Psychotria goniocarpa* (Baill.) Guillaumin**	L. Barrabe 586 (NOU)	New Caledonia	KF675940	KF675838	-	-	KF676029	-	KF676115	-
***Psychotria hawaiiensis* (A.Gray) Fosberg**	Y. Pillon 1425 (NOU)	Hawaii	KF675941	KF675840	-	-	KF676030	-	KF676116	-
***Psychotria hivaoana* Fosberg**	Meyer 3071 (PAP)	French Polynesia	KF675942	KF675841	-	-	KF676031	-	KF676117	-
***Psychotria insularum* A.Gray**	Y. Pillon 909 (NOU)	Wallis & Futuna	KF675943	KF675842	-	-	KF676032	-	KF676118	-
***Psychotria iteophylla* Stapf**	Axelius 303 (S)	Borneo	-	-	-	-	-	-	AF410726	-
***Psychotria loniceroides* Sieber ex DC.**	L. Barrabe & Rigault 1042 (NOU)	Australia	KF675945	KF675846	-	-	KF676033	-	KF676120	-
***Psychotria lorentzii* Valeton**	Puradyatmika 10460 (K)	Papua New Guinea	KF675946	KF675847	-	-	KF676034	-	KF676121	-
***Psychotria micralabastra* (Lauterb. & K.Schum.) Valeton**	Takeuchi 16163 (K)	Papua New Guinea	KF675949	KF675851	-	-	KF676036	-	KF676124	-
***Psychotria micrococca* (Lauterb. & K.Schum.) Valeton**	Drozd & Molem s.n. (PSF)	Papua New Guinea	KF675951	KF675853	-	-	KF676038	-	KF676126	-
***Psychotria microglossa* (Baill.) Baill. ex Guillaumin**	L. Barrabe 585 (NOU)	New Caledonia	KF675950	KF675852	-	-	KF676037	-	KF676125	-
***Psychotria monanthos* (Baill.) Schltr.**	Y. Pillon 1370 (NOU)	New Caledonia	KF675953	KF675855	-	-	KF676040	-	KF676128	-
***Psychotria poissoniana* (Baill.) Guillaumin**	J. Munzinger 5156 (NOU)	New Caledonia	KF675958	KF675861	-	-	KF676045	-	KF676133	-
***Psychotria pritchardii* Seem.**	L. Barrabe et al 1124 (NOU)	Fiji	KF675992	KF675903	-	-	KF676078	-	KF676165	-
***Psychotria raivavaensis* Fosberg**	Meyer 3088 (PAP)	French Polynesia	KF675960	-	-	-	KF676047	-	KF676135	-
***Psychotria submontana* Domin**	L. Barrabe et al. 1044 (NOU)	Australia	KF675988	KF675899	-	-	-	-	KF676168	-

***Psychotria temehaniensis* J.W.Moore**	Mouly 403 (P)	French Polynesia	KF675989	KF675900	-	-	KF676075	-	KF676162	-
***Psychotria trisulcata* (Baill.) Guillaumin**	L. Barrabe et al. 902 (NOU)	New Caledonia	KF675990	KF675901	-	-	KF676076	-	KF676163	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (SUVA) HOLOTYPE	Taveuni, Bouma falls, Lavena	KU586339	KU586358	KU586427	KU586376	KU586388	KU586406	KU586436	KU586417
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 47 (M)	Vanua Levu, Waisali forest park	-	-	-	KU586372	-	KU586402	KU586432	KU586413
***Squamellaria guppyana* (Becc.) Chomicki, comb. nov.**	G. Chomicki 123 (M)	Cultivated, origin Solomons	KU586345	-	-	-	KU586396	-	-	-
***Squamellaria huxleyana* Chomicki sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 48 (SUVA) HOLOTYPE	Fiji, Vanua Levu, road between Savusavu to	KU586336	KU586355	KU586425	KU586373	KU586385	KU586403	KU586433	KU586414
***Squamellaria imberbis* (A. Gray) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	Fiji, Vanua Levu, track to vodaphone tower.	KU586337	KU586356	-	KU586374	KU586386	KU586404	KU586434	KU586415
***Squamellaria jebbiana* Chomicki, sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	Fiji, Taveuni, Mt Manuca area.	KU586342	KU586361	-	KU586379	KU586391	KU586408	KU586438	KU586419
***Squamellaria kajewskii* (Merr. & L.M.Perry) Chomicki, comb. nov.**	G. Chomicki 122 (M)	Cultivated, origin Solomons	KU586335	-	-	-	KU586384	-	-	-
***Squamellaria major* A.C. Sm.**	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586338	KU586357	KU586426	KU586375	KU586387	KU586405	KU586435	KU586416
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	-	-	KU586430	KU586381	KU586393	KU586410	KU586440	KU586421
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	KU586343	KU586362	KU586431	KU586382	KU586394	KU586411	-	KU586422
***Squamellaria thekii* Jebb**	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586340	KU586359	KU586428	KU586377	KU586389	KU586407	KU586437	KU586418
***Squamellaria vanuatuensis* (Jebb & Huxley) Chomicki, comb. nov.**	McPherson 19437 (P)	Vanuatu	JX155078	-	-	-	-	-	JX155170	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki,**	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	Fiji, Vanua Levu, Waisali forest park.	-	-	KU586429	KU586380	KU586392	KU586409	KU586439	KU586420
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki,**	G. Chomicki, J. Aroles, A. Naikatini 49 (M)	Fiji, Vanua Levu, Waisali forest park.	-	KU586364	-	-	-	-	-	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki,**	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	Fiji, Vanua Levu, Waisali forest park.	KU586344	KU586363	-	KU586383	KU586395	KU586412	KU586441	KU586423
***Squamellaria wilsonii* (Horne ex Baker) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586341	KU586360	-	KU586378	KU586390	-	-	KU586424

Supplementary Movies

Movie S1. Computed-tomography-based 3D reconstruction of a facultative ant-plant domatium (*Squamellaria tenuiflora*). Distinct colors show independent (unconnected) cavities. The first (oldest) cavity is shown in red.

Movie S2. Computed-tomography-based 3D reconstruction of an obligate ant-plant domatium (*Squamellaria wilsonii*). The first (oldest) cavity is shown in red.

Supplementary references

21. Katoh, K. and Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772-780.
22. Maddison, W.P. and Maddison, D.R., 2010. Mesquite: a modular system for evolutionary analysis. 2011; Version 2.75. *Available at: mesquiteproject. org/mesquite/download/download.html*.
23. Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313.
24. Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61: 539-542.
25. Revell, L.J., 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3: 217-223.
26. Bollback, J.P., 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC bioinformatics 7*: 1.

27. Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A. and Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537.

28. Barrabé, L., Maggia, L., Pillon, Y., Rigault, F., Mouly, A., Davis, A.P. and Buerki, S., 2014. New Caledonian lineages of Psychotria (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Molecular Phylogenetics and Evolution* 71: 15-35.

29. Rambaut, A., 2014. FigTree 1.4. 2 software. Institute of Evolutionary Biology, Univ. Edinburgh.

| Chapter 8 |

The assembly of Australasian ant-gardens over the past 10 million years: specialization via host broadening

Guillaume Chomicki, Milan Janda and Susanne S. Renner

Unpublished manuscript

Invited manuscript in *Proceedings of the Royal Society B: Biological Sciences*, special feature on “Ant interactions with their biotic environment”

.

Title: **The assembly of Australasian ant-gardens over the past 10 million years: specialization via host broadening**

Authors: Guillaume Chomicki*[1], Milan Janda[2,3], and Susanne S. Renner[1]

Affiliations:

[1]Systematic Botany and Mycology, University of Munich (LMU), Menzinger Str. 67, 80638 Munich. [2]Biology Centre of the Czech Academy of Sciences, Branisovska 31, 37005 Ceske Budejovice, Czech Republic. [3]Department of Biology, University of Guanajuato, Guanajuato, Mexico.

Author for correspondence:

Guillaume Chomicki

Email: guillaume.chomicki@gmail.com

Abstract [220]

Ant-gardens (AGs) are ant-plant mutualisms in which ants farm epiphytes in return for nest stabilization and food rewards. They occur in the Neotropics and Australasia, but not in Africa, and their assembly over evolutionary time has been a long-standing mystery. We here use phylogenetic frameworks for key ant and plant AG lineages in Australasia, namely the ant genera *Philidris* and *Anonychomyrma* and domatium-bearing clades of ferns [*Lecanopteris*]), Apocynaceae [*Hoya*, *Dischidia*], and Rubiaceae [*Myrmecodia*, *Hydnophytum*, *Anthorrhiza*, *Myrmephytum* and *Squamellaria*]. We show that the first AG partners were species of *Philidris* and nesting site-offering Rubiaceae, with the relevant species groups apparently originating in the Mid-Miocene in Papua New Guinea. With range expansions and long-distance dispersal of *Philidris* from Papua New Guinea to Sundaland and Fiji, diaspore dispersal by ants evolving at least 13 times in the three plant clades. The farming of non-domatium bearing epiphytes in the same AGs suggests that the broadening of host use by the ants may have driven the evolution of additional domatium-bearing AG-epiphytes and thus the specialization of pre-adapted host lineages. Consistent with this, we found a statistical correlation between the evolution of diaspore dispersal by ants and domatia in all three lineages. An unexpected result from our study is that the fern lineage *Lecanopteris* became an ant-plant only after the Rubiaceae, but concurrently to the Apocynaceae.

Keywords: Ants, plants, farming mutualism, ant-gardens, evolution, ecology.

1. Introduction

Mutualisms between ants that act as seed dispersers and plants with suitable propagules have been documented or inferred for thousands of species in 77 families of flowering plants (Sernander, 1908; Lengyel et al., 2010). Typically, these interactions involve ants gathering seeds to feed on lipid- and protein-rich seed appendages (elaiosomes) and abandoning the seeds within 0.1 to 77 meters from where the ants collected them (Gomez and Espadaler, 1998). In the Neotropics, ants disperse certain epiphytes by placing their seeds inside their carton nests, where they germinate and eventually form so-called ant-gardens (AGs) (Ule, 1901, 1905, 1906). The epiphytes benefit by being dispersed to sites far above the ground and by being planted in nutrient-rich carton nests (often enriched with vertebrate feces); the ants benefit through the scaffold formed by epiphyte roots that stabilize their nests and by nutritional rewards, such as extrafloral nectar (Davidson, 1988). Seed recognition is mediated by chemical cues, and convergent odors have apparently driven the assembly of Neotropical AGs (Davidson and Epstein, 1989; Davidson et al., 1990; Seidel et al., 1990; Youngsteadt et al., 2008). Neotropical AGs typically lack domatium-bearing plants. Ant garden formation represents a solution to the problem that arboreal ants face in solidifying their nests, with fungus cultivation inside carton nest providing further nest strengthening (Kaufmann, 2002). Different from Neotropical AGs, Australasian AGs are dominated by domatium-bearing epiphytes, which means that in these mutualisms, the ants are provided nesting space *within* their cultivated plants, not between their roots or bracts. Whether the assembly of AGs began with the cultivation of plants for food (i.e., elaiosomes; extrafloral nectar) or with nest strengthening (via roots or bracts of the cultivated epiphytes) is currently unclear.

The domatium-bearing epiphytic species of Southeast Asia have long attracted researchers' attention (Beccari, 1886; Huxley, 1978; Kaufmann, 2002; Chomicki and Renner, 2015), and it has been shown that AGs In Southeast Asia involve species from five genera of ants (*Philidris*, *Crematogaster*, *Pheidole*, *Camponotus*, *Diacamma*) and 17 genera of plants that build carton nests (Kaufmann, 2002). Frequent AG species in Australasia include species of the Rubiaceae genera *Hydnophytum* or *Myrmecodia* (subtribe Hydnophytinae), the Apocynaceae genera *Hoya* and *Dischidia*, and the polypod genus *Lecanopteris* (Fig. 1). The Hydnophytinae have the largest number of ant-epiphytes and are also the largest clade of ant-plants worldwide (Chomicki and Renner, 2015). To trace the evolutionary history of Australasian ant-gardens, we generated phylogenetic frameworks for *Lecanopteris*, *Hoya*, *Dischidia*, and the Hydnophytinae, as well as their most commonly associated ant genus, *Philidris*. Based on these clock-dated phylogenies, we wanted to answer the following questions: (*i*) When, where, and in which sequence did AG epiphytes and AG-forming *Philidris* ants originate? (*ii*) Did AG-forming *Philidris* lineages diversify and specialize on particular host plant groups or did ants instead broaden their host use over time by using species from an increasing number of host genera? And (*iii*) which trait combinations favored the evolution of AGs in Australasia.

2. Materials and Methods

(a) Taxon sampling, DNA extraction and phylogenetic analyses

Species boundaries in the dolichoderine genus *Philidris* are poorly understood, and the phylogeny proved hard to resolve with Sanger sequencing. At this time, nine species are generally accepted in *Philidris* (Shattuck, 1992; Zhou and Zeng, 1998). Our sampling of *Philidris* comes from three sets of field trips. Eva Kaufmann collected numerous AG-associated *Philidris* for her doctoral dissertation in Thailand, Borneo, Peninsular Malaysia and Java between 1998 and 2001. Milan Janda, in a series of fieldtrips in Papua New Guinea in 2004-2014, collected both plant-associated and free-living *Philidris*. Finally, Guillaume Chomicki collected *Philidris* in Fiji during three fieldtrips in 2014-2016. We sequenced four nuclear loci (EFαF1, EFαF2, CAD, LW Rh) and the mitochondrial marker COI, following standard methods for PCR and sequencing (Clouse et al., 2015). In the absence of statistically supported (ML BS > 70%) conflict between nuclear and mitochondrial data, we concatenated the datasets, which yielded an alignment of 2906 base pairs (bp). Voucher information is reported in Table S1.

For *Lecanopteris*, we used the dataset of Haufler et al. (2003), relying on two plastid markers, rbcL and the trnL-trnF spacer, and sampling 12 of the 13 known species. The concatenated matrix had a length of 1522 bp. Voucher information is reported in Table S2.

For *Hoya* and *Dischidia*, we used the dataset of Wanntorp et al. (2006), using two plastid markers (atpB-rbcL spacer and trnL-trnF region), together yielded a matrix of 2582 bp for 43 of the estimated 200 species in these two genera, including 6 of the 18 *Dischidia* and *Hoya* species with domatia (Chomicki and Renner, 2015). Voucher information is reported in Table S3.

For the Hydnophytinae, we generated a matrix of six markers (nuclear ITS and ETS and plastid *ndhF*, *psbA-trnH*, *trnL* intron and *trnL-trnF* spacer), sampling 76 species out of *ca.*102 Hydnophytinae species. We sampled all 12 *Squamellaria* species recognized by Chomicki and Renner (2016), 5 of the 8 species of *Anthorrhiza* recognized by Huxley and Jebb (1991a), 32 of the 51 *Hydnophytum* species recognized by Jebb and Huxley in an unpublished revision, 4 of the 5 described species of *Myrmephytum* (Huxley and Jebb, 1991b), and 19 of the 26 *Myrmecodia* species recognized by Huxley and Jebb (1993). These numbers include four species that were previously synonymized but stand as good species in light of molecular data. There are also a few yet unnamed species of *Hydnophytum* to which we refer with their preliminary scientific names proposed by Mathew Jebb, followed by voucher information (name of collector and collection number). Jebb's nomina nuda are also listed in supplementary Table S4. A sampling of outgroups (in the tribe Psychotrieae) was selected based on Barrabé et al. (2014). Voucher information is reported in Table S4. In the absence of statistically supported incongruence (i.e., maximum likelihood bootstrap (BS) support >75) between the plastid and nuclear data partitions), we concatenated all markers, which yielded an alignment of 3055 bp.

Total genomic DNA was extracted from c. 20 mg of leaf tissues, using a commercial plant DNA extraction kit (NucleoSpin; Macherey–Nagel, Düren, Germany) according to manufacturer protocols. Polymerase chain reaction (PCR) was performed using Taq DNA polymerase (New England Biolabs, Cambridge, MA, USA) and a standard protocol (39 cycles, annealing temperature 56^0C). PCR products were purified using the ExoSap clean-up kit (Fermentas, St Leon-Rot, Germany), and sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 automated sequencer (Applied Biosystems, Perkin-Elmer). Sequences were edited in Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). All new sequences were BLAST-searched in GenBank. Sequence alignment was performed in MAFFT v. 7 in the online server (http://mafft.cbrc.jp/alignment/server) (Katoh and Standley, 2013) under standard parameters except for the ITS region, which was aligned under Q-INS-i optimization, which takes rRNA secondary structure into consideration. Minor alignment errors were corrected manually in Mesquite v. 2.75 (Maddison and Maddison, 2011). Maximum-likelihood (ML) inference relied on RAxML v8.0 (Stamatakis, 2014) and the GTR + Γ substitution model, with empirical nucleotide frequencies and 25 gamma rate categories; bootstrap support was assessed from 100 replicates under the same model. We also conduced Bayesian inference in MrBayes v. 3.2 (Ronquist et al., 2012) under the substitution model selected by jmodeltest2 (Darriba et al., 2012) and using the program's default two runs and four chains (one cold and three heated), with the uniform default priors. We set a 10X10^6 MCMC chain, sampling trees every 1000th generation. Split frequencies approaching zero indicated convergence. We used the 50% consensus tree to assess posterior probabilities for nodes of interest.

(b) Molecular clock dating

Molecular dating analyses relied on BEAST v. 2 (Bouckaert et al., 2014) and uncorrelated lognormal relaxed clock models unless otherwise stated. We used the GTR + G substitution model with four rate categories and a Yule tree prior. For both our plant and ant trees, MCMCs were run for 20 million (*Lecanopteris*) or 40 million (*Philidris*, *Hoya-Dischidia*, Hydnophytinae) generations, with parameters and trees sampled every 10,000 generations. We used Tracer v. 1.6 (Rambaut and Drummond, 2007) to check that the effective sample size (ESS) of all parameters was >200, indicating that runs had converged. After discarding 20% as burn-in, trees were summarized in TreeAnnotator v. 1.8 (part of the BEAST package) using the options 'maximum clade credibility tree', which is the tree with the highest product of the posterior probability of all its nodes. The final tree was visualized in FigTree v. 1.4 (Rambaut, 2012). To calibrate our trees, we relied on secondary calibrations and rates since no fossils were available in the respective groups.

For *Philidris*, we used the fossil-calibrated study of the ant subfamily Dolichoderinae to which *Philidris* belongs (Ward et al., 2010), setting the tree root to 23 ± 6 million years (Ma) with a normal prior and a standard deviation of 2 Ma, and the stem age of *Philidris* to 17 ± 5 Ma using a normal prior with a standard deviation of 2 Ma. Because *P. nagasau* contains an endemic clade from the island of Taveuni, which has been dated to 0.8 Ma (Rodda and Kroenke, 1984; Rodda, 1994), we used this age as a geological maximal constraint for the age of this clade, using a uniform prior with a 0.8-0 Ma bound. We also constrained the entire *P. nagasau* clade to a maximal age of 4 Ma, the age of the oldest island it inhabits (Rodda and Kroenke, 1984; Rodda, 1994), using a uniform prior with a 4-0 Ma bound. Since some species boundaries between *Philidris* samples were unclear, our tree likely comprises many representatives of the same species, which can bias divergence estimates towards older ages (Drummond and Bouckaert, 2015). We thus ran a first analysis with only two terminals per cluster that may represent a species, and used it to constrain our analysis of the larger matrix.

For the Hydnophytinae, we constrained the age of the root, i.e., the split between the Pacific clade and the so-called *Psychotria* clade IV in the fossil-calibrated study of Barrabé et al. (2014), to 22 ± 7 Ma, based on the age of this node estimated by these authors, using a normal prior and a standard deviation of 4 Ma, corresponding to the 95% confidence interval of Barrabé et al. (2014). For *Hoya-Dischidia*, we followed our previous strategy (Chomicki and Renner, 2015), using rates of 2.5×10^{-9}, 3.5×10^{-9} and 4.5×10^{-9} substitutions/site/year consistent with non-coding plastid in other herbaceous perennials (Manen & Natali, 1995; Richardson et al., 2001; Kay et al., 2006). The tree shown in Fig. 2 is the one that uses a rate of 3.5×10^{-9} substitutions/site/year. For *Lecanopteris*, we used a secondary calibration from a larger fossil-calibrated study of Schneider et al. (2010), setting the stem age of *Lecanopteris* to 10 ± 5 Ma.

(c) Ancestral area reconstruction

We defined nine operational units: (A) Fiji and Vanuatu (since there was a single species from Vanuatu); (B) the Solomon Islands; (C) the Bismarck archipelago, including New Ireland, New Britain, Normanby Island and D'Entrecasteaux Islands; (D) Northern Papua New Guinea inclusive of and limited by the Maoke range in Indonesian Papua and the Bismarck range in Papua New Guinea; (E) South Papua New Guinea (south of the Maoke range in Indonesian Papua and the Bismarck range in Papua New Guinea) and Australia; (F) Sulawesi, Moluccas and the lesser

Indonesian Islands (limited by Java in the west); (G) the Philippines; (H) Sundaland (including Indochina, Sundaland, Borneo, Java, Sumatra that were connected until the Quaternary (Hall, 2009); we also include continental Asia as far west as India since our focal clades are younger than 20 Ma, a time during which these area were constantly connected; only two species are found in area H; and (I) Pacific islands (Hawaii, Wallis and Futuna, French Polynesia), in which we also included New Caledonia because only two outgroups are native from this island.

