Morphological homogeneity, phylogenetic heterogeneity and systematic complexity in species-rich groups: a case study of floral evolution in Myrteae (Myrtaceae)

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‘ I, Thais Nogales da Costa Vasconcelos, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

ABSTRACT

Myrteae is the most diverse tribe in the species-rich angiosperm family Myrtaceae. Myrteae species play a critical ecological role in tropical forests and savannas, biomes with some of the highest biodiversity on earth. Hence there is a growing interest in its use as a model for evolutionary, ecological and conservation studies. However, morphologically homogeneous reproductive structures cause taxonomic instability and jeopardize modelling and conservation initiatives. This study demonstrates how evolutionary patterns are underpinned by floral traits in Myrteae. Aims are approached using combined phylogenetic and morphological analyses in two work packages (WP): WP1 increases understanding of systematics and floral evolution in Myrteae based on multiloci molecular matrices for a near complete generic sample. The framework is used to interpret biogeography, diversification and over-arching patterns of floral morphology and development; data are reciprocally combined to illuminate those processes. WP2 presents four case studies using floral development and multidimensional trait analysis to address questions related to systematic complexity, phylogenetic heterogeneity and theoretical cladistics concepts, such as evolution of homoplastic traits. Results harness Myrteae as a model group to address relevant questions in plant evolution and systematics; the applicability of this approach to similar questions in other diverse tropical angiosperm groups is discussed.

Key words: diversification; macro-evolution; Myrtaceae; phenotypic evolution; phylogenetics; systematics.

This thesis is dedicated to the

kindness

of countless people

.

.

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Thanks!

“The biologists who enter this field must resign themselves to the fact that they can never achieve certainty. Their end point must always be a judgement as to which several hypothesis appears to be most plausible on the basis of presently available factors.”

G.L. Stebbins (“Flowering Plants: evolution above the species level”; 1974, p.viii)

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Notes

Chapters 1 to 7 of this study correspond to manuscripts already, or to be, submitted for publication individually. Manuscripts have been adapted to form integral parts of the thesis, but individual introductions were not changed and contain some repeated content. Slight inconsistencies in figure format between chapters reflect rules of target journals for publication.

Introduction: An Overview of Myrteae

All taxa discussed in this thesis (with the exception of some genera and species studied in Chapter 4) form a single monophyletic group of flowering plants: tribe Myrteae DC**.** Basic knowledge of general aspects of Myrteae systematics and evolution are desirable to understand the relevance of this study. This introduction summarises information on this topic and highlights the questions and hypotheses that are addressed in this thesis.

I.1 What is Myrtaceae?

Myrtaceae Juss. is a family of flowering plants comprising around 5500 species of shrubs and trees with highest diversity in Australasia and South America (WCSP, 2017). Myrtaceae is the third most species-rich family of angiosperms in number of tree species (Beech et al., 2017). Among its main characteristics are aromatic leaves with entire margins, white polystemonous flowers, oil gland dots and flaky bark (Wilson, 2011). Some of the best known Myrtaceae include eucalypt trees (the tallest angiosperms in the world; Ashton, 1958), the clove (*Syzygium aromaticum*), the tea tree (*Melaleuca*) and edible fleshy fruits that are cultivated around the world, such as “pitangas”, “guavas” and “jambos” (*Eugenia, Psidium* and *Syzygium*, respectively).

I.2 What is Myrteae?

Myrteae is one of the major Myrtaceae lineages, described at the taxonomic level of tribe by De Candolle (1828), circumscribed by traditional cladistic methods based on morphological characters (Briggs and Johnson, 1979) and supported as a monophyletic group by molecular data (Wilson et al., 2001, 2005; Lucas et al., 2007). Myrteae is the richest tribe in Myrtaceae both in number of genera (c. 50) and species (c. 2500), making up over half of the family’s biodiversity. Myrteae is almost entirely restricted to tropical areas (see examples in Fig. I.1), with highest diversity (c. 2000 species, 80% of the total) in the Neotropics. Significant biodiversity is also found in New Caledonia (c. 200 species), Southeast Asia and Australia (c. 150 species) and Africa and Madagascar (c. 150 species), although in the latter it is represented by a single genus, *Eugenia*. The only European genus, *Myrtus*, completes the tribe’s geographical distribution. Morphological traits that characterise Myrteae are all those described above for Myrtaceae plus opposite leaves, brochidodromous venation, inferior ovaries and fleshy berries (Fig. I.2).

Figure I.1 (next page): Examples of habitats where Myrteae occurs. (A) “Igapó” Amazonian forest; (B) “Terra firme” Amazonian forest; (C) High altitude humid forests; (D) “Restinga” coastal vegetation; (E) Montane atlantic rainforest; (F) New Caledonian valleys and mountains; (G) “Caatinga” dry forest; (H) Brazilian savanna or “Cerrado” *sensu stricto*; (I) “Campo rupestre” high altitude savanna. All photos taken during field expeditions between 2014 and 2016.

I.3 Brief History of Myrteae Systematics

Myrteae is a group of historical taxonomic complexity. Linnaeus described the first four genera of Myrteae in the *Species Plantarum* (1753): the Neotropical *Eugenia*, *Plinia*, *Psidium* and the European *Myrtus*, the last of which was taken as the nomenclatural type of Myrteae (De Candolle, 1828), the family Myrtaceae (Jussieu, 1789) and the order Myrtales (Reichenbach, 1828). The first comprehensive treatments of Myrteae genera, however, came years later as substantial numbers of Myrtaceae collections were sent from Latin America to European herbaria by naturalists during the 18th and 19th century. The work of De Candolle (1826) and O. Berg (1855) represents the first complete efforts to address all Myrteae biodiversity comparatively in a single piece of work. De Candolle's major contribution to Myrteae systematics, besides giving the tribe its name, was the description of embryo traits that are still relevant as diagnostic characters at the infra-tribal level (e.g. see Landrum and Kawasaki, 1997; Lucas et al., 2007). Berg's major contribution to Myrteae systematics are the detailed descriptions of flower morphology, including placentation, number of ovary locules and number of ovules. Berg was also first to note the importance of the calyx and hypanthium in separating Myrteae genera and to use inflorescence morphology to distinguish sections in the giant genus *Eugenia* (see discussion in Mazine et al., 2014). Together, De Candolle and Berg described c. 2300 Myrteae taxa (IPNI, 2017), among genera, species and subspecies, shaping the tribe as a mega-diverse tropical plant group.

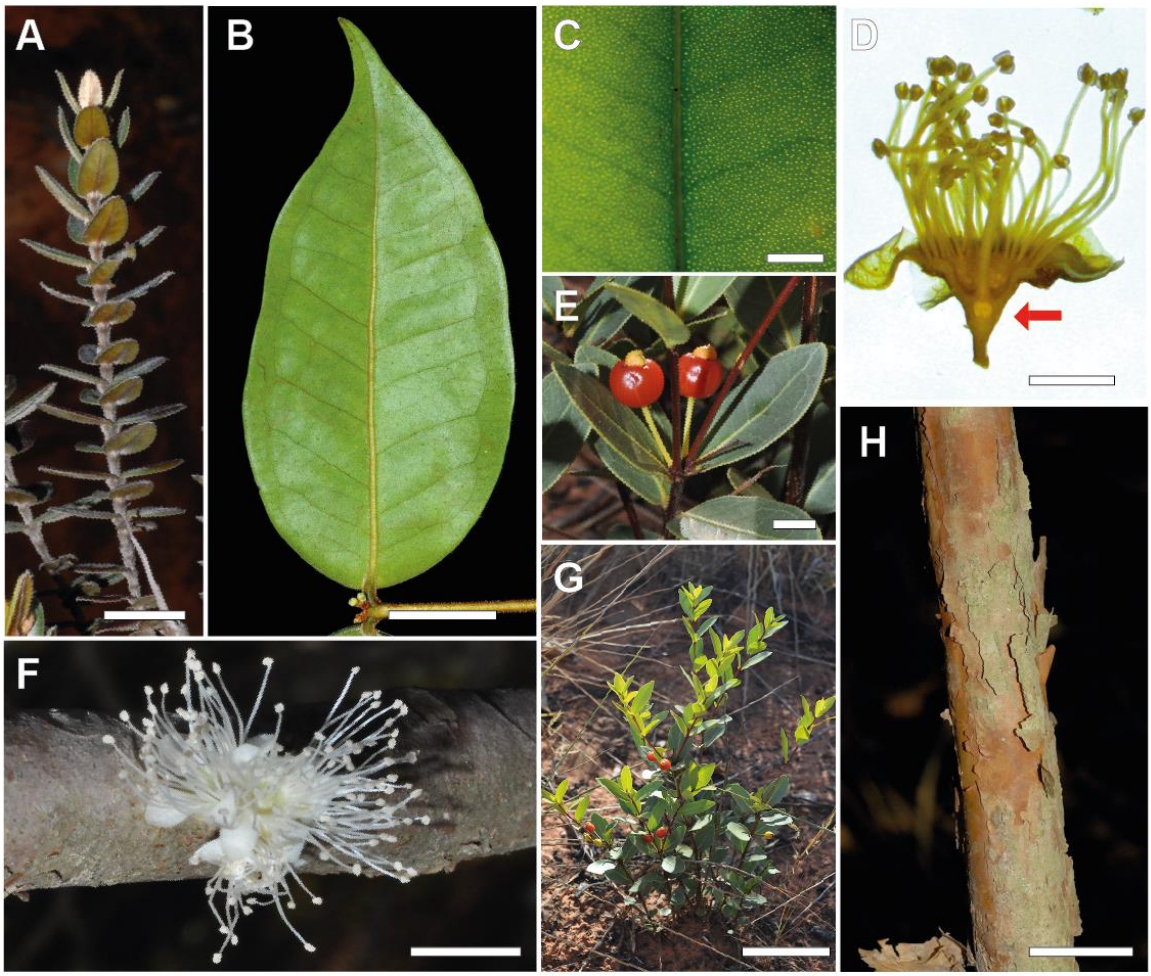

Figure I.2: Myrteae morphological features. (A) Opposite leaves in *Myrcia sp*. (B) Simple leaf with entire margins and brochidodromous venation of *Psidium sp.* (C) Translucid glands spread throughout the leaf blade of *Myrcia neuwiedeana*. (D) Arrow pointing to the inferior ovary of *Myrcia rubella*. (E) Fleshy berries of *Eugenia punicifolia*. (F) White polyandrous flowers of *Plinia cauliflora*. (G) As "E", showing plant habit. (H) Flaky bark of *Eugenia sp.* Scale: 5mm (C,D,F), 1cm (A,B,E), c. 20cm (G,H). All photos taken during field expeditions between 2014 and 2016.

At this point, the limited access to collections (due to distribution often restricted to tropical areas with difficult access) and morphological complexity (caused by widespread homoplastic traits) was already a clear drawback to fully understanding evolutionary relationships within Myrteae. While other prolific taxonomists continued to describe and reorganize Myrteae species names, more than a century passed after Berg until all tribal diversity was treated systematically again. In this sense, the studies of Kausel (1956) and Mc Vaugh (1968) were the most comprehensive, and their focus on large Neotropical groups with difficult circumscriptions represented a significant advance in Myrteae systematics. Neither Kausel nor McVaugh, however, included the c. 20% of Myrteae species with Australasian distributions in their studies. One of Mc Vaugh's quotes stresses the challenge in interpreting Myrteae relationships in light of its morphological homogeneity:

"The species of American Myrtaceae are distressingly alike in aspect and in most individual characters, making identification and classification of both genera and species a correspondingly difficult and tedious matter. The leaves are essentially all opposite and entire, and of similar venation throughout; the inflorescences are of a few basic types; the flowers are all much alike except for occasional species in which the stamen-number is drastically reduced, or those relatively few which are set apart by some strong morphological character like the calyptrate calyx of *Calyptranthes*. […] In the absence of more obvious distinctions, the taxonomist of the Myrtaceae is often constrained to consider details of ovarian structure, of placentation, and of ovule number, but even these sometimes fail to provide the evidence necessary for a satisfactory conclusion.**"**

(Mc Vaugh, 1968, p. 359)

After a long period of accumulation of taxonomic uncertainty, Myrteae systematics resurfaced in the last 30 years. Chronologically, this phase started with the important works of Landrum in the Neotropics (e.g. 1981, 1984, 1986, 1988a, 1988b), and Scott (e.g. 1978, 1979a, 1979b, 1985) and Snow (Snow 2000, 2004, 2007; Snow and Guymer, 2001; Snow et al., 2003) in the Australasian region. Focusing on small genera or groups of species with somewhat restricted geographical distribution, these systematic reviews provided detailed morphological analysis and highlighted several circumscription problems. Resulting taxonomic inflation and deflation during this period led to a significant number of new genera to be described from species that could not be fitted into any system (e.g. *Accara*, Landrum, 1990; *Chamguava*, Landrum, 1991; *Curitiba*, Salywon & Landrum, 2007; *Gossia* and *Lenwebbia*, Snow et al., 2003; *Kanakomyrtus*, Snow, 2009). Most of these genera are also shown to be distinct by molecular data (see Chapter 1 of this thesis).

In the 21st century, Myrteae systematics arrived in the molecular era. The first significant works in this sense are those of Wilson (et al., 2001, 2005), who updated the Myrteae circumscription in the context of the whole Myrtaceae, and Lucas (et al., 2005, 2007), who produced the first comprehensive Myrteae phylogenetic trees. These studies also tackled the largest genera and provided infra-generic structure allowing smaller monophyletic groups to be treated separately (e.g. Snow et al., 2011; Mazine, et al., 2014; Staggemeier et al., 2015; Santos et al., 2016; Bünger et al., 2016). Alpha-taxonomists, often working in collaboration with molecular systematists, also illustrated Myrteae mega-diversity in the Neotropics; Sobral, for example, has described more than 150 species in the last 15 years (IPNI, 2017).

The studies of the last decades have consolidated current systematic understanding of Myrteae to the extent that it has been lifted from almost complete neglect to a particularly data-rich tropical tribe. The significant increase in Myrteae data available has given rise to research in other areas to address hypotheses related to conservation, ecology and evolution, turning Myrteae into an important model group, especially in the Neotropics (e.g. Biffin et al., 2010; Staggemeier et al., 2010; Thornhill et al., 2012; Lucas and Bünger, 2015; Giaretta et al., 2015; Murillo-A et al., 2016).

I.4 Morphological vs. Phylogenetic heterogeneity

Contrary to expectations however, newly available information and more studies has brought more questions than answers to Myrteae systematic and evolutionary understanding, especially in the dichotomy between molecular and morphological evidence. Even though most generic delimitations are supported by molecular data, many traits traditionally used to diagnose

natural groups are shown to be highly artificial (see discussions in Lucas et al., 2005, 2007, and throughout this thesis). Consequentially, traditional classifications of suprageneric (i.e. subtribes) and infrageneric (i.e. sections) relationships often feature para- and polyphyletic groups in phylogenetic trees after molecular analysis (e.g. Lucas et al., 2007, 2011; Snow et al., 2011; Mazine et al., 2014). Such ambiguity is not restricted to Myrteae and is frequently found in other diverse tropical plant groups (e.g. *Miconia*, Michelangeli et al., 2004; *Croton*, Berry et al., 2005; *Mimosa*, Simon et al., 2011).

Contrast of molecular vs. morphological evidence is a central component of this thesis, where Myrteae is assumed to be an effective model of such idiosyncrasies in plant systematics. There are two main motives for the focus of this work on the relevance of plant morphology in the molecular era. The first is practical; morphology diagnoses natural groups in the field and herbaria, correctly places fossils in phylogenies (see Saraswati and Srinivasan, 2015) and supplements molecular information for taxonomic decision making, allowing a holistic understanding of biodiversity. The second is theoretical, as morphological (or phenotypical) changes result from natural selection and provide footprint evidence of interactions between organisms and their environment over the long term (Darwin, 1859). It is for this reason that combination of phenotypic information with phylogenetic trees is so powerful for understanding the evolutionary history of organisms and their biomes (e.g. Simon et al., 2009; Crips et al., 2011; Bacon et al., 2015). Despite its morphological homogeneity, Myrteae shows high disparity in species diversity per clade with almost 80% of species grouped in two gigantic genera (*Myrcia* and *Eugenia*), leaving the remaining c. 50 genera relatively species poor. Myrteae therefore, with its unusual morphological homogeneity and phylogenetic heterogeneity, provides a valuable example of discreet/cryptic phenotypical adaptations radically changing plant lineage fitness and increasing or decreasing diversification rates.

Fleshy-fruits have been proposed as the key-innovation responsible for the disparity between Myrteae's mega-diversity and other Myrtaceae tribes (Biffin et al., 2010). Fruits however, cannot explain the heterogeneous distribution of species diversity within the tribe as they are all fleshy berries. Myrteae flowers are also superficially similar but possess subtle variations never fully explored in the context of systematics and macroevolutionary dynamics. The studies presented here consider floral phenotype in different ways, each addressing the phenotypic and ecological factors that generate the documented high levels of species disparity described.

I.5 Thesis Objectives

This thesis sets phylogenetic and floral phenotype information of Myrteae in a comparative framework. The main objectives are three:

1) Reconstruct the evolutionary framework of Myrteae.

The most comprehensive Myrteae phylogeny was published 10 years ago (Lucas et al., 2007). Since then, new analytical tools are available and newly described fossils can be used to time-calibrate the phylogeny. Additionally, c.15 genera remained phylogenetically unplaced and will be included in a reconstructed Myrteae phylogeny based on the broadest molecular matrix and

generic sample to date. The resultant topology is used to explain chronological, biogeographical and diversification patterns in the tribe (Chapter 1).

2) Describe floral phenotypic variation in Myrteae

A systematic review of floral morphology and development in Myrteae illuminates generic relationships and potentially adaptive features (Chapters 2 and 3).

3) Angiosperm evolution: phylogeny vs. morphology

Case studies show how clarification of morphological homology improves phylogenetic signal estimation (Chapter 4); how reproductive strategies in morphologically homogeneous groups are modified by heterochrony (Chapter 5); how interpretation of convergence and parallelism leads to uncertainty for the systematics of complex and/or large genera (Chapter 6); and how phenotypic homogeneity enhances the general success of Neotropical tree lineages (Chapter 7).

Work Package I – Systematics and flower evolution of Myrteae

Comparative analysis of molecular data is currently the most reliable way to infer natural relationships between organisms. Chapter 1 presents an almost generically complete molecular phylogeny of Myrteae, alongside dating analysis , biogeography and diversification patterns; Chapter 2 demonstrates implications of stamen posture for interpretation of these phylogenetic results; Chapter 3 presents a systematic survey of Myrteae floral diversity, highlighting evolutionary patterns and characters that can be further explored for diagnosis of natural lineages.

Chapter 1: Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species rich tribe of Myrtaceae

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- T.N.C.Vasconcelos contributions: development of hypotheses, design of experiments, collection of samples, DNA sequencing, phylogenetic and statistical analyses and writing of manuscript.

ABSTRACT

Myrteae (c. 2500 species; c.50 genera) is the largest tribe of Myrtaceae and an ecologically important group of angiosperms in the Neotropics. Systematic relationships in Myrteae are complex, hindering conservation initiatives and jeopardizing evolutionary modelling. A well-supported and robust phylogenetic hypothesis was targeted towards a comprehensive understanding of the relationships within the tribe. The resultant topology was used as a base for key evolutionary analyses such as age estimation, historical biogeography and diversification rate patterns. One nuclear (ITS) and seven chloroplast (*psbA-trnH*, *matK*, *ndhF*, *trnl-trnF*, *trnQ-rps16*, *rpl16* and *rpl32-trnL*) DNA regions for 115 taxa representing 46 out of the 51 genera in the tribe were accessed and analysed using maximum likelihood and Bayesian inference tools for phylogenetic reconstruction. Dates of diversification events were estimated and contrasted using two distinct fossil sets (macro and pollen) in BEAST. The subsequent dated phylogenies were compared and analysed for biogeographical patterns using BioGeoBEARS and diversification rates using BAMM. Myrteae phylogeny presents strong statistical support for three major clades within the tribe: Australasian group, *Myrtus* group and Main Neotropical Lineage. Dating results from calibration using macrofossil are an average of 20 million years older and show an early Paleocene origin of Myrteae, against a mid-Eocene one from the pollen fossil calibration. Biogeographic analysis shows the origin of Myrteae in Zealandia in both calibration approaches, followed by a widespread distribution throughout the still-linked Gondwana continents and diversification of Neotropical endemic lineages by later vicariance. Best configuration shift indicates three points of acceleration in diversification rates, all of them occurring in the Main Neotropical Lineage. Based on the reconstructed topology, several new taxonomic placements were recovered, including: the relative position of *Myrtus communis*, the placement of the *Blepharocalyx* group, the absence of generic endemism in the Caribbean, and the paraphyletism of the former *Pimenta* group. Distinct calibration approaches affect biogeography interpretation, increasing the number of necessary long distance dispersal events in the topology with older nodes. It is hypothesised that biological intrinsic factors such as modifications of embryo type and polyploidy might have played a role in accelerating shifts of diversification rates in Neotropical lineages. Future perspectives include formal subtribal classification, standardization of fossil calibration approaches and better links between diversification shifts and trait evolution.

Key words: *Eugenia*, evolution, *Myrcia*, *Myrtus*, *Psidium*, systematics.

INTRODUCTION

1.1 Myrteae systematics and diversity

Myrtaceae is a large family of woody flowering plants represented by around 5500 accepted species, classified in 144 genera and 17 tribes (Wilson et al., 2005; Wilson, 2011; WCSP, 2016). Myrtaceae represents an old, mid-Cretaceous lineage within the order Myrtales (c. 85 million years old, Berger et al., 2016) and is characterized by a strong southern-hemisphere, Gondwanan distribution (Thornhill et al., 2015). Myrtaceae is an important floristic component in the areas where it is most species diverse, especially in the forests of Southeast Asia, Australia and South America (e.g. Johnson and Briggs, 1981; Kochummen et al., 1990; Oliveira-Filho and Fontes, 2000; Flora of Brazil, 2016). In Neotropical environments, all Myrtaceae diversity (excluding a single species from tribe Metrosidereae, *Metrosideros stipularis*, restricted to Chile; Pillon et al., 2015) is represented by a sole lineage: tribe Myrteae (Wilson et al., 2005; Lucas et al., 2007). Myrteae is the most diverse tribe within Myrtaceae both in number of species (c. 2500) and genera (51), representing half of the family's biodiversity (Wilson, 2011; WCSP, 2016). Myrteae species are ecologically important in many Neotropical environments due to the fleshy berries eaten by birds and mammals and the white generalist flowers that supply pollen and resources to a variety of bee species (Mori et al., 1983; Nic Lughadha and Proença, 1996; Gressler et al., 2006; see Fig. 1.1). Due to its ecological importance, a growing interest has been addressed by researchers using Myrteae as a model group for evolutionary, ecological and conservation studies in Neotropical biomes (e.g. Murray-Smith et al., 2009; Lucas and Bünger, 2015; Staggemeier et al., 2015; Giaretta et al.; 2015).

A common barrier encountered by those wishing to study Myrteae is the problematic systematics of the group. The homogeneous morphology of flowers, fruits and vegetative characters between even distantly related Myrteae species makes taxonomy in the tribe a tiresome process even for specialists and until recently resulted in its neglect (McVaugh, 1968; Landrum and Kawasaki, 1997; Lucas et al., 2005). Recent phylogenetic systematic studies and taxonomic revision of individual clades within the tribe has improved the understanding of relationships and characterization of smaller groups (e.g. Landrum, 1981, 1986; Proença, 1990; Grifo, 1992; Lucas et al., 2011; Murillo-A et al., 2012; Mazine et al., 2014; Staggemeier et al., 2015). However, narrower distributed genera not sampled at the molecular level until now remain phylogenetically unplaced. To place such taxa in a broader phylogenetic system is central to improve the understanding of relationships and evolution within this ecologically important tribe.

Although morphologically similar, Myrteae lineages have an uneven, heterogeneous distribution of biodiversity in terms of species per genus. Two thirds of the diversity of described species occurs in only two genera, *Eugenia* s.l. (sensu Mazine et al., 2014) and *Myrcia* s.l (sensu Lucas et al., 2011), which are also two of the largest angiosperm genera (Frodin, 2004) with c. 1000 and 700 species, respectively (WCSP, 2016). Furthermore, these two genera have been consistently proved to be sister to species poor lineages in the tribe (Lucas et al., 2007; this study), increasing the extant diversity disparity between closely related clades.

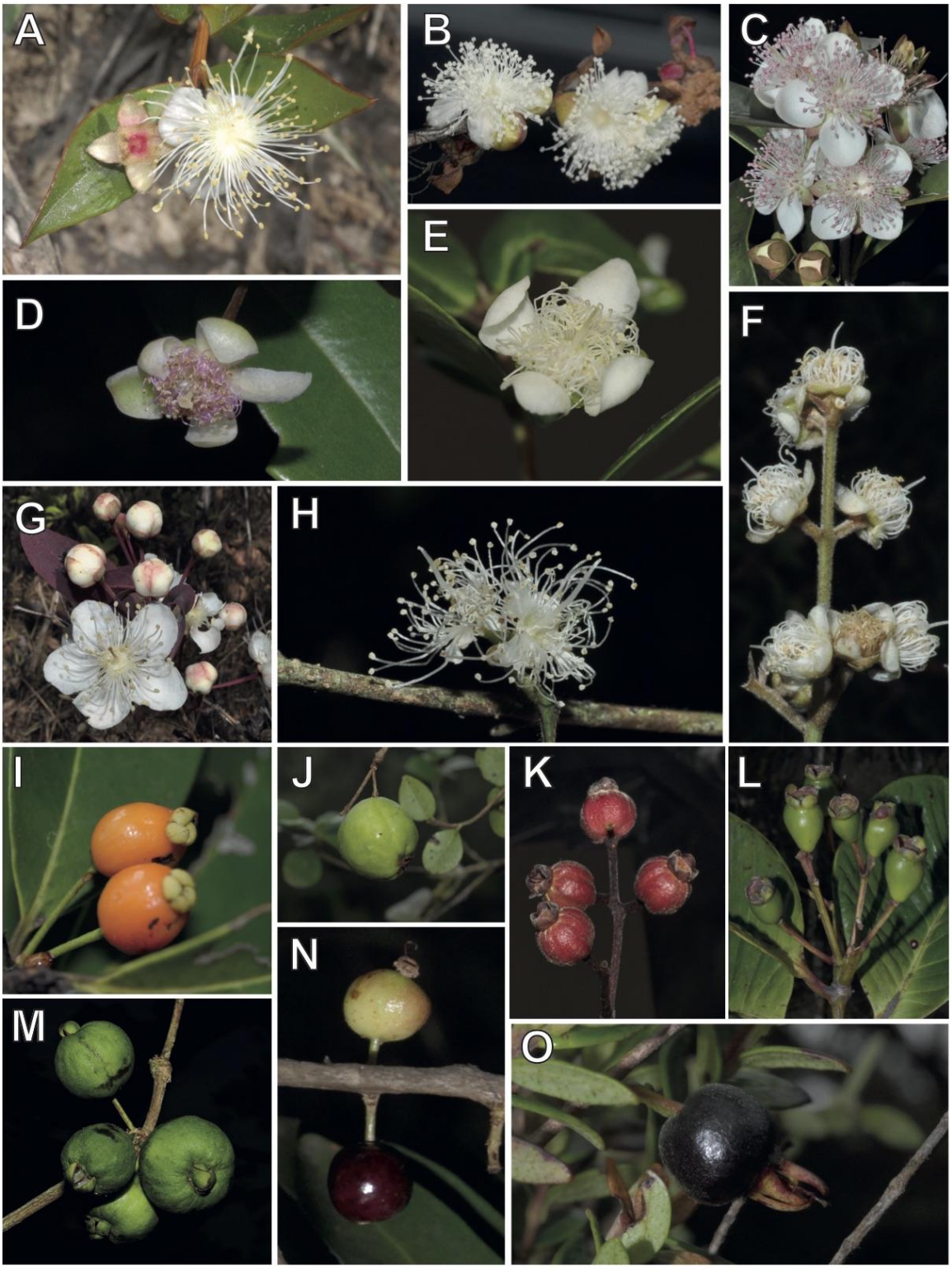


Figure 1.1: Biodiversity of Myrteae represented by the characteristic polystemonous white flowers (A -H) and fleshy, berry-like fruits (I - O). (A) *Accara elegans*; (B) *Calyptrogenia cuspidata*; (C) *Eugenia involucrata*; (D) *Archirhodomyrtus turbinata*; (E) *Luma apiculata*; (F) *Myrcia splendens*; (G) *Campomanesia adamantium*; (H) *Myrciaria floribunda*; (I) *Eugenia punicifolia*; (J) *Hottea neibensis*; (K) *Myrcia sp1* (voucher T. Vasconcelos 307); (L) *Gossia clusioides*; (M) *Chamguava schippii*; (N) *Siphoneugena densiflora* (O) *Myrtastrum rufopunctatum*. Size of reproductive structures varies between c. 0.5 to 3cm. (all photos taken during field expeditions between 2014 and 2016)

1.2 Myrteae global geographic distribution

Although most extant biodiversity of Myrteae is restricted to the Neotropics, at least 15 genera (Wilson, 2011) and c. 450 species are found in other continents. These are predominantly from Southeast Asia, Northeast Australia and the Pacific islands, including New Caledonia and New Zealand (Scott, 1978; Snow, 2000; Wilson, 2009; Snow et al., 2011; WCSP, 2016). A few species of *Eugenia* are also found in Africa, Madagascar and Mauritius (Van Wyk, 1982, van der Merwe et al. 2005, Snow, 2008) and an additional genus, *Myrtus*, represents the only European/Northern African lineage (Lucas et al., 2007; Migliore et al., 2011). On the American continent, most species diversity is found in the rainforests and savannah of central and eastern Brazil, the Guiana shield and Caribbean (McVaugh, 1968; Mori et al., 1983; Oliveira-Filho and Fontes, 2000; Holst, 2003, Murray-Smith et al.; 2009); less but still significant biodiversity is found in continental Central America and the low-land Amazon basin (Landrum, 1992; WCSP, 2016). Species diversity is relatively low in the subtropical and temperate areas of southern South-America (Patagonia) and the high altitude Andes, but these areas boast a significant array of endemic genera (e.g. *Ugni*, *Amomyrtus*, *Legrandia*, *Luma*; Landrum, 1981, 1986, 1988). Previous phylogenetic analyses consistently showed *Myrtus* representing a sister clade to all of the extant Myrteae (Lucas et al., 2005, 2007; Biffin et al., 2010; Thornhill et al., 2015). In these studies, most Australasian genera also group in a distinct clade, sister to the that containing all Neotropical clades (Lucas et al., 2005, 2007). The relative position of these clades in the tribe, in addition to biogeographical analysis in a broader Myrtaceae context (Thornhill et al., 2015) shows that Australia represents the most likely ancestral range in the family and that Neotropical genera are likely a result from a more recent event of vicariance between Australia and South America, while the distribution of *Myrtus* is attributed either to a previous wider distribution of the tribe or to an old long distance dispersion and establishment (henceforward coined LDDE) event.

1.3 Study aims

Despite recent progress in understanding relationships within Myrteae using molecular tools (e.g. Lucas et al., 2011; Snow et al., 2011; Murillo-A et al., 2012; Mazine et al., 2014; Staggemeier et al., 2015; Santos et al., 2016), available studies have focused mainly on smaller clades and still lack complete generic sampling, ultimately preventing proper examination of relationships within the tribe. Improving taxonomic and DNA sampling when building phylogenetic trees is known to solve controversial relationships in plants (e.g. APG IV, 2016). Results from such improved phylogenies are key to elucidating systematic problems and also to detect consistent evolutionary patterns as low statistically supported and unbalanced phylogenetic trees may present unreliable branching patterns, branch lengths and substitution models, all of which are ultimately misleading when estimating dates or any other subsequent analysis. Improved phylogenetic resolution in Myrteae will allow more reliable systematic, biogeographic and evolutionary hypotheses of diversity in the tribe. Therefore, the aims of this study are to:

1) Develop a well-supported and robust phylogenetic chronogram for Myrteae including all main lineages (46 out of 51 genera and all main clades within large genera).

2) Propose a biogeographical hypothesis of evolution of the tribe allowing detection of variation (shifts) in ancestral geographical ranges within a global perspective.
3) Estimate diversification rate variation to understand the evolution of heterogeneous diversity among closely related lineages.

MATERIALS AND METHODS

1.4 Taxonomic sampling

The selected sample includes a large range of lineages and geographical distributions within Myrteae. In the case of the mega-diverse genera *Myrcia s.l.* and *Eugenia s.l.*, at least one species was sampled from each informal group (soon to be recognized as formal sections, Mazine et al. 2016; Lucas et al. in revision.) in each genus, following the clade classifications of Lucas et al. (2011) for the nine *Myrcia* s.l. clades and Mazine et al. (2014) and Bünger et al. (2016) for the ten *Eugenia* s.l. clades (clades 1 to 9 and section *Speciosae*). Fieldwork was conducted in Brazil, Jamaica, Costa Rica, Dominican Republic, New Caledonia, Singapore and Malaysia to collect missing taxa for DNA extraction. Sample was supplemented from the living collection of the Royal Botanic Gardens Kew (K). Duplicate vouchers were deposited in local herbaria and in the Kew herbarium.

The final sample comprises 115 terminals representing 114 species. These include 99 species representing 46 of the 51 genera of Myrteae, 16 genera more than the previous published sample (Lucas et al., 2007). *Blepharocalyx salicifolius* was sampled twice, due to inconsistent placement in past studies (Lucas et al., 2005, 2007; Murillo-A et al., 2012; de-Carvalho, 2013). Fifteen species were chosen as outgroups based on previous phylogenetic works (Lucas et al., 2007; Biffin et al., 2010; Thornhill et al., 2015). These represent five tribes of Myrtaceae: Leptospermeae (*Leptospermum scoparium*, defined as the furthermost outgroup in all analysis), Eucalypteae (*Eucalyptus perriniana*), Metrosidereae (*Metrosideros perforata, M. stipularis* and *M. nervulosa*), Tristanieae (*Xanthomyrtus compacta* and *X. montivaga*) and Syzygieae (*Syzygium jambos, S. maire, S. gustavioides, S. buxifolium, S. paniculatum, S. amplifolium, S. muellerii* and *S. guineense*). Previous studies provide evidence that Metrosidereae, Syzygieae and Tristanieae are closely related to Myrteae (part of the BKMMST clade *sensu* Biffin et al., 2010). See Appendix 1.1 for a full list of sampled species and vouchers.

1.5 Extraction and Sequencing

DNA extraction followed the CTAB extraction protocol for long term DNA storage (Doyle and Doyle, 1987, with modifications following Lucas et al., 2007, and Staggemeier et al., 2015). Approximately 200 milligrams of leaf tissue were used for each extraction. Eight DNA regions were selected for sequencing based on their informative quality evidenced in previous Myrtaceae studies (Lucas et al., 2005, 2007; Snow et al., 2011; Murillo-A et al., 2012; Mazine et al., 2014; Staggemeier et al., 2015). These are the nuclear region ITS and seven chloroplast regions: *psbA-trnH*, *matK*, *ndhF*, *trnl-trnF*, *trnQ-rps16*, *rpl16* and *rpl32-trnL*. Sequencing was performed using traditional Sanger sequencing protocol, following Lucas et al. (2007). Information on primers and PCRs conditions are available in Appendices 1.2 and 1.3. Raw sequences were imported and assembled using Geneious (v. 9, Kearse et al., 2012). Resulting contigs were aligned separately for each

region using Muscle (Edgar, 2004) implemented in Geneious and adjusted manually. A total of 535 new sequences were generated in this study. Sequences sourced from Genbank are listed in Appendix 1.1.

1.6 Phylogenetic Analysis

The seven chloroplast regions were concatenated resulting in a matrix of 6453 base pairs, hereafter referred to as the 'cpDNA dataset'. This and the 'nuclear dataset', including only the ITS region (916 base pairs), were used to run two independent Bayesian Inference (BI) phylogenetic analysis**.** The best evolutionary model was estimated prior to phylogenetic reconstruction using jModelTest 2 (Darriba et al., 2012). Estimation resulted in a best model of GTR gamma+inv for both nuclear and cpDNA datasets. Models were then implemented in MrBayes on XSEDE V. 3.2.6 (Ronquist and Huelsenbeck, 2003) executed in Cipres and run for 15,000,000 generations using default parameters. After visual comparison between phylogenies based on nuclear and cpDNA datasets separately (see section *Phylogenetic tree analysis - Grouping and Main lineages*, p.), both nuclear and cpDNA matrices were concatenated resulting in a final matrix of 7369 base pairs, hereafter referred to as the 'combined dataset'. For this matrix, Maximum Likelihood (ML) and BI were run independently to compare topologies and node support (bootstrap vs. posterior probabilities, respectively). For the ML analysis, the final concatenated alignment (available as Supplementary Material in the online article) was converted into a simplified Nexus file in Mesquite v3.04 (Maddison and Maddison, 2015) and sourced as input to RAxML-HPC2 (Stamatakis, 2014) analysis implemented in Cipres (Miller et al., 2010). Outputs of all phylogenetic analysis were read using Figtree v1.4.2 (Rambaut, 2014).

1.7 Fossil calibration and Dating

Dates of Myrteae diversification events are controversial. Myrtaceae and Myrteae phylogenies have been dated using fossil calibration and molecular clock approaches in at least seven previous studies (Sytsma et al., 2004; Biffin et al., 2010; Thornhill et al., 2012a, 2015; Murillo-A et al., 2016; Staggemeier et al., 2015; Berger et al., 2016 – see Appendix 1.4). Except on the occasions where studies were conducted by the same research group, most obtain different dates for similar nodes, sometimes extremely (e.g. Berger et al. [2016] date the crown node of Myrteae at 18 million years old, whilst Murillo-A et al. [2016] date the same node at 92 million years old). The differences in dates appear partially related to phylogeny sample size and balance, but distinctly dependent on the fossils selected and their position in calibration analysis. Because phylogenetic node age is key to interpretation of historical biogeography, reliable fossil selection, calibration and dating analysis is critical; it is discouraging to realise that these decisions are so subjective and open to interpretation. In dating estimation using fossil calibration the standard protocol is to place the estimate minimum date of a fossil on the stem node of a related extant monophyletic taxa in the phylogeny (Forest, 2009). A survey of the oldest fossil records with affinity to Myrteae was conducted and a relatively good fossil record was found assigned to the tribe in the literature. Many fossil descriptions tentatively link them to modern genera (see Appendix 1.5) however, in reality it is very difficult to identify individual Myrteae genera based on only a few morphological characters. For this reason, the safest approach is to choose the oldest fossil

remains confidently described as any genus in Myrteae and place them in the deepest nodes of the tribe.

The oldest fossil records of Myrteae are represented by macrofossil from the upper Cretaceous of Antarctica and represent remains of wood (*Myrceugenelloxylon antarcticus*) and leaves (*Myrciophyllum santacruzensis*) that are similar to extant *Luma* and *Myrcia* respectively (Berry, 1939; Poole et al., 2003). Other wood and leaf fossils from the Paleocene at extreme southern latitudes show affinity in form and distribution to modern genera (e.g. Ragonese, 1980; Troncoso et al., 2002). The most popular fossil from this period used for calibration of Myrteae studies, however, is *Paleomyrtinae*, a fossil fruit with affinity to *Psidium* or *Mosiera* recorded far from any other Myrteae records, in Northern North America (Pigg et al., 1993). Recently, another Paleocene/Eocene macrofossil from the northern hemisphere was described and placed in Myrteae: *Myrtineoxylon maomingensis*, from China (Oskolski et al., 2013). This is stated to be similar to extant Australasian group genera (*sensu* Lucas et al., 2007). Macrofossils assigned to Myrteae found in Eocene deposits are also common and show similar distribution to modern Myrteae (see Appendix 1.5).

Pollen fossil in Myrteae is, contrariwise, only found in more recent, mid-late Eocene deposits. Myrtaceae pollen fossil (represented by the genus *Myrtacedeites*) was recently reviewed by Thornhill and Macphail (2012) and even though these are found in deposits as old as the Cretaceous, only one species, *M. verrucosus*, shows morphology that undoubtedly places it as Myrteae. Myrteae pollen morphology is conservative (Thornhill et al., 2012b) and in this sense, *Myrtacedeitees verrucosus* represents the most reliable fossil record for Myrteae. At least two varieties of *Myrtaceideites verrucosus* are found in late Eocene deposits of Australia, New Zealand, Patagonia and Panama, suggesting Myrteae was an already widespread and diverse group during that period. *Myrtacedeites verrucosus* is not however, found in deposits of earlier periods (Thornhill and Macphail, 2012).

An important and antagonistic reasoning arises here; pollen fossil of Myrtaceae was recently reviewed and is found to be up to 90 million years old (Thornhill and Macphail, 2012), however, the morphotype that closely matches Myrteae only appears and apparently diversifies in mid Eocene deposits. Added to the hypothesis that pollen is usually the first structure to fossilize when an angiosperm group diversifies (Sauquet et al., 2012), it appears that Myrteae had not diversified before the mid Eocene. Alternatively, if identification of the late cretaceous and Paleocene macrofossils assigned to Myrteae are correct, then Myrteae has to be older than the dates showed by fossil pollen. Furthermore, it is not possible to combine pollen and macrofossil datasets in this case, because they would be placed on similar nodes or represent paradoxal calibration (e.g. if the fossil *Myrceugenia chubutenses* is used to calibrate the stem node of *Myrceugenia* at 66mya, the oldest *Myrtacedeites verrucosus* remains cannot be used to calibrate the whole of the Neotropical Myrteae at 37mya, because the first represents a shallower node in the phylogeny than the second).The solution adopted by this study is to compare two calibration approaches using two distinct fossil sets: a macrofossil set, based on the oldest fossil remains assigned to Myrteae in the literature; and a pollen fossil set, based on different records of *Myrtacedeites verrucosus* remains. The macrofossil approach referred to as Approach A,

considered three fossil records: *Myrceugeneloxylon antarticus*, the oldest fossil in Myrteae, was placed on the crown node of Myrteae calibrating it at 66 million years ago (mya). The following fossils were placed based on their geographical distribution: the crown of the Australasian group was calibrated at 41mya, based on the minimum age estimate of *Myrtineoxylon maomingensis*, a fossil remain from China with affinity to *Octamyrtus*. *Paleomyrtineae princetonensis* from the Paleocene was used to calibrate the crown node of the *Myrtus* group+Main Neotropical Lineage clade at 56mya, given its reported affinities to modern *Psidium* and *Mosiera* and its distribution closer to extant Neotropical Myrteae.

The second approach is referred to as Approach B and considers three distinct records of *Myrtacedeites verrucosus* (revised by Thornhill and Macphail, 2012) and additional secondary calibration points. The placement of the three remains of *M. verrucosus* was geographically based, following a similar protocol to that of Thornhill et al. (2012a). The oldest record of the pollen in the Neotropics (*Myrtacedeites verrucosus* from the mid-Eocene of Panama and Argentina) was placed on the crown node of the *Myrtus* group+Main Neotropical Lineage clade, calibrating it at 37mya. The oldest *Myrtacedeites verrucosus* recorded for Australia was placed on the crown node of the Australasian group, calibrating it at 35 mya. Finally, *Myrtacedeites verrucosus* remains found in New Zealand from 23mya was used to calibrate the crown node of the *Myrteola* group, the only clade currently found in New Zealand (Lucas et al., 2007, this study). Secondary calibration points from the broader Myrtaceae analysis of Thornhill et al. (2012a, 2015) were used to calibrate the crown of Myrteae at 41mya and the crown of the BKMMST clade (Myrteae + sister tribes, sensu Biffin et al., 2010) at 66 mya. In both approaches A and B, the root of the family was constrained to be no older than 85 mya (following Berger et al. 2016). A summary of the calibration points used and the rate parameters applied in Beast are summarized in Table 1.1. Both approaches A and B were used to produce dated phylogenies using a lognormal relaxed clock set for Birth-Death speciation and 50,000,000 generations in BEAST v.1.8.3. (Drummond et al., 2012). Two analyses were run for each approach, results were checked for convergence in Tracer v1.6.0 (Rambaut et al., 2013), burnin was selected as 0.1% of total trees and final chronograms (dated phylogenies) were visualised in Figtree v1.4.2 (Rambaut, 2014).

1.8 Historical Biogeography Inference

BioGeoBEARS (Matzke, 2013) implemented in R (R Core Team, 2016) was used to analyze ancestral geographical range variation over resulting chronograms (Approaches A and B). BioGeoBEARS allows implementation of a third free parameter “j” (founder event/jump speciation) that permits a daughter lineage to have a different area from the direct ancestor a feature that improves the log likelihood of resulting inferences of ancestral areas in comparison to a model with only two free parameters (e.g. dispersion/extinction only in Lagrange; Ree and Smith, 2008). BioGeoBEARS does not work well when many possible ancestral areas are implemented unless the maximum number of areas any species may occupy is reduced. Range area per terminal in the phylogeny was therefore coded in relation to species distributions, not genera. In this way, most terminals are restricted to single area. Area coding aimed to consider the current distribution of the group and historical geology and tectonics. The seven areas chosen were: (A) South America, (B) Central+North America (including the greater Antilles in the Caribbean), (C) Australia and New

Guinea (referred to as Australia+NG), (D) New Caledonia and New Zealand (referred to as NCNZ, representing the Zealandia plate; Trewick et al., 2007), (E) Africa (here including Madagascar), (F) Mediterranean Europe and (G) Southeast Asia (referred to as SEAsia). Distribution ranges, time slice matrices and values of area adjacency through time are available in Appendix 1.6.

Approach A: Macrofossil	Node	Age (in million years ago)	Rate
Myrceugenelloxylon antarcticus	Myrteae crown	66 (late-Cretaceous)	Lognormal
Myrtineoxylon maomingensis	Australasian group crown	40 (Mid-Eocene)	Lognormal
Paleomyrtinae princetonensis	Neotropical lineage crown	56 (late-Palaeocene)	Lognormal
Approach B – Pollen fossil			
Secondary calibration point – Thornhill et al. (2012)	Crown BKMST	63.1 (early-Paleocene)	Normal
Secondary calibration point – Thornhill et al. (2012)	Crown Myrteae	41 (early-Eocene)	Normal
Myrtaceideites verrucosus (Panama, Argentina)	Neotropical lineage crown	37.2 (late-Eocene)	Lognormal
Myrtaceideites verrucosus (Australia)	Australasian group crown	35 (late-Eocene)	Lognormal
Myrtaceideites verrucosus (New Zealand)	*Myrteola* group crown	23 (late-Oligocene)	Lognormal
Both approaches:			
Secondary calibration point – Berger et al. (2016)	Myrtaceae crown	85 (Cretaceous)	Normal

Table 1.1: Summary of two fossil sets and secondary calibration points selected to estimate diversification rates in Myrteae. Rate (normal or lognormal) is based on Beast parameters. For fossil reference see Supplementary Material 5.

1.9 Diversification Rates Analysis

Configuration shifts in diversification rates were calculated using speciation/extinction model type analysis in BAMM (Rabosky et al., 2014). BAMM works with incomplete phylogenetic datasets and allows a certain degree of phylogenetic uncertainty (see BAMM documentation). Missing taxa per tip or clade in the phylogenetic tree was estimated using previously published works (Wilson et al., 2005; Wilson, 2011; Lucas et al., 2007, 2011; Mazine et al., 2014; Staggemeier et al., 2015; Santos et al., 2016; WCSP, 2016). In the largest genera, *Myrcia* s.l. and *Eugenia s.l.*, the numbers of species per clade was estimated by specific studies (Mazine et al., 2014) and unpublished data (Lucas et al., in revision; Faria, 2014; Bünger, 2015). Priors for the BAMM control file were generated using the dated phylogenetic tree input into the function *setBAMMpriors* in the package *BAMMtools* v2.5.2 implemented in R (R Core Team, 2016), estimating 2500 species in Myrteae. The control file was set for 100,000,000 generations and the analysis was run twice as

recommended (see BAMM documentation), giving similar results. Resultant MCMC Log likelihoods were tested against generation number for convergence using the *coda* package implemented in R (R Core Team, 2016). All other outputs contained in the "*event_data*" file were analysed using *BAMMtools* in R. A recent paper casted doubt in the reliability of results produced by BAMM (Moore et al., 2016), but the criticism concerning the priors used by the software were adjusted in the latest version (see BAMM documentation). Other problems cited by that study can be applied to most macroevolutionary methods (e.g. estimation of extinct clades) and in this sense BAMM was not considered better or worse than similar softwares. Priors and proportion of samples per clade are given in Appendix 1.7.

RESULTS

1.10 Phylogenetic tree analysis - Grouping and Main lineages

Phylogenetic analysis shows Myrteae to be a coherent, well defined group with >0.95 posterior probability and 100% bootstrap support in cpDNA, nuclear and combined datasets analyses (node A, Fig. 1.2, Appendices 1.8 and 1.9). The next deepest node in the tribe's phylogeny (node B, Fig. 1.2) is poorly supported by all datasets while the two following nodes (nodes C and D, Fig. 1.2) are recovered with strong posterior probability (>0.95) and high bootstrap support (>70) in the combined and cpDNA datasets. Four lineages result from divergences at these four nodes (A, B, C and D). One of them represents a single, ungrouped monotypic genus (*Myrtastrum*) and the other three are here informally coined: the Australasian group, the *Myrtus* group and the Main Neotropical Lineage (colour coded in Fig. 1.2 as orange, blue and green respectively).

The backbone of the Main Neotropical Lineage is poorly supported in all dataset analyses, but eight major clades with high bootstrap (>70) and/or posterior probability (>0.95) supports are recovered in the combined dataset and here informally named: the *Eugenia*, *Pimenta*, *Myrteola*, *Myrceugenia*, *Myrcia*, *Plinia*, *Blepharocalyx* and *Psidium* groups. These eight clades are also recognized with similar representing taxa and support in the cpDNA dataset analysis (Appendix 1.8). The nuclear dataset analysis presents poor support for most of the deepest nodes in the phylogeny and is mostly non-informative to analyse relationship between and within these clades. The relationship between *Plinia* sp1 as sister to *Myrrhinium atropurpureum* is the only strongly supported arrangement in the nuclear dataset analysis that differs from the cpDNA and combined datasets (Appendix 1.9). In the next sections, relationships within each of the ten clades (the eight clades within the Main Neotropical Lineage plus *Myrtus* and Australasian groups) and two ungrouped genera (*Myrtastrum* and *Amomyrtus*) are discussed based on the combined dataset (Fig. 1.2). Diversity estimates per clade are taken from WCSP (2016) and Wilson (2011).

1.11 The Australasian group

The Australasian group (in orange, Fig. 1.2) has similar configuration to the informal Australasian group *sensu* Lucas et al. (2007). It is positioned as sister to the *Myrtus* group+Main Neotropical lineage clade and includes species within the genera *Gossia*, *Uromyrtus*, *Rhodamnia*, *Austromyrtus*, *Decaspermum*, *Octamyrtus*, *Rhodomyrtus*, *Kanakomyrtus*, *Pilidiostigma* and *Archirhodomyrtus*. This lineage comprises genera restrictedly distributed in Southeast Asia, Australia and Pacific islands (Fig. 1.3A) and an estimated c. 250 accepted species. Supports both

from ML and BI analysis are high (>70 bootstrap and/or 0.95 posterior probability) for most internal nodes in the clade, except for the positions of *Austromyrtus*.

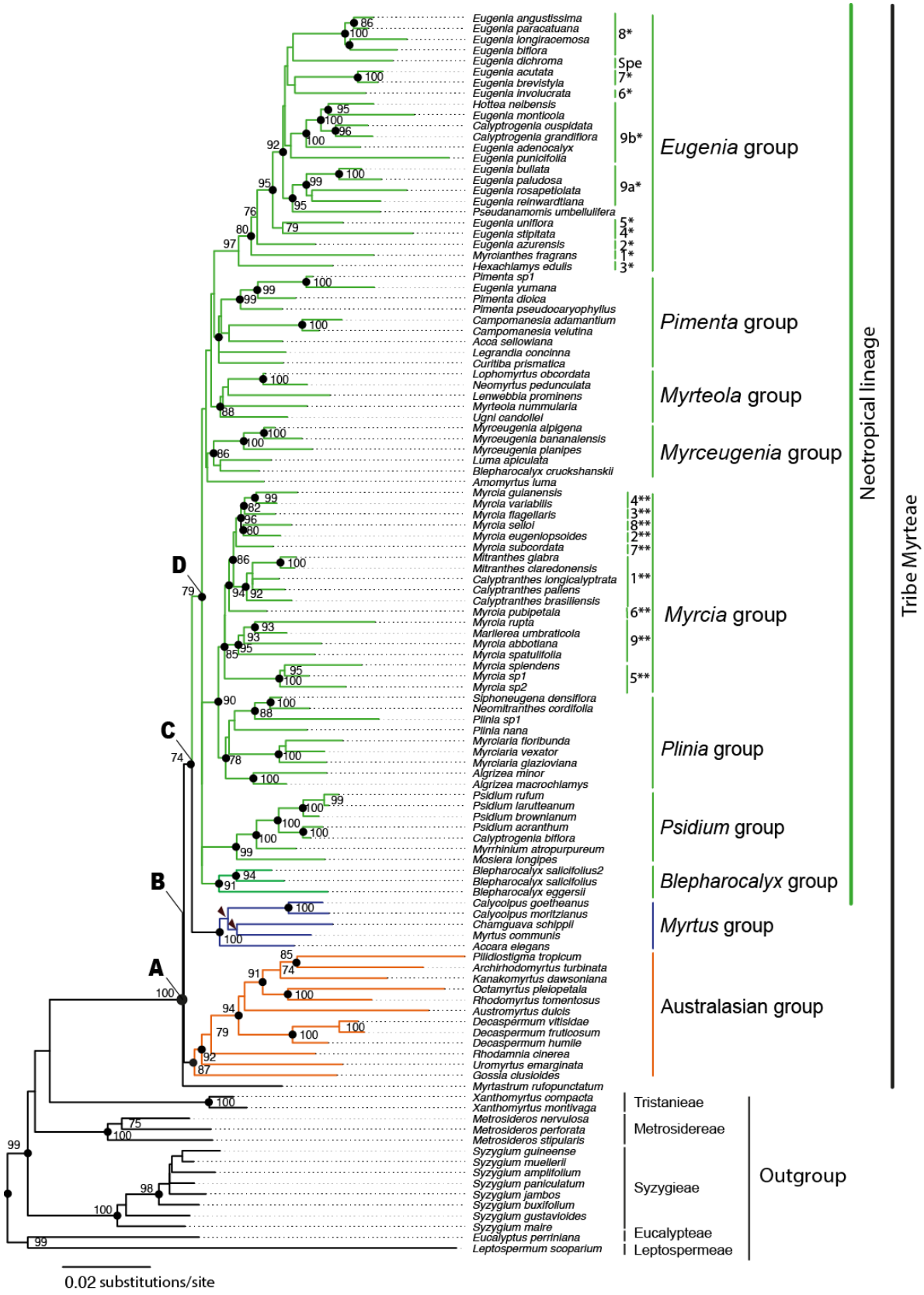


Figure 1.2 (previous page): Myrteae ML phylogenetic tree resulting from the combined dataset analysis. Bootstrap percentages greater than 50 are shown above branches; clades receiving posterior probabilities greater than 0.95 in equivalent BI analysis are indicated by black circles. Arrows indicate clades that were not recovered in BI analysis. *Clade numbers *sensu* Mazine et al. (2014). **Clade numbers *sensu* Lucas et al. (2011). 'Spe': section Speciosae sensu Bünger et al. (2016).

1.12 The *Myrtus* group

The *Myrtus* group (in blue, Fig. 1.2) contains the only European genus *Myrtus* and three Neotropical genera: *Accara*, *Chamguava* and *Calycolpus*. This group is recovered in all molecular dataset analyses, although relationships within the group vary slightly depending on the dataset under examination and the type of phylogenetic analysis (ML or BI). The main distinction is the placement of *Accara* and *Myrtus* that swap positions between sister to the rest of the group or to *Chamguava*. The two species of *Calycolpus* always appear as a strong supported group. Based on these results, *Myrtus* group present a peculiar discontinuous distribution throughout Mediterranean and Neotropical areas (Fig. 1.3B) and an estimated diversity of c. 20 species.

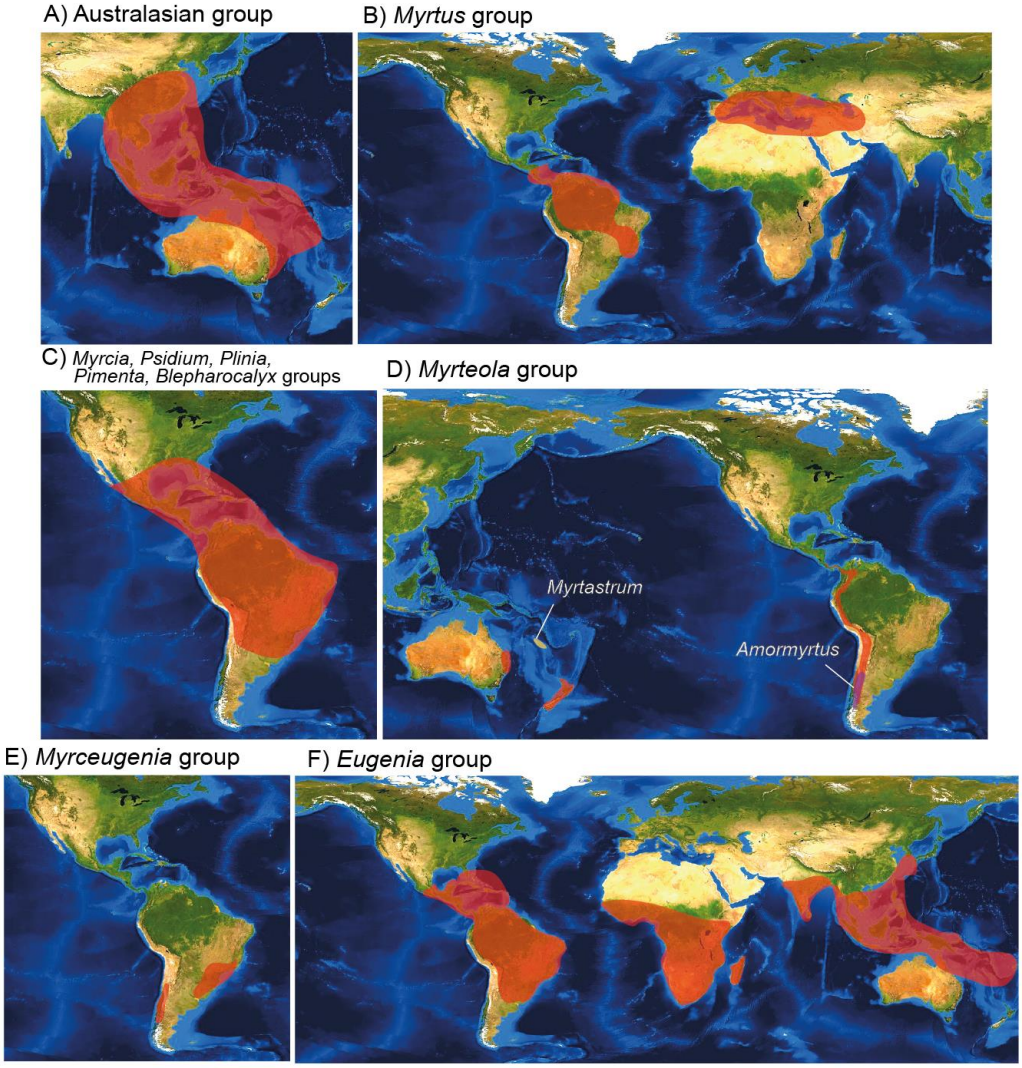


Figure 1.3: Global species distribution of Myrteae, as sourced from the WCSP (2016).

1.13 Main Neotropical lineage

The Main Neotropical Lineage (in green, Fig. 1.2) presents eight well supported (PP >0.95, BS >70) clades: the *Blepharocalyx*, *Psidium*, *Pimenta*, *Myrteola*, *Myrceugenia*, *Plinia, Myrcia*, *Eugenia* groups. The latter five are very similar to the circumscription of Lucas et al. (2007). With the exception of the consistently well supported relationship between the *Plinia* and *Myrcia* groups, the relationship between these groups is poorly resolved within the Neotropical lineage. The *Blepharocalyx* group is endemic to the Neotropics (Fig. 1.3C) and includes *Blepharocalyx salicifolius* and *B. eggersii*. *Blepharocalyx* is a genus of only four accepted species and future additions to the phylogeny may also place *Blepharocalyx myriophyllus* (the only unsampled *Blepharocalyx* species in this study) in this group increasing diversity to three accepted species. Currently accepted *Blepharocalyx cruckshanksii* is nested in the *Myrceugenia* group. The *Psidium* group includes the genera *Mosiera*, *Myrrhynium*, *Psidium* and at least one species of the polyphyletic *Calyptrogenia* (*C. biflora*).

The *Pimenta* group includes the genera *Curitiba*, *Acca* (*A. sellowiana*), *Campomanesia*, *Legrandia*, *Pimenta* and at least one species of *Eugenia* (*Eugenia yumana*), nested within *Pimenta*. Taken in this sense, the group is endemic to the Neotropics (Fig. 1.3C) and includes an estimated c. 50 species. The *Myrteola* group includes the genera *Lophomyrtus*, *Neomyrtus*, *Myrteola*, *Ugni* and *Lenwebbia*, and contains c. 15 species. This group presents an atypical geographical distribution within the tribe, with two genera (*Ugni* and *Myrteola*) endemic to Patagonia and the alpine biomes of South and Central America, one genus endemic to Australia (*Lenwebbia*) and two genera endemic to New Zealand (*Neomyrtus* and *Lophomyrtus*) (Fig. 1.3D). The *Myrceugenia* group includes the genera *Luma*, *Myrceugenia* and one species of the polyphyletic *Blepharocalyx* (*B. cruckshanksii*); an estimated c. 50 species are assigned here. This group presents a somewhat restricted distribution to sub-temperate and subtropical biomes of South America, mainly Chile and Southern Brazil (Fig. 1.3E). The *Plinia* group includes the genera *Plinia* (emerging paraphyletic), *Algrizea*, *Myrciaria*, *Siphoneugena* and *Neomitranthes* and an estimated diversity of c. 120 species. The *Myrcia* group includes four genera: *Mitranthes, Myrcia*, *Marlierea* and *Calyptranthes.* This group is estimated to include around 700 species. Both *Plinia* and *Myrcia* groups are endemic to the Neotropics (Fig. 1.3C). The *Eugenia* group includes the genera *Myrcianthes, Hottea, Pseudanamomis*, and *Calyptrogenia*. Clade 9 (sensu Mazine et al., 2014) appears polyphyletic in our analysis with all old world species (including *Eugenia roseopetiolata*, *E. reinwardtiana*, *E. bullata* and *E. paludosa*, here defined as clade 9a) appearing monophyletic in an unrelated, well supported clade. The *Eugenia* group is the most diverse and widespread group in Myrteae, with around 1000 species and a pantropical distribution (Fig. 1.3F).

1.14 Ungrouped genera: *Myrtastrum* and *Amomyrtus*

Two genera, *Myrtastrum* and *Amomyrtus*, appear ungrouped in the combined dataset. *Myrtastrum*, a monotypic genus endemic to New Caledonia (shown in orange, Fig. 1.3D), appears either isolated as sister to all extant Myrteae in the combined and nuclear datasets, or as sister to *Myrtus* group+Main Neotropical lineage, in the cpDNA dataset analysis. *Amomyrtus*, a genus of two species endemic to Patagonia (shown in purple, Fig. 1.3D), appears as sister to *Myrceugenia* group in both the cpDNA and combined dataset, though this relationship presents a poor support

in the latter. This relationship is not supported by the nuclear dataset, where it appears as sister to *Legrandia,* again with a low support.