To infer the ancestral areas of *Philidris*-inhabited AG plants and to probe whether ancestral areas of *Philidris* match those of their plant hosts, we used ancestral range reconstruction as implemented in the R package BioGeoBEARS (Matzke, 2012; 2014) on the BEAST chronograms. BioGeoBEARS infers ancestral geographic ranges and permits comparison of three biogeographic models, namely dispersal-extinction-cladogenesis (DEC), dispersal-vicariance (DIVALIKE), and BAYAREA (BAYAREALIKE). Founder-event speciation is modeled via a speciation parameter j that can be added to each of the models. We selected the best model based on LogLikelihood values as well as the Akaike Information Criterion (ΔAICc). Biogeobears statistics are shown in Table S5.

(d) Interactions

Data on interactions between dolichoderine ants and plant species in our three focal clades came from the field observations of Eva Kaufman (Kaufman, 2002), Milan Janda (unpublished), and Guillaume Chomicki (unpublished). A few additional links came from field observations by Matthew Jebb in Papua New Guinea (including Indonesian Papua) (Jebb, 1985, table 10.2) or from matching geography and morphological traits; the latter are shown as dashed lines in Fig. 2. Where the ant phylogeny includes multiple accessions per species, we only added putative links to a single specimen.

(e) Correlated evolution of myrmecochory with other mutualistic traits

To test whether the evolution of dispersal by ants correlates with other mutualistic traits, we used BayesTraits v.2 (Pagel and Meade, 2014), which allows detecting correlated evolution between pairs of discrete binary traits. We studied the following traits, all treated as binary: *ant inhabitants* (score ‘0’ when no associations with ants are formed or only facultative associations with generalist ants, ‘1’ for consistent association with one or few specialized ant partners), *domatium growth* (scored '0' for diffuse growth, and '1' for apical growth), *entrance holes* (scored '0' when absent or smaller than 1 cm in diameter), *warts*, absorptive structures inside Hydnophytinae domatia (Huxley, 1978), (scored '0' when present, and '1' when absent), *first domatium cavity* (scored '0' when it enlarges throughout development, '1' when its development is determinate), *post-anthetic sugar rewards* (scored '0' when absent, and '1' when present), seed dispersal (scored '0' for birds, other animals or gravity alone and '1' for dispersal by ants). Trait states were coded based on Huxley (1978), Jebb (1985, 1991), Davidson and Epstein (1989), Huxley and Jebb (1991a, 1991b, 1992, 1993), Maeyama and Matstumoto (2000), Chomicki and Renner (2016), and Chomicki et al. (2016), an unpublished revision of *Hydnophytum* from M. Jebb and C.R. Huxley, and personal observations by G.C., personal communications to G.C. from M.H.P. Jebb (March 2015) and Camilla Huxley-Lambrick (October 2015). We tested the correlation of every pair of traits. We used the maximum clade credibility (MCC) tree from BEAST but pruned the outgroups and first ran a model of independent trait evolution and estimated the four-transition rate parameters $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, wherein double transitions from state 0,0 to 1,1 or from 0,1 to 1,0 are set to zero. We then ran a model of dependent trait evolution with eight parameters (q12, q13, q21, q24, q31, q34, q42, q43). To compare these non-nested models, we calculated the Bayes Factor score.

3. Results

(a) Phylogenetic relationships in Australasian dolichoderines and their host plants

The 5-genes phylogeny of Dolichoderine ants showed the genus *Philidris* as monophyletic with maximal statistical support while the internal topology was poorly resolved (Fig. S1). Nevertheless, we found strong biogeographic signal. Phylogenetic relationships in *Lecanopteris* and *Hoya-Dischidia* are discussed in previous studies (Hauffler et al., 2003; Wanntorp et al., 2006). Our 6-gene Hydnophytinae phylogeny yielded a strongly supported tree (Fig. S2) and implies a single origin of the characteristic modified hypocotyl tuber (Fig. 1a,d,f). The ant-dispersed lineages are recovered with high support: *Myrmecodia* (ML BS = 89%, pp = 0.99), *H. formicarum* (71/0.99), *H. moseleyanum* (100/1), *Myrmephytum arfakianum* (99/1), *Philidris* *nagasau*-inhabited *Squamellaria* clade (85/1), except for the *Anthorrhiza chrysacantha*/*A. caerulea* clade (60/0.55), likely because of missing data for *A. chrysacantha*.

(b) Times of origin of ant-dispersed epiphytes and their dispersers

The MRCA of *Philidris* originated at 12.4 ± 7 Ma, older than the age of oldest ant-dispersed Hydnophytinae lineage (*Myrmecodia*) (6.3 ± 3 Ma), but the *Philidris cordata* clade, a lineage of Australian and Papuan epiphyte-disperser dates from 4 ± 3 Ma, matching the crown age of its main host lineage, *Myrmecodia*. The Fijian *P. nagasau* clade dates from 3.5 ± 2 Ma, matching the age of its obligate host clade within *Squamellaria* dating to 2.55 ± 1.5 Ma. Epiphyte seed dispersal independently evolved in *Anonychomyrma* more recently, some 2.6 ± 2 Ma.

(c) Biogeographic analysis

The biogeographic model comparison yielded the DEC + J model as best fitting for *Philidris* (-42.7 vs. -53.73 for DEC), the *Hoya-Dischida* data (-79.44 vs. -91.46 for DEC), the Hydnophytinae (LnL = -130.53 vs. -170.05 for DEC), and *Lecanopteris* (-25.53 vs. -27.07) (Table S5). *Philidris* likely originated in Australia because during the mid Miocene, Southern Papua New Guinea was still submerged (Hall, 2009). The decrease in sea level in the late Miocene resulted in the connection of Australia to the emerging Southern Papua New Guinea (Hall, 2009), enabling *Philidris* to expand its range and providing a stepping-stone for the colonization of Northern Papua New Guinea (Fig. 2), which at the time formed several disconnected landmasses (Hall, 2009). From Northern Papua New Guinea, *Philidris* colonized Sundaland Fig. 2), including today's Borneo, peninsular Malaysia, Sumatra, Java and continental Asia (Hall, 2009). Fiji was apparently colonized by long-distance dispersal from Northern Papua New Guinea (Fig. 2), and since *P. nagasau* in endemic from Vanua Levu and Taveuni, the maximal age of the colonization is 4 Ma, the time of emergence of Vanua Levu, and Taveuni only emerged 0.8 Ma ago (Rodda and Kroenke, 1984; Rodda, 1994).

The *Hoya-Dischidia* clade apparently originated in continental Asia, and underwent minimally two long-dispersal events to Southern Papua New Guinea/Australia in the Pliocene and Quaternary (Fig. 2). The Philippines were recurrently colonized from Sundaland during the same period (Fig. 2).

The presence of widespread species precludes identifying the possible ancestral area of *Lecanopteris* (Fig. 2).

The Hydnophytinae apparently originated in Fiji some 15.5 Ma, which implies that the group was present in Viti Levu, the oldest island of the archipelago, dating back to ca. 30 Ma Rodda and Kroenke, 1984; Rodda, 1994). The Solomon Islands were colonized by dispersal, not vicariance since the Solomon arc had drifted from the Fiji-Vanuatu arc some 12 Ma (Rodda and Kroenke, 1984; Rodda, 1994). Papua New Guinea was thus apparently colonized from Fiji, where the Hydnophytinae radiated (predominantly in the Northern part), although support for this scenario is low. Alternatively, the Hydnophytinae could have originated in Northern Papua New Guinea, which at the time formed a disconnected archipelago (Hall, 2009), and underwent an early dispersal event to Fiji. While most of the radiation (ca. 75%) is endemic from Papua New Guinea, a number of dispersal events to islands off Papua New Guinea, Moluccas, Philippines, Sulawesi and Sundaland occurred during the last 5 million years.

These these ancestral area analyses indicate that both *Philidris* and the Hydnophytinae clade originated in Papua Guinea at matching times, and that most ant-dispersed plants originated in areas matching that of their ant dispersers, including the highly specialized obligate *Squamellaria* clade and *Philidris nagasau* in Fiji (Chomicki and Renner, 2016; Chomicki et al., 2016).

(e) Correlated evolution of mutualistic traits

Of the 21 pairwise correlations between each of the seven binary traits (ant inhabitants, domatium growth type, entrance holes size, warts presence or absence, first cavity enlargement, post-anthetic sugar rewards presence or absence, dispersal type), 11 were very strongly correlated (Bayes Factor >10), one was strongly correlated (5< Bayes Factor <10), three were positively correlated (Bayes Factor >2) and six were non-correlated (Fig. 3). Dispersal type (presence or absence of dispersal by ants) was correlated with all mutualistic traits except entrance hole size.

4. Discussion

(a) The spatio-temporal assembly of Australasian ant-gardens

Our analyses reveal the assembly of ant-gardens in Australasia over the last 10 million years. Ancestral area reconstructions for the most species rich groups of epiphyte-dispersing ants (*Philidris*) and ant-dispersed epiphytes (Hydnophytinae) both point to an origin in Papua New Guinea in the Middle Miocene. However, we cannot exclude that unspecialized ant-gardens originated in several areas simultaneously or even before. Consistently with an earlier recruitment of Hydnophytinae by *Philidris*, many species of Hydnophytinae occur as mono-cultivated taxa (Fig. 1f; Chomicki and Renner, in revision). This suggests that some Australasian ant-gardens derive from an ant/epiphyte symbiosis via broadening of the host range, meaning that certain ant populations began 'importing' new epiphytes into existing gardens and that this occurred with sufficient frequency to set up selection on morphological traits in these plants. In particular, ant-gardens found in Sundaland involve young lineages (~ 3 species of Hydnophytinae that are not sisters), *Dischidia* and *Lecanopteris* species, and it is clear from the biogeographic analyses that they originated as during range expansions of *Philidris* and the Hydnophytinae. Key pre-adaptations for the convergent evolution of AG host lineages probably were convergent chemical cues in the seeds (Davidson, 1990; Kaufmann, 2002), originally present as a by-product unrelated to its current function of cue for ants-removal. Another important pre-adaptation in of ants to cultivate plants is polydomy (Davidson, 1988), the capacity to form several interlink nests, which allows to negotiate the lag-time between seed-planting and rewards (domatia or food).

(b) Mutualism specialization via host broadening

The striking correlation between the evolution of dispersal by ants and ant domatia (Figs. 2, 3), together with the finding of a large number of non-domatium bearing plants in Australasia AGs (Fig. 1; Kaufmann, 2002; Kaufmann and Maschwitz, 2006), and the inference that the oldest ant-dispersed epiphyte lineages were domatium bearing, suggests that the first Australasian AGs might have involved domatium-based symbioses. This implies that the recurrent recruitment by ants of pre-adapted epiphyte lineages (for example with food rewards or particular seed surface chemistry), and the co-cultivation of non-domatium- and domatium-bearing epiphytes, promoted the evolution of domatia, probably to enhance the plants' ability to take up ant-derived nutrients (Laube and Zotz, 2003), a strategy that has proved efficient in many AG lineages (Huxley, 1978; Gay, 1993; Treseder et al., 1995; Chomicki and Renner, in revision). Thus, plant lineages that were secondarily recruited into AG communities probably increased their level of specialization, from having a set of pre-adaptations to forming domatia (Fig. 1). This leads to the counter-intuitive conclusion that the broadening of host ranges (hence generalization) by AG ants led to the specialization of plant hosts.

(c) Correlated evolution of dispersal by ants and other mutualistic traits

The statistical correlation between myrmecochory and post-flowering nectar rewards detected here suggests that such rewards are required for symbiosis with polydomous dolichoderines (Jebb, 1985; Chomicki et al., 2016). While the small number of evolutionary events did not permit statistical analysis, dispersal by ants is also correlated with domatium presence in *Lecanopteris* and *Hoya-Dischidia*. This is consistent with the hypothesis above that co-cultivation of initially non-domatium bearing plants selected on domatium evolution in *Dischidia* and *Lecanopteris*.

Conclusion

Our study reveals the assembly of Australasia ant-gardens through time and space. We inferred a minimum of 13 independent origins of AG plants during the last 10 million years. The matching ages and area of AG-forming *Philidris* pinpoints an origin of Australasian AGs in Papua New Guinea some 10 Ma. This suggests that AGs originated from domatium-based symbiosis and that host broadening by *Philidris* resulted in the recurrent entry of diverse pre-adapted plant lineages into the AG 'adaptive zone' (sensu Simpson, 1953), thus resulting in specialization of hosts. Perhaps unexpectedly, the *Philidris*/Rubiaceae AGs preceded the (evolutionary) incorporation of *Lecanopteris* ferns into AGs. More generally, host-broadening by ant symbionts has been an important driver of AG evolution, similarly to terrestrial ant/plant symbioses that do not involve seed dispersal (Chomicki et al., 2015).

Acknowledgements

We thank Eva Kaufmann for *Philidris* samples, Matthew Jebb and Camilla Huxley-Lambrick for discussion, Andreas Wistuba for samples of cultivated plants and Jeremy Aroles for proofreading the manuscript. This work was supported by a grant from the German Research Foundation (DFG), RE 603/20, and grants from the Society of Systematic Biologists and the American Association of Plant Taxonomy to GC.

References

Barrabé L, Maggia L, Pillon Y, Rigault F, Mouly A, Davis AP, Buerki S. 2014 New Caledonian lineages of *Psychotria* (Rubiaceae) reveal different evolutionary histories and the largest documented plant radiation for the archipelago. *Mol. Phylogenet. Evol.* **71**, 15-35. (doi:10.1016/j.ympev.2013.10.020)

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014 BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**, e1003537. (http://dx.doi.org/10.1371/journal.pcbi.1003537)

Chomicki G, Renner SS. 2015 Phylogenetics and molecular clocks reveal the repeated evolution of ant‐plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytol*. **207**, 411-424. (Doi: 10.1111/nph.13271)

Chomicki G, Ward PS, Renner SS. 2015 Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. *Proc. R. Soc. B* **282**, 20152200). (Doi: 10.1098/rspb.2015.2200)

Chomicki G, Staedler YM, Schönenberger J, Renner SS. 2016 Partner choice through concealed floral sugar rewards evolved with the specialization of ant–plant mutualisms. *New Phytol*. (Doi: 10.1111/nph.13990)

Chomicki G, Renner SS. 2016 Evolutionary relationships and biogeography of the ant-epiphytic genus *Squamellaria* (Rubiaceae: Psychotrieae) and their taxonomic implications. *PloS one* **11**, p.e0151317. (http://dx.doi.org/10.1371/journal.pone.0151317)

Clouse RM, Janda M, Blanchard B, Sharma P, Hoffmann BD, Andersen AN, Czekanski-Moir JE, Krushelnycky P, Rabeling C, Wilson EO, Economo EP, 2015 Molecular phylogeny of Indo-Pacific carpenter ants (Hymenoptera: Formicidae, *Camponotus*) reveals waves of dispersal and colonization from diverse source areas. *Cladistics* **31**: 424-437. (Doi: 10.1111/cla.12099)

Davidson DW, Epstein WW. 1989 Epiphytic associations with ants. In *Vascular plants as epiphytes* (pp. 200-233). Springer Berlin Heidelberg.

Davidson DW. 1988 Ecological studies of neotropical ant gardens. *Ecology* 69:

pp.1138-1152. (Doi: 10.2307/1941268)

Davidson DW, Seidel JL, Epstein WW. 1990 Neotropical ant gardens II. Bioassays of seed compounds. *J. Chem Ecol.* **16**, 2993-3013. (doi: 10.1007/BF00979490)

Darriba D, Taboada GL, Doallo R, Posada D. 2012 jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9: 772-772. (doi:10.1038/nmeth.2109)

Drummond AJ, Bouckaert RR. 2015 *Bayesian evolutionary analysis with BEAST*. Cambridge University Press, Cambridge, UK.

Gay H. 1993 Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Biol. J. Linn. Soc.* 50: 221-233. (Doi: 10.1111/j.1095-8312.1993.tb00928.x)

Gomez C, Espadaler X. 1998 Myrmecochorous dispersal distances: a world survey. *J. Biogeogr.* 25: 573–580. (Doi: 10.1046/j.1365-2699.1998.2530573.x)

Ivens AB. 2015 Cooperation and conflict in ant (Hymenoptera: Formicidae) farming mutualisms-a review. *Myrmecol. News* **21**, 19-36.

Hall R. 2009 Southeast Asia's changing palaeogeography. *Blumea* **54**, 148-161.

Haufler CH, Grammer WA, Hennipman E, Ranker TA, Smith AR, Schneider H. 2003 Systematics of the ant-fern genus *Lecanopteris* (Polypodiaceae): testing phylogenetic hypotheses with DNA sequences. *Syst. Bot.***28**, 217-227. (doi: http://dx.doi.org/10.1043/0363-6445-28.2.217)

Huxley CR. (1978) The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytol.* **80**, 231-268. (doi: 10.1111/j.1469-8137.1978.tb02285.x)

Huxley CR, Jebb MHP. (1991) The tuberous epiphytes of the Rubiaceae 3: A revision of *Myrmephytum* to include *Myrmedoma*. *Blumea* **36**, 43-52.

Huxley CR, Jebb MHP. (1991) The tuberous epiphytes of the Rubiaceae 2: the new genus *Anthorrhiza*. *Blumea* **36**, 21-41.

Huxley CR, Jebb MHP. (1993) The tuberous epiphytes of the Rubiaceae 5. A revision of *Myrmecodia*. *Blumea* **37**, 271-334.

Jebb MHP. (1985) *Taxonomy and tuber morphology of the rubiaceous Ant-Plants*. Doctoral dissertation, University of Oxford.

Kaufmann E, Maschwitz U. 2006 Ant-gardens of tropical Asian rainforests. *Naturwissenschaften* **93**, 216-227. (Doi: 10.1007/s00114-005-0081-y)

Kaufmann E. 2002 Southeast Asian Ant-Gardens: Diversity, ecology, ecosystematic significance, and evolution of mutualistic ant-epiphyte associations. Doctoral dissertation, Johann Wolfgang Goethe-Universität, Frankfurt.

Laube S, Zotz G. 2003 Which abiotic factors limit vegetative growth in a vascular epiphyte? *Funct. Ecol.* **17**, 598-604. (Doi: 10.1046/j.1365-2435.2003.00760.x)

Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2010 Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: a global survey. *Perspect. Plant Ecol. Evol. Syst.* **12**, 43-55. (doi:10.1016/j.ppees.2009.08.001)

Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2009 Ants sow the seeds of global diversification in flowering plants. *PLoS One* **4**, e5480. (http://dx.doi.org/10.1371/journal.pone.0005480)

Maeyama T, Matsumoto T. 2000 Genetic relationship of myrmecophyte (*Anthorrhiza caerulea*) individuals within and among territories of the arboreal ant (*Dolichoderus sp.)* detected using random amplified polymorphic DNA markers. *Austral Ecology* **25**, 273-282. (Doi: 10.1046/j.1442-9993.2000.01034.x)

Matzke NJ. 2012 Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in dispersal–extinction–cladogenesis DEC analyses. *Front. Biogeogr.* **4**, 210.

Matzke NJ. 2014 Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol.* **63**, 951–970. (doi:10.1093/sysbio/syu056)

Pagel M, Meade A. 2013 Bayes Traits V2. *Computer program and documentation. Available at: http://www.evolution.rdg.ac.uk/BayesTraits.html*

Rambaut A, Drummond AJ. 2007 Tracer version 1.4. (*Available at http://tree. bio. ed. ac. uk/software*)

Rambaut A. 2012 FigTree version 1.4. 0. *Available at http://tree.bio.ed.ac.uk/software/figtree*.

Rodda P, Kroenke L. 1984 Fiji: a fragmented arc. In: Cenozoic Tectonic Development of the Southwest Pacific (ed. L. Kroenke), pp. 87-110. U.N. ESCAP,CCOP/SOPAC.

Rodda P. 1994 Geology of Fiji. South Pacific Applied Geoscience Commission (SOPAC) *Technical Bulletin* **8**, 131-151.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012 MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539-542. (doi: 10.1093/sysbio/sys029)

Seidel JL, Epstein WW, Davidson DW. 1990 Neotropical ant gardens I. Chemical constituents. *J. Chem. Ecol*. **16**, 1791-1816. (Doi: 10.1007/BF01020495)

Sernander R. 1906 Entwurfeiner Monographieder europäischen Myrmekochoren. *Kunglica Svenska Vetenskapsakademien Handlingar* **41**, 1–410.

Shattuck SO. 1992 Review of the dolichoderine ant genus *Iridomyrmex* Mayr with descriptions of three new genera (Hymenoptera: Formicidae). *Aust. J. Entomol.* **31**, 13-18. (Doi: 10.1111/j.1440-6055.1992.tb00453.x)

Schneider H, Kreier HP, Janssen T, Otto E, Muth H, Heinrichs J. 2010 Key innovations versus key opportunities: identifying causes of rapid radiations in derived ferns. In *Evolution in action* (pp. 61-75). Springer Berlin Heidelberg.

Stamatakis A. 2014 RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312-1313. (doi: 10.1093/bioinformatics/btu033)

Treseder KK, Davidson DW, Ehleringer JR. 1995 Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature* **375**, 137-139. (doi: 10.1038/375137a0)

Wanntorp L, Kocyan A, Renner SS. 2006 Wax plants disentangled: A phylogeny of *Hoya* (Marsdenieae, Apocynaceae) inferred from nuclear and chloroplast DNA sequences. *Mol. Phylogenet. Evol*. **39**, 722-733. (doi: 10.1016/j.ympev.2006.01.022)

Youngsteadt E, Nojima S, Häberlein C, Schulz S, Schal C. 2008 Seed odor mediates an obligate ant–plant mutualism in Amazonian rainforests. *Proc. Nat. Acad. Sci. USA* **105**, 4571-4575. (doi: 10.1073/pnas.0708643105)

Zhou S, Zheng Z. 1998 *Philidris* (Hymenoptera: Formicidae), a genus new to China, with description of a new species. *Insect Sci*. **5**, 136-138. (Doi: 10.1111/j.1744-7917.1998.tb00311.x)

Figure legends

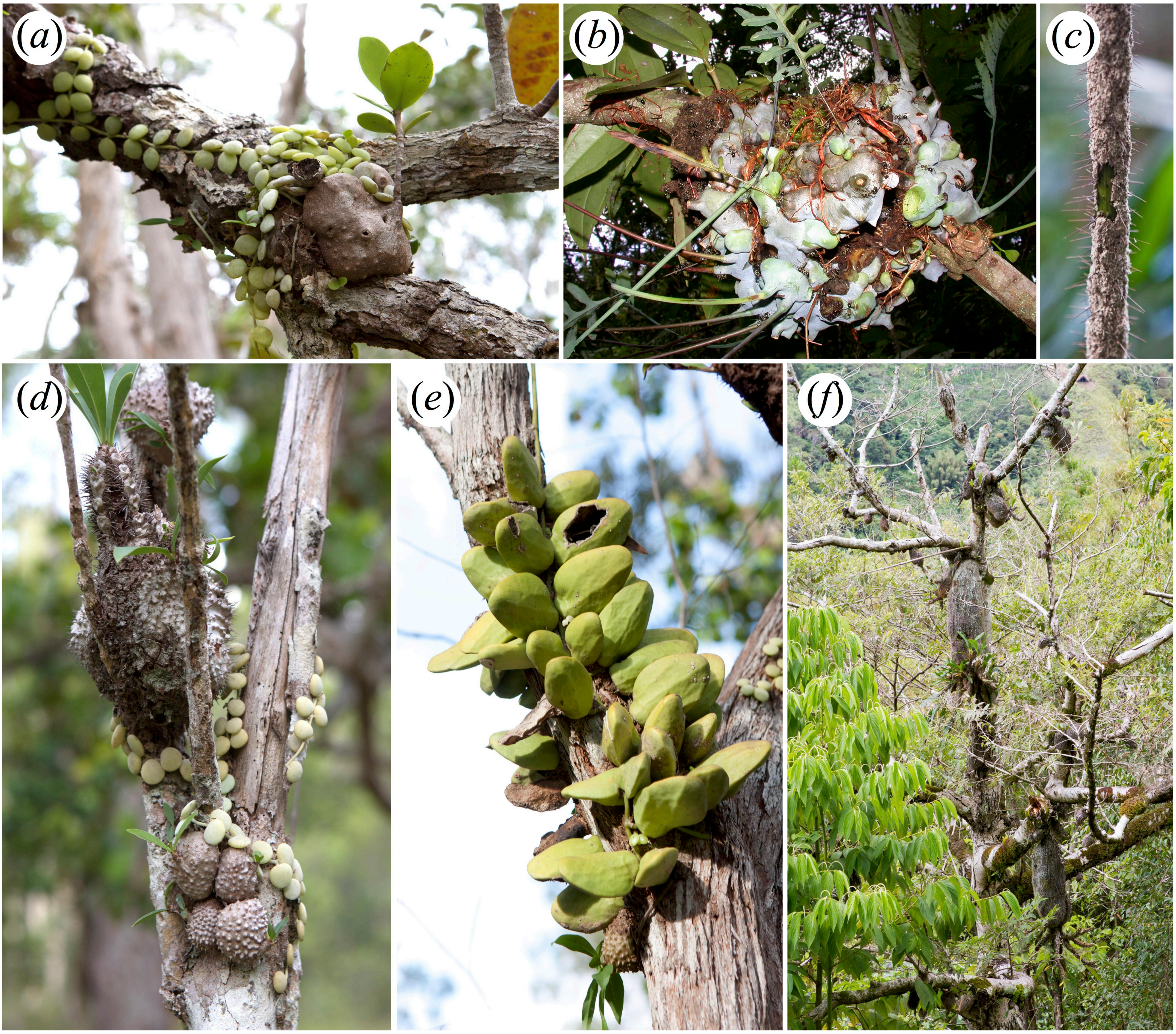
Figure 1. Examples of Australasian ant-gardens. (*a*) *Hydnophytum moseleyanum* and *Dischidia nummularia* cultivated by *Philidris cordata* in Papua New Guinea. (*b*) *Lecanopteris carnosa* cultivated by a *Polyrhachis* species from the *sericata* group, Papua New Guinea. (*c*) Example of carton nest freshly formed by *Philidris myrmecodiae* on a *Korthalsia* ocrea, Papua New Guinea. (*d*) *Myrmecodia platytyrea* subsp. *antoinii* and *Dischidia nummularia* cultivated by *Philidris cordata*, Papua New Guinea. (*e*) *Dischidia major*, *D. nummularia* and *Myrmecodia* cultivated by *Philidris cordata*, Papua New Guinea. (*f*) Dozens of *Myrmecodia schlechteri* on the same tree, cultivated by *Anonychomyrma*.

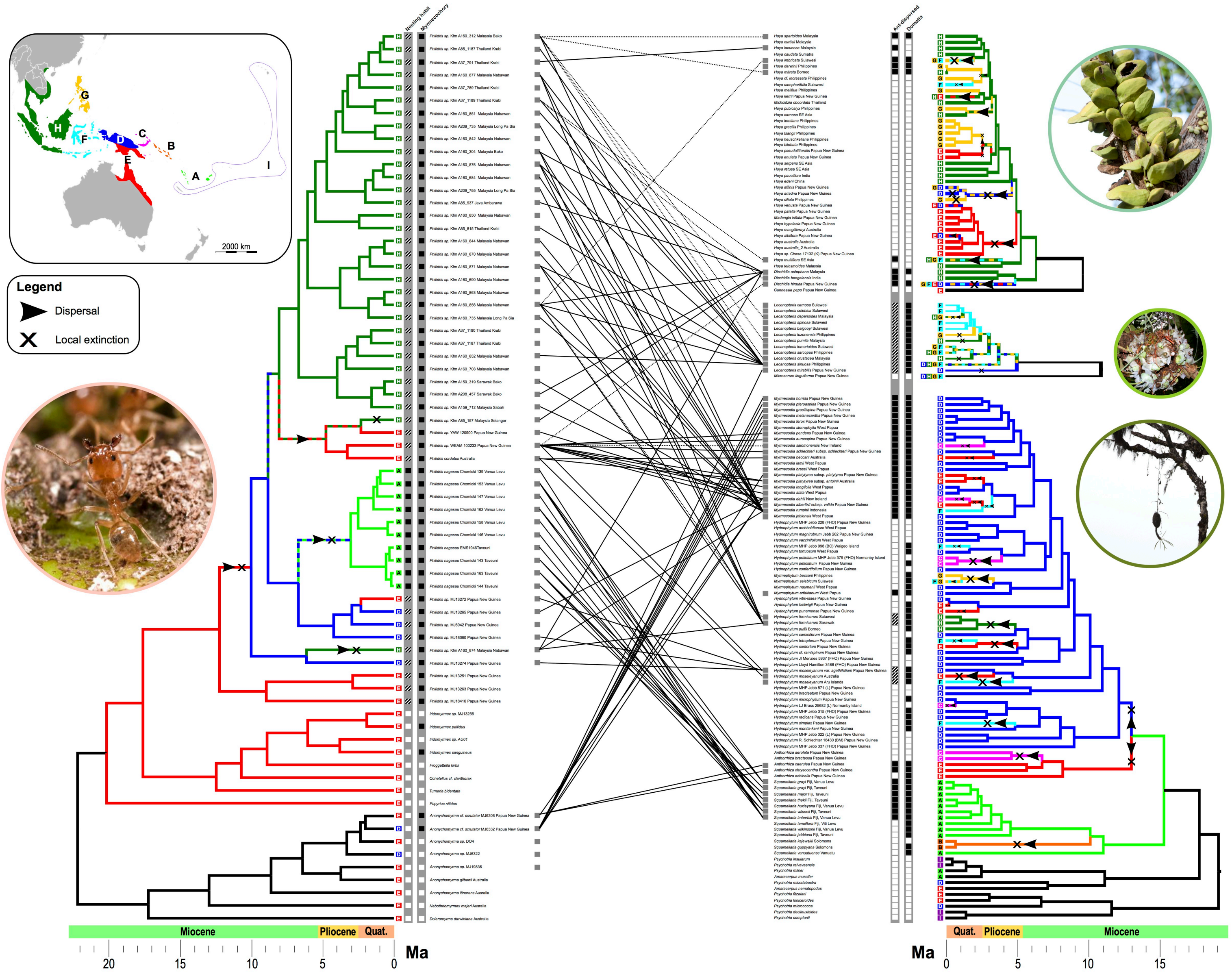
Figure 2. Dated phylogenies and ancestral area reconstructions for *Philidris* ants (left), and *Hoya-Dischidia*, *Lecanopteris* and Hydnophytinae plant hosts. Color-coding on the branches indicates ancestral areas as explained on the inset map.

Figure 3. Correlates of dispersal by ants in the Hydnophytinae. Binary correlations using Pagel's (1994) approach (*Materials and Methods*) between seven plant traits, number in the boxes are Bayes Factor deriving from the comparison of non-correlated and correlated models of trait evolution. Support from Bayes Factor is reported by the following color coding: very strong support (BF > 10): red, strong support (5 < BF < 10): orange, positive support (BF > 2): yellow, no support (BF < 2): white. Phylogenies show ancestral state reconstructions for these seven traits using stochastic mapping (phytools), under an equal rate (ER) and all rate dependent (ARD) models of evolution. For all analyses, 1,000 character simulations were computed.

Supplementary materials:

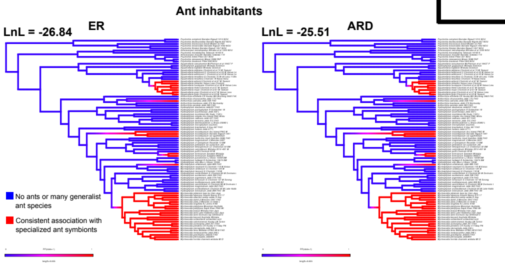
Figures S1-S2.
Tables S1-S5

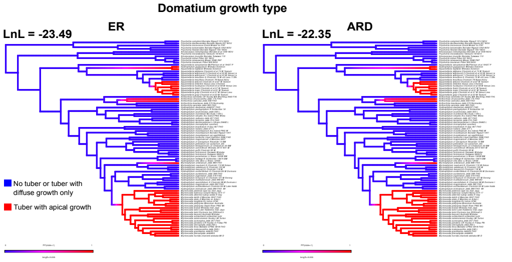

(a)
(b)
(c)
(d)
(e)
(f)

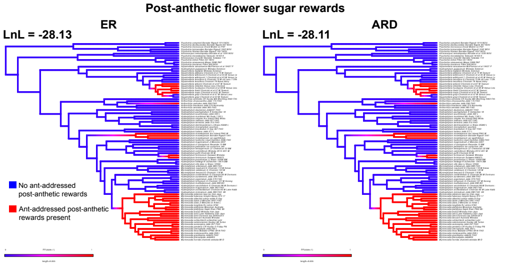


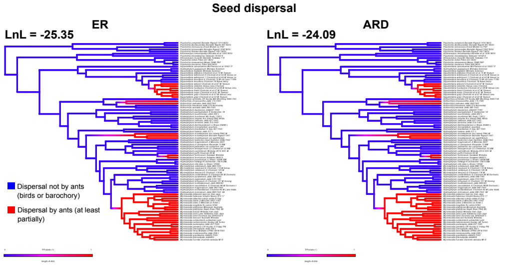
Nesting habit
Myrmecochory
Ant-dispersed
Domatia
G
H
F
D
C
B
E
A
I
2000 km
Legend
Dispersal
Local extinction
Miocene
Pliocene
Quat.
Ma
20
15
10
5
0
Ma
0
5
10
15
Quat.
Pliocene
Miocene

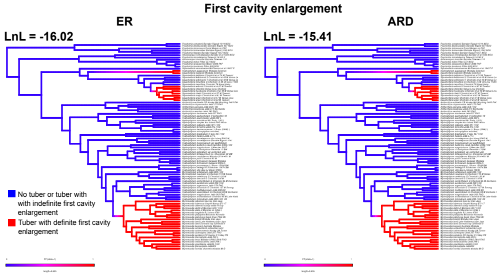
Ant inhabitants	Domatium growth	Entrance holes	Warts	First cavity	Post-anthetic sugar rewards	Seed dispersal	
	86.62	-27.17	2.74	43.81	39.34	24.82	Ant inhabitants
		-59.77	-70.61	75.58	57.13	69.45	Domatium growth
			8.36	-67.39	3.87	-41.13	Entrance holes
				2.73	-27.35	10.96	Warts
					64.93	59.96	First cavity
						81.10	Post-anthetic sugar rewards
							Seed dispersal

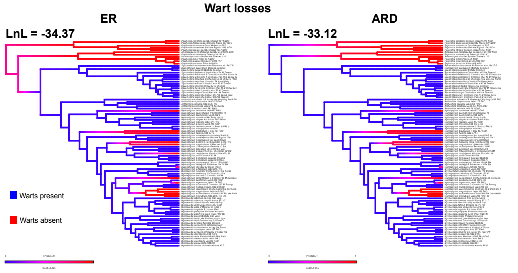

Ant inhabitants


Domatium growth type


Post-anthetic flower sugar rewards


Seed dispersal


First cavity enlargement


Wart losses

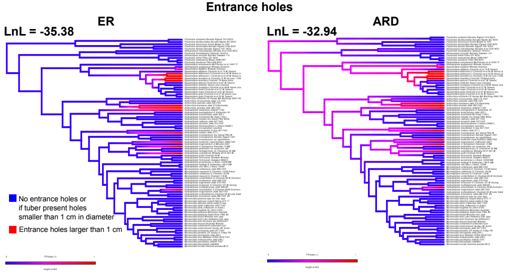

Entrance holes

Figure S1. Maximum likelihood tree for the Hydnophytinae from RAxML. Numbers above branch show the bootstrap from 100 replicates, and the posterior probabilities from MrBayes.

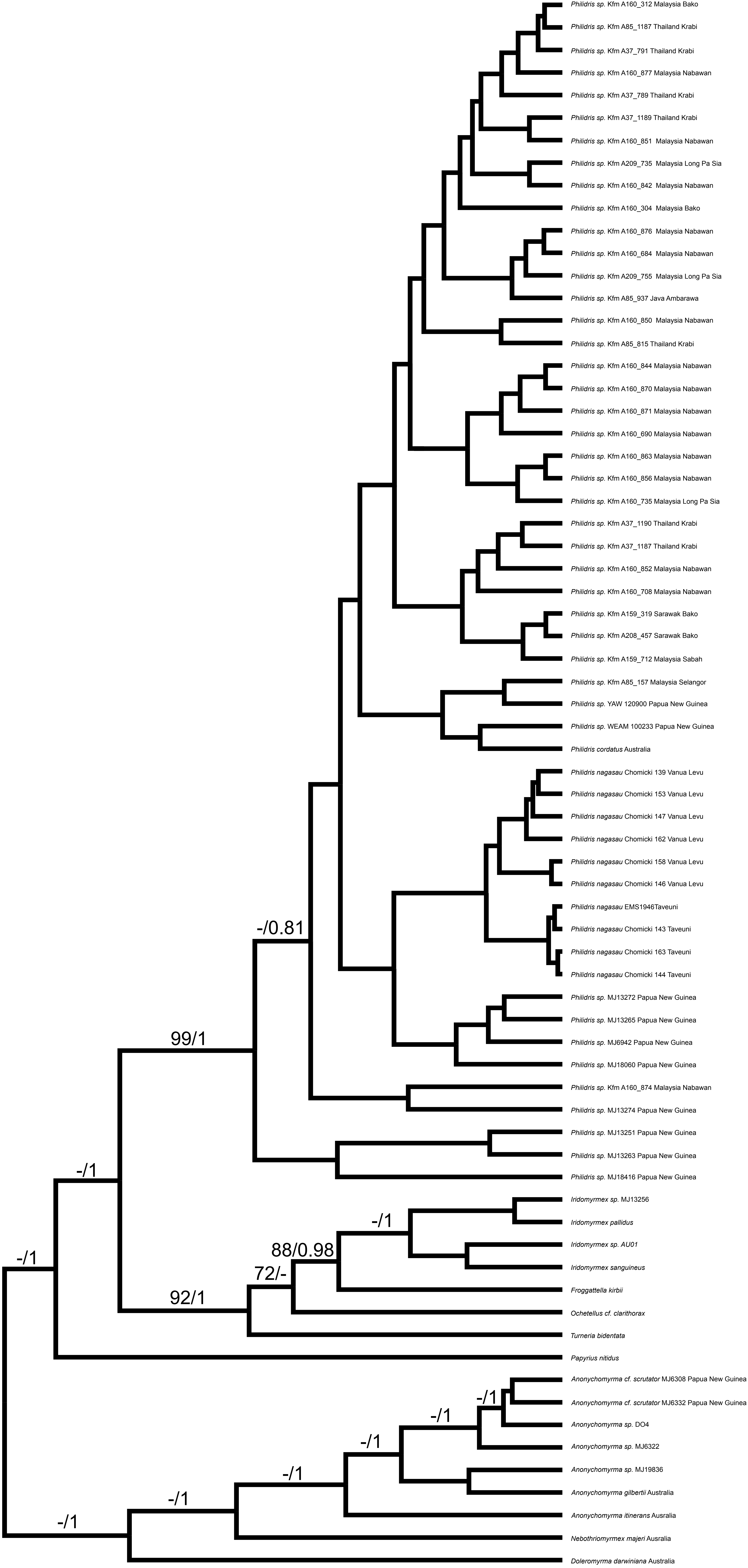


Fig. S1. Phylogeny of *Philidris* ants, with support values from likelihood (RAxML) and Bayesian analyses (BEAST).

Table S1. Ant material included in this study. Information for CASENT vouchers can be found in ant web: https://www.antweb.org.

Taxon	Voucher	Geographic origin	CAD	EFαF1	EFαF2	Long Wave Rhodopsin (LR)	COI
Philidris sp.	KfmA85_157	Malaysia, Selangor	-	Submitted	-	Submitted	-
Philidris sp.	KfmA160_312	Malaysia, Bako National Park	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA85_1187	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA37_791	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_877	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA37_789	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA37_1189	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_851	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA209_735	Malaysia, Long Pa Sia	Submitted	Submitted	Submitted	Submitted	-

Philidris sp.	KfmA160_842	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_304	Malaysia, Bako	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_876	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_684	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA209_755	Malaysia, Long Pa Sia	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA85_937	Java, Ambarawa	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_850	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA85_815	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_844	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_870	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
Philidris sp.	KfmA160_871	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-

***Philidris* sp.**	KfmA160_690	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_863	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_856	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_735	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA37_1190	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA37_1187	Thailand, Krabi	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_852	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_708	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_874	Malaysia, Nabawan	Submitted	Submitted	Submitted	Submitted	-
***Philidris* sp.**	KfmA160_735	Malaysia, Sarawak	-	Submitted	Submitted	Submitted	-
Philidris cordata	CASENT0106011	Australia, Queensland	FJ939937	EF013320	EF013482	EF013618	DQ353313

Philidris sp.	MJ13251	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	MJ13263	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	Kfm85_815	Thailand	-	Submitted	-	Submitted	Submitted
Philidris sp.	MJ18060	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	MJ13272	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	WEAM100233	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	KfmA208_457	Malaysia, Sarawak	-	Submitted	-	Submitted	Submitted
Philidris sp.	KfmA159_319	Malaysia, Sarawak	-	Submitted	-	Submitted	Submitted
Philidris sp.	KfmA159_712	Malaysia, Sabah	-	Submitted	-	Submitted	Submitted
Philidris sp.	EY YAW 120900	Papua New Guinea	AF071988	Submitted	Submitted	Submitted	Submitted
Philidris sp.	MJ13265	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted

Philidris sp.	MJ6942	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC146	Fiji, Vanua Levu	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC153	Fiji, Vanua Levu	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC147	Fiji, Vanua Levu	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC162	Fiji, Vanua Levu	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC158	Fiji, Vanua Levu	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC144	Fiji, Taveuni	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	GC163	Fiji, Taveuni	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris nagasau Mann (1921)	EMS 1946	Fiji, Taveuni	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	MJ13274	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted
Philidris sp.	MJ18416	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted

Iridomyrmex pallidus	CASENT0106152	Australia, Queensland	FJ939922	FJ940066	FJ939969	Submitted	Submitted
Iridomyrmex sp.	MJ13256	Papua New Guinea	Submitted	Submitted	Submitted	-	-
Iridomyrmex sp.	AU01	Australia	FJ939921	FJ940065	FJ939968	FJ940000	-
Iridomyrmex sanguineus	AIMI	Australia	FJ939923	FJ940067	FJ939970	FJ940002	JN134882
Froggattella kirbii	CASENT0009944	Australia, Queensland	FJ939919	FJ940063	FJ939966	FJ939998	-
Ochetellus cf. clarithorax	CASENT0106166	Australia, Queensland	FJ939935	FJ940071	FJ939974	FJ940006	-
Turneria bidentata	CASENT0106019	Australia, Queensland	FJ939946	EF013365	EF013527	EF013656	-
Papyrius nitidus	CASENT0106012	Australia, Queensland	FJ939936	EF013314	EF013476	FJ161859	FJ161756
Anonychomyrma gilberti	CASENT0106003	Australia, Queensland	FJ939895	EF013222	EF013384	EF013543	-
Anonychomyrma itinerans	CASENT0009959	Australia, Western Australia	FJ939896	FJ940044	FJ939947	FJ939979	-
Nebothriomyrmex majeri	CASENT0106174	Australia, Western Australia	FJ939933	FJ940070	FJ939973	FJ940005	-