1.15 Dating inference

Figure 1.4 contrasts results from calibration using the two fossil datasets (approaches A and B). Relationships between the *Eugenia*, *Pimenta* and *Myrteola* groups receive high statistical support (PP >0.95) in the chronograms compared to the lower support returned from the ML and BI analysis. Other aspects of the topology, including outgroup relationships, show discreet differences between chronograms where node support is low.

Because the macrofossil ages are older, approach A returns older dates for all nodes within Myrteae. In this analysis, the stem node of Myrteae (Fig. 1.4A"a") is estimated as being from the late-Cretaceous (80.72 mya) and the crown node (Fig. 1.4A"b") from the Cretaceous-Paleocene boundary (KT boundary, 65.55 mya). Approach A also suggests that the three major clades within Myrteae (the Australasian group, *Myrtus* group and the Main Neotropical Lineage) split soon after initial Myrteae diversification, in the Paleocene and early-Eocene, between 63 mya and 53 mya (highlighted in Fig. 1.4A). The diversification of all major clades within the Main Neotropical Lineage are estimated in this analysis to have taken place in the Eocene, between 52 and 39 mya. The oldest crown nodes in this analysis are: the Australasian group (59.05 mya), the *Eugenia* group (44.42 mya) and the *Pimenta* group (44.41 mya). The youngest crown nodes in this analysis are: the *Plinia* group (39.61 mya), the *Myrcia* group (39.19 mya) and the *Psidium* group (39.12 mya).

	Approach A (Macrofossil) Age (95% HPD) in million of years		**Approach B (Microfossil) Age (95% HPD) in million of years**	
Clade	**Stem**	**Crown**	**Stem**	**Crown**
Myrteae	80.72 (76.64 – 84.27)	65.55 (65.03 – 66.80)	58.96 (53.00 – 64.07)	40.76 (40.03 – 42.76)
Australasian Lineage (Australasian group)	63.73 (59.25 – 66.24)	59.05 (52.80 – 63.96)	40.09 (38.01 – 42.22)	36.88 (34.16 – 39.62)
Myrtus group	57.09 (55.06 – 61.68)	42.34 (33.20 – 51.04)	37.56 (36.27 – 39.73)	27.78 (21.80 – 33.60)
Psidium group	52.03 (46.33 – 57.60)	39.12 (30.75 – 47.47)	35.01 (32.34 – 37.70)	25.62 (20.14 – 31.07)
Blepharocalyx group	52.03 (46.33 – 57.60)	40.15 (28.49 – 49.95)	35.36 (32.80 – 38.03)	26.38 (19.64 – 32.90)
Myrcia group	42.85 (36.57 – 48.76)	39.19 (33.04 – 45.17)	27.99 (23.83 – 31.98)	25.58 (21.32 – 29.73)
Myrceugenia group	49.00 (41.84 – 55.34)	41.40 (31.72 – 49.42)	32.32 (27.85 – 35.86)	27.33 (20.83 – 32.62)
Plinia group	42.85 (36.57 – 48.76)	39.61 (33.35 – 46.00)	27.99 (23.83 – 31.98)	25.86 (21.66 – 29.93)
Eugenia group	48.36 (44.01 – 53.22)	44.42 (39.58 – 49.17)	31.93 (29.16 – 34.63)	29.29 (26.55 – 32.29)

Table 1.2: Median age estimations and 95% confidence intervals (CI) for dates of the main Myrteae nodes based on BEAST analysis.

Myrteae pollen fossil is younger than the macrofossils and consequently ages estimated from this fossil set (approach B, Fig. 1.4B) are younger than those from approach A. In this approach, the stem node of Myrteae (Fig. 1.4B"a") is estimated from the late-Paleocene (58.96 mya) and the crown node (Fig. 1.4B"b") dates to the mid-late Eocene (40.76 mya), around 25 mya younger than the same nodes in approach A. In approach B the three major clades within Myrteae (Australasian and *Myrtus* groups and the Main Neotropical Lineage) again split immediately after initial Myrteae diversification (highlighted in Fig. 1.4B) but these events are estimated to have occurred between 40 mya and 35 mya, in the late Eocene. In this approach the diversification of all major clades within the Main Neotropical Lineage are estimated to have taken place between the late-Eocene and Oligocene. The oldest and youngest crown nodes in this analysis are similar to approach A but between 15 mya and 20 mya younger. The oldest groups in this analysis are: the Australasian group (36.88 mya), the *Pimenta* group (29.40 mya) and the *Eugenia* group (29.29 mya). The youngest crown nodes in this analysis are: the *Psidium* group (25.62 mya), the *Myrcia* group (25.58 mya) and the *Myrteola* group (23.39 mya). Median age estimates and 95% confidence intervals (CI) for diversification dates of the main nodes of both analysis are plotted and contrasted in Table 1.2.

1.16 Biogeographical patterns

BioGeoBEARS was applied to chronograms resulting from both calibration approaches (Fig. 1.5). In each case results indicate a higher value of log likelihood for three parameters (DEC+j, LnL= −156.72 and LnL = −161.48 for approaches A and B respectively) in comparison to two parameters (DEC, LnL= −202.75 and LnL= −207.92 for approaches A and B respectively) showing jump speciation (i.e. dispersal between non-adjacent areas) as an important pattern in range variation of Myrteae. The most probable ancestral areas for the stem and crown nodes of Myrteae (Fig. 1.5 "a", "b" respectively) is NCNZ in both analyses. In the Australasian group the ancestral range of the crown node also has high probability of being NCNZ in both dating approaches but subsequent nodes show multiple shifts from NCNZ to Australia+NG and SEAsia and back to NCNZ. These shifts are estimated to date from the Eocene-Oligocene (shifts 2-7, Fig. 1.5A) in approach A and from the Oligocene to late Miocene (shifts 2-7, Fig. 1.5B) in approach B. The clade composed of the *Myrtus* group + Main Neotropical Lineage share a most likely ancestral area of South America for both approaches shifting from a previous NCNZ range (shift 1, Fig. 1.5) during the Paleocene (approach A) or the late-Eocene (approach B). The estimate of ancestral range for the stem and crown node of the *Myrtus* group presents an important difference between approaches A and B. In approach A an early South American range shifts to Central+North America range during the late Paleocene (shift 8, Fig. 1.5A) influenced by the distribution of *Chamguava* on the latter tectonic plate. This then shifts to the Mediterranean during the mid-Eocene for *Myrtus* (shift 9, Fig. 1.5A) and to South America for *Calycolpus* and *Accara* in the late-Eocene to early-Oligocene (shifts 10 and 11, Fig. 1.5A). In dating approach B, the crown node of the *Myrtus* group presents high probability of ancestral range in South America, shifting from there to the Mediterranean area during the late Oligocene for *Myrtus* (shift 8, Fig. 1.5B) and to Central+North America in the early Miocene for *Chamguava* (shift 9, Fig. 1.5B).

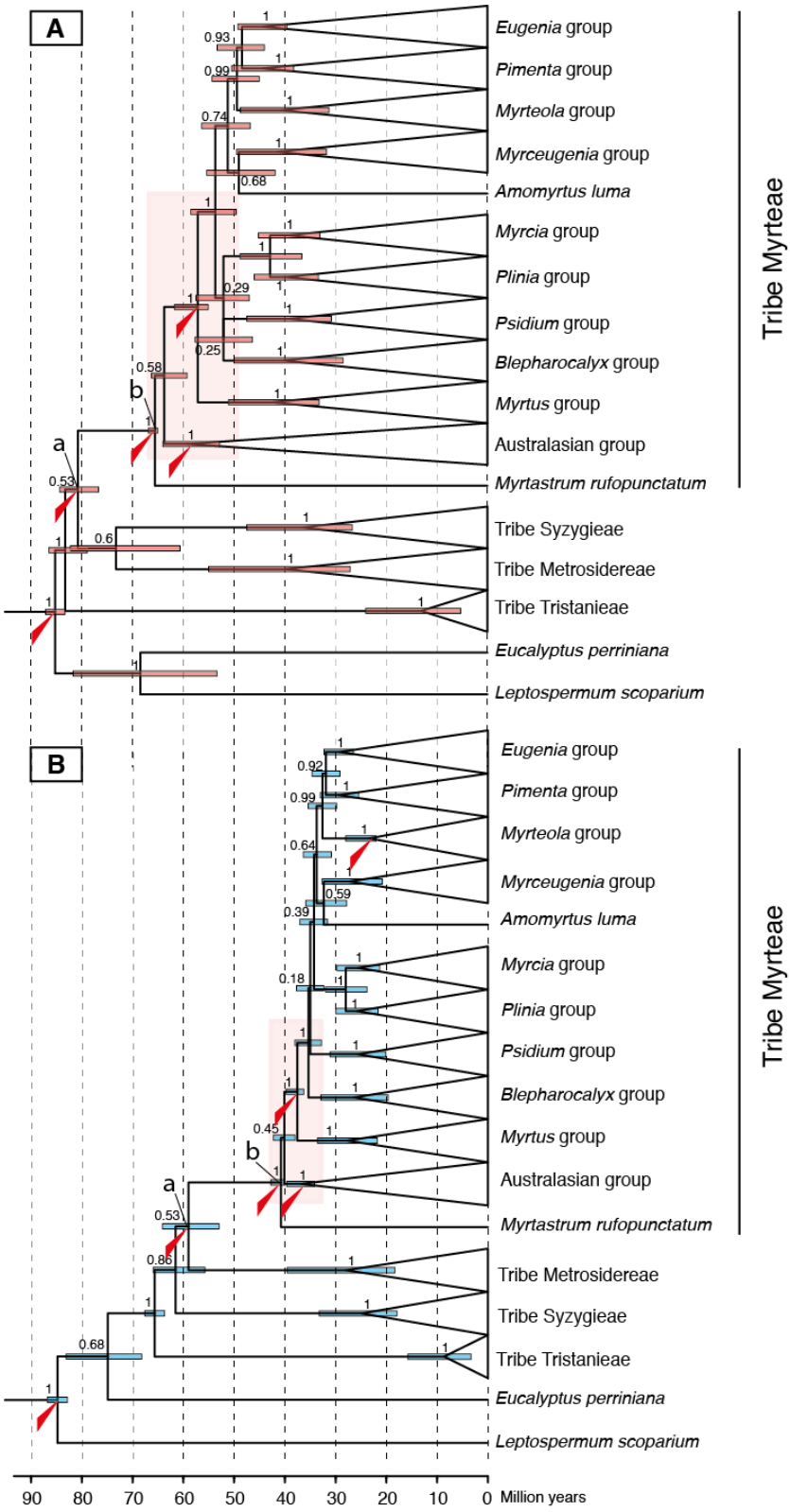


Figure 1.4: Comparative dating analysis in Myrteae generated by Beast and based on two distinct fossil sets. (A) Calibration using macrofossil dataset (approach A). (B) Calibration using microfossil dataset (approach B). “a” and “b” indicate Myrteae stem and crown nodes respectively. Highlighted areas show divergence between the three major clades (Australasian and *Myrtus* groups and the Main Neotropical lineage) in each calibration. Fossil placements used to calibrate each chronogram are marked with red arrows and refer to estimations presented in Table 1.1.

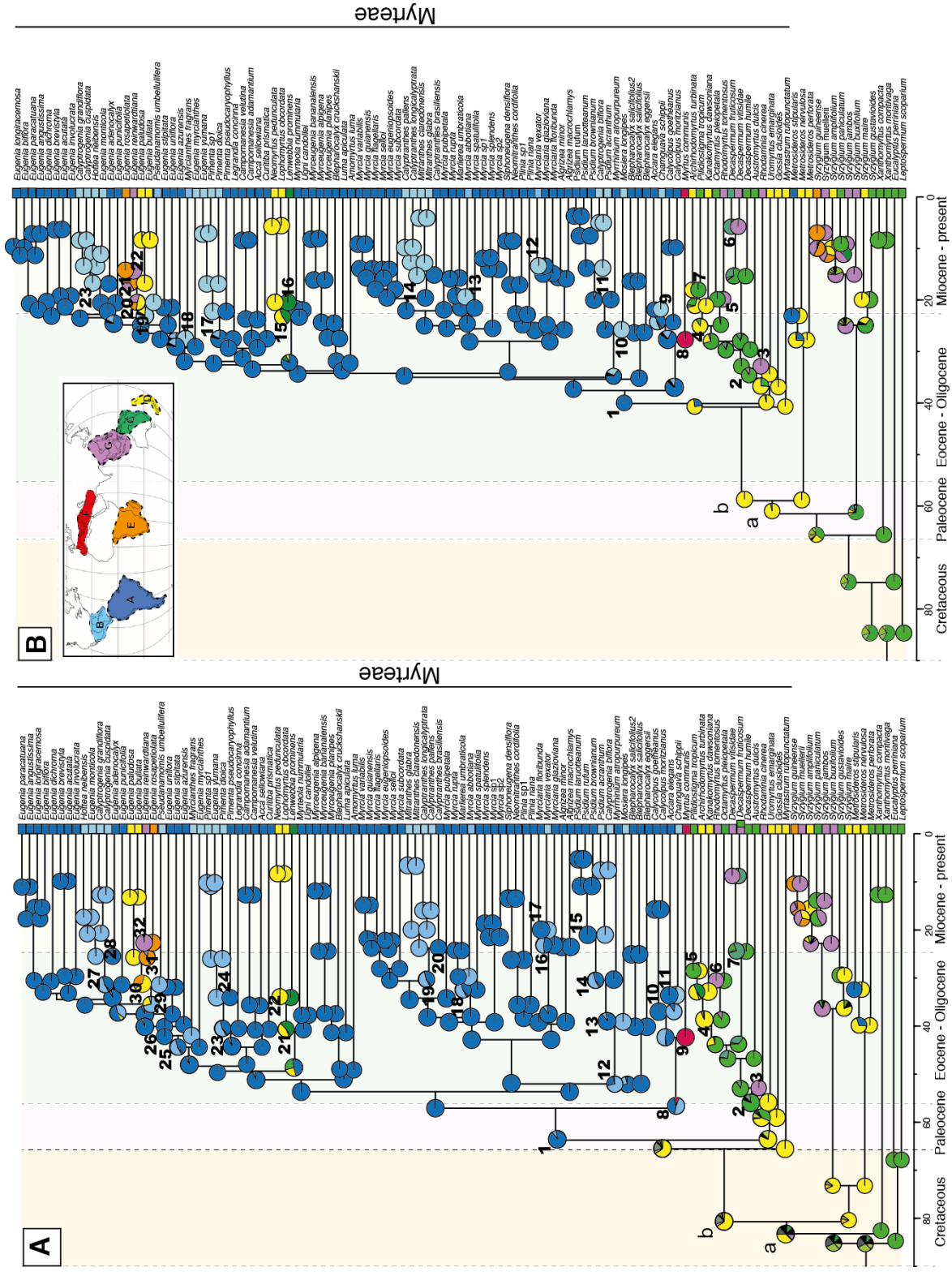


Figure 1.5: Biogeographic inference recovered from BioGeoBEARS analysis in phylogenies dated with (A) Macrofossil dataset (j=0.0574; LnL=−156.72), and (B) pollen fossil data set (j=0.055; LnL=−161.48). “a” and “b” represent Myrteae stem and crown node respectively. Range shifts are numerated above pie charts.

In the Main Neotropical Lineage the most likely areas of ancestral range for both Approaches A and B is South America. In approach A, nine shifts from South to Central+North America (shifts 12, 14, 16, 18, 19, 23, 25, 27, 29, Fig. 1.5A) and seven shifts back to South America (shifts 13, 15, 16, 20, 24, 26, 28, Fig. 1.5A) are detected in this lineage. These occurred during the Eocene-Oligocene time slice and are observed in all clades with the exceptions of the *Myrceugenia* and *Myrteola* groups. In approach B, the same nine shifts from South to Central+North America are detected in the same groups (shifts 10, 11, 12, 13, 14, 17, 18, 19, 23, Fig 1.5B). In approach B however, these shifts are no older than the early Miocene and no shifts back to South America are observed. Events of dispersion from the Neotropics (areas A and B) to the region of Australia+NG and NCNZ (areas C and D) are observed in the *Myrteola* and in *Eugenia* groups. In the *Myrteola* group this event is estimated in approach A to have occurred from South America to Australia+NG in the late Eocene (in *Lenwebbia*, shift 21, Fig. 1.5A) and afterwards to NCNZ (in *Neomyrtus* + *Lophomyrtus,* shift 22, Fig. 1.5A). In approach B, the same event is estimated to have occurred in the late Oligocene and with a higher probability for the route NCNZ to Australia+NG than the other way around (shifts 15 and 16, Fig. 1.5B).

The *Eugenia* group presents a more complex series of dispersion events. In both approaches A and B, a shift from the Central+North America region to NCNZ is observed in the common ancestor of the clade containing the Australasian and African species (shift 29 in Fig. 1.5A and 20 in Fig. 1.5B). This lineage subsequently disperses to Africa+Madagascar (represented by *Eugenia rosapetiolata,* shift 30 in Fig. 1.5A and 21 in Fig. 1.5B) and to Southeast Asia (represented by *Eugenia reinwartdiana,* shift 31 in Fig. 1.5A and 22 in Fig. 1.5B). Even though the geographic sequence of events in this *Eugenia* clade is the same, the estimated date for these dispersion events in approach A is the late Oligocene, while in approach B it is at least 10 million years later, in the Miocene.

1.17 Diversification Rate Shifts

Number of configuration shifts and log likelihood were higher than 1000 (significantly more than the recommended minimum of 200) after burnin for all BAMM analyses. Convergence between log likelihood and number of generations was observed in analysis with both callibrations (Approach A and B). The 95% credible set of rate shift configurations sampled with BAMM included 91 distinct shift configurations for approach A and 73 for approach B, of which the configurations with the highest probability included two or three shifts for both approaches. Posterior probability for a null model (i.e. no diversification rate shifts) was lower than could be estimated in both cases, therefore a Bayes factor was not calculated (see BAMM documentation). Thus, diversification rate heterogeneity is clear in the dataset. Mean phylorate through time is plotted for both chronograms in Figure 1.6. In both approaches, the best configuration shift indicates three points of increasing diversification rates, all of which occur in the Main Neotropical Lineage. The highest shift configuration probability shows three shifts towards acceleration of diversification rates positioned in similar branches in the two analyses: one in the common ancestor of most extant species of *Eugenia*, (Fig. 1.6Aa, Ba), one in the crown node of *Psidium* (Fig. 1.6Ab, Bb) and one in the

common ancestor between *Plinia* and *Myrcia* groups (Fig. 1.6Ac, Bc). In approach A, shifts in the *Eugenia* and *Plinia+Myrcia* groups occurred at the mid or late-Eocene, while that in *Psidium* occurred at the Oligocene/Miocene boundary. In approach B, both shifts in the *Eugenia* and *Plinia+Myrcia* groups occurred at the Oligocene, while the one in *Psidium* dates to the mid-Miocene. Due to its younger dating estimation, approach B presents higher diversification rates through the tribe than approach A.

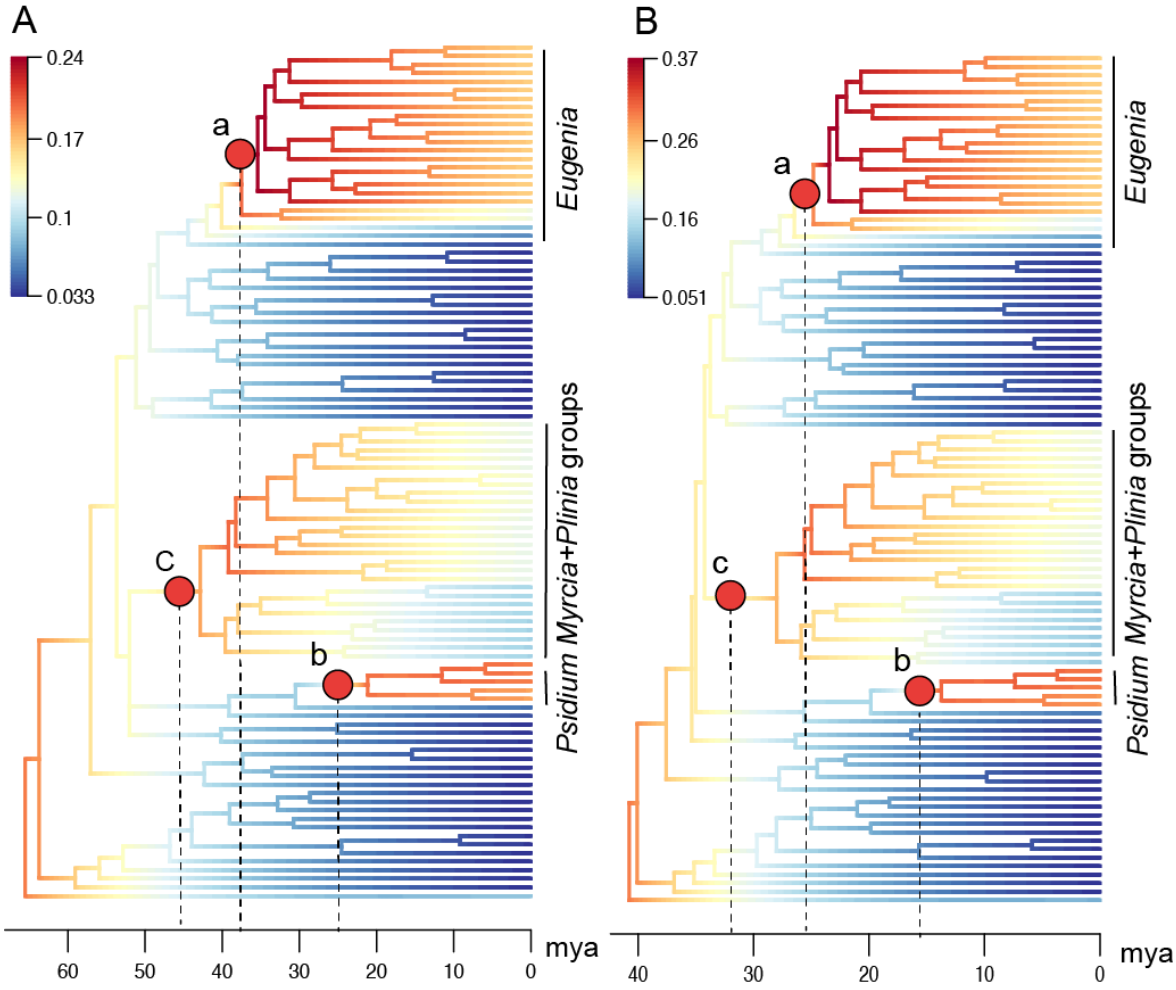


Figure 1.6: Phylorate showing the single best shift configuration recovered from BAMM in chronograms resulting from (A) macrofossil calibration and (B) pollen fossil calibration. Three accelerating shifts on diversification rates (marked by “a”, “b” and “c”) are detected in each case. Color coding (blue to red) is in scale of species per million years.

DISCUSSION

1.18 Systematic Implications

The phylogeny of Myrteae resulting from the combined dataset was reconstructed by a more informative molecular matrix and has considerably broader lineage sampling and higher statistical support in the deep nodes than those in previous works (e.g. Wilson et al., 2005; Lucas et al., 2005, 2007; Murillo-A et al., 2012; Thornhill et al., 2015) and can be used to understand the systematics, evolution and ecology of the tribe more accurately. Low support in most branches from the nuclear database makes it difficult to evaluate potential incongruence between nuclear and

cpDNA trees. There is not enough evidence to detect, for example, the role of ancient hybridization events in Myrteae history, usually noted by incongruence between these genomes (e.g. Soltis and Kuzoff, 1995). The only clear incongruence, the position of *Plinia* sp1 as sister to *Myrrhinium atropurpureum*, has to be investigated but may be an artefact of the sequencing process (e.g. contamination).

One of the main differences between this and previous phylogenetic hypotheses is the relative position of the three main lineages: the Australasian and *Myrtus* groups and the Main Neotropical Lineage. In the first phylogenetic works focused on the tribe (Lucas et al., 2005, 2007), *Myrtus communis* appeared as the sister lineage to all extant Myrteae and the Australasian clade appeared sister to the equivalent Main Neotropical Lineage clade. With this broader sample however, it is evident that *Myrtus* forms part of a predominantly Neotropical lineage. Within the Main Neotropical lineage, novel subtribal relationships are the inclusion of the *Blepharocalyx* group, formally ungrouped (Lucas et al., 2005, 2007; Murillo-A et al., 2012) or placed next to *Pimenta* (de-Carvalho, 2013) and the position of *Algrizea*, previously unplaced (Lucas et al., 2007), within *Plinia* group (also shown but not discussed in Staggemeier et al., 2015). Another novelty is the division of the former *Pimenta* group genera (sensu Lucas et al., 2007) into two groups, the *Pimenta* group and the new *Psidium* group, and one ungrouped species *Amomyrtus luma*. The placement of *Amomyrtus luma* fluctuates, but the high support of the relationship between *Amomyrtus* and the *Myrceugenia* group in the cpDNA sataset, in addition to similar geographical distribution, might mean that this genus will be treated as *Myrceugenia* group in the future. Further analysis to better place this genus within Myrteae is desirable.

Genera that will require nomenclatural adjustment include: *Hottea*, *Pseudanamomis* (both nested inside *Eugenia*), *Calyptrogenia* (polyphyletic, with species nested in *Eugenia* and *Psidium*), *Mitranthes* (nested within *Myrcia* s.l.), *Eugenia* (polyphyletic, with at least one species nested in *Pimenta*) and *Plinia* (paraphyletic). *Blepharocalyx* is known to be polyphyletic since the first molecular works in the tribe, likely requiring the resurrection of the genus *Temu* for *Blepharocalyx cruckshanksii* (see Lucas et al., 2007). *Calyptrogenia biflora* is noted to strongly resemble the continental America species *Psidium amplexicaule* Pers., but formal synonimization is required. A further important result from this phylogenetic topology is that it seems that the Caribbean, previously considered home to four endemic genera, apparently has no generic endemism in Myrteae, *as Hottea, Calyptrogenia, Mitranthes, and Pseudanamomis* are all nested inside larger widespread genera.

Of the five here unsampled, accepted genera in Myrteae (based on Wilson, 2011), *Meteroromyrtus* has recently been shown to be nested in *Eugenia* (Wilson and Heslewood, 2016). The remaining four (*Myrtella* from New Guinea, Andean *Amomyrtella*, *Lithomyrtus* from Australia and *Stereocaryum* from New Caledonia) are still to be placed. These four genera present straight stamens in the bud, so based on this consistent morphological character it is likely that their positions will be other than within the *Myrcia*, *Plinia* or *Blepharocalyx* groups, in which stamens are consistently incurved (Vasconcelos et al., 2015 [Chapter 2 in this study]). These results, in addition to the already proven polyphyletism of the classical subtribal classification based on embryo

morphology (Lucas et al., 2007) brings consistency to the current understanding of Myrteae and its classification.

1.19 Comparative Dating analysis

Results from comparative fossil calibration show important distinctions between estimated crown node ages using different approaches. Thornhill et al. (2012a) also contrast macro and microfossil calibration in Myrtaceae, combining the two fossil sets in a third calibration analysis. The fossils selected in the study presented here however, had to be placed on the same nodes so a combined dataset was not possible. Since calibration was performed with fossils of different ages on similar nodes in each approach, the resulting date distinction is expected but it is useful to demonstrate subjectivity when choosing fossil placement and how this influences interpretation of dates. Even though dates stabilize towards shallower nodes, especially when considering confidence intervals, overlap between dates from approaches A and B is still low (see Fig. 1.7).

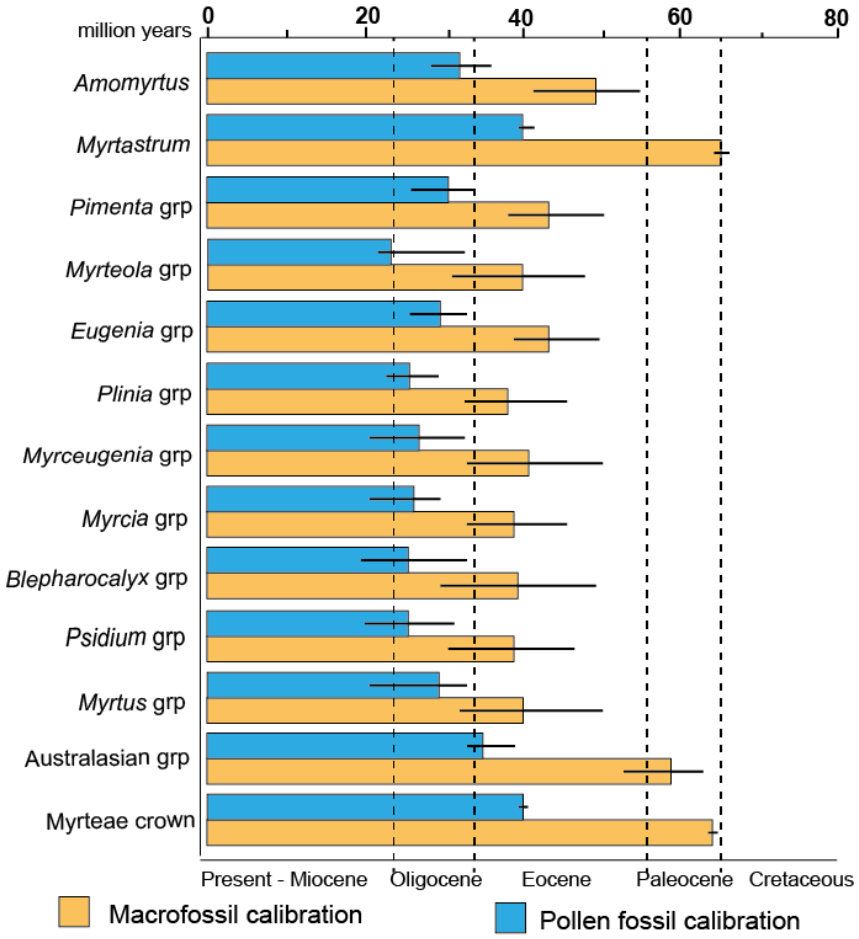


Figure 1.7: Graph comparing crown node ages of macrofossil calibration (orange) and pollen fossil calibration (blue). Bars show confidence intervals per node.

Approach A, using only macrofossil data finds estimated dates similar to Sytsma et al. (2004) and Staggemeier et al. (2015), suggesting a first event of Myrteae diversification in the Paleocene. An estimated age near the KT boundary might link increased Myrteae species diversity to increased mammal and bird diversity following dinosaur extinction (Cracraft, 2001; Penny and

Phillips, 2004). A preference of mammals and birds for fleshy berries may have provided a selective advantage over the capsular fruits of closely related tribes of Myrtaceae (Friis, 1987; Biffin et al., 2010). On the other hand, approach B finds a similar dates to Biffin et al. (2010) and Thornhill et al. (2012a), suggesting a first event of Myrteae diversification in the Eocene. In this approach, the explanation for the KT boundary above could be applied to the BKMSST clade (Myrteae and sister tribes; *sensu* Biffin et al., 2010) as this clade has other fleshy fruited Myrtaceae tribes and appears in approach B to date from the KT boundary (Thornhill et al., 2012a). In further support of approach B, the younger dates returned better explain the current distribution of Myrteae with less necessary LDDE events (see section below).

1.20 Biogeographical inference

The biogeographical analyses presented here provides a hypothesis of how Myrteae acquired its present Pantropical geographical distribution. Thornhill et al. (2015) and Berger et al. (2016) using a smaller Myrteae sample, recovered Australia as the most likely ancestral area of early diversification for Myrtaceae. The present study infers NCNZ as the ancestral range of Myrteae, with high probability in both approaches A and B (Fig. 1.5"a","b"). There is evidence, however, that large portions of Zealandia, including New Caledonia and New Zealand, were underwater between the Eocene and Oligocene (Gibbs, 2004), casting doubt on a potential NCNZ Eocene origin suggested by the more recent dates of approach B. Some hypothesis, however, indicate that other adjacent land portions of the Zealandia continent were above sea level when NCNZ was submerged; these neighbouring islands could have acted as refugia, preserving representative biodiversity in Zealandia from lineages that have since undergone extinction in other continents (e.g. Australia) even when NCNZ was submerged (e.g. Condamine et al., 2016). This pattern would explain the survival and present distribution of *Myrtastrum*, a monotypic genus endemic to New Caledonian and sister to the rest of Myrteae. Even though a possible NCNZ origin can be explained, the safest conclusion may be that Myrteae shows an eastern Gondwana ancestral area that today is represented by NCNZ and also Australia+NG. Reasons for this include the proximity of the Zealandia and Australian plate during that period (Trewick et al., 2007), the possibility that NCNZ species diversity observed today is a relict of more widespread lineages (as reasoned above) and the possibility that incomplete sampling of some deeper-node genera is biasing the analysis (*Gossia* and *Uromyrtus*, for instance are also diverse in Australia+NG [WCSP, 2016] but area coding according to species distribution influenced the reconstruction towards NCNZ).

Approaches A and B show similar area shifts (numbered in Fig. 1.5), but occurring during distinct time periods. The older age estimation of approach A causes it to present more area shifts (32 in comparison with 23 from approach B), perhaps due to area adjacencies of different time slices (see Appendix 1.6). The dating divergences between approaches also affect the number of LDDE events necessary to explain the current distribution in Myrteae (see summary in Table 1.3). Although events of LDDE are an important process in angiosperm biogeography (Crisp et al., 2011), long transmarine diversification events are considered less likely than short distance dispersion and diversification by vicariance or continental population isolation (Howe and Smallwood, 1982). The first area shift recorded in both approaches A and B is the transition from NCNZ to South America

from the stem to the crown node of the clade containing *Myrtus* group and the Main Neotropical Lineage (shift 1, Fig. 1.5A,B). LDDE is unlikely here as until around 40 mya, South America was still linked to portions of eastern Gondwana, forming a single continent connected by Antarctica (McLoughlin, 2001). It is possible that, after initial diversification in eastern Gondwana, Myrteae became widespread throughout Antarctica and South America; there is evidence that global temperature was much warmer in the early Cenozoic (Huber et al., 1995) and that rainforest vegetation covered Antarctica until around 30 mya (Francis and Poole, 2002; Francis et al., 2008). Abundant Myrtaceae fossil records found at high latitudes in South America, southern Patagonia and nearby Antarctica (Appendix 1.5; Eklund, 2003; Hayes et al., 2006; Francis et al., 2008) also provide evidence for this hypothesis. The scenario of a widespread Myrteae throughout these continents, followed by their late-Eocene disconnection (McLoughlin, 2001) and Miocene Antarctica glaciation (Kennett et al., 1975) with consequent vicariance between the Australasian group and *Myrtus* group+Main Neotropical Lineage on distinct sides of the globe is likely in both dating scenarios.

In the Australasian group, most area shifts between SE Asia, Australia+NG and NCNZ, in both approaches, occurred in a period range where proximity between these continents did not require LDDE events. The only exception is *Rhodamnia cinerea* that shifts from Australia+NG to SE Asia (shift 3, Fig. 1.5A,B) in the Eocene to early Oligocene; this may only be explained by LDDE, given the distance between these areas in that period (McLoughlin, 2001). In both approaches A and B, there is evidence for a quick northerly vertical expansion into the whole of South America soon after initial diversification in that continent. In approach A, a series of shifts back and forth South America and Central+North America are observed occurring mostly from the early Eocene to the late Oligocene. Such area shifts, however, would require multiple LDDE events, because these two continents were too far apart during that period (McLoughlin, 2001). Similar area shifts in approach B are estimated to have occurred much more recently, mostly during the Miocene, when South and North America were closer together or connected by the Panama Isthmus (Montes et al., 2015) suggesting short distance dispersion events. The only exception is the diversification of *Myrcianthes fragrans* to the greater Antilles that would require an LDDE event in both approaches.

Table 1.3 (following page): Summary of most likely events responsible for area shifts in Myrteae based on age period and confidence intervals. LDDE events were considered when distance between areas are recorded as 0.1 or 0.5 for the time slice (see Appendix 1.6)

Shift Number	Approach A shifts (Fig 1.5A)	Area shift	Age (CI 95%)	Geological time	Likely nature of event inferred by period age
1	Neotropical stem - crown	From NCNZ to South America	63.73 (59.25 - 66.24)	early-Paleocene	Land migration and vicariance
2	Australasian group - first shift to Australia	From NCNZ to Australia+NG	55.93 (49.52 - 61.56)	early-Eocene	Short distance dispersal and/or vicariance
3	Australasian group - *Rhodamnia*	From Australia+NG to SE Asia	52.89 (46.14 - 58.78)	early-Eocene	LDDE only
4	Australasian group - shift to Zealandia	From Australia+NG to NCNZ	43.96 (37.16 - 50.39)	mid-Eocene	Short distance dispersal and/or vicariance
5	Australasian group - second shift to Australia	From NCNZ to Australia+NG	28.64 (20.27 - 36.84)	early-Oligocene	Short distance dispersal and/or vicariance
6	Australasian group - *Rhodomyrtus*	From NCNZ to SE Asia	30.76 (22.17 - 38.85)	early-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and/or vicariance
7	Australasian group - *Decaspermum*	From Australia+NG to SE Asia	24.52 (15.79 - 33.66)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and/or vicariance
8	*Myrtus* group - North American shift	From South America to Central+North America	57.08 (55.06 - 61.68)	late-Paleocene	Short distance dispersal and/or vicariance
9	*Myrtus* group - *Myrtus*	From South America to Mediterranean area	42.34 (33.19 - 51.04)	mid-Eocene	Short distance dispersal and/or vicariance (via North America)
10	*Myrtus* group - South American shift (*Calycolpus)*	From Central+North to South America	37.37 (28.58 - 46.19)	late-Eocene	LDDE only
11	*Myrtus* group - South American shift (*Accara)*	From Central+North to South America	33.56 (24 - 42.78)	early-Oligocene	LDDE only
12	*Psidium* group - stem	From South to Central+North America	52.03 (46.33 - 57.6)	early-Eocene	LDDE, but upper CI limit also allows short distance dispersal and/or vicariance
13	*Psidium* group - first shift to South America	From Central+North to South America	39.12 (30.75 - 47.47)	mid-Eocene	LDDE only

14	*Psidium* group - Caribbean *Psidium*	From South to Central+North America	30.5 (22.7 - 38.74)	early-Oligocene	LDDE, but lower CI limit also allows short distance dispersal or vicariance
15	*Psidium* group - second shift to South America	From Central+North to South America	21.15 (14.66 - 28.9)	early-Miocene	Short distance dispersal or vicariance
16	*Plinia* group - *Myrciaria*	From South to Central+North America	23.15 (15.89 - 31.29)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal or vicariance
17	*Plinia* group - *Myrciaria*	From Central+North to South America	20.23 (12.97 - 28.33)	early-Miocene	Short distance dispersal and/or vicariance
18	*Myrcia* group - first North American shift	From South to Central+North America	32.98 (26.47 - 40.14)	early-Oligocene	LDDE only
19	*Myrcia* group - shift to South America	From Central+North to South America	30.59 (22.72 - 37.25)	early-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
20	*Myrcia* group - second North American shift	From South to Central+North America	23.79 (16.89 - 30.79)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
21	*Myrteola* group - New Zealand	From South America to NCNZ	40.64 (31.28 - 48.68)	mid-Eocene	Short distance dispersal and/or vicariance (via Antarctica)
22	*Myrteola* group - Australia	From NCNZ to Australia+NG	34.14 (23.40 - 43.89)	late-Eocene	Short distance dispersal and/or vicariance
23	*Pimenta* group - North American shift	From South to Central+North America	41.58 (34.48 - 48.24)	mid-Eocene	LDDE only
24	*Pimenta* group - *Pimenta pseudocaryophyllus*	From Central+North to South America	34.08 (26.07 - 41.98)	late-Eocene	LDDE only
25	*Eugenia* group - *Myrcianthes*	From South to Central+North America	44.42 (39.58 - 49.17)	mid-Eocene	LDDE only
26	*Eugenia* group - shift back South America	From Central+North to South America	42.01 (37.38 - 46.86)	mid-Eocene	LDDE only

27	*Eugenia* group - shift clade 9b to Caribbean area	From South to Central+North America	31.38 (26.55 - 36.41)	early-Oligocene	LDDE only
28	*Eugenia* group - shift clade 9b back to South America	From Central+North to South America	25.7 (20.33 - 30.93)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
29	*Eugenia* group - *Pseudanamomis*	From South to Central+North America	35.42 (31.02 - 39.08)	late-Eocene	LDDE only
30	*Eugenia* group - NCNZ	From Central+North America to NCNZ	31.24 (25.69 - 36.73)	early-Oligocene	LDDE only
31	*Eugenia* group - Africa	From NCNZ to Africa	25.72 (20.04 - 31.55)	late-Oligocene	LDDE only
32	*Eugenia* group - SA Asia	From Africa to SE Asia	22.75 (16.15 - 28.88)	early-Miocene	Land migration
Shift Number	**Approach B shifts (Fig 1.5B)**	**Nature and timing of tested geological event**	**Age (HPD 95% interval)**	**Geological time**	**Likely nature of event inferred by age**
1	Neotropical stem - crown	From NCNZ to South America	40.09 (38.01 - 42.21)	late-Eocene	Land migration and vicariance
2	Australasian group - first Australia shift	From NCNZ to Australia+NG	35.15 (31.99 - 38.61)	late-Eocene	Short distance dispersal and/or vicariance
3	Australasian group - *Rhodamnia*	From Australia+NG to SE Asia	33.37 (29.81 - 36.96)	early-Oligocene	LDDE only
4	Australasian group - shift to Zealandia	From Australia+NG to NCNZ	25 (21.07 - 29)	late-Oligocene	Short distance dispersal and/or vicariance
5	Australasian group - *Rhodomyrtus*	From Australia+NG to SE Asia	19.85 (14.64 - 24.64)	early-Miocene	Short distance dispersal and/or vicariance
6	Australasian group - *Decaspermum*	From Australia+NG to SE Asia	5.87 (2.75 - 9.9)	late-Miocene	Short distance dispersal and/or vicariance
7	Australasian group - *Pilidiostigma*	From NCNZ to Australia+NG	18.23 (13.35 - 23.15)	early-Miocene	LDDE, but upper CI limit also allows short distance dispersal and vicariance
8	*Myrtus* group - *Myrtus*	From South America to Mediterranean area	27.78 (21.79 - 33.60)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance (via North America)

9	*Myrtus* group - *Chamguava*	From South to Central+North America	22.03 (15.88 - 28.22)	early-Miocene	Short distance dispersal and/or vicariance
10	*Psidium* group - *Mosiera*	From South to Central+North America	25.62 (20.14 - 31.07)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
11	*Psidum* group - Caribbean *Psidium*	From South to Central+North America	13.73 (9.38 - 18.58)	mid-Miocene	Short distance dispersal and/or vicariance
12	*Plinia* group - *Myrciaria*	From South to Central+North America	13.55 (8.38 - 18.86)	mid-Miocene	Short distance dispersal and/or vicariance
13	*Myrcia* group - *Mycia abbotiana*	From South to Central+North America	19.59 (14.70 - 24.39)	early-Miocene	Short distance dispersal and/or vicariance
14	*Myrcia* group - *Calyptranthes*	From South to Central+North America	12.73 (8.27 - 17.35)	mid-Miocene	Short distance dispersal and/or vicariance
15	*Myrteola* group - Australia	From South America to Australia+NG	23.39 (22.04 - 28.02)	late-Oligocene	Land migration and vicariance (via Antarctica)
16	*Myrteola* group - New Zealand	From Australia+NG to NCNZ	20.45 (14.55 - 26.16)	early-Miocene	LDDE, but upper CI limit also allows short distance dispersal and vicariance
17	*Pimenta* group - North American shift	From South to Central+North America	22.52 (17.52 - 27.46)	early-Miocene	Short distance dispersal and/or vicariance
18	*Eugenia* group - *Myrcianthes*	From South to Central+North America	27.72 (24.83 - 30.71)	late-Oligocene	LDDE only
19	*Eugenia* group - *Pseudanamomis*	From South to Central+North America	23.44 (21.88 - 27.99)	late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
20	*Eugenia* group - NCNZ	From Central+North America to NCNZ	20.69 (17.24 - 24.1)	early-Miocene	LDDE only
21	*Eugenia* group - Africa	From NCNZ to Africa	16.87 (12.07 - 20.43)	early-Miocene	LDDE only
22	*Eugenia* group - SE Asia	From Africa to SE Asia	14.96 (10.82 - 19.06)	mid-Miocene	Land migration
23	*Eugenia* group - Clade 9b	From South to Central+North America	16.93 (13.58 - 20.36)	early-Miocene	Short distance dispersal and/or vicariance

Based on past phylogenic position and northern hemisphere distribution, past studies proposed that the current geographical range of *Myrtus* might be a relic from a much wider distribution of Myrteae (Berry, 1915; Thornhill et al., 2015). However, the highly supported sister relationship of *Myrtus* to exclusively Neotropical genera, including Central American *Chamguava*, provides evidence of vertical movement through the American continents towards the Mediterranean, perhaps by relatively short distance dispersal via what is today Greeenland and northern Europe, under a warmer paleo-climatic regime (Zachos et al., 2001). Possible evidence for this event is the presence of the *Paleomyrtineae* fossil from this period in North Dakota (Pigg et al., 1993). The diversification of the *Myrtus* group from South to Central+North America in the Paleocene as estimated by approach A (shift 8, Fig. 1.5A) is possible without LDDE events due to the Nicoya island complex, which linked present day Ecuador and Central America during that period (Dengo, 1975; Gentry, 1982). In approach B, the shift between South America to Central+North America in the stem node of the *Myrtus* group is not recovered. In this approach, the estimated shift occurs from South America straight to Mediterranean Europe (shift 8, Fig. 1.5B). Nevertheless, much later dates for this shift in this approach means that a similar route from South to Central+North America and Europe would be possible without LDDE events, because of the proximity of these continents in the Miocene. *Myrtus* genetic diversification varies however, from the east to west of its range (Migliore et al., 2011), not congruent with vertical movement through the American continent. This complex pattern requires future research.

Two clades (*Myrteola* and *Eugenia* groups) within the Main Neotropical Lineage also have representatives in Australia+NG, SE Asia and Africa, but these colonisation events likely occurred in different periods and by different processes. Antarctica remained habitable and in proximity to NCNZ and South America until the late Oligocene (Francis et al., 2008). In both approaches A and B (when considering upper confidence interval limits), the shift in ancestral area in the *Myrteola* group from South America to NCNZ and Australia+NG occurred before this bridge was severed by ice-sheet formation, suggesting the possibility of terrestrial migration or Antarctic colonization followed by vicariance, giving the *Myrteola* group a *Nothofagus*-like distribution (van Stenis, 1971; Swenson et al., 2001). Adaptations that may have allowed this group to achieve this range and survival in Antarctica until later than sister lineages even in colder climates, include their shrubby habit, winter seed dormancy (Smith-Ramirez et al., 1998) and likely frost resistant wood anatomy (Schmid and Baas, 1984), uncommon in other Myrteae (Lucas et al., 2007).

Due to stabilization of dates at the shallower nodes and considering the confidence intervals, Australasian and African *Eugenia* events of dispersion are estimated to have occurred at similar dates, around the late Oligocene-early Miocene, in both dating approaches. Considering an ancestral area of Central+North America for the clade and that Antarctica was already covered by ice-sheets and no longer habitable (Zachos et al., 1991, Ivany et al., 2006) at the Miocene, the only scenario possible to explain ***Eugenia's*** current pantropical distribution is a series of LDDE events (similar to other plant groups such as *Psychotria*, Matzke, 2013; and Simaroubaceae, Clayton et al., 2009). The picture proposed by the results of biogeographic analysis is that this event was towards the east, from the Caribbean (in *Pseudanamomis*) colonizing first NCNZ, then Africa and

lastly SE Asia, but a larger *Eugenia* sample from these regions may prove otherwise. Particular abilities of the *Eugenia* lineage that underwent long-distance dispersal, to cross marine boundaries, might explain why species of this group are also found in many islands of the Indian and Pacific oceans. Many (possibly all) South African species of *Eugenia* are cryptically dioecious, a character unrecorded for the genus out of Africa (van der Merwe et al., 2005; Vasconcelos pers. obs.; see also Chapter 3). Dioecy is linked to small green or white flowers, generalistic pollination systems and to island floras where in extreme cases, such as Hawaii, over a quarter of the species can be dioecious (Bawa, 1980). It is possible that dioecy of extant South African *Eugenia* species is a legacy of island-hopping ancestors. Further research focused on innovative reproductive characteristics necessary for such dispersal, such as co-evolution with migratory birds, seed resistance and self-compatibility (Baker, 1955) will be necessary to better understand the unique distribution patterns of this group.

1.21 Changes in diversification rates, key innovations and mega-diverse genera

This study demonstrates heterogeneity of diversification rates in Myrteae. Both dating approaches return similar results in this case: the three main accelerating shifts of diversification rates occurred in the Main Neotropical lineage. This explains why species diversity of the tribe in this continent is ten times higher than in the Old World (Lucas et al., 2007; WCSP 2016). In evolutionary biology, some of the most plausible explanations for changes in diversification rates are related to acquisition of new biological traits in the lineage (e.g. key-innovations; Donoghue, 2005). This is a reasonable hypothesis for Myrteae: differences in characters related to embryo morphology in *Myrcia, Plinia* and *Eugenia* have been proposed as adaptive advantages for these groups (Landrum, 1986; Landrum and Stevenson, 1986). The *Plinia* and *Eugenia* groups, with independent origins, present homogeneous cotyledons that have been related to seedling starch storage (Landrum, 1986) while *Myrcia* have leaf-like, well developed embryos that allow faster germination. These embryo forms are different from extant Myrteae that do not exhibit these specialisations.

The accelerating diversification rate shift in *Psidium* however, is less likely to be linked to the embryo as in this group it is similar to those found in the Australasian and *Pimenta* groups (Landrum and Stevenson, 1986). A possible explanation for the success of *Psidium* may be linked to cytogenetic events: *Psidium* is the Myrteae lineage with the highest documented cases of polyploidy (Costa et al., 2008), frequently associated with increased fitness (Wood et al., 2009; Madlung, 2013). The bony *Psidium* testa opening via an operculum (a synapomorphy of the genus) through which germination occurs (Landrum and Stevenson, 1986) may also be a factor, promoting mechanical seed dormancy conducive to success in seasonal environments. It is also notable that all invasive species of Myrteae are *Psidium* (Richardson and Rejmanek, 2011), showing adaptive features of this lineage that might be linked to its higher diversification rate.

The analysis of diversification rate shifts in Myrteae provides an unexpected result. Evidence presented here shows that species richness in the Myrcia and Plinia group lineages result from a single acceleration of diversification rates. There is a tendency to assume that taxonomic levels such as genus and species reflect different evolutionary units (reviewed by Frodin, 2004) and that lineages that are more diverse are more successful (e.g. Raikow, 1986).

Based on this, it can be assumed that the Myrcia group is more successful than the Plinia group (700 vs. 100 species respectively). Thus, interpretation of evolutionary success in each lineage depends on where the taxonomic line is drawn, emphasizing that taxonomic ranking can mislead evolutionary understanding.

CONCLUSIONS

This chapter provides an up to date phylogeny to be used as a base for further systematic and modelling studies in Myrteae. The dating, biogeography and diversification patterns analyses clarify the evolutionary picture of the most diverse tribe in Myrtaceae, but also raise a number of avenues for future studies. These include, for instance: a better resolution for the relationships in the backbone of the main Neotropical lineage; nomenclatural changes in poly and paraphyletic genera; formalization of subtribal nomenclature; detailed biogeographical analysis of individual clades; the importance of high southern latitudes in early Myrteae diversification events; and better links between acceleration shifts in diversification rates and trait evolution (see also Chapter 7). Results from the comparative dating approaches using macro and microfossil separately show how the choice of fossil set and placement interpretation affects all interpretation of subsequent evolutionary analysis. Calibration using pollen fossil evidence (approach B) requires less LDDE events to explain current Myrteae distribution. This, in addition to the reasoning provided in the *1.7 Fossil calibration and Dating* section suggests that this dating approach is more reliable and should be preferred by future studies in Myrteae.

APPENDIX

Appendix 1.1: Sample list, collection localities and Genbank accession numbers for the species used in the Myrteae phylogenetic analysis. *Accession numbers represent different vouchers from those indicated in the voucher column (see Genbank for more information). Blank spaces represent missing data in the molecular matrix.

			Molecular markers (DNA region)							
Species	**Voucher**	**Collection locality**	**ITS**	***matK***	***ndhF***	***psbA-trnH***	***rpl16***	***rpl32-trnL***	***trnL-trnF***	***trnQ-rps16***
Acca sellowiana (O.Berg) Burret	E. Lucas 205	RBG Kew (cultivated)	AM234067	AM489973		AM489807			MF954134	
Accara elegans (DC.) Landrum	T. Vasconcelos 485	Brazil (Minas Gerais)	MF954013	MF954518	MF954431	MF954271	MF954309	MF954197		
Algrizea macrochlamys (DC.) Proença & NicLugh.	A. Giulietti 1648	Brazil (Bahia)	AM234126	AM489975	MF954432	AM489809	MF954310	MF954198	JN091320	KP722283
Algrizea minor Sobral, Faria & Proença	J.E.Q. Faria 4157	Brazil (Bahia)	MF954014		MF954433	MF954272	MF954311	MF954199		MF954078
Amomyrtus luma (Molina) D.Legrand & Kausel	RBGE 1996-1065	RBG Edinburgh (cultivated)	AM234073	KM065305*	MF954434	AM489811		MF954200	MF954135	
Archirhodomyrtus turbinata (Schltr.) Burret	J. Soewarto HB 11	New Caledonia	MF954015		MF954435	MF954273	MF954312	MF954201	MF954136	MF954079
Austromyrtus dulcis (C.T.White) L.S.Sm.	S. Belsham M77	Australia (Queensland)	MF954016	AM489977	MF954436	AM489813				MF954080
Blepharocalyx cruckshanksii (Hook. & Arn.) Nied. in H.G.A.Engler & K.A.E.Prantl	RBGE 1998-073D; [a]Murillo 4219	RBG Edinburgh (cultivated)	AM234070	AM489978	MF954437	AM489814	JN660956[a]	JN661055[a]		JN661105[a]
Blepharocalyx eggersii (Kiaersk.) Landrum	T. Vasconcelos 458	Brazil (Bahia)	MF954017	MF954519	MF954438	MF954274	MF954313	MF954202	MF954137	MF954081
Blepharocalyx salicifolius (Kunth) O.Berg	E. Lucas 78	Brazil (São Paulo)	AM234084	AM489979	MF954439	AM489815	JN660984*	JN661083*	MF954138	JN661133*

Blepharocalyx salicifolius (Kunth) O.Berg	T. Vasconcelos 482	Brazil (Minas Gerais)	MF954018	MF954520	MF954440	MF954275	MF954314		MF954139	MF954082
Calycolpus goetheanus (Mart. ex DC.) O.Berg	T. Vasconcelos 332	Brazil (Amazonas)	MF954019	MF954521	MF954441	MF954276	MF954315	MF954203	MF954140	MF954083
Calycolpus moritzianus (O.Berg) Burret	(all from GenBank)	Colombia	KU945986	KU945991		KU945999				
Calyptranthes brasiliensis Spreng.	E. Lucas 930	Brazil (Espirito Santo)	MF954020		MF954443	MF954277	MF954317	MF954205		
Calyptranthes longicalyptrata B.Holst & M.L.Kawas.	T. Vasconcelos 523	Costa Rica			MF954444		MF954318		MF954142	MF954085
Calyptranthes pallens Griseb.	T. Vasconcelos 534	Costa Rica	MF954021		MF954445	MF954278	MF954319		MF954143	
Calyptrogenia biflora Alain	T. Vasconcelos 565	Dominican Republic	MF954022		MF954446	MF954279	MF954320	MF954206	MF954144	MF954086
Calyptrogenia cuspidata Alain	T. Vasconcelos 593	Dominican Republic	MF954023		MF954447	MF954280	MF954321	MF954207	MF954145	MF954087
Calyptrogenia grandiflora Burret	T. Vasconcelos 588	Dominican Republic	MF954024		MF954448	MF954281	MF954322	MF954208	MF954146	MF954088
Campomanesia adamantium (Cambess.) O.Berg	T. Vasconcelos 474	Brazil (Minas Gerais)	MF954025		MF954449	MF954282	MF954323	MF954209	MF954147	MF954089
Campomanesia velutina (Cambess.) O.Berg	T. Vasconcelos 507	Brazil (Distrito Federal)	MF954026		MF954450	MF954283	MF954324	MF954210	MF954148	MF954090

Chamguava schippii (Standl.) Landrum	D. Aguilar 9833	Costa Rica	MF954027	MF954523	MF954451	MF954284	MF954325	MF954211	MF954149	MF954091
Curitiba prismatica (D.Legrand) Salywon & Landrum	D.F. Lima 551	Brazil (Paraná)	MF954028	MF954524	MF954452	MF954285	MF954326	MF954212	MF954150	MF954092
Decaspermum fruticosum J.R.Forst. & G.Forst	T. Vasconcelos 730	Malaysia (Sabah)	MF954029		MF954453	MF954286	MF954327	MF954213		MF954093
Decaspermum humile (Sweet ex G.Don) A.J.Scott	S. Belsham M82	RGB Melbourne (cultivated)	AM234128		AY498780*	AM489824	MF954328		MF954151	
Decaspermum vitis-idaea Stapf	T. Vasconcelos 729	Malaysia (Sabah)	MF954030		MF954454	MF954287	MF954329	MF954214	MF954152	
Eucalyptus perriniana F.Muell. ex Rodway	E. Lucas 283	RBG Kew (cultivated)	AM234139	AM489985	MF954455	AM489825	MF954330	MF954215	MF954153	MF954094
Eugenia acutata Miq.	T. Vasconcelos 506	Brazil (Distrito Federal)	MF954031		MF954456	MF954288	MF954331	MF954216		MF954095
Eugenia adenocalyx DC.	A. Giaretta 1441	Brazil (Roraima)	MF954042		MF954470	MF954299	MF954342	MF954219		MF954105
Eugenia angustissima O.Berg	T. Vasconcelos 405	Brazil (Goias)	MF954032		MF954457	MF954289	MF954332	MF954217	MF954154	MF954096
Eugenia azurensis O.Berg	J.E.Q. Faria 4186	Brazil (Bahia)	MF954033		MF954458	MF954290	MF954333	MF954423	MF954155	
Eugenia biflora (L.) DC.	F.F. Mazine 1075	Brazil	KJ187610	MF954525	MF954459	KJ469659			MF954156	
Eugenia brevistyla D.Legrand	F.F. Mazine 993	Brazil	KJ187614		MF954460	KJ469663			MF954157	
Eugenia bullata Pancher ex Guillaumin	T. Vasconcelos 608	New Caledonia	MF954034		MF954461	MF954291	MF954334	MF954424	MF954158	MF954097

Eugenia bunchonsiifolia Nied.	T. Vasconcelos 466	Brazil (Espirito Santo)	MF954041		MF954469	MF954298	MF954341	MF954218		MF954104
Eugenia involucrata DC.	T. Vasconcelos 256	Brazil (Distrito Federal)	MF954035		MF954462	MF954292	MF954335	MF954425	MF954159	MF954098
Eugenia longiracemosa Kiaersk.	T. Vasconcelos 310	Brazil (Amazonas)	MF954036		MF954463	MF954293	MF954336	MF954426		MF954099
Eugenia monticola (Sw.) DC**.**	T. Vasconcelos 566	Dominican Republic	MF954037	JQ588481*	MF954464	MF954294	MF954337	MF954427	MF954160	MF954100
Eugenia myrcianthes Nied.	Savassi ESA 85681	Brazil	KJ187652	MF954526	AY498784	KJ469702	MF954346	MF954223		MF954108
Eugenia paludosa Pancher ex Brongn. & Gris	T. Vasconcelos 646	New Caledonia	MF954038		MF954465	MF954295	MF954338	MF954428	MF954161	MF954101
Eugenia paracatuana O.Berg	P.O. Rosa 1399	Brazil (Goias)	MF954039			MF954296	MF954339	MF954429		MF954102
Eugenia punicifolia (Kunth) DC.	F.F. Mazine 1065	Brazil (Mato Grosso)			MF954466	AM489827*			MF954162	
Eugenia reinwardtiana (Blume) DC.	B. Holst 8870	MSBG (cultivated)		KM894685*	MF954467		AY463131*		MF954163	
Eugenia roseopetiolata N.Snow & Cable	T. Vasconcelos s.n.	RBG Kew (cultivated)	MF954040		MF954468	MF954297	MF954340	MF954430	MF954164	MF954103
Eugenia stipitata McVaugh	T. Vasconcelos 677	Singapore BG (cultivated)	MF954043		MF954471	MF954300	MF954343	MF954220	MF954165	
Eugenia uniflora L.	E. Lucas 207	RBG Kew (cultivated)	AM234088	AM489986	MF954472	AM489828	AF215627*		KP722326	KP722202

Eugenia yumana Alain	T. Vasconcelos	Dominican Republic	MF954044		MF954473	MF954301	MF954344	MF954221	MF954166	MF954106
Gossia clusioides (Brongn. & Gris) N.Snow	J. Soewarto HB 14	New Caledonia	MF954045		MF954474	MF954302	MF954345	MF954222	MF954167	MF954107
Hottea neibensis Alain	T. Vasconcelos 590	Dominican Republic	MF954046		MF954476	MF954303	MF954347	MF954224	MF954168	MF954109
Kanakomyrtus dawsoniana N.Snow	T. Vasconcelos 639	New Caledonia	MF954047		MF954477	MF954304	MF954348	MF954225		
Legrandia concinna (Phil.) Kausel	RBGE 1999-0656	RBG Edinburgh (cultivated)	AM234072	AM489990	MF954478	AM489839				
Lenwebbia prominens N.Snow & Guymer	N. Snow 7463	Australia (Queensland)	MF954048	AY521538*		MF954305		MF954226		
Leptospermum scoparium J.R.Forst. & G.Forst.	E. Lucas 284		AM234142	AM489991	AM235423	AM489840	AM235459	MF954227	KF591267	
Lophomyrtus obcordata (Raoul) Burret	S. Belsham M41	New Zealand	AM234146	AM489993	MF954480	AM489842	MF954349	MF954228		
Luma apiculata (DC.) Burret	E. Lucas 208	RBG Kew (cultivated)	AM234101	AM489995	AY498795	AM489843	JN660959*	MF954229	KP722331	KP722209
Marlierea umbraticola (Kunth) O.Berg	M.A.D. Souza s.n.	Brazil (Amazonas)	KP722392		KP722470	KP722300	MF954350	MF954230	KP722350	KP722246
Metrosideros nervulosa C.Moore & F.Muell.	(all from GenBank)		JF950784	DQ088535	AY498802		DQ088395		JF950929	
Metrosideros perforata (J.R.Forst. & G.Forst.) Druce	E. Lucas 209	RBG Kew (cultivated)	AM234141	AM489998	MF954481	AM489848	MF954351	MF954231	MF954169	
Metrosideros stipularis (Hook. & Arn.) Hook.f.	(all from GenBank)		AM234071	AF368222		AM489884				
Mitranthes clarendonensis (Proctor) Proctor	T. Vasconcelos 511	Jamaica	MF954049		MF954482	MF954306	MF954352		MF954170	MF954110
Mitranthes glabra Proctor	E. Lucas 1224	Jamaica	MF954050		MF954483	MF954307	MF954353	MF954232	MF954171	MF954111

Mosiera longipes (O.Berg) Small	Salywon 1183	U.S.A. (Florida)	MF954051		MF954484	MF954308	MF954354	MF954251	MF954172	
Myrceugenia alpigena (DC.) Landrum	E. Lucas 167	Brazil (Minas Gerais)	AM234098	JN660991	KP722441	AM489854	JN660941.	MF954252	KP722376	JN661090
Myrceugenia bananalensis Bezerra & Landrum	J.E.Q. Faria 4049	Brazil (Distrito Federal)	MF954052		MF954485	MF954309	MF954355	MF954253	MF954173	MF954112
Myrceugenia planipes (Hook. & Arn.) O.Berg	L. Landrum s.n.	Chile	MF954053	JN661027*	MF954486	MF954310	MF954356	MF954254		
Myrcia abbotiana (Urb.) Alain	T. Vasconcelos 571	Dominican Republic	MF954054				MF954357	MF954255		
Myrcia rupta M.L.Kawas. & B.Holst	T. Vasconcelos 311	Brazil (Amazonas)	MF954055		MF954487	MF954311	MF954358	MF954256		MF954113
Myrcia eugeniopsoides (D.Legrand & Kausel) Mazine	E. Lucas 61	Brazil (Sao Paulo)	AM234107	AM489996	KP722429	AM489845	MF954359	MF954257	JN091327	KP722205
Myrcia flagellaris (D.Legrand) Sobral	E. Lucas 83	Brazil (Sao Paulo)	AM234113	AM489989	KP722430	AM489836	MF954360	MF954258	JN091350	KP722206
Myrcia guianensis (Aubl.) DC.	Harley 50307	Brazil	JN091225						JN091351	
Myrcia pubipetala Miq.	E. Lucas 86	Brazil (Sao Paulo)	AM234114	AM490001	KP722426	AM489855	MF954361	MF954259	JN091364	KP722273.
Myrcia selloi (Spreng.) N.Silveira	E. Lucas 110	Brazil	JN091240	JN091315	KP722436	JN091431	MF954363	MF954261	JN091371	KP722212
Myrcia sp2	J.E.Q. Faria 4193	Brazil (Bahia)	MF954057		MF954489	MF954313	MF954364	MF954262		
Myrcia sp1	T. Vasconcelos 307	Brazil (Amazonas)	MF954056		MF954488	MF954312	MF954362	MF954260	MF954174	MF954114
Myrcia spathulifolia Proença	J.E.Q. Faria 4214	Brazil (Bahia)	MF954058		MF954490	MF954314	MF954365	MF954263		MF954115
Myrcia splendens (Sw.) DC.	T. Vasconcelos 587	Dominican Republic	MF954059		MF954491	MF954315	MF954366	MF954264	MF954175	

Myrcia subcordata DC.	M. Santos 586	Brazil (Minas Gerais)	MF954060		MF954492	MF954316	MF954367	MF954265	MF954176	MF954116
Myrcianthes fragrans (Sw.) McVaugh	B. Holst 8862	Guyane	KJ187655	KJ772955	AY498803*	KJ469705				
Myrciaria floribunda (H.West ex Willd.) O.Berg	T. Vasconcelos 388	Brazil (Amazonas)	MF954062		MF954494	MF954318		MF954267	MF954178	MF954118
Myrciaria glazioviana (Kiaersk.) G.M.Barroso ex Sobral	T. Vasconcelos 413	Brazil (Bahia)	MF954061		MF954493	MF954317	MF954368	MF954266	MF954177	MF954117
Myrciaria vexator McVaugh	T. Vasconcelos 709	Singapore BG (cultivated)	MF954063	AY521544*	MF954495	MF954319		MF954268	MF954179	MF954119
Myrrhinium atropurpureum Schott in K.P.J.Sprengel	Costa, I.R. 594	Brazil (Rio de Janeiro)	MF954064		MF954496	MF954320		MF954269	MF954180	MF954120
Myrtastrum rufopunctatum (Pancher ex Brongn. & Gris) Burret	J. Soewarto HB 10	New Caledonia	MF954065	MF954527	MF954497	MF954321		MF954270	MF954181	MF954121
Myrteola nummularia (Lam.) O.Berg	RBGE 1996-1096	RBG Edinburgh (cultivated)	AM234068	AM490008	MF954498	AM489871		MF954419	MF954182	MF954122
Myrtus communis L.	E. Lucas 211	RBG Kew (cultivated)	AM234149	AM490009	MF954499	AM489872	JN660939*	MF954420	KP722327	KP722221
Neomitranthes cordifolia (D.Legrand) D.Legrand	Forster 1011	Brazil	AM489410			AM489569		MF954421	JN091386	MF954123
Neomyrtus pedunculata (Hook.f.) Allan	S. Belsham M42	New Zealand	AM234144	AM490010		AM490637	MF954369			
Octamyrtus pleiopetala Diels	R. Johns s.n.	New Guinea	AM234130		MF954500	AM489873	MF954370	MF954422	MF954183	
Pilidiostigma tropicum L.S.Sm.	Forster 27636	Australia (Queensland)	MF954066		MF954501	MF954322		MF954233		MF954124
Pimenta dioica (L.) Merr.	E. Lucas 212	RBG Kew (cultivated)	AM234081	AM490011	MF954502	AM489874	MF954371		MF954184	
Pimenta pseudocaryophyllus (Gomes) Landrum	E. Lucas 161	Brazil	AM234083	AM490013	MF954503	AM489876	MF954372	MF954234	MF954185	MF954125

Pimenta sp1	T. Vasconcelos 576	Dominican Republic	MF954067			MF954323	MF954373	MF954235	MF954186	MF954126
Plinia nana Sobral	F.F. Mazine 662	Brazil (Minas Gerais)	MF954068		MF954504	MF954324	MF954374	MF954236	MF954187	
Plinia sp1	B. Holst 9482	French Guiana	MF954069		MF954505	MF954325	MF954375	MF954237	MF954188	
Pseudanamomis umbellulifera (Kunth) Kausel	T. Vasconcelos 572	Dominican Republic	MF954070		MF954506	MF954326	MF954376	MF954238	MF954189	MF954127
Psidium acranthum Urb.	T. Vasconcelos 578	Dominican Republic	MF954073		MF954509	MF954329	MF954379	MF954240		MF954129
Psidium brownianum Mart. ex DC.	T. Vasconcelos 465	Brazil (Bahia)	MF954071		MF954507	MF954327	MF954377	MF954239	MF954190	MF954128
Psidium laruotteanum Cambess.	J.E.Q. Faria 2362	Brazil (Bahia)		MF954522	MF954442	MF954277	MF954316	MF954204	MF954141	MF954084
Psidium rufum Mart. ex DC.	J.E.Q. Faria 4270	Brazil (Minas Gerais)	MF954072		MF954508	MF954328	MF954378	MF9542	MF954191	
Rhodamnia cinerea Jack	T. Vasconcelos 672	Singapore	MF954074	KJ709064*	MF954510	MF954330	MF954380	MF954241	MF954192	MF954130
Rhodomyrtus tomentosa (Aiton) Hassk	T. Vasconcelos 678	Singapore BG (cultivated)	MF954075	AF105093*	MF954511	MF954331	MF954381	MF954242	MF954193	MF954131
Siphoneugena densiflora O.Berg	F.F. Mazine 1050	Brazil	AM489412		KP722444	AM489571	MF954382	MF954243	JN091389	KP722220
Syzygium amplifolium L.M.Perry	(all from GenBank)		EF026620	DQ088556	DQ088381		DQ088416			
Syzygium buxifolium Hook. & Arn.	(all from GenBank)		KP093045	KP093852	DQ088491	KJ687225	DQ088424		AB817604	

Syzygium guineense (Willd.) DC.	(all from GenBank)		EF026628	DQ088581	DQ088500		DQ088432			
Syzygium gustavioides (F.M.Bailey) B.Hyland	(all from GenBank)		AY187194	DQ088582	DQ088501		DQ088433			
Syzygium jambos (L.) Alston in H.Trimen	E. Lucas 214	RBG Kew (cultivated)	AM234135	AM490017	MF954512	AM489882	DQ088434*	MF954244	MF954194	
Syzygium muellerii (Miq.) Miq.	(all from GenBank)		EF026634	DQ088593	DQ088511		DQ088439			
Syzygium maire (A.Cunn.) Sykes & Garn.-Jones	NZFRI29089	New Zealand	KM064865	KM065310	DQ088508	AM489883	DQ088438			
Syzygium oblatum (Roxb.) Wall. ex A.M.Cowan & Cowan	(all from GenBank)		KR532632	AB924759		KR532989				
Syzygium paniculatum Gaertn.	(all from GenBank)		KM065112	KM065271	DQ088515		DQ088441			
Ugni candollei (Barnéoud) O.Berg	T. Vasconcelos s.n.	RBG Kew (cultivated)	MF954076	MF954528	MF954513	MF954332	MF954383	MF954245	MF954195	MF954132
Uromyrtus emarginata (Pancher ex Baker f.) Burret	T. Vasconcelos 628	New Caledonia	MF954077	MF954529	MF954514	MF954333	MF954384	MF954246		
Xanthomyrtus compacta (Ridl.) Diels	P. Edwards 4214A	New Guinea	AM234148		MF954515	AM489887	MF954385	MF954247	MF954196	MF954133
Xanthomyrtus montivaga A.J.Scott	E. Lucas 16	New Guinea	AM234147		MF954516	AM489886	MF954386	MF954248		

Appendix 1.2: Primers used for sequencing. (F) Forward; (R) Reverse.

DNA Regions	Primers	Sequence 5' – 3'	Reference
ITS	AB101 (F)	ACGAATTCATGGTCCGGTGAAGTGTTCG	Sun et al., 1994
	AB102 (R)	GAATTCCCCGGTTCGCTCGCCGTTAC	Sun et al., 1994
psbA-trnH	PsbA (F)	CGAAGCTCCATCTACAAATGG	Hamilton, 1999
	trnH (GUG) (R)	ACTGCCTTGATCCACTTGGC	Hamilton, 1999
Rpl16 (intron)	Rpl16-F71 (F)	GCTATGCTTAGTGTGTGACTCGTTG	Jordan et al., 1996
	Rpl16-R1516 (R)	CCCTTCATTCTTCCTCTATGTTG	Jordan et al., 1996
Rpl32-trnL	trnL(UAG) (R)	CTGCTTCCTAAGAGCAGCGT	Shaw et al., 2007
	Rpl32(F)	CAGTTCCAAAAAAACGTACTTC	Shaw et al., 2007
	MYtrnL(UAG) (R)	CGTTTTCGTAGTTTATGCTCTCCT	Faria, 2014
	MYrpl32 (F)	ACAAGATGTTCAGTTCAGGCCA	Faria, 2014
trnQ-rps16	trnQ(UUG) (F)	GCGTGGCCAAGYGGTAAGGC	Shaw et al., 2007
	Rps16x1 (R)	GTTGCTTTYTACCACATCGTTT	Shaw et al., 2007
	MYtrnQ (R)	AGTTGATGTAAAGGAAGATTTAGACTC	Murillo-A et al., 2012
	MYrps16 (F)	GCGTAAAAWGAGGAAATGCTTAATG	Murillo-A et al., 2012
trnL-trnF	B49317 (R)	CGAAATCGGTAGACGCTACG	Taberlet *et al.,* 1991
	A50272 (F)	ATTTGAACTGGTGACACGAG	Taberlet *et al.,* 1991
ndhF	1252 (F)	GATGAAATTMTTAATGATAGTTGGT	Biffin *et al.,* 2006
	2063 (R)	CATTTGGAATTCCATCAATTA	Biffin *et al.,* 2006
matK	390 (F)	CGATCCTTTCATGCATT	Johnson and Soltis, 1994
	1326 (R)	GTATTAGGGCATCCCATT	Johnson and Soltis, 1994

Appendix 1.3: PCR conditions.

DNA region	PCR conditions
ITS	94°C/2min, 30x cycle[94°C /1min, 50°C /1min, 72°C /1.5min], 72°C /4min
psbA-trnH	80°C /5min, 35x cycle[95°C /1min, 48°C /1min, 65°C /5min] 65°C /4min
trnQ-rps16	95°C /3min, 20x cycle[95°C /1min, 60°C *-0.5°C /1min, 65°C /1min], 20x [95°C /1min, 50°C /1min, 65°C /1min] 64°C /4min
rpl32-trnL	80°C /5min, 35 [95°C /1min, 50°C /1min, 65°C /5min] 65°C /4min
rpl16	80°C /5min, 35x cycles[95°C /1min, 50°C /1min, 65°C /5min] 65°C /4min
ndhF	80°C /5min, 35x cycles[95°C /1min, 50°C /1min, 65°C /5min] 65°C /4min
trnL-trnF	95°C /3min, 35x cycles[95°C /1min, 50°C /1min, 65°C /1.5min], 65°C /4min
matK	80°C /5min, 35x cycles[95°C /1min, 50°C /1min, 65°C /5min], 65°C /4min

Appendix 1.4: Previous studies with Myrteae dating estimates.

Dates estimation for Myrteae based on previous studies			
Reference	**Myrteae crown age (million years ago)**	**95% Confidence Interval**	**Obs.**
Sytsma et al., 2004	56	NA	Focused on Myrtales. Narrower Myrteae sample.
Biffin et al., 2010	28	34 - 22	Too recent to consider macro fossil evidence.
Staggemeier et al., 2015	56	doesn't say	Sample focused on *Myrcia*. Only one fossil considered.
Thornhill et al., 2012 (pollen fossil only)	41	37.5 – 46.5	Narrower Myrteae sample; too recent to consider macro fossil evidence.
Thornhill et al., 2012 (macrofossil only)	51	50 – 54.6	Narrower Myrteae sample; too recent to consider macro fossil evidence.
Thornhill et al., 2012 (combined fossil set)	50.9	50 – 53.6	Narrower Myrteae sample; too recent to consider macro fossil evidence.
Thornhill et al., 2015	50.7	50 – 51.4	Narrower Myrteae sample; too recent to consider macro fossil evidence.
Berger et al., 2016	18.4	doesn't say	Narrower Myrteae sample; too recent to consider macro fossil evidence.
Murillo-A et al., 2016	92.09	82.32 – 101.69	Narrower Myrteae sample; too old to consider late Cretaceous crown of Myrtaceae.

Appendix 1.5: Myrteae fossil survey (Cretaceous to Eocene). This is not an extensive list, but represents the diversity of fossil records in Myrteae by period.

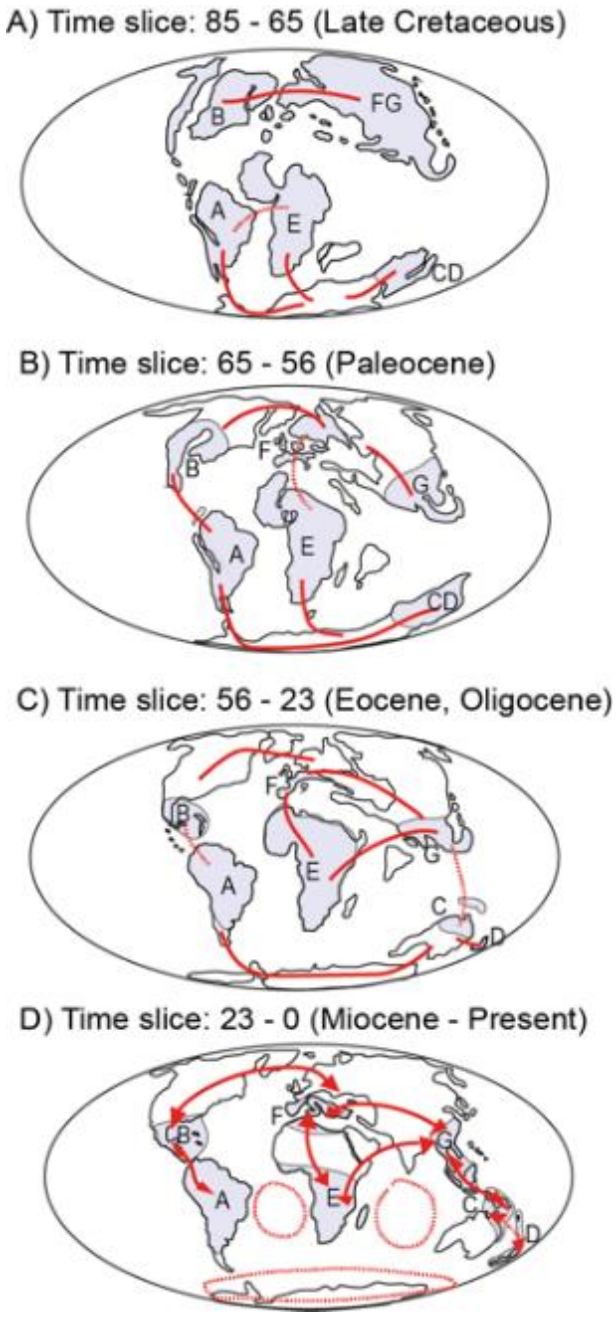
Fossil	Age (mya)	Modern taxa affinity	Location	Reference	Obs:
Upper Cretaceous					
Myrceugenelloxylon antarcticus (wood)	72.1 - 66	*Luma*	Antarctica (Seymour Island)	Poole et al. (2003)	Seems OK (according to Oskolki et al., 2013).
Myrciophyllum santacruzensis (leaves)	84.9 - 66	*Myrcia*	Antarctica (King George Island)	Dutra and Batten (2000)	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Eugenia camparabilis (leaves)	66	*Eugenia*	Venezuela	Berry (1939)*	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Paleocene					
Myrceugenia chubutense (wood)	65 - 56	*Myrceugenia*	Chile	Ragonese (1980)	Wood characters also present in *Melaleuca* (according to Oskolki et al., 2013)
Myrcia cf. *reticulato-venosa* (leaves)	61 – 47	*Myrcia*	Chile	Troncoso et al. (2002)	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Palaemyrtinae princetonensis (fruit)	56	*Mosiera, Psidium*	USA (North Dakota)	Pigg et al. (1993)	Seems OK and it is a popular choice for calibration analysis; fruit characters are, however, also common in Lythraceae.
Eocene					
Myrtineoxylon maomingensis (wood)	56 - 40	*Calycolpus* or *Octamyrtus*, "Australasian group"	China	Oskolski et al. (2013)	Seems OK. The only extant Myrteae with such distribution are *Decaspermum*, *Rhodamnia* and *Rhodomyrtus*. *Octamyrtus* and *Rhodomyrtus* are sister groups.
Eugenia sp. (leaves)	55.8 - 33.9	*Eugenia*	Venezuela	Berry (1936)*	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Calyptranthes myrtifolia (leaves)	48.6 to 37.2	*Calyptranthes*	USA (Florida)	MacGinitie (1941)	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Myrcia chubutense (leaves)	52	*Myrcia*	Chile	Wilf et al. (2005)	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.

Myrtaceae (leaves)	55.8 - 33.9	*Eugenia, Myrcia, Psidium, Myrcianthes*	Argentina	Panti (2014)	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Myrtaceidites verrucosus (pollen)	33.9 mya	Myrteae	Australia	Reviewed by Thornhill and Macphail (2012)	Seems OK. Placed by geographical location at the crown node of “Australasian group”
Myrtaceidites verrucosus (pollen)	23 mya	Myrteae	New Zealand	Reviewed by Thornhill and Macphail (2012)	Seems OK. Placed by geographical location at the crown node of “Myrteola group”
Myrtaceidites verrucosus (pollen)	Around 37	Myrteae	Argentina	Reviewed by Thornhill and Macphail (2012)	Seems OK. Placed by geographical location at the crown node of “Myrtus group”+ Main Neotropical Lineage
Myrtaceidites verrucosus (pollen)	Around 37	Myrteae	Panama	Reviewed by Thornhill and Macphail (2012)	Seems OK. Placed by geographical location at the crown node of “Myrtus group”+ Main Neotropical Lineage
Myrtaceidites oceanicus (pollen)	33 – 28 mya	Myrteae	South Africa, Ninetyeast Ridge	Reviewed by Thornhill and Macphail (2012)	Can also be assigned to other Myrtaceae tribes.
Rhodomyrtus australis (leaves)	48.6 to 23.03	*Rhodomyrtus*	Western Australia (near Perth)	Hill and Merrifield. (1993)	Fossil leaves were not considered due to the lack of morphological characters that can assign them confidently into Myrteae.
Myrceugenelloxylon pseudoapiculatum (wood)	Eocene - Oligocene	*Luma*	Chile (Mocha Island)	Nishida (1984)	Seems OK (according to Oskolki et al., 2013), it was not used because would have similar calibration placement as *Myrceugenelloxylon antarcticus*.
Myrceugenellites maytenoides (wood)	Eocene - Oligocene	*Luma*	Chile	Nishida (1988)	Seems OK (according to Oskolki et al., 2013), it was not used because would have similar calibration placement as *Myrceugenelloxylon antarcticus*.

Appendix 1.6: BioGeoBEARS supporting data.

Comments on area adjacency

Most area dispersion probabilities are based on Buerki et al. (2011). Some 0.5 dispersion probability (dashed line) were added, based on the distance between continents during times slices under consideration (see Figure below). LDDE event is considered whenever a shift occurs between areas that present either 0.5 or 0.1 dispersion probability. Continuous line: dispersion probability 1; Dashed line: dispersion probability 0.5; All other connections between areas (not indicated by lines) correspond to 0.1 dispersion probability.



BioGeoBEARS matrices

######AREA_CODE_TIP

115 7 (A B C D E F G)
Eugenia_longiracemosa 1000000
Eugenia_biflora 1100000
Eugenia_paracatuana 1000000
Eugenia_angustissima 1000000
Eugenia_acutata 1000000
Eugenia_brevistyla 1000000
Eugenia_involucrata 1000000
Eugenia_monticola 0100000
Hottea_neibensis 0100000
Calyptrogenia_cuspidata 0100000
Calyptrogenia_grandiflora 0100000
Eugenia_adenocalyx 1000000
Eugenia_punicifolia 1000000
Eugenia_dichroma 1000000
Eugenia_bullata 0001000
Eugenia_paludosa 0001000
Eugenia_reinwardtiana 0000001
Eugenia_rosapetiolata 0000100
Pseudanamomis_umbellulifera 0100000
Eugenia_uniflora 1000000
Eugenia_stipitata 1000000
Eugenia_azurensis 1000000
Hexachlamys_edulis 1000000
Myrcianthes_fragrans 0100000
Eugenia_yumana 0100000
Pimenta_sp1 0100000
Pimenta_dioica 0100000
Pimenta_pseudocaryophyllus 1000000
Campomanesia_adamantium 1000000
Campomanesia_velutina 1000000
Acca_sellowiana 1000000
Legrandia_concinna 1000000
Curitiba_prismatica 1000000
Lophomyrtus_obcordata 0001000
Neomyrtus_pedunculata 0001000
Lenwebbia_prominens 0010000
Myrteola_nummularia 1000000
Ugni_candollei 1000000
Psidium_brownianum 1000000
Psidium_larutteanum 1000000

Psidium_rufum 1000000
Calyptrogenia_biflora 0100000
Psidium_acranthum 0100000
Myrrhinium_atropurpureum 1000000
Mosiera_longipes 0100000
Calyptranthes_longicalyptrata 0100000
Calyptranthes_pallens 0100000
Mitranthes_claredonensis 0100000
Mitranthes_glabra 0100000
Calyptranthes_brasiliensis 1000000
Myrcia_subcordata 1000000
Myrcia_flagellaris 1000000
Myrcia_selloi 1000000
Myrcia_eugeniopsoides 1000000
Myrcia_pubipetala 1000000
Myrcia_rupta 1000000
Marlierea_umbraticola 1000000
Myrcia_abbotiana 0100000
Myrcia_spatulifolia 1000000
Myrcia_splendens 1000000
Myrcia_sp1 1000000
Myrcia_sp2 1000000
Myrcia_guianensis 1000000
Myrcia_variabilis 1000000
Myrciaria_vexator 0100000
Myrciaria_floribunda 1000000
Myrciaria_glazioviana 1000000
Algrizea_macrochlamys 1000000
Algrizea_minor 1000000
Neomitranthes_cordifolia 1000000
Siphoneugena_densiflora 1000000
Plinia_sp1 1000000
Plinia_nana 1000000
Myrceugenia_bananalensis 1000000
Myrceugenia_alpigena 1000000
Myrceugenia_planipes 1000000
Luma_apiculata 1000000
Blepharocalyx_cruckshanskii 1000000
Blepharocalyx_salicifolius2 1000000
Blepharocalyx_salicifolius 1000000
Blepharocalyx_eggersii 1000000
Amomyrtus_luma 1000000
Chamguava_schippii 1000000
Calycolpus_goetheanus 1000000
Calycolpus_moritzianus 1000000
Accara_elegans 1000000
Myrtus_communis 0000110
Archirhodomyrtus_turbinata 0001000

Pilidiostigma_tropicum 0010000
Kanakomyrtus_dawsoniana 0001000
Rhodomyrtus_tomentosus 0000001
Octamyrtus_pleiopetala 0010000
Decaspermum_vitisidae 0000001
Decaspermum_fruticosum 0010001
Decaspermum_humile 0010000
Rhodamnia_cinerea 0000001
Uromyrtus_emarginata 0001000
Gossia_clusioides 0001000
Austromyrtus_dulcis 0010000
Myrtastrum_rufopunctatum 0001000
Xanthomyrtus_montivaga 0010000
Xanthomyrtus_compacta 0010000
Metrosideros_nervulosa 0001000
Metrosideros_perforata 0001000
Metrosideros_stipularis 1000000
Syzygium_guineense 0000100
Syzygium_muellerii 0000001
Syzygium_amplifolium 0001000
Syzygium_paniculatum 0010000
Syzygium_jambos 0000001
Syzygium_buxifolium 0000001
Syzygium_gustavioides 0010000
Syzygium_maire 0001000
Eucalyptus_perriniana 0010000
Leptospermum_scoparium 0010000

####DISTANCE

A	B	C	D	E	F	G
1	0.1	1	1	0.5	0.1	0.1
0.1	1	0.1	0.1	0.1	1	1
1	0.1	1	1	1	0.1	0.1
1	0.1	1	1	1	0.1	0.1
0.5	0.1	1	1	1	0.1	0.1
0.1	1	0.1	0.1	0.1	1	1
0.1	1	0.1	0.1	0.1	1	1

A	B	C	D	E	F	G
1	1	1	1	0.1	0.1	0.1
1	1	0.1	0.1	0.1	1	0.1
1	0.1	1	1	1	0.1	0.1
1	0.1	1	1	1	0.1	0.1
0.1	0.1	1	1	1	0.5	0.1
0.1	1	0.1	0.1	0.5	1	1
0.1	0.1	0.1	0.1	0.1	1	1

A	B	C	D	E	F	G
1	0.5	1	0.1	0.1	0.1	0.1
0.5	1	0.1	0.5	0.1	1	0.1
1	0.1	1	1	0.1	0.1	0.5
0.1	0.5	1	1	0.1	0.1	0.1
0.1	0.1	0.1	0.1	1	1	1
0.1	1	0.1	0.1	1	1	1
0.1	0.1	0.5	0.1	1	1	1

A	B	C	D	E	F	G
1	1	0.5	0.5	0.5	0.1	0.1
1	1	0.1	0.1	0.1	1	0.1
0.5	0.1	1	0.5	0.5	0.1	1
0.5	0.1	0.5	1	0.1	0.1	0.1
0.5	0.1	0.5	0.1	1	1	1
0.1	1	0.1	0.1	1	1	1
0.1	0.1	1	0.1	1	1	1

END

#####TIME_SLICE_MYA

23
56
65
90

Appendix 1.7: BAMM analysis supporting information and matrices.

#sample size
1.0
Eugenia_longiracemosa race 0.066
Eugenia_biflora race 0.066
Eugenia_paracatuana race 0.066
Eugenia_angustissima race 0.066
Eugenia_acutata clyc 0.07
Eugenia_brevistyla clyc 0.07
Eugenia_involucrata phyl 0.05
Eugenia_monticola umbl 0.0127
Hottea_neibensis umbl 0.0127
Calyptrogenia_cuspidata umbl 0.0127
Calyptrogenia_grandiflora umbl 0.0127
Eugenia_adenocalyx umbl 0.0127
Eugenia_punicifolia umbl 0.0127
Eugenia_dichroma went 0.125
Eugenia_bullata oldc 0.024
Eugenia_paludosa oldc 0.024
Eugenia_reinwardtiana oldc 0.024
Eugenia_rosapetiolata oldc 0.024
Pseudanamomis_umbellulifera oldc 0.024
Eugenia_uniflora sten 0.05
Eugenia_stipitata dich 0.05
Eugenia_azurensis clad 0.05
Hexachlamys_edulis hexa 0.125
Myrcianthes_fragrans myct 0.029
Eugenia_yumana pime 0.25
Pimenta_sp1 pime 0.25
Pimenta_dioica pime 0.25
Pimenta_pseudocaryophyllus pime 0.25
Campomanesia_adamantium camp 0.064
Campomanesia_velutina camp 0.064
Acca_sellowiana acca 1
Legrandia_concinna legr 1
Curitiba_prismatica curi 1
Lophomyrtus_obcordata loph 0.666
Neomyrtus_pedunculata loph 0.666
Lenwebbia_prominens lenw 0.5
Myrteola_nummularia mytl 0.333

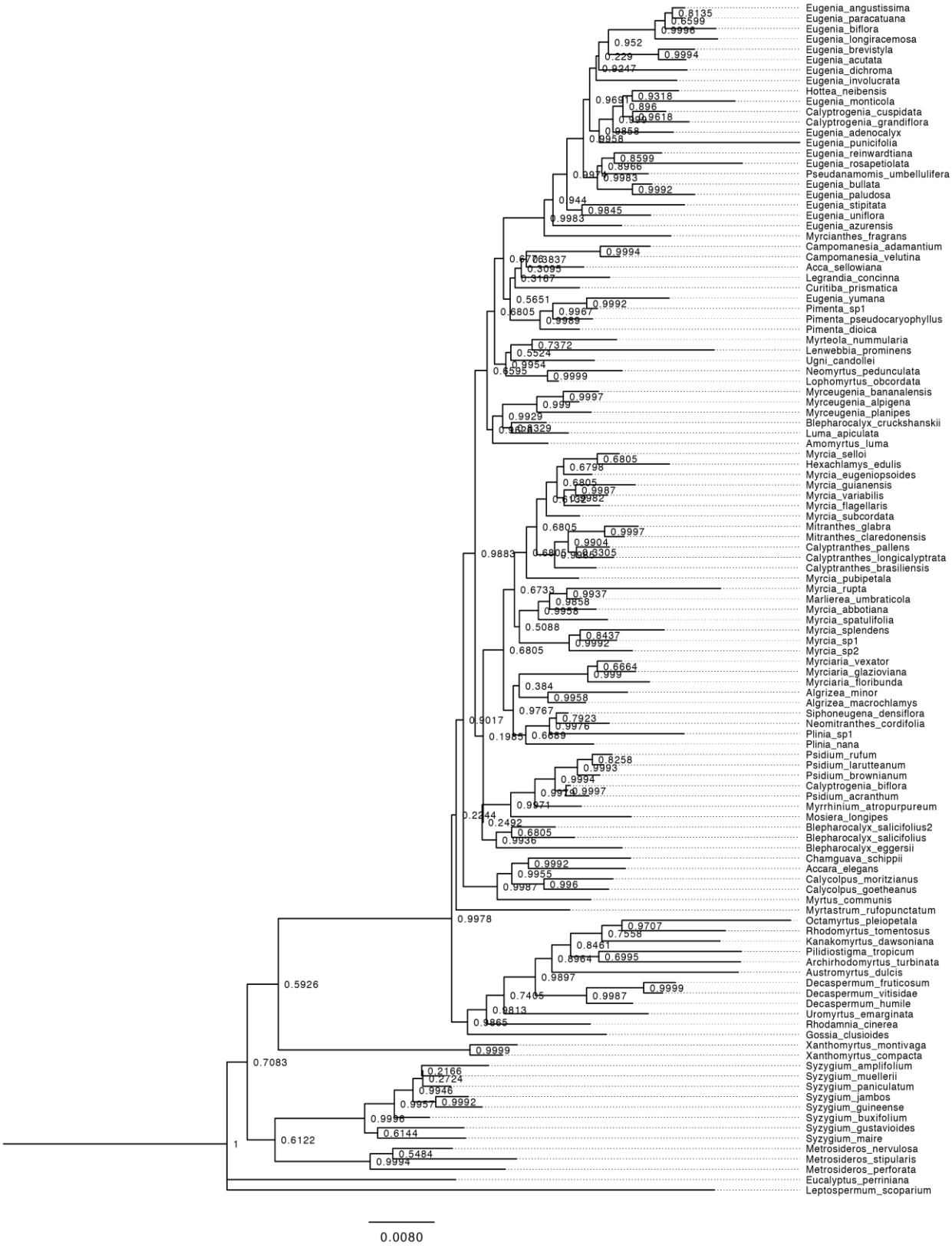
Ugni_candollei	ugni	0.25	
Psidium_brownianum	psid	0.0625	
Psidium_larutteanum	psid	0.0625	
Psidium_rufum	psid	0.0625	
Calyptrogenia_biflora	psid	0.0625	
Psidium_acranthum	psid	0.0625	
Myrrhinium_atropurpureum	myrr	0.333	
Mosiera_longipes	mosi	0.031	
Calyptranthes_longicalyptrata	clpt	0.019	
Calyptranthes_pallens	clpt	0.019	
Mitranthes_claredonensis	clpt	0.019	
Mitranthes_glabra	clpt	0.019	
Calyptranthes_brasiliensis	clpt	0.019	
Myrcia_subcordata	symp	0.037	
Myrcia_flagellaris	gomi	0.016	
Myrcia_selloi	tome	0.833	
Myrcia_eugeniopsoides	eugn	0.045	
Myrcia_pubipetala	pubi	0.05	
Myrcia_rupta	aulb	0.05	
Marlierea_umbraticola	aulb	0.05	
Myrcia_abbotiana	aulb	0.05	
Myrcia_spatulifolia	aulb	0.05	
Myrcia_splendens	sple	0.03	
Myrcia_sp2	sple	0.03	
Myrcia_sp1	sple	0.03	
Myrcia_guianensis	gui	0.06	
Myrcia_variabilis	gui	0.06	
Myrciaria_vexator	myri	0.136	
Myrciaria_floribunda	myri	0.136	
Myrciaria_glazioviana	myri	0.136	
Algrizea_macrochlamys	algr	1	
Algrizea_minor	algr	1	
Neomitranthes_cordifolia	plin	0.043	
Siphoneugena_densiflora	plin	0.043	
Plinia_sp1	plin	0.043	
Plinia_nana	plin	0.043	
Myrceugenia_bananalensis	myrc	0.07	
Myrceugenia_alpigena	myrc	0.07	
Myrceugenia_planipes	myrc	0.07	
Luma_apiculata	luma	0.5	
Blepharocalyx_cruckshanskii	blcr	1	

Blepharocalyx_salicifolius2	blep	1	
Blepharocalyx_salicifolius	blep	1	
Blepharocalyx_eggersii	blep	1	
Amomyrtus_luma	amom	0.333	
Chamguava_schippii	cham	0.333	
Calycolpus_goetheanus	caly	0.25	
Calycolpus_moritzianus	caly	0.25	
Accara_elegansacra	1		
Myrtus_communis	mytu	0.5	
Archirhodomyrtus_turbinata	arch	0.2	
Pilidiostigma_tropicum	pili	0.166	
Kanakomyrtus_dawsoniana	kana	0.166	
Rhodomyrtus_tomentosus	rhmy	0.05	
Octamyrtus_pleiopetala	octa	0.166	
Decaspermum_vitisidae	deca	0.088	
Decaspermum_fruticosum	deca	0.088	
Decaspermum_humile	deca	0.088	
Rhodamnia_cinerea	rhod	0.028	
Uromyrtus_emarginata	urom	0.043	
Gossia_clusioides	goss	0.027	
Austromyrtus_dulcis	aust	0.125	
Myrtastrum_rufopunctatum	mtrm	1	

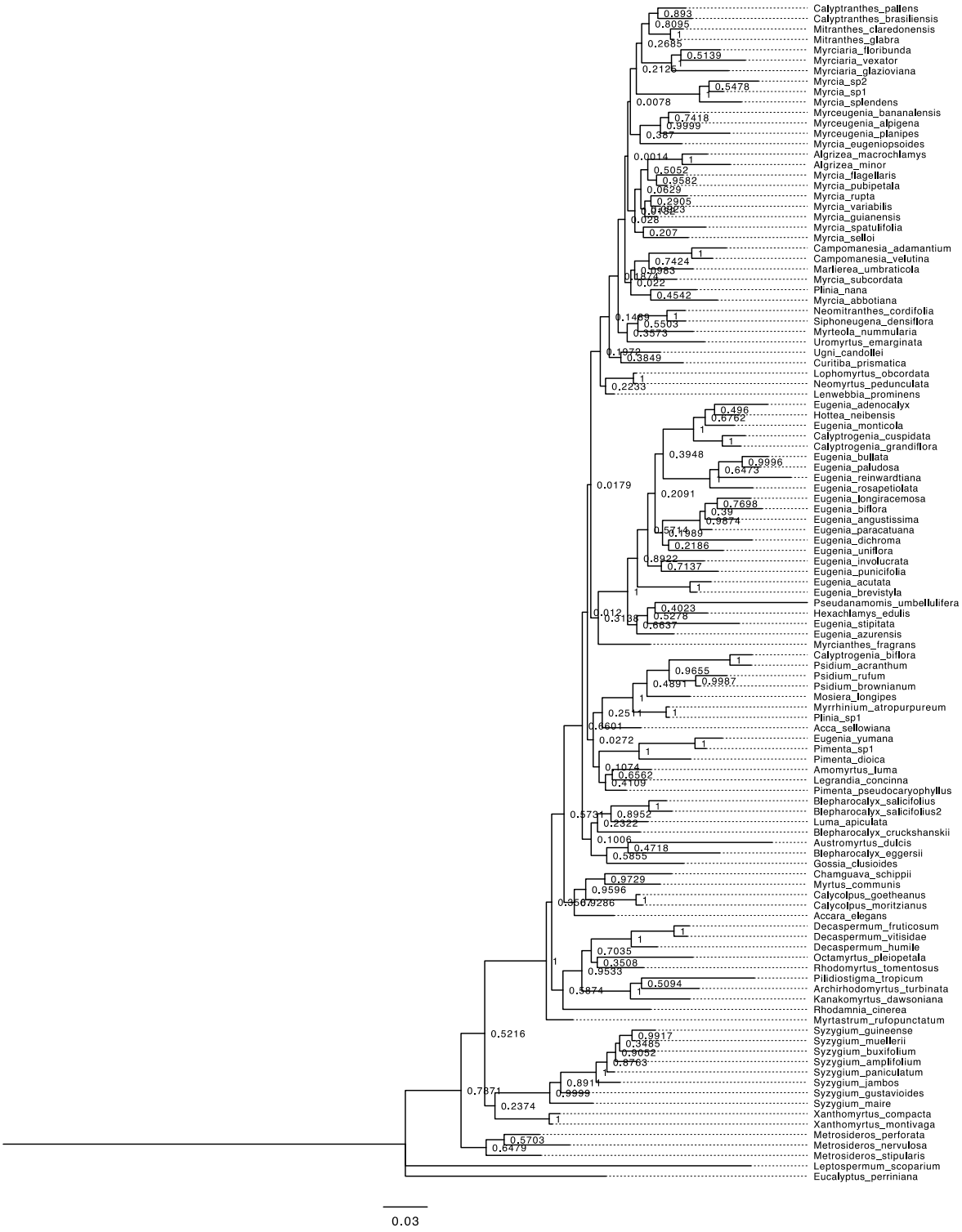
################
PRIORS (Approach A)
################
expectedNumberOfShifts = 1.0
lambdaInitPrior = 1.83848722468205
lambdaShiftPrior = 0.0175634891749598
muInitPrior = 1.83848722468205
lambdaIsTimeVariablePrior = 1

################
PRIORS (Approach B)
################
expectedNumberOfShifts = 1.0
lambdaInitPrior = 1.14331354209679
lambdaShiftPrior = 0.028242690460734
muInitPrior = 1.14331354209679
lambdaIsTimeVariablePrior = 1

Appendix 1.8: BI phylogeny based on cpDNA dataset.



Appendix 1.9: BI phylogeny based on nuclear (ITS) dataset.



Chapter 2: Systematic and evolutionary implications of stamen posture in Myrteae (Myrtaceae)

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- T.N.C.Vasconcelos contributions: development of hypotheses, design of experiments, collection of samples, genaration of SEM images and writing of manuscript.

ABSTRACT

As previously discussed, taxonomy of Myrteae is notoriously difficult. Although the phylogeny has been improved, the morphological characteristics that support its cladistic configuration are still unknown. The present study evaluates stamen posture and anthesis type as characters of systematic and evolutionary relevance. 69 species from 41 genera across the tribe were checked using herbarium material and spirit collections. Results recognize three patterns of stamen posture in the pre-anthetic bud: straight, semi-curved and strongly incurved. The three patterns of stamen posture correspond to the phylogenetic structure of the tribe, supporting the topology of the clades. Incurving of stamens along the evolutionary history of Myrteae appears linked to hypanthium extension and leads to different anthesis types that may be related to shifts in pollination strategy. The accessibility of stamen posture and its systematic consistence makes this character a useful tool for field and herbarium identification and allows inference of relationships for taxa not yet sampled in molecular analyses.

Key-words: anthesis, evolution, *Eugenia,* flower development, *Myrcia*, Myrtales, pollination.

2.1 Myrteae taxonomic complexity and absence of diagnostic characters

Myrtaceae (c. 5500 species; Wilson, 2011) is one of the ten most species-rich angiosperm families (Stevens, 2001 onwards; Wilson, 2011). Members of Myrtaceae are particularly diverse in the tropical biomes of America and Asia and throughout Australia, always contributing to a significant proportion of species composition (Govaerts et al., 2008). Many of these areas of highest diversity of Myrtaceae are also home to some of the highest biodiversity on Earth and are under high environmental threat from deforestation (Achard et al., 2002; Geist and Lambin, 2002). Molecular phylogenetic studies in Myrtaceae classified the family into two subfamilies: Psiloxyloideae, with two monospecific tribes, and Myrtoideae, with 15 tribes (Wilson et al., 2005). Among these tribes, Myrteae is the richest in terms of species (c. 2500) and genera (c.50), representing more than half of the family diversity (Wilson, 2011). With the exception of the a single species of *Metrosideros* (tribe Metrosidereae), Myrteae is also the only tribe in the family that naturally occurs in the New World (Wilson et al., 2005; Lucas et al., 2007; Wilson, 2011).

Taxonomy of Myrteae is notoriously difficult resulting in routine mis-naming or no-naming of species in floristic inventories that often underpin conservation initiatives (Mc Vaugh, 1968; Kawasaki, 1989; Barroso, 1994; e.g. Carvalho and Braga, 2007 in Atlantic Rainforest; Moro et al., 2014 in Caatinga). The consequences of this problem are exacerbated given that Myrteae represents 10 - 15% of tree species diversity in Brazilian savannas and Atlantic forests (Sobral et al., 2014) which are habitats under most acute pressure from deforestation (Mori et al., 1983; Oliveira-Filho and Fontes, 2000).

Prior to the first DNA-based phylogenies, tribe Myrteae was classified into three sub-tribes based on characters of the embryo (Berg, 1855-56, 1857-59). Preliminary molecular phylogenetic analysis demonstrated that these sub-tribes are not monophyletic and characters of the embryo are not congruent with the sub-tribal classification (Lucas et al., 2005). Lucas et al. (2007) recovered seven morphologically cohesive clades within Myrteae and informally named them: *Plinia* group, *Myrcia* group, *Myrceugenia* group, *Myrteola* group, *Eugenia* group, *Pimenta* group and Australasian group. Three species remained ungrouped: *Algrizea macrochlamys* (DC.) Proença & NicLugh, *Blepharocalyx salicifolius* (Kunth) O.Berg and *Myrtus communis* L.. This initial study has been revisited by Costa (2009), De-Carvalho (2013) and in this study (Chapter 1). These added more molecular information (i.e. DNA regions and taxa) and recognized overall a similar phylogenetic structure, with two main clades for the Neotropical lineages consistent throughout the studies: one clade formed by *Plinia* Group, *Algrizea* and *Myrcia* group (henceforward PAM clade), which appears as sister of *Myrceugenia* group in all studies, and the other formed by *Myrteola*, *Eugenia* and *Pimenta* groups (the latter including *Psidium* group, following Lucas et al., 2007; henceforward MEP clade) (Fig. 2.1). The most significant changes between the topologies of Lucas et al. (2007), Costa (2009) and De-Carvalho (2013) relate to the relationship inside the PAM and MEP clades, the position of *Blepharocalyx salicifolius* within the tribe (discussed by De-Carvalho, 2013) and the position of *Myrtus communis* as sister to the Australasian group.

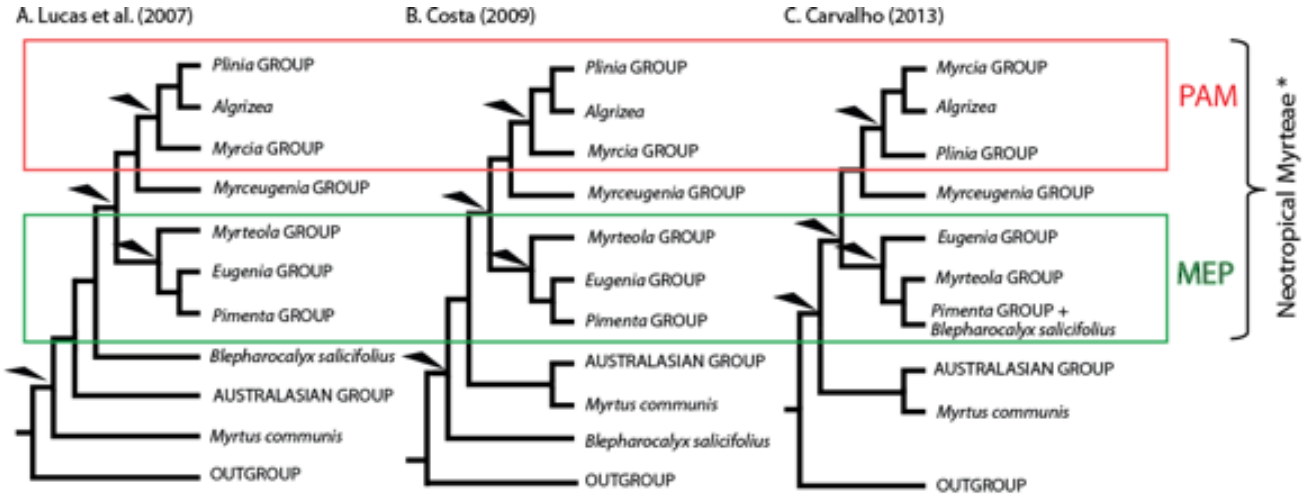


Figure 2.1. Comparison of three Myrteae molecular phylogenies, all three using data both from nuclear and chloroplast sequences. Arrows indicate Bayesian probabilities greater than 0.95. * all Neotropical with exception of c. 10% of the species in *Eugenia* and the New Zealand genera *Neomyrtus* and *Lophomyrtus* (*Myrteola* group).

The supra-generic groups of Myrteae are poorly understood in an evolutionary point of view and only few morphological characters support the phylogenetic structures. Recent studies on the evolution in the tribe have struggled to score morphological characters into homologous states for phylogenetic reconstruction and character optimization (e.g. Lucas et al., 2007, 2011); results demonstrate low phylogenetic signal from these characters and high levels of homoplasy. To understand the tribal evolution and relationship is crucial revisiting morphological aspects. A better understanding of Myrteae evolutionary aspects may contribute to ecological studies in Neotropical biomes in which they are most diverse. Such a framework can then be used in conjunction with dating, historical biogeography and identification of diversification rate shifts to provide insight into the origins of the biomes in which they are found.

In Myrtales flowers, the hypanthium often extends into a cup which can influence the development of the androecium, i.e. stamens, filaments and anthers (Ronse DeCraene and Smets, 1991; see also Chapter 3). Descriptive studies on the development of the hypanthium and stamen behaviour in the bud show differences in these characters in various species within Myrteae (Proença and Gibbs, 1994; Belsham and Orlovich, 2002, 2003). Proença and Gibbs (1994) also observed differences in anthesis among different genera in Myrteae, possibly as a consequence of the different arrangement of the stamen in the bud. However, these studies were produced before any molecular phylogeny was available and were based on few species without detailed systematic and evolutionary discussion.

The pattern of stamen posture in the bud and the anthesis type are easy characters to access in the field or herbarium material. Incurved stamens in the bud are considered a synapomorphy in the angiosperm order Myrtales (Stevens, 2001 onwards), with exceptions recorded in Vochysiaceae, Onagraceae and the Myrtaceae subfamily Psiloxyloideae (Johnson and Briggs, 1984; Dahlgren and Thorne, 1984). However, this character has never before been considered for Myrteae as a feature of systematic importance. The aims of this study were to

investigate patterns of stamen posture in the buds of the main clades of Myrteae, correlate this character to the anthesis type in Myrteae and associate it to the most recent phylogenetic hypotheses and evolution in Myrteae.

MATERIALS AND METHODS

2.2 Sampling

At least one species from 41 genera in Myrteae was sampled. *Heteropyxis natalensis* Harv. (Psiloxyloideae) was also sampled to represent the exceptional character of straight stamens in Myrtales. Buds were sampled from herbarium material, spirit collection, and from field collections (more details in the next sections). Buds were analysed pre-anthesis, i.e. mature buds in the final stage before flower opening. This standardised the observations and maximized the sampling because most herbarium material was found to be at this stage. When available, younger buds were also studied. Species were analysed using a stereo microscope, scanning electron microscope and field photography. The mega species-rich genera *Eugenia* (c. 1000 spp.) *sensu* Mazine *et al*. (2014, including *Calycorectes*), and *Myrcia* (c. 700 spp.) *sensu* Lucas *et al*. (2011), were represented by at least one species per subgeneric clade reported by those studies. List of analysed specimens is available in Appendix 3.1 (p.).

2.3 Herbarium material

Samples were preferentially taken from the vouchers from the Lucas et al. (2007) phylogeny. When these vouchers did not have buds, or when they were unavailable, material identified by specialists was used. Buds from each sample were rehydrated in boiling water and dissected using fine tweezers, a razor blade and a dissecting microscope. Buds were analysed in two ways: (1) a frontal view from above after removing sepals and petals and (2) a longitudinal cut of the whole bud. Aims when dissecting were: (1) to verify the visibility of anthers in top view within the bud and (2) to determine the nature of the filaments (straight or incurved). Digital images were taken with a Nikon coolpix 4500 digital camera mounted on a Leica WILD M3Z binocular microscope. Images were used as basis for schematic drawings using Adobe Illustrator CS5. The analysed buds were returned to the herbarium voucher. All vouchers, besides Faria J.E.Q. collections, are deposited at the Royal Botanic Gardens, Kew (K). Faria J.E.Q. collections are deposited at the Universidade de Brasilia herbarium (UB).

2.4 SEM analyses

SEM analysis of alcohol preserved material was carried out in parallel to the study on herbarium samples. At least one species of each informal group of Myrteae sensu Lucas *et al*. (2007) was included as well as the still not firmly placed *Blepharocalyx salicifolius* and the ungrouped *Myrtus* and *Algrizea.* The SEM images of pre-anthesis buds were analysed in the same way as the herbarium dissections. Material was taken from the spirit collections of the following herbaria: K, MO, NY and US. Additional material was added from the living collection at the Royal Botanic Gardens Kew and field collections made in Brazil. Vouchers for field collections are deposited at K and UB herbaria.

Material was dissected in 70% ethanol, dehydrated through an alcohol series to absolute ethanol, and critical-point dried using an Autosamdri-815B critical-point dryer (Tousimis Research,

Rockville, Maryland, USA). Dried material was further dissected and mounted onto specimen stubs using nail polish, coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinsted, UK) and examined with a Hitachi cold field emission SEM S-4700-II (Hitachi High Technologies, Tokyo, Japan).

2.5 Anthesis type observation

Anthesis pattern of different genera was observed and photographed during fieldworks in Brazil between September and November of 2014. Fieldwork was carried out in the Brazilian Amazon (Amazonas and Roraima states), "Caatinga" dry forests (Bahia state), "Cerrado" savanna vegetation (DF, Goias, Bahia and Minas Gerais states) and Atlantic Rainforest (Bahia, Espirito Santo, Minas Gerais and Sao Paulo states). Flowers in anthesis stage were selected and photographed using a digital Nikon D200 camera with a 60mm macrolens.

RESULTS

2.6 Stamen posture

Results show different stamen posture in pre-anthetic buds for different Myrteae groups in both herbarium and SEM analysis. In all analysed species of the Australasian Group (*Archirhodomyrtus beckleri*, *Decaspermum parviflorum*, *Gossia bidwillii, Octamyrtus arfakensis*, *Pilidiostigma tropicum*, *Rhodamnia dumetorum*, *Rhodomyrtus tomentosa*; see *Rhodomyrtus* sp. and *Octamyrtus* sp. Fig. 2.2A, B) and in *Myrtus communis* (Fig. 2.2C, E) straight filaments with anthers visible from the top in pre-anthetic buds were recorded. The same pattern was found in the *Eugenia* group and was consistent in all analysed species of *Eugenia s.l.* (*Eugenia adenocalyx* (Fig. 2.2D), *E. florida*, *E. involucrata*, *E. klotzschiana, E. pluriflora*, *E. pyriformis*, *E. uniflora* (Fig. 2.2F), *E. myrcianthes* and *Calycorectes bergii*) and in *Myrcianthes fragrans*. Straight stamens were also found consistently in all samples of the *Myrteola* group, (*Ugni candollei* (Fig. 2.2G), *Lophomyrtus obcordata*, *Neomyrtus pedunculata* and *Myrteola nummularia*). Most species of the *Pimenta* group also show straight pre-anthesis filaments with anthers visible from the top. However, the pattern is not consistent in this group. Stamens are completely straight in *Acca sellowiana* (Fig. 2.3A, B) and in all analysed species of *Psidium* (see *P.guineense,* Fig. 2.3C, D) and *Campomanesia.* However, there was variation in the position of stamens in *Pimenta* and *Blepharocalyx salicifolius*. While *Pimenta racemosa* and *Pimenta dioica* (herbarium material only) show semi-curved stamens similar to those in the *Myrceugenia* group (discussed in the next paragraph; see also Chapter 3), *Pimenta pseudocaryophyllus* (Fig. 2.3E) and *Blepharocalyx salicifolius* (Fig. 2.3F) show strongly incurved stamens in the pre-anthesis bud, with anthers touching the bottom of the floral disc. In these species, the filaments develop from the rim of the hypanthium cup formed by the hypanthium extension.

A semi-curved pattern was found consistently in the *Myrceugenia* group (*Luma apiculata,* Fig. 2.3G; *Myrceugenia alpigena*, *M. planipes,* Fig. 2.3H; *M. bananalensis,* Fig. 2.3I). Species with this pattern show straight to slightly incurved outer filaments. The hypanthium is also extended in these species and the inner filaments are strongly incurved. The anthers remain facing downwards during development, touching the bottom of the hypanthial cup. On removal of the calyx and corolla, only anthers from the outer whorls are visible from above. *Blepharocalyx* cruckshanksii, also within

Myrceugenia group, was only available as herbarium material and was difficult to interpret. Filaments were clearly incurved, however, it was not clear if stamens were strongly curved as in *Blepharocalyx salicifolius* or semi-curved as in other taxa of the *Myrceugenia* group.

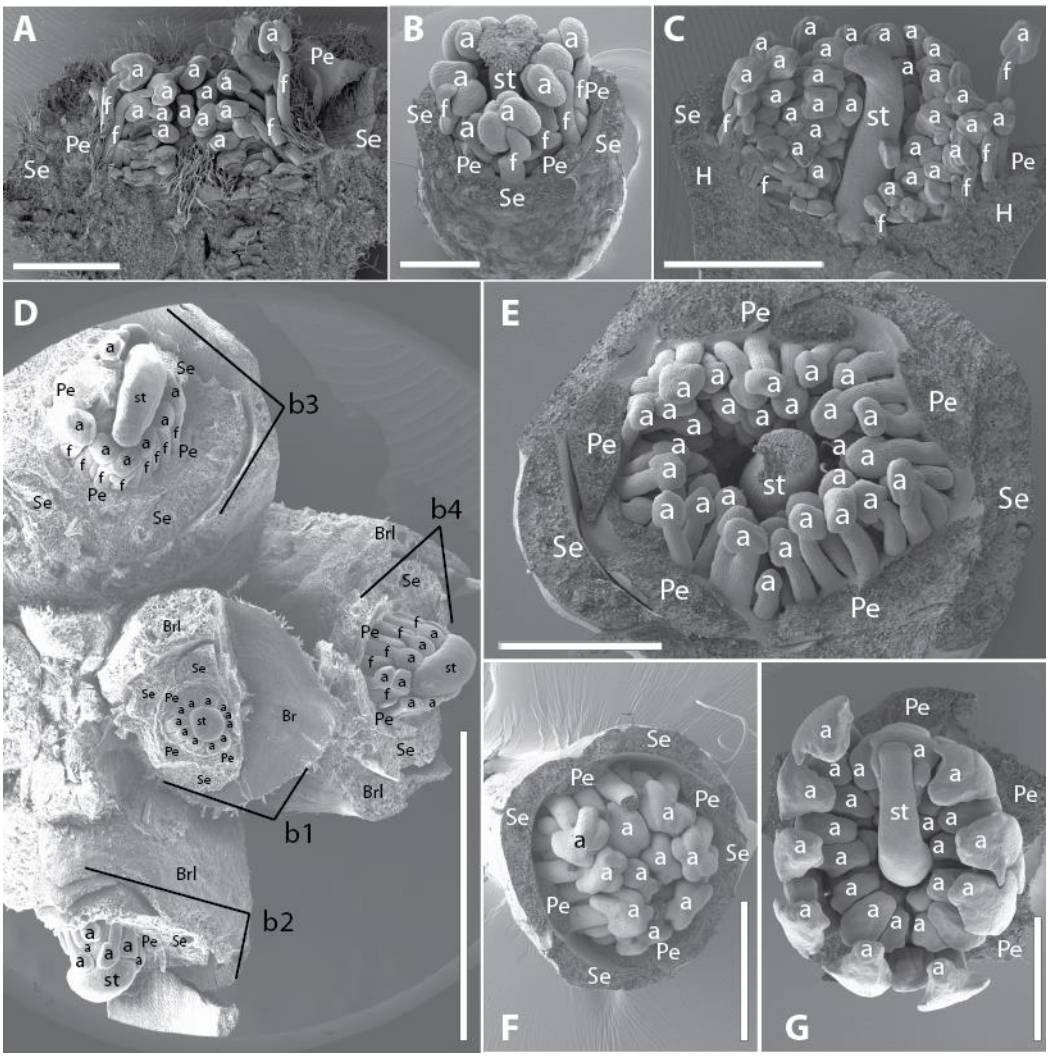


Figure 2.2: SEM images of buds of species from the Australasian group, *Eugenia* group and *Myrtus*. Perianth removed in all. (A) *Rhodomyrtus* sp. (Australasian group), showing straight filaments with anthers facing upwards. (B) *Octamyrtus* sp. (Australasian group) showing anthers visible from above in the bud. (C) and (E) *Myrtus communis* in two different developmental stages: (C) A nearly pre-anthetic bud with straight filaments growing from a slightly extended hypanthia and anthers facing upwards. (E) Anthers already growing upwards in a young bud. (D) Inflorescence of *Eugenia adenocalyx* (*Eugenia* group) with buds in different developmental stages showing filaments always straight and anthers visible from above. (F) *Eugenia uniflora* (*Eugenia* group) showing anthers visible from above. (G) *Ugni candollei* (*Myrteola* group) in pre-anthetic stage, showing anthers visible from above. b1-b4, flower buds from the youngest to oldest; a, anther; Br, bracts; Brl, bracteoles; f, filament; Pe, petal scar; Se, sepal scar; st, style. Scale: 250µm (B), 500µm (E,F), 1mm (A,C,G), 2mm (D).

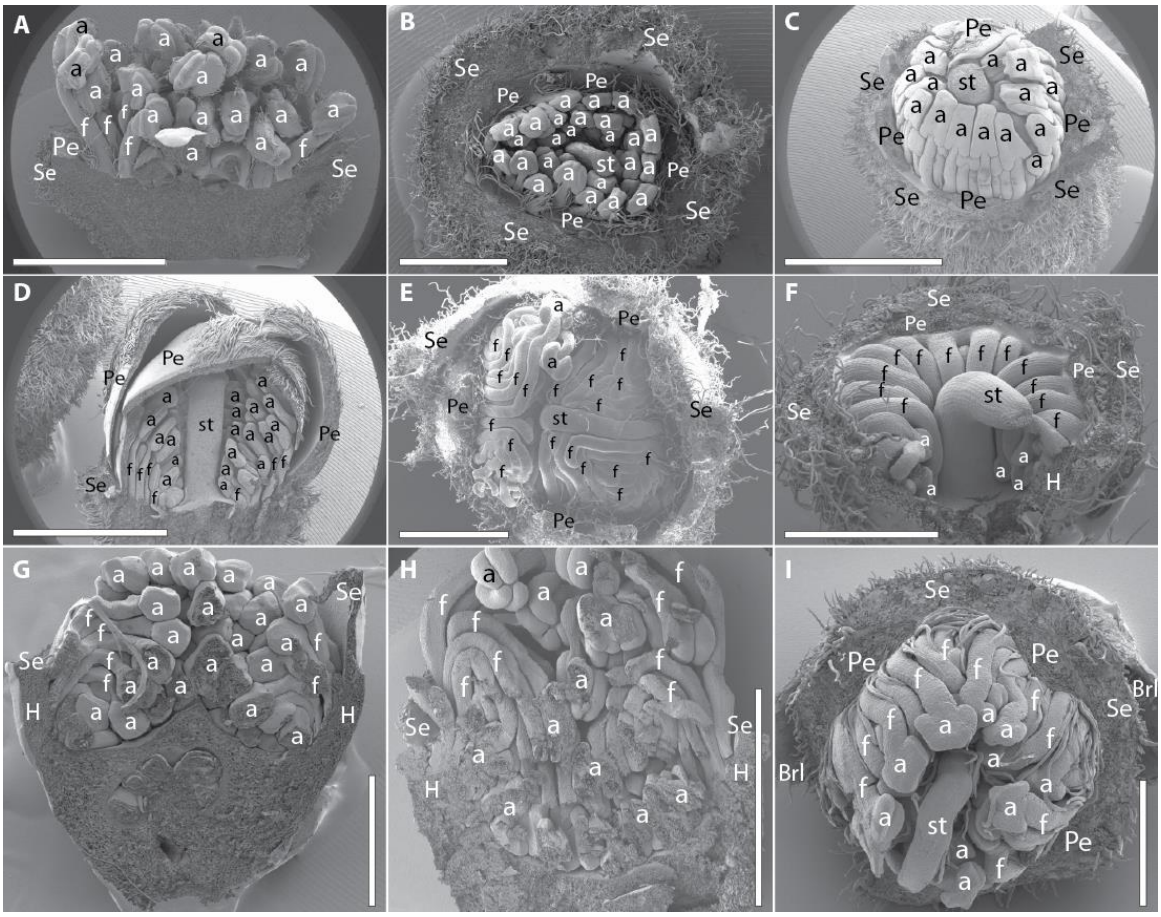


Figure 2.3: SEM images of buds of *Pimenta* group and *Myrceugenia* group species. Perianth completely or partly removed in all. (A) and (B) *Acca sellowiana* (*Pimenta* group). (A) Bud in a nearly pre-anthetic stage, with straight filaments and anthers upwards. (B) Bud in an earlier developmental stage, with anthers already visible from above. (C) and (D) *Psidium guineense* (*Pimenta* group) buds in pre-anthetic stage. (C) Anthers visible from above. (D) Straight filaments with anthers touching inner surface of petals (Pe). (E) *Pimenta pseudocaryophyllus* (Pimenta group). Exceptional incurved filaments in the pre-anthetic bud with most anthers not visible from above. (F) *Blepharocalyx salicifolius* (*Pimenta* group) also showing the exceptional incurved filaments, with anthers touching the bottom of the hypanthia cup formed by the hypanthial extension in a longitudinal view. (G) *Luma apiculata* (*Myrceugenia* group) with inner filament whorls curved and outer whorls straight. Only the anthers from the inner whorls touch the bottom of the hypanthia cup. (H) *Myrceugenia planipes* (*Myrceugenia* group) in a pre-anthetic stage, showing outer filaments straight and inner filaments curved, with anthers from the later touching the bottom of the hypanthial cup. (I) *Myrceugenia bananalensis* (*Myrceugenia* group) showing only anthers from the outer staminal whorls visible from above. a, anther; Br, bracts; Brl, bracteoles; f, filament; Pe, petal scar; Se, sepal scar; st, style. Scale: 500µm (F,I), 1mm (B,E,G,H), 2mm (A,C,D).