Doleromyrma darwiniana	CASENT0009949	Australia, AUST ACT	FJ939908	FJ940055	FJ939958	FJ939990	-

Table S2. *Lecanopteris* material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Species	**Voucher**	**Location**	**rbcL**	**trnL-trnF**
***Lecanopteris carnosa* (Reinw.) Blume**	D. Klein s.n. (L)	Sulawesi	AF470322	AY083625
***Lecanopteris celebica* Hennipman**	Hennipman s.n. (L)	Sulawesi	AF470323	AY083626
***Lecanopteris deparioides* (Ces.) Baker**	Hennipman 7865 (U)	Malaysia	AF470324	AY083627
***Lecanopteris spinosa* Jermy & Walker**	Jaarsma s.n. (U)	Sulawesi	AF470327	AY083630
***Lecanopteris balgooyi* Hennipman**	D. Klein s.n. (L)	Sulawesi	AF470328	AY083631
***Lecanopteris luzonensis* Hennipman**	Hennipman 7820 (U, L)	Philippines	AF470325	AY083628
***Lecanopteris pumila* Blume**	Woodhams 551 (L)	Malaysia	AF470331	AY083634
***Lecanopteris lomarioides* (Kunze ex Mett.) Copel.**	Hennipman s.n. (L).	Sulawesi	AF470326	AY083629
***Lecanopteris sarcopus* (Teijsm. & Binn.) Copel.**	RBGE 171 (E)	Cultivated, Royal Botanical Gardens Edinburgh	EU482935	EU483030
***Lecanopteris crustacea* Copel.**	Franken and Roos 341 (L)	Malaysia	AF470329	AY083632
***Lecanopteris sinuosa* (Hook.) Copel.**	Hennipman 7821 (U)	Philippines	AF470321	AY083624
***Lecanopteris mirabilis* (C. Chr.) Copel.**	Hennipman s.n. (U)	Papua New Guinea	AF470330	AY083633
***Microsorum linguiforme* Copel.**	T. Ranker 1776 (UC)	Papua New Guinea	AF470334	AY083637

Table S3. *Hoya*/*Dischidia* material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Species	**Voucher information**	**Location**	***trnL-F* region (trnL region and trnL-trnF spacer**	***atpB-rbcL* spacer**
***Absolmsia spartioides* (Benth.) Kunze (Genus type) D *Hoya spartioides* (Benth.) Kloppenburg**	Wanntorp L. 592 (S), Sipitang, Borneo	Borneo, Sipitang	DQ334549	DQ334591
***Dischidia astephana* Scort. ex King & Gamble**	Wanntorp L. 562 (S), Cameroon Highland, Pahang, Malaysia	Malaysia, Pahang, Cameroon Highland	DQ334534	DQ334576
***Dischidia bengalensis* Colebr.**	920392 (CONN)	-	AF214189; AF214343	-
***Dischidia hirsuta* Decne.**	Wanntorp L. 563 (S),	Cultivated, Stockholm University	DQ334531	DQ334573
***Gunnessia pepo* P.I. Forster (Genus type)**	P.I.F. Forster PIF6465 (BRI), Queensland, Australia	Australia, Queensland	DQ334528	DQ334570
***Hoya affinis* Hemsl.**	Chase 17128 (K), RBG-Kew, Liv. Coll. 1983-4478	Cultivated, Royal Botanic Gardens, Kew	DQ334546	DQ334588
***Hoya albiflora* Zipp. Ex Blume**	Wanntorp L. 584 (S), L20000646	Cultivated	DQ334555	DQ334597
***Hoya anulata* Schltr.**	Wanntorp L. 585 (S), IPPS 8603, L990438	Cultivated	DQ334550	DQ334592
***Hoya ariadna* Decne.**	Chase 17125 (K), RBG-Kew, Liv. Coll. 1983-4474	Cultivated, Royal Botanic Gardens, Kew	DQ334559	DQ334602

***Hoya australis* R.Br. ex Traill**	Wanntorp L. 564 (S), ex hort Departm. Bot., Stockholm University	Cultivated	DQ334527	DQ334569
***Hoya australis* 1 R.Br. ex Traill**	Wanntorp L. 565 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334524	DQ334566
***Hoya bilobata* Schltr**	Chase 17129 (K), RBG-Kew, Liv. Coll. 1983-4481	Cultivated, Royal Botanic Gardens, Kew	DQ334554	DQ334596
***Hoya camphorifolia* Warburg**	Wanntorp L. 590 (S), Philippines, Quezon National Park.	Philippines, Quezon National Park	DQ334539	DQ334581
***Hoya carnosa* R.Br. (Genus type)**	Wanntorp L. 566 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334535	DQ334577
***Hoya caudata* Hook. f.**	Wanntorp L. 587 (S), ex hort, Departm. Bot., Stockholm University	Cultivated	DQ334548	DQ334590
***Hoya ciliata* Elmer ex C.M.Burton**	Wanntorp L. 586 (S), IPPS 3071, L920785	Cultivated	DQ334562	DQ334605
***Hoya curtisii* King & Gamble**	Wanntorp L. 578 (S), 1998-3180, Uppsala Bot. Gar	Cultivated	DQ334544	DQ334586
***Hoya* cf. *darwinii* Loher**	Chase 17135 (K), RBG-Kew, Liv. Coll. 1984-2899	Cultivated	DQ334542	DQ334584
***Hoya edeni* King ex Hook.f.**	Wanntorp L. 579 (S), IPPS 8292	Cultivated	DQ334540	DQ334582
***Hoya gracilis* Schltr.**	Wanntorp L. 567 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334426	DQ334568
***Hoya heuschkeliana* Kloppenb.**	Wanntorp L. 568 (S), ex hort. Departm. Bot., Stockholm	Cultivated	DQ334529	DQ334571

	University			
***Hoya hypolasia* Schltr.**	Wanntorp L. 588 (S), IPPS 7006, L901824	Cultivated	DQ334538	DQ334580
***Hoya imbricata* Decne.**	Wanntorp L. 569 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334545	DQ334587
***Hoya* cf. *incrassata* Elmer ex Merr.**	Chase 17136 (K), RBG-Kew, Liv. Coll. 1984-3340, Philippines, Palawan	Philippines, Palawan	DQ334561	DQ334604
***Hoya kentiana* C.M. Burton**	Wanntorp L. 570 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334522	DQ334564
***Hoya kerrii* Craib**	Chase 17123 (K), RBG-Kew, Liv. Coll. 1982-2786.	Cultivated, Royal Botanic Gardens, Kew	DQ334533	DQ334575
***Hoya lacunosa* Blume**	Wanntorp L. 571 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334557	DQ334599
***Hoya macgillivrayi* F.M.Bailey**	Wanntorp L. 572 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334553	DQ334595
***Hoya meliflua* Merr.**	Wanntorp L. 591 (S), Philippines, Mindoro Occidental, Puerto Galera	Philippines, Mindoro Occidental, Puerto Galera	DQ334525	DQ334567
***Hoya mitrata* Kerr.**	Wanntorp L. 589 (S), IPPS 7684, L914643	Cultivated	DQ334558	DQ334600
***Hoya multiflora* Blume**	Wanntorp L. 573 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334552	DQ334594
***Hoya patella* Schltr.**	Wanntorp L. 575 (S), ex hort. Departm. Bot., Stockholm	Cultivated	DQ334556	DQ334598

	University			
***Hoya pauciflora* Wight**	Wanntorp L. 574 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334536	DQ334578
***Hoya pseudolittoralis* C. Norman**	Wanntorp L. 582 (S), IPPS 4551	Cultivated	DQ334543	DQ334585
***Hoya pubicalyx* Merr.**	Wanntorp L. 576 (S), ex hort. Departm. Bot., Stockholm University	Cultivated	DQ334530	DQ334572
***Hoya retusa* Dalz.**	Wanntorp L. 580 (S), 1998-3127, Rosendal Uppsala, Uppsala Bot. Gar.	Cultivated	DQ334532	DQ334574
***Hoya serpens* Hook. f.**	Chase 17118 (K), RBG-Kew	Cultivated, Royal Botanic Gardens, Kew	DQ334547	DQ334589
***Hoya telosmoides* R. Omlor**	Wanntorp L. 577 (S), Mount Kinabalu, Sabah, Malaya	Malaya, Sabah, Mount Kinabalu	DQ334551	DQ334593
***Hoya tsangii* C.M. Burton**	Wanntorp L. 581 (S), 1998-3136, Uppsala Bot. Gar.	Cultivated	DQ334523	DQ334565
***Hoya venusta* Schltr.**	Wanntorp L. 583 (S), IPPS 3773	Cultivated	DQ334560	DQ334603
***Hoya*–Chase 17132**	Chase 17132 (K), RBG-Kew, Liv. Coll. 1983–4484	Cultivated, Royal Botanic Gardens, Kew	DQ334537	DQ334579
***Madangia inflata* P.I. Forst., D.J. Liddle & I.M. Liddle (Genus type)**	I.M. Liddle IML1076 (BRI), Madang Province, New Guinea	New Guinea, Madang Province	DQ334541	DQ334583
***Marsdenia carvalhoi* G.Morillo & Carnevali**	Chase 17115 (K), RBG-Kew, Liv. Coll. 1982-1949, Brazil, Bahia.	Brazil, Bahia	DQ334521	DQ334563

Table S4. Hydnophytinae material included in this study, with species authors, vouchers and their geographic origin, GenBank accession numbers for all sequences. Herbarium acronyms follow the *Index Herbariorum* (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon	**Voucher**	**Geographic origin**	**ITS**	**ETS**	**trnL intron**	**trnL-trnF spacer**	**ndhF**	**trnH-psbA**
***Amaracarpus muscifer* A.C.Sm.**	L. Barrabé & M. Tuiwawa 1109 (NOU)	Fiji	KF675907	KF675790	-	-	KF675995	-
***Amaracarpus nematopodus* (F.Muell.) P.I.Forst.**	L. Barrabé et al. 1030 (NOU)	Australia	JX155060	KF675791	-	-	JX155105	-
***Anthorrhiza aerolata* Huxley & Jebb**	M.P.H. Jebb 383 (FHO)	Papua New Guinea, Normanby island	Submitted	Submitted	-	-	-	Submitted
***Anthorrhiza caerulea* Huxley & Jebb**	M.P.H. Jebb 358 (FHO)	Papua New Guinea	KU586349	KU586368	-	-	-	Submitted
***Anthorrhiza chrysocantha* Huxley & Jebb**	M.P.H. Jebb 175 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Anthorrhiza bracteosa* Huxley & Jebb**	M.P.H. Jebb 374 (FHO)	Papua New Guinea, Normanby island	Submitted	Submitted		-	-	Submitted
Anthorrhiza echinella	G. Chomicki 83 (M)	Cultivated Oxf. Bot Gard., origin New Guinea	KU586350	KU586369	-	-	-	Submitted
***Hedstromia latifolia* A.C.Sm.**	L. Barrabé et al 1090 (NOU)	Fiji	KF675911	KF675795	-	-	KF675999	-
***Hydnophytum formicarum* Jack**	G. Chomicki 87 (M)	Cultivated, origin Malaysian region	KU586346	KU586365	-	-	KU586397	Submitted
***Hydnophytum formicarum* Jack**	G. Chomicki 90 (M)	Cultivated, origin Sumatra	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum simplex* Becc.**	G. Chomicki 94 (M)	Cultivated, origin Aru Island, Papua New Guinea	KU963311	KU963332	KU963350	KU963362	KU963377	Submitted
***Hydnophytum montis-kani* Valeton**	M.J. Sudo 1193 (L)	Papua New Guinea	Submitted	Submitted	-	-	-	Submitted
***Hydnophytum sp. 1* (=*H. dentrecastense* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	L.J. Brass 25682 (L)	Papua New Guinea	KU963312	Submitted	-	-	-	Submitted
***Hydnophytum sp. 2* (=*H. orichalcum* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	L.J. Brass 28248 (L)	Papua New Guinea	KU963313	Submitted	-	-	-	Submitted

Hydnophytum sp. 3 **(=*H. terrestris* in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	M.P.H. Jebb 315 (FHO)	Papua New Guinea	KU963314	-	-	KU963376	-	Submitted
***Hydnophytum hellwigii* Warb.**	R. Schlechter 13674 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum radicans* Becc.**	M.H.P. Jebb 427 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum punamense* Lauterb.**	L.J. Brass 15008 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum vitis-idaea* Merr. & L.M.Perry**	L.J. Brass 12046 (BM)	Papua New Guinea	KU963316	KU963337	KU963351	KU963363	-	Submitted
Hydnophytum sp. (Hydnophytum petiolatum var. argentatum **in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	M.H.P. Jebb 379 (FHO)	Normanby island, Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum sp. (Hydnophytum petiolatum var. auridemens **in Jebb and C.R. Huxley unpublished *Hydnophytum* revision)**	M.H.P. Jebb 398 (FHO)	Missima island, Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum sp. (Hydnophytum dauloense **in Jebb and C.R. Huxley unpublished *Hydnophytum***	M.H.P. Jebb 337 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum tortuosum* Becc.**	G. Chomicki 127 (M)	Cultivated, origin Indonesian Papua	KU963318	KU963339	KU963352	KU963364	KU963379	Submitted
***Hydnophytum archboldianum* Merr. & L.M.Perry**	G. Chomicki 95 (M)	Cultivated, origin Indonesian Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum sp. (Hydnophytum minirubrum **in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 288 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum sp. (Hydnophytum magnirubrum **in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 262 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum sp. **(*Hydnophytum multituberosum* in M.H.P. Jebb and C.R. Huxley's unpublished revision)**	M.H.P. Jebb 998 (BO)	Waigeo Island, Indonesia	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum tetrapterum* Becc.**	L.E. Cheesman 32 (BM)	Papua New Guinea	KU963319	KU963340	-	-	-	Submitted
***Hydnophytum ramispinum* Merr. & L.M.Perry**	Recende 13 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted

***Hydnophytum confertifolium* Merr. & L.M.Perry**	G. Chomicki 85 (M)	Cultivated, origin West Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum contortum* Merr. & L. M. Perry**	M.P.H Jebb 310 (FHO)	Papua New Guinea	KU963321	KU963342	-	KU963375	-	Submitted
***Hydnophytum sp. (Hydnophytum fusiforme* in M.H.P. Jebb and C.R. Huxley’s unpublished revision)**	Lloyd Hamilton 3486 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum magnirubrum* in M.H.P. Jebb and C.R. Huxley’s unpublished revision)**	J. I. Menzies 5937 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum trichomanes* in M.H.P. Jebb and C.R. Huxley’s unpublished revision)**	R. Schlechter 18430 (BM)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum puffii* Low, Sugau & Wong**	G. Chomicki 93 (M)	Cultivated, origin Borneo	KU963322	KU963343	KU963354	KU963366	KU963381	Submitted
***Hydnophytum sp. (Hydnophytum acuminicalyx* in M.H.P. Jebb and C.R. Huxley’s unpublished revision)**	M.H.P. Jebb 322 (L)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum caminiferum* Wistuba, U.Zimm., Gronem. & Marwinski**	A. Wistuba 2014-001 (M)	West Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
Hydnophytum moseleyanum* Becc. *‘agathifolium’	C.R. Huxley 5902 (FHO)	Papua New Guinea	KU963323	KU963344	KU963355	KU963367	-	Submitted
***Hydnophytum moseleyanum* Becc.**	L. Barrabeé & Rigault 1041 (NOU)	Australia	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum moseleyanum* Becc.**	G. Chomicki 92 (M)	Cultivated, origin Aru Island	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum bracteatum* Valeton**	H. Gay 467 (FHO)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum sp. (Hydnophytum hailans* in M.H.P. Jebb and C.R. Huxley’s unpublished revision)**	M.H.P. Jebb 571 (L)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum microphyllum* Becc.**	L0105910 (L)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Hydnophytum vaccinifolium* P.Royen**	G. Chomicki 98 (M)	Cultivated, origin Papua	KU963324	KU963345	KU963356	KU963368	KU963382	Submitted
***Myrmecodia beccarii* Hook f.**	G. Chomicki 99 (M)	Cultivated, origin Australia	KU586347	KU586366	-	-	KU586398	Submitted

***Myrmecodia salomonensis* Becc.**	C. R. Huxley and L. M. Turton 3442 (FHO)	Solomons	KU586351	KU586370	-	-	-	Submitted
***Myrmecodia dahlii* K.Schum.**	J.I. Menzies 5947 (FHO)	Papua New Guinea	KU586348	KU586367	KU963357	KU963369	KU586399	Submitted
***Myrmecodia rumphii* Becc.**	G. Chomicki 115 (M)	Cultivated	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia alata* Becc.**	J.I. Menzies s.n. (L)	Papua	Submitted	Submitted	-	-	-	Submitted
***Myrmecodia jobiensis* Becc.**	G. Chomicki 101 (M)	Cultivated, origin Papua	KU963326	KU963347	KU963358	KU963370	KU963384	Submitted
***Myrmecodia albertisii* Becc. subsp. *valida* C.R.Huxley & Jebb**	H.J. Gay 901 (FHO)	Papua New Guinea	-	KU963327	-	-	-	Submitted
***Myrmecodia schlechteri* subsp. *schlechteri* var. *schlechteri* C.R.Huxley & Jebb**	H. J. Gay 488 (FHO)	Papua New Guinea	AF071988	-	JN643394	JN643394	-	Submitted
***Myrmecodia pendens* Merr. & L.M.Perr.**	C.R. Huxley and J. Friday 5938 (FHO)	Papua New Guinea	-	KU963328	-	-	-	Submitted
***Myrmecodia aureospina* C.R.Huxley & Jebb**	M.P.H. Jebb 257 (FHO)	Papua New Guinea	-	KU963335	-	-	-	Submitted
***Myrmecodia sterrophylla* Merr. & L.M. Perry**	M.P.H. Jebb 240 (L)	Papua New Guinea	KU963330	-	-	-	-	Submitted
***Myrmecodia lamii* Merr. & L.M.Perry**	G. Chomicki 102 (M)	Cultivated, origin Indonesian Papua, Doorman’	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia lamii* Merr. & L.M.Perry**	G. Chomicki 103 (M)	Cultivated, origin Indonesian Papua, Lake Habbema	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia brassii* Merr. & L.M.Perry**	G. Chomicki 125 (M)	Cultivated, origin Indonesian Papua, Doorman’	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia platytyrea subsp. platytyrea* (Becc.) C.R.Huxley & Jebb**	G. Chomicki 104 (M)	Cultivated, origin Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia platytyrea subsp. antoinii* (Becc.) C.R.Huxley & Jebb**	G. Chomicki 105 (M)	Cultivated, origin Australia	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmecodia ferox* C.R.Huxley & Jebb**	C.R. Huxley & Matiabe UPNG 5818 (FHO)	Papua New Guinea	-	KU963334	-	-	-	Submitted
***Myrmecodia longifolia* Valeton**	R.J. Johns 9760 (NSW)	Papua New Guinea	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted

***Myrmecodia melanacantha* C.R.Huxley & Jebb**	M.P.H. Jebb 248 (L)	Papua New Guinea	KU963331	Submitted	-	-	-	Submitted
***Myrmecodia horrida* C.R.Huxley & Jebb**	G. Chomicki 100 (M)	Cultivated, origin Papua New Guinea	KU963329	KU963338	KU963359	KU963371	KU963385	Submitted
***Myrmecodia gracilispina* C.R.Huxley & Jebb**	M.P.H. Jebb 35 (FHO)	Papua New Guinea	-	KU963333	-	-	-	Submitted
***Myrmecodia pteroaspida* C.R.Huxley & Jebb**	M.P.H. Jebb 804 (FHO)	Papua New Guinea	KU963325	KU963346	-	-	-	Submitted
***Myrmephytum selebicum* (Becc.) Becc.**	G. Chomicki 120 (M)	Cultivated, origin Papua	KU963320	KU963341	KU963360	KU963372	KU963386	Submitted
***Myrmephytum beccarii* Elmer**	G. Chomicki 118 (M)	Cultivated, origin Philippines	KU586353	KU586354	KU963361	KU963373	KU586401	Submitted
***Myrmephytum naumanii* (Warb.) Huxley & Jebb**	G. Chomicki 119 (M)	Cultivated, origin West Papua	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Myrmephytum arfakianum* (Becc.) Huxley & Jebb**	M.H.P. Jebb 889 (FHO)	West Papua, Arfak Mountains	Submitted	Submitted	Submitted	Submitted	Submitted	Submitted
***Psychotria comptonii* S.Moore**	L. Barrabé & Rigault 1014 (NOU)	New Caledonia	KF675927	KF675823	-	-	KF676015	-
***Psychotria dallachiana* Benth.**	L. Barrabé & Rigault 1048 (NOU)	Australia	KF675928	KF675824	-	-	KF676016	-
***Psychotria declieuxioides* S.Moore**	L. Barrabé & Nigote 937 (NOU)	New Caledonia	KF675932	KF675828	-	-	KF676020	-
***Psychotria faguetii* (Baill.) Schltr.**	L. Barrabé et al. 820 (NOU)	New Caledonia	KF675934	KF675831	-	-	KF676023	-
***Psychotria fitzalanii* Benth.**	L. Barrabé & Rigault 1057 (NOU)	Australia	KF675935	KF675832	-	-	KF676024	-
***Psychotria goniocarpa* (Baill.) Guillaumin**	L. Barrabé 586 (NOU)	New Caledonia	KF675940	KF675838	-	-	KF676029	-
***Psychotria hawaiiensis* (A.Gray) Fosberg**	Y. Pillon 1425 (NOU)	Hawaii	KF675941	KF675840	-	-	KF676030	-
***Psychotria hivaoana* Fosberg**	Meyer 3071 (PAP)	French Polynesia	KF675942	KF675841	-	-	KF676031	-
***Psychotria insularum* A.Gray**	Y. Pillon 909 (NOU)	Wallis & Futuna	KF675943	KF675842	-	-	KF676032	-
***Psychotria iteophylla* Stapf**	Axelius 303 (S)	Borneo	-	-	-	-	-	-
***Psychotria loniceroides* Sieber ex DC.**	L. Barrabé & Rigault 1042 (NOU)	Australia	KF675945	KF675846	-	-	KF676033	-