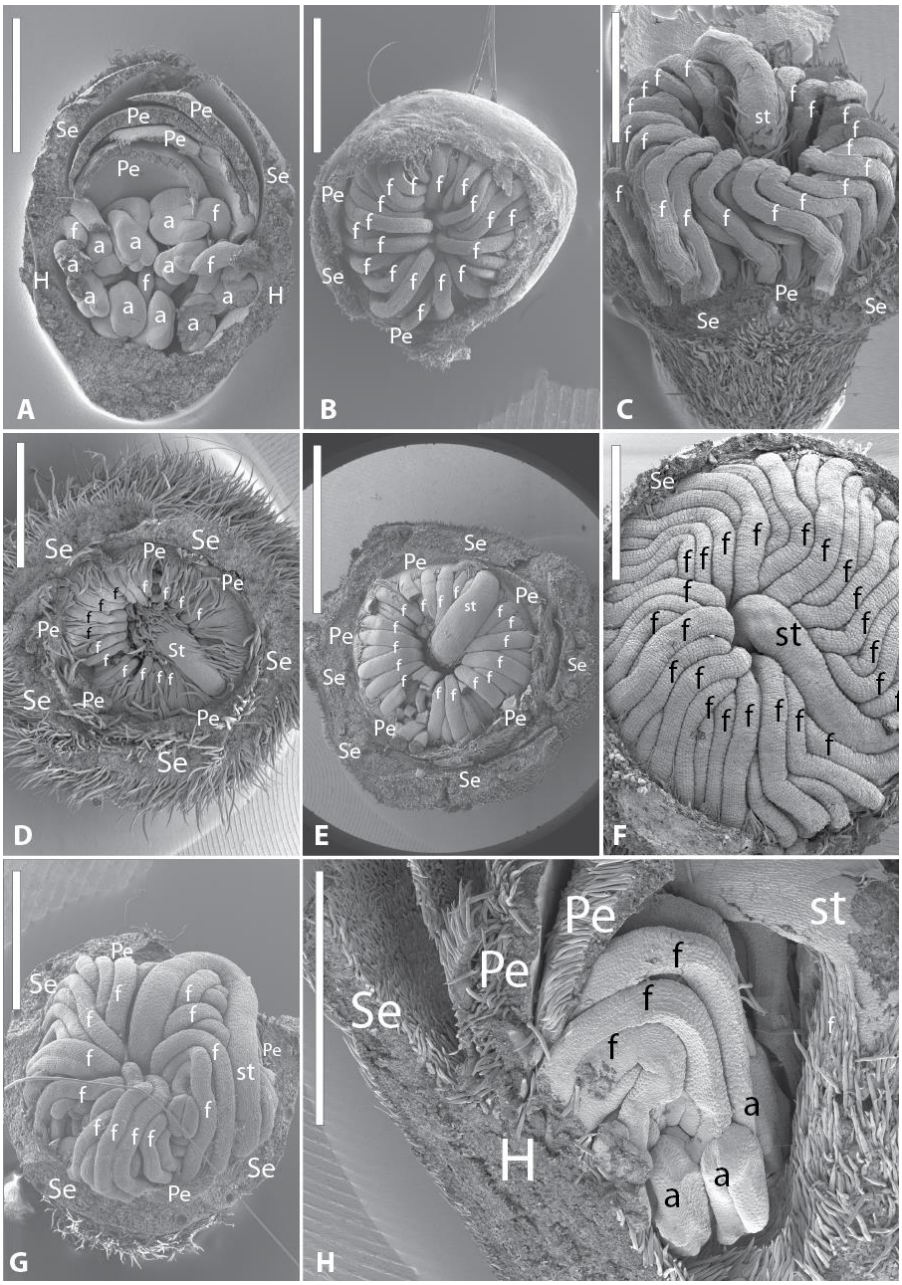


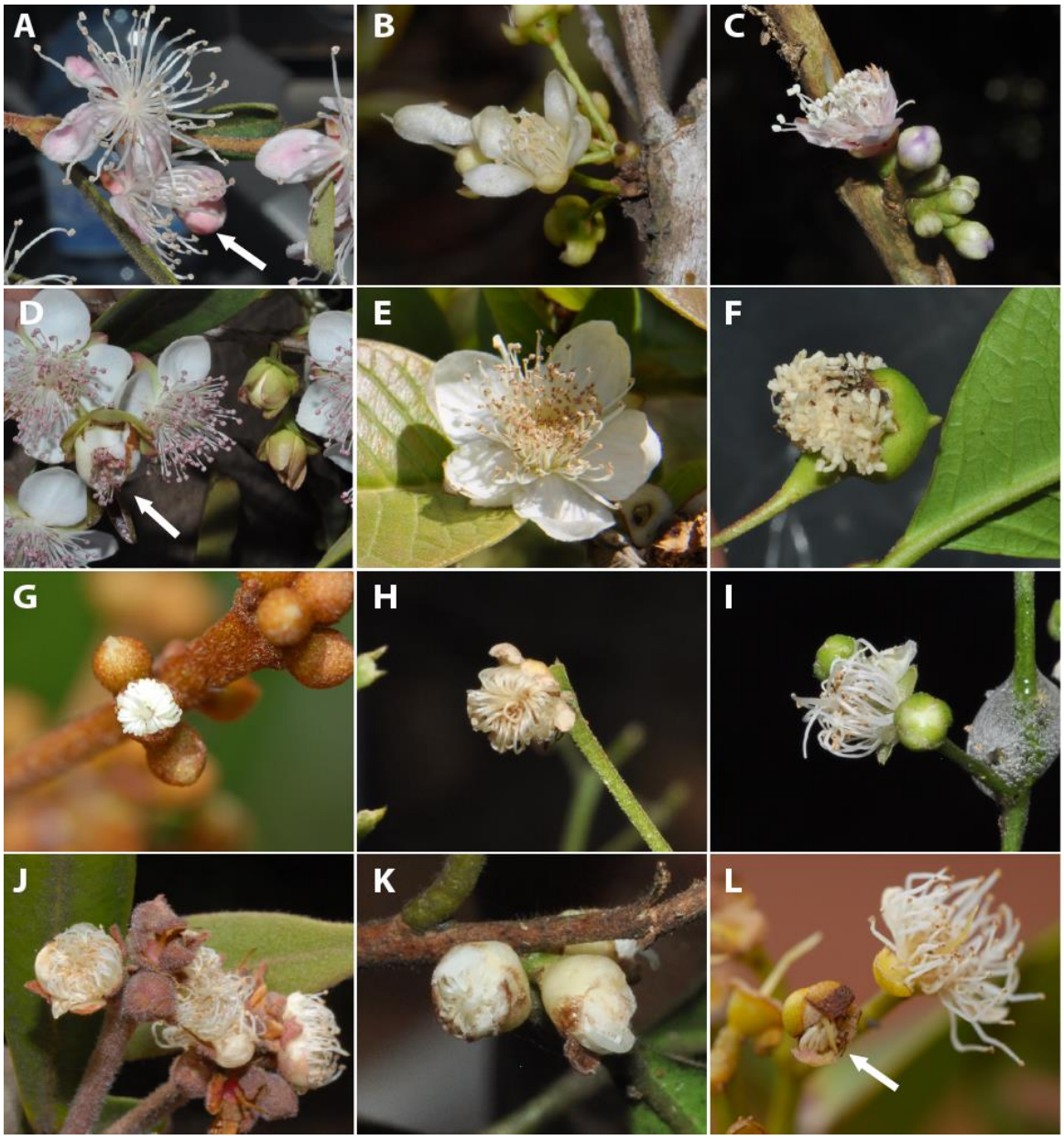
Figure 2.4: SEM images of buds of species from the *Plinia* and *Myrcia* group and in *Algrizea minor*. Perianth completely or partly removed in all. (A) *Plinia cauliflora* (*Plinia* group). Longitudinal section of nearly pre-anthetic bud, showing strongly incurved filaments and anthers facing downwards. Some of them do not fit inside the hypanthial cup due to the reduced size of the bud. Petals and sepals were not removed. (B) *Myrciaria floribunda* (*Plinia* group). Pre-anthetic bud from above, with strongly incurved filaments and anthers not visible. (C) *Myrcia sylvatica*, (D) *Gomidesia* sp., (E) *Myrcia spectabilis* and (F) *Calyptranthes aff. blanchetiana* (all *Myrcia* group) and (G) *Algrizea minor* (ungrouped, sister to *A. macrochlamys*) in pre-anthetic stage, showing filaments strongly incurved with anthers not visible from above. (H) Detail of strongly incurved stamens in mid-development in *Myrcia spectabilis* (*Myrcia* group). Filaments are strongly incurved and force the anthers to the bottom of the hypanthial cup which is formed by the extended hypanthium. a, anther; Br, bracts; Brl, bracteoles; f, filament; Pe, petal scar; Se, sepal scar; st, style. Scale bars = 500µm (A,C), 1mm (B,F,G,H), 2mm (D,E).

In all samples of the *Plinia* group (*Plinia cauliflora,* Fig. 2.4A; *Myrciaria floribunda,* Fig. 2.4B; *Siphoneugena densiflora, Neomitranthes obscura* and *N. cordifolia*), filaments were strongly incurved in the bud, a characteristic that is visible even with the naked eye. *Plinia cauliflora* is exceptional - it has very small buds and even though the filaments are strongly incurved, the anthers are sometimes visible from above because they do not physically fit in the bud and they are therefore pushed outside (Fig. 2.4A). In all samples of the *Myrcia* group (*Calyptranthes aff. Blanchetiana,* Fig. 2.4F*; Gomidesia* sp., Fig. 2.4D; *Myrcia amplexicaulis*, *M. aff. eriopus, M. laxiflora, M. pubipetala, M. splendens, M. trimera, M. truncada, M. spectabilis,* Fig. 2.4E,H; *M. sylvatica,* Fig. 2.4C) and in *Algrizea* (Fig. 2.4G) the stamens are also strongly incurved in the pre-anthetic bud, and the anthers are never visible from above. In both *Plinia* and *Myrcia* groups, as well as in *Algrizea*, the hypanthium is often extended forming a hypanthial cup. In all samples analysed from buds in early stages, the filaments remain strongly incurved throughout the development of the bud forcing the anthers downwards to the bottom of the hypanthial cup (Fig. 2.4H).

2.7 Anthesis type

Species of different genera within *Eugenia* group, *Pimenta* group (MEP clade), *Myrcia* group and *Plinia* group (PAM clade) were found with anthetic buds and photographed in the field. All species of the genera *Eugenia* and *Myrcianthes* (*Eugenia* group) as well as *Acca*, *Campomanesia* and *Psidium* (*Pimenta* group) presented a similar type of anthesis. In these species, the anthers are the first organs to appear after anthesis, and their filaments seem to have a continuous growth during and after bud opening (Fig. 2.5A–F). On the other hand, all species of *Myrcia* s.l. (*Myrcia* group), *Myrciaria*, *Plinia* (*Plinia* group) as well as *Blepharocalyx salicifolius* and *B. eggersii* (*Pimenta* group) showed a different type of anthesis. In these species, the filaments (which were strongly incurved before anthesis) are the first part of the androecium to appear, and it has to be unfolded during anthesis to expose the anthers for pollination (Fig. 2.5G–L).

Figure 2.5 (next page). Different anthesis types in Myrteae. Taxa with straight stamens in the bud and anthers which emerge first from the bud found in (A) *Eugenia cristaensis* (*Eugenia* group), (B) *E. stictosepala* (*Eugenia* group), (C) *E. adenocalyx* (*Eugenia* group), (D) *E. involucrata* (*Eugenia* group), (E) *Psidium guajava* (*Pimenta* group) and (F) *P. acutangulum* (*Pimenta* group). Taxa with incurved stamens in which the filaments have to straighten first before the anthers face outwards found in (G) *Calyptranthes brasiliensis* (*Myrcia* group), (H) *Myrcia sect. Aulomyrcia* (*Myrcia* group), (I) *M. splendes* (*Myrcia* group), (J) *M. subavenia* (*Myrcia* group), (K) *Myrciaria floribunda* (*Plinia* group) and (L) *Blepharocalyx eggersii* (*Pimenta* group). (All photos taken during field expeditions between 2014 and 2016).



DISCUSSION

2.8 Systematic Implications of Stamen Posture and Anthesis Type in Myrteae

Results show clear differences in pre-anthetic stamen posture in different genera of Myrteae. In our analyses, we found three different patterns of stamen posture in the bud (Fig. 2.6). (1) Straight stamens. Species with this pattern show straight to slightly curved filaments. Removal of calyx and corolla reveals the anthers of almost all staminal whorls visible from above. Pre-anthesis anthers touch the inner surface of the corolla (Fig. 2.6A). This pattern was found in *Myrtus communis* and the Australasian, *Eugenia*, *Myrteola* and *Pimenta* groups. (2) Semi-curved stamens. Species with this pattern show straight to slightly incurved outer filaments. The inner filaments on the other hand, present strongly incurved stamens and anthers facing downwards. On removal of the calyx and corolla, only anthers from the outer whorls are visible from above (Fig. 2.6B). This pattern was found consistently in the Myrceugenia group (*Luma apiculata*, *Myrceugenia alpigena*, *M. planipes*, *M. bananalensis.* and *Blepharocalyx cruckshanksii*). (3) Strongly incurved stamens.

Species with this pattern show strongly incurved pre-anthetic stamens. Here, all filaments are acutely curved down towards the centre of the bud and all anthers touch the bottom of the hypanthial cup. After removal of the calyx and corolla, anthers are obscured by filament tissue on the view from above (Fig. 2.6C).

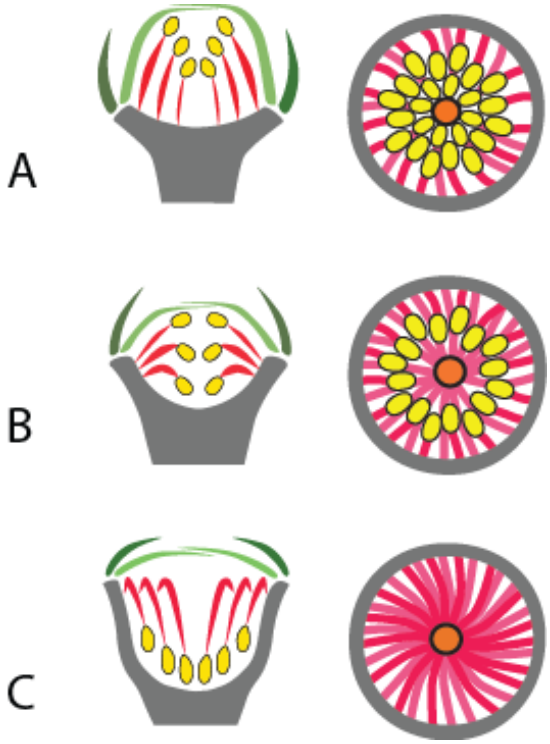


Figure 2.6: Variation in stamen posture in Myrteae. Position of filaments and anthers in longitudinal section (left) and anther visibility from above in the bud after removing petals and sepals (right). (A) Straight stamens pattern. (B) Semi-curved stamens pattern. (C) Strongly incurved stamens pattern. Colours indicate: grey = hypanthium, red = filaments, yellow = anthers, light green = petals, dark green = sepals, orange = gynoecium.

Regarding the type of anthesis, the two different extremes of stamen posture, straight stamens and the strongly incurved stamens, also seem to be related to differences of anthesis types. In the first type, anthers are presented first and the latter filaments have to unfold to anther exposition. Proença and Gibbs (1994) already reported differences in stamen behaviour during anthesis in different Myrteae species. Those authors classified species in which anthers are upright during this phase as "*Psidium* like" (corresponding to 'straight stamens' as defined here) and those where filaments unfold at anthesis to expose the anthers, (corresponding to 'strongly incurved stamens' as defined here) were classified as "*Myrcia* like".

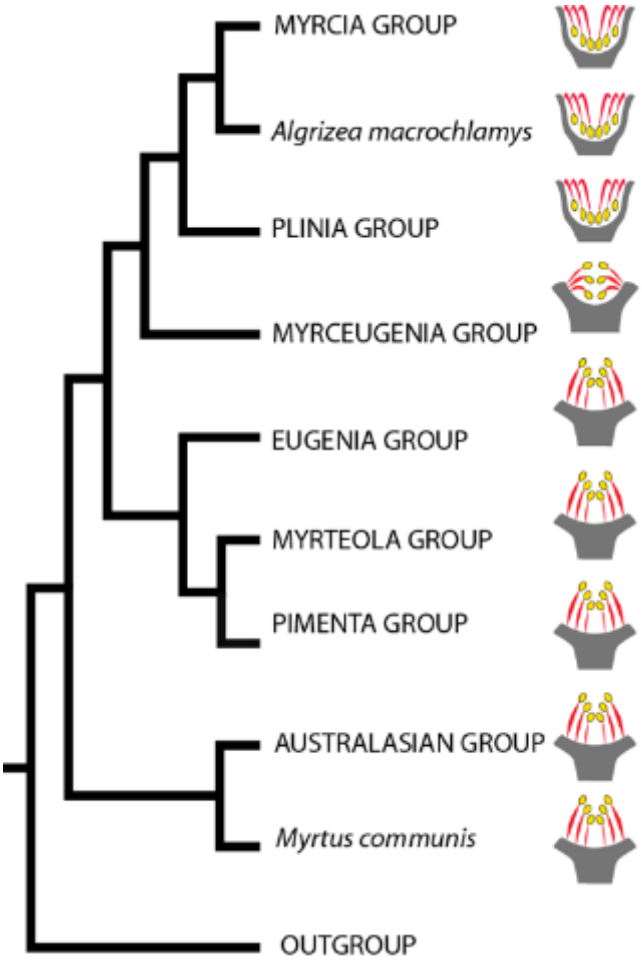


Figure 2.7: Summary tree of Myrteae phylogeny with stamen patterns per clade. Colours indicate same structures as Fig. 2.6.

An examination of pre-anthesis stamen posture and consequently anthesis type against a summary of the phylogenetic Myrteae hypothesis (De-Carvalho, 2013) demonstrates that these characters are congruent with the phylogenetic topology (Fig. 2.7). Straight pre-anthesis stamens with *Psidium* like anthesis appears to be a pleisiomorphic state in Myrteae. This character is found in the *Myrtus communis* + Australasian group clade at the base of the tribe and most of the MEP clade, except *Pimenta* and *Blepharocalyx salicifolius*. The incurved stamens and *Myrcia* like anthesis of the PAM clade as well as in *Pimenta* and *Blepharocalyx salicifolius* may have a secondary evolutionary origin. The PAM clade is recovered in all phylogenetic works with high bayesian and bootstrap support (Lucas *et al*., 2007; Costa, 2009; De-Carvalho, 2013) and incurved stamens appear to be synapomorphic for this clade. The *Myrceugenia* group at the base of the PAM clade presents semi-curved stamens and it is tempting to interpret this as an intermediate stage between the straight stamens of the MEP clade and the strongly incurved stamens of the PAM clade (see also Chapter 3). Strongly incurved stamens occur in *Blepharocalyx salicifolius* and in *Pimenta,* providing support for a possible placement of *B. salicifolius* within the *Pimenta* group (P.S. De-Carvalho, personal communication).

2.9 Relationship between stamen posture and hypanthium extension

As inferred by previous studies (Proença, 1992; Belsham and Orlovich, 2002, 2003), our study demonstrates a relationship between pre-anthesis stamen posture and hypanthium extension. In general, species with an extended hypanthium have incurved stamens developing just below the hypanthial rim suggesting that hypanthial extension has 'carried' the stamens upwards (also suggested by C.E.B. Proença, in personal communication). On the other hand, species with no hypanthial extension (i.e. a flat floral base) generally have straight stamens and those with short hypanthial extension have a semi-curved pattern.

Nevertheless, exceptions exist to these rules. Belsham and Orlovich (2002, 2003) studied androecium and hypanthium ontogeny in the *Myrteola* group. These studies found *Lophomyrtus* and *Neomyrtus*, two closely related New Zealand genera, to initially have a short hypanthium cup (exceptional within genera with straight stamens) with laterally and slightly incurved stamens resembling *Luma apiculata* (*Myrceugenia* group). In pre-anthesis bud however, *Lophomyrtus* and *Neomyrtus* stamens assume the straight position as the other species of *Myrteola* group. This also seems to occur in *Campomanesia* (*Pimenta* group), where the stamens are straight but the hypanthial extension is also variable.

In the *Myrcia* group, where the strongly incurved stamens occur consistently, one clade (*Myrcia* sect. *Myrcia*, sensu Lucas *et al*., 2011) does not have an extended hypanthium. Interestingly, results for this group also show strongly incurved pre-anthesis stamens despite the non-extended hypanthium (Fig. 2.4C). This suggests that the flat hypanthium may be a secondary condition that arose from the extended-hypanthium state with the remaining curved stamens a 'relictual' arrangement.

2.10 Evolutionary Implications of Hypanthial Extension, Stamen Posture in the Bud and Floral Ecology

The present study concludes that floral evolution played a role in the divergence of Myrteae, together with other aspects associated with dispersal such as embryo type (Landrum and Kawasaki, 1997). Stamens are recognized to be a variable condition in Myrtales (Decraene and Smets, 1991) and the evolution of these characteristics within Myrteae may have been a driving force in its diversification, especially in the Neotropical lineages where stamen posture is a character that separates the two big clades (i.e. the MEP from the PAM + *Myrceugenia* group).

Differences in hypanthium extension (leading to stamen posture in the bud) and anthesis type may be linked to shifts in pollination strategy. Proença (1992) noted that Myrteae species with extended hypanthia often show pollen collection via "buzz-pollination", and the hypanthium extension may assist the bees grip of flowers during vibration. Species of *Myrcia* s.l. (*Myrcia* Group) and *Siphoneugena* (*Plinia* group) with extended hypanthia attract bees that use buzz strategies, while in *Campomanesia*, *Psidium* (*Pimenta* group) and *Eugenia* (*Eugenia* group), species with flat hypanthia, buzzing was not reported (Proença, 1992). Exceptionally, *Myrcia linearifolia*, a species without extended hypanthia (as *Myrcia sylvatica* – Fig. 2.4C), was the only *Myrcia* that did not attract buzz-pollinators. This provides further support for the suggestion that hypanthia are implicated in buzz-pollination. However, Fidalgo and Kleinert (2009) also observed buzz-pollination in species of

Myrteae without hypanthial extension. Further field as well as experimental studies are required to clarify the function of the hypanthium in connection with pollen collecting bees.

An alternative hypothesis of the adaptive advantage of hypanthial extension is linked to the transition from pollen to nectar as the main floral reward. Hypanthial extension ultimately leads to the formation of a hypanthial cup which is frequently linked to nectar production (Harder and Cruzan, 1990). Myrteae flowers are known to be almost exclusively pollen-reward flowers (Nic Lughadha and Proença, 1996). Nevertheless, there are records of nectar presence in flowers of *Plinia* (*Plinia* group; Malerbo *et al.*, 1991; Pirani and Cortopassi-Laurino, 1993), a genus with a hypanthial cup, and also in flowers with hypanthial cups in other tribes of Myrtaceae as Syzygieae (Lack and Kevan, 1984; Crome and Irvine, 1986; Abe, 2006) and Eucalypteae (Bond and Brown, 1979) suggesting that these two characteristics may be linked in the family.

Adaptative advantages in having a flat hypanthium and straight stamens can also be hypothesized. *Eugenia* and *Psidium*, two genera with straight stamens and "*Psidium* like" anthesis have filaments and styles that grow continuously after anthesis (Proença & Gibbs, 1994; Silva & Pinheiro, 2007). Under this scenario, pollen is immediately available at anthesis (Silva and Pinheiro, 2007) which may lead to a higher degree of pollination success. In addition, Silva and Pinheiro (2007) analysed reproductive biology of six species of *Eugenia* and noted that the straight, continuously growing style might help with self-pollination by making contact with mature anthers during growth. Furthermore, Fidalgo and Kleinert (2009) compared *Eugenia speciosa* to five other Myrtaceae species from other genera and found it was the only species with significant fruit production when pollinators were excluded. Proença and Gibbs (1994) also found *Eugenia dysenterica* and *Psidium firmum* to be completely self-compatible, with *Psidium firmum* having an even higher percentage of fruit-set in self-pollinated flowers. More information about the relationship between stamens, hypanthium and Myrteae evolution are presented in Chapters 3 and 5.

2.11 Systematic Implication for Straight Stamens in Myrtales

Classic works (Jonson and Briggs, 1984; Dahlgren and Thorne, 1984) cite incurved stamens in the bud as a synapomorphy of the order Myrtales with exceptions in Onagraceae, Vochysiaceae, *Heteropyxis* and *Psiloxylon*. The present study extends the exception to most taxa of the tribe Myrteae. Buds of *Heteropyxis* were also checked to understand these authors' definition of "straight stamens". Despite *Heteropyxis* having only 5 stamens, these stamens strongly resemble the straight stamens recorded here in polystemonous Myrteae flowers.

Molecular phylogenetic studies in Myrtales in conjunction with character evolution interpretations showed incurved stamens as the likely plesiomorphic state in Myrtales, with straight stamens evolving independently in Onagraceae, Vochysiaceae, *Heteropyxis* and *Psiloxylon* (Johnson and Briggs, 1984; Conti et al., 1997). Conti et al. (1997) further hypothesized that straight stamens are the plesiomorphic condition for the Vochysiaceae + Myrtaceae clade and that this character was lost in the subfamily Myrtoideae. Our results challenge this hypothesis, by suggesting that straight stamens are plesiomorphic in the tribe Myrteae, even though in other tribes of Myrtaceae pre-anthesis stamens are mostly strongly incurved (Drinnan and Ladiges, 1991; Orlovich et al., 1999; Bohte and Drinnan, 2005; Drinnan and Carrucan, 2005). Further character

reconstruction studies are required in order to better understand the evolution of this character across Myrtales (see Chapter 5).

CONCLUSIONS

This study reveals a previously undetected morphological pattern within Myrteae that consolidates taxonomic understanding in the tribe and provides means for specimen identification to genus level. Pre-anthesis buds are the most common phase found in herbarium specimens (T.N.C. Vasconcelos, personal observation) and can be easily manipulated to verify if anthers are visible from above when the perianth is removed. This, aligned with other traditional characteristics can be used as a complementary identification tool in the field and herbarium. At tribal level, stamen pattern is more consistent than the inflorescence, embryo, placentation, number of locules per ovary, number of sepals, and other characteristics that have been used in Myrteae systematics before. Congruent characters are rare in Myrteae, although recent work on the development of the gynoecium (Pimentel et al., 2014) has found other positively correlated characters, indicating that characters of the flower development might be important to understand evolution in Myrteae.

Chapter 3: A systematic overview of floral diversity in Myrteae (Myrtaceae)

Manuscript – to be submitted to *Taxon*

- T.N.C.Vasconcelos contributions: collection of samples, morphological analyses, generation of SEM images, literature review and writing of manuscript.

ABSTRACT

Myrteae is the largest tribe of Myrtaceae and one of the most diverse groups of flowering plants in the tropical Americas. In light of recent systematics adjustments, the present study is a review and provides new insights into floral diversity and evolution of Myrteae. General aspects of floral ontogeny and morphology for c.40 accepted genera plus all accepted sections within the large genera *Eugenia* and *Myrcia* are described and discussed, and systematic relevance is examined. Results and discussion provide a broader understanding of the floral diversity across the tribe, highlighting developmental modes, ecological traits and specializations in reproductive strategies.

Key words: androecium, evolution, gynoecium, perianth, morphology, ontogeny.

INTRODUCTION

Myrtaceae is a species rich angiosperm family of which half of the biodiversity (c. 2500 species; WCSPF, 2017) occurs within a single monophyletic group with main distribution in the Neotropics: tribe Myrteae (sensu Wilson et al., 2005). Myrteae comprises some of the highest tree species diversity in South American forests and savannas (Oliveira-Filho and Fontes, 2000; Beech et al., 2017) where it has a critical ecological role as a dominant flower and fruit supplier, sustaining associated fauna during the whole year (Staggemeier et al., 2010, 2017). Consequently, recent studies have advocated Myrteae as a useful model group for understanding biodiversity ecology, evolution and conservation in Neotropical environments (Murray-Smith et al., 2009; Lucas and Bünger, 2015; Staggemeier et al., 2015; Giaretta et al., 2015).

Despite its crucial role in Neotropical ecology, Myrteae has been a group of notoriously complicated systematics due to its highly homoplastic traits and superficially homogeneous morphology (Mc Vaugh, 1968; Landrum and Kawasaki, 1997). Initial molecular phylogenetic studies (Lucas et al., 2005, 2007) showed that traditionally used characters, such as the embryo type (Berg, 1855-56), had little power to accurately explain relationships in Myrteae and that morphological characters with which to characterise natural groups are few and poorly understood. In light of recent systematic rearrangements (Lucas et al., 2007; Chapter 1), a search for characters that explain relationships and diagnose natural lineages is required. Chapter 2 highlights stamen posture as a valuable diagnostic characters, demonstrating that dispite its apparent homogeneous morphology floral traits can explain systematics and evolution in Myrteae. Descriptive works are currently out of vogue but reassessment of traits in light of newly available phylogenetic frameworks, in a process of reciprocal illumination, will be of utmost importance for future studies of systematics, ecology and evolution.

This study provides a thorough documentation of floral diversity across Myrteae genera. Information is assembled from literature, herbarium material, floral ontogeny and field observations to propose more reliable diagnostic characters, stimulate debate of form and function and generate new hypothesis to be tested by future studies.

MATERIAL AND METHODS

Buds and flowers were collected in the field, from living collections or sampled from herbarium material. Fruits were analysed in some cases. c.40 genera of Myrteae were surveyed (see Appendix 3.1). For the largest genera (*Myrcia* c. 700 spp, Lucas et al., 2011; Eugenia, c. 1000 spp, Mazine et al., 2016), specimens representing all accepted sections were also analysed. Herbarium specimens surveyed were those identified by specialists and were re-hydrated for dissection. Descriptions of anthesis are based on comparison of buds and open flowers from herbarium specimens and field observations during field expeditions between 2013 and 2016 or occasionally based on pictures sent by specialists. Ontogenetic discussion is based exclusively on specimens collected in ethanol 70% or FAA in the field. For SEM preparation, buds were dissected and passed through an ethanol series until full dehydratation, critical point dried using an Autosamdri-815B critical-point dryer, mount into stubs, platinum coated using a Quorum Q-150-T sputter coater and analysed under a Hitachi cold field emission SEM S-4700-II. The total list of analysed specimens is given in Appendix 3.1.

3.1 Perianth (calyx and corolla)

Perianth as calyx and corolla are usually treated together (e.g. Endress, 1994) even though they may have distinct evolutionary histories (Ronse DeCraene, 2008). In Myrteae, there is a historical taxonomic interest in the variation of perianth characters (e.g. Landrum, 1984; Lucas et al., 2011), especially the calyx, due to its frequent persistence even at fruiting stage (since Linnaeus 1779, who divided *Myrtus* from *Eugenia* based on merism). The majority of flower diversity in Myrteae can be divided into two main calyx organisations: pentamerous, with classic imbricate quincuncial aestivation (i.e. two sepals without, two sepals within and one in between, Fig.3.1Ai-iv); or tetramerous, with two pairs of sepals developing decussately (Fig.3.1Bi-iv). Dimerous flowers are rare but are the rule for at least one species of *Blepharocalyx* (*B. eggersii*, Fig.1Ci-iv, Fig.2G). Flowers in opposite position in an inflorescence have opposite directions of perianth development (clockwise and anticlockwise, Fig.3.1D).

Sepals are usually of the same size when buds are mature, but both pentamerous and tetramerous flowers may have sepals slightly to strongly unequal in size even at late stages. Such size distinctions carry taxonomic relevance in some groups (e.g. cited in Scott, 1979, for *Rhodomyrtus*, and in Sobral, 2005, for *Eugenia inversa*; see Fig. 3.1E) but usually do not significantly change overall floral symmetry. Calyx fusion is a common trend observed in a few to several species in different lineages (Fig. 3.1I,J; e.g. Landrum, 1984, in *Myrceugenia*; Parra, 2016, in *Myrcianthes*). This is achieved mostly by post-genital fusion, when the base of initially free sepals become a homogeneous tissue just after calyx initiation (see Chapters 4 and 6 for evolutionary interpretation of this character). Anthetic behaviour of this structure varies from a “calyptra” to irregular tearing (as in Fig.3.1J), patterns with historical taxonomic relevance (Mc Vaugh, 1968; Wilson et al., 2016; see also Chapter 6).

The corolla develops after the calyx. Petals are always alternisepalous and are usually present in the same number as sepals. Flowers with five sepals develop five petals in alternate positions, following the same imbricate quincuncial aestivation pattern (Fig.3.1Aiv). Flowers with four sepals tend to have four petals that are almost simultaneously initiated, in contrast to the decussate pattern of the sepals (Fig.3.1Biv). Petals are either rounded or elliptic and are attached to the hypanthium by a very narrow base, making them easily caducous. There are few exceptions in petal arrangement among all the c.50 genera. The most remarkable ones are in the tetramerous genus *Octamyrtus*, where a second corolla whorl and sometimes two extra petals develop (summing up to 10 petals in total; Svott, 1978; Craven, 2006); and *Myrtus* that commonly presents particularly narrow petals developing in somewhat indefinite whorls (Mulas and Fadda, 2004).

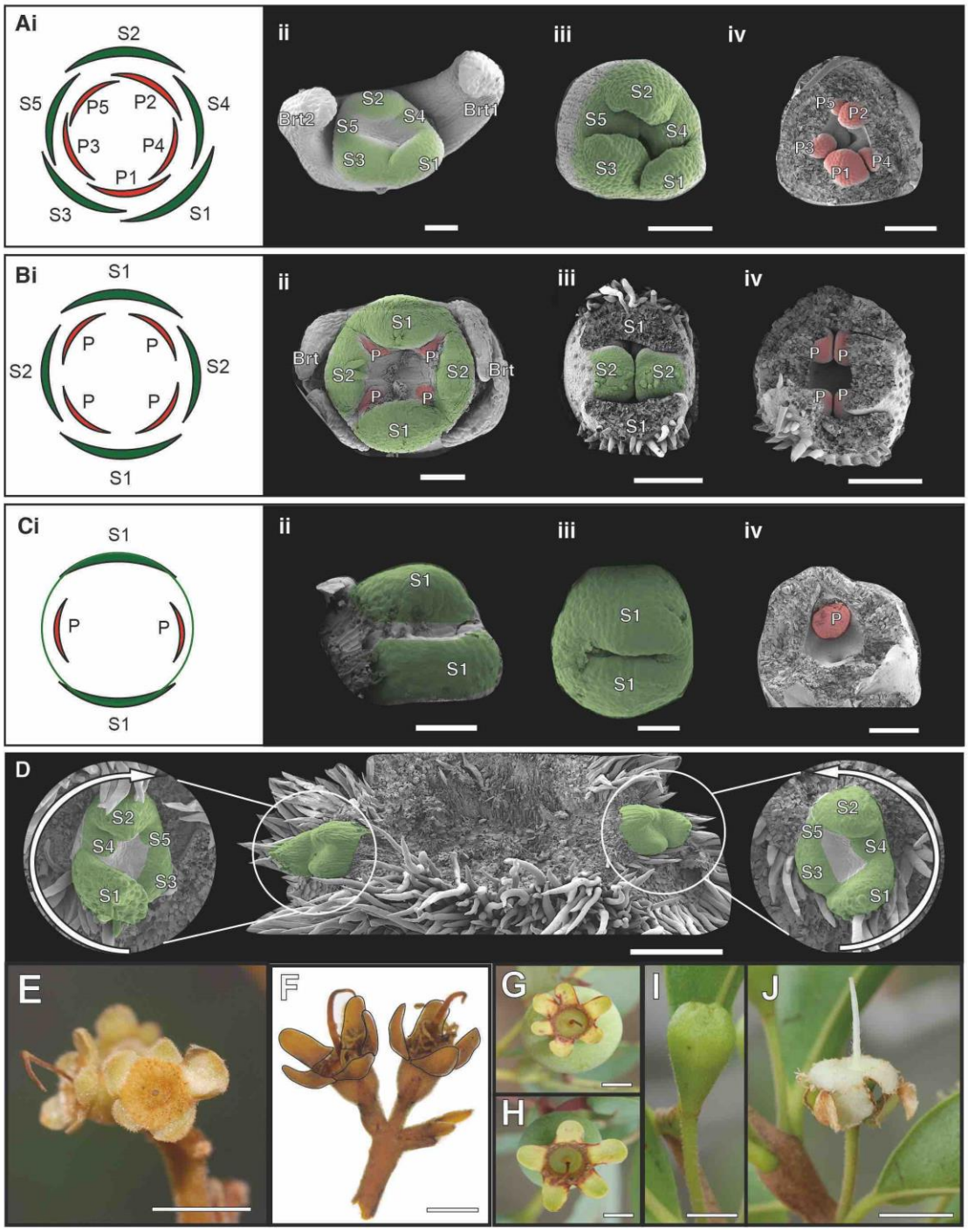


Figure 3.1: Patterns of perianth arrangement in Myrteae. (A) Pentamerous flowers with imbricate quincuncial arrangement: (Ai) Floral diagram and (Aii-Aiv) ontogenetic sequence in *Myrcia* cf. *guianensis* (*Myrcia* sect. *Aguava*). (B) Tetramerous flowers with decussate arranged sepals and four equal petals: (Bi) Floral diagram and (Bii-Biv) ontogenetic sequence in *Eugenia stipitata* (*Eugenia* sect. *Pilothecium*); (C) Bimerous flowers blabla: (Ci) Floral diagram and (Cii-Civ) ontogenetic sequence in *Blepharocalyx eggersii*. (D) Clockwise and anticlockwise direction of perianth development in opposite flowers of *Myrcia spectabilis* (*Myrcia* sect. *Gomidesia*). (E) Unequal sepals in old flowers of *Myrcia splendens* (*Myrcia* sect. *Myrcia*). (F) Variation of merism in two flowers of same inflorescence in *Algrizea minor* and (G,H) in the same individual of *Campomanesia adamantium*. (I,J) Calyx fusion in *Psidum* sp, showing torn calyx after anthesis. S,

sepals; P, petals. Scale: 50µm (Aii,Bii,Cii,Ciii), 100µm (Aiii,Aiv,Biii,Civ), 150µm (Biv,D), 5mm (E,F, G,I,J). (E-I photos taken during field expeditions between 2014 and 2016).

Shifts back and forth between tetramerous and pentamerous flowers are likely to have occurred multiple times in Myrteae. Variation between four and five perianth parts is commonly observed at infrageneric and even at infraspecific levels (e.g. Fig.3.1F-H). The norm, however, is that the lower the taxonomic level the more stable is merism. Therefore, it is difficult to estimate with precision which pattern is the plesiomophic state for the tribe, but merism is still an important component of generic identification in Myrteae (e.g. keys in Landrum and Kawasaki, 1997; Sobral 2003; Mazine et al., 2014).

3.2 Androecium and hypanthium extension

The androecium has been neglected in Myrteae systematics. The almost invariable polystemonous flowers produce no superficially noticeable variation so specific references to the relevance of the androecium is virtually absent in classical Myrteae taxonomic literature (e.g. even in extensive reviews such as Mc Vaugh 1968 and Landrum and Kawasaki, 1997). However, variations discussed in Chapter 2 show that the androecium harbours valuable taxonomic characters, especially when considered alongside hypanthium development (e.g. Belsham and Orlovich, 2002, 2003; see also Chapter 5).

The definition of hypanthium is somewhat obscure in the literature. General floral morphologists define the hypanthium as a cup-shaped structure that involves the ovary in perigineous and epigineous flowers (Weberling 1989, Endress 1994). Werbeling's hypanthium is also somewhat tangled with his definition of the floral receptacle, for which he states that "[perianth and androecium] appear inserted on the edge of the hypanthium, or so called receptacle" (Weberling, 1989, p. 20). While some authors prefer to use the term "receptacle" (e.g. Ronse DeCraene and Smets, 1992, 1993), most Myrtaceae literature adopts the term "hypanthium" to refer to this tissue between perianth and gynoecium (e.g. Proenca et al., 2006; Snow and Wilson, 2010; Amorim and Alves, 2012; Martos et al., 2017). It is on the surface of this tissue that the stamen primordia appear and the stamens of the polyandrous androecium develop (Ronse DeCraene and Smets, 1991; Belsham and Orlovich, 2002, 2003). In this sense, it is impossible to fully separate androecium from hypanthium when discussing floral morphology of Myrteae. In Myrteae, mature flowers present two main hypanthium types: these can be either extended above the ovary, forming a hypanthium tube (e,g, in *Myrcia* and *Siphoneugena*) or flat, non-extended above the ovary (e.g. *Eugenia*). Development of these two patterns is very similar during floral ontogeny; the difference is mainly the extent to which stamen primordia cover the tissue during early stages of development.

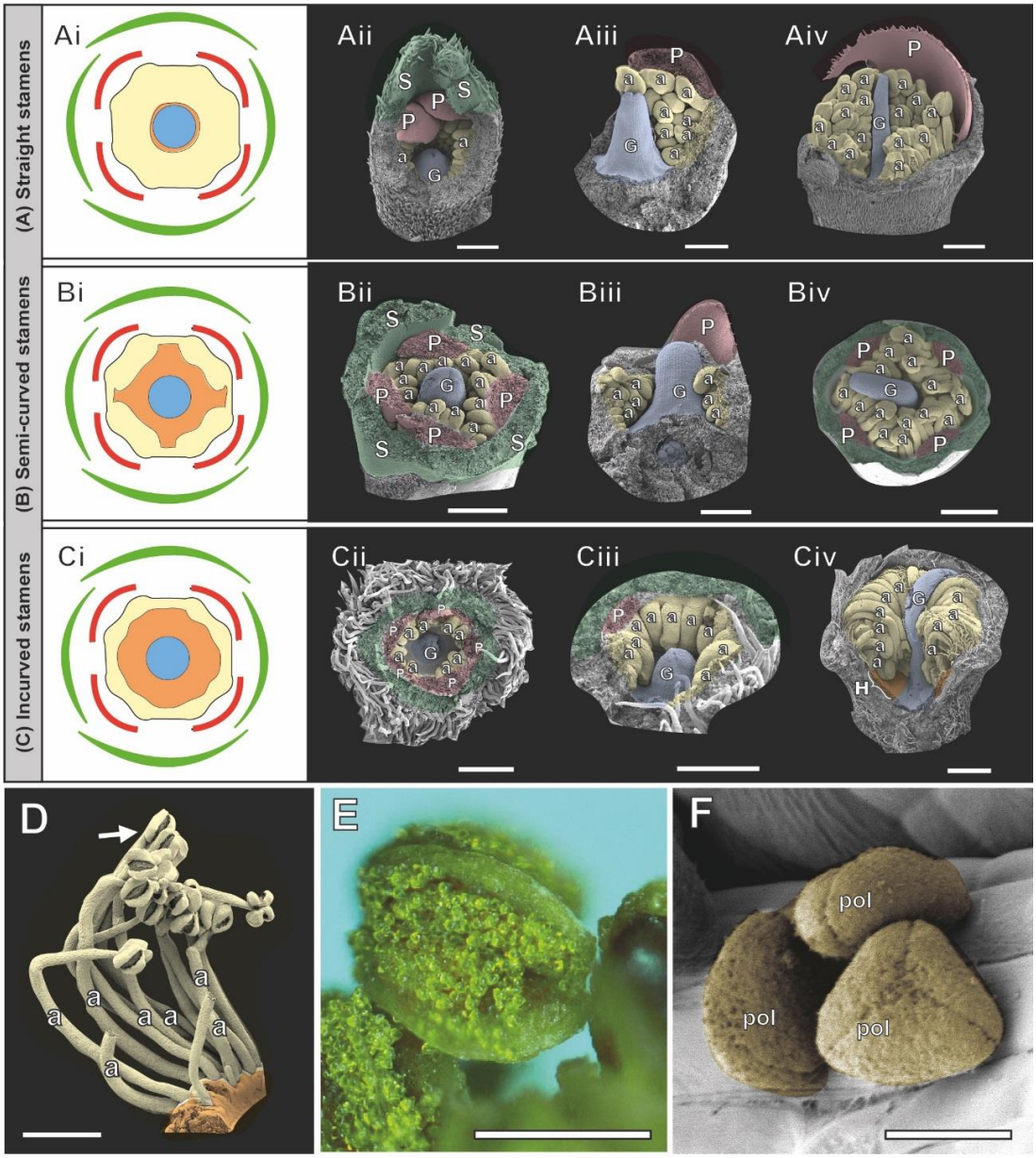
Early androecium development in Myrteae occurs by the appearance of two (or more) stamen primordia at the flanks of each petal (Belsham and Orlovich, 2002, 2003; Chapter 5). Sequentially, more primordia develop forming the first staminal ring. This is contrary to other Myrtaceae where secondary polyandry is clear from the appearance of obhapolostemonous first stamen primordia (e.g. *Melaleuca*; Carrucan and Drinnan, 2000); in Myrteae there is no clear definition between primary and secondary stamen primordia (as described for other Myrtaceae by

Ronse Decraene and Smets, 1991). After the first staminal ring is formed, more stamen primordia initiate centripetally. The degree to which these primordia cover the inner hypanthial surface determines the final position of the stamens within the flower (as observed in Chapter 2). When the stamen primordia cover the whole hypanthium tissue up to the stylar base, stamens in the bud appear straight and no hypanthial tube is observed (Fig. 3.2A). When the stamen primordia cover just a restricted area at the hypanthium rim during development, stamens bend into the area provided by the "bare" hypanthium tissue, resulting in curved stamens at anthesis and a hypanthium tube of variable length (Fig. 3.2C). Straight vs. curved stamens proved to be a trait with high systematic relevance, explaining some intrageneric relationships recovered by the molecular phylogeny of Myrteae (Chapter 2). A third variation is the discontinuous androecium observed in *Lewenbbia*, *Luma*, *Myrceugenia* and some *Pimenta* species (Belsham and Orlovich, 2003; Snow et al., 2000). In these flowers, the first stamens develop from primordia below each petal whilst only subsequent stamens form the continuous ring (Fig. 3.2B; Belsham and Orlovich, 2003). This discontinuous development gives the stamens a position that can be coarsely described as "semi-folded" in the bud (Chapter 2).

Anthers are always tetrasporangiate, consisting of four pollen sacs that differentiate at later stages of floral development at the distal portion of each filament. Abaxial pollen sacs are usually smaller than adaxial ones, and laterose dehiscence occurs by a simple longitudinal slit (Fig. 3.2D), with the tearing of the thin tissue between each pair of pollen sacs (as in most eudicots; Endress, 1994). Anthers are dorsifixed, except in *Ugni* and *Uromyrtus* where they are somewhat basifixed (Snow and Cantler, 2010; Wilson, 2011). During anthesis or even slightly before, tissue that connects each pair of pollen sac tears. At this point, the walls of all four locules retract completely, giving an opening of c. 180 degrees for each lateral pair of pollen sacs (Fig.3.2E). Specialisations in this dehiscence behaviour do occur and examples include apiculate connectives in some species of *Campomanesia* (Landrum, 1986); reticulate pollen sacs in some species of Eugenia (B.S.Amorim pers. com) and disproportionally long anthers with slightly dislocated pollen sacs of *Myrcia* sect. *Gomidesia* (Lucas et al., 2011). In the latter, possibly due to an uneven growth of the connective, locules wall retraction is not always complete, giving a somewhat poricidal aspect that is associated with buzz pollination (Proenca, 1992; Nic Lughadha, 1998). Pollen grains are small to medium sized, triangular shaped and brevicolpate, with very little variation between Myrteae lineages (see recent review by Thornhill et al., 2012).

Figure 3.2 (next page): Three main patterns of stamen development along the hypanthium and common system of anther dehiscence and pollen exposure in Myrteae. (A) Straight stamens developmental pathway, where stamen primordia cover the whole hypanthial tissue; (Ai) Floral diagram and (Aii-iv) ontogenetic sequence in *Eugenia dichroma*. (B) Semi-curved stamen pathway, where stamen primordia is form discontinuous rings on hypanthial tissue; (B) Floral diagram and (Bii-iv) ontogenetic sequence in *Luma apiculata*. (C) Folded stamen developmental pathway, where stamen primordia are restricted to the rims of the hypanthial tissue. (C) Floral diagram and (Cii-iv) ontotogenetic sequence in Myrcia subcordata. (D) Anther dehiscence in *Blepharocalyx salicifolius*, showing most common longitudinal laterorse pattern of dehiscence (arrow). (E) Anther completely open exposing pollen grains in *Myrcia eugeniopsoides*. (F) Small triangular-shaped pollen

characteristic of Myrteae, exemplified by *Eugenia involucrata*. A, androecium; G, gynoecium; P, petal S, sepal; 'pol', pollen grain. Scale: 10µm (F) 250µm (Aii,Aiii,Bii,Biii,Cii,Ciii,E), 500µm (Aiv,Biv,Civ), 1mm (D).



3.3 Gynoecium

The gynoecium is the most variable floral organ complex in Myrteae. Characters related to the gynoecium are present in buds, flowers and fruits (when well dissected, locules and aborted ovules can be seen against the ovary wall; e.g. Fig. 3.8). Traits of the gynoecium are usually infragenerically consistent and intragenerically variable, highlighting the convenience of this character for taxonomic diagnosis (as discussed by Bentham, 1869; and Kausel, 1956). During the last decades, several genera were described based mainly on gynoecium characters (e.g. *Accara*, Landrum, 1990; *Chamguava*, Landrum, 1991; *Gossiam,* Snow et al., 2003). Overall gynoecium morphology also has a strong evolutionary component, as it affects the width of the stigma (e.g. Fig. 3.5), the length of the style, and possibly the number of ovules that can be fertilized (see sections 3.7 and 3.8 in the following pages). Variation in the morphology of this structure is,

however, difficult to record. The position of the inferior ovary and distinct patterns of ovule arrangement, placentation and carpel fusion create a complex system of tunnels and chambers. Consequently, distinct arrangements can only be appreciated when information from transversal, longitudinal and tangential sections are combined (see Figs 3.3 and 3.4).

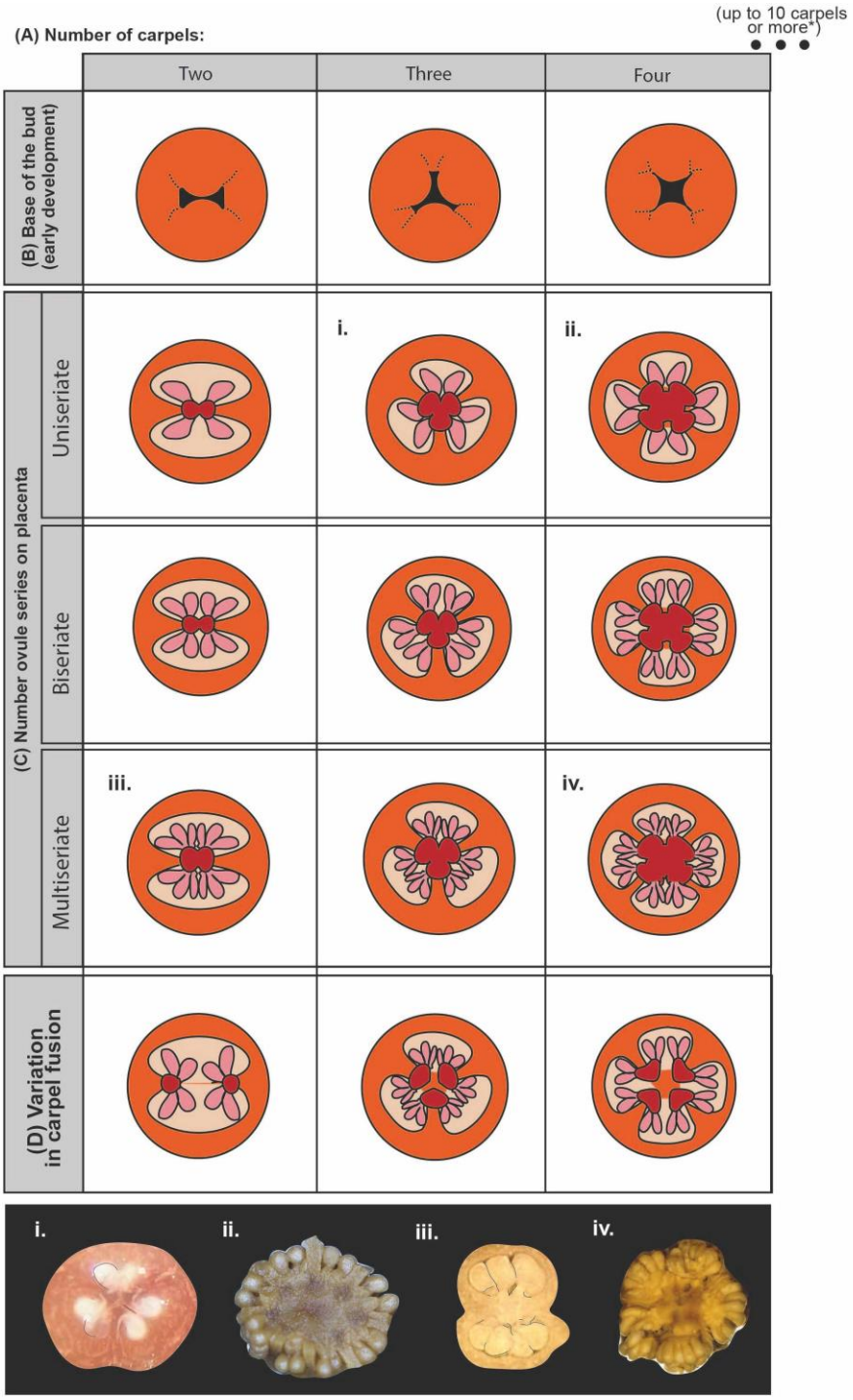


Figure 3.3– Diagrams of transversal cuts in ovaries of Myrteae; showing variation in number of locules, number of ovule series on placenta and carpel fusion. (A) Number of carpels usually relates to the number of locules, unless fusion is not complete (few exceptions). (B) Fomat of depression left on the base of the bud at early developmental stages, suggesting post genital fusion of carpels. (C) Arrangement of ovules on placenta varies from uniseriate to multiseriate. (D) Examples of when

closure of carpels is not complete (as it happens in *Rhodamnia*, *Myrtus* and *Acca*). (i – iv) Examples of (i) trilocular ovary with uniseriate ovule arrangement on placenta (exemplified by *Rhodomyrtus tomentosa*); (ii) multilocular (eight locules) ovary with uniseriate ovule arrangement on placenta (exemplified by *Campomanesia adamantium*); (iii) bilocular ovary with multiseriate ovule arrangement on placenta (exemplified by *Eugenia uniflora*); (iv) multilocular (five locules) ovary with multiseriate ovule arrangement on placenta (exemplified by *Calycolpus goetheanus*). Color code: orange = ovary and locule wall; red = placenta; pink = ovule.

In Myrteae, early stages of gynoecium development are visible by a depression that appears in the centre of the flower base, simultaneous to androecium initiation. Carpel fusion is a combination of congenital fusion at the base and post-genital fusion at the top, so that it is usually possible to recognize how many locules are formed by the shape of the initial depression (Fig. 3.3B, Fig.3.5A,F; see also Chapters 5 and 6) or when the ventral slits are still visible. Sequentially, tissues around the depression swell to form a proto-stigma (Fig.3.5A,F). In species with multiple locules, the depression is larger and consequently the proto-stigma is broader, forming a stigma that is capitate or peltate, contrasting to a simple one in species with fewer locules (Fig.3.5). Meanwhile during early ovary development, each locule forms an individual chamber around the center of the ovary. At this point, a cauline axis protrudes from the base whilst an apical septum elongates from the apex, forming a central septum and the locule walls (Pimentel et al. 2014, Hartmann, 2016). The point where the cauline axis and apical septum meet can be very tightly closed, or slightly to completely open, providing connection between locules in some genera (e.g. *Myrtus*, Pimentel et al., 2014, Hartmann, 2016; Fig.3.3D). Placentation is axial and the placenta can develop either directly from the “cauline axis” (in *Myrcia*, *Plinia*, *Eugenia* and *Blepharocalyx* groups; “caulicine placentation” sensu Pimentel et al., 2014) or along the edges of the points where locule walls meet at the centre of the ovary (all other groups; “carpellar placentation” sensu Pimentel et al., 2014). Ovules develop attached to a “u” shape placenta protuberance that bypass the ventral slits on each locule. Ovules can be either organised in a single series around the placenta on each locule (uniseriate), two series (biseriate) or in series of ovules without clear organization (multiseriate). (See Fig.3.3C and Fig.3.4).

Number of ovules per locule and number of locules are commonly variable at lower taxonomic levels. Placenta format, a character cited as important in some studies (Landrum 1991,1992; Snow 2000), may be misleading because it distorts when the number of locules changes. Nevertheless, the systematic relevance of the ovary can be assessed by: 1) How many locules are there? 2) Are the locule septa completely closed or are the ventral slits still distinct? 3) At which point of the septum does the placenta protrude? 4) How many series of ovules exist on the placenta (uniseriate, biseriate, multiseriate)? The answers to these questions usually allow identification to a genus or group of genera with reasonable confidence (see Table 3.1).

3.4 Hairs and trichomes

Pubescence, or the presence of hairs, is a characteristic of most Myrteae flowers. These are mostly single-celled trichomes (Fig 3.6A) that give a silky appearance to the tissue where they grow. The presence or absence of hairs and where they occur on the floral surface is often

taxonomically consistent and thus useful for systematics. Examples include silky appearance of *Myrcia* sect. *Myrcia* buds in contrast to other *Myrcia* sections (Berg, 1855; Lucas et al., 2011), pubescent flowers of the Australasian group that distinguish them from other sympatric Myrtaceae (Low pers.com; Ashton, 2011); dibrachiate hairs that occur in *Myrceugenia* and *Myrcia* (Landrum 1981a,b); hairs on the locule walls in *Eugenia* sect. *Pillothecium* and some *Pimenta* (Fig.3.6B, Faria, 2014). The evolutionary significance for the presence of these hairs is not clear, but similar indumenta are associated with protection against predators (e.g. Breedlove and Ehrlich, 1972; Fig.3.6C) and reflective properties against solar radiation (Miller, 1986).

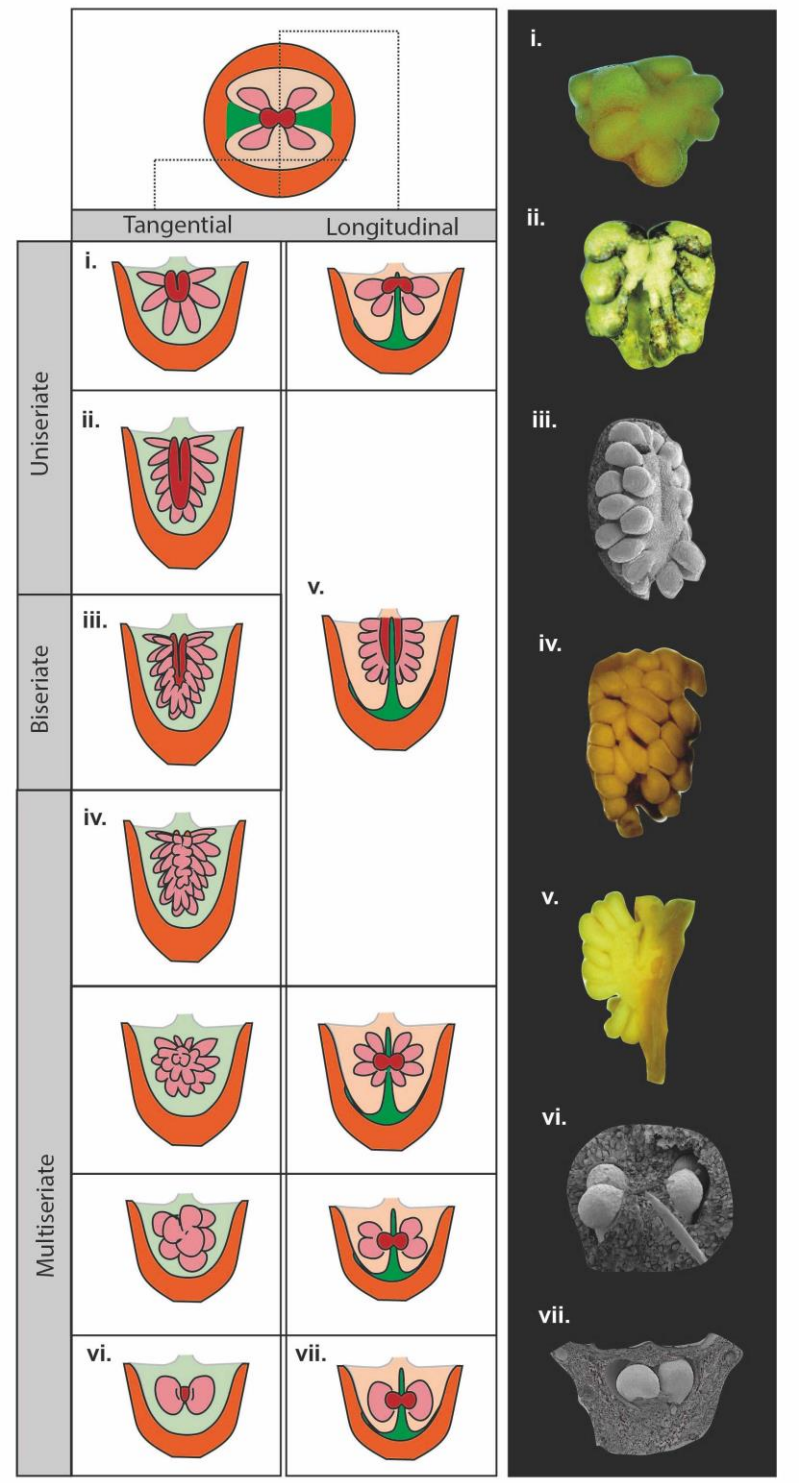


Figure 3.4 (previous page): Diagrams of tangential and longitudinal cuts in ovaries of Myrteae; showing variation in number of ovule series on placenta length. Note that variation in number of ovule series on placenta can only be noticed by tangential cuts; (ii –iv) have distinct ovule arrangements but present the same aspect in longitudinal cuts (v).). (i – vii) Examples of (i) uniseriate ovule arrangement on short placenta (exemplified by *Pimenta pseudocaryophyllus*); (ii) uniseriate ovule arrangement on medium sized placenta (exemplified by *Lophomyrtus obcordata*); (iii) biseriate ovule arrangement on medium sized placenta (exemplified by *Ugni candolei*); (iv) multiseriate ovule arrangement on medium sized placenta (exemplified by *Chamguava shippii*); (v) longitudinal cut in species with medium sized placenta (exemplified by *Myrtus communis*); (vi) multiseriate ovule arrangement on short placenta (exemplified by *Myrcia subcordata*); (vii) longitudinal cut in species with short placenta (exemplified by *Blepharocalyx eggersii*). Color code: orange = ovary; red = placenta; pink = ovule; green = locule wall.