***Psychotria lorentzii* Valeton**	Puradyatmika 10460 (K)	Papua New Guinea	KF675946	KF675847	-	-	KF676034	-
***Psychotria micralabastra* (Lauterb. & K.Schum.) Valeton**	Takeuchi 16163 (K)	Papua New Guinea	KF675949	KF675851	-	-	KF676036	-
***Psychotria micrococca* (Lauterb. & K.Schum.) Valeton**	Drozd & Molem s.n. (PSF)	Papua New Guinea	KF675951	KF675853	-	-	KF676038	-
***Psychotria microglossa* (Baill.) Baill. ex Guillaumin**	L. Barrabé 585 (NOU)	New Guinea	KF675950	KF675852	-	-	KF676037	-
***Psychotria monanthos* (Baill.) Schltr.**	Y. Pillon 1370 (NOU)	New Caledonia	KF675953	KF675855	-	-	KF676040	-
***Psychotria poissoniana* (Baill.) Guillaumin**	J. Munzinger 5156 (NOU)	New Caledonia	KF675958	KF675861	-	-	KF676045	-
***Psychotria pritchardii* Seem.**	L. Barrabé et al 1124 (NOU)	Fiji	KF675992	KF675903	-	-	KF676078	-
***Psychotria raivavaensis* Fosberg**	Meyer 3088 (PAP)	French Polynesia	KF675960	-	-	-	KF676047	-
***Psychotria submontana* Domin**	L. Barrabé et al. 1044 (NOU)	Australia	KF675988	KF675899	-	-	-	-
***Psychotria temehaniensis* J.W.Moore**	Mouly 403 (P)	French Polynesia	KF675989	KF675900	-	-	KF676075	-
***Psychotria trisulcata* (Baill.) Guillaumin**	L. Barrabé et al. 902 (NOU)	New Caledonia	KF675990	KF675901	-	-	KF676076	-
***Squamellaria grandiflora* (Becc.) Chomicki, comb. nov.**	S. Vodonaivolu DA2128 (SUVA)	Fiji, Kadavu Island	-	KU963388	-	-	-	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 53 (SUVA) HOLOTYPE	Taveuni, Bouma falls, Lavena	KU586339	KU586358	KU586376	KU586376	KU586388	-
***Squamellaria grayi* Chomicki & Wistuba sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 47 (M)	Vanua Levu, Waisali forest park	-	-	KU586372	KU586372	-	-
***Squamellaria guppyana* (Becc.) Chomicki, comb. nov.**	G. Chomicki 123 (M)	Cultivated, origin Solomons	KU586345	-	-	-	KU586396	-
***Squamellaria huxleyana* Chomicki sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 48 (SUVA)	Fiji, Vanua Levu, road between Savusavu to	KU586336	KU586355	KU586373	KU586373	KU586385	-
***Squamellaria imberbis* (A. Gray) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 50 (M)	Fiji, Vanua Levu, track to vodaphone tower	KU586337	KU586356	KU586374	KU586374	KU586386	-
***Squamellaria jebbiana* Chomicki, sp. nov.**	G. Chomicki, J. Aroles, A. Naikatini 74 (M)	Fiji, Taveuni, Mt Manuca area.	KU586342	KU586361	KU586379	KU586379	KU586391	-
***Squamellaria kajewskii* (Merr. & L.M.Perry) Chomicki, comb. nov.**	G. Chomicki 122 (M)	Cultivated, origin Solomons	KU586335	-	-	-	KU586384	-
***Squamellaria major* A.C. Sm.**	G. Chomicki, J. Aroles, A. Naikatini 61 (M)	Fiji, Taveuni, road to DesVoeux peak	KU586338	KU586357	KU586375	KU586375	KU586387	-

***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 75 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	-	-	KU586381	KU586381	KU586393	-
***Squamellaria tenuiflora* (Becc.) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 78 (M)	Fiji, Viti Levu, Colo-i-Suva forest park.	KU586343	KU586362	KU586382	KU586382	KU586394	-
***Squamellaria thekii* Jebb**	G. Chomicki, J. Aroles, A. Naikatini 57 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586340	KU586359	KU586377	KU586377	KU586389	-
***Squamellaria vanuatuensis* (Jebb & Huxley) Chomicki, comb. nov.**	McPherson 19437 (P)	Vanuatu	JX155078	-	-	-	-	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 43 (M)	Fiji, Vanua Levu, Waisali forest park.	-	-	KU586380	KU586380	KU586392	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 49 (M)	Fiji, Vanua Levu, Waisali forest park.	-	KU586364	-		-	-
***Squamellaria wilkinsonii* (Horne ex Baker) Chomicki, comb. nov.**	G. Chomicki, J. Aroles, A. Naikatini 45 (M)	Fiji, Vanua Levu, Waisali forest park.	KU586344	KU586363	KU586383		KU586395	-
***Squamellaria wilsonii* (Horne ex Baker) Becc.**	G. Chomicki, J. Aroles, A. Naikatini 67 (M)	Fiji, Taveuni, road to DesVoeux peak.	KU586341	KU586360	KU586378		KU586390	-

Table S5. BioGeoBEARS statistics for biogeographic model testing applied to ant and plant clades. Details see *Materials and Methods*.

(a) Hydnophytinae

Model	LnL	numparams	d	e	j	AICc	AICc_wt_vBest	rellike_AICc
DEC	-120.82	2	0.0058	0.0031	0	245.77	1.85e-12	1.85e-12
DEC+J	**-93.42**	**3**	**0.0004**	**1,00E-12**	**0.0167**	**193.12**	**0.50**	**0.50**
DIVALIKE	-108.51	2	0.0059	1,00E-12	0	221.15	4.13e-07	4.13e-07
DIVALIKE+J	-93.64	3	0.0005	1,00E-12	0.0167	193.56	0.40	0.40
BAYAREALIKE	-149.24	2	0.0066	0.0510	0	302.62	8.41e-25	8.41e-25
BAYAREALIKE+J	-95.12	3	0.0003	1,00E-07	0.0174	196.53	0.09	0.09

(b) *Hoya* and *Dischidia*

	LnL	Numparams	d	e	j	AICc	AICc_wt_vBest	rellike_AICc
DEC	-91.46	2	0.0386	0.0216	0	187.21	1.18e-05	1.18e-05
DEC+J	**-79.44**	**3**	**0.0208**	**1.0E-12**	**0.0508**	165.49	0.6174	0.6174
DIVALIKE	-87.24	2	0.0435	2.0e-08	0	178.78	0.0008	0.0008
DIVALIKE+J	-79.94	3	0.0256	1.0E-12	0.0430	166.50	0.3735	0.3735
BAYAREALIKE	-101.90	2	0.0305	0.1973	0	208.10	3.4616e-10	3.46e-10
BAYAREALIKE+J	-83.77	3	0.0184	1,00E-07	0.0708	174.16	0.0081	0.0081

(c) *Lecanopteris*

	LnL	numparams	d	e	j	AICc	AICc_wt_vBest	rellike_AICc
DEC	-27.07	2	0.0390	1,00E-12	0	59.35	0.3809	0.3809
DEC+J	**-25.53**	**3**	**0.0339**	**1,00E-12**	**0.1315**	**59.72**	**0.3165**	**0.3165**
DIVALIKE	-28.37	2	0.05	2.30e-09	0	61.94	0.1045	0.1045
DIVALIKE+J	-26.67	3	0.0390	1,00E-12	0.1181	62.00	0.1011	0.1011
BAYAREALIKE	-33.11	2	0.0557	0.0819	0	71.42	0.0009	0.0009
BAYAREALIKE+J	-26.72	3	0.0231	1,00E-07	0.2223	62.11	0.0959	0.0959

(d) *Philidris*

	LnL	numparams	d	e	j	AICc	AICc_wt_vBest	rellike_AICc
DEC	-53.73	2	0.0080	1,00E-12	0	111.66	1.65e-05	1.65e-05
DEC+J	**-42.70**	**3**	**1.00E-12**	**1,00E-12**	**0.0303**	**91.79**	**0.3424**	**0.3424**
DIVALIKE	-51.07	2	0.0114	1,00E-12	0	106.33	0.0002	0.0002
DIVALIKE+J	-42.74	3	1.00E-12	1,00E-12	0.0302	91.87	0.3287	0.3287
BAYAREALIKE	-82.22	2	0.0127	0.0518	0	168.63	7.03e-18	7.03e-18
BAYAREALIKE+J	-42.74	3	1.00E-07	1,00E-07	0.0297	91.87	0.3285	0.3285

| General discussion |

General discussion

Macroevolution of ant/plant symbioses

Ant/plant symbioses are a conspicuous feature of tropical ecosystems across the Neotropics, Africa, and Australasia (Davidson and McKey, 1993; Chapter 1). In addition to the long-known hotspot of myrmecophytes in the Neotropics (Davidson and McKey, 1993; McKey and Davidson, 1993), my meta-analysis of all described species with morphological structures for housing ants revealed that Australasia is equally rich in ant-plants (Chapter 1). The Australasian species differ from the Neotropical ones, however, in that the majority of these species are epiphytes, not trees or shrubs and that they engage primarily in nutritional, rather than defensive, mutualisms (Chapter 1). The vast spectrum of ant-plants, including 684 species of angiosperms and ferns from 159 genera in 50 families and their pantropical distribution led me to ask how frequently domatia have evolved and were lost, and at what (estimated) geological time this occurred on each of the continents where ant plants are found. That I discovered three new ant-plant species during the first two weeks of my first fieldtrip in Fiji (Chapter 3) suggests that an estimate of 1,140 ant plants (Chapter 1), almost twice the known number (684), with the program CatchAll (Bunge, 2011), which implements distinct capture-release models, may well be correct. For non-taxonomists it may be important to stress that recognizing the new species required morphological study of relevant herbarium collections in visits to Leiden (L), Oxford (FHO and OFX), Kew (K), Dublin (DUB), Sydney (NSW), Suva (SUVA), British Museum (BM), Paris (P) and Munich (M) to be able to distinguish known species from new species (Chapter 3).

The origins of ant/plant symbioses

My updated world list of domatium-bearing plants together with a 1,200-species molecular phylogeny, allowed determining the phylogenetic distribution of ant-plants (Chapter 1). Ant domatia evolved minimally 158 times and were subsequently lost 43 times. Molecular-clock dating on >56% of all ant/plant lineages revealed an assembly of domatium-bearing lineages over the last 19 million years, with African ant-plants potentially being as young as 5 million years, suggesting that climatic fluctuations in tropical Africa during the late Miocene and Pliocene (van Zinderen Bakker and

Mercer, 1986; Jacobs, 2004) either limited the diversification or fostered the extinction of African ant-plants (Chapter 1). Based on phylogenies (Richardson et al., 2001; Hearn, 2006; Dunn et al., 2007; Lengyel et al., 2009; Marazzi and Sanderson, 2010) and fossils (Pemberton, 1992; Nucete et al., 2012), and also my results (chapter 1), it appears that ant/plant symbioses are the latest ant/plant mutualisms to have evolved during the Miocene, postdating dispersal by ants (myrmecochory) in the late Cretaceous and extrafloral nectary mutualisms in the Oligocene. These results are in line with the dynastic-succession hypothesis of Wilson and Hölldobler (2005), which emphasized the importance of angiosperm-dominated forest canopies where ants could no longer rely on insect predation and shifted to tending honeydew-producing homoptera and plant extrafloral nectaries (Davidson et al., 2003). Living in domatia represents one step further in this succession, and indeed, obligate plant-ants evolved from generalist arboreal ants (Chapter 2). The recurrent Miocene origin of at least 158 ant-plant lineages across the World's tropics likely reflects intense competition for nesting sites. Such ant nesting limitation has been shown within primary and secondary tropical forest ecosystems (Wilson, 1959; Philpott and Foster, 2005) but also among and within obligate plant-ant species (Davidson et al., 1989; Fonseca, 1993, 1999; Stanton et al., 1999). Miocene radiations in rainforest canopies (Brady et al., 2006; Moreau et al., 2006; Moreau and Bell, 2013) may have played an important role in nesting saturation, leading to pressure to use alternative nesting sites, such as living in plant cavities.

Stages and diversification in ant/plant symbioses

The strong asymmetry in species richness between plant-ants and ant-plants (113 vs. 684 species), and most importantly that between the number of evolutionary origins of plant-ants and plant-ants (40-60 vs. 158) raises the question of the origination of new ant-plant lineages. I explored this question using a Neotropical system (*Pseudomyrmex*/ plant symbioses, Chapter 2) and an Australasian system (ant-garden forming *Philidris* and ant-garden epiphytes, Chapter 8). The emerging theme is that host broadening, not switching, has played a major role in generating new ant-plant lineages. This differs from the situation in parasitic and some mutualistic systems including in ant/plant symbioses, where host switching, but rarely broadening occurs (e.g. Zietera and Lumme, 2002; Sorenson et al., 2003; Page et al., 2004; Quek et al., 2004; Ricklefs et al., 2004; Mu et al., 2005; Bruyndonckx et al., 2009; Cruaud et al., 2012).

Plant clades of different ages, but inhabited by the same plant-ant lineage, indicate colonization events and recruitment of new myrmecophyte lineages (Chapters 2, 8). I also found evidence of symbiont broadening, when an ant group secondarily colonized an already existing ant-plant lineage, thus competing with its existing plant-ants and increasing the symbiont pool of the ‘older’ ant-plants (Chapter 2). Interestingly, secondary colonization from generalist ancestors appears to have been the pathway used by specialized and generalist parasites to invade mutualistic ant/plant symbioses (Chapter 2), thus providing a further example of parasitism not evolving from mutualism as theory had predicted (Axelrod and Hamilton, 1981). I did find evidence for co-diversification (but not cospeciation) for the ant-acacias and the *Pseudomyrmex ferrugineus* group, but another obligate mutualism (*P. concolor* group and *Tachigali*) was found to have discordant ant and plant ages that suggested complete partner replacement, further highlighting the dynamic nature of ant/plant symbioses assembly. In Australasian ant-gardens, host broadening is also the dominant process (Chapter 8). In Australasian ant-gardens, the farming of epiphytes without domatium together with domatium-bearing Hydnophytinae may have led to the evolution of domatia and thus the specialization of the hosts, while the symbionts were broadening (i.e. generalizing) their host range. Thus, the broadening of symbiont’s host use may have led to host specialization (Chapter 8).

The breakdown of ant/plant symbioses

An important result of this thesis is the finding of a common pathway for mutualism breakdown in ant/Hydnophytinae symbioses (Chapter 6). Chapter 6 provides the first support from comparative data for the prediction that partner scarcity can drive mutualism breakdown (Vandermeer and Boucher, 1978; Keeler, 1985; Schemske and Lande, 1985; Holland et al., 2004; Fosters and Wenseleers, 2006; Sachs and Simms, 2006). The recurrent shifts to high altitude environments where ants are both species-poor and scarce (Longino et al., 2014; Gilette et al., 2015), including in Papua New Guinea (Maurice Leponce, pers. comm. to G.C. May 2016), where most losses of mutualisms occurred, provide strong support for this prediction. Future experimental work on laboratory-tractable symbioses, such as legume/*Rhizobium*, could determine hosts’ responses to partner’ abundance at a micro-evolutionary level.

Morphological evolution tracks mutualistic strategies

The finding that morphological evolution of traits involved in mutualisms tracks mutualistic strategies (Chapter 6) has several implications. First, it confirms the expectation of stabilizing selection in specialized mutualism (Thompson, 2005; Kopp and Gavrilets, 2006; Yoder and Nuismer, 2010; Raimundo et al., 2014), which functions in maintaining the traits required for the interactions. It also confirms a corollary expectation that mutualism abandonment should be associated with increased morphological evolutionary rate linked to the relaxation from stabilizing selection (Chapter 6). My results, in line with results of Davis et al. (2014) on greater morphological flower diversity in Malpighiaceae once the oil bee pollination mutualism is lost, have implications for macroevolutionary theory regarding the correlates and perhaps general rules of morphological evolution. Darwinian gradualism assumes that morphological changes are proportional to time, a prediction with some support from comparative data (Harmon et al., 2010). By contrast, the punctuated equilibrium theory posits that burst of diversifications should be correlated with burst of morphological evolution, with subsequent periods of evolutionary stasis as inferred from the fossil record (Eldredge and Gould, 1972). This prediction has received considerable support from comparative data either comparing rates of morphological change with species richness (Ricklefs, 2004; Rabosky and Adams, 2012) or with diversification rates (Rabosky et al., 2013). Finer scale studies have shown that during adaptive radiation, the decrease in ecological opportunity mirrors a decrease in rate of morphological change (Mahler et al., 2010). However, the finding that there is no significant correlation between diversification and morphological evolution (Adams et al., 2009; Fig. 1) pinpoints that we should not expect any causal relationships between the two, unless changes in the morphological traits of interest mediate reproductive isolation.

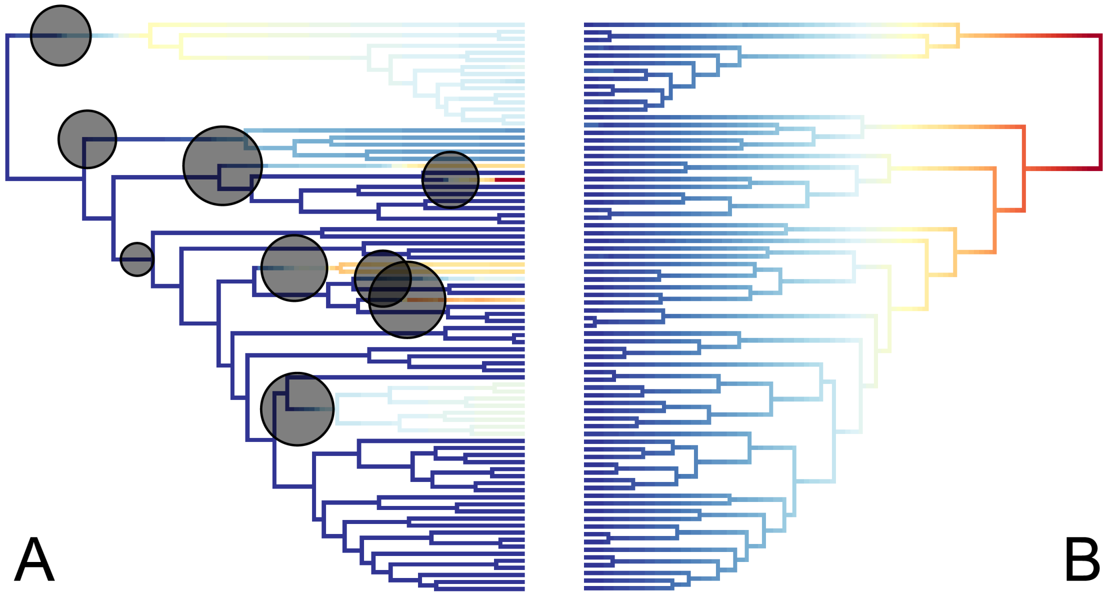


Figure 1. Morphological evolution does not track diversification in the Hydnophytinae. (A) Best shift configuration of Hydnophytinae entrance hole diameter evolution inferred using a reverse-jump Bayesian approach in a software program called BAMM (Bayesian Analysis of Macroevolutionary Mixtures; Rabosky, 2014). The size of the grey circles is proportional to the frequencies of shifts recovered. (B) Best shift configuration of a diversification analysis of the Hydnophytinae, supporting a constant (zero shift) diversification.

I draw two conclusions from these findings: (i) first, mutualism strategies control the pace of morphological change in mutualism-related traits through variation in the level of stabilizing selection and (ii) second, in single species, traits are uncoupled, implying that expectations on morphological evolution should be formulated with regard to particular traits. Overall, this suggests that contrary to diversification, which has been the subject of intense theoretical and comparative studies over the last decade (e.g. Rosenzweig, 1995; Hubbel, 2001; Whittaker et al., 2001; Ricklefs, 2006), a macroevolutionary paradigm of morphological evolution remains to be established with phylogenies and trait data across many clades of the tree of life. Species interactions likely play key roles in shaping organisms morphological evolution.

The Fijian *Squamellaria*: a new system in which to study mutualism specialization and farming

An important – and unexpected – finding of this thesis is that of a new type of ant/plant mutualism, amenable to experimentation, presenting unique opportunities for studying the evolution of mutualism specialization as well as the evolution of an analogue to agriculture in non-human species. This system – the Fijian *Squamellaria* clade – consists of 9 species, a grade of three species that form facultative associations with generalist ant species and a clade of six of which form obligate mutualisms with a single species of ants, the dolichoderine *Philidris nagasau* (Chapters 3, 4, 5, 7). The opportunity to study a recently evolved clade of several species within a relatively small area, together with the Hydnophytinae context in which multiple specializations and losses have occurred (the precondition for inferring trait correlations), makes the Fijian *Squamellaria* system uniquely suited for investigating mutualism evolution. Chapters 4, 5 and 7 focus on this topic. The specialized *Squamellaria* clade presents a new type of ant/plant mutualism: *P. nagasau* disperses the seeds of its obligate *Squamellaria* hosts and plants them under tree bark, high in the canopy and only on certain 'ant-beneficial' trees. *Philidris nagasau* workers constantly patrol the tiny domatium of *Squamellaria* seedlings and defecate inside, providing nitrogen fertilization essential for epiphyte seedlings (Chapter 5). This can be seen as another instance of the evolution of agriculture in ants, arguably comparable in complexity to that of fungi by attine ants.

Squamellaria/*Philidris nagasau*: a new farming mutualism

Farming is a mutualism wherein a species cultivates another on which it relies for food. Some of the most specialized and best-studied farming mutualisms involve ants, namely the attine/fungi and Homoptera tending (Ivens, 2015). I describe the obligate mutualism between *Squamellaria* and *P. nagasau* in detail in Chapter 5 and am only briefly summarizing the key aspects here. *Philidris nagasau* farms *Squamellaria* (planting, fertilizing, and tending the seeds) and feeds on the sugary post-anthetic rewards (Chapter 4), but the ants also live inside their 'crop' (inside the plants' domatia). In a way, the ants 'farm' their future houses. The loss of the sugary post-anthetic rewards in one *Squamellaria* species (*S. grayi*, Chapter 4) does not seem to have affected the farming by *P. nagasau*, either implying that *P. nagasau* does not primarily farm *Squamellaria* for sugary rewards or that the farmer has fallen in a coevolutionary trap where the crop is in control. Both explanations may apply: *P. nagasau* forages on other food sources, in particular the host trees with extrafloral nectaries (EFNs) or sugary fruits where they selectively plant *Squamellaria*, but also occasionally insects. On the other hand, my experiments showed that the recognition and seed planting mechanisms are most likely coevolved, and recognition may rely on chemical cues (Chapter 5).

Farming by *Philidris nagasau* allows *Squamellaria* to achieve large, often single-species, clustered aggregations and to thrive as dominant epiphytes in some Fijian forest communities. Their great abundance in disturbed forests seems to be the result of the preference of *P. nagasau* for pioneer species of the Euphorbiaceae genus *Macaranga*, on whose sugary EFN rewards and stipules it feeds. Resilience of other ant/plant symbioses (Passmore et al., 2012) may also relate to the competitiveness of some mymecophytes in high light environments.

Theory predicts three arenas of conflicts in farming mutualisms: (i) the mode of reproduction of the 'crop', (ii) its transmission and dispersal, and (iii) the diversity of organisms farmed (Frank, 1996a, 1996b; Herre et al., 1999; Mueller, 2002; Ivens, 2015). Typically, resource allocation towards sexual reproduction of the farmed organism implies a conflict of interest with the farmer (Ivens, 2015), which led some 'farmers' (ants) to sterilize their crops and propagate them asexually, such as in the leafcutter ant/fungus mutualism (Mueller, 2002). In the *Squamellaria/ P. nagasau* farming mutualism, this conflict is resolved because the food rewards are old flowers and thus allocation to sex parallels allocation to *P. nagasau*-directed food rewards. Transmission and dispersal of the crop can also be a source of conflict since if transmission is horizontal; the crop can 'escape' from its farmer but on the other hand, the 'farmer' can stop cultivating whenever there is no need (Ivens, 2015). Vertical transmission can thus align crops and farmers' interests and promote their coevolution. No conflict in transmission is apparent in the *Squamellaria/ P. nagasau* farming mutualism, since *P. nagasau* workers (farmers) disperse the seeds of their hosts on the same tree or nearby trees, creating mega-colonies with highly related *Squamellaria*. The transmission is not fully vertical because the queen has no role in it, contrary to the attine ant/fungus mutualism (Mueller, 2015). However, the impossibility of full vertical transmission in this system (because *Squamellaria* is longer lived than *P. nagasau* individuals) could be balanced by the potential partially asexual propagation of *P. nagasau* colonies, which may split or bulge to form new colonies. This would result in *Squamellaria* aggregations on different trees shared by a single *P. nagasau* colony. While this hypothesis requires future population genetic work, such a mechanism exists in similar, albeit less specialized, systems involving ants and Hydnophytinae (Maeyama and Matsumoto, 2000a, 2000b; Kaufmann, 2002). Farmers may 'prefer' monocultures to increase productivity, but crops may 'prefer' diversity to avoid competing with close relatives (Frank, 1996b; Bot et al., 2001; Ivens, 2015). In the *Squamellaria/ P. nagasau* farming mutualism, most epiphyte clusters consist of single species of *Squamellaria*. A few, however, comprise two or three *Squamellaria* species, but then always obligate hosts (i.e., species occupied by *P. philidris*). The seeds of non-specialized *Squamellaria* species are discriminated against by the ants and hence not planted (Chapter 5). The altruistic behavior that *P. nagasau* has towards the seedlings of specialized *Squamellaria*, which it plants and fertilizes, suggests limited competition among kin as found by Aanen et al. (2009) in fungi cultivated by termites. In that system, there was more competition among fungi in polycultures than in monocultures. Whether there is competition among *Squamellaria* species on Fiji when cultivated in polycultures remains to be investigated.

***Squamellaria/Philidris nagasau*: a new type of ant/plant mutualism**

As laid out in the introduction, ants engage in a myriad of transportation, protection, and nutrition mutualisms with a large spectrum of organisms. Ant/plant mutualisms involve transportation mutualisms (myrmecochory), nutritional mutualisms, or defense mutualisms (which may be symbiotic or not). Australasian ant-gardens (AGs) combine dispersal and nutritional ant/plant symbioses (Kaufman, 2002; Kaufman and Maschwitz, 2006) and thus are a form of proto-farming. So how does the *Squamellaria/Philidris nagasau* mutualism differ from Australasian AGs? Several features make it a unique type of ant/plant mutualism. First, *P. nagasau* has lost the ability to build carton nest and thus inserts the seeds right inside tree bark (Chapter 5), different from all other AGs (Davidson, 1988; Kaufman, 2002; Kaufman and Maschwitz, 2006). This results in *P. nagasau* nesting inside domatia, while in other Southeast-Asian AGs, a large part of each colony nests in carton runways, not in domatia (Kaufman, 2002; Kaufman and Maschwitz, 2006). The most important distinguishing feature is the obligatory nature of this mutualism, together with species specificity that leads to *Squamellaria* monocultures, again starkly contrasting with Australasian AGs. Another unique trait is the altruistic act of seedling fertilization. This contrasts with AGs where fertilization occurs only via detritus and fecal matter present in carton nests for epiphytes, and, for the case of domatium-bearing AG epiphytes, via defecation and the transport of nutrients into domatia (Benzing, 1970; Janzen, 1974; Huxley, 1978; Rickson, 1979; Rico-Gray et al., 1989; Gay, 1993; Treseder et al., 1995; Gegenbauer et al., 2012). Finally, the aggressiveness of *P. nagasau* could imply that it plays a role in protection, too, as suggested for other *Philidris*-inhabited Hydnophytinae (Huxley, 1978). Overall, the *Squamellaria*/ *Philidris nagasau* mutualism represents an unique type of advanced farming, with a number of apparently coevolved traits, such as the hypocotyl foot linked to the loss of carton nest building and the recognition of, and defecation on, hyper-absorptive warts, and thus is a novel type of ant/plant mutualism.

Maximizing net benefits

Theory predicts that mutualists are tempted to maximize net benefits by defection. The question of how partners both maximize benefits is thus central. In chapter 7, I address this question. A combination of experiments with ^{15}N nitrogen revealed that in obligate interactions, coevolution of *P. nagasau* behavior and *Squamellaria* domatium physiology results in a partitioning of domatium function. This occurs because ants defecate on hyper-absorptive structures inside *Squamellaria* domatia, the warts (Chapter 7, Fig. 2).

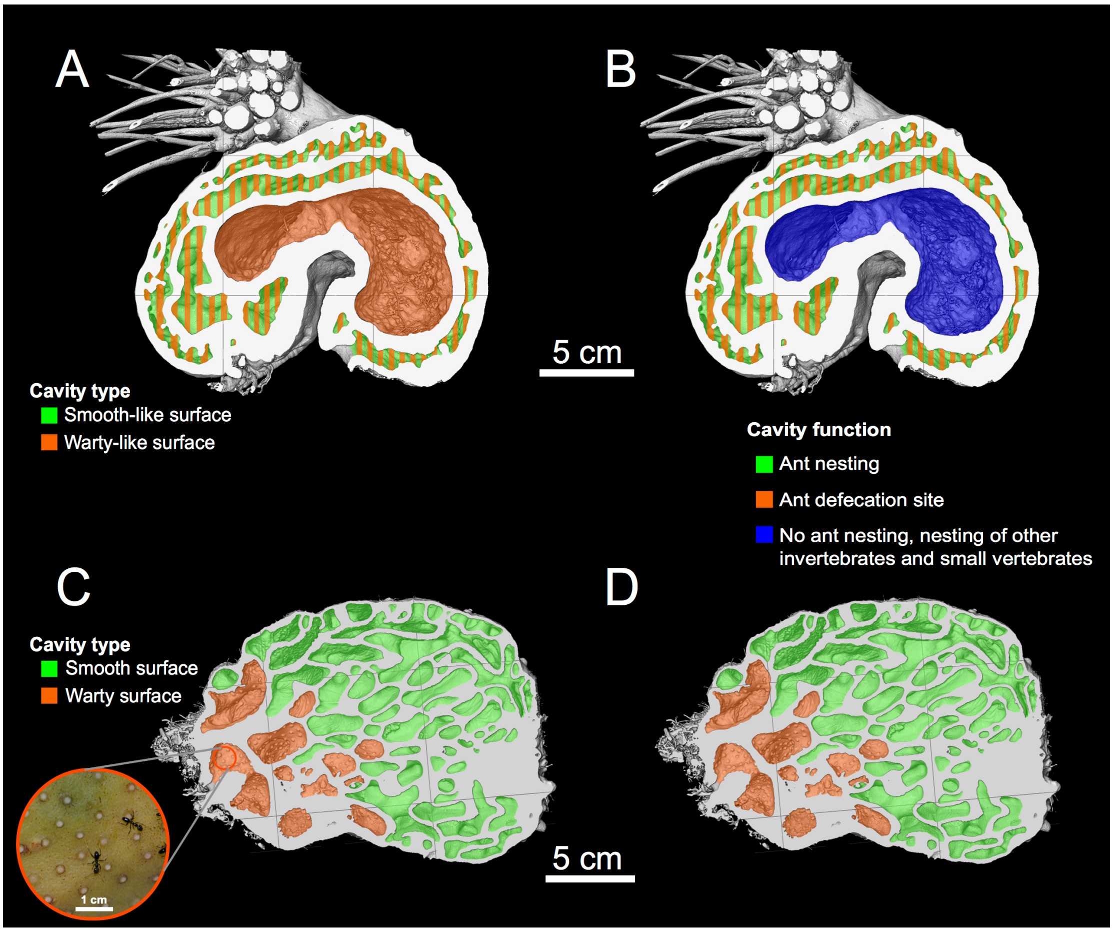


Figure 2. Structure and function of domatia in the facultative ant-plant *Squamellaria tenuiflora* and the obligately-*Philidris nagasau* occupied *S. wilsonii*. In the latter, the warts (round inset) are structures with extremely high nutrient uptake capacity (Chapter 7), which are recognized by *P. nagasau* and act as exclusive defecation sites inside the domatia. The physical partitioning of domatia reduces competition among different ant colonies (Chapter 7) but the functional partitioning could also reduce the spread of diseases in brood cavities of specialist hosts.

The facultative plant mutualists, whose ant occupants lack the behavior of selective defecation on the cavity warts, have domatia with unlinked cavities with separate entrance holes (Chapter 7; Fig. 2). This modular inner structure of the domatia leads to a mismatch between colony size and domatium size because cavity size limits colony growth (the generalist ants were monodomous). It appears that a modular domatium structure reduces the probability of strong competition between founding colonies, which allows facultative hosts to maximize time with large ant colonies despite turnover in the colonies or even species of ants occupying a particular domatium, which I estimate may live for several decades. This suggests that in facultative mutualisms characterized by high rate of symbiont turnover, maximizing benefits to hosts requires adapting to this turnover. Relying on a modeling approach, we showed that partitioning domatia reduces competition and thus constitutes such an adaptation.

How are facultative and obligate ant/*Squamellaria* mutualisms maintained?

Facultative symbioses between *Squamellaria* and generalist ant species are archetypal examples of by-product reciprocity: domatia evolved to benefit generalist ants, which in turn increase the by-product benefits to the plant. Cheating cannot evolve from the symbiont side, and likewise, exploitation is not possible since the host obtains by-product benefits (defecation) by opportunistic nesting. Obligate *Squamellaria*/*P. nagasau* symbioses are much more complex. Firstly, the domatium structure, with an all-linked cavity with high surface-to-volume ratio, implies the linking of colony size and domatium volume, thus partner fidelity feedback (Chapter 7). Food robbers are a strong threat in specialized ant/plant symbioses, which selects for partner choice and partner manipulation mechanisms to exclude them (Heil and McKey, 2003; Heil et al., 2005; Orona-Tamayo et al., 2013; Heil et al., 2014; Chapter 4). In obligate *Squamellaria*, I discovered that a concealed, and thus exclusive, reward (post-anthetic sugars in tissues only accessible to the mutualistic ant species) also acts as a partner choice mechanism by concealing the sugar under a thick epidermis, efficiently excluding exploiters (Chapter 4). Moreover, the near-vertical transmission and the location of food rewards in floral nectaries together resolve the conflict over reproduction (since the ants have an interest in abundant host flowering as only flowers offer food rewards). The actual functioning of the nutritional mutualism is what I here term a ‘two-way byproduct reciprocity’ meaning that both partners coevolved (plant physiology and ant behavior), but the functioning is that of a byproduct mutualism wherein the key function (fertilization by defecation) is cost-free. The multitude of stabilizing mechanism in this specialized mutualism suggests that the stability of mutualism decreases as mutualism complexity increases, implying the need for more stabilizing mechanisms during mutualism specialization.

The specialization of mutualisms

A still poorly understood issue is how a network of generalist mutualists evolves into one-to-one specialized mutualisms? The first requirement for the evolution of mutualism specialization is that some partners are more beneficial than others (Schemske and Horvitz, 1984). In this regard, specialization is a way to increase and homogenize benefits from mutualism and increase complexity in cooperative systems (Waser et al., 1996; Chapters 4, 7), which is essential for community functioning (Thrall et al., 2007). Nevertheless, specialization is a risky move that increases the dependency on a single partner, thus being sensitive to changes in partners' abundances, for instance driven by climate change (Kiers et al., 2010), and can ultimately lead to co-extinction of partners (Rezende et al., 2007). However, by increasing interaction network modularity, specialization implies that specialists' extinction has milder effects on the community than extinction of generalists (Albert et al., 2000; Bascompte and Stouffer, 2009).

The danger of co-extinction as one partner fluctuates has several implications. In pollination mutualisms where partners form time-limited associations and can easily sustain fluctuations in partner abundance, specialization is associated with life history traits such as perennially or ease to propagate vegetatively. Annual plants, by contrast, tend to be generalists (Bond, 1994; Waser et al., 1996). Temporally unpredictable partners (for example, between seasons or years) also favor mutualism generalization (Waser et al., 1996). In plants, sexual systems could have an impact on mutualism specialization. Bawa and Opler (1975) suggested a correlation between dioecy and generalist pollination by small insects, arguing that they could promote inbreeding in hermaphrodites, but a meta-analysis showed that this was wrong at least in tropical angiosperms, where occurrence in hyperdiverse rainforest with low population densities and far apart partners requires specialization (Renner and Feil, 1993). Specialization towards an obligate symbiotic mutualism may also be costly when it 'enforces' a particular association even when it might not be needed (Ivens, 2015). While differential efficiency of partners is essential for specialization to be selected by natural selection, having several co-occurring partners of similar efficiency may preclude specialization (Thompson and Pellmyr, 1992). Below I outline the conditions that might be required for mutualism specialization and suggest mechanisms for how such specialization can occur, based on the work presented in chapters 4 and 7.

When do mutualisms specialize?

At the community level, it has been hypothesized that mutualisms may specialize when both resource availability and biotic complexity are relatively low (Thrall et al., 2007). The predictions are supported by analyses of interaction networks that suggest a global trend towards more generalized networks in the tropics (Schleuning et al., 2009) and experiments with mycorrhiza and legume/*Rhizobium* mutualisms showing that when soil fertility is high, net benefits decrease in the mutualism (Zahran et al., 1999; Jones and Smith, 2008). But this may be largely dependent on the type of mutualism considered. For instance, ant/plant mutualisms may be more specialized in the tropics than in temperate regions (Blüthgen et al., 2007; Chapter 1), and extreme nutrient limitation can lead to the breakdown of mycorrhizal mutualism (Treseder and Allen, 2002). At the species' level, mutualisms may become more specialized if the net benefits of interacting with a subset of preferred partners are higher than those gained when interacting with a broader range of partners. Because this becomes risky when partners become less abundant or vanish, life history likely plays a crucial role in favoring specialization in species that have outside options, such as vegetative reproduction for plants. Host specialization may thus depend on the pool of partners available. For instance, the moth *Greya politella* is exclusively dependent on a few Saxifragaceae flowers for brood sites. In sites where the only host for *G. pollitella* is *Lithophragma parviflorum*, abundance of equally good co-pollinators (such as bombylid flies) seems to prevent the reciprocal evolutionary specialization of *G. pollitella* and *L. parviflorum* (Thompson and Pellmyr, 1992). Such context-dependency will play a large role in mutualism specialization.

How do mutualisms become specialized?

The analysis of large-scale mutualistic networks (focused on pollination and seed dispersal networks) has revealed that at the community level, specialized mutualisms form nested networks, where specialists interact with a subset of generalists rather than with other specialists (Bascompte et al., 2003; Vásquez and Aiden, 2004; Joppa et al., 2009). This was in line with Waser et al.'s (1996) prediction that plant specialization should occur when pollinator abundances fluctuate little, suggesting that specializing on abundant generalists rather than on rare specialists is a safer strategy (Vásquez and Aiden, 2004). Specialization by definition involves small set of interacting partners, with the highest degree of specialization involving so-called one-to-one pairs. The process of specialization involves that one partner 'drives' (is a limiting resource for the other) and that this is either followed by evolutionary responses from the other partner, leading to a coevolved mutualism, or by asymmetric dependency and specialization as apparently is frequent in pollination and dispersal mutualisms. The starting point is a pool of generalists varying in their abundances, predictability, and in the benefits that they bring to the host. Choice of the best partners (i.e., stronger selective interactions) implies increasing the level of trade exchange to ensure fidelity. An increased level of rewarding, however, attracts opportunists, which then requires a *simultaneous* change in the regime of mutualism stabilization, which can be achieved by making the rewards inaccessible except for the desired partners (Chapter 4, Federle et al., 1997; Heil et al., 2005; Orona-Tamayo et al., 2013), which in turn requires the preferred (main) partner to coevolve. Thus it appears that the difficulty in the evolution of specialized cooperation among species lies in fostering partner fidelity via increased rewarding, while excluding opportunists (Chapter 4). In contrast to theory, my empirical findings point to no increase in cheating with high rewards for exclusive partners.

Two reasons may explain this: (i) firstly, specialization implies the selection of the partner providing the best service, and thus cheating would imply that the best partner deteriorates in its behavior and (ii) secondly, this reduction in service provisioning (deterioration in behavior) would occur while the other (choosing) partner increases the rewards (the benefits for the early-stage cheater), which would immediately be selected against by natural selection. This model has been strongly influenced by the results reported in Chapter 4 of the correlation between partner choice and mutualism specialization in the Hydnophytinae. Partner choice seems to be essential for the filtering required in mutualism specialization, and it would thus appear that partner-fidelity feedback alone cannot drive the evolution of highly specialized mutualism.

Outlook

This thesis provides a framework for addressing a number of outstanding questions on the evolution of mutualism. In particular, the Fijian ant/plant system described in chapters 3, 4, 5 and 7 provides a unique opportunity to study mutualism specialization. Three questions that I am planning to answer in the next few years (and for which I have already submitted grant proposals) include: (i) What is the chemical basis of *Squamellaria*/*Philidris nagasau* communication? (ii) What gene networks have controlled *Squamellaria* specialization and how has the expression of key genes shifted during specialization? And (iii) how has the evolution of farming affected population structure?

For my research question (i), chapters 4, 5, and unpublished data suggest that key aspects in the *Squamellaria*/*Philidris nagasau* mutualism are mediated by chemical signaling. Such signaling plays a role in host/symbiont recognition. The evidence comes for this from a 're-colonization experiment', where I placed 10 ant-free facultative *Squamellaria*, and 10 ant-free obligate *Squamellaria* around a large obligate *Squamellaria* with a *P. nagasau* colony. *Philidris nagasau* rapidly re-colonized all obligate hosts, not the closely related facultative hosts, suggesting that they sense chemical cues present in obligate but not in facultative hosts. Chemical signalling also plays a role in seed recognition by workers. This is implied by the unique farming mechanism described in Chapter 5. Next, it plays a role in the repellence of ants by open flowers. This is based on the observation that *P. nagasau* seem to avoid open flowers, while patrolling on corolla-free flowers for sugar rewards (Chapter 4). And lastly, chemical signalling plays a role in the recognition of the warts on the inner cavity surface and inducement to defecate. This is based on the unique fertilization mechanism described in chapter 7. To identify the compounds involved, I will perform hexane extraction, followed by GC-MS and behavioural assays with either extract and target compounds identified by GC-MS, following the methodology of Youngsteadt et al. (2009). This will be followed by electroantennography to test for *P. nagasau* electrophysiological activity in response to particular compounds.