3.5 Oil glands and elaiophores

Myrtaceae are renowned for their oil glands (Evert, 2006), and Myrteae flowers are no exception. Oil glands are present in all floral tissues, but can be particularly prominent on the anther and connective (Fig. 3.6F,G; e.g. Landrum and Kawasaki, 1997). These glands lack stomata or clear secretory specialization, but may present some systematic or ecological relevance (suggested by Landrum and Bonilla, 1996). Correlation of this character with environmental variables is, however, weakly supported (see Chapter 7). A number of species present small cavities on the surface of the floral receptacle, around the stylar base (Fig.3.6D). These are at a similar position to nectary tissue in other Myrtaceae (O'Brien et al., 1996; Ronse DeCraene, 2010), but lack clear secretory structures (Fig.3.6E; see also Chapter 5). Furthermore, there is no strong support for nectar production in Myrteae, even in genera with a hypanthium tube (Gressler et al., 2006). Such cavities were shown to be elaiophores and suggested as nectary relics that are now only phenolic producers (Ciccarelli et al., 2008). These serve to attract pollinators, and although no evidence for scents acting as reward has ever been documented, some Neotropical bees that visit Myrteae species (e.g. *Euglossini*, Nic Lughadha and Proença, 1996) are known to be phenolic foragers (Cameron, 2004).

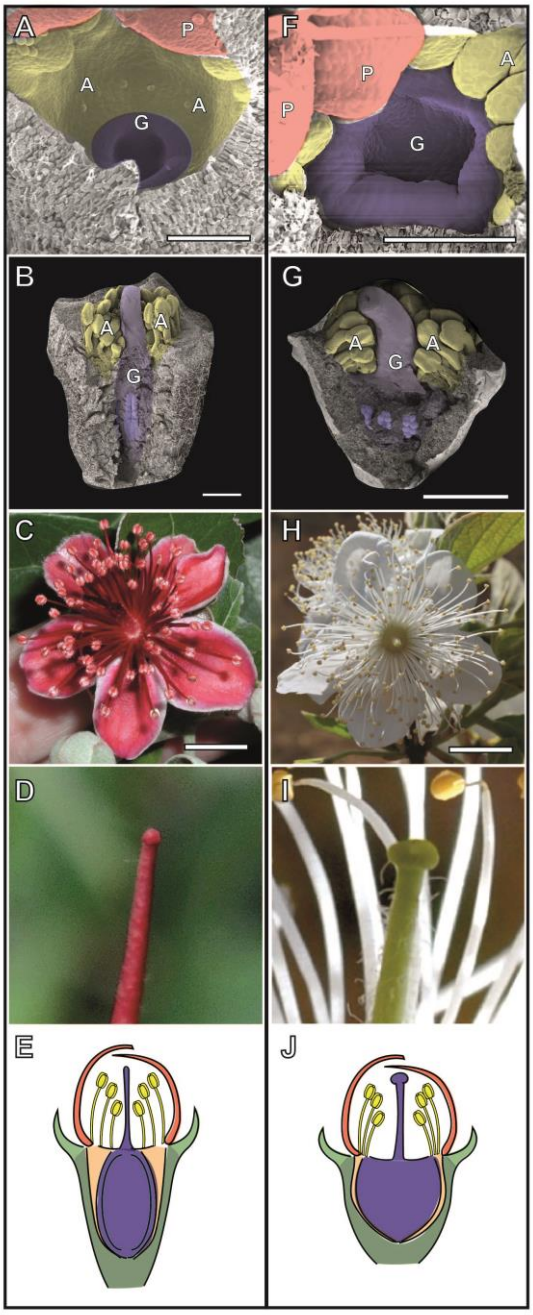


Figure 3.5: Distortions in flower architecture caused by differential development of gynoecium in two closely related genera. (A-E) *Acca sellowiana*; (F-J) *Campomanesia adamantium*; (A,F) early flower development, showing larger ovary depression in (F) *C. adamantium*; (B,G) longitudinal cut in mature bud; note stamens slightly dislocated upwards in "G" due to robust gynoecium; (C,H) flowers post-anthesis; (D,I) comparison between (D) simple stigma of *A. sellowiana* and (I) capitate stigma of *C. adamantium*; (E, J) diagram of longitudinal cut in mature bud showing changes in architecture resultant from variation in gynoecium development. A, androecium; G, gynoecium; P, petal. Scale: 50µm (A, F) 250µm (B); 500µm (G) c.5mm (C, H). Color code: light green = sepals; red = petals, yellow = androecium; blue = gynoecium. (H,D,I photos taken during field expeditions between 2014 and 2016; C from Google images).

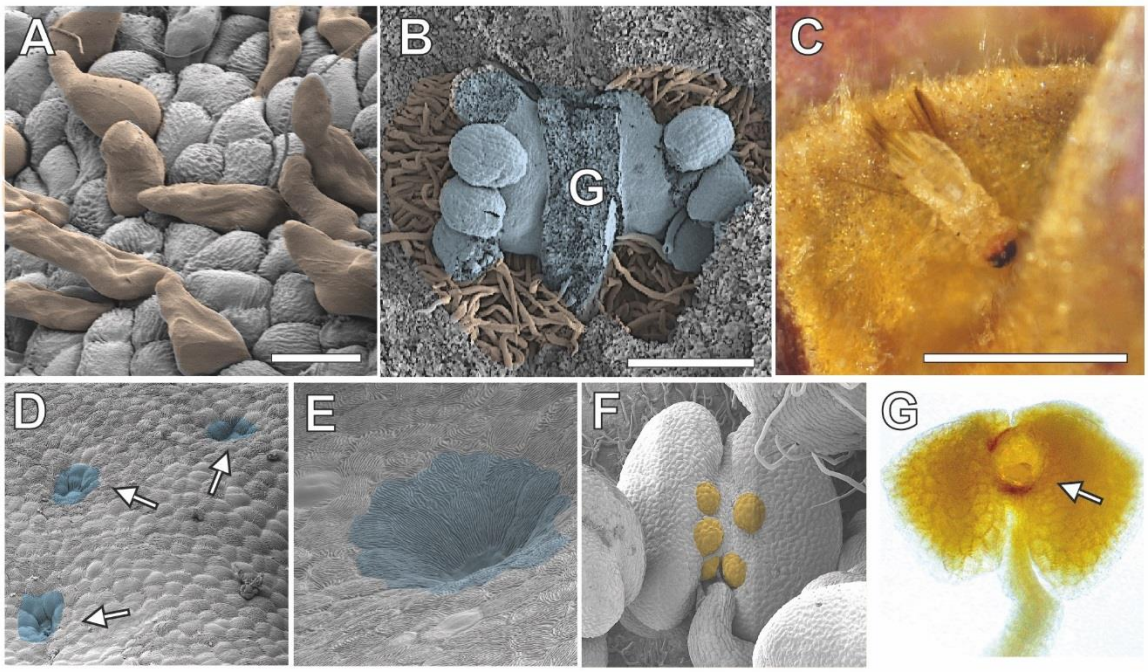


Figure 3.6: Trichomes, elaiophores and anther glands in Myrteae flowers. (A) single celled trichomes (hairs) developing on external surface of a *Myrcia splendens* bud; (B) similar trichomes growing on the surface of the locule wall in *Eugenia itajurensis*. (C) Termite inside a pubescent bud of *Myrcia* sect. *Gomidesia*. (D,E) Elaiophores in (D) *Pimenta dioica* (indicated by arrows) and in (E) *Rhodamnia cinerea*. (F,G) Anther oil gland in (F) Rhodomyrtus tomentosa and (G) Myrcia rubella (arrow). G, gynoecium. Scale: 25µm (A), 50µm (E,D), 250µm (B,F,G), 3mm (C).

3.6 Andromonoecy: more common than acknowledged

Most Myrteae flowers are hermaphrodite, with an androecium and a gynoecium developing as previously described. In a few species, however, individual plants within a population bear hermaphrodite flowers while others bear only male flowers. This trend known as andromonoecy is fairly common in Myrtaceae (see also Ashton 2011, for *Syzygium*). Andromonoecious species are present in at least seven Myrteae genera (*Pimenta, Psidium, Myrcia, Eugenia, Decaspermum and Kanakomyrtus*, *Myrtastrum*; Nic Lughada and Proenca, 1996; Snow et al., 2003; Wilson, 2011; pers. obs.) and their broad phylogenetic distribution indicates that this trend might be more common than previously appreciated. Plants bearing male flowers produce buds that either have an atrophied gynoecium (Fig. 3.7) or an additional whorl of stamens at the equivalent position to the gynoecium (pers.obs.). In two genera (*Pimenta* and *Decaspermum*), there is evidence that the breeding system is functionally dioecious, where apparently hermaphrodite plants do not produce viable pollen (Chapman, 1964; Kevan and Lack, 1985).

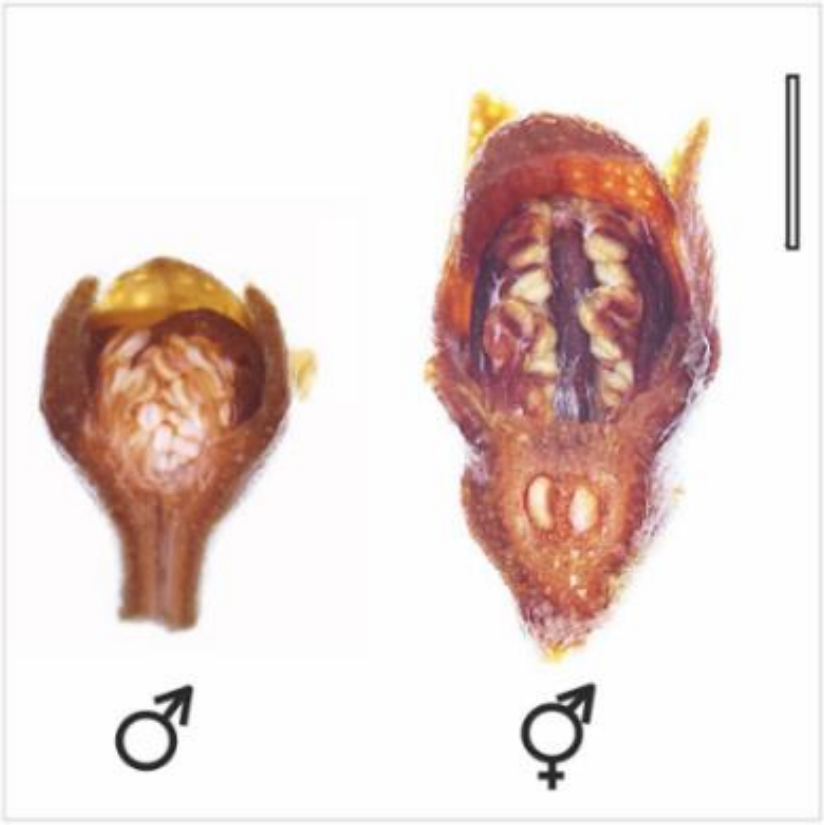


Figure 3.7: Andromonoecy in *Decaspermum parviflorum*. Flower on the left hand side has aborted gynoecium (staminate flower), while the one on the right hand side has both gynoecium and androecium present (hermaphrodite). Scale bar = 10mm.

3.7 Ovule oversupply

Even though number of ovules varies within a genus, most genera present a standard range of seed number. For most *Eugenia*, *Myrcia*, *Blepharocalyx* and *Luma* species for example, seed number is one or two regardless of the number of ovules produced (Fig. 3.8A,B; Berg, 1855-56; Lucas et al., 2007; Staggemeier et al. 2017). On the other hand, *Plinia*, *Myrceugenia* and *Myrtus* produce few seeds (less than ten, Fig. 3.8C) while *Psidium*, *Campomanesia* and *Acca* produce multiple seeds (Landrum and Kawasaki, 1997). In this way, ovule oversupply, i.e. the production of more ovules than will be fertilized (Rosenheim et al., 2016), occurs at different levels throughout Myrteae. Taxa that are single or few seeded are concentrated in lineages with “caulicine placentation” while multiple seeded taxa are more common in genera with “carpelate placentation”. It is possible that the shift from carpellar (the plesiomorphic state; Pimentel et al., 2014) to “caulicine placentation” has constrained the number of ovules that can be fertilized. This may give an advantage to certain lineages, allowing the development of larger seeds that are better adapted to certain environments (e.g. rainforests; Foster, 1986) conferring a shift in quality vs. quantity strategy (Schupp, 1993).

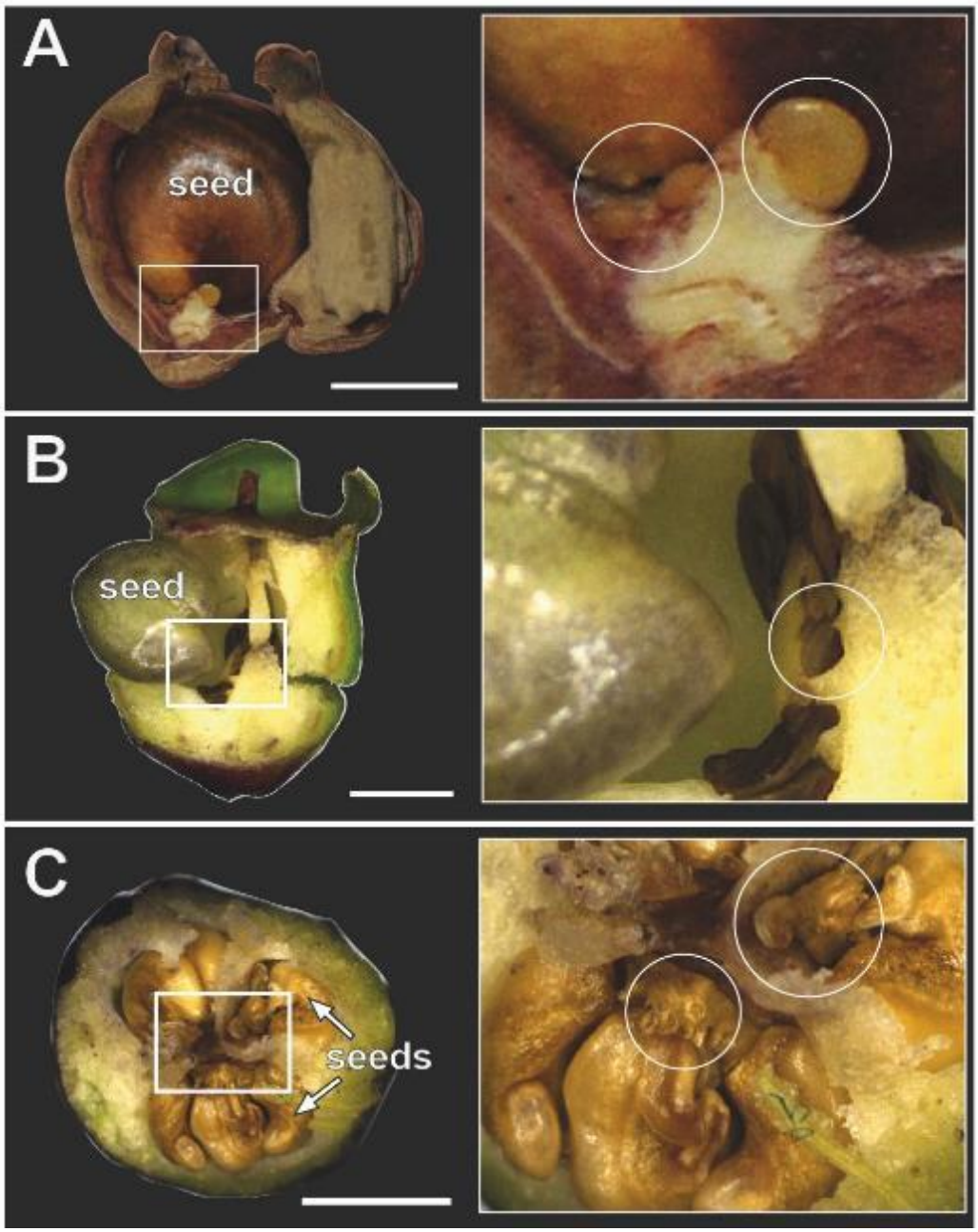


Figure 3.8: Distinct degrees of ovule oversupply in fruits of similar size. Distinct lineages present from a few to several aborted ovules in the mature fruit. (A) *Myrcia spectabilis* showing three aborted ovules and one seed; (B) *Luma apiculata* showing several aborted ovules and one seed; and (C) *Myrtus communis*, showing several aborted ovules and several seeds. Scale bar = c.5mm.

3.8 Herkogamy and strategies to avoid selfing

Distinct genera and groups of genera present different strategies to avoid selfing. In species with folded stamens, the style straightens earlier than the stamens, thus presenting discreet protogyny that may help avoid self-pollination (Fig. 3.9A; most Myrteae are self-incompatible, Nic Lughadha and Proenca, 1996). Species with straight stamens usually have both stamens and style at the same height after anthesis (moment when flower opens), with pollen presentated ready for collection as soon as the flower opens, increasing the chances of self-pollination (see discussion in Chapter 2). Some *Eugenia* and *Psidium* species avoid this by presenting style gigantism, where

the style stands twice as high as the anthers during anthesis (see Chapter 5 for *Eugenia*). This strategy may be linked with higher diversification rates in these groups (Chapter 1), with further evidences from similar trends in other plant groups (de Vos, 2014). Heterostyly is not evident in any species, but cannot be discarded until more extensive surveys are carried out.

3.9 Common pollination strategies

Most pollination biology studies in Myrteae show a similar strategy. Anthesis commonly occurs just before sunrise and is concentrated in the months between September and December (spring in the southern hemisphere; Staggemeier et al., 2010). Myrteae flowers offer pollen as the main or sole reward (Gressler et al., 2006), and are visited by a range of insects, with bees considered the most general and effective pollinators (Nic Lughadha and Proenca, 1996; Gressler et al., 2006). Most Myrteae flowers can be loosely classified into two subcategories based on display. The first is a stamen-dependent display (also called brush blossom, Johnson & Briggs, 1981), where stamens are the main component of floral visual attraction (Fig. 3.9C). In this display, perianth reflexes backwards after anthesis and is thought to play a less important role than the stamens in pollinator attraction. The second trend is a petaloid display and in this case the larger non reflexed petals represent the most obvious component of floral attraction (Fig.3.9D); filaments are commonly shorter than in more stamen dependent displays. Many intermediaries are observed but even closely related groups may represent extremes in this continuum (e.g. *Calycolpus* vs. *Myrtus*). A similar variation between stamen-dependent and petaloid display is also observed in other Neotropical pollen-flowers, such as Solanaceae and Melastomataceae (Buchmann and Cane, 1989; Kriebel and Zumbado, 2014), and may be related to sub-syndromes of pollen-gathering bee pollination.

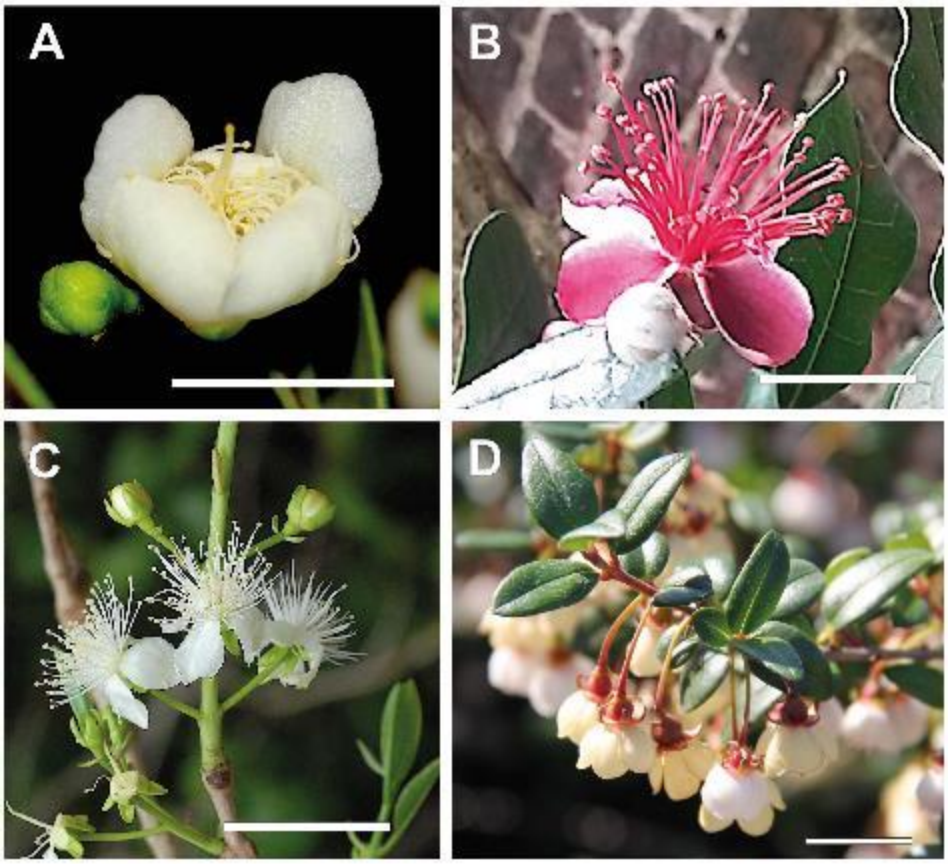


Figure 3.9: Diversity of floral display strategies in Myrteae. (A) Protogy during anthesis of *Luma apiculata*; (B) Red showy flowers of the bird pollinated *Acca sellowiana*; (C) Brush-blossom display in *Eugenia dichroma*.; (D) Petaloid display in *Ugni candollei*. Scale bar = c.10mm. (Photos taken during field expeditions between 2014 and 2016).

3.10 Uncommon pollination strategies

Pollination by vertebrates is rare in Myrteae, but exists in at least two genera. The bird pollinated *Myrrhinium* and *Acca* show similar floral strategies: decreased numbers of stamens, increased filament length, red coloured display and thick-sweet petals (Fig. 3.9B), the latter being the main reward for pollinators (Roitman, 1997). Reduced number of stamens is especially extreme in *Myrrhinium*, where just four to six stamens develop (Landrum, 1986). The fact that *Acca* and *Myrrhinium* represent species-poor clades (one and three species respectively) of relativelely old crown node ages (Chapter 1) suggests that specialization towards bird pollination has not been advantageous in Myrteae. It is important to reinforce that Myrteae flowers usually do not produce nectar, and thus cannot benefit from the most successful bird pollinators in the Neotropics, hummingbirds (as other diverse and sympatric bird pollinated groups such as Bignonieae (Alcantara and Lohman, 2010) and *Costus* (Kay et al., 2005) have; see also Rocca and Sazima, 2010). Other flowers with unusual shapes and somewhat tubular display and long filaments that resembles a bird or bat pollination syndromes are observed in the Australasian group (e.g. *Octamyrtus*; White 1951, Craven, 2006) and *Eugenia* from the Pacific region (section *Jossinia*; e.g. *Eugenia bullata*), but pollinator data is not available for these taxa.

SYSTEMATIC OUTLINE

As stated previously, Myrteae flowers present strong morphological conservatism (Mc Vaugh, 1968). Homoplasy, possibly due to parallelisms (Scotland, 2011), is common and similar structures are found distributed throughout the tribe without a strong phylogenetic context (e.g. merism, fused calyx, trichomes, locule and ovule number; see also Chapters 4 and 6). Single apomorphic traits for individual clades are almost absent and therefore observation of a single organ is usually systematically irrelevant. Combinations of traits, however, can identify a genus or a group of genera with fairly confidence (Table 3.1). The overall floral pattern found in each phylogenetic group is described below.

3.11 Australasian group

The Australasian group is the only clade in Myrteae with distribution restricted to the Australasian and Pacific geographic regions (Lucas et al., 2007; Chapter 1). Flowers commonly present a pinkish display (Fig 3.10G,H), distinct from the usually white Neotropical groups. Possibly due to its old age and broad geographical distribution (Chapter 1), morphological characters that are exclusive and constant enough to be defined as diagnostic are few. In general, pentamery is the most common perianth arrangement, although tetramerous flowers are found in *Octamyrtus*, *Rhodamnia* and some *Decaspermum* species (Scott, 1978b, 1979a; Snow, 2000). The fused calyx, a common trend in Neotropical Myrteae, is almost absent and reported only for the male flowers of *Kanakomyrtus dawsoniana* (Snow, 2003). Staminal primordia are spread over the entire hypanthium, with stamens in a straight position in the bud (Chapter 2). Locule number is variable, but the most common pattern is trilocular. The unilocular ovary of *Rhodamnia* (Scott, 1979a) is formed by incomplete fusion of the bi or tri-carpellar ovary (Figs 3.3D and 3.10I). Ovule organization on the placenta is mostly uniseriate, in bilocular species giving a lamelliform aspect to the placenta (terminology used by Snow et al., 2003). *Gossia* is aberrant in the sense that it presents a multiseriate arrangement (Snow et al., 2003). The clade formed by *Rhodomyrtus*, *Octamyrtus*, *Kanakomyrtus*, *Pilidiostigma* and *Archirhodomyrtus* (“K+A+R+P+O” clade; supported by PP>0.95 and bootstrap >90; Chapter 1; Snow et al., 2011) has several shared floral modifications. These include a tissue called ‘pseudo-septum’ (term coined by Scott, 1978b,c) between layers of ovules on the placenta and peltate stigmas. *Octamyrtus* flowers are similar to those of *Rhodomyrtus* in general morphology, the main difference being an additional whorl of longer petals that gives its display a characteristic tubular appearance (Craven et al., 2016).

3.12 *Myrtus* group

The close relationship between the only European Myrtaceae, the genus *Myrtus*, and a group of Neotropical Myrteae has been recently clarified (see Chapter 1). These four genera (*Myrtus*, *Calycolpus*, *Accara* and *Chamguava*) share multiseriate ovule organization and somewhat elongated placentas, in contrast to other sympatric Myrteae genera with multiseriate ovules attached to a minute placenta (e.g. *Eugenia*) (Landrum, 1990; 1991; Landrum and Kawasaki, 1997). Perianth is pentamerous in *Myrtus* (Fig.3.10F) and *Calycolpus* and tetramerous in *Accara* (Fig.3.10E) and *Chamguava*. *Myrtus* frequently has an additional but somewhat reduced whorl of petals (Mulas and Fadda, 2004).

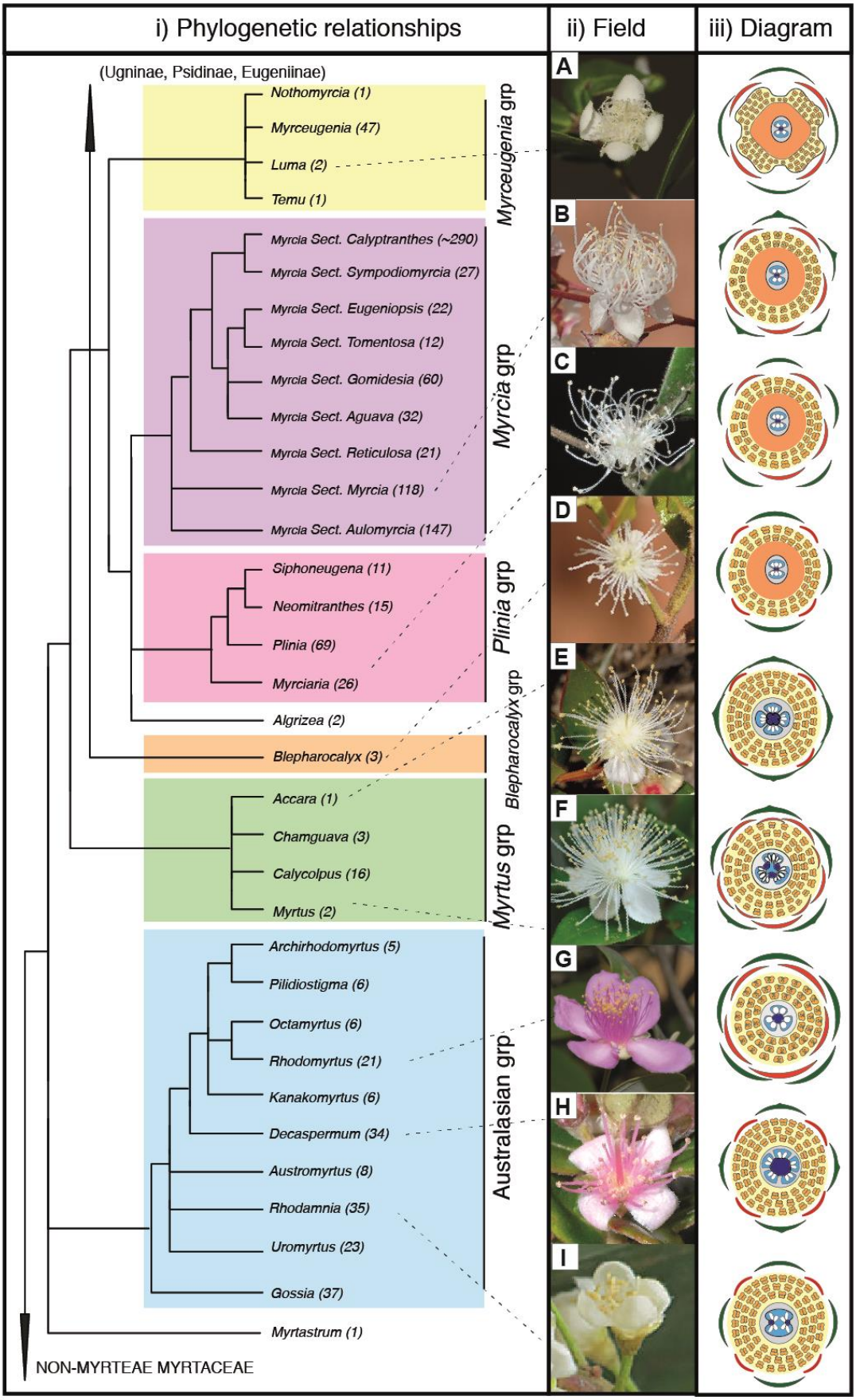
3.13 *Blepharocalyx*, *Myrcia* and *Plinia* groups

The *Blepharocalyx* group, consisting of *Blepharocalyx* as the sole genus, has historically been closely related to the *Pimenta* and *Psidium* groups based mainly on embryo morphology (Landrum, 1986). In terms of floral architecture, however, *Blepharocalyx* flowers are similar to those of the *Myrcia* and *Plinia* groups (Fig. 3.10B-D). Characters shared by all three groups include strongly folded stamens (Chapter 2), “caulicine placentation” (sensu Pimentel et al., 2014), multiseriate ovule arrangement on placenta and low number of ovules per ovary (this study, Lucas et al., 2007). Locules are usually two, less commonly three. Within the *Plinia* and *Myrcia* groups, flowers are very homogeneous and variation that diagnoses infrageneric groups come from traits such as hairs, calyx fusion, hypanthium elevation and thickness of the staminal ring (see Chapters 6 and 7).

3.14 *Myrceugenia* group

Myrceugenia has been historically associated with *Myrcia* based on of embryo characters (McVaugh, 1968) but in recent phylogenetic studies it has grouped with *Luma* (Lucas et al., 2007; Murillo-A et al., 2012; Chapter 1), *Blepharocalyx cruckshanksii* (or *Temu*, Berg 1855-57) and *Nothomyrcia* (Murillo-A and Ruiz, 2011). All four genera share common floral traits, including tetramery, discontinuous staminal rings that gives a semi-folded aspect prior to anthesis, two-four locular ovaries with uniseriate ovule organization (Fig.3.10A). The style is long and folds on top of the anthers.

Figure 3.10 (next page): Simplified phylogeny of Myrteae (i), field pictures (ii) and floral diagrams (iii) of selected species. Topology is a summary of total molecular evidence (nuclear and plastidial) found by Lucas et al. (2005, 2007, 2011); Costa (2009); De-Carvalho (2013); Mazine et al., (2014); Bunger et al. (2015), Santos et al., (2017) and Chapter 1. Represented clades are those supported with strong PP and bootstrap by the majority of studies. Poorly supported relationships are collapsed into polytomies. Numbers between brackets represent estimated species diversity. (A) *Luma apiculata*; (B) *Myrcia linearifolia*; (C) *Myrciaria floribunda*; (D) *Blepharocalyx salicifolius*; (E) *Accara elegans*; (F) *Myrtus communis*; (G) *Rhodomyrtus tomentosa*; (H) *Decaspermum vitis-idea*; (I) *Rhodamnia cinerea*. "grp” = group. Color code in floral diagrams: green = sepals, red = petals, yellow = androecium, orange = hypanthium, blue = gynoecium (dark blue = placenta, light blue = locule, white = ovules and ovary walls). (Photos taken during field expeditions between 2014 and 2016).


i) Phylogenetic relationships
ii) Field
iii) Diagram
(Ugninae, Psidinae, Eugeniinae)
Nothomyrcia (1)
Myrceugenia (47)
Luma (2)
Temu (1)
Myrceugenia grp
Myrcia Sect. Calyptranthes (~290)
Myrcia Sect. Sympodiomyrcia (27)
Myrcia Sect. Eugeniopsis (22)
Myrcia Sect. Tomentosa (12)
Myrcia Sect. Gomidesia (60)
Myrcia Sect. Aguava (32)
Myrcia Sect. Reticulosa (21)
Myrcia Sect. Myrcia (118)
Myrcia Sect. Aulomyrcia (147)
Myrcia grp
Siphoneugena (11)
Neomitranthes (15)
Plinia (69)
Myrciaria (26)
Plinia grp
Algrizea (2)
Blepharocalyx (3)
Blepharocalyx grp
Accara (1)
Chamguava (3)
Calycolpus (16)
Myrtus (2)
Myrtus grp
Archirhodomyrtus (5)
Pilidiostigma (6)
Octamyrtus (6)
Rhodomyrtus (21)
Kanakomyrtus (6)
Decaspermum (34)
Austromyrtus (8)
Rhodamnia (35)
Uromyrtus (23)
Gossia (37)
Australasian grp
Myrtastrum (1)
NON-MYRTEAE MYRTACEAE
A
B
C
D
E
F
G
H
I

3.17 *Eugenia* group

The *Eugenia* group consists of only two genera, *Myrcianthes* and *Eugenia*. While the first is a small sized genus mostly from the Andean region (Grifo, 1992), the latter is the largest and most widespread genus of Myrteae, with c. 1000 species spread throughout the Neotropics, New Caledonia, Madagascar, Continental Africa and India (Mazine et al., 2014). In general aspects, *Myrcianthes* is pentamerous while almost all *Eugenia* are tetramerous (Mazine et al., 2016). In both genera staminal primordia cover the whole hypanthium during flower development resulting in straight stamens in the bud (Chapter 2; see also Chapter 5). Ovaries are mostly bilocular, with a small central placenta that is cauline in origin and attached to a single point in the septum ovules are multiseriate (Landrum and Kawasaki, 1997).

In general aspects, floral morphology is homogeneous throughout the vast majority of *Eugenia* species (Figs. 3.11A,B) and, traditionally, morphological characters that separate sections and clades within the genus are related to non-floral aspects (e.g. seeds and inflorescences; Mazine et al., 2014). However, some fundamental differences in the gynoecium were observed in two lineages arising from the deepest nodes of *Eugenia*. *Eugenia* Sect. *Pseudeugenia* and sect. *Pilothecium* have somewhat uniseriate placentation and frequently more locules than the bilocular rule (Faria, 2014; Mazine et al., 2016). Other floral variation includes developmental rate (see Chapter 5), presence of trichomes and where they occur (Faria, 2014), the length of the style (Chapter 5) and calyx modifications (aspect and fusion; Bunger et al., 2015). These variations may have systematic relevance. Section *Pilothecium*, for example, can be easily identified by the presence of hairs in the ovary (a character shared with some *Pimenta* group genera; Faria, 2014) while most *Eugenia* sect. *Umbellatae* species have styles that are twice as long as in other clades (see Chapter 5). Furthermore, sect. *Phyllocalyx* is recognizable by the leafy aspects of sepals, which are morphologically similar to the bracteoles (Berg, 1855-57; Bunger et al., 2015).

3.15 *Pimenta* group

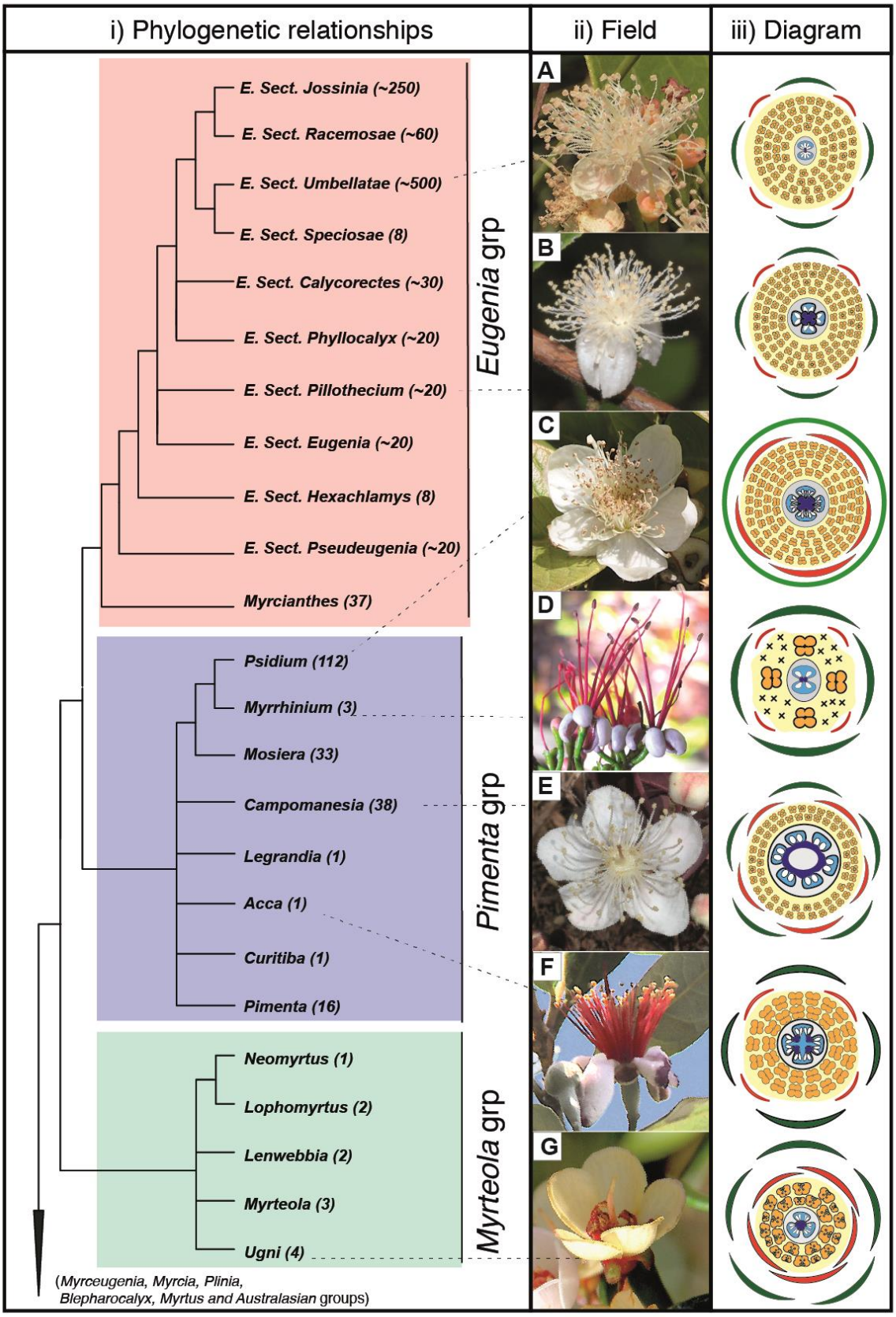
The *Pimenta* group genera present possibly the broadest relative flower diversity. Flowers are either tetramerous or pentamerous, but variation is common even at the species level. Locularity goes from bilocular to multilocular, sometimes reaching 18 locules in some *Campomanesia* (Landrum, 1986). Stamens are mostly straight in the bud, with the exception of *Pimenta*, where stamen primordia develop in a somewhat discontinuous ring (Chapter 2, similar to the *Myrceugenia* group). Stigmas are mostly capitate and this provides a good character to separate the *Pimenta* group from other sympatric Myrteae (Bentham, 1869; Landrum and Kawasaki 1997). *Psidium*, *Myrhhinium* and *Mosiera* form a strongly supported clade (Chapter 1), but differ in some fundamental aspects of flower organization. *Mosiera* and some *Psidium* (e.g. *Psidium guajava*, *P. guineense*; Fig. 3.11C), not all, have multiseriate ovule arrangement on placenta and locules that can be only partially fused (Landrum, 1992). Other *Psidium* species (e.g. *P. brownianum*, pers. obs.) present uniseriate ovule arrangement and in this sense are most similar to all other remainder genera in the *Pimenta* group (e.g. *Campomanesia*, Fig.3.11E). *Myrrhinium* presents strong reduction in number of stamens (Fig.3.11D; Landrum and Kawasaki, 1997). In this genus, structures resembling staminodes were observed on the base of the hypanthium and may

represent aborted filaments (crosses in floral diagram, Fig.3.11D). *Acca* flowers are very distinct from the *Pimenta* group and Myrteae. They present hairy anthers, not observed in any other genus, and distinct multilocular ovaries where the central axis is not fused, giving the impression of a unilocular chamber (Fig.3.11F; Dettori and Gaetano, 1991).

3.16 *Myrteola* group

The Myrteola group is the smallest clade treated here. It represents c. 15 species distributed in five small genera (Lucas et al., 2007; Wilson 2011; Chapter 1). Merism is useful for generic delimitation: *Ugni* and *Neomyrtus* are pentamerous while *Myrteola*, *Lophomyrtus* and *Lenwebbia* are mostly tetramerous (Landrum, 1988). Stamens are mostly straight in the bud (Chapter 2). Ovaries are either bi- or tri-locular and ovule arrangement is uni- or bi-seriate, never multiseriate. *Ugni* has an overall distinct floral morphology, with a campanulate corolla formed by relatively large petals, resembling some Ericaceae (Fig. 3.11G; Wilson, 2011); the red anthers are sagittate and longitudinally covered in oil glands. *Lenwebbia* has an unusual androecium morphology. The staminal base is slightly fused and the discontinuous rings are similar to those of the *Myrceugenia* group, giving the stamens a semi-folded aspect in the bud (Chapter 2). *Myrteola*, a genus with a Patagonian distribution, presents small flowers with few stamens but a non-reduced number of ovules, increasing the ovule/pollen ratio that characterize a possible change in breeding system (Cruden, 1977). *Neomyrtus* and *Lophomyrtus*, the only Myrteae native to New Zealand, are positioned together in the phylogeny (Lucas et al., 2007; Chapter 1). In overall flower aspect, however, *Neomyrtus* is like *Ugni* (larger glandulous anthers, biseriate ovules on the placenta), whilst *Lophomyrtus* resembles *Lenwebbia* (somewhat semi-folded stamens in bud, uniseriate ovules on the placenta) (Belsham and Orlovich, 2002).

Figure 3.11 (next page): Simplified phylogeny of Myrteae (i), field pictures (ii) and floral diagrams (iii) of selected species. Topology is a summary of total molecular evidence (nuclear and plastidial) found by Lucas et al. (2005, 2007, 2011); Costa (2009); De-Carvalho (2013); Mazine et al., (2014); Bunger et al. (2015), Santos et al., (2017) and Chapter 1. Represented clades are those supported with strong PP and bootstrap by the majority of studies. Poorly supported relationships are collapsed into politomies. Numbers between brackets represent estimated species diversity. (A) *Eugenia bimarginata*; (B) *Eugenia stipitata*; (C) *Psidium guajava*; (D) *Myrrhinium atropurpureum*; (E) *Campomaneia adamantium*; (F) *Acca sellowiana*; (G) *Ugni candolei*. "grp" = group. Color code in floral diagrams: green = sepals, red = petals, yellow = androecium, orange = hypanthium, blue = gynoecium (dark blue = placenta, light blue = locule, white = ovules and ovary walls). (Photos taken during field expeditions between 2014 and 2016; except D (Google images)).


i) Phylogenetic relationships
ii) Field
iii) Diagram
E. Sect. Jossinia (~250)
E. Sect. Racemosae (~60)
E. Sect. Umbellatae (~500)
E. Sect. Speciosae (8)
E. Sect. Calycorectes (~30)
E. Sect. Phyllocalyx (~20)
E. Sect. Pillothecium (~20)
E. Sect. Eugenia (~20)
E. Sect. Hexachlamys (8)
E. Sect. Pseudeugenia (~20)
Myrcianthes (37)
Eugenia grp
Psidium (112)
Myrrhinium (3)
Mosiera (33)
Campomanesia (38)
Legrandia (1)
Acca (1)
Curitiba (1)
Pimenta (16)
Pimenta grp
Neomyrtus (1)
Lophomyrtus (2)
Lenwebbia (2)
Myrteola (3)
Ugni (4)
Myrteola grp
(Myrceugenia, Myrcia, Plinia,
Blepharocalyx, Myrtus and Australasian groups)
A
B
C
D
E
F
G

3.17 *Incertae sedis*

Five genera are here considered as *incertae sedis* (unplaced): *Algrizea*, *Myrtastrum*, *Amomyrtella*, *Myrtella* and *Lithomyrtus*. The first two have an uncertain phylogenenetic relationships with other groups (Lucas et al., 2005; 2007; Vasconcelos et al., 2017; Murillo-A et al., 2012); the last three are still to be included in molecular analysis so their phylogenetic position in uncertain. *Algrizea* is a small genus of two species that present many morphological similarities with *Myrcia* and *Plinia* groups, including folded stamens in the bud, bilocular ovaries with "caulicine placentation" (sensu Pimentel et al., 2014) and reduced number of multisseriate organized ovules (Proenca et al., 2006). *Algrizea* appears either as sister to *Myrcia* group (Lucas et. al, 2007) or sister to/nested within *Plinia* group (Staggemeier et al., 2015, Chapter 1), but its unstable position and somewhat mixed morphology makes it difficult to include in either one or the other (Proenca et al., 2006; Sobral et al., 2010). *Myrtastrum*, a mono-specific genus endemic to New Caledonia, has an unusual floral structure relative to other extant Myrteae. The stigma is capitate but shorter than the anthers (protoandry), a pattern not observed elsewhere in the tribe. Petals are shorter than sepals, restricting the degree to which the corolla reflexes (Snow, 2000). The gynoecium is three locular with incomplete fusion and ovule arrangement and has been described as biseriate (Scott, 1979), but seems to be in fact uniseriate. *Amomyrtella*, a genus from the Andes, is described as a genus with morphologically distinct flowers (Landrum and Morocho, 2011), with anthers up to 2mm and trilocular ovaries with biseriate ovule arrangement on placenta. Such descriptions place the genus closest to *Ugni* and predict that *Amomyrtella* will ultimately be positioned in the *Myrteola* group. *Myrtella* and *Lithomyrtus* have general Australasian group traits: straight stamens in the bud, bi-tetralocular ovaries and a uniseriate ovule arrangement on placenta (Scott, 1978, 1979; Snow and Guymer, 1999). The lack of a capitate/peltate stigma and pseudoseptum between ovules suggest that their position will be other than within the K+A+R+P+O clade.

Table 3.1: Floral formulae, general ground plan and diagnostic characters in Myrteae clades.

Clade	Genus	Floral formulae	Ovule arrangement on placenta	Stigma	Position of stamens in the bud
Australasian	*Archirhodomyrtus*	♂K5* C5* A∞* Ĝ(2-3)┼ Vx∞	Uniseriate	Capitate	Straight
	Austromyrtus	♂K5* C5* A∞* Ĝ(2)┼ Vx10-20	Apparently biseriate	Simple	Straight
	Decaspermum	♂ ♀ K4-5* C4-5* A∞* Ĝ(5)┼ Vx10	Uniseriate	Capitate	Straight
	Gossia	♂K4-5*C4-5*A∞* Ĝ(2)┼ Vx5-20	Multiseriate	Simple	Straight
	Kanakomyrtus	♂ ♀ K4-5* C4-5* A∞* Ĝ(2-4)┼ Vx∞	Uniseriate	Capitate (with linear lobes)	Straight to semi-curved
	Octamyrtus	♂K4* C4*+4*+2↓ A15-20* Ĝ(3)┼ Vx∞	Uniseriate	Capitate	Straight
	Pilidiostigma	♂K4-5*C4-5*A∞* Ĝ(1-3) ┼Vp-Vx∞	Uniseriate	Capitate	Straight
	Rhodamnia	♂K4-5*C4-5*A∞* Ĝ(2-3) ┼Vp10-20	Uni- or biseriate	Simple or capitate	Straight
	Rhodomyrtus	♂K5* C5* A∞* Ĝ(3)┼ Vx∞	Uniseriate	Capitate	Straight
	Uromyrtus	♂K5*C5*A∞* Ĝ(3) ┼Vx10-20	Uniseriate	Simple	Straight
General ground plan		**♂ K4-5*C4-5*A∞* Ĝ(2-3) ┼Vx5-∞**	**Uniseriate**	**Simple or capitate**	**Straight**
Blepharocalyx	*Blepharocalyx eggersii*	♂K2*C2*A∞* Ĝ(2) ┼Vx4-8	Multiseriate	Simple	Strongly incurved
	Blepharocalyx salicifolius	♂K4*C4*A∞* Ĝ(2) ┼Vx4-16	Multiseriate	Simple	Strongly incurved
General ground plan		**♂ K2-4*C2-4*A∞* Ĝ(2) ┼Vx4-16**	**Multiseriate**	**Simple**	**Strongly incurved**
Eugenia	*Eugenia*	♂♀K4*C4*A∞* Ĝ(2) ┼Vx4-∞	Multiseriate	Simple	Straight
	Myrcianthes	♂K5*C5*A∞* Ĝ(2) ┼Vx∞	Multiseriate	Simple	Straight
General ground plan		**♂ K4*C4*A∞* Ĝ(2) ┼Vx4-∞**	**Multiseriate**	**Simple**	**Straight**
Myrceugenia	*Luma*	**♂ K4*C4*A∞* Ĝ(2) ┼Vx∞**	Uniseriate	Simple	Semi-curved

	Myrceugenia	♂ **K4*C4*A∞* Ĝ(2) ┼Vx∞**	Uniseriate	Simple	semi-curved
	Nothomyrcia	♂ **K4*C4*A∞* Ĝ(2) ┼Vx∞**	Uniseriate	Simple	semi-curved
	Temu (Blepharocalyx cruckshanksii)	♂ **K4*C4*A∞* Ĝ(2) ┼Vx∞**	Uniseriate	Simple	semi-curved
General ground plan		♂ **K4*C4*A∞* Ĝ(2) ┼Vx∞**	Uniseriate	Simple	semi-curved
Myrcia	*Myrcia*	♂ **K5*C5*A∞* Ĝ(2-3) ┼Vx4-6**	**Multiseriate**	**Simple**	**Strongly incurved**
Myrteola	*Lenwebbia*	♂ K4*C4*A∞* Ĝ(3) ┼Vx∞	Uniseriate	Simple	Semi-curved
	Lophomyrtus	♂ K4*C4*A∞* Ĝ(2-3) ┼Vx∞	Uniseriate	Simple	Semi-curved
	Myrteola	♂ K4-5*C4-5*A10-30* Ĝ(2-3) ┼Vx∞	Uniseriate	Simple	Straight
	Neomyrtus	♂ K5*C5*A∞* Ĝ(2) ┼Vp∞	Biseriate	Simple	Straight
	Ugni	♂ K5*C5*A20-30* Ĝ(3) ┼Vx∞	Biseriate	Simple	Straight
General ground plan		♂ **K4-5*C4-5*A20-∞* Ĝ(2-3) ┼Vx∞**	**Uni- or Biseriate**	**Simple**	**Straight or Semi-curved**
Myrtus	*Accara*	♂ K(4)*C4*A∞* Ĝ(4) ┼Vx∞	Multiseriate	Simple	Straight
	Calycolpus	♂ K5* C5* A∞* Ĝ(4-6) ┼Vx∞	Multiseriate	Simple to slightly capitate	Straight
	Chamguava	♂ K(4)* C4* A∞* Ĝ(2) ┼Vx∞	Multiseriate	Simple	Straight
	Myrtus	♂ K5* C5* A∞* Ĝ(3) ┼Vx∞	Multiseriate	Simple	Straight
General ground plan		♂ **K4-5* C4-5* A∞* Ĝ(2-4) ┼Vx∞**	**Multiseriate**	**Simple**	**Straight**
Pimenta	*Acca*	♂K5* C4–5* A15-30* Ĝ(4)┼Vx∞	Biseriate	Simple	Straight
	Campomanesia	♂K5* C5* A∞* Ĝ(2-18)┼Vx ∞	Uniseriate	Capitate	Straight
	Curitiba	♂K4* C4* A∞* Ĝ(2)┼Vx∞	Uniseriate	Simple	Straight
	Legrandia	♂K4* C4* A∞* Ĝ(2-3)┼Vx∞	Uniseriate	Simple	Straight
	Mosiera	♂K4* C4* A∞* Ĝ(2-3)┼Vx∞	Multiseriate	Capitate	Straight

	Myrrhinium	♂K4*C4*A4–6:∞^{0}∂ Ĝ(2)┼Vx7–15	Uniseriate	Simple	Straight
	Pimenta	♂♀ K4–5* C4–5* A∞* Ĝ(2)┼Vx 1–8	Uniseriate	Simple	Semi-curved
	Psidium	♀K(4–5)* C4–5* A∞* Ĝ(2–5)┼Vx∞	Multiseriate	Capitate	Straight
General ground plan		**♀K4–5* C4–5* A∞* Ĝ(2–∞)┼Vx∞**	**Uni- or Multiseriate**	**Simple or Capitate**	**Straight**
Plinia	*Myrciaria*	♀K4* C4* A∞* Ĝ(2)┼Vx4-6	Multiseriate	Simple	Strongly incurved
	Neomitranthes	♀K(4)* C4^{r} * A∞* Ĝ(2)┼ Vx4-8	Multiseriate	Simple	Strongly incurved
	Plinia	♀K4* C4* A∞* Ĝ(2)┼Vx6-10	Multiseriate	Simple	Strongly incurved
	Siphoneugena	♀K4* C4 * A∞* Ĝ(2)┼ Vx4-8	Multiseriate	Simple	Strongly incurved
General ground plan		**♀K4* C4* A∞* Ĝ(2)┼Vx4-8**	**Multiseriate**	**Simple**	**Strongly incurved**

CONCLUSION

The general ground plan of the clades does not differ significantly and is similar to that of other Myrtaceae tribes (Wilson, 2011), but combination of floral traits points with failry high confidence to individual clades. In terms of systematic relevance, the general sequence in order of floral character stability, from higher to lower taxonomic levels is: androecium structure (stamen primordia distribution over the hypanthium and consequent position in the pre-anthetic bud); gynoecium structure (origin of placenta and ovule arrangement), and lastly perianth structure (number of parts and degree of fusion).

In uniform groups such as this, careful morphological studies that reveal discrete changes responsible for flexibility of reproductive strategies are the most relevant. In Myrteae, these include subtle herkogamic effects, changes from brush-blossom to a petaloid display (and vice-versa) and poorly understood evolutionary trends such as andromonoecy and ovule oversupply. The gynoecium, a hidden and difficult structure to analyse, appears to be especially meaningful in the evolution of Myrteae. Ovary development appears to influence the number of seeds, the development of the embryos and to balance inbreeding vs. outbreeding (by strong style elongation in some groups) and pollen competition due to distinct compitum arrangements (Mulcahy and Mulcahy, 1987). Deeper studies of ovary structure and evaluation of its role in these processes will be profitable. Furthermore, fine changes in one floral whorl lead to spatial changes that affect the development of the next whorl (e.g. Fig.3.5), showing the importance of considering the whole flower system in conjunction as a single unit under natural selection.

APPENDIX

Appendix 3.1: List of analysed specimens in Chapters 2 and 3. All vouchers deposited in herbarium K. Species name and authorship according to the WCSP (2017). Clade names according to Figs.3.10 and 3.11.

Clade	Species	Voucher	Collection locality
Australasian	*Archirhodomyrtus beckleri (F.Muell.) A.J.Scott*	B. Gray 1548	Australia (Queensland)
Australasian	*Archirhodomyrtus turbinata (Schltr.) Burret*	J. Soewarto HB 11	New Caledonia
Australasian	*Archirhodomyrtus turbinata (Schltr.) Burret*	PS Green 1258	New Caledonia
Australasian	*Austromyrtus dulcis (C.T.White) L.S.Sm.*	S. Belsham M77	Australia (Queensland)
Australasian	*Decaspermum parviflorum (Lam.) A.J.Scott*	T. Vasconcelos 728	Malayisia (Sabah)
Australasian	*Decaspermum parviflorum (Lam.) A.J.Scott*	T. Vasconcelos 730	Malayisia (Sabah)
Australasian	*Decaspermum vitis-idaea Stapf*	T. Vasconcelos 729	Malayisia (Sabah)
Australasian	*Gossia bidwillii (Benth.) N.Snow & Guymer*	L.S. Smith 4516a	Australia (Queensland)
Australasian	*Kanakomyrtus longipetiolata N.Snow*	H.S. Mackee 32732	New Caledonia
Australasian	*Octamyrtus arfancensis Kaneh. & Hatus. ex C.T.White*	P. Van Royen 7925	New Guinae
Australasian	*Octamyrtus pleiopetala Diels*	D.R. Pleyte 209	New Guinae
Australasian	*Octamyrtus sp.*	Johns 9885	New Guinae
Australasian	*Pilidiostigma tropicum L.S.Sm.*	S.F. Kajewski 1265	Australia (Queensland)
Australasian	*Pilidiostigma tropicum L.S.Sm.*	PiF 27636	Australia (Queensland)
Australasian	*Rhodamnia cinerea Jack*	T.Vasconcelos 672	Singapore
Australasian	*Rhodamnia dumetorum (DC.) Merr. & L.M.Perry*	Schanzer I. et al. 148c	Australia
Australasian	*Rhodomyrtus tomentosa (Aiton) Hassk.*	T. Vasconcelos 726	Malayisia (Sabah)
Australasian	*Rhodomyrtus tomentosa (Aiton) Hassk.*	Amin and Francis SAN116159	NA (from US spirit collection)
Australasian	*Rhodomyrtus tomentosa (Aiton) Hassk.*	T. Vasconcelos 678	Singapore
Australasian	*Rhodomyrtus tomentosa (Aiton) Hassk.*	Eyde 4/79	NA (from MO spirit collection)
Australasian	*Uromyrtus archboldiana (Merr. & L.M.Perry) A.J.Scott*	P. Puradyatmika 7425	New Guinae

Australasian	*Uromyrtus emarginata (Pancher ex Baker f.) Burret*	T. Vasconcelos 605	New Caledonia
Australasian	*Uromyrtus emarginata (Pancher ex Baker f.) Burret*	T. Vasconcelos 628	New Caledonia
Blepharocalyx	*Blepharocalyx eggersii (Kiaersk.) Landrum*	B. W. Nelson 923	Brazil (AM)
Blepharocalyx	*Blepharocalyx eggersii (Kiaersk.) Landrum*	T.Vasconcelos 458	Brazil (BA)
Blepharocalyx	*Blepharocalyx salicifolius (Kunth) O.Berg*	J.A. Ratter 5984	Brazil (MS)
Blepharocalyx	*Blepharocalyx salicifolius (Kunth) O.Berg*	T.R.S. Silva 13494	Brazil (MG)
Blepharocalyx	*Blepharocalyx salicifolius (Kunth) O.Berg*	J.E.Q Faria 4050	Brazil (DF)
Eugenia	*Myrcianthes fragrans (Sw.) McVaugh*	T.Vasconcelos 535	Costa Rica
Eugenia	*Myrcianthes fragrans (Sw.) McVaugh*	R. Chaco'n 350	NA
Eugenia	*Myrcianthes pungens (O.Berg) D.Legrand*	J.E.Q. Faria 4277	Brazil (DF)
Eugenia (sect. Calycorectes)	*Calycorectes acutatus (Miq.) Toledo*	T. Vasconcelos 506	Brazil (DF)
Eugenia (sect. Calycorectes)	*Calycorectes bergii Sandwith*	J.G. Myers 5955	French Guiana
Eugenia (sect. Eugenia)	*Eugenia ligustrina (Sw.) Willd.*	Hamilton M.A. 570	British Virgin Islands
Eugenia (sect. Eugenia)	*Eugenia ligustrina (Sw.) Willd.*	TV 570	Dominican Republic
Eugenia (sect. Eugenia)	*Eugenia uniflora L.*	T. Vasconcelos s.n.	RBG Kew living collection (native to Brazil)
Eugenia (sect. Hexachlamys)	*Eugenia splendens O.Berg*	J.E.Q.Faria 4196	Brazil (BA)
Eugenia (sect. Hexachlamys)	*Hexachlamys edulis (O.Berg) Kausel & D.Legrand*	T.M. Pedersen 2756	Brazil (SP)
Eugenia (sect. Jossinia)	*Eugenia malangensis (O.Hoffm.) Nied.*	Robson 342	South Africa
Eugenia (sect. Jossinia)	*Eugenia malangensis (O.Hoffm.) Nied.*	Brenan 7962	South Africa
Eugenia (sect. Jossinia)	*Eugenia malangensis (O.Hoffm.) Nied.*	Brenan 8024	South Africa
Eugenia (sect. Jossinia)	*Eugenia malangensis (O.Hoffm.) Nied.*	Greenway 8129	South Africa
Eugenia (sect. Jossinia)	*Eugenia bullata Pancher ex Guillaumin*	T.Vasconcelos 608	New Caledonia
Eugenia (sect. Jossinia)	*Eugenia paludosa Pancher ex Brongn. & Gris*	T.Vasconcelos 646	New Caledonia
Eugenia (sect. Jossinia)	*Eugenia roseopetiolata N.Snow & Cable*	T. Vasconcelos s.n.	RBG Kew living collection (native to Madagascar)

Eugenia (sect. Phyllocalyx)	*Eugenia involucrata DC.*	J.E.Q. Faria 4047	Brazil (DF)
Eugenia (sect. Phyllocalyx)	*Eugenia involucrata DC.*	T.Vasconcelos 734	Brazil (DF)
Eugenia (sect. Phyllocalyx)	*Eugenia involucrata DC.*	T.Vasconcelos 256	Brazil (DF)
Eugenia (sect. Pilothecium)	*Eugenia itajurensis Cambess.*	J.E.Q. Faria 4250	Brazil (BA)
Eugenia (sect. Pilothecium)	*Eugenia klotzschiana O.Berg*	Heringer et al. 1975	Brazil (GO)
Eugenia (sect. Pilothecium)	*Eugenia pohliana DC.*	J.E.Q. Faria 4184	Brazil (BA)
Eugenia (sect. Pilothecium)	*Eugenia stipitata McVaugh*	T.Vasconcelos 677	Singapore (cultivated, native to Brazilian Amazon
Eugenia (sect. Pilothecium)	*Eugenia victoriana Cuatrec.*	T.Vasconcelos 717	Singapore (cultivated, native to Colombia)
Eugenia (sect. Pseudeugenia)	*Eugenia azurensis O.Berg*	T.Vasconcelos 433	Brazil (BA)
Eugenia (sect. Pseudeugenia)	*Eugenia azurensis O.Berg*	J.E.Q. Faria 4186	Brazil (BA)
Eugenia (sect. Pseudeugenia)	*Eugenia pyriformis Cambess.*	L.M. Borges 1090	Brazil (RJ)
Eugenia (sect. Pseudeugenia)	*Eugenia pyriformis Cambess.*	Reitz & Klein 11341	Brazil (RJ)
Eugenia (sect. Racemosae)	*Eugenia angustissima O.Berg*	D.F.Lima 489	Brazil (GO)
Eugenia (sect. Racemosae)	*Eugenia biflora (L.) DC.*	T.Vasconcelos 589	Dominican Republic
Eugenia (sect. Racemosae)	*Eugenia longiracemosa Kiaersk.*	T.Vasconcelos 310	Brazil (AM)
Eugenia (sect. Racemosae)	*Eugenia modesta DC.*	TV 476	Brazil (MG)
Eugenia (sect. Racemosae)	*Eugenia modesta DC.*	TV 478	Brazil (MG)
Eugenia (sect. Racemosae)	*Eugenia paracatuana O.Berg*	PO Rosa 1399	Brazil (GO)
Eugenia (sect. Speciosae)	*Eugenia dichroma O.Berg*	T. Vasconcelos 466	Brazil (ES)
Eugenia (sect. Umbellatae)	*Calyptrogenia cuspidata Alain*	E. Lucas 1125	Dominican Republic
Eugenia (sect. Umbellatae)	*Eugenia bahiensis DC.,*	J.E.Q. Faria 4229	Brazil (BA)
Eugenia (sect. Umbellatae)	*Eugenia coffeifolia DC. Vel. Eugenia adenocalyx DC.*	A. Giaretta 1441	Brazil (RR)
Eugenia (sect. Umbellatae)	*Eugenia pluriflora DC.*	Hatschbach 19022	Brazil (PR)
Eugenia (sect. Umbellatae)	*Eugenia protenta McVaugh*	T.Vasconcelos 350	Brazil (AM)

Eugenia (sect. Umbellatae)	*Eugenia punicifolia (Kunth) DC.*	T.Vasconcelos 475	Brazil (MG)
Eugenia (sect. Umbellatae)	*Eugenia punicifolia (Kunth) DC.*	J.E.Q. Faria 4051	Brazil (DF)
Eugenia (sect. Umbellatae)	*Eugenia punicifolia (Kunth) DC.*	T.Vasconcelos 284	Brazil (GO)
Eugenia (sect. Umbellatae)	*Eugenia punicifolia (Kunth) DC.*	J.E.Q. Faria 4237	Brazil (ES)
Eugenia (sect. Umbellatae)	*Eugenia aff. schunkei McVaugh*	A.Giaretta 1419	Brazil (AM)
Eugenia (sect. Umbellatae)	*Eugenia stictosepala Kiaersk.*	J.E.Q. Faria 4269	Brazil (ES)
Eugenia (sect. Umbellatae)	*Hottea ekmanii (Urb.) Borhidi*	E. L. Ekman 2502c	Dominican Republic
Incertae sedis	*Algrizea macrochlamys (DC.) Proença & NicLugh*	E. Melo 4496	Brazil (BA)
Incertae sedis	*Algrizea minor Sobral, Faria & Proença*	J.E.Q. Faria 4157	Brazil (BA)
Incertae sedis	*Amomyrtus luma (Molina) D. Legrand & Kausel*	RBGE 1996- 1065	RBG Edimburg living collection (native to Chile)
Incertae sedis	*Myrtastrum rufopunctatum (Pancher ex Brongn. & Gris) Burret*	M.W. Callmander 796	New Caledonia
Myrceugenia	*Blepharocalyx cruckshanksii (Hook. & Arn.) Nied.*	M.F. Gardner 4193	Chile
Myrceugenia	*Luma apiculata (DC.) Burret*	T. Vasconcelos s.n.	RBG Kew living collection (native to Chile)
Myrceugenia	*Myrceugenia alpigena (DC.) Landrum*	J.E.Q. Faria 4264	Brazil (MG)
Myrceugenia	*Myrceugenia alpigena (DC.) Landrum*	T.Vasconcelos 489	Brazil (MG)
Myrceugenia	*Myrceugenia bananalensis Gomes-Bezerra & Landrum*	JEQ Faria 4048	Brazil (DF)
Myrceugenia	*Myrceugenia bananalensis Gomes-Bezerra & Landrum*	JEQ Faria 4049	Brazil (DF)
Myrceugenia	*Myrceugenia planipes (Hook. & Arn.) O.Berg*	E. J. Lucas s.n.	RBG Kew living collection (native to Chile)
Myrcia (clade Aulomyrcia)	*Calyptranthes multiflora Poepp. ex O.Berg*	A.Giaretta 1429	Brazil (AM)
Myrcia (clade Aulomyrcia)	*Calyptranthes multiflora Poepp. ex O.Berg*	A.Giaretta 1431	Brazil (AM)
Myrcia (clade Aulomyrcia)	*Calyptranthes multiflora Poepp. ex O.Berg*	T.Vasconcelos 379	Brazil (AM)
Myrcia (clade Aulomyrcia)	*Myrcia strigipes Mart.*	J.E.Q. Faria 6303	Brazil (RJ)
Myrcia (clade Aulomyrcia)	*Marlierea excoriata Mart.*	T.Vasconcelos 493	Brazil (MG)

Myrcia (clade Aulomyrcia)	*Marlierea glabra Cambess.*	JEQ Faria 4246	Brazil (ES)
Myrcia (clade Aulomyrcia)	*Marlierea neuwiedeana (O.Berg) Nied.*	TV 467	Brazil (ES)
Myrcia (clade Aulomyrcia)	*Marlierea umbraticola (Kunth) O.Berg*	TV 311	Brazil (AM)
Myrcia (clade Aulomyrcia)	*Myrcia rubella Cambess.*	D.F.Lima 495	Brazil (GO)
Myrcia (clade Aulomyrcia)	*Myrcia amazonica DC.*	T. Vasconcelos 591	Brazil (SP)
Myrcia (clade Aulomyrcia)	*Myrcia hirtiflora DC.*	TV 440	Brazil (BA)
Myrcia (clade Calyptranthes)	*Calyptranthes aff. blanchetiana O.Berg*	E. Lucas 1208	Brazil (BA)
Myrcia (clade Calyptranthes)	*Calyptranthes brasiliensis Spreng.*	J.E.Q. Faria 4244	Brazil (BA)
Myrcia (clade Calyptranthes)	*Calyptranthes chytraculia (L.) Sw.*	KC 18-16	Jamaica
Myrcia (clade Calyptranthes)	*Calyptranthes grammica (Spreng.) D.Legrand*	T.Vasconcelos 483	Brazil (MG)
Myrcia (clade Calyptranthes)	*Calyptranthes lucida Mart. ex DC.*	T.Vasconcelos 259	Brazil (DF)
Myrcia (clade Calyptranthes)	*Calyptranthes pallens Griseb.*	T.Vasconcelos 534	Costa Rica
Myrcia (clade Calyptranthes)	*Calyptranthes thomasiana O.Berg*	T.Vasconcelos s.n.	RBG Kew living collection (native to British Virgin Islands)
Myrcia (clade Calyptranthes)	*Mitranthes clarendonensis (Proctor) Proctor,*	T.Vasconcelos 510	Jamaica
Myrcia (clade Calyptranthes)	*Mitranthes ottonis O.Berg*	E. Otto 272	Jamaica
Myrcia (clade Eugeniopsis)	*Marlierea laevigata (DC.) Kiaersk.*	J.E.Q. Faria 4236	Brazil (ES)
Myrcia (clade Eugeniopsis)	*Myrcia multipunctata Mazine*	T.Vasconcelos 801	Brazil (MG)
Myrcia (clade Gomidesia)	*Myrcia fenzliana O.Berg*	E. Nic-Lughada H50637	Brazil (BA)
Myrcia (clade Gomidesia)	*Myrcia sp.1*	T. Vasconcelos 500	Brazil (MG)
Myrcia (clade Gomidesia)	*Myrcia spectabilis DC.*	E. Lucas 1214	Brazil (BA)
Myrcia (clade Gomidesia)	*Myrcia spectabilis DC.*	E. Lucas 1210	Brazil (BA)
Myrcia (clade Guianensis)	*Myrcia guianensis (Aubl.) DC.*	D.F.Lima 463	Brazil (MG)
Myrcia (clade Guianensis)	*Myrcia laxiflora Cambess.*	E. Lucas 1221	Brazil (BA)
Myrcia (clade Guianensis)	*Myrcia nivea Cambess.*	D.F. Lima 492	Brazil (GO)
Myrcia (clade Guianensis)	*Myrcia sp.2*	D.F. Lima 483	Brazil (MG)

Myrcia (clade Myrcia)	*Myrcia aff. eriopus DC.*	E. Lucas 1205	Brazil (BA)
Myrcia (clade Myrcia)	*Myrcia cardiaca O.Berg*	T.Vasconcelos 274	Brazil (GO)
Myrcia (clade Myrcia)	*Myrcia linearifolia Cambess.*	P.O. Rosa 1402	Brazil (GO)
Myrcia (clade Myrcia)	*Myrcia paivae O.Berg*	T.Vasconcelos 516	Costa Rica
Myrcia (clade Myrcia)	*Myrcia paivae O.Berg*	T.Vasconcelos 298	Brazil (AM)
Myrcia (clade Myrcia)	*Myrcia splendens (Sw.) DC.*	T.Vasconcelos 250	Brazil (DF)
Myrcia (clade Myrcia)	*Myrcia splendens (Sw.) DC.*	T.Vasconcelos 587	Dominican Republic
Myrcia (clade Myrcia)	*Myrcia splendens (Sw.) DC.*	T.Vasconcelos 753	Brazil (ES)
Myrcia (clade Myrcia)	*Myrcia splendens (Sw.) DC.*	G.C. Herrera 9932	NA
Myrcia (clade Myrcia)	*Myrcia sylvatica (G.Mey.) DC.*	E. Lucas 1222	Brazil (BA)
Myrcia (clade Reticulosae)	*Myrcia pubipetala Miq.*	E. Lucas 477	Brazil (SP)
Myrcia (clade Sympodiomyrcia)	*Myrcia amplexicaulis (Vell.) Hook.f.*	E. Lucas 1207	Brazil (BA)
Myrcia (clade Sympodiomyrcia)	*Myrcia mucugensis Sobral*	TV 441	Brazil (BA)
Myrcia (clade Sympodiomyrcia)	*Myrcia mucugensis Sobral*	JEQ Faria 4197	Brazil (BA)
Myrcia (clade Sympodiomyrcia)	*Myrcia subcordata DC.*	JEQ Faria 4257	Brazil (ES)
Myrcia (clade Sympodiomyrcia)	*Myrcia trimera Sobral*	E. Lucas 1219	Brazil (BA)
Myrcia (clade Sympodiomyrcia)	*Myrcia truncada Sobral*	E. Lucas 1216	Brazil (BA)
Myrcia (clade Tomentosa)	*Myrcia laruotteana Cambess.*	J.E.Q. Faria 4046	Brazil (DF)
Myrcia (clade Tomentosa)	*Myrcia tomentosa (Aubl.) DC.*	T.Vasconcelos 262	Brazil (DF)
Myrcia (clade Tomentosa)	*Myrcia tomentosa (Aubl.) DC.*	PO Rosa 1379	Brazil (DF)
Myrteola	*Lenwebbia prominens N.Snow & Guymer*	L. Bird AQ424632	Australia (Queensland)

Myrteola	*Lenwebbia prominens N.Snow & Guymer*	G.P. Guymer AQ424641	Australia (Queensland)
Myrteola	*Lophomyrtus obcordata (Raoul) Burret*	spirit collection 10291	New Zealand
Myrteola	*Lophomyrtus obcordata (Raoul) Burret*	Cult Lord Headfort (Kew id:16201)	New Zealand
Myrteola	*Lophomyrtus obcordata (Raoul) Burret*	Melville 5751	New Zealand
Myrteola	*Myrteola nummularia (Lam.) O.Berg*	M.F. Gardner 3579	Falklands
Myrteola	*Myrteola nummularia (Lam.) O.Berg*	G.T.Prance 28535	Falklands
Myrteola	*Neomyrtus pedunculata (Hook.f.) Allan*	B.H.Macmillan 76/102	New Zealand
Myrteola	*Neomyrtus pedunculata (Hook.f.) Allan*	Colens 1714	New Zealand
Myrteola	*Ugni candolei (Barnéoud) O.Berg*	T.Vasconcelos s.n.	RBG Kew living collection (native to Chile)
Myrteola	*Ugni myricoides (Kunth) O.Berg*	T.Vasconcelos 533	Costa Rica
Myrtus	*Accara elegans (DC.) Landrum*	T.Vasconcelos 485	Brazil (MG)
Myrtus	*Calycolpus goetheanus (Mart. ex DC.) O.Berg*	T.Vasconcelos 332	Brazil (AM)
Myrtus	*Chamguava schippii (Standl.) Landrum*	D.Aguilar 9833	Costa Rica
Myrtus	*Chamguava schippii (Standl.) Landrum*	P.H. Gentle 8354	Costa Rica
Myrtus	*Myrtus communis L.*	E. Lucas 211	RBG Kew living collection (native to Mediterranean region)
Myrtus	*Myrtus communis L.*	T. Vasconcelos s.n.	RBG Kew living collection (native to Mediterranean region)
Pimenta	*Acca sellowiana (O.Berg) Burret*	T.Vasconcelos s.n.	RBG Kew living collection (native to southern Brazil)
Pimenta	*Acca sellowiana (O.Berg) Burret*	Spirit collection 14462	RBG Kew living collection (native to southern Brazil)
Pimenta	*Campomanesia adamantium*	T.Vasconcelos 474	Brazil (GO)

Pimenta	*Campomanesia adamantium*	T.Vasconcelos 293	Brazil (GO)
Pimenta	*Campomanesia guazumifolia (Cambess.) O.Berg*	A Lobao 1372	Brazil (SP)
Pimenta	*Campomanesia simulans*	T.Vasconcelos 472	Brazil (MG)
Pimenta	*Campomanesia velutina*	T.Vasconcelos 507	Brazil (DF)
Pimenta	*Capomanesia adamantium (Cambess.) O.Berg*	T. Vasconcelos 273	Brazil (DF)
Pimenta	*Legrandia concinna (Phil.) Kausel*	Germain s.n.	Chile
Pimenta	*Mosiera longipes (O.Berg) Small*	M.A. Hamilton 630	Sadle 186 turks and caicos islands
Pimenta	*Myrrhinium atropurpureum*	A. Stadinik s.n.	Brazil (RJ)
Pimenta	*Myrrhinium atropurpureum*	C. Farney 2265	Brazil (RJ)
Pimenta	*Myrrhinium atropurpureum Schott*	G. Hatchbachi 61056	Brazil (RJ)
Pimenta	*Pimenta dioica (L.) Merr.*	T.Vasconcelos 534	Costa Rica
Pimenta	*Pimenta pseudocaryophyllus (Gomes) Landrum*	H.S. Irwin 19844	Brazil (GO)
Pimenta	*Pimenta pseudocaryophyllus (Gomes) Landrum*	A.P. Duarte 8722	Brazil (SP)
Pimenta	*Pimenta pseudocaryophyllus (Gomes) Landrum*	H.C. de Lima 3453	Brazil (SP)
Pimenta	*Pimenta pseudocaryophyllus (Gomes) Landrum*	E. Lucas 193	Brazil (SP)
Pimenta	*Pimenta pseudocaryophyllus (Gomes) Landrum*	T. Vasconcelos 403	Brazil (MG)
Pimenta	*Pimenta racemosa (Mill.) J.W.Moore*	F. Axelrod 7796	Dominican Republic
Pimenta	*Pimenta sp.1*	T.Vasconcelos 578	Dominican Republic
Pimenta	*Psidium acranthum Urb.*	T.Vasconcelos 579	Dominican Republic
Pimenta	*Psidium laruotteanum Cambess*	J.E.Q. Faria 4276	Brazil (GO)
Pimenta	*Psidium brownianum Mart. ex DC.*	T.Vasconcelos 465	Brazil (BA)
Pimenta	*Psidium firmum O.Berg*	T.Vasconcelos 290	Brazil (GO)
Pimenta	*Psidium friedrichsthalianum (O.Berg) Nied.*	T.Vasconcelos 522	Costa Rica
Pimenta	*Psidium guajava L.*	T.Vasconcelos 585	Dominican Republic (cultivated)
Pimenta	*Psidium guajava L.*	T.Vasconcelos 389	Brazil (DF) (cultivated)

Pimenta	*Psidium guineense Sw.*	T.Vasconcelos 279	Brazil (GO)
Pimenta	*Psidium guineense Sw.*	B.S.Amorim 1913	Brazil (PE)
Pimenta	*Psidium myrsinites DC.*	T.Vasconcelos 503	Brazil (GO)
Pimenta	*Psidium myrtoides O.Berg*	T.Vasconcelos 402	Brazil (SP)
Pimenta	*Psidium oligospermum Mart. ex DC.*	F.F.Mazine 1346	Brazil (ES)
Pimenta	*Psidium oligospermum Mart. ex DC.*	F. Franca 5431	Brazil (BA)
Pimenta	*Psidium riparium Mart. ex DC.*	J.E.Q. Faria 4107	Brazil (TO)
Pimenta	*Psidium rufum Mart. ex DC.*	J.E.Q. Faria 4271	Brazil (MG)
Plinia	*Myrciaria floribunda (H.West ex Willd.) O.Berg,*	T.Vasconcelos 380	Brazil (AM)
Plinia	*Myrciaria aff. glazioviana (Kiaersk.) G.M.Barroso ex Sobral*	T.Vasconcelos 413	Brazil (BA)
Plinia	*Myrciaria floribunda (H.West ex Willd.) O.Berg*	R.M. Harley 54895	Brazil (BA)
Plinia	*Myrciaria glanduliflora (Kiaersk.) Mattos & D.Legrand*	T.Vasconcelos 479	Brazil (BA)
Plinia	*Neomitranthes cordifolia (D.Legrand) D.Legrand*	M.C. Souza 550	Brazil (RJ)
Plinia	*Neomitranthes obscura (DC.) N.Silveira*	A.M. Carvalho 816	Brazil (SP)
Plinia	*Plinia cauliflora (Mart.) Kausel*	T.Vasconcelos 388	Brazil (DF) (cultivated)
Plinia	*Plinia nana Sobral*	A Stadnik s.n.	Brazil (MG)
Plinia	*Siphoneugena delicata Sobral & Proença*	T.Vasconcelos 760	Brazil (ES)
Plinia	*Siphoneugena densiflora O.Berg*	G. Martinelii 11939	NA
NON MYRTEAE	*Heteropyxis natalensis Harv.*	M.F. Correia 594	South Africa

Work Package II – Case studies on systematics and floral evolution of Myrteae

Due to its role in reproduction, floral variability is intimately related to oscillations in reproductive success and adaptive value of a lineage. Understanding development and morphological diversity in these organs is an important tool for comprehension of plant evolution. Work Package II demonstrates how floral development and diversity in Myrteae can help answering general questions in evolution, ecology and systematics of angiosperms. Chapter 4 reassesses homoplastic characters in a phylogenetic context after homologous categories are clarified, observing improved phylogenetic signal and general understanding of a trait's adaptive features. Chapter 5 highlights the importance of parallelisms as a source of systematic strife in large groups. Chapter 6 demonstrates how heterochronies can lead to subtle morphological changes that affect reproductive success even in apparent morphologically homogeneous groups. Finally, Chapter 7 describes an atypical case of conservative flower evolution and macro-evolutionary dynamics in the tropics, using the mega-diverse genus *Myrcia* as an example of long lasting stability in ecological-evolutionary systems.

Chapter 4: Augmenting Phylogenetic Signal for Homoplastic Traits: The Evolutionary History of Perianth Fusion in Myrtaceae Flowers

Manuscript – to be submitted to *Cladistics*

- T.N.C.Vasconcelos contributions: development of hypotheses, design of experiments, collection of samples, generation of SEM and LM images, morphological analyses, phylogenetic analyses and writing of manuscript.

ABSTRACT:

Molecular phylogenies often reveal that traits once credited as systematically useful are often strongly homoplastic. Perianth fusion in Myrtaceae flowers is an example of such a trend, having evolved independently multiple times in the family's evolutionary history. Here we re-visit the homology of the fused perianth in Myrtaceae and use the results as a case study to demonstrate how careful morphological investigation can improve the phylogenetic signal of homoplastic traits. We describe and compare calyptra development and anatomical characteristics in distinct lineages using SEM and LM. Results show equivocality in usage of the terms 'fused perianth' and 'calyptra', as these structures correspond to at least three anatomically and ontogenetically distinct structures. Perianth fusions by the same process are identified and noted in different lineages; despite this, phylogenetic signal increases from $\lambda = 0.634$, when presence/absence of calyptra is plotted without any discrepancy, to $\lambda = 0.651$, $\lambda = 0.782$ and $\lambda = 1$ when homologous calyptra formations are analysed individually against the Myrtaceae phylogeny. This supports the hypothesis that characters previously discarded as systematically irrelevant can actually present a strong phylogenetic signal, once their structural details are clarified and re-assessed under an existing phylogenetic framework. We discuss the recurrence of the calyptrate flower in Myrtaceae in light of functional traits and the possible adaptive role of the structure in distinct niches. We encourage morphological reassessment of homoplastic characters in light of well-known phylogenetic frameworks. Unidentified homoplasy can hide relatively high phylogenetic signals that are key to appreciate underlying evolutionary patterns when trait evolution of individual lineages is analysed at high taxonomic rank.

Key words: calyx, convergence, corolla, functional traits, underlying homology.

4.1 Trait homoplasy in the molecular era

Cladistic studies traditionally rely on the search for synapomorphies, i.e. phenotypic traits shared by organisms decedent from common ancestors, to identify and characterize single evolutionary units (Duncan and Stuessey, 1984). During most of the 20th century, this process was based on annotation of morphological traits, starting from fundamental differences at higher taxonomic ranks to very fine ones at species level systematics (Stebbins, 1974). This approach is, however, often prone to inaccuracies due to the subjective nature of trait classification (see review in Hillis, 1987). Molecular phylogenies, the standard contemporary approach to relationship reconstruction of organisms, are also sensitive to errors (e.g. Palmer, 1992), but they have the advantage in providing larger datasets and a less artificial interpretation of evolutionary history based on DNA level homologies (Felsestein, 2004). Despite their advantages, molecular systematics often do not identify recurring homoplastic traits in phylogenetic trees (i.e. appearance of similar characters in non-related lineages) (Sanderson and Hufford, 1996).

Various processes lead to the appearance of homoplastic traits, including convergences, parallelisms and reversals. Structural convergences appear when ontogenetically distinct structures are superficially similar due to a similar functional role (e.g. Abrahão et al., 2014). Parallelisms and reversals result from ontogenetically identical structures in phylogenetically related but non-sister taxa (Scotland, 2011). These two processes can metaphorically be described as “the most closely related groups of organisms wander in the most similar (adaptive) landscapes […and…] there are certain easy tracks where there is constant going (parallel evolution) and coming back (evolutionary reversals)” (Endress, 1996a, p.303-304). Prioritizing molecular over morphological data as the basis of systematics has driven the latter into a secondary position (Scotland et al., 2003). However, morphological re-examination of homoplastic traits in an era where algorithms are most heavily relied on to understand nature (Mooi and Gill, 2010) reveals semantic issues and still unexplored evolutionary patterns.

4.2 Perianth fusion in Myrtaceae

In Weberling’s (1989) definition of the “perfect flower”, the perianth corresponds to the outmost floral part and is formed by two whorls of leaf-like organs. The external whorl, the calyx, is formed by sepals and the internal whorl, the corolla, is formed by petals, which in spite of the leaf-like appearance are sometimes regarded to be evolutionarily closer to the androecium (Ronse De Craene, 2007). Across angiosperms, the calyx most commonly has the role of protection, covering the sensitive sexual organs during flower ontogeny. The corolla, frequently showy, is usually linked to pollinator attraction (Endress, 1994). Nevertheless, variations of these more common functions are commonly observed. A perianth that appears partially or completely fused in the bud, for instance, is a common trend in some angiosperm families (e.g. Euphorbiaceae, Esser, 1999; Solanaceae, D’Arcy, 1986) and specific terminology exists to describe behaviour of these structures during anthesis. “Calyptrate” or “operculate” flowers are designated as such when a perianth appears completely fused in bud, detaching at the base and falling off as a single “cap like” structure during anthesis (e.g. McVaugh, 1956; Fig. 4.1).

Calyptrate flowers are observed in many lineages across angiosperms, such as Vitaceae (Soejima and Wen, 2006), Eupomatiaceae (Endress, 2003), and in the order Myrtales (e.g.

Goldenberg and Meirelles, 2011; Kriebel et al., 2015). In the latter, calyptrate flowers are an especially common trend in Myrtaceae, appearing in at least 17 out of the 144 genera (Wilson, 2011). Drinnan and Ladiges (1980) provide a thorough description and systematic discussion of perianth fusion in *Eucalyptus*, but some of the most diverse Myrtaceae lineages with calyptrate flowers remain to be studied. In spite of its clear homoplastic pattern, this character has not yet been examined in light of the phylogenetic history of the family.

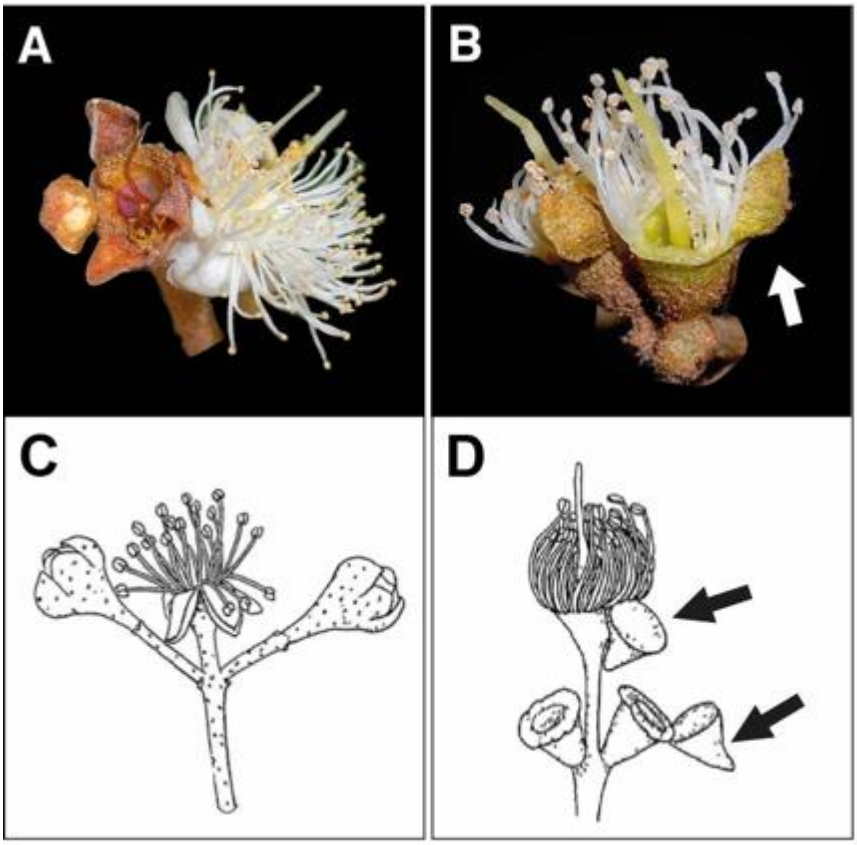


Figure 4.1: Calyptrate and non-calyptrate flowers in Myrtaceae (illustration). “A” and “B” show closely related *Myrcia* and *Calyptranthes* flowers, the former with four free sepals and petals; the latter with a calyptrate perianth (arrow). “C” and “D” are illustrations representing the same trend in *Blepharocalyx* (A and B photos taken during field expeditions between 2014 and 2016; illustration reproduced from Landrum and Kawasaki, 1997).

4.3 Aims

The calyptra in Myrtaceae is a good example of the omission of potentially informative characters from careful study due to an apparent lack of correspondence with the phylogenetic framework. However, careful morphological examination and posterior reassessment of traits on phylogenetic trees increases understanding of underlying evolutionary processes that lead to homoplastic patterns. In this sense, the aims of this study are: 1) To test the homology of the calyptrate flower in Myrtaceae by surveying perianth development and anatomy of distinct calyptrate lineages; 2) To adjust phylogenetic signal for calyptrate flowers in Myrtaceae by re-coding the trait after homologous categories are identified; and 3) To use the results to discuss the function of the closed perianth and its role in Myrtaceae evolution.

4.4 Study group and sampling approach

Calyptrate flowers are distributed in 17 of the 144 accepted genera of Myrtaceae, distributed in four tribes: 11 in Myrteae, one in Xanthostemonae two in Syzygieae, one in Metrosidereae and two in Eucalypteae. Genera and species were assessed for possession of a calyptra and fieldwork expeditions were conducted in Brazil, Central America, SE Asia and New Caledonia, to collect a broad sample of Myrtaceae species with calyptrate flowers. In the case of *Eucalyptus*, samples were collected mainly from the living collection of the Royal Botanic Gardens Kew. Samples were collected in alcohol 70% and FAA. Perianth behaviour during anthesis was also observed and photographed in the field. The list of species and vouchers analysed for ontogeny, anatomy and anthesis behaviour is presented in Appendix 4.1. Perianth ontogeny and anatomy was recorded for calyptrate species and compared with their closest relatives with free perianths.

4.5 Ontogenetic study

Floral buds and flowers were dissected in 70% ethanol, dehydrated through an alcohol series to 100% ethanol, and critical-point dried using an Autosamdri-815B critical-point dryer (Tousimis Research, Rockville, Maryland, USA). Dried material was mounted onto specimen stubs, coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinsted, UK) and examined with a Hitachi cold field emission SEM S-4700-II (Hitachi High Technologies, Tokyo, Japan). Key stages of perianth development such as organ initiation, point of fusion and degree of fusion at anthesis were noted and described. Distinct types of calyptra are described based the nature of perianth tissue at these stages. These were then classified into three categories of calyptrate flowers (see *Results* sections 4.9-4.12)

4.6 Anatomical survey

Selected closely related taxa were anatomically profiled. Anatomical sectioning was conducted on pre-anthetic buds. For histology, samples passed through a series of alcohol to histoclear (100%) and were then embedded in wax (paraplast 100%) or, when the material was too hard, in resin. Wax was changed every other day for three weeks until the samples were full embedded. Sectioning was performed using a microtome (Leica RM2155) and slides were stained with safranin red and alcian blue. These stains colour lignified tissues in red and cellulose in blue. Samples embedded in resin passed through the same process of staining, but the colours react differently and thus are not comparable to the ones in wax. Slides are accessible in the slide collection of Royal Botanic Gardens Kew.

4.7 Phylogenetic assessment and placement of *Pleurocalyptus*

The most up-to-date phylogeny of Myrtaceae of Thornhill et al. (2015) was used to test phylogenetic signal. Most lineages with calyptrate buds are represented in this cladogram based on three molecular markers for 199 tips, sourced from the supplementary material of the original publication. The function *bind.tip* (package *phytools* in R; Revell, 2012) was used to add four further taxa, providing a more complete representation of the character under study. These were placed according to phylogenetic positions determined by independent studies (Lucas et al., 2007; Chapter 1). These extra tips comprise a calyptrate species of *Eugenia* (*Calyptrogenia cuspidata,* placed

sister to *Eugenia sulcata*), a calyptrate species of *Blepharocalyx* (*Blepharocalyx eggersii*, placed sister to *B. salicifolius*), *Neomitranthes* (*Neomitranthes spp.*, placed sister to *Myrciaria vexator*) and *Pleurocalyptus* (*Pleurocalyptus pancherii*, placed sister to *Xanthostemon chrysanthus*). For unequivocal placement the four tips were placed at a branch length of half distance between the sister species and the common ancestor node. *Pleurocalyptus pancherii*, a species with calyptrate flowers from New Caledonia was thought by Wilson (2011) to be a calyptrate version of *Xanthostemon*. To confirm its position, ITS and *ndhf*, two of the three molecular markers included by Thornhill et al. (2015), were sequenced for *Pleurocalyptus pancherii* (using methods described in Chapter 1, section 1.5; voucher T.Vasconcelos 622). Blast results match both sequences to *Xanthostemon chrysanthus* with c. 98% similarity, corroborating its position in tribe Xanthostemonae (see Appendix 4.2).

4.8 Character reconstruction and estimates of phylogenetic signal

All categories of calyptrate flowers (see *Results* sections 4.9-4.12) can be easily recognized using herbarium material once homologous states are understood. Following ontogenetic survey of the sample selected, categories of calyptra were assigned and extrapolated to all phylogenetic tips. Some *Syzygium* species, particularly those with small buds, were difficult to access due to fragile perianths post-herborisation. As a result, some *Syzygium* species were excluded because no category could confidently be assigned (species marked with "NA", Appendix 4.1). Calyptrate categories in some *Eucalyptus* were coded from the literature (Carr and Carr, 1962; Drinnan and Ladriges, 1988, 1989, 1991; Brooker, 2000).

Characters were reconstructed on the phylogeny using function *ace* (package *ape* in R software; Paradis et al., 2004; R core team, 2017) to map appearance and reversal of the calyptrate state. Phylogenetic signal was measured as the tendency of two closely related species to resemble each other more than two random species, using the function *fitDiscrete* from package *geiger* in R (Harmon et al., 2008). This provides a value of comparable log-likelihood and AIC values that are further corrected into a score from 0 to 1 (*lambda*, or "λ") based on Brownian motion. The higher the value, the more likely that the phylogenetic framework is affecting the distribution of the character in the tree. In all estimates, traits were coded into simple binary states (presence or absence), first for all calyptrate taxa without distinction of calyptra categories, and then using homologous calyptrate states. In species with combinations of calyptrate categories, each category was coded separately. Scores received from each analysis were compared to assess changes in phylogenetic signal for a given homoplastic trait depending on interpretation of homology.

RESULTS

4.9 Calyptra homology

Ontogenetic and anatomical analyses of selected species confirm that the myrtaceous calyptra cannot be treated as a homologous structure. Closed perianths occur via at least three distinct developmental patterns that involve different organs (calyx or corolla) and types of fusion.

4.10 The calycine calyptra

The calycine calyptra is formed by the calyx, the outmost floral whorl, the usually four lobes of which, initiate free following a decussate pattern (Fig. 4.2A). After a short period of elongation the base of the four sepals fuse into a homogeneous calycine tissue (post-genital fusion; Fig.

4.2C,H,I,J). During this process, the free sepal tips meet or overlap slightly (Fig. 4.2C,E). The now gamosepalous structure continues its development fused until pre-anthesis. At this point, signs of the initial free lobes remain as inconspicuous scars at the top of the buds, characterising this developmental mode (Fig. 4.2F; Fig 4.3A,F). During anthesis, pressure from within the bud tears the calycine tissue at the weakest spot, frequently the base, resulting in a “cap-like” structure that often remains attached to the side of the flower (Fig. 4.2N,O; Fig 4.3G,H). Calycine calyptras may appear in conjunction with all other varieties of corolla development. These include calycine calyptras on top of coralline calyptras (common in Eucalypteae, see below); calycine calyptras on top of free reduced or showy petals (as in Myrteae Fig. 4.3B-D; and Xanthostemoneae Fig. 4.3H) or calycine calyptras on top pseudocalyptras (common in Syzygieae, Fig.4.2M).

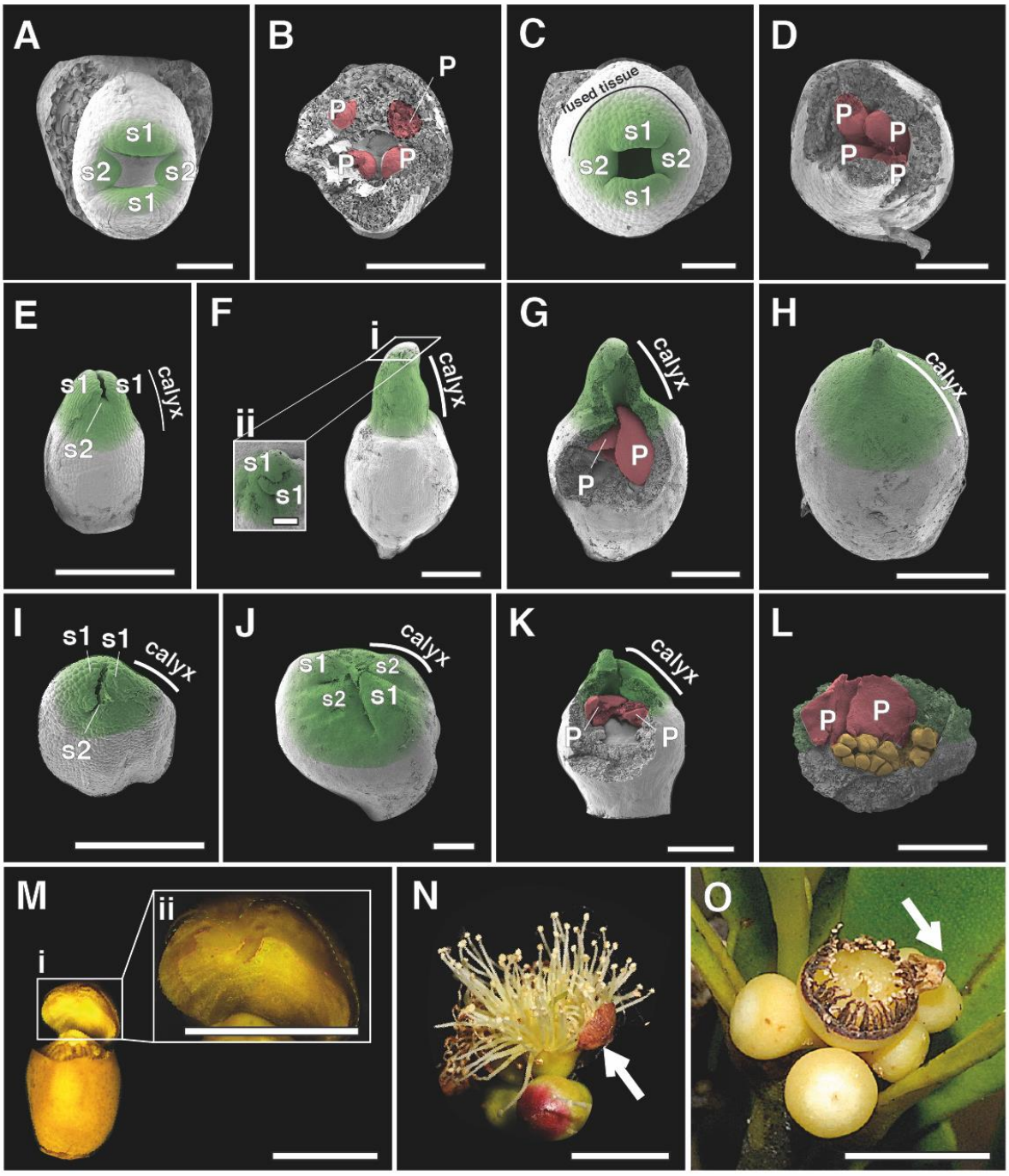


Figure 4.2 (previous page): Calycine calyptra in Syzygieae. (A-H, M, N): perianth development in *Syzygium nervosum*. (I-L, O): perianth development in *Piliocalyx* sp. (voucher T.Vasconcelos 651). (A) Decussate development of four free sepals followed by (B) simultaneous initiation of four free petals in the axils of each sepal. (C) Calyx undergoing post genital fusion while (D) petals remain free and overlap. (E) Sepal tips meet and overlap leaving a (F) scar on the top of the bud. (G-H) Continuous development of the bud with fused calyx and free petals. (I, J) Sepal tips meeting and overlapping and (K-L) continuous development of fused sepals and free petals in *Piliocalyx* sp. (M, N) Anthesis in *Syzygium nervosum* highlighting (Mii) petals attached to the calycine calyptra (arrow in N). (O) Arrow showing old calyptra in a *Piliocalyx* sp. flower. S: sepals; P: petals. Scale: 50µm (A,C), 100µm (D,Fii), 150µm (B), 250µm (E,Fi,G,I), 400µm (L), 500µm (K), 1mm(H), 5mm (Mi,Mii,N,O). ('N' photo by A. Lambrianides; 'O' photo taken during field expeditions in 2015)

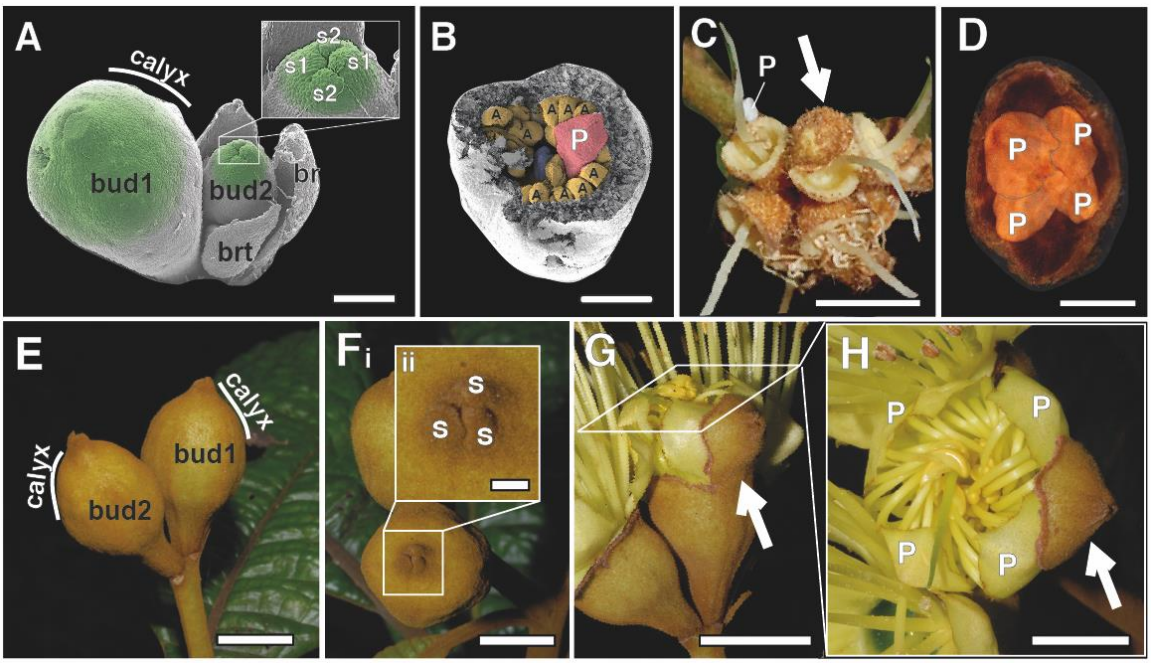


Figure 4.3: Calycine calyptra in Myrteae and Xanthostemoneae. (A) Young inflorescence of *Myrcia aulomyrcioides* showing scar left by the previous free sepals on the top of the bud. (B) Dissected bud showing free petal. (C) Old inflorescence of *Calyptranthes pallens*, showing calyptra attached to the flower (arrow) and remaining reduced petal. (D) An upside-down dissected calyptra of *Calyptranthes pallens*, showing four free, somewhat reduced, petals. (E) Pre-anthetic flowers of *Pleurocalyptus pancherii* showing fused calyx and (F) scar from sepal tips on the top of the bud. (G) Arrow indicating calycine calyptra in *Pleurocalyptus pancherii* flower on top of (H) four free showy petals. S: sepals; P: petals; A: androecium. Scale: 250µm (A,B), 1mm (Fii), 5mm (C, H), 1cm (D, E, Fi, G). (C,E,F,G,H photos taken during field expeditions between 2014 and 2016)

4.11 The corolline calyptra

The corolline calyptra is formed from usually four, fused petals that are free at initiation (Fig. 4.4D) but subsequently fuse and elongate as a homogeneous tissue (Fig. 4.4E,F). This structure is similar to the calycine calyptra in the sense that organs undergo post-genital fusion (Fig.4A-F). The corolline calyptra is only found in Eucalypteae where it often develops in association

with a calycine calyptra (Fig. 4.4A-C). During anthesis both structures tear at the base and fall as a single, inseparable, unit (Fig.4G).

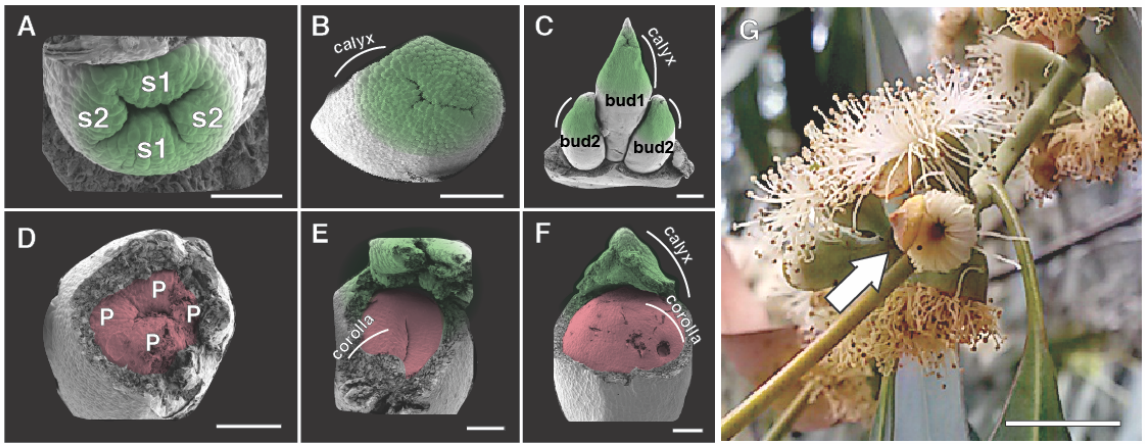


Figure 4.4: Calycine and coralline development in Eucalypteae (all images from *Eucalyptus perriniana*). (A) Early development of four free sepals in a decussate fashion followed by (B) post-genital fusion of sepals. (C) Young inflorescence showing calycine calyptras in different stages. (D) Early petal development, showing the still recognizable four petals, followed by (E) post-genital fusion and (F) formation of an almost completely homogeneous tissue in mature flowers. (G) Flower at anthesis with two-layered calyptra (arrow). S: sepals, P: petals. Scale: 50µm (A), 100µm (B, D, E, F), 250µm (C), 1cm (G). ('G' photo taken from living collection at Royal Botanic Gardens Kew).

4.12 The pseudocalyptra

Perianth development of pseudocalyptrate species is identical to that of species with a free perianth (Fig. 4.5). The four sepals initiate in a decussate fashion followed by four petals that initiate simultaneously or almost simultaneously in the radius of sepals and soon overlap (Fig 4.5A,B,J-L), forming a tissue of four layers (Fig. 4.5D). Both petals and sepals elongate and develop with no fusion until anthesis (Fig.4.5A-I). However, in species where a pseudocalyptra occurs, sepals either stop developing early or elongate at a slower rate, so that at pre-anthesis the four sepals are barely visible. The surfaces of the four still free, layered petals are strongly attached so that the whole corolla detaches as a single unit at anthesis, remaining attached to the flower as does a calycine or coralline calyptra (Fig. 4.5I). In some species, this pseudocalyptra is associated with calycine calyptras (Fig. 4.2M). In species with a fully free-perianth, sepals and petals never fall as a single unit and can be easily identified at anthesis (Fig. 4.5M-O).

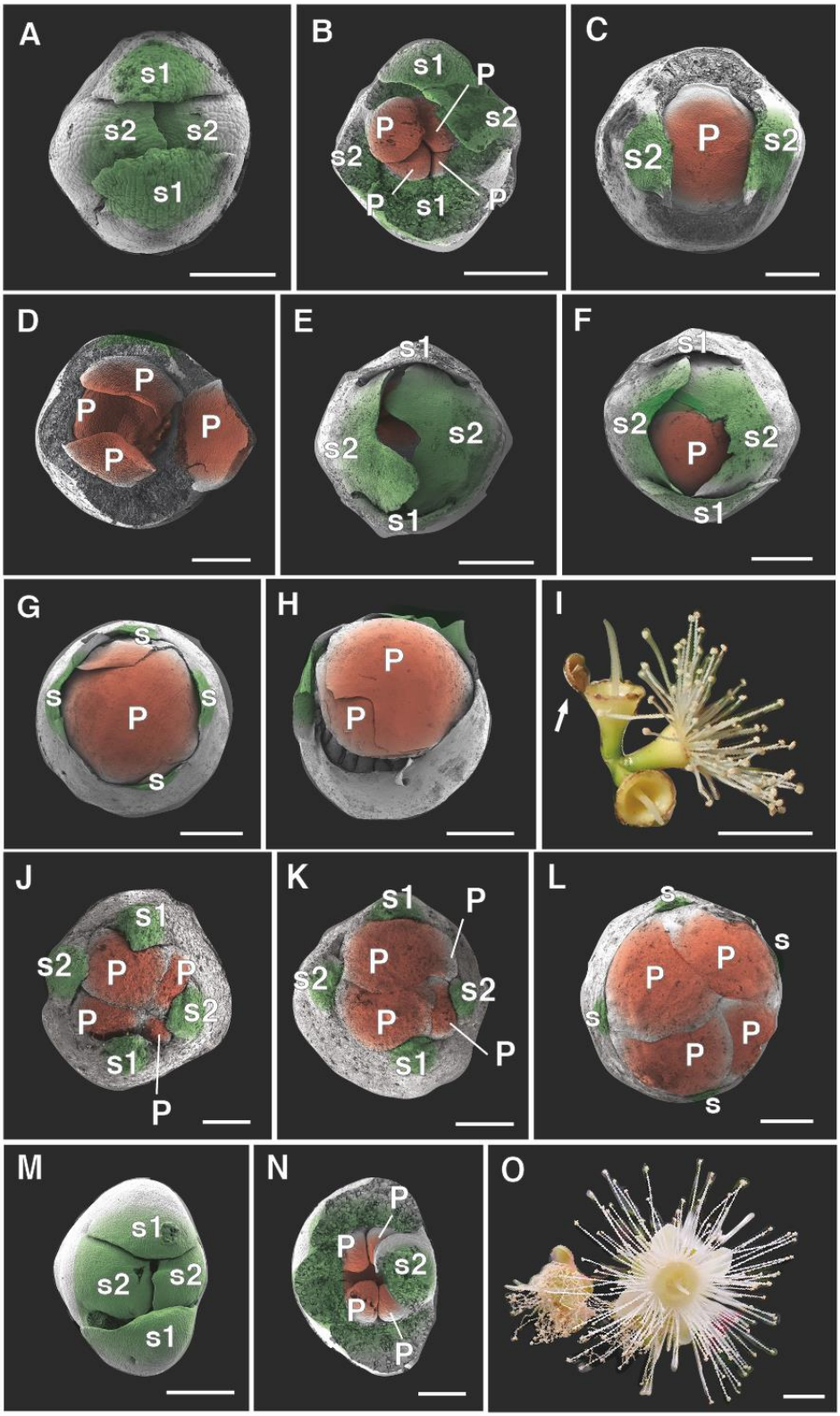


Figure 4.5: Pseudocalyptra and free-perianth development in Syzygieae and Eucalypteae. (A-I) *Syzygium cumini*; (G-L) *Syzygium paniculatum*; (M-O) *Eucalyptus curtisii*. (A) Early development of four free sepals in a decussate fashion. (B) As "A", but further dissected to show the four petals in early stages of development. (C) Slightly older bud with both S1 dissected, showing already overlapping petals. (D) Same as "C", but all sepals dissected and petals forced using tweezers to highlight their free condition. (E-G) Sequential bud development, showing sepals that stop developing and are very reduced in the mature bud. (H) Bud at anthesis, highlighting petals

detaching from the base of the bud as a single unit. (I) Old inflorescence indicating the pseudocalyptra formed by the four layers of petals. (J-L) Sequential bud development in *Eucalyptus curtisii*, highlighting sepals that stop developing and reduced in the mature bud. (M, N) Early development and (O) anthesis in the free-perianth *Syzygium paniculatum*, showing four sepals and petals clearly identifiable at anthesis. S: sepals; P: petals. Scale: 150µm (A, F), 200µm (B), 250µm (C, D, J, K, M), 500µm (E, F, N), 1mm (G, O), 2mm (L), 5mm (I).

4.13 Calyptra anatomical profile and functional traits

Anatomical profiles of calyptrate species of distinct lineages illustrate the organs involved in formation of different calyptras. Histological differences between similar organs are also revealed, especially regarding tissue thickness and cellular structure. Calycine calyptras are observed to be either strongly glandulous or lignified, with variations even within the same lineage (e.g. *Calyptranthes* spp. in Fig.4.6 F,G). In one case in tribe Myrteae, the inner side of the thickened calycine calyptra is observed to be covered in strongly lignified trichomes (Fig. 4.6E,ii). In *Syzygium nervosum*, the upmost petal adheres strongly to the abaxial side of the calyptra (Fig. 4.6iv), which may explain why during anthesis the corolla detaches with the calyptra (Fig. 4.2M). Tissue thickness frequently increases from bottom to top in calycine calyptras, leaving the thinnest layer of tissue at the calyptra base.

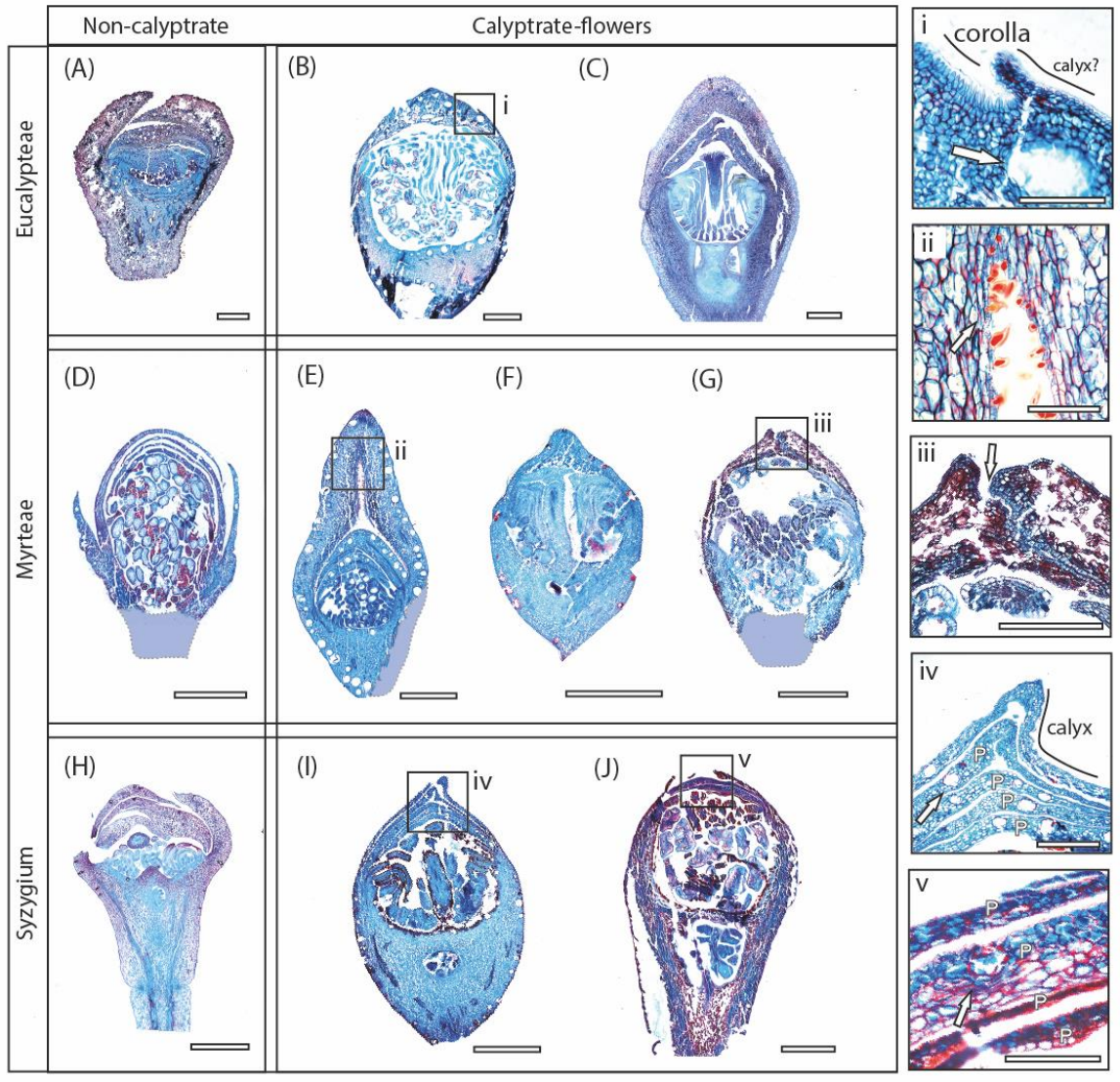


Figure 4.6 (previous page): Anatomical profiles of three species with free perianth and seven species with calyptrate flowers in three distinct Myrtaceae lineages. (A) *Arillastrum gumifferum*; (B) *Eucalyptus pauciflora;* (C) *Corymbia* sp. (voucher TVasconcelos 681, resin embedded); (D) *Decaspermum parviflorum*; (E) *Psidium brownianum*; (F) *Myrcia aulomyrcioides* (G) *Calyptranthes pallens* (H) *Syzygium paniculatum* (I) *Syzygium nervosum* (J*) Syzygium cumini*. Inserts: (i) Arrow indicates gland lumen in *Eucalyptus pauciflora* and possible division between coralline calyptra and calyx. (ii) Internal surface of calycine calyptra in *Psidium brownianum*; arrow shows strongly lignified single celled trichomes. (iii) Apex of calycine calyptra in *Calyptranthes pallens*, highlighiting sepal fusion and strongly lignified tissue. (iv) Apex of calycine calyptra and pseudocalyptra in *Syzygium nervosum*, highlighting the upmost petal strongly adhered to the calycine calyptra and strongly glandular tissue. (v) Apex of pseudocalyptra in *Syzygium cumini* showing four layers of lignified petals. Scale: 150μm (ii, iii), 200μm (i), 250μm (iv), 500μm (v), 1mm (A-J).

4.14 Re-coding characters and adjustment of phylogenetic signal

42 out of 199 tips (21 %) are scored as “calyptrate-flower” present (state = 1). Flowers with free perianth (state = 0) constitute the ancestral state of Myrtaceae with calyptrate flowers appearing at least 22 times independently: once in Xanthostemoneae (origin of *Pleurocalyptus* – Fig.7C), three times in Eucalypteae, seven times in Myrteae and eleven times in Syzygieae. Syzygieae also shows three likely reversals to the ancestral state (blue triangles, Fig.4.7). In Myrteae, Metrosidereae and Syzygieae, transitions from non-calyptrate to calyptrate occurred mainly in the Miocene or earlier (Fig. 4.8A). In Eucalypteae, transition to calyptrate appear as far back as the Paleocene (e.g. Fig. 4.7, shift 4, in *Eucalyptus*).

Analysis of phylogenetic signal for calyptrate flowers in Myrtaceae show low a priori phylogenetic signal ($\lambda = 0.634$, Fig. 4.8A) indicating weak phylogenetic correlation. When calycine calyptras are distinguished the phylogenetic signal is higher than when calyptrate taxa are coded without distinction ($\lambda = 0.782$, Fig. 4.8B). Similarly, when coralline calyptras are coded separately the phylogenetic signal is maximum ($\lambda = 1$, Fig.4.8C). The Pseudocalyptra, however, presents phylogenetic signal as low as when calyptrate flowers are coded without distinction ($\lambda = 0.651$, Fig.4.8D).

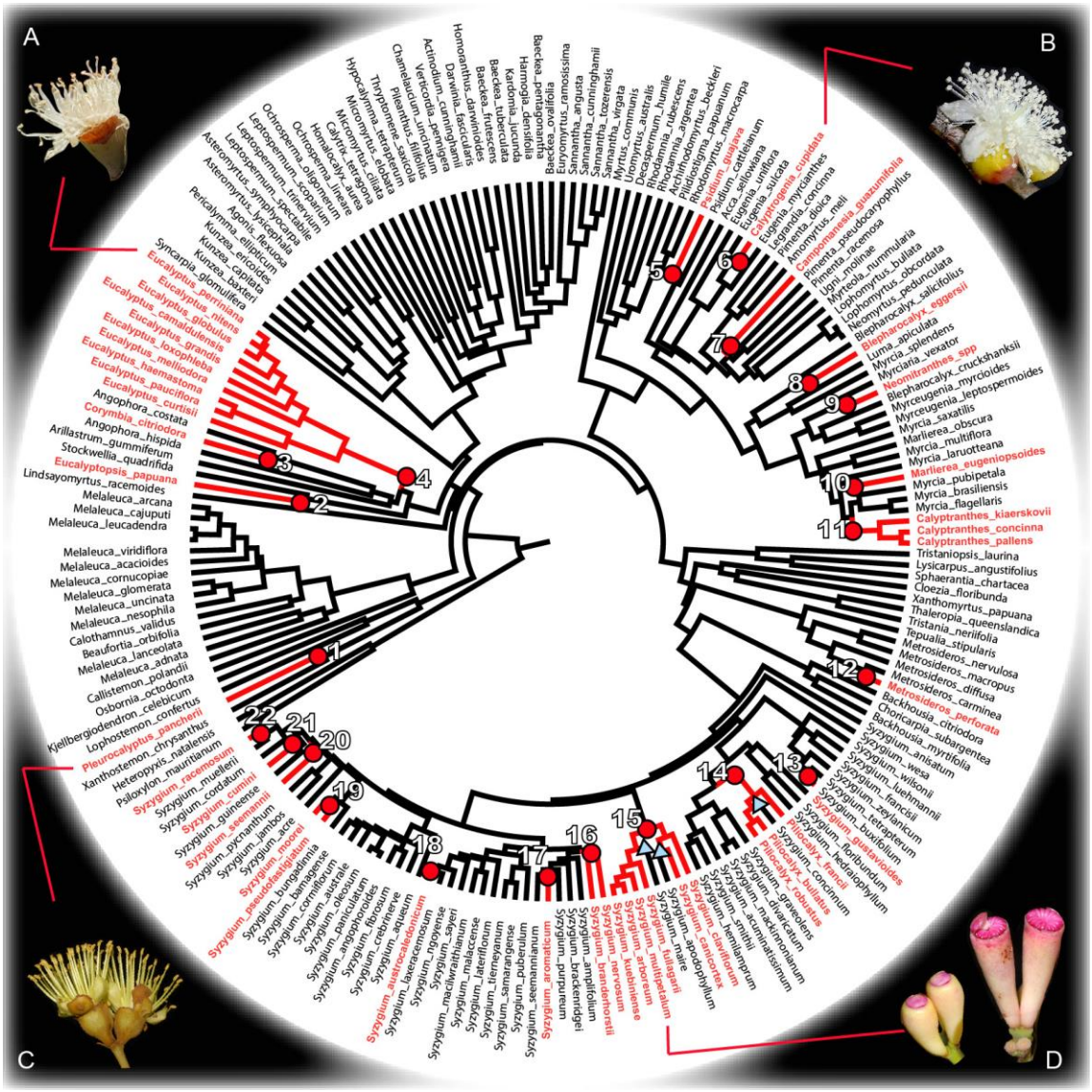


Figure 4.7: Calyptrate flowers mapped on to the Myrtaceae phylogeny. Ancestral character reconstruction shows calyptrate flowers appearing 22 times (red circles) independently in Myrtaceae, with three reversals (triangles) in Syzygieae. (A) *Eucalyptus perriniana*; (B) *Calyptrogenia cuspidate*; (C) *Pleurocalyptus pancheri;* (D) *Syzygium multipetalum.* (All photos taken during field expeditions between 2014 and 2016).