For my future research question (ii), I expect to find key differences between facultative and obligate hosts in the expression levels of genes involved in the biosynthetic pathway of the compounds that mediate chemical signalling (see above) and root development that should be up-regulated in warty chambers and down-regulated in smooth chambers of obligate hosts, since roots sometimes ectopically replaced warts in specialized species, and frequently in facultative species. This indicates that the root 'developmental toolkit' (Petricka et al., 2012) has been recruited inside the domatium to mediate a specialized nutritional mutualism. I will use RNAseq to survey a broad sample of expressed genes, using the coffee genome as reference (Denoeud et al., 2014). I will extract RNA from specific tissues of all 9 obligate and facultative plant hosts (e.g. smooth chambers, warts from warty chambers, and non-warts for comparison) and use the latest tools for the analysis of differential expression of RNAseq data and coexpression network analysis (Trapnell et al., 2012; Hong et al., 2013). To refine further investigation of the genetic basis of trait differences, I will also sample *S. grayi*, a probable polyploid with one specialized and one facultative ant plant as parents (based on mitochondrial and nuclear DNA). Because it has a mixture of traits of both specialist and facultative plant hosts, it will provide an independent test of the association between particular differentially expressed genes and the traits of interest.

For future research question (iii), I aim to test the effect of dispersal by ants on population structure. Chapter 5 suggests that there is a verticalized transmission of hosts by *P. nagasau* workers, which implies that there is a high relatedness of *Squamellaria* within a *P. nagasau* colony. Pedigree analysis using population genomic markers, such as RAD-sequencing, will allow quantifying the extent of vertical host transmission. Moreover, this population genomic approach will allow testing the hypothesis that new *P. nagasau* colonies form by colony splitting. Vertical transmission is known from endosymbionts or endoparasite transmission, but is not known in insect/plant interactions.

References

Aanen D.K., Henrik H., Debets A.J., Kerstes N.A., Hoekstra R.F., Boomsma J.J. (2009). High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites. *Science* 326: 1103-1106.

Adams D.C., Berns C.M., Kozak K.H., Wiens J.J. (2009). Are rates of species diversification correlated with rates of morphological evolution? *Proceedings of the Royal Society B: Biological Sciences* 276: 2729–2738.

Albert R., Jeong H., Barabasi A. (2000). Error and attack tolerance of complex networks. *Nature* 406: 378–382.

Axelrox R.M. and Hamilton W.D. (1981). The evolution of cooperation. *Science* 211: 1390-1396.

Bascompte J., Stouffer D.B. (2009). The assembly and disassembly of ecological networks. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 364: 1781-1787.

Bascompte J., Jordano P., Melián C.J., Olesen J.M. (2003). The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences of the USA* 100: 9383-9387.

Bawa K.S., Opler, P.A. (1975). Dioecism in tropical forest trees. *Evolution* 29: 167-179.

Benzing D.H. (1970). An investigation of two bromeliad myrmecophytes: *Tillandsia butzii* Mez, *T. caput-medusae* E. Morren, and their ants. *Bulletin of the Torrey Botanical Club* 97: 109-115.

Blüthgen N., Menzel F., Hovestadt T., Fiala B., Blüthgen N. (2007). Specialization, constraints, and conflicting interests in mutualistic networks. *Current biology* 17: 341-346.

Bond W.J. (1994). Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 344: 83-90.

Boomsma J.J. (2011) Evolutionary biology: farming writ small. *Nature* 469: 308-309.

Brady S.G., Schultz T.R., Fisher B.L., Ward P.S. (2006). Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proceedings of the National Academy of Sciences of the USA* 103: 18172–18177.

Bruyndonckx N., Dubey S., Ruedi M., Christe P. (2009). Molecular cophylogenetic relationships between European bats and their ectoparasitic mites (Acari, Spinturnicidae). *Molecular Phylogenetics and Evolution* 51: 227-237.

Bunge J. (2011). Estimating the number of species with CatchAll. *Pacific Symposium on Biocomputing* 2011: 121–130.

Cruaud A., Rønsted N., Chantarasuwan B., Chou L.S., Clement W.L., Couloux A., Savolainen V. (2012). An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Systematic Biology* 61: 1029–1047.

Davidson D.W., McKey D. (1993). The evolutionary ecology of symbiotic ant/plant relationships. *Journal of Hymenopteran Research* 2: 13–83.

Davidson D.W. (1988). Ecological studies of Neotropical ant gardens. *Ecology* 69: 1138-1152.

Davidson D.W., Cook S.C., Snelling R.R., Chua T.H. (2003). Explaining the abundance of ants in lowland tropical rainforest canopies. *Science 300*: 969-972.

Davidson D.W., Snelling R.R., Longino J.T. (1989). Competition among ants for myrmecophytes and the significance of plant trichomes. *Biotropica* 21: 64-73.

Davis C.C., Schaefer H., Xi Z., Baum D.A., Donoghue M.J., Harmon L.J. (2014). Long-term morphological stasis maintained by a plant–pollinator mutualism. *Proceedings of the National Academy of Sciences of the USA* 111: 5914-5919.

Denoeud F., Carretero-Paulet L., Dereeper A., Droc G., Guyot R., Pietrella M., Zheng C., Alberti A., Anthony F., Aprea G., Aury J.M. (2014). The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. *Science* 345: 1181-1184.

Dunn R.R., Gove A.D., Barraclough T.G., Givnish T.J., Majer J.D. (2007). Convergent evolution of an ant-plant mutualism across plant families, continents, and time. *Evolutionary Ecology Research* 9: 1349-1362.

Eldredge N., Gould S.J. (1972). Punctuated equilibria: an alternative to phyletic gradualism. In T.J.M. Schopf, ed., *Models in Paleobiology*. San Francisco: Freeman Cooper. pp. 82-115.

Federle W., Maschwitz U., Fiala B., Riederer M., Hölldobler B. (1997). Slippery ant-plants and skilful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). *Oecologia* 112: 217-224.

Fonseca C.R. (1993). Nesting space limits colony size of the plant-ant *Pseudomyrmex* concolor. *Oikos* 67: 473-482.

Fonseca C.R. (1999). Amazonian ant–plant interactions and the nesting space limitation hypothesis. *Journal of Tropical Ecology* 15: 807-825.

Foster K.R., Wenseleers T. (2006). A general model for the evolution of mutualisms. *Journal of Evolutionary Biology* 19: 1283-1293.

Frank S.A. (1996a). Host control of symbiont transmission: The separation of symbionts into germ and soma. *The American Naturalist* 148: 1113-1124.

Frank S.A. (1996b). Host-symbiont conflict over the mixing of symbiotic lineages. *Proceedings of the Royal Society B: Biological Sciences* 263: 339-344.

Gay H. (1993). Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Biological Journal of the Linnean Society* 50: 221-233.

Gegenbauer C., Mayer V.E., Zotz G., Richter A. (2012). Uptake of ant-derived nitrogen in the myrmecophytic orchid *Caularthron bilamellatum*. *Annals of Botany* 110: 757-766.

Gillette P.N., Ennis K.K., Domínguez Martínez G., Philpott S.M. (2015). Changes in species richness, abundance, and composition of arboreal twig-nesting ants along an elevational gradient in coffee landscapes. *Biotropica* 47: 712-722.

Harmon L.J., Losos J.B., Jonathan Davies T., Gillespie R.G., Gittleman J.L., Bryan Jennings W., Kozak K.H., McPeek M.A., Moreno-Roark F., Near T.J., Purvis A. (2010). Early bursts of body size and shape evolution are rare in comparative data. *Evolution* 64: 2385-2396.

Hearn D.J. (2006). *Adenia* (Passifloraceae) and its adaptive radiation: phylogeny and growth form diversification. *Systematic Botany* 31: 805–821.

Heil M., McKey D. (2003). Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution, and Systematics* 34: 425-453.

Heil M., Barajas-Barron A., Orona-Tamayo D., Wielsch N., Svatos A. (2014). Partner manipulation stabilises a horizontally transmitted mutualism. *Ecology letters* 17: 185-192.

Heil M., Rattke J., Boland W. (2005). Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308: 560-563.

Herre E.A., Knowlton N., Mueller U.G., Rehner S.A. (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14: 49-53.

Holland J.N., DeAngelis D.L., Schultz S.T. (2004). Evolutionary stability of mutualism: interspecific population regulation as an evolutionary stable strategy. *Proceedings of the Royal Society B: Biological Sciences* 271: 1807–1814.

Hong S., Chen X., Jin L., Xiong, M. (2013). Canonical correlation analysis for RNA-seq co-expression networks. *Nucleic acids research 41*: e95.

Hubbell S.P. (2001). *The unified neutral theory of biodiversity and biogeography (MPB-32)* (Vol. 32). Princeton University Press, Princeton, New Jersey, USA.

Huxley C.R. (1978). The ant-plants *Myrmecodia* and *Hydnophytum* (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology. *New Phytologist* 80: 231-268.

Ivens A.B. (2015). Cooperation and conflict in ant (Hymenoptera: Formicidae) farming mutualisms-a review. *Myrmecological News* 21: 19-36.

Jacobs B.F. (2004). Palaeobotanical studies from tropical Africa: relevance to theevolution of forest, woodland and savannah biomes. *Philosophical Transactions of the Royal Society B 359*: 1573–1583.

Janzen D.H. (1974). Epiphytic myrmecophytes in Sarawak: mutualism through the feeding of plants by ants. *Biotropica* 6: 237-259.

Jones M.D., Smith S.E. (2004). Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms? *Canadian Journal of Botany* 82: 1089-1109.

Joppa L.N., Bascompte J., Montoya J.M., Sole R.V., Sanderson J., Pimm S.L. (2009). Reciprocal specialization in ecological networks. *Ecology letters* 12: 961-969.

Kaufmann E., Maschwitz U. (2006). Ant-gardens of tropical Asian rainforests. *Naturwissenschaften* 93: 216-227.

Kaufmann E. (2002). Southeast Asian Ant-Gardens: Diversity, ecology, ecosystematic significance, and evolution of mutualistic ant-epiphyte associations. Doctoral dissertation, Johann Wolfgang Goethe-Universität, Frankfurt.

Keeler K.H. (1985). Cost:benefit models of mutualism. In *The Biology of Mutualism, Ecology and Evolution* (Boucher, D.H., ed.), pp. 100–127, Oxford University Press, New York, USA .

Kiers T.E., Palmer T.M., Ives A.R., Bruno J.F., Bronstein J.L. (2010). Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters* 13: 1459-1474.

Kopp M., Gavrilets S. (2006). Multilocus genetics and the coevolution of quantitative traits. *Evolution 60*: 1321-1336.

Lengyel S., Gove A.D., Latimer A.M., Majer J.D., Dunn R.R. (2009). Ants sow the seeds of global diversification in flowering plants. *PLoS ONE* 4: e5480.

Longino J.T., Branstetter M.G., Colwell R.K. (2014). How ants drop out: ant abundance on tropical mountains. *PloS one* 9(8): e104030.

Maeyama T., Matsumoto T. (2000a). Genetic relationship of myrmecophyte (*Anthorrhiza caerulea*) individuals within and among territories of the arboreal ant (*Dolichoderus sp.*) detected using random amplified polymorphic DNA markers. *Austral Ecology 25*: 273-282.

Maeyama T., Matsumoto T. (2000b). Colonial system of *Philidris* ants (Formicidae; Dolichoderinae) occupying epiphytic myrmecophytes in a tropical mangrove forest. *Tropical Ecology* 41: 209-216.

Mahler D.L., Revell L.J., Glor R.E., Losos J.B. (2010). Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution* 64: 2731-2745.

Marazzi B., Sanderson M.J. (2010). Large-scale patterns of diversification in the widespread legume genus *Senna* and the evolutionary role of extrafloral nectaries. *Evolution* 64: 3570–3592.

McKey D., Davidson D.W. (1993). Ant–plant symbioses in Africa and the Neotropics: history, biogeography and diversity. In: Goldblatt P, ed. Biological relationships between Africa and South America. Yale University Press, Yale, CT, USA. pp. 568–606.

Moreau C.S., Bell C.D., Vila R., Archibald S.B., Pierce N.E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. *Science* 312: 101–104.

Moreau C.S., Bell C.D. (2013). Testing the 'museum versus cradle' tropical biological diversity hypothesis: phylogeny, diversification, and ancestral biogeographic range evolution of the ants. *Evolution* 67: 2240–2257.

Mu J., Joy D.A., Duan J., Huang Y., Carlton J., Walker J., Barnwell J., Beerli P., Charleston M.A., Pybus O.G., Su, X.Z. (2005). Host switch leads to emergence of *Plasmodium vivax* malaria in humans. *Molecular Biology and Evolution 22*:

1686-1693.

Mueller U.G. (2015). The attine ant-fungus mutualism. In *Mutualism. Ed. J.L. Bronstein. Oxford University Press, Oxford, UK*, pp.78-79.

Mueller U.G. (2002). Ant versus fungus versus mutualism: Ant cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. *The American Naturalist* 160: S67-S98.

Nucete M., Van Konijnenburg-van Cittert J.H.A., Van Welzen P.C. (2012). Fossils and palaeontological distributions of *Macaranga* and *Mallotus* (Euphorbiaceae). *Palaeogeography, Palaeoclimatology, Palaeoecology* 353: 104–115.

Orona-Tamayo D., Wielsch N., Blanco-Labra A., Svatos A., Farías-Rodríguez R., Heil M. (2013). Exclusive rewards in mutualisms: ant proteases and plant protease inhibitors create a lock–key system to protect *Acacia* food bodies from exploitation. *Molecular Ecology* 22: 4087-4100.

Page R.D., Cruickshank R.H., Dickens M., Furness R.W., Kennedy M., Palma R.L., Smith V.S. (2004). Phylogeny of “*Philoceanus complex*” seabird lice (Phthiraptera: Ischnocera) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 30: 633-652.

Passmore H.A., Bruna E.M., Heredia S.M., Vasconcelos H.L. (2012). Resilient networks of ant-plant mutualists in Amazonian forest fragments. *PloS one* 7: e40803.

Pemberton R.W. (1992). Fossil extrafloral nectaries, evidence for the ant-guard antiherbivore defense in an Oligocene *Populus*. *American Journal of Botany* 79: 1242–1246.

Petricka J.J., Winter C.M., Benfey P.N. (2012). Control of *Arabidopsis* root development. *Annual review of plant biology* 63: 563-590.

Philpott S.M., Foster P.F. (2005). Nest-site limitation in coffee agro-ecosystems: artificial nests maintain diversity of arboreal ants. *Ecological Applications* 15: 1478–1485.

Quek S.P., Davies S.J., Itino T., Pierce N.E. (2004). Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). *Evolution* 58: 554–570.

Rabosky D.L. (2014). Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PloS one* 9: e89543.

Rabosky D.L., Adams D.C. (2012). Rates of morphological evolution are correlated with species richness in salamanders. *Evolution* 66: 1807-1818.

Rabosky D.L., Santini F., Eastman J., Smith S.A., Sidlauskas B., Chang J., Alfaro M.E. (2013). Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nature Communications* 4: 1958.

Raimundo R.L., Gibert J.P., Hembry D.H., Guimaraes Jr. P.R. (2014). Conflicting selection in the course of adaptive diversification: the interplay between mutualism and intraspecific competition. *The American Naturalist* 183: 363-375.

Renner S.S., Feil J.P. (1993). Pollinators of tropical dioecious angiosperms. *American Journal of Botany* 80: 1100-1107.

Rezende E.L., Lavabre J.E., Guimarães P.R., Jordano P., Bascompte J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature* 448: 925-928.

Richardson J.E., Pennington R.T., Pennington T.D., Hollingsworth P.M. (2001). Rapid diversification of a species-rich genus of Neotropical rain forest trees. *Science* 293: 2242–2245.

Ricklefs R.E. (2004). Cladogenesis and morphological diversification in passerine birds. *Nature* 430: 338-341.

Ricklefs R.E. (2006). The unified neutral theory of biodiversity: do the numbers add up?. *Ecology* 87: 1424-1431.

Ricklefs R.E., Fallon S.M., Bermingham E. (2004). Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology*, 53: 111-119.

Rickson F.R. (1979). Absorption of animal tissue breakdown products into a plant stem-the feeding of a plant by ants. *American Journal of Botany* 66: 87-90.

Rico-Gray V., Barber J.T., Thien L.B., Ellgaard E.G., Toney J.J. (1989). An unusual animal-plant interaction: feeding of *Schomburgkia tibicinis* (Orchidaceae) by ants. *American Journal of Botany* 76: 603-608.

Rosenzweig M.L. (1995). *Species diversity in space and time*. Cambridge University Press, Cambridge, UK.

Sachs J.L., Simms E.L. (2006). Pathways to mutualism breakdown. *Trends in Ecology and Evolution* 21: 585-592.

Schemske D.W., Lande R. (1985). The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39: 41-52.

Schemske D.W., Horvitz C.C. (1984). Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* 225: 519-521.

Schleuning M., Fründ J., Klein A.M., Abrahamczyk S., Alarcón R., Albrecht M., Andersson G.K., Bazarian S., Böhning-Gaese K., Bommarco R., Dalsgaard B. (2012). Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Current Biology* 22: 1925-1931.

Sorenson M.D., Sefc K.M., Payne R.B. (2003). Speciation by host switch in brood parasitic indigobirds. *Nature* 424: 928-931.

Stanton M.L., Palmer T.M., Young T.P., Evans A., Turner M.L. (1999). Sterilization and canopy modification of a swollen thorn acacia tree by a plant-ant. *Nature* 401: 578-581.

Thompson J.N., Pellmyr O. (1992). Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology* 73: 1780-1791.

Thompson J.N. (2005). *The geographic mosaic of coevolution*. University of Chicago Press.

Thrall P.H., Hochberg M.E., Burdon J.J., Bever J.D. (2007). Coevolution of symbiotic mutualists and parasites in a community context. *Trends in Ecology and Evolution* 22: 120-126.

Trapnell C., Roberts A., Goff L., Pertea G., Kim D., Kelley D.R., Pimentel H., Salzberg S.L., Rinn J.L., Pachter L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols 7*: 562-578.

Treseder K.K., Allen M.F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist* 155: 507-515.

Treseder K.K., Davidson D.W., Ehleringer J.R. (1995). Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte. *Nature 375*: 137-139.

van Zinderen Bakker E.M., Mercer J.H. (1986). Major late Cenozoic climatic eventsand palaeoenvironmental changes in Africa viewed in a world wide context. *Palaeogeography, Palaeoclimatology, Palaeoecology* 56: 217–235.

Vandermeer J.H., Boucher D.H. (1978). Varieties of mutualistic interaction in population models. *Journal of Theoretical Biology* 74: 549–558.

Vázquez D.P., Aizen M.A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology 85*: 1251-1257.

Waser N.M., Chittka L., Price M.V., Williams N.M., Ollerton J. (1996). Generalization in pollination systems, and why it matters. *Ecology* 77: 1043-1060.

Whittaker R.J., Willis K.J., Field R. (2001). Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography* 28: 453-470.

Wilson E.O. (1959). Some ecological characteristics of ants in New Guinea rainforests. *Ecology* 40(3):437-447.

Wilson E.O., Hölldobler B. (2005). The rise of the ants: a phylogenetic and ecological explanation. *Proceedings of the National Academy of Sciences of the United States of America* 102: 7411-7414.

Yoder J.B., Nuismer S.L. (2010). When does coevolution promote diversification? *The American Naturalist* 176: 802-817.

Youngsteadt E., Nojima S., Häberlein C., Schulz S., Schal C. (2008). Seed odor mediates an obligate ant–plant mutualism in Amazonian rainforests. *Proceedings of the National Academy of Sciences* 105: 4571-4575.

Zahran H.H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and molecular biology reviews* 63: 968-989.

Zietara M.S., Lumme J. (2002). Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). *Evolution*, *56*(12), pp.2445-2458.

Acknowledgements

First and foremost, I would like to thank Susanne for the outstanding supervision that she has offered me over the past three years. I deeply admire Susanne's kicking passion, knowledge, insights and critical thinking, and I am immensely grateful to Susanne's unrivalled help and availability, direction, freedom in research, and opportunity. I am very proud to be able to say, "I am a Renner student", and even more of what it means.

I want to thank the love of my life, Jeremy, for supporting me throughout these three years, for agreeing to live in Munich and thus to be in a distance relationship with his university across the Channel and thus avoiding us being in a distance relationship, and for being an indispensable field-worker and driver in Fiji, making tree climbing and experiments possible. Je t'aime!

I also want to thank my parents, Françoise and Philippe, who have always encouraged my passion for plants and Science, supported my early days of plant collecting and experimentation (and this started as early as I can remember), and as a teenager allowed me to have sacred lotus in my bedroom in winter to demonstrate the lotus effect in the classroom or when my turn to cacti implied spines all around the house!

I thank my brother Benoît (aka dinde farfelue) for being fun and helping me with movie editing.

I dedicate this thesis to Jeremy, Françoise, Philippe and Benoît.