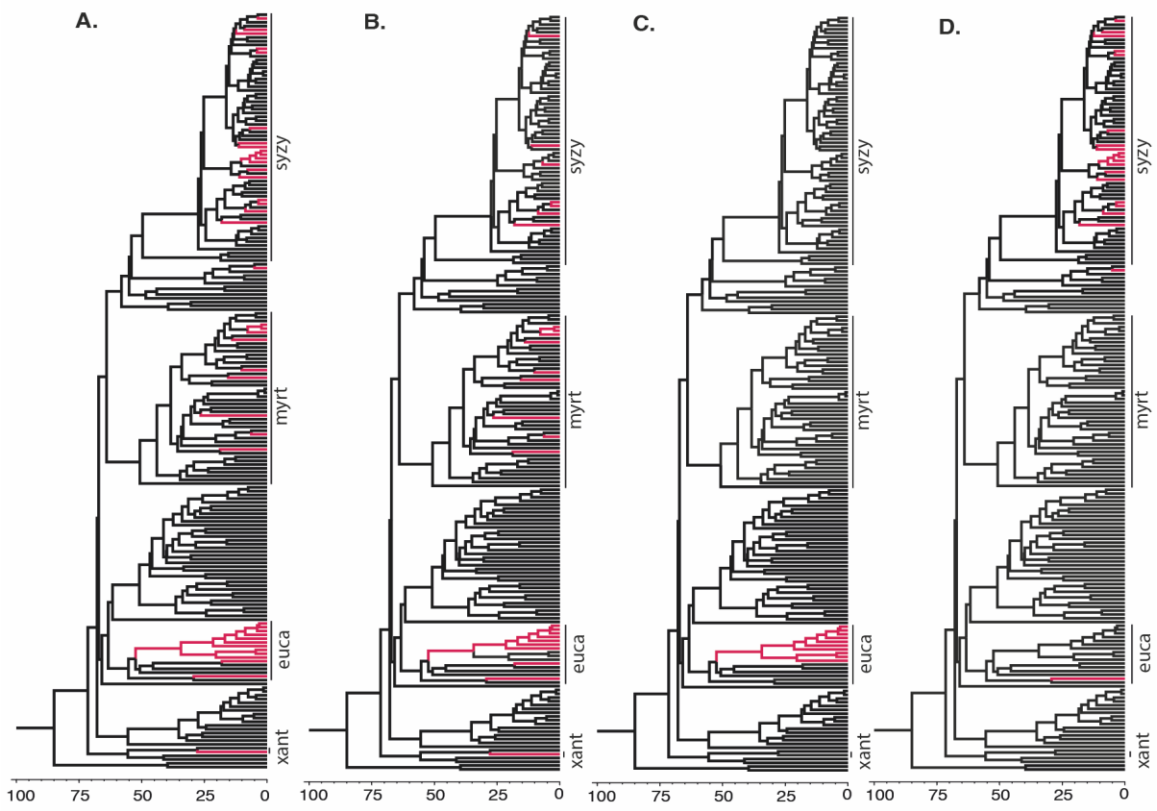


Figure 4.8: Changes in phylogenetic placement of calyptrate flowers when levels of homology are clarified. Red branches represent species with calyptrate flowers. (A) All calyptrate flowers; (B) Calycine calyptra only; (C) Corolline calyptra only; (D) Pseudocalyptra only. “xant”, tribe Xanthostemonae; “euca”, tribe Eucalypteae; “myrt”, tribe Myrteae; “syzy”, tribe Syzygieae. X axis represents age in million years.

DISCUSSION

4.15 A matter of semantics: standardising the terminology for calyptrate structures in Myrtaceae

The structure traditionally referred to as a calyptra in Myrtaceae corresponds to at least three distinct, non-homologous structures. This highlights an issue of semantics aggravated by the favouring by authors of slightly different terms to describe the same structure in similar taxa. Even though the impetus in standardising calyptra terminology began as part of the original description of *Eucalyptus* (L’Héritier, 1788), there is not yet terminological consensus for the structural variation in the family. A series of studies of *Eucalyptus* floral development, for instance, describes the combination of fused calyx and corolla as an “operculum” (Drinnan and Ladiges, 1989), whilst a similar structure is referred to as “calyptrate calyx and corolla” in Syzygieae (Ashton, 2011), or as “petaline opercular structures [that] consist of the imbricate petals that cohere and fall as a unit” in Syzygieae (Wilson, 2011, p.216).

Given that a fused perianth is a characteristic that can re-occur throughout Myrtaceae, it is necessary and important to standardise a structurally and evolutionarily coherent terminology. We advocate that a calyptra *sensu stricto* consists of homogeneous tissue formed by post-genital fusion of sepals or petals. Thus, most Syzygieae, some *Metrosideros* and *Eucalyptus curtissii*, described as calyptrate (e.g. Ashton, 2011) are not calyptrate, because their perianth shares the same

developmental pattern as species with a free perianth. Their anthetic behaviour where imbricate petals fall as a unit can still be used as a trait of taxonomic value (see also next section and Chapter 6), but should not be treated as homologous to “true” Myrtaceae calyptras and would be better defined as “pseudocalyptras”. Furthermore, the designation of a distinct term such as “operculum” for adhering calycine and coralline calyptras is also inadequate, since homologous coralline and calycine structures may appear independently of each other in the family’s evolution.

4.16 Reciprocal illumination: the calyptra in Myrtaceae systematics

Calyptrate flowers have been traditionally used a character of taxonomic relevance in Myrtaceae (e.g. Landrum, 1984). It is here demonstrated (as in other studies, e.g. Biffin et al., 2005) that calyptrate flowers cannot be considered true synapomorphies for any Myrtaceae lineage, as the trait commonly re-occurs during evolution. However, it is noteworthy that phylogenetic signal improves when morphological homologies are clarified. The reason for this is that independent recurrence of calyptrate flowers results from parallelisms that are, in theory, lineage related (Cronk 2002; Scotland 2011). In other words, these trends are phylogenetically linked even though a certain level of homoplasy still persists. It is also noticeable that most reversals from calyptrate to non-calyptrate disappear when homologous calyptras are identified (reversal towards the pseudocalyptra in *Eucalyptus curtisii* may be persistent due to the low support of that clade; Thornhill et al., 2015). This corroborates the hypothesis that when true calyptras appear in evolution they can be quite stable in a lineage, also increasing phylogenetic signal. The only occasion where phylogenetic signal does not increase when homology is clarified is when pseudocalyptras (developmentally identical to a free perianth) are distinguished. This is because the pseudocalyptra type results from convergence, which in cladistics is defined as a character misinterpretation or a mistake in trait coding (Coddington, 1994), rather than a true developmental variation.

Systematists also note that calyptrate flower anthesis varies between “a perfect dehiscence line at the base of the perianth during anthesis” and “a fused perianth that tears irregularly” (e.g. Lucas et al., 2011). These trends have some taxonomic value but should not be prioritised over the mode of development. Variation in the point or mode of perianth tearing during anthesis is observed to occur even within an individual (Fig.9) and likely depends on the point of post-genital fusion initiation and anatomical idiosyncrasies.

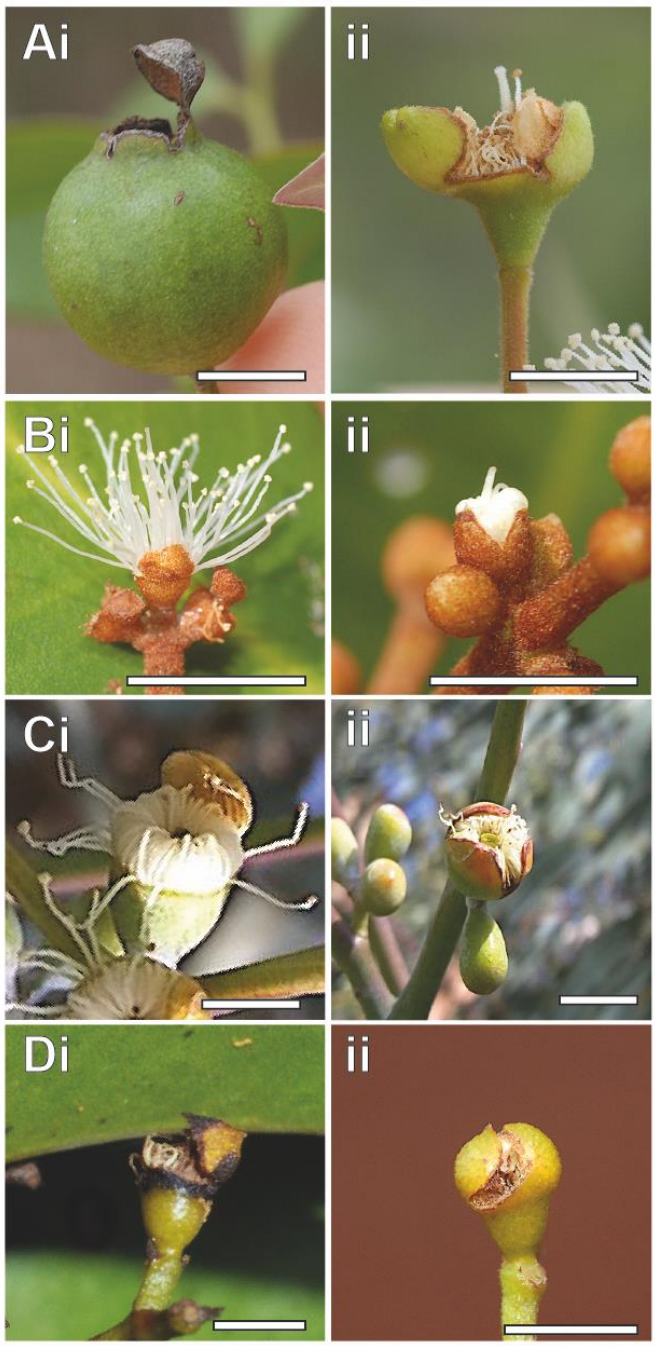


Figure 4.9: Variation in perianth tearing within individuals. Column “i” shows a perfect calyptra, column “ii” shows irregular tearing. (A) *Psidium* sp; (B) *Calyptranthes brasiliensis*; (C) *Eucalyptus perriniana*; (D) *Blepharocalyx eggersii*. Scale bar: c.5mm. (Photos taken during field expeditions between 2014 and 2016 and from living collection at the Royal Botanic Gardens Kew).

4.17 Functional advantage of repeated selection of calyptrate flowers

Recurrence and persistence of traits in evolution is usually related to two factors: 1) the trait increases adaptive value (i.e. positive effect on survival and reproductive success rates) for the lineage in a given niche; and/or 2) the trait does not bring any negative effect that would lead to a higher extinction risk for the lineage in a given niche (Futuyma, 2009). If a calyptrate perianth appears in Myrtaceae both by processes of parallelism and convergence, assumptions are that certain external pressures are positively selecting this structure multiple times. As previously desribed, sepals and petals protect the flower (mainly sepals) and enhance pollination (mainly

petals) (Endress, 1994). An extra resistant layer that completely covers the floral bud until anthesis can be intuitively associated with protection (e.g. as in *Rosmarinus*, Bottega and Corsi, 2000; and *Spatophea*, Carlson and Harms, 2007). Nevertheless, evidence that calyptrate species appear at distinct times and environments during Myrtaceae evolution (Thornhill et al., 2015, this study) suggests that protection conferred by this structure is not necessarily related to the same environmental pressure.

In this sense, anatomical components of Myrtaceae calyptras, that show high flexibility even within the same genus (e.g. Fig 4.6F-G, I-J), can give directions on functional aspects of the calyptra. These are exemplified by the presence of glandular cavities, trichomes and lignified tissues (Fig.4.6). Oil glands are common in all Myrtaceae tissues, including sepals and petals (Evert, 2006) and the essential oil produced by these glands creates a chemical barrier against predators (Batish et al., 2008). An extra layer of oil glands granted by the calyptra to the bud may be useful to enhance bud survival in certain niches. The presence of lignified tissues could be related to the same function, but these probably also confer physical protection against harsh environments rather than just herbivory. In *Eucalyptus*, for example, calyptras are evidenced in the fossil record since the Palaeocene (Gandolfo et al, 2011). A strongly lignified bud coverage may have been key to their long survival in dry and fire predisposed environments of Australia (Crisp et al., 2011), especially due to their particularly extensive flowering period (Birtchnell and Gibson, 2006). This hypothesis is also supported by the restricted distribution of *Angophora*, a much species poorer non-calyptrate genus sister to *Eucalyptus*, native to more humid environments of eastern Australia (Ladiges et al., 2003). In the Neotropics, a similar tendency is observed in species from the semi-arid regions of Northeast Brazil. *Psidium brownianum*, for example, presents both a very thick calyptra and abundance of strongly lignified hairs on its inner surface (Fig.4.6E,iii). This kind of adaptation is similarly found in leaves of plants from very arid environments (e.g. *Ammophila,* Poaceae) and are related to minimising transpiration by retaining air over the stomata (Purer, 1942).

But if a calyptrate perianth has so many advantages, why do most Myrtaceae species still have an open, non-fused perianth? The reason may be related to the fact that calyptrate flowers often have reduced petals (in the case of calycine calyptra, see e.g. *Calyptranthes* Fig.4.3CD and Chapter 6) or they lose the corolla completely at anthesis (as it is the case of coralline and pseudocalyptras). In many cases, the attraction of Myrtaceae flowers to pollinators relies on the brush-blossom in which the polyandrous androecium is the main showy structure (Johnson and Briggs, 1984; Chapter 3). This system relieves selective pressure for pollinator attraction from the perianth, making the corolla somewhat dispensable from pollinator attraction and thus better used for protection (in a “transference of function”, as coined by Corner, 1958). In this way, a shift to a calyptrate perianth may be favourable and thus common. However, this strategy may also represent a two-edged sword, since the acquisition of a calyptra may constrain a lineage to occupy niches where pollinator attraction is perianth dependent (e.g. *Myrrhinium*, Roitman et al., 1997), especially because the acquisition of a calyptrate flower is definite in a lineage (shown by the lack of reversals).

4.18 Reinforcing the importance of morphological studies in phylogenetically well-known groups

For any homoplastic trait, low phylogenetic signal is expected *a priori* (Revell et al., 2008). Owen (1843) was the first naturalist to define homology in his studies of invertebrate animals. In his definition, homology corresponds to an organ of similar embryological and anatomical origin in distinct taxa, independent of its function. Using these criteria to augment phylogenetic signal *a posteriori* highlights the arbitrariness of trait definition and is useful to clarify underlying evolutionary pattern. Phylogenetic signal may increase once true homologies are categorised, indicating that those traits are lineage related, even though a certain degree of homoplasy still persists. Thus, underlying homologies (i.e. parallelisms) tend to have higher phylogenetic signal than convergences, which is not necessarily lineage related, when a trait is analysed in a large scale. Reassessing morphology to infer trait-function aspects of a structure also allows understanding of the relationship of that lineage to their environment. This tool is especially efficient when dated phylogenies and biogeographical hypothesis are already available, so structural changes can be inserted in the right eco-evolutionary context for the lineage (see e.g. Renner and Schaefer, 2010). This is the case, as exemplified here, of diversity of anatomical characters present in apparently similar calyptrate flowers of Myrtaceae.

CONCLUSION

Identification of underlying homologies in superficially homoplastic characters clarifies systematic and evolutionary interpretation of individual lineages. A step-by-step homology test as presented here can be summarised as: 1) definition of terminology, as distinct authors refer to similar traits in different ways and vice-versa; 2) definition of organs involved; 3) definition of homologous and non-homologous categories; and 4) trait re-coding and adjustment of phylogenetic signal. This homology test shows that characters previously discarded as systematic and evolutionary irrelevant actually have a strong phylogenetic signal once their morphological patterns are clarified and re-assessed under a phylogenetic framework. In Myrtaceae, both parallelisms and convergences are responsible for calyptrate flowers. The recurrence of a perianth that is completely closed in the bud and “disposable” at anthesis is probably linked to selective pressures towards protection and reliance on brush blossoms with the androecium as the main floral display.

APPENDIX

Appendix 4.1: *Pleurocalyptus pancherii* ITS and *ndh*F sequences and BLAST search evidencing relationship with *Xanthostemon* (as suggested by Wilson, 2011).

>its_tv622_consensus_sequence

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ACCGGTAACGAACTCGATGGGGACGGCGGGCTCTCCGCCCGACGTCCCTCGACGCTCGGATTGCGCG
GGCGCCCAGAGCGTCGGGCTTTCCGGGCGGCACAACGAACCCCGGCGCGGAACGCGCCAAGGAACT
CGAACGAAGAGAGCGTTGCTCCCACCGCCCCAGACCTGGTGCGCGCGTGGGATGCCATGCGATCTCC
TATTTATCCATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAACTG
CGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGC
CATTCGGTCGAGGGCACGTTTGCCTGGGTGTCACACACGGCGTTGCCCCTAATCCCTCGCCTCGATCG
GGCGGGCGGGACCTGGGTGCGTACGTTGGCCTCCCGCGACGACCTCGTCCCGGTTGGCCCAAAATTG
AGCGTCGGAGCGATTAGCACCGCGACATTCGGTGGTTGATGAGACCCCCAACGTTGAAATGTCGCGCT
TGCCGCTCACGCACGTGCTCCGCGAATCTACTCCTCACCAATCGCGACCCCCATCAAGCGAGGCTACC
CGCTGAGTTTAAGCATATCAATAAGGCG

>ndhf_tv622_consensus_sequence

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CAATAAAAATACTTTCTCTTATCCTCATGAATCGGACAATACTATGCTATTTTCCATGGTTATATTAGTGCT
ATTTACTTTGTTTGTTGGAGTCGTAGGAATTCCCTTTCCTTTTAATCAAGAAGGAATTCATTTGGATATAT
TATCCAAATTGTTAAATCCGTCTATAAACCTTTTACATCAGAATTCAAATAATTCTATGGATTGGTATGAAT
TTGTGACAAATGCAAGTTTTTCTGTTAGTATAGCCTTTTTCGGAATATTTATAGCGTCTTTTTTATATAAGC
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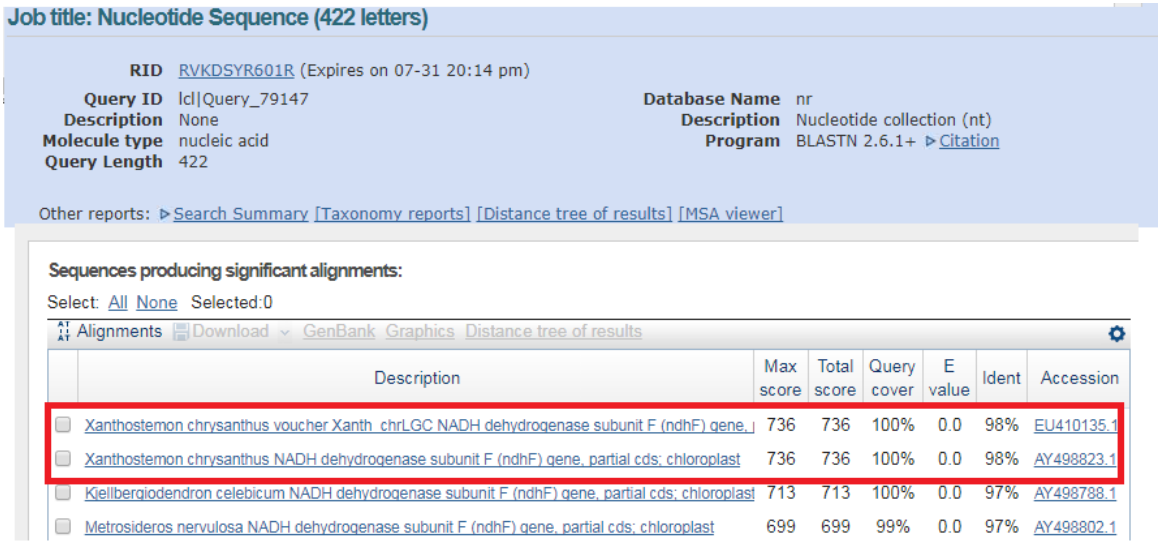

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RID RVKDSYR601R (Expires on 07-31 20:14 pm)

Query ID lcl|Query_79147
Description None
Molecule type nucleic acid
Query Length 422

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.6.1+ ▷Citation

Other reports: ▷Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

Sequences producing significant alignments:

Select: All None Selected:0

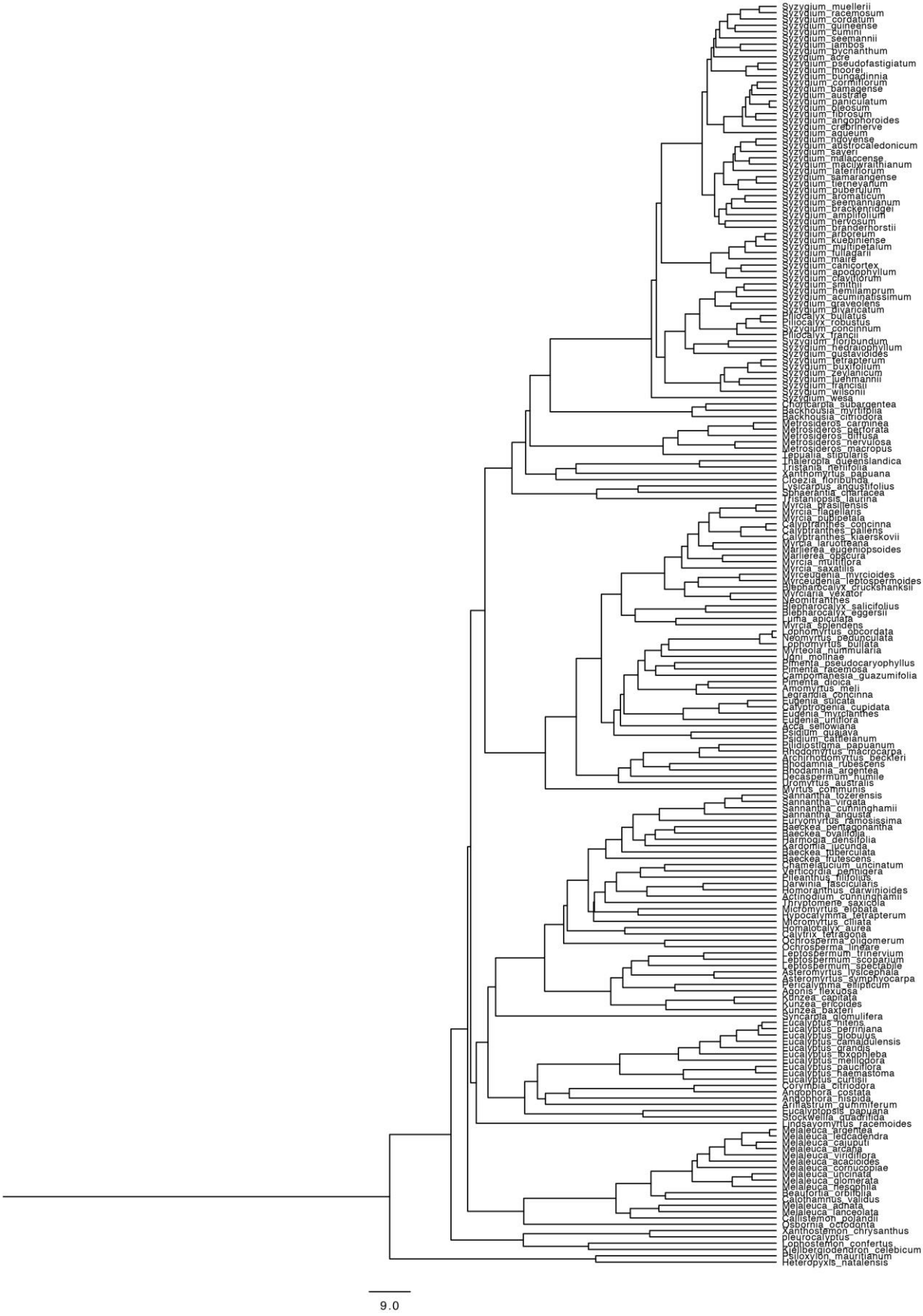
Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Xanthostemon chrysanthus voucher Xanth_chrLGC NADH dehydrogenase subunit F (ndhF) gene,	736	736	100%	0.0	98%	EU410135.1
Xanthostemon chrysanthus NADH dehydrogenase subunit F (ndhF) gene, partial cds; chloroplast	736	736	100%	0.0	98%	AY498823.1
Kjellbergiodendron celebicum NADH dehydrogenase subunit F (ndhF) gene, partial cds; chloroplast	713	713	100%	0.0	97%	AY498788.1
Metrosideros nervulosa NADH dehydrogenase subunit F (ndhF) gene, partial cds; chloroplast	699	699	99%	0.0	97%	AY498802.1

Appendix 4.2: Vouchers used in ontogenetic and anatomical analysis of calyptrate flowers of Myrtaceae. All deposited at K herbarium. Names follow the WSPF (2017).

Tribe	Species	Voucher	Collection locality
Eucalypteae	*Arillastrum gumiferum* (Brongn. & Gris) Pancher ex Baill.	Mc Pherson 4422	New Caledonia
Eucalypteae	*Corymbia sp.*	T.Vasconcelos 681	Singapore (cultivated)
Eucalypteae	*Eucalyptus curtisii* Blakely & C.T.White	BGQLD0805	Australia (Queensland)
Eucalypteae	*Eucalyptus pauciflora* Sieber ex Spreng.	T.Vasconcelos s.n.	RBG Kew living collection (native to Australia)
Eucalypteae	*Eucalyptus perriniana* F.Muell. ex Rodway	T.Vasconcelos s.n.	RBG Kew living collection (native to Australia)
Myrteae	*Calyptranthes multiflora* Poepp. ex O.Berg	A.Giaretta 1429	Brazil (AM)
Myrteae	*Calyptranthes pallens* Griseb.	T.Vasconcelos 534	Costa Rica
Myrteae	*Decaspermum parviflorum* (Lam.) A.J.Scott	T.Vasconcelos 728	Malaysia Sabah
Myrteae	*Psidium brownianum* Mart. ex DC.	T.Vasconcelos 465	Brazil (BA)
Myrteae	*Psidium myrsinites* DC.	T.Vasconcelos 503	Brazil (GO)
Syzygieae	*Piliocalyx sp.*	T.Vasconcelos 651	New Caledonia
Syzygieae	*Syzygium alatum* (Lauterb.) Diels	Barker 115	New Guinea
Syzygieae	*Syzygium cumini* (L.) Skeels	T.Vasconcelos 296	Brazil (cultivated)
Syzygieae	*Syzygium longifolium* (Brongn. & Gris) J.W.Dawson	T.Vasconcelos 610	New Caledonia
Syzygieae	*Syzygium ngoyense* (Schltr.) Guillaumin	T.Vasconcelos 598	New Caledonia
Syzygieae	*Syzygium paniculatum* Gaertn.	T.Vasconcelos s.n.	RBG Kew living collection (native to Australia)
Syzygieae	*Syzygium sp.*	T. Vasconcelos 642	Singapore (cultivated)
Syzygieae	*Syzygium sp.*	T.Vasconcelos 629	New Caledonia
Syzygieae	*Syzygium sp.*	T.Vasconcelos 632	New Caledonia
Xanthostemonae	*Pleurocalyptus pancheri* (Brongn. & Gris) J.W.Dawson	T. Vasconcelos 627	New Caledonia
Xanthostemonae	*Pleurocalyptus pancheri* (Brongn. & Gris) J.W.Dawson	T.Vasconcelos 622	New Caledonia
Xanthostemonae	*Xantostemon sp.*	T.Vasconcelos 687	Singapore (cultivated)

Appendix 4.3: Myrtaceae phylogeny (modified from Thornhill et al., 2015).



Appendix 4.4: Trait coding per tip for each phylogenetic signal analysis of calyptrate flowers in Myrtaceae. Tip names according to phylogeny in Appendix 4.3.

species	modeTotal	modeCalyc	modeCorol	modePseu
Psiloxylon_mauritianum	0	0	0	0
Heteropyxis_natalensis	0	0	0	0
Lophostemon_confertus	0	0	0	0
Kjellbergiodendron_celebicum	0	0	0	0
Xanthostemon_chrysanthus	0	0	0	0
Pleurocalyptus	1	1	0	0
Callistemon_polandii	0	0	0	0
Melaleuca_adnata	0	0	0	0
Melaleuca_lanceolata	0	0	0	0
Beaufortia_orbifolia	0	0	0	0
Calothamnus_validus	0	0	0	0
Melaleuca_nesophila	0	0	0	0
Melaleuca_uncinata	0	0	0	0
Melaleuca_glomerata	0	0	0	0
Melaleuca_cornucopiae	0	0	0	0
Melaleuca_acacioides	0	0	0	0
Melaleuca_viridiflora	0	0	0	0
Melaleuca_argentea	0	0	0	0
Melaleuca_leucadendra	0	0	0	0
Melaleuca_cajuputi	0	0	0	0
Melaleuca_arcana	0	0	0	0
Eucalyptopsis_papuana	1	1	0	0
Stockwellia_quadrifida	0	0	0	0
Syncarpia_glomulifera	0	0	0	0
Lindsayomyrtus_racemoides	0	0	0	0
Osbornia_octodonta	0	0	0	0
Arillastrum_gummiferum	0	0	0	0
Angophora_hispida	0	0	0	0
Corymbia_citriodora	1	1	1	0
Angophora_costata	0	0	0	0
Eucalyptus_curtisii	1	0	1	0
Eucalyptus_pauciflora	1	0	1	0
Eucalyptus_haemastoma	1	0	1	0
Eucalyptus_melliodora	1	1	1	0
Eucalyptus_loxophleba	1	1	1	0
Eucalyptus_grandis	1	1	1	0
Eucalyptus_camaldulensis	1	1	1	0
Eucalyptus_globulus	1	1	1	0
Eucalyptus_nitens	1	1	1	0
Eucalyptus_perriniana	1	1	1	0
Kunzea_baxteri	0	0	0	0
Kunzea_capitata	0	0	0	0
Kunzea_ericoides	0	0	0	0

Pericalymma_ellipticum	0	0	0	0
Agonis_flexuosa	0	0	0	0
Asteromyrtus_lysicephala	0	0	0	0
Asteromyrtus_symphyocarpa	0	0	0	0
Leptospermum_spectabile	0	0	0	0
Leptospermum_trinervium	0	0	0	0
Leptospermum_scoparium	0	0	0	0
Ochrosperma_oligomerum	0	0	0	0
Ochrosperma_lineare	0	0	0	0
Homalocalyx_aurea	0	0	0	0
Calytrix_tetragona	0	0	0	0
Micromyrtus_ciliata	0	0	0	0
Micromyrtus_elobata	0	0	0	0
Hypocalymma_tetrapterum	0	0	0	0
Thryptomene_saxicola	0	0	0	0
Pileanthus_filifolius	0	0	0	0
Chamelaucium_uncinatum	0	0	0	0
Verticordia_pennigera	0	0	0	0
Actinodium_cunninghamii	0	0	0	0
Darwinia_fascicularis	0	0	0	0
Homoranthus_darwinioides	0	0	0	0
Baeckea_frutescens	0	0	0	0
Baeckea_tuberculata	0	0	0	0
Kardomia_jucunda	0	0	0	0
Harmogia_densifolia	0	0	0	0
Baeckea_pentagonantha	0	0	0	0
Baeckea_ovalifolia	0	0	0	0
Euryomyrtus_ramosissima	0	0	0	0
Sannantha_angusta	0	0	0	0
Sannantha_cunninghamii	0	0	0	0
Sannantha_tozerensis	0	0	0	0
Sannantha_virgata	0	0	0	0
Myrtus_communis	0	0	0	0
Uromyrtus_australis	0	0	0	0
Decaspermum_humile	0	0	0	0
Rhodamnia_rubescens	0	0	0	0
Rhodamnia_argentea	0	0	0	0
Archirhodomyrtus_beckleri	0	0	0	0
Pilidiostigma_papuanum	0	0	0	0
Rhodomyrtus_macrocarpa	0	0	0	0
Psidium_guajava	1	1	0	0
Psidium_cattleianum	0	0	0	0
Acca_sellowiana	0	0	0	0
Eugenia_uniflora	0	0	0	0
Eugenia_sulcata	0	0	0	0
Eugenia_myrcianthes	0	0	0	0

Calyptrogenia_cupidata	1	1	0	0
Legrandia_concinna	0	0	0	0
Pimenta_dioica	0	0	0	0
Amomyrtus_meli	0	0	0	0
Campomanesia_guazumifolia	1	1	0	0
Pimenta_pseudocaryophyllus	0	0	0	0
Pimenta_racemosa	0	0	0	0
Ugni_molinae	0	0	0	0
Myrteola_nummularia	0	0	0	0
Lophomyrtus_bullata	0	0	0	0
Lophomyrtus_obcordata	0	0	0	0
Neomyrtus_pedunculata	0	0	0	0
Blepharocalyx_eggersii	1	1	0	0
Blepharocalyx_salicifolius	0	0	0	0
Luma_apiculata	0	0	0	0
Myrcia_splendens	0	0	0	0
Myrciaria_vexator	0	0	0	0
Neomitranthes	1	1	0	0
Blepharocalyx_cruckshanksii	0	0	0	0
Myrceugenia_myrcioides	0	0	0	0
Myrceugenia_leptospermoides	0	0	0	0
Myrcia_saxatilis	0	0	0	0
Marlierea_obscura	0	0	0	0
Myrcia_multiflora	0	0	0	0
Myrcia_laruotteana	0	0	0	0
Marlierea_eugeniopsoides	1	1	0	0
Myrcia_pubipetala	0	0	0	0
Myrcia_brasiliensis	0	0	0	0
Myrcia_flagellaris	0	0	0	0
Calyptranthes_kiaerskovii	1	1	0	0
Calyptranthes_concinna	1	1	0	0
Calyptranthes_pallens	1	1	0	0
Tristaniopsis_laurina	0	0	0	0
Lysicarpus_angustifolius	0	0	0	0
Sphaerantia_chartacea	0	0	0	0
Cloezia_floribunda	0	0	0	0
Xanthomyrtus_papuana	0	0	0	0
Thaleropia_queenslandica	0	0	0	0
Tristania_neriifolia	0	0	0	0
Tepualia_stipularis	0	0	0	0
Metrosideros_nervulosa	0	0	0	0
Metrosideros_macropus	0	0	0	0
Metrosideros_diffusa	0	0	0	0
Metrosideros_carminea	0	0	0	0
Metrosideros_perforata	1	0	0	1
Backhousia_citriodora	0	0	0	0

Choricarpia_subargentea	0	0	0	0
Backhousia_myrtifolia	0	0	0	0
Syzygium_wesa	0	0	0	0
Syzygium_wilsonii	0	0	0	0
Syzygium_luehmannii	0	0	0	0
Syzygium_francisii	0	0	0	0
Syzygium_zeylanicum	0	0	0	0
Syzygium_tetrapterum	0	0	0	0
Syzygium_buxifolium	0	0	0	0
Syzygium_gustavioides	1	1	0	0
Syzygium_floribundum	0	0	0	0
Syzygium_hedraiophyllum	0	0	0	0
Piliocalyx_francii	1	1	0	0
Syzygium_concinnum	0	0	0	0
Piliocalyx_bullatus	1	1	0	0
Piliocalyx_robustus	1	1	0	0
Syzygium_graveolens	0	0	0	0
Syzygium_divaricatum	0	0	0	0
Syzygium_acuminatissimum	0	0	0	0
Syzygium_smithii	0	0	0	0
Syzygium_hemilamprum	0	0	0	0
Syzygium_claviflorum	1	0	0	1
Syzygium_canicortex	1	0	0	1
Syzygium_apodophyllum	0	0	0	0
Syzygium_maire	0	0	0	0
Syzygium_fullagarii	1	1	0	1
Syzygium_multipetalum	1	0	0	1
Syzygium_arboreum	1	0	0	1
Syzygium_kuebiniense	1	0	0	1
Syzygium_nervosum	1	1	0	1
Syzygium_branderhorstii	1	0	0	1
Syzygium_amplifolium	0	0	0	0
Syzygium_brackenridgei	0	0	0	0
Syzygium_aromaticum	1	0	0	1
Syzygium_seemannianum	0	0	0	0
Syzygium_puberulum	0	0	0	0
Syzygium_samarangense	0	0	0	0
Syzygium_tierneyanum	0	0	0	0
Syzygium_lateriflorum	0	0	0	0
Syzygium_malaccense	0	0	0	0
Syzygium_macilwraithianum	0	0	0	0
Syzygium_sayeri	0	0	0	0
Syzygium_ngoyense	0	0	0	0
Syzygium_austrocaledonicum	1	0	0	1
Syzygium_aqueum	0	0	0	0
Syzygium_crebrinerve	0	0	0	0

Syzygium_fibrosum	0	0	0	0
Syzygium_angophoroides	0	0	0	0
Syzygium_paniculatum	0	0	0	0
Syzygium_oleosum	0	0	0	0
Syzygium_australe	0	0	0	0
Syzygium_cormiflorum	0	0	0	0
Syzygium_bamagense	0	0	0	0
Syzygium_bungadinnia	0	0	0	0
Syzygium_pseudofastigiatum	1	0	0	1
Syzygium_moorei	1	0	0	1
Syzygium_acre	0	0	0	0
Syzygium_jambos	0	0	0	0
Syzygium_pycnanthum	0	0	0	0
Syzygium_seemannii	1	1	0	0
Syzygium_guineense	0	0	0	0
Syzygium_cumini	1	0	0	1
Syzygium_cordatum	0	0	0	0
Syzygium_muellerii	0	0	0	0
Syzygium_racemosum	1	0	0	0

Chapter 5: Floral heterochrony promotes lability of reproductive strategies in the morphologically homogeneous genus *Eugenia* (Myrtaceae)

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- T.N.C.Vasconcelos contributions: development of hypotheses, design of experiments, collection of samples, generation of SEM images, morphological analyses, phylogenetic analyses and writing of manuscript.

ABSTRACT

Comparative floral ontogeny represents a valuable tool to understand angiosperm evolution. Such an approach may elucidate subtle changes in development that discretely modify floral architecture and underlie reproductive lability in groups with superficial homogeneous morphology. This study presents a comparative survey of floral development in *Eugenia* (Myrtaceae), one of the largest genera of angiosperms, and shows how previously undocumented ontogenetic trends help to explain the evolution of its megadiversity in constrast to its apparent flower uniformity. Using SEM, selected steps of the floral ontogeny of a model species (*Eugenia punicifolia*) are described and compared with 20 further species representing all ten major clades in the *Eugenia* phylogenetic tree. Additional floral trait data are contrasted for correlation analysis and character reconstructions perfomed against a published phylogenetic tree. *Eugenia* flowers show similar organ arrangement patterns: radially symmetric, (most commonly) tetramerous flowers with variable numbers of stamens and ovules. Despite a similar general organisation, heterochrony is evident from size differences between tissues and structures at similar developmental stages. These differences underlie variable levels of investment in protection, subtle modifications to symmetry, herkogamic effects and independent androecium and gynoecium variation, producing a wide spectrum of floral display and contributing to fluctuations in fitness. During *Eugenia*’s bud development, the hypanthium (as defined here) is completely covered by stamen primordia, unusual in other Myrtaceae. This is the likely plesiomorphic state for Myrteae and may have represented a key evolutionary novelty in the tribe. Floral evolution in *Eugenia* depends on heterochronic patterns rather than changes in complexity to promote flexibility in floral strategies. The successful early establishment of Myrteae, previously mainly linked to the key-innovation of fleshy-fruit, may also have benefitted from changes in flower structure.

Key words: androecium, gynoecium, hypanthium, Myrteae, ontogeny, perianth.

5.1 Floral ontogeny in studies of systematics and evolution

Flower organs (i.e. calyx, corolla, androecium and gynoecium) and associate tissues are responsible for two main functions in the angiosperm life cycle. The primary function of these organs is forming male and female gametes and their connection for sexual reproduction. The secondary function is to enhance and protect this process, as well as balancing in- and out-breeding (Endress, 1994). In this way, evolutionary changes of floral traits affect reproductive success and promote fitness fluctuations in individual lineages (e.g. de Jager and Ellis, 2013; Antiqueira and Romero, 2016) and comparative floral developmental studies are a useful tool to comprehend evolution in angiosperms (e.g. Endress, 2002, 2006; Rudall and Bateman, 2004). By comparing floral ontogeny in distinct but closely related taxa, changes in rates of organ initiation and development (i.e. heterochronies) are documented, explaining differences in flower architecture (Endress, 1994; Tucker, 2003; Prenner, 2004; Prenner *et al*., 2008). These alterations in developmental rythyms promote differential investments in organs implicated in adapative features for plant reproduction (e.g. changes in breeding system; see review in Li and Jonhston, 2000).

5.2 Deficit of floral development data for large tropical genera

Such comparative surveys of floral ontogeny are often hampered by a lack of systematic understanding and the difficulty of finding suitable material for analysis of the group of interest (i.e. spirit collections of floral buds in different developmental stages). For that reason, studies on large, tropical and/or taxonomically complicated taxa are rare in comparison to relatively species poor (e.g. Endress, 2003) and/or temperate plant groups (e.g. Webster and Gilmartin, 2003). Systematic complexity in large genera is often a result of morphological homogeneity (e.g. Briggs and Johnson, 1979). The absence of comparative ontogenetic surveys in these groups mean that remarkable but discreet patterns that are key to explain evolutionary trends and diversification patterns are overlooked.

The tropical Myrtaceae genus *Eugenia* is an example of this deficit. *Eugenia*, with around 1000 species (WCSP, 2017), is one of the largest angiosperm genera, the second most diverse tree genus (Beech *et al*., 2017) and listed among the genera with highest diversity of species in threatened Neotropical biomes (Mori *et al*., 1983; Oliveira-Filho and Fontes, 2000). Being so huge and ecologically important, it is surprising that there is so little information available on the evolution of its floral structure. Flowers of *Eugenia* are known to display a series of Myrtaceae features: they are epigynous, radially symmetric and polyandrous (Fig. 5.1; see Ronse De Craene and Smets, 1991; Belsham and Orlovich, 2002, 2003; Chapter 3). However, they differ from other Myrtaceae flowers in presenting straight stamens in the bud, a character shared by other related genera within tribe Myrteae (Chapter 2). Certain histogenetic aspects have been described for a few species of *Eugenia* in isolated studies (Schmid, 1972; Pimentel et al., 2014; Martos *et al*., 2017); these focus on the highly similar vascular structure and a lack of infrageneric variation, reinforcing the homogeneous aspects of the genus' floral morphology. The absence of information regarding floral evolution in *Eugenia* is aggravated by sample inaccessability (due to it usually being a tree with a tropical distribution) and, until recently, the absence of a phylogenetic framework (available in Mazine *et al*., 2014) with which to study an evolutionary coherent sample.

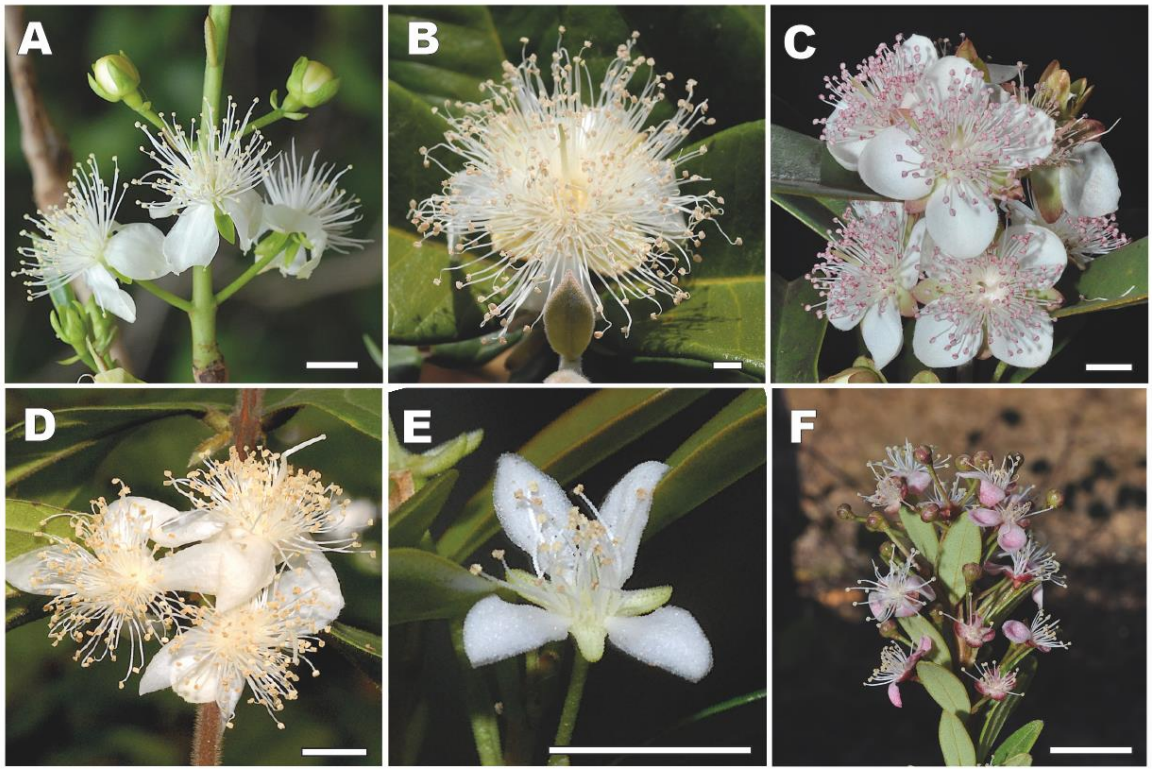


Figure 5.1: Field pictures of flowers in distinct Eugenia clades. (A) Eugenia dichroma (Sect. Speciosae); (B) E. azurensis (Sect. Pseudeugenia); (C) E. involucrata (Sect. Phyllocalyx); (D) E. stipitata (Sect. Pilothecium); (E) E. ligustrina (Sect. Eugenia); (F) E. angustissima (Sect. Racemosae). Scale bar: c.5mm. (all photos taken during field expeditions between 2014 and 2016).

In this study, it is hypothesised that large groups with morphologically homogeneous flowers such as *Eugenia*, rely on heterochronies to promote lability of reproductive strategies. This hypothesis is here tested by documenting floral ontogeny in a phylogenetically representative sample of *Eugenia*. Dimensions of organs and tissues at selected stages are compared to observe changes in developmental rates. Developmental differences are discussed in the context of flexibility of functional traits in the flower (e.g. protection and breeding system). *Eugenia* floral development data is also provides understanding of changes in floral structure associated with heterochrony and their influence on stamen posture in Myrteae, the most species rich tribe of Myrtaceae.

MATERIALS AND METHODS

5.3 Sampling

A complete ontogenetic sequence for *Eugenia punicifolia* (*Eugenia* Sect. *Umbellatae*) is described and used as a base for comparing variation in selected developmental stages between samples from twenty species representing ten consistent clades (i.e. clades that re-occur in independent phylogenetic analysis, Mazine *et al.*, 2014, 2016; Bunger *et al*., 2016; Vasconcelos *et al.,* 2017b). A list of all analysed species and the clades to which they belong is available in Table I. *Eugenia punicifolia* represents the most common floral phenotype for the genus (tetramery, bilocular ovaries and multiple ovules) and is a common, widespread shrub in South America.

Additional samples for stage specific comparison of development and correlation between ovule number/stamen number and size were selected to represent both phylogenetic variation and geographic distribution of *Eugenia*.

Flower samples of *Eugenia* were collected mainly from their natural environments during field expeditions to South America, the Caribbean and New Caledonia. In a few cases, samples were taken from cultivated collections in botanic gardens. Samples of young inflorescence shoots, flower buds and open flowers were collected and fixed in FAA (formalin, acetic acid and ethanol) or 70% ethanol in falcon tubes; field pictures at anthesis were also registered. Herbarium vouchers for all collections are deposited at the Royal Botanic Garden Kew (K) with duplicates in local herbaria from where the collections originate.

5.4 Ontogenetic examination

Flower buds in different developmental stages were selected and dissected in 50% or 70% ethanol to expose structures of interest, then dehydrated through an ethanol series to 100% ethanol. Critical-point drying was performed using an Autosamdri-815B critical-point dryer (Tousimis Research, Rockville, Maryland, USA). Dried material was mounted onto metal specimen stubs using a carbon stick disk and coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinstead, UK). Stubs were examined and distinct floral developmental stages were documented using a Hitachi cold field emission SEM S-4700-II (Hitachi High Technologies, Tokyo, Japan).

5.5 Flower measurements and correlation analysis

Additional measurements were taken to analyse correlation and disparity in the number of floral parts as a consequence of changes in developmental patterns. These were: floral receptacle diameter (the base of the flower), total number of stamens and total number of ovules. All measurements were taken in mature, pre-anthetic buds or recently opened flowers and annotated as an average of observations from at least three buds per sample. Missing data correspond to samples that only presented buds in inadequate stages for reliable measurements (e.g. receptacle diameter was not recorded for samples that did not present open flowers, because the staminal ring appears to continuously expand in later stages of development and anthesis). All resulting measurements are presented in Table 5.1.

Linear regressions between flower receptacle diameter and total number of stamens and ovules were performed using the *lm* function in the *stats* package in R (R core team, 2017). This analysis was executed to test correlation between investment in receptacle diameter and formation of male (stamens) and female (ovules) reproductive structures.

5.6 Supporting analysis of character reconstruction

Ancestral state reconstruction analysis was conducted to interpret stamen posture and evolution of the combined androecium-hypanthium development in Myrtaceae. The Myrtaceae cladogram presented is based on the phylogenetic hypothesis published by Thornhill et al. (2015, see Appendix 4.3); it was used to reconstruct the characters as: (1) stamen primordia forming along the whole hypanthial surface; and (2) stamen primordia forming only on the edges of the hypanthial surface. The tree was trimmed to include only the Myrtoideae subfamily (all Myrtaceae except the monotypic and non-polyandrous *Psyloxylon* and *Heteropyxis*). Reconstruction was performed using

the function *ace* in the R package *ape* (R core team, 2017). The character matrix and associated references are available in the Appendix 5.1.

RESULTS

5.7 Floral Structure in *Eugenia*

Flowers of *Eugenia* are variable in size, reaching from 5 mm to over 30 mm in diameter when open. Most analysed flowers of *Eugenia* share the same general floral ground-plan and formula (Fig. 5.2; see also Chapter 3). The *Eugenia* calyx and corolla are tetramerous, with decussate aestivation. *Eugenia myrcianthes* is exceptional in its pentamery and imbricate quincuncial aestivation (i.e. two external sepals, two internal and one intermediate; see Appendix 5.2 - Plate 1). Symmetry is radial to slightly asymmetric. The androecium is polyandrous, with stamen number varying from c.30 to c.350 (see Table 5.1). Stamens are free throughout flower development. The ovary is inferior with two (most common phenotype) to three or four locules (see see Appendix 5.2 - Plate 2). Ovules are attached radially to an axillary placenta positioned at a single point on each locule wall of the ovary septum. Number of ovules per locule varied between 2 and 50 in analysed species. The complete ontogenetic sequence of *Eugenia punicifolia* is described below.

5.8 Flower development in *Eugenia punicifolia*

The complete floral ontogenetic sequence of *Eugenia punicifolia* is divided into five main stages (Stages 1 to 5) and seven substages (Stages 1a, 1b, 2a, 2b, 3, 4, 5), according to meristematic differentiation, from sepal initiation to anthesis. Stages can be summarized as: Stage 1a - calyx initiation, Stage 1b - corolla initiation, Stage 2a - androecium and gynoecium initiation, Stage 2b - hypanthium elongation/expansion, Stage 3 - differentiation of ovules and anthers, Stage 4 - pre-anthetic bud enlargement and final maturation of sexual organs, Stage 5 - anthesis.

At Stage 1a, the first two sepals initiate almost simultaneously in a median position (“S1” and “S1*”, Fig. 5.3A) with the abaxial (lower) sepal appearing slighly older than the adaxial one. Shortly after, two sepals form simultaneously in transversal positions, decussate to the first two sepals (“S2”, Fig. 5.3A,B). During early bud elongation, the first pair of sepals overlaps the second (Fig. 5.3B,C). At this point, the difference in initiation timing between the two sepals from the first pair (“S1” and “S1*”, Fig. 5.3A) is almost indistinguishable (“S1”, Fig. 5.3B). Single celled hairs appear on the tips of the sepals at this very early stage. These keep the edges of each pair of sepals tightly closed against each other and act like “eye lashes”, protecting the young bud during early floral development (arrow, Fig. 5.3C). Sepals are free throughout flower development.

Table 5.1: Analysed species, vouchers, collection location and selected traits averaged for three flowers per collection of *Eugenia* (except when standard deviation is absent). [1]Nomenclature follows Mazine *et al*., (2016), except for clade *Jossinia*.

Section [1]	Species	Analysed voucher	Collection locality	Diameter [mm]	Stamen number	Ovule number	Ovary locule
Umbellatae	*Eugenia punicifolia* (Kunth) DC.	J.E.Q. Faria 4051	Brazil (Distrito Federal)	2.1 (±0.1)	88 (±3.3)	23.3 (±0.5)	2
Umbellatae	*Eugenia citrifolia* Poir.	A. Giaretta 1441	Brazil (Roraima)	4.36 (±0.5)	101 (±3.4)	50 (±2.2)	2
Umbellatae	*Eugenia flavescens* DC.	J.E.Q. Faria 4168	Brazil (Bahia)	2.47 (±0.1)	92 (±4.5)	16 (±1.5)	2
Umbellatae	*Eugenia sp.*	T. Vasconcelos 350	Brazil (Amazonas)	1.88 (±0.2)	69 (±3.3)	17 (±0.8)	2
Clade *Jossinia*	*Eugenia gacognei* Montrouz.	T. Vasconcelos 595	New Caledonia				
Clade *Jossinia*	*Eugenia paludosa* Pancher ex Brongn. & Gris	T. Vasconcelos 646	New Caledonia	3.66 (±0.03)	267 (±5.2)	107.3 (±14.1)	2 or 3
Racemosae	*Eugenia inversa* Sobral	J.E.Q. Faria 4230	Brazil (Espirito Santo)	1.19 (±0.1)	60 (±2.1)	6.4 (±1.4)	2
Racemosae	*Eugenia angustissima* O.Berg	D.F.Lima 490	Brazil (Goiás)	1.65 (±0.1)	33 (±3.4)	7.7 (±2.1)	2
Racemosae	*Eugenia longiracemosa* Kiaersk.	T. Vasconcelos 310	Brazil (Amazonas)	2.02 (±0.1)	67 (±4.9)	21 (±2.1)	2
Eugenia	*Eugenia uniflora* L.	T. Vasconcelos s.n.	RBG Kew (cultivated - originally from Brazil)	2.06 (±0.2)	43 (±4.2)	19 (±4.9)	2
Eugenia	*Eugenia ligustrina* (Sw.) Willd.	T. Vasconcelos 570	Dominican Republic	1.5(0.5)	44 (±6.2)	7 (±2.3)	2
Pilothecium	*Eugenia stipitata* McVaugh	T. Vasconcelos 677	Singapore (cultivated - originally from Brazil)	3.1 (±0.4)	149 (±13.9)	21 (±8.5)	3 or 4

Pilothecium	*Eugenia itajurensis* Cambess.	J.E.Q. Faria 4250	Brazil (Espirito Santo)	6.2 (±0.3)	180 (±8.7)	18 (±3.2)	2
Pilothecium	*Eugenia pohliana* DC.	J.E.Q. Faria 4184	Brazil (Bahia)	3.75 (±0.05)	121 (±9.4)	5 (±1.4)	2
Pseudoeugenia	*Eugenia azurensis* O.Berg	J.E.Q. Faria 4186	Brazil (Bahia)	10.44 (±0.7)	354 (±33.2)	35 (±6.9)	2 or 3
Pseudeugenia	*Eugenia splendens* O.Berg	J.E.Q. Faria 4196	Brazil (Bahia)	4.05 (±0.3)	149 (±2.6)	34.3 (±4.5)	2
Hexachlamys	*Eugenia myrcianthes* Nied.	J.E.Q. Faria 6547	Brazil (Brasilia)	5.4 (±0.3)	150 (±8.3)	4 (±0)	2
Calycorectes	*Eugenia acutata* Miq.	T. Vasconcelos 506	Brazil (Distrito Federal)	5.2 (±0.2)	168 (±14)	32 (±8.3)	2
Phyllocalyx	*Eugenia involucrata* DC.	T. Vasconcelos 256	Brazil (Distrito Federal)	6.02 (±0.3)	218(±20.1)	66 (±3.5)	2
Speciosae	*Eugenia dichroma* O.Berg	T. Vasconcelos 466	Brazil (Espirito Santo)	3.86 (±0.1)	130 (±5.7)	34.7 (±4.5)	2

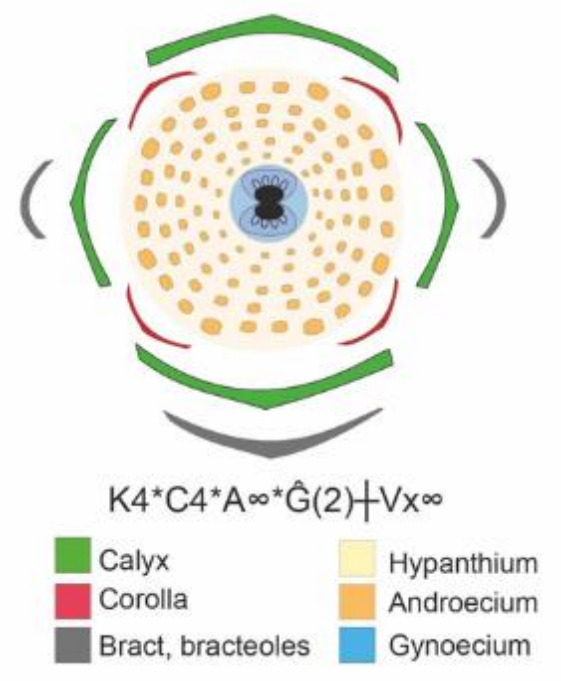


Figure 5.2. Floral diagram of *Eugenia punicifolia*, showing the most common floral ground-plan and floral formula for the genus (bilocular ovaries). For floral formulae interpretation see Prenner et al. (2010). Colour coding: sepals=green, corolla=red, androecium=yellow, gynoecium=blue.

The corolla is the second whorl to develop, during early floral ontogenetic stages. In Stage 1b, four petals initiate almost simultaneously as bulges in alternate positions to the sepals on the inner slopes of the developing hypanthium ("P", Fig. 5.3D). The four petals enlarge, eventually touch each other in the centre of the bud (Fig. 5.3E,F) and overlap in the next stages (Fig. 5.3G-H), providing a cover of four layers of tissue below the calyx on the top of the bud. Because of the nearly simultaneous initiation of the four petals there is no clear pattern of aestivation, even within the same species (see Appendix 5.2 - plate 3). Petals are free throughout flower development.

Stage 2a starts with the initiation of the androecium and gynoecium. The development of the first staminal ring occurs on the hypanthial tissue just underneath each petal ("A", Fig. 5.3G) where two primary staminen primordia are formed flanking each petal ("A1(1^{st})" Fig. 5.3H,I). The first ring continues to develop laterally and after a longer plastochron, secondary stamen primordia appear between the primary ones ("A1(2^{nd})", Fig. 5.3I) resulting in a complete first staminal ring. The time gap (plastochron) between the appearance of the first group of stamen primordia and the appearance of the second group of stamen primordia in the first whorl is noticeable at this stage (see also Appendix 5.2 – plate 4) and as the flower continues to develop, this size distinction almost disappears so that the dissimilarity in age between stamens is barely visible in later stages (e.g. Fig. 5.3J,K). The gynoecium originates as a depression that appears on the apical surface of the flower base simultaneously with the appearing of the first androecial primordia ("G", Fig. 5.3G,H).