I also thank my second parents, Corinne and André, for continued love and support.

I am very grateful to numerous colleagues and collaborators that helped me in various ways, in particular Alivereti Naitatini and Marika Tuiwawa for permission and assistance in the field in Fiji, Christoph Mayr for help with IR-MS, Veronika Mayer for advice and material for ^{15}N experiments, Bernhardt Ruthensteiner for teaching me how to analyze CT scanning data, my collaborators from Vienna Yannick Staedler, Jürg Schoënenberger, Dirk Metzler for agreeing to model *Squamellaria*'s optimal cavity number, Camilla Huxley-Lambrick for numerous samples, stimulating discussions on the Hydnophytinae, and accommodation in Oxford, and Matthew Jebb for invaluable taxonomic advice in the Hydnophytinae, and various samples for DNA and morphological analyses and Andreas Wistuba for numerous samples of cultivated Hydnophytinae.

I am indebted to Yasumin Sophia Lermer for drawing the wonderful *Squamellaria* plates for Chapter 3, and fascinating discussion at the boundary of Art and Science.

Un agradecimiento muy especial a mi buen amigo Oscar, quien me ayudó muchas veces y que puso un ambiente latino en el edificio. ¡Ahorita pienso a nuestros colaboraciones de puta madre!

Special thanks go to Constantin, for induction to Bayesian Hierarchical Modelling, and a peaceful 3-year office share, to Martina Silber, for teaching, discussion and fun in the lab and beyond, to Alexander, and several former members from the lab, including Sidonie, Fernanda, Stefan, Juliana who provided a super friendly atmosphere when I joined the lab.

Finally, I would like to thank all participants from the "*Ants 2016: ant interactions with fungi, microbes, other insects, and plants*" conference that Susanne and myself organized, for fascinating discussions on ant/plant mutualism.

July 2016

Curriculum vitae
Guillaume Chomicki
University of Munich (LMU)

Institute for Systematic Botany and Mycology (Office 229b)
Department of Biology
University of Munich (LMU)
Munich 80638
Germany
Email: guillaume.chomicki@gmail.com
Website: https://guillaumechomicki.wordpress.com
Researchgate: https://www.researchgate.net/profile/Guillaume_Chomicki
Born on 16 October 1989, Neuville-aux-Bois, France

EDUCATION

July 2013- July 2016

PhD in Biology, University of Munich (LMU), Germany
Doctoral Thesis: "*Ant/plant symbioses: evolution, specialization and breakdown*"
Supervised by Prof. Susanne S. Renner

2012 **B.Sc. (Hons) Plant Science with Research Experience** First Class Honours
The University of Manchester, UK
Honour Thesis: "*SPIRAL2 complexes mobility controls Katanin's severing frequency underpinning microtubule cortical array morphology*" Supervised by Prof. Simon R. Turner

RESEARCH SKILLS

Microscopy: Scanning electron microscopy (SEM), confocal microscopy, epifluorescent microscopy, light microscopy, image analysis (imageJ). **Phylogenetics and data analysis**: Sequence alignment, phylogenetics reconstruction (RAxML, MrBayes, BayesPhylogenies), **Divergence time estimation** (BEAST), Character evolution (Mesquite, BayesTraits, Ace (Ape), Phytools), statistics (R), basic geometric morphometrics (MorphoJ). **Diversification analysis**: BAMM. **Historical biogeography**: Lagrange, BioGeoBEARS. **Molecular biology and**

biochemistry: PCR; qRT-PCR; Sanger sequencing; DNA and RNA extraction; *Physcomitrella patens* (moss) and *Arabidopsis thaliana*, *Saccharomyces cerevisiae* (yeast) and *E. coli* transformation; restriction-based molecular cloning, directional molecular cloning (D-TOPO), Yeast one-hybrid (Y1H) screening for protein-DNA interactions; HPLC-MS. **Field experiments**: Ant monitoring, cafeteria experiments, ^{15}N isotope feeding and uptake experiments, exclusion experiments. **Other**: architectural analysis of plants; analysis of 3D image data from X-ray Computed Tomography scanning data using Amira.

AWARDS AND GRANTS

2016	**Martin Fellowship**, Naturalis, Leiden, Netherlands. Grant to travel to Leiden to write a grant proposal with Dr. Vincent Merckx. This funds a three-week stay at Naturalis to co-write a grant proposal to the Dutch Organisation for Scientific Research (NWO), on the specialization of ant/plant mutualisms, using genomic, transcriptomics and metabolomics approaches to understand mutualism specialization, using my ant/plant system in Fiji.
2016	**Guest editor of *Proceedings of the Royal Society B: Biological Sciences*.** Special issue on ant interactions with their biotic environment, relating to the conference "Ants 2016" I organize with S.S.R. (http://sysbot.de/ants), to be published in 2017.
2016	**Conference organizer for the symposium "Ants 2016"**: Ants in their biotic environment, jointly with S.S.R.
2016	**German Science Foundation grant** (DFG) RE 603/23-1 written by G.C. and S.S.R. (awarded to S.S.R.), funding for the conference Ants 2016, Munich 6-9 May 2016. (13,800 Euros).
2015	**German Science Foundation grant** (DFG) RE 603/20 written by G.C. and S.S.R. (awarded to S.S.R.), "Field observations, morphology, allometry, and phylogenetic approaches to illuminate the evolution of a species-rich ant/plant system: Hydnophytinae (Rubiaceae) and their *Philidris* ants (Dolichoderinae)" (126,410 Euros).
2014	**Graduate student research award**, Society of Systematic Biologists ($2000)
2014	**Graduate Research Award**, American Association of Plant Taxonomy ($800)
2013	Elected **Fellow of the Linnean Society of London**

2012 **DH Valentine Plant Science Programme Prize**, (top plant science student), awarded by the Faculty of Life Sciences, University of Manchester (£100).

2012 **Conference Travel Award**, awarded by the American Society of Plant Biology ($575 plus ASPB annual meeting fee waiver).

2011 **Young Botanist Award**, awarded by the Botanical Society of America.

2010-2011 Funding provided by Dr. Jill Harrison, University of Cambridge (UK), in the context of my third year research placement to work on the development of a gene enhancer trapping system to mark stem cell in the moss *Physcomitrella patens*. (£8,122).

2010-2012 **Sainsbury Undergraduate Studentship**, awarded by the Gatsby charitable Foundation for plant science (UK) (£4,000). Part of this award funded a summer research project in Prof. Jane Langdale lab, University of Oxford, on '*Effect of polar auxin transport in microphyll and ligule development in the Lycopsid Selaginella kraussiana*'.

2009 **National Tropical Botanical Garden (US) scholarship** for the Biodiversity of Tropical Plants Harvard summer course and travel expenses (US$1,550).

2009 Funding provided by Prof. Jay-Allemand, University of Montpellier II (France), (1200€), Research assistant to Dr. Luc P. R. Bidel.

CONFERENCES

2016 **Chomicki G.** *Specialization and loss of mutualism between the Hydnophytinae and ants*. Talk at the "Ants 2016" symposium, May 5-8th, Munich.

2016 **Chomicki G.** *Regulation of the plant microtubule cortical array*. Invited seminar Lecture, Systematic Botany and Mycology, University of Munich, November.

2015 **Chomicki G.** *Evolution and maintenance of symbioses between ants and plants*. Invited seminar Lecture, School of Biological Sciences, University of Sydney, December.

2015 **Chomicki G.** *Evolution and maintenance of symbioses between ants and Epiphytic Rubiaceae*. Invited seminar Lecture, Systematic Botany and Mycology, University of Munich, November.

2015 **Chomicki G.** *Mutualism evolution, maintenance and dissolution in ant/plant symbioses.* Contributed talk at the Systematics Association Biennial 2015 meeting, 26-29 August, Oxford.

2015 **Chomicki G.** *The evolution and functional specialization in ant-plant symbioses*. Invited lecture at the Natural History Museum, Paris and CNRS, UMR ISYEB. February 3rd.

2015 **Chomicki G.** and Renner S. (Jan 2015). *Biogeographic patterns underlying the specialization or loss of symbiotic association between ant and plants*. Poster presented at the 7the biennial conference of the International society of biogeography, Bayreuth, Germany.

2014 **Chomicki G.** *Phylogenetic distribution and specialization in ant plant symbioses*. Invited seminar lecture, University of Vienna, December.

2014 **Chomicki G.** *The evolution of plant ants and ant plants*. Invited seminar Lecture, Systematic Botany and Mycology, University of Munich, October.

2014 **Chomicki G.**, Bidel L.P.R., Feng M., Coiro M., Zhang X., Wang Y., Baissac Y., Jay-Allemand C. and Renner S. S. *A lineage-specific Chalcone Synthase clade underpins UV-B avoidance in epiphytic orchid roots, but has been lost following a major transition in habit during the Cenozoic*. Presented (by L.P.R. Bidel) at the XXVIth conference on Polyphenols, 2-6 Sept 2014, Nagoya, Japan.

2014 **Chomicki G.** and Renner S. *The staggered evolution of ant-plant symbioses in the World's intertropical regions during the last 15 million years*. Poster presented at the Radiation meeting, Zurich, Switzerland, June. Paper published in *New Phytologist*, special edition on evolutionary radiations.

2014 **Chomicki G.** *Root photoprotection in epiphytic orchids: a new function for the velamen*. Invited seminar Lecture, Systematic Botany and Mycology, Munich, May 2014.

2012 **Chomicki G.**, Bidel LPR., Baissac Y. and Jay-Allemand C. "*Inducible epidermal asymmetric flavonoid distribution protects the root cortex of epiphytic orchids from UV-B light*". July 20-23th presented at the Annual meeting of the American Society of Plant Biologists, Austin, Texas. Two published papers (*New Phytologist* and *Flora*).

2010 **Chomicki G.**, Sanders HL. and Langdale JA. "*Auxin's basic function is conserved in the lycophyte Selaginella kraussiana but has been recruited in different developmental contexts*". Poster presented at the Gatsby Plant Science network meeting, 9-10 sept. 2010, Pembroke College, University of Oxford. Contribution acknowledged in Sanders and Langdale, *New Phytologist* 198(2): 419-428 (2013).

2009 **Chomicki G.** "*Introduction to the biology of Winteraceae*" talk given to the Harvard Summer School (BIOS 111 course) Class of 2009, Miami.

PUBLICATIONS l13 published l 1 in revision l 5 in preparation with full manuscript (Citations 77; h-index 5; Sum of impact factor 65.93)

*** Corresponding author; § joint first author**

19. **Chomicki G.***, Metzler D., Renner S.S. Domatium 3D structure maximizes benefits in facultative and obligate nutritional mutualisms. (in prep., <u>manuscript in preparation can be provided</u>).

18. **Chomicki G***, Janda M., Renner S.S. The assembly of South-East Asian ant gardens: specialization via host broadening. Invited manuscript in ***Proceedings of the Royal Society of London B: Biological Sciences*** special issue on ‘Ants in their biotic environment’. (in prep., <u>manuscript in preparation can be provided</u>).

17. **Chomicki G.***, Renner S.S. Altitude drives mutualism breakdown, leading to accelerated morphological evolution. **(**in prep. <u>manuscript in preparation can be provided</u>).

16. **Chomicki G.§***, Perez O.A.§, Matzke N.J., Renner S.S. Biogeographic models and the global history of Orchids. (in prep. <u>manuscript in preparation can be provided</u>).

15. Perez O.A.§, **Chomicki G.§***, Condamine F.L., Matzke N.J., Silvestro D., Antonelli A. Mountain uplift triggered the diversification of Neotropical orchids. **(**in prep. <u>manuscript in preparation can be provided</u>).

14. **Chomicki G.***, Renner S.S. Active and exclusive planting and fertilization by ants of their hosts’ offspring. ***Nature Plants*** (Submitted 21 May 2016, <u>accepted pending revisions 21 June 2016</u>).

13. **Chomicki G.**, Wightman R.W., Turner S.R. A specific class of short treadmilling microtubules enhances cortical microtubule ordering. ***Molecular Plant*** (DOI: 10.1016/j. molp. 2016.05. 008).

12. **Chomicki G.***, Staedler Y., Schönenberger J., Renner S.S. (2016). Partner choice through concealed floral sugar rewards evolved with the specialization of ant/plant mutualisms. ***New Phytologist*** (DOI: 10.1111/nph.13990). **Featured on http://blog.willyvanstrien.nl/2016/05/31/geheime-snoeppot/**

11. **Chomicki G.***, Renner S.S. (2016). Evolutionary relationships and history of the ant-epiphytic genus *Squamalleria* (Rubiaceae: Psychotrieae) and their taxonomic implications. ***PLoS ONE*** 11(3): e0151317.

10. **Chomicki G.***, Ward P.S., Renner S.S. (2015). Macroevolutionary assembly of ant/plant symbioses: *Pseudomyrmex* ants and their ant-housing plants in the Neotropics. ***Proceedings of the Royal Society of London B: Biological Sciences*** **282: 20152200**. (Accepted 19 Oct. 2015). http://dx.doi.org/10.1098/rspb.2015.2200. **The paper made the front cover. Paper featured, see the University of Munich press release: http://www.uni-muenchen.de/forschung/news/2015/renner_ameisen.html**
9. Bidel L. P. R., **Chomicki G.**, Bonini F., Mondolot-Cosson L., Soulé J., Baissac Y., Petit V., Loiseau A., Cerovic Z. G. and Jay-Allemand C. (2015). Distinct accumulation dynamics of leaf epidermal flavonols govern UV-B acclimation in *Centella asiatica* (Apiaceae). ***Planta*** 242(3): 545-559. **Part of a special issue on Polyphenols: biosynthesis and function in plants and ecosystems.**
8. **Chomicki G.*** and Renner S.S. (2015). Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. ***New Phytologist*** 207(2): 411-424. **Part of a special issue on evolutionary radiations.**
7. **Chomicki G.*** §, Bidel L.P.R.§, Ming F.§, Coiro M., Zhang X., Wang Y., Baissac Y., Jay-Allemand C., Renner S.S. (2015). The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. ***New Phytologist*** 205(3): 1330-1341. **Paper featured in AoB Blog (https://aobblog.com/2015/03/plant-parts-doing-unexpected-things-part-2-or-root-research-all-up-in-the-air/)**
6. **Chomicki G.***, Renner S.S. (2015). Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. ***New Phytologist*** 205(2): 526-532. **Paper featured in the German Newspaper Suddeusche Zeitung on Oct. 29th 2014 ("Die Familienbande der Wassermelone"). Also featured in Phys.org (http://phys.org/news/2014-11-melon-melange.html), also featured in (http://www.laboratoryequipment.com/news/2014/11/research-corrects-80-year-old-watermelon-mistake) and see also the University of Munich press release (http://www.en.uni-muenchen.de/news/newsarchiv/2014/renner_melone.html)**

5. Renner S.S., **Chomicki G.**, Greuter W. (2014). Proposal to conserve *Momordica lanata*, the basionym of *Citrullus lanatus* (watermelon, Cucurbitaceae), with a conserved type and against an earlier synonym. ***Taxon*** 63(4): 941-942.
4. **Chomicki G.***, Bidel L.P.R., Baker,W. J., Jay-Allemand C. (2014). Palm snorkelling: leaf bases as aeration structures in the mangrove palm (*Nypa fruticans*). ***Botanical Journal of the Linnean Society*** 174(2): 257-270. **Paper featured: See press article in *Kew Magazine* entitled 'snorkelling palms' written by Stephanie Pain.**
3. **Chomicki G.***, Bidel L.P.R., Jay-Allemand C. (2014). Exodermis structure controls fungal invasion in the leafless epiphytic orchid *Dendrophylax lindenii* (Lindl.) Benth. ex Rolfe. ***Flora*** 209: 89-94.
2. Wightman R.§, **Chomicki G.§,** Kumar M., Carr P., Turner S.R. (2013). SPIRAL2 determines plant microtubule organization by modulating microtubule severing. ***Current Biology*** 23(19): 1902-1907. **The paper made the front cover of the journal. Paper featured: See BBSRC press release: (http://www.bbsrc.ac.uk/news/fundamental-bioscience/2013/130919-pr-cell-scaffold-key-plant-growth/)**
1. **Chomicki G.*** (2013). Analysis of rhizome morphology of the Zingiberales in Payamino (Ecuador) reveals convergent evolution of two distinct architectural strategies. ***Acta Botanica Gallica*** 160(3-4): 239-254.

PEER REVIEWED ACTIVITIES

Annals of Botany (1 ms), *American Journal of Botany* (1 ms), *The American Naturalist* (1 ms), Cambridge University Press (1 ms), *Journal of Biogeography* (1 ms), *New Phytologist* (1 ms), *Plos ONE* (1 ms), *Planta* (1 ms, invitation rejected), *Journal of Horticulture* (1 ms), *African Journal of Biotechnology* (1 ms).

OTHER

Reviewer for mini-ARTs grants, Society of Systematic Biologists.

TEACHING EXPERIENCE

2013-2015	Evolution of plants and fungi (WP10). Teaching assistant
2013-2015	Flora and plant systematics, plant identification. Teaching assistant

SUPERVISION EXPERIENCE

October 2014-February 2015. Christian Feregrino. Intensive Research Training 1 (Master). Project: *The evolution and biogeography of Tachigali (Fabaceae).*

October 2014-February 2015. Anna Farré Orteu. Intensive Research Training 1 (Master). Project: *The evolution and biogeography of Philidris (Dolichoderinae) ants.*

April 2014-September 2014. Rosa Elena Andrade. Intensive Research Training 2 (Master). Project: *The evolution of Pseudomyrmecinae-associated plants with a focus on Tachigali.*

LANGUAGES

French (Native Speaker), English (Fluent), Spanish (Proficient)

RESEARCH EXPERIENCE (additional to BSC and PhD thesis lab work)

2015	Fieldwork in Fiji (March) funded by the DFG.
2014	Fieldwork in Fiji (September) funded by the Society of Systematic Biologists and the American Association of Plant Taxonomy.
2013	eFLOWER summer school, University of Vienna (July, 10 days).
2013	University of Warwick, Warwick Crop Science Centre. Project title: *Transcriptome-wide Yeast-one hybrid screen for the identification of transcription factors that regulate expansins and GA oxidases during germination* (3 months). Supervised by Dr. Katherine Denby.

2010-2011	University of Cambridge, Department of Plant Science, Research placement (Ten months): “*Development of a gene enhancer trap to mark stem cell in the moss Physcomitrella patens*”. Supervised by Dr. Jill Harrison.
2010	University of Manchester, Faculty of Life Sciences, Tropical biology field course in Ecuador (One month), Project title: “*Comparative morphology of Zingiberales rhizomes*”. Supervised by Prof. Richard Preziosi.
2010	University of Oxford, Department of Plant Sciences, Sainsbury undergraduate student, summer internship, Project title: “*Effect of polar auxin transport in microphyll and ligule development in the Lycopsid Selaginella kraussiana*” (Six weeks). Supervised by Prof. Jane Langdale and Dr. Heather Sanders
2009-2010	University of Manchester, Faculty of Life Sciences, Research assistant in Prof. Simon Turner lab, Project title: “*Characterization of spiral2 mutant phenotype using dynamic live imaging of microtubules in Arabidopsis thaliana*” (October-May). Supervised by Prof. Simon Turner and Dr. Raymond Wightman.
2009	University of Montpellier II, Faculty of Sciences, Research assistant in Prof. Christian Jay-Allemand lab, Project Titles: “*Roles of flavonols and hydroxycinnamic acids in leaf acclimation to UV light in Centella asiatica*” And “*Functions of flavonoids in the root of the leafless orchid Dendrophylax lindenii*”(One month). Supervised by Dr. Luc Bidel and Prof. Jay-Allemand.
2009	Harvard University, Harvard Summer School, Summer course BIOS S-111: Biodiversity of Tropical Plants (4 credits, A grade). Project title: “*Aspects of branching in two dichotomous palms (Nannorrhops ritchiana and Nypa fruticans) and anatomical investigations of a new function for the Nypa fruticans leaf base*.” (One month). Supervised by Prof. (emeritus) P. Barry Tomlinson.
2009	University of Montpellier II, Faculty of Sciences. Extracurricular completion of the third year unit “Plant architecture and Morphogenesis” (research based, one week). Project title: “*Architecture of exotic Conifers”* Supervised by Dr. Claude Edelin. (Grade ranking first of the cohort)
2009	University of Montpellier II, Faculty of Sciences. Research assistant in Prof. Christian Jay-Allemand lab (October-May). Project title: *Induction of saponin biosynthesis by methyljasmonate induction in Centella asiatica*” Supervised by Dr. Luc Bidel and Prof. Jay-Allemand.

2008	James Cook University, Australian Tropical Herbarium (Cairns). Summer Placement in taxonomic management (one week). Supervised by Prof. Darren Crayn.
2008	Sydney Royal Botanic Gardens, NSW Herbarium. Summer Placement in taxonomic management, focusing on Proteaceae (one week). Supervised by Dr. Peter Weston.

REFEREES

Prof. Susanne S. Renner
Chair of Systematic Botany
Director of Munich Herbarium (M)
Director of Munich Botanic Garden
University of Munich
renner@lrz.uni-muenchen.de

Prof. Naomi E. Pierce
Hessel professor of Biology
Department of Organismic &
Evolutionary Biology
Harvard University
npierce@oeb.harvard.edu

Prof. Philip S. Ward
Professor of Entomology
Department of Entomology and Nematology
University of California, Davis
psward@ucdavis.edu

Prof. Martin Heil
Group leader in Plant Ecology
Cinvestav, Mexico
mheil@ira.cinvestav.mx