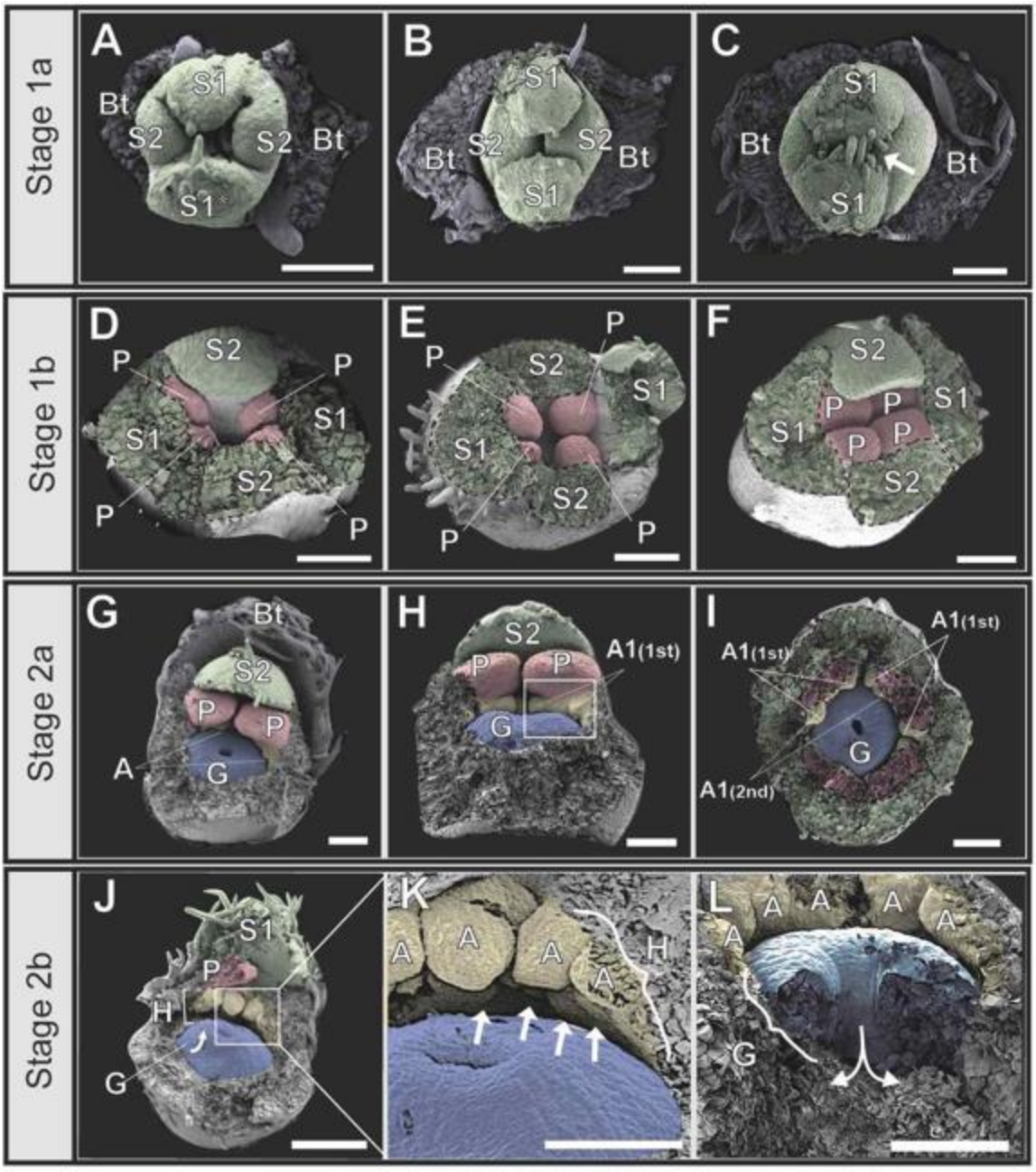


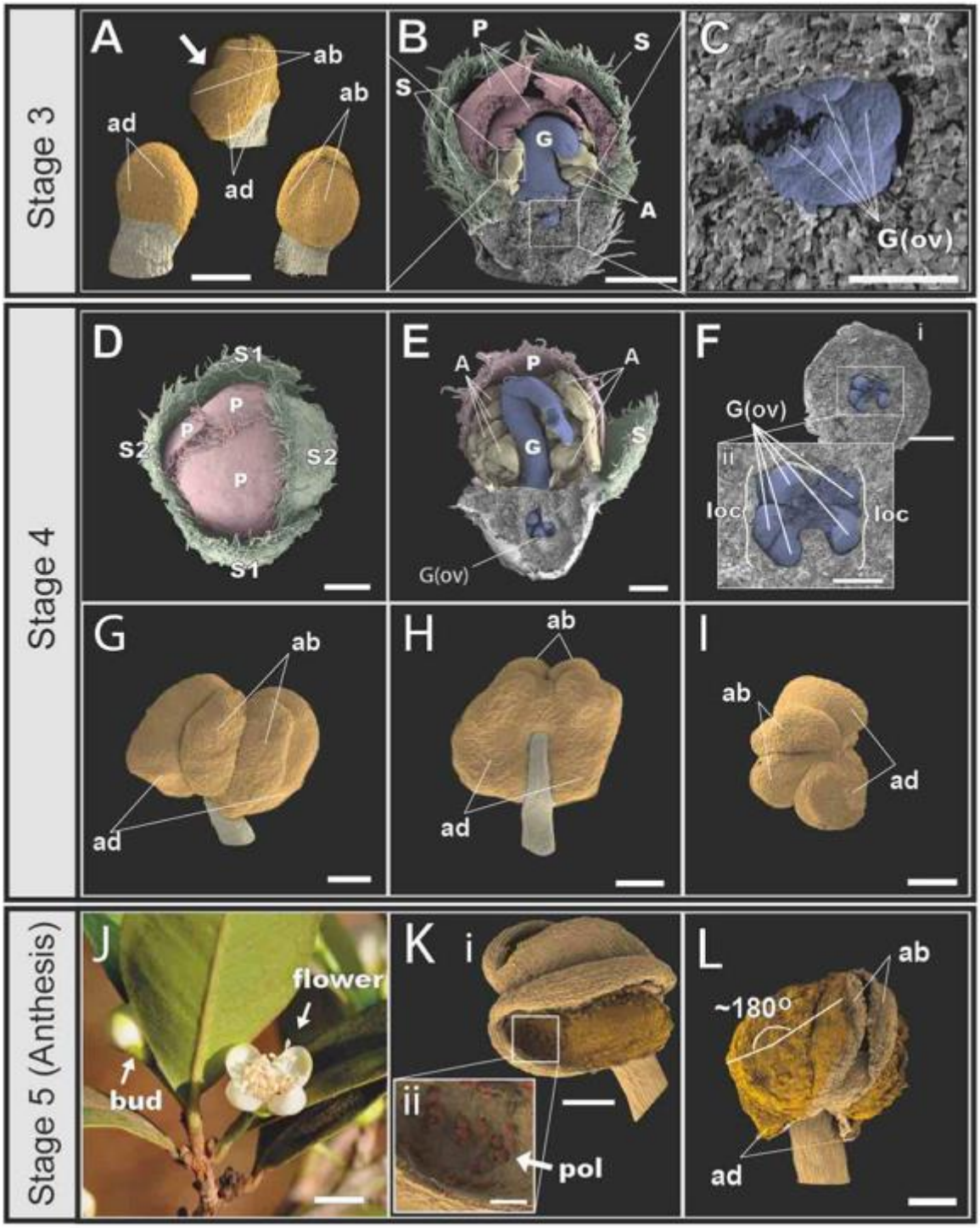
Figure 5.3. Early stages of floral ontogeny in *Eugenia punicifolia*. Stage 1a: (A) Early sepal development showing transverse bracteoles (removed), and first and second pair of sepals. S1* appears to be slightly older, a discrepancy only noticable at this stage. (B, C) Early sepal development; sepals enclose the bud; single-celled hairs develop at the tips of the sepals (arrow). Stage 1b: (D) Petal initiation; four petals arising simultaneously as bulges alternate to sepals. (E, F) Continuously growing petals eventually meeting in the middle of the bud. Stage 2a: (G, H) Petals overlap; one sepal and one bracteole left to highlight proportion between organs. (H) Lateral view, showing first stamen initiation (A1(1st)) flanking the petals. (I) As ”G” and “H”, but in frontal view; calyx and corolla removed; primary stamen primordia prominent. Stage 2b: (J) Proto-style developing upwards (arrow) and initiation of second staminal whorl; stamens of the first whorl similar in size. (K) Detail of J, showing stamen primordia covering the hypanthium below the first staminal girdle. (L) Same stage as “J”, but further dissected and in lateral view; gynoecium depression (ovary) expands downwards while proto-style grows upwards. Bt, bracteole; S, sepals;

P, Petals; A, androecium; G, gynoecium; H, hypanthium. Bracteoles removed in all. Scale: 50µm (K); 100µm (A, B, C, D, E, G, H, I, L); 250µm (F, J). Colour coding in online version: sepals=green, corolla=red, androecium=yellow, gynoecium=blue.

In Stage 2b, the hypanthium tissue expands ("H", Fig. 5.3J,K). Simultaneously, the androecium continues to develop as centripetal and concentric loosely distributed stamen primodia originating along the inner surface of the hypanthium, covering the whole area below the first staminal ring to the gynoecium (arrow in Fig. 5.3K, see Figs. 5.6 and 5.7 below, for other species). During this process, the gynoecium starts to form a proto-style ("G", Fig. 5.3J), whereas the initial depression, now a pore, represents the proto-stigma. As the proto-style develops upwards, the two ovary locules are formed (Fig. 5.3L).

After all organs are formed, the floral bud continually enlarges toward anthesis (Stages 3 and 4). In Stage 3, the stamens differentiate each into a proximal filament and a distal anther. The tetrasporangiate anthers start to differentiate as sagitate structures (Fig. 5.4A) and a longitudinal depression appears in the middle of the abaxial side when the pollen sacs start to form (arrow in Fig. 5.4A). During this process, the style reaches the inner surface of the corolla and bends sidewards on top of the developing anthers ("G", Fig. 5.4B,E). Ovules start to develop at this stage, as protuberances on the axial placentas in both locules ("G(ov)", Fig. 5.4C).

Figure 5.4 (next page). Late stages of floral ontogeny in *Eugenia punicifolia*. (A-C) Initiation of anthers and ovules. Note style bending in "B". (D) Exposure of the corolla prior to anthesis. (E) Longitudinal section of pre-anthetic bud showing maturation of anthers and ovules. Note that the style is sharply bent downwards. (F) Detail of ovule maturation in both ovary locules. (G - I) Mature pollen sacs in pre-anthetic anther. (J) Bud with exposed corolla (arrow) and recently opened flower of *Eugenia punicifolia* (field image). (K, L) thecae opening during anthesis of *Eugenia dichroma*; thecae are reflexed 180 degrees to expose pollen grains. S, sepals; P, Petals; A, androecium; G, gynoecium; G(ov), ovules; A(ant), anther; loc, locule; ad, adaxial pollen sac; ab, abaxial pollen sac; pol, pollen grain. Scale: 50µm (Kii); 100µm (A, C, G, H, I, Ki, L) 200µm (Fii); 500µm (B, D, E, Fi); 5mm (J). Colour coding in online version: sepals=green, corolla=red, androecium=yellow, gynoecium=blue.



In Stage 4, the sexual organs (androecium and gynoecium) finish pre-anthetic development, producing mature ovules and dorsifixed pollen sacs. Mature ovules are organized in loose series on the placenta ("G(ov)", Fig. 5.4E,F). Counts in mature flowers show distinct ovule numbers per locule, apparently reflecting a short plastochron between each locule. The abaxial pollen sacs of each anther ("ab", Fig. 5.4G-I) are slightly smaller than the adaxial pollen sacs ("ad", Fig. 5.4G-I). The stigma is thin and simple, with single celled papillae. During Stage 4, sexual organs mature faster than the perianth elongates (calyx and corolla). As a consequence, the corolla that until this point remains covered by the calyx lobes is pushed upwards and exposed (Fig. 5.4D). This exposure of the corolla is the last step before anthesis. Also at this point, the sepal pairs ("S1"

and “S2”) approach in size producing four sepals of similar proportions. This process occurs either by developmental acceleration of “S2”, slow-down of “S1” or both (“S1” and “S2”, Fig. 5.4D).

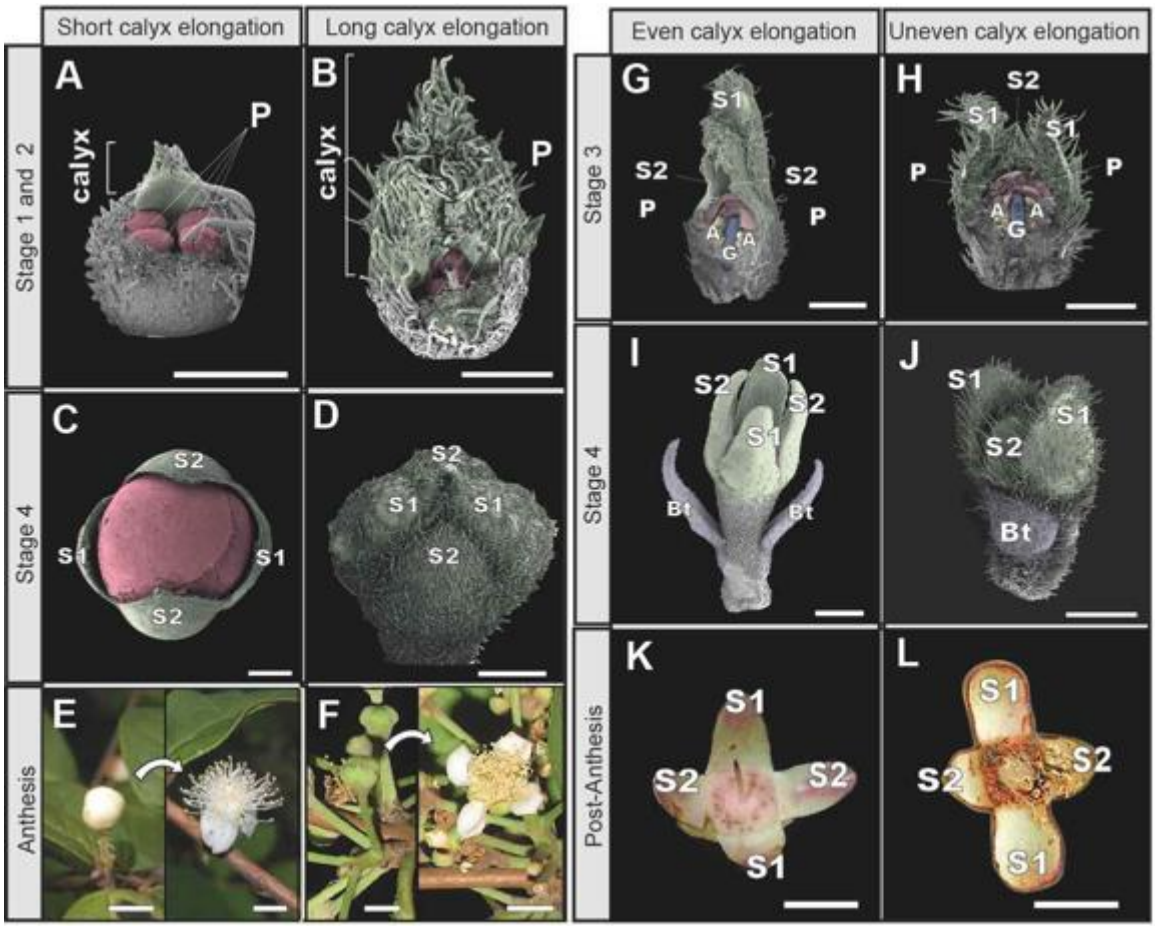
Stage 5 represents anthesis. During this process (Fig. 5.4J), the perianth opens, the style straightens and the anthers are exposed. Tissue between each pollen sac (anther locules) opens longitudinally and laterorsely (Fig. 5.4K,L) until the thecae are held at nearly 180 degrees to expose the pollen (“pol” in Fig. 5.4K; Fig. 5.4L). The flower is then ready for pollination.

5.9 Heterochronical pattern 1: Perianth growth rate

Using *Eugenia punicifolia* as a reference, it is possible to compare organ size proportions at similar stages of development between species to infer changes in development rate (heterochronies). The first clear heterochronical pattern is observed when comparing perianth development between species. Most analysed samples showed a similar rate of perianth development as *Eugenia punicifolia*. However, in at least three species (*Eugenia involucrata*, *E. acutata* and *E. dichroma*) sepal enlargement occurs at a noticeably faster rate (Fig. 5.5A) in early developmental stages. In these taxa the sepals elongate at least twice as fast as in similar stages in other *Eugenia* (see contrast between Fig. 5.5A and B). As a consequence, in these species the calyx covers the whole bud until developmental Stage 4, with no corolla exposure prior to anthesis (see contrast between Fig. 5.5C and D and Fig. 5.5E and F).

Another distinct pattern of sepal development was observed in *Eugenia inversa* and *E. splendens* (Fig. 5.5G,I,K). In all other species, the ultimate size of the second pair of sepals (“S2”) is similar to the first one (“S1”) in developmental Stage 4, producing a radially symmetric calyx at anthesis (Fig. 5.5G, I, K). In contrast, in *Eugenia inversa* and *E. splendens* the size difference between sepals S1 and S2 is constant during and after anthesis, resulting in unequal sepals and a disymmetric calyx, still evident in post-anthetic stages (highlighted in Fig. 5.5L).

Figure 5.5 (next page): Variation of perianth developmental rate in *Eugenia*. (A) Early development of *Eugenia* stipitata, showing short calyx contrasting to (B) extremely elongated calyx of *Eugenia acutata.* (C) Pre-anthetic stages in *Eugenia protenta*, showing corolla exposition prior to anthesis; (D) same stage in *Eugenia acutata*, showing sepals that cover the whole buds prior to anthesis. (E) Anthesis in *Eugenia stipitata*, highlighting how exposed corolla is in the pre-anthetic stage; (F) Anthesis in *Eugenia acutata*, showing sepals that cover the whole bud prior to anthesis. (G) Stage 3 bud in Eugenia involucrata and in (H) Eugenia inversa, showing S1 more developed than S2. (I) Pre-anthetic buds of *Eugenia dichroma*, with S1 and S2 equally developed in contrast to same stage in (J) Eugenia inversa, where S1 is still more developed than S2. (K) Calyx from post-anthetic flower of Eugenia involucrata, showing all sepals the same size contrasted to (L) post-anthetic flower of Eugenia splendens, showing disymmetric calyx with distinctly larger S1 than S2 sepal pairs. Bt, bracteole; S, sepal; P, petal; A, androecium; G, gynoecium. Scale: 250µm (A, B); 500µm (C, D, H); 1mm (G, I, J); 5 mm (E, F, K, L); Colour coding in online version: sepals=green, corolla=red, androecium=yellow, gynoecium=blue. Picture in (F) by Augusto Giaretta.



5.10 Heterochronical pattern 2: Style gigantism in *Eugenia* sect. *Umbellatae*

A second heterochronical pattern is found in the rate of stylar growth. Two main patterns of style elongation are observed across the sampled species. In species within Sect. *Umbellatae* (here represented by *Eugenia punicifolia, E. citrifolia*, *E. flavescens* and *E. protenta*), the style develops faster, reaching the inner surface of the closed corolla early in Stage 3, bending to one side and resting upon the anthers ("Sect. *Umbellatae*" column, Figs. 5.6A, C, E, G, I). In these species, the long style is twice the length of the androecium in anthetic flowers with a visible mark in the middle where it was folded (highlighted in Fig. 5.6K). In all other analysed species, the rate of style development is slower than in *Eugenia punicifolia* ("Other clades" Figs. 5.6 B, D, F, H, J) and the style never bends over the androecium in the pre-anthetic bud (Fig. 5.6J). After anthesis, the style in these species has the same length as the stamens (arrow, Fig. 5.6J). This variation was observed to be particular to each species, with no infraspecific variation that would characterize heterostyly detected.

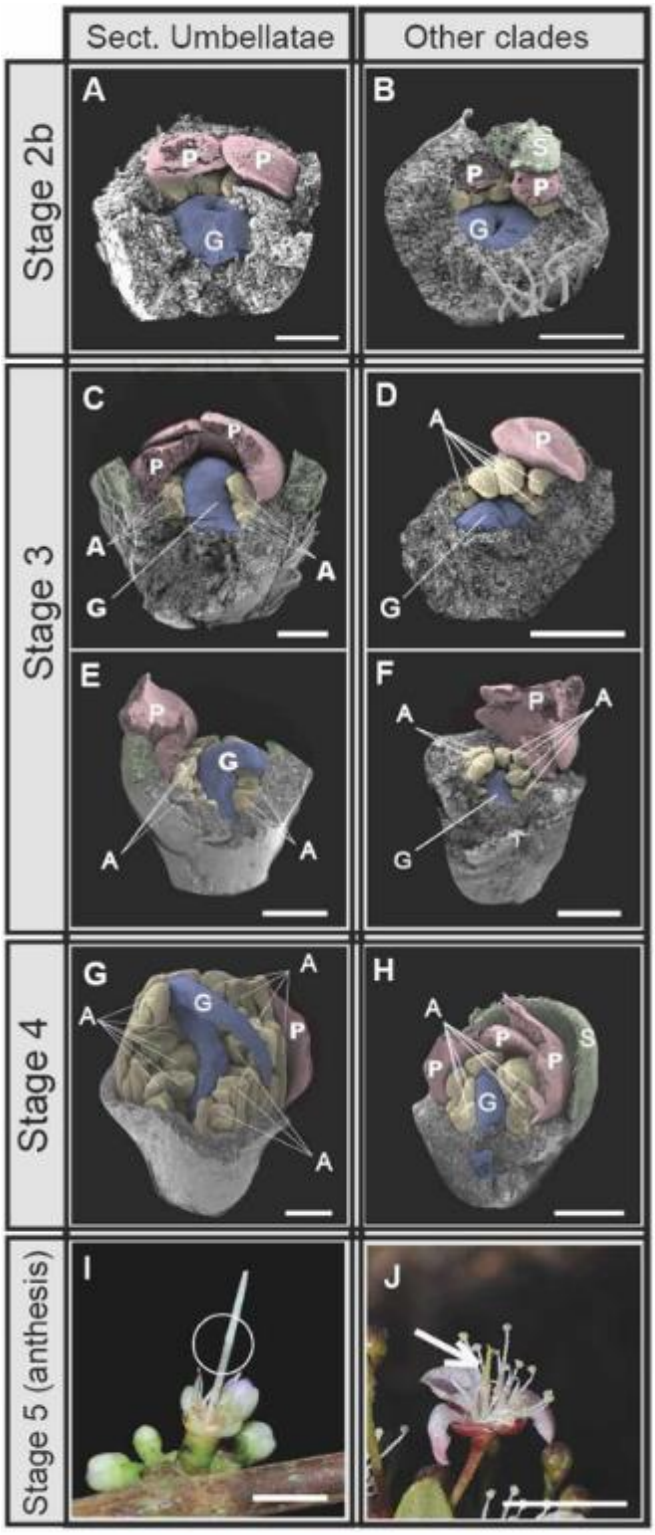


Figure 5.6: Comparative style development in *Eugenia* Sect. *Umbellatae* and other clades. (A) Swollen proto-style in *Eugenia punicifolia*, contrasted to (B) flat proto-style in same stage of *Eugenia angustissima*. (C and E) continuous development of style in *Eugenia punicifolia* and (E) *Eugenia protenta*, showing style bending on top of the anthers (D and F) continuous development of style of *Eugenia angustissma*, showing comparativelely shorter style than in "C" and "E". (G) Pre anthetic bud in *Eugenia protenta*, showing long style that folds on top of the anthers in contrast to (H) *Eugenia angustissima*, where the style is always shorter than the stamens. (I) Open flowers of *Eugenia citrifolia*, with highlighted folding mark in the middle of the style, in constrast to (J) open

flower of *Eugenia angustissima*, showing style at roughly the same height as stamens. Colour coding in online version: sepals=green, corolla=red, androecium=yellow, gynoecium=blue. 150µm (A, B); 250µm (C, D, E, F); 500µm (G, H); 5mm (I, J).

5.11 Heterochronical pattern 3: Hypanthium elongation and androecium development

A third heterochronical pattern concerns early hypanthium elongation and its effects on the initiation and morphogenesis of the androecium. Androecium development is similar in all analysed species: initially, two stamen primordia appear below each petal followed by a continuous sequence of newly appearing stamen primordia in between, forming the first rings of stamens in Stage 2a (Fig. 5.7, see additional images in Appendix 5.2 – plate 4). Sequentially, the hypanthium broadens and stamen primordia cover the entire surface of the hypanthium tissue, from corolla to the stylar base, in Stage 2b. The degree of early hypanthial elongation varies between species and thus the number of stamens also varies from species to species (see Table 5.1). Stamens can be distributed in two to eight or nine rings (Figs. 5.7A-I) depending on the width of the available surface as a result of hypanthium expansion. An additional pattern was observed in the New Caledonian species *Eugenia paludosa* and *E. gacognei* (clade *Jossinia*). In these species, hypanthial expansion occurs later in development, after the first staminal whorl is already prominent (Fig. 5.7J,K). This results in a clearer plastochron between the first and following staminal whorls; the first is already well developed when the later primordia appear. In this case, stamens in the first whorl end up folding slightly towards the centre of the bud in the available cavity (Fig. 5.7L).

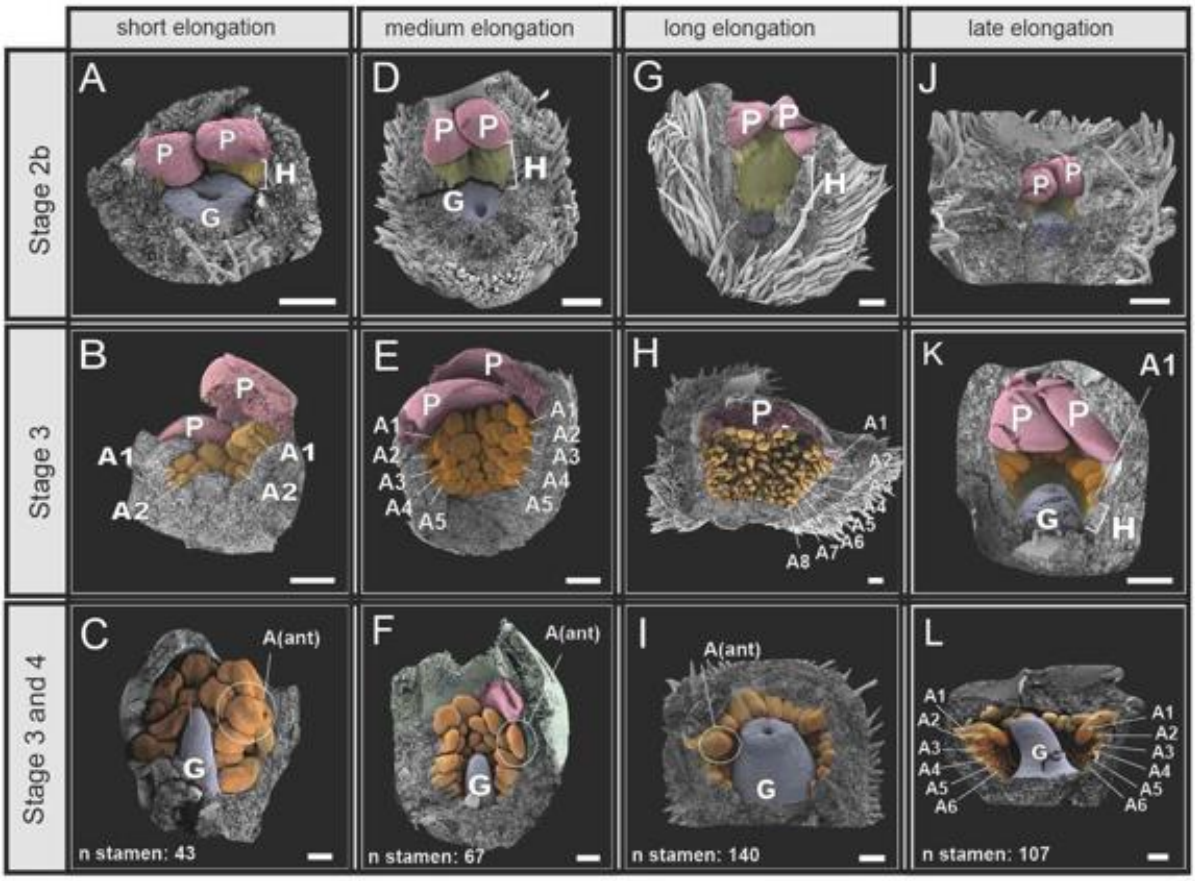


Figure 5.7 (previous page): Comparative hypanthium and androecium development in *Eugenia* and differential rate of pollen sac maturation (in orange) according to number of stamens per flower. (A, B) Androecium initiation and hypanthium development of *Eugenia angustissima*, showing two loose stamen whorls forming on the expanded hypanthium. (C) Early maturation of pollen sacs in *Eugenia uniflora*. (D, E) Androecium initiation and hypanthium development of *Eugenia dichroma*, showing five loose stamen whorls forming on the hypanthium. (F) Pollen sacs in maturation process in *Eugenia longiracemosa*. (G, H) Androecium initiation and hypanthium development of *Eugenia azurensis*, showing eight loose stamen whorls forming on the hypanthium. (I) Late pollen sac maturation in *Eugenia stipitata*. (J, K) Androecium initiation and hypanthium development of (J) *Eugenia gacognei* and (K) *E. paludosa*, showing a gap between the development of the first and 5 following stamen whorls. (L) Pollen sac maturation in *Eugenia paludosa*, showing anthers from the first staminal whorl in a more advanced state of development. A(ant), anther; G, gynoecium; S, sepal; P, petal; H, hypanthium. Scale: 100µm (A, B, C, D, F, G, I, J, K, L), 200µm (E, H). Colour coding in online version: sepals=green, corolla=red, androecium=yellow, gynoecium=blue.

Observed variation in androecium development is also responsible for a clear difference in the rate of anther maturation among analysed species. In flowers with fewer stamens, the whole androecium matures faster in comparison to flowers with a higher number of stamens. As a result, flowers at apparently similar stages of development present anthers in different maturation stages according to stamen number (Fig. 5.7C,F,I,L).

5.12 Hypanthial heterochrony effects on androecium/gynoecium proportion

The relative hypanthial expansion in *Eugenia* flowers is also responsible for the final size of the floral receptacle. Therefore, species with longer initial hypanthial expansion (e.g. *Eugenia azurensis*, *E. itajurensis*, *E. paludosa*) have larger floral receptacles in comparison to those with short hypanthial expansion (e.g. *Eugenia angustissima*, *E. ligustrina*, *E. punicifolia*) (see Table 5.1). Since the production of stamen primordia is continuous throughout hypanthium expansion, stamen number is directly linked to the growth of the hypanthium. This relationship of dependance strongly correlates stamen number with floral receptacle size ($p<0.001$, $r^2>0.7$, Fig. 5.8A). Thus, larger flowers bear more stamens and consequently more pollen sacs (reproductive male parts). Curiously, however, the same is not true for the relationship between floral receptacle and number of ovules (reproductive female parts). Because the hypanthium expands above the ovary, changes in hypanthium expansion rate have little influence on the number of ovules. Therefore, size of floral receptacle is not significantly correlated with total ovule number per flower ($p = 0.214$, $r^2<0.1$, Fig. 5.8B), meaning that larger flowers do not necessarily bear more ovules than smaller ones. There is a slight difference in ovule size (see Appendix 5.2 – plate 5) but most variation appears to result from differential investment in receptacle tissue. These results suggest that shifts in rate of the hypanthial development, responsible for the total number of stamens formed, affect the final size of the flower and the production of male structures (androecium) but not female floral parts (gynoecium) in *Eugenia*.

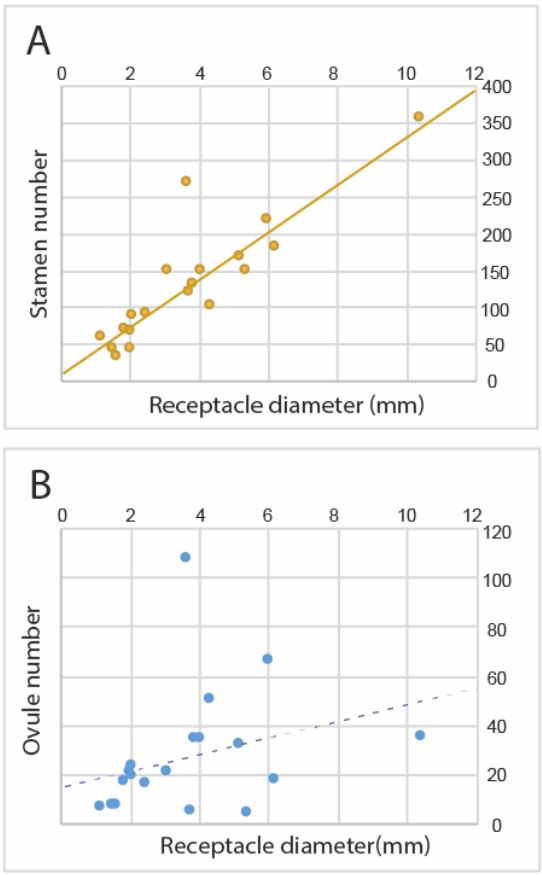


Figure 5.8: Correlation between total diameter of the floral receptacle and: (A) total stamen number per flower ($p < 0.001$, r2), (B) total ovule number per flower ($p = 0.2140$, r2).

DISCUSSION

5.13 Eugenia flower development in the context of Myrtaceae

Flower morphology in *Eugenia* is similar to that in other Myrtaceae and Myrtales. The tetramerous-decussate phenotype is very frequent in other Myrtaceae (e.g. *Eucalyptus*, *Syzygium*) and even the variation between tetramerous-decussate and pentamerous-quincuncial aestivation can be found in other closely related genera (e.g. *Myrcia*, see Chapters 3 and 6).

Polyandry is the most frequent androecium arrangement in Myrtaceae (Ronse De Craene and Smets, 1991) and is common in other core eudicot families (e.g. Prenner et al., 2008; Prenner, 2011; Paulino et al., 2014) and in Magnoliales (e.g. Ronse De Craene and Smets, 1998). Eudicots differ from the latter, however, in presenting whorled rather than spiral stamen formation (Ronse De Craene and Smets, 1992, 1998), having evolved from ancestral oligandrous arrangements, i.e. secondary polyandry (Endress, 1996).

The acquisition of secondary polyandry is not as evident in *Eugenia* as it is in other Myrtaceae (e.g. *Melaleuca*, Orlovich et al., 1999), but some heterogenety in the appearance of the first and second group of stamen primordia suggest this pathway in the genus. During androecium initiation, a first group of staminal primordia is formed in an antepetalous position, so that the flower is initially obhaplostemonous. This pattern may represent a relic from a plesiomorphic stage, where these areas would have shown a more apparent primary primordia that would further divide into secondary primordia and sequential rings (Ronse De Craene and Smets, 1992; Endress, 1994).

Diplostemony is hypothesized to be the plesiomorphic state for Myrtales (Dahlgren and Thorne, 1984) but there is no evidence for this state in *Eugenia* or Myrtaceae (Ronse De Craene and Smets, 1995) because even though two primary primordia are flanking each petal, these are arranged in a single whorl.

5.14 Heterochronic trends and adaptative features

When very few changes in complexity are observed within the morphologically homogeneous flowers of *Eugenia* (see Appendix 5.2), lability of reproductive strategies must rely on an alternative strategy. In this sense, heterochronies are an important component of secondary flower function (see definition in the *Introduction* section *5.1*). Examples of how heterochronies may affect fitness (i.e. the the efficiency of the flower as reproductive organ) in *Eugenia* are observed in all floral organs. In the perianth development for instance, early calyx elongation in *Eugenia acutata*, *E. dichroma* and *E. involucrata* may protect the bud in late development stages, hiding the reproductive organs until anthesis (as reported in *Calyptranthes* and *Marlierea*, see Chapter 6). Likewise, the constant disparity between the first and second pair of sepals in *Eugenia inversa* and *E. splendens* causes the open flower to be slightly disymmetric instead of actinomorphic (the most common arrangement in the genus), which in turn may affect pollinator behaviour (Endress, 1999).

Regarding the gynoecium, hyper-style elongation present in all four observed species of *Eugenia* sect. *Umbellatae* creates a spatial gap between the stigma and the anthers after anthesis, i.e. herkogamy, a trait not observed in the other species (Fig. 5.9). Herkogamy is traditionally thought to increase the ratio of cross pollination, by avoiding accidental self-pollination (Webb and Lloyd, 1986). Although flowers of *Eugenia* present a certain degree of self-compatibility (Proença and Gibbs, 1994; Silva and Pinheiro, 2007), higher levels of cross-pollination are related to higher diversification rates (with abundant examples in flowering plants e.g. Ferrer and Good, 2012; de Vos *et al*., 2014). The systematic consistency of this character and its relationship to the most diverse section of *Eugenia* may implicate this innovation in the accelerated diversification rates found in *Eugenia* Sect. *Umbellatae* (one of the highest in tribe Myrteae; see Chapter 1).

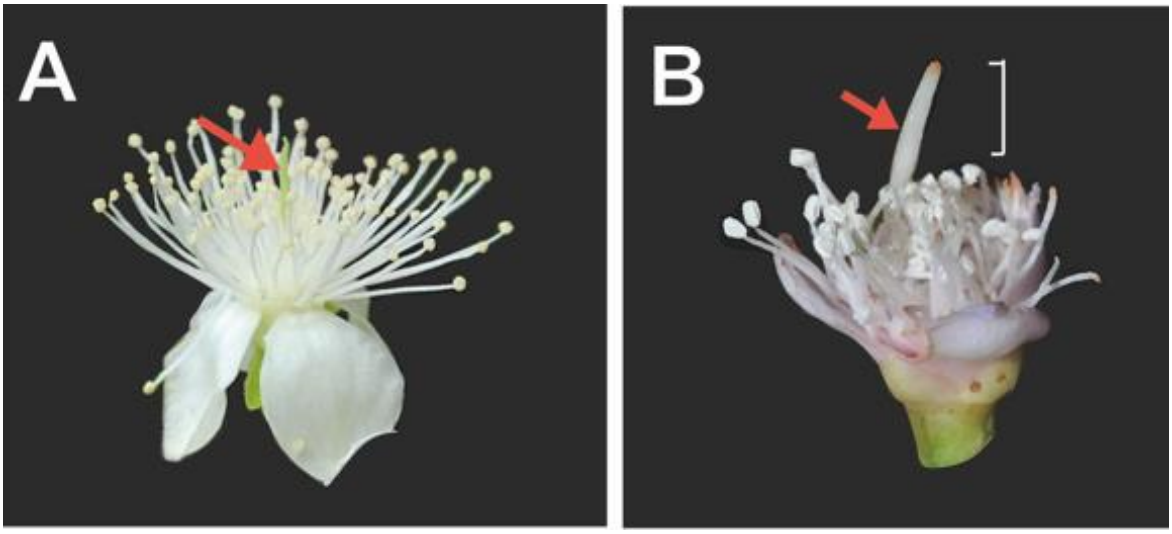


Figure 5.9 Style gigantism and resulting herkorgamy of *Eugenia* sect. *Umbellatae*. (A) Flower of *Eugenia dichroma* (Sect. *Speciosae*), in which the style is at the same height as stamens in the open flower (arrow) and (B) Flower of *Eugenia adenocalyx* (Sect. *Umbellatae*), showing style almost

twice as long as the stamens (arrow). (Photos taken during field expeditions between 2014 and 2016)

5.15 Hypanthium vs. androecium: space matters

The most prominent effects of heterochrony in *Eugenia* flowers are seen in the development of the androecium. Changes in the rate of early hypanthial development are shown to affect the final diameter of the floral receptacle and consequently the number of stamens formed. Variation in stamen number is especially likely to affect aspects of reproductive strategies in *Eugenia*. It has been shown, for example, that the bottle-brush appearance that results from the large number of stamens in Myrtaceae flowers is the main agent of floral display and pollinator attraction (Proença and Gibbs, 1994; Willmer 2011) so changes in stamen number could be related to variations within this syndrome. It is also clear that smaller flowers with fewer stamens undergo faster anther maturation, suggesting that the whole flower might have a faster rate of development. This could relate to a trade-off between investment in receptacle size and number of stamens (floral display) vs. faster maturation with consequences for flowering phenology (Primack 1985, 1987).

An alternative (or additional) hypothesis for variation in stamen number is that these changes affect the proportion of male and female parts in the flower and consequently relate to changes in breeding systems (Cruden, 1977; Charlesworth, 2006). An indication of this is that stamen and ovule numbers respond independently to variations in size of floral receptacle (resulting from hypanthium expansion) in different species (Fig. 5.8). While stamens and anther numbers are highly dependendent on space available after hypantial expansion (similar development of corona size in Passifloraceae; Claßen-Bockhoff and Meyer, 2016), gynoecium configuration is more clade specific, with lower number of ovules characteristic of certain clades (e.g. Faria, 2014). If hypanthium extension rate disparity affects the number of male but not of female parts, this heterochronic pattern might drive, or be implicated in, a labile reproductive system and increased adaptative value of the genus throughout evolution. Pollen counts and ovule viability tests are required to fully test the importance of this character (Harder and Barret, 1993).

5.16 Relevance of hypanthium/androecium dependency for early Myrteae evolution

Even though polyandry is a configuration shared by most members of the Myrtoideae subfamily, the trait varies between lineages. Recent systematic survey shows that *Eugenia*, alongside other related genera within tribe Myrteae, are exceptions within Myrtales in presenting straight (as opposed to folded) stamens in the bud (Chapter 2). Comparison of hypanthium and androecium development in *Eugenia* with that of other Myrtoideae genera (e.g. Drinnan and Ladiges, 1991; Orlovich *et al*., 1999; Bohte and Drinnan, 2005; see list in Appendix 5.1) suggests the distinction between straight and folded stamens in the bud is related to the area occupied by stamen primordia over the expanded hypanthium.

Eugenia (and related genera) produces an indeterminate number of staminal primordia that cover the whole hypanthial tissue up to the stylar base during androecium development (Fig. 5.4C-J). Conversely, Myrtaceae genera with folded stamens in the bud (including some *Eucalyptus* species with slightly straight stamens in the bud; e.g. McDonald et al., 2009) present staminal primordia development only on a restricted area of the hypanthial rim, below the corolla (Drinnan and Ladiges, 1991; Orlovich et al., 1999; Bohte and Drinnan, 2005; see Chapter 3). The restricted

development of stamen primordia on the hypanthial rim during bud development creates an open space below the youngest staminal ring when the hypanthium expands (in red, Fig. 5.10A), forming a hypanthial cup. This explains the position of the stamens in Myrtaceae buds as a physical matter: gravitropy folds the stamens down when adequate space is available (Fig. 5.10A). Meanwhile, *Eugenia* species (and related genera, e.g. *Acca*, *Ugni*; see Belsham and Orlovich, 2003; see Chapters 2 and 3) do not present any open space during early bud development to allow stamens to fold, as the whole hypanthial tissue is covered by stamen primordia (Fig. 5.10B). This leaves no space for folding and causes stamens to develop in a straight position.

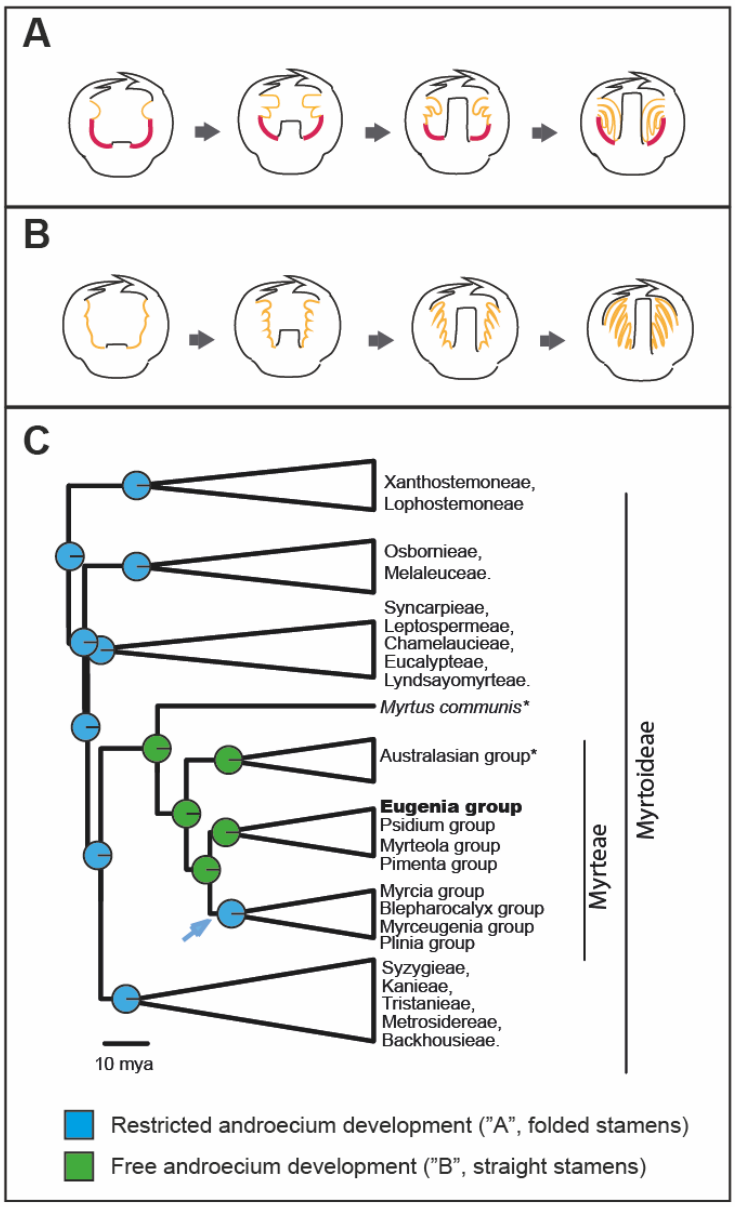


Figure 5.10: Evolution of androecium development in *Eugenia* and related taxa. (A) Restricted androecium development, where stamen primordia appear just in the apical part of the hypanthium leading to folded stamens in the bud. (B) Free androecium development, where stamen primordia cover the whole hypanthium tissue, leading to straight stamens in the bud. Stamens are shown in yellow and "empty" hypanthial tissue in red. Other floral organs were kept the same size to help

interpretation. (C) Reconstruction of stamen posture in the bud on the Myrtaceae phylogeny. Arrow shows reversal to plesiomorphic state in Myrcia, Plinia, Blepharocalyx and Myrceugenia groups. * The position of the Myrtus and Australasian groups are swapped in more recent phylogenies (Chapter 1)

Therefore, the “folded stamen in the bud” trait indicates that androecium development is restricted to the hypanthial rim while “straight stamens in the bud” trait indicates unrestricted androecium development over the hypanthium (as discussed in Chapter 3). By ploting these traits on the Myrtaceae phylogenetic hypothesis, a shift from restricted to unrestricted androecium development is estimated to have occurred at the crown node of tribe Myrteae (Fig. 5.10C). This shift may also be related to the loss of nectar production: while nectaries are present in many Myrtaceae (e.g. Beardsell et al., 1993; see also Appendix 5.2 – plate 6) favoured also by the hypanthium cup, where the nectar can accumulate (as in other Myrtales, Ronse DeCraene and Smets, 1991), they are absent in most Myrteae.

The unrestricted development state appears then to have reversed to a plesiomorphic restricted development state in *Myrcia* and related genera (Arrow, Fig. 5.10C), the clade in Myrteae with folded stamens (Chapter 2). This shift and the consequent lability of reproductive strategies provided by the association of unrestricted stamen formation on the hypanthium expansion (Fig. 5.8) may have been important in the early evolution of tribe Myrteae. The high acceleration in diversification rates associated with the early evolution of the tribe (Biffin et al., 2010; Berger et al., 2016) have been traditionally linked to the key-innovation of the fleshy-fruit (Biffin et al., 2010), but this study provides evidence that not only fruit, but also adaptations of the flower may have contributed to early establishment of tribe Myrteae.

CONCLUSION

The present study shows that *Eugenia* flowers present diversity in floral strategies despite its morphological similarity. These are mainly driven by subtle changes in developmental rates that altered proportions between floral organs throughout the evolutionary history of the group. Heterochronies in *Eugenia* are shown to be implicated in subtle breeding system changes (affecting differential production of male and female parts), phenology (floral development rate changes) and unbalanced clade diversity (in the case of the style in *Eugenia* sect. *Umbellatae*). This study also provides insights into the evolution of characteristic Myrtaceae polyandry by indicating unrestricted primordia initiation throughout the hypanthium to have been an evolutionary novelty in Myrteae. Recognition that superficially homogeneous flowers may present an array of possible reproductive strategies by fine tuning developmental rhythms is a step forward from traditional deterministic concepts in plant reproductive biology. Future directions include field hypothesis testing and trait dependent diversification rate analyses, particularly regarding longer styles in the mega-diverse *Eugenia* sect. *Umbellatae*.

APPENDIX

Appendix 5.1 Character matrix for reconstruction of androecium evolution in Myrtoideae (based on the phylogeny of Thornhill *et al*., 2015). 1) stamen primordia developing throughout the hypanthium. 2) stamen primordia restricted to the apical area of the hypanthium.

species	stam
Lophostemon_confertus	2
Kjellbergiodendron_celebicum	2
Callistemon_polandii	2
Melaleuca_adnata	2
Melaleuca_lanceolata	2
Beaufortia_orbifolia	2
Calothamnus_validus	2
Melaleuca_nesophila	2
Melaleuca_uncinata	2
Melaleuca_glomerata	2
Melaleuca_cornucopiae	2
Melaleuca_acacioides	2
Melaleuca_viridiflora	2
Melaleuca_argentea	2
Melaleuca_leucadendra	2
Melaleuca_cajuputi	2
Melaleuca_arcana	2
Eucalyptopsis_papuana	2
Stockwellia_quadrifida	2
Arillastrum_gummiferum	2
Angophora_hispida	2
Corymbia_citriodora	2
Angophora_costata	2
Eucalyptus_curtisii	2
Eucalyptus_pauciflora	2
Eucalyptus_haemastoma	2
Eucalyptus_melliodora	2
Eucalyptus_loxophleba	2
Eucalyptus_grandis	2
Eucalyptus_camaldulensis	2
Eucalyptus_globulus	2
Eucalyptus_nitens	2
Eucalyptus_perriniana	2
Kunzea_baxteri	2
Kunzea_capitata	2
Kunzea_ericoides	2
Pericalymma_ellipticum	2

Agonis_flexuosa	2
Asteromyrtus_lysicephala	2
Asteromyrtus_symphyocarpa	2
Leptospermum_spectabile	2
Leptospermum_trinervium	2
Leptospermum_scoparium	2
Ochrosperma_oligomerum	2
Ochrosperma_lineare	2
Homalocalyx_aurea	2
Calytrix_tetragona	2
Micromyrtus_ciliata	2
Micromyrtus_elobata	2
Hypocalymma_tetrapterum	2
Thryptomene_saxicola	2
Pileanthus_filifolius	2
Chamelaucium_uncinatum	2
Verticordia_pennigera	2
Actinodium_cunninghamii	2
Darwinia_fascicularis	2
Homoranthus_darwinioides	2
Baeckea_frutescens	2
Baeckea_tuberculata	2
Kardomia_jucunda	2
Harmogia_densifolia	2
Baeckea_pentagonantha	2
Baeckea_ovalifolia	2
Euryomyrtus_ramosissima	2
Sannantha_angusta	2
Sannantha_cunninghamii	2
Sannantha_tozerensis	2
Sannantha_virgata	2
Myrtus_communis	1
Uromyrtus_australis	1
Decaspermum_humile	1
Rhodamnia_rubescens	1
Rhodamnia_argentea	1
Archirhodomyrtus_beckleri	1
Pilidiostigma_papuanum	1
Rhodomyrtus_macrocarpa	1
Psidium_guajava	1
Psidium_cattleianum	1
Acca_sellowiana	1
Eugenia_uniflora	1

Eugenia_sulcata	1
Eugenia_myrcianthes	1
Legrandia_concinna	1
Pimenta_dioica	1
Amomyrtus_meli	1
Campomanesia_guazumifolia	1
Pimenta_pseudocaryophyllus	1
Pimenta_racemosa	1
Ugni_molinae	1
Myrteola_nummularia	1
Lophomyrtus_bullata	1
Lophomyrtus_obcordata	1
Neomyrtus_pedunculata	1
Blepharocalyx_salicifolius	2
Luma_apiculata	2
Myrcia_splendens	2
Myrciaria_vexator	2
Blepharocalyx_cruckshanksii	2
Myrceugenia_myrcioides	2
Myrceugenia_leptospermoides	2
Myrcia_saxatilis	2
Marlierea_obscura	2
Myrcia_multiflora	2
Myrcia_laruotteana	2
Marlierea_eugeniopsoides	2
Myrcia_pubipetala	2
Myrcia_brasiliensis	2
Myrcia_flagellaris	2
Calyptranthes_kiaerskovii	2
Calyptranthes_concinna	2
Calyptranthes_pallens	2
Tristaniopsis_laurina	2
Lysicarpus_angustifolius	2
Sphaerantia_chartacea	2
Cloezia_floribunda	2
Xanthomyrtus_papuana	2
Thaleropia_queenslandica	2
Tristania_neriifolia	2
Tepualia_stipularis	2
Metrosideros_nervulosa	2
Metrosideros_macropus	2
Metrosideros_diffusa	2
Metrosideros_carminea	2

Metrosideros_perforata	2
Backhousia_citriodora	2
Choricarpia_subargentea	2
Backhousia_myrtifolia	2
Syzygium_anisatum	2
Syzygium_wesa	2
Syzygium_wilsonii	2
Syzygium_luehmannii	2
Syzygium_francisii	2
Syzygium_zeylanicum	2
Syzygium_tetrapterum	2
Syzygium_buxifolium	2
Syzygium_gustavioides	2
Syzygium_floribundum	2
Syzygium_hedraiophyllum	2
Piliocalyx_francii	2
Syzygium_concinnum	2
Piliocalyx_bullatus	2
Piliocalyx_robustus	2
Syzygium_graveolens	2
Syzygium_divaricatum	2
Syzygium_mackinnonianum	2
Syzygium_acuminatissimum	2
Syzygium_smithii	2
Syzygium_hemilamprum	2
Syzygium_claviflorum	2
Syzygium_canicortex	2
Syzygium_apodophyllum	2
Syzygium_maire	2
Syzygium_fullagarii	2
Syzygium_multipetalum	2
Syzygium_arboreum	2
Syzygium_kuebiniense	2
Syzygium_nervosum	2
Syzygium_branderhorstii	2
Syzygium_amplifolium	2
Syzygium_brackenridgei	2
Syzygium_purpureum	2
Syzygium_aromaticum	2
Syzygium_seemannianum	2
Syzygium_puberulum	2
Syzygium_samarangense	2
Syzygium_tierneyanum	2

Syzygium_lateriflorum	2
Syzygium_malaccense	2
Syzygium_macilwraithianum	2
Syzygium_sayeri	2
Syzygium_ngoyense	2
Syzygium_laxeracemosum	2
Syzygium_austrocaledonicum	2
Syzygium_aqueum	2
Syzygium_crebrinerve	2
Syzygium_fibrosum	2
Syzygium_angophoroides	2
Syzygium_paniculatum	2
Syzygium_oleosum	2
Syzygium_australe	2
Syzygium_cormiflorum	2
Syzygium_bamagense	2
Syzygium_bungadinnia	2
Syzygium_pseudofastigiatum	2
Syzygium_moorei	2
Syzygium_acre	2
Syzygium_jambos	2
Syzygium_pycnanthum	2
Syzygium_seemannii	2
Syzygium_guineense	2
Syzygium_cumini	2
Syzygium_cordatum	2
Syzygium_muellerii	2
Syzygium_racemosum	2
Xanthostemon_chrysanthus	2
Osbornia_octodonta	2
Syncarpia_glomulifera	2
Lindsayomyrtus_racemoides	2

Appendix 5.2 Floral ontogenetic aspects that are not linked to heterochronies in *Eugenia*. All plates colour coded as: sepals=green, corolla=red, androecium=yellow, gynoecium=blue.

Plate 1: Merism in *Eugenia*. Comparison of the most common tetramerous phenotype exemplified by *Eugenia citrifolia* (A - C) with the pentamerous phenotype typical of Sect. *Hexachlamys* shown in *E. myrcianthes* (D – F). (A, D) Mid-stage development with perianth removed to show base of sepals and petals; (B, E) Frontal view of mature bud; (C, F) ground-plan diagrams. S, sepal; P, petal. Scales: 250µm (A), 500µm (B, D, E).

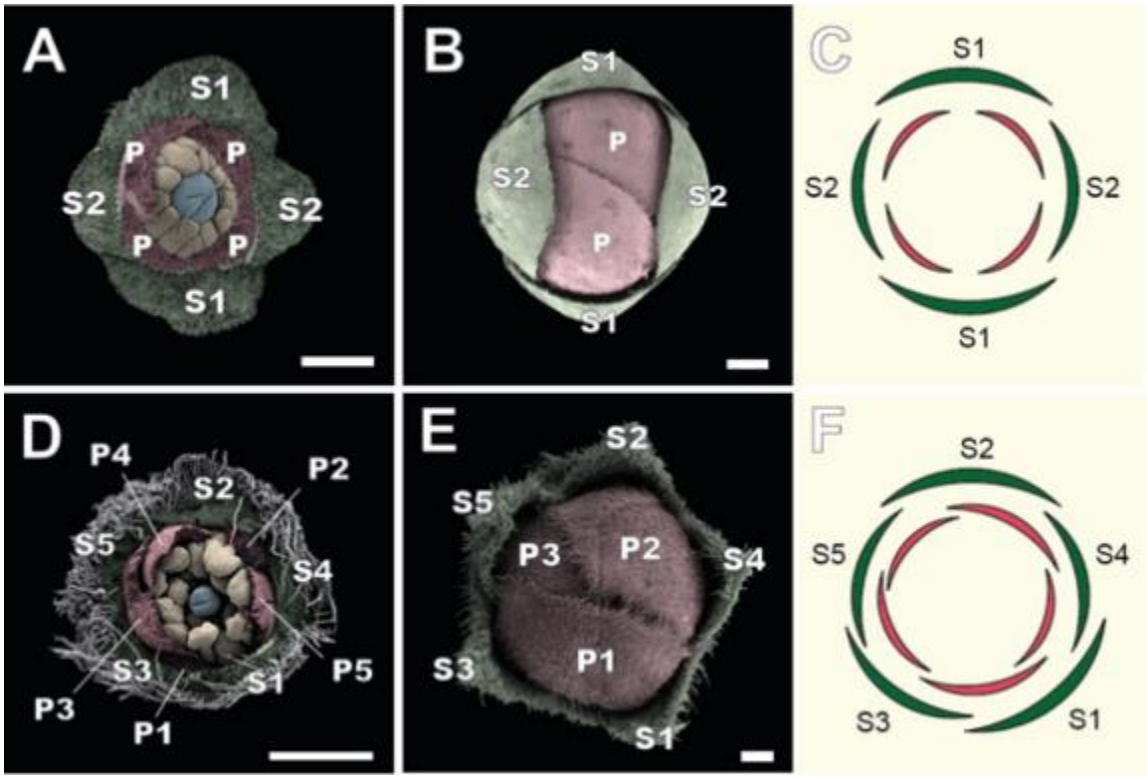


Plate 2: Gynoecium configurations in *Eugenia*. (A, B) Bilocular and trilocular ovaries found in two flowers of the same plant of *E. paludosa*. (C, D) Tetralocular ovaries of *Eugenia stipitata*, showing (C) initiation by four primordia and (D) expansion and fusion of initial primordia, forming a proto-style with a “cross” scar formation. P, petal; loc, ovary locule; G, gynoecium. Scale: 100µm (A, B, C), 250µm (D).

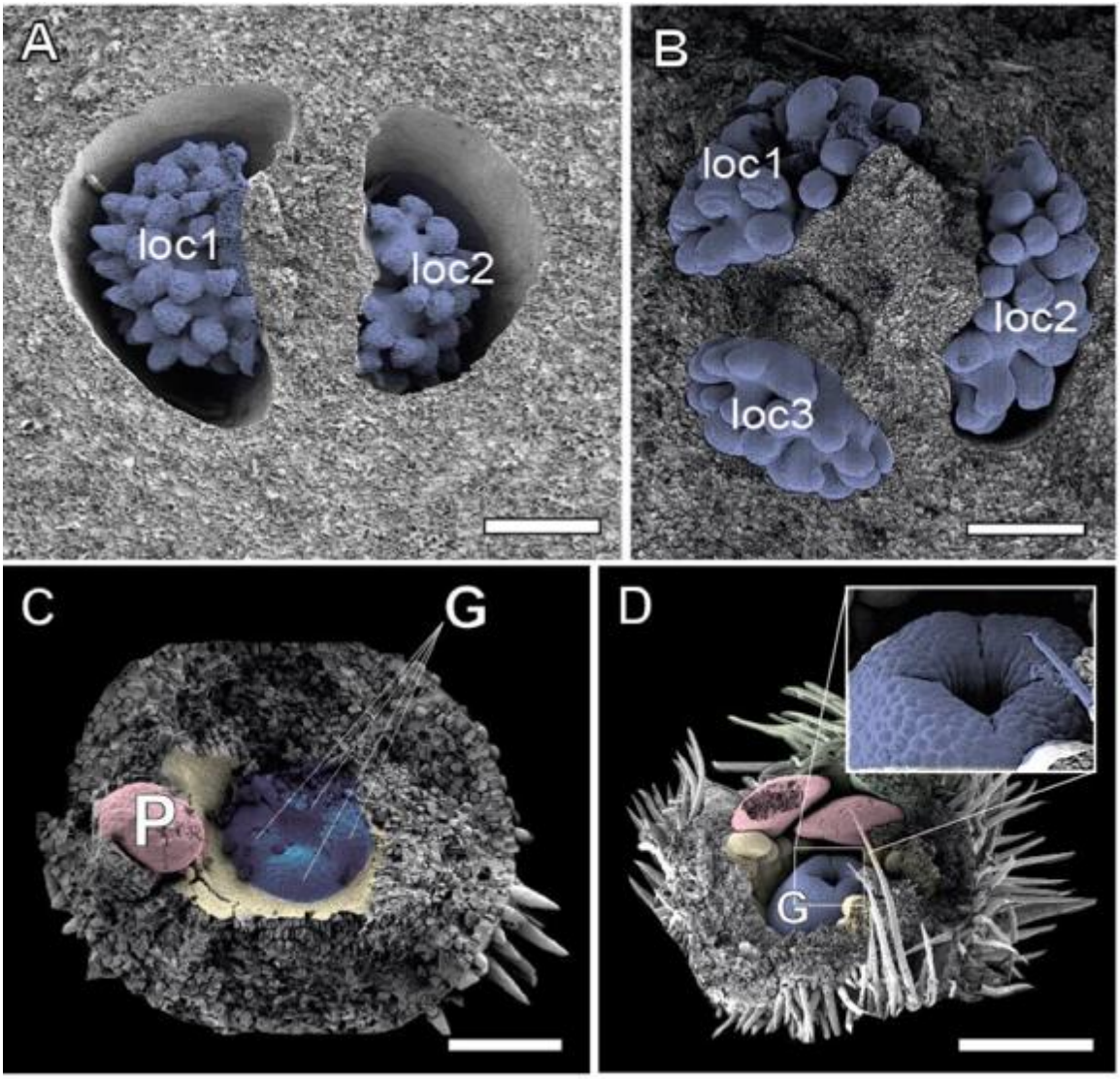


Plate 3: Random corolla aestivation in *Eugenia*. White dot indicates adaxial side. (A) *Eugenia dichroma*; (B) *Eugenia angustissima*; (C) *Eugenia pohliana*. S, sepal; P, petal. Scale: 150µm (C), 250µm(A, B).

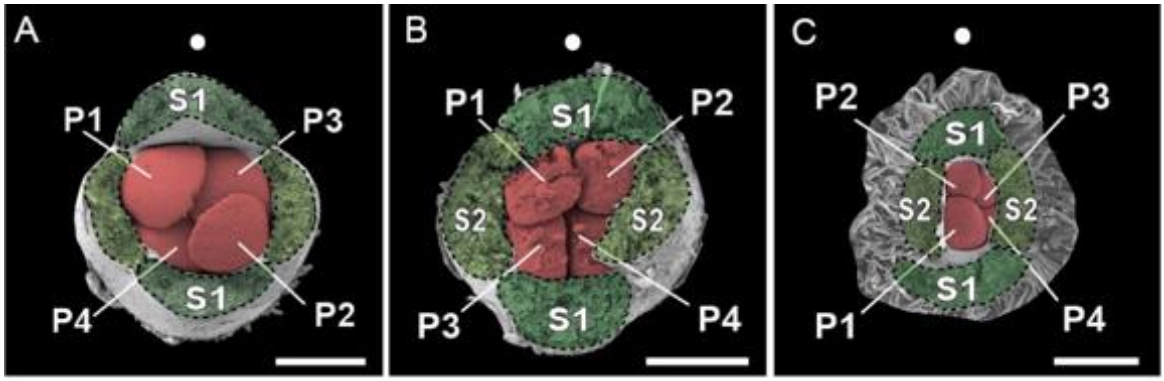


Plate 4: Detail of obhaplostemonous stamen initiation in *Eugenia*. (A) Early androecium development in *Eugenia stipitata*, showing initiation of primary primordia "A1(1st)" and secondary primordia "A1(2nd)" on the apical portion of the hypanthium. (B) Petal and hypanthium portion removed from *Eugenia itajurensis,* showing relative positions of primary stamen primordia "A1(1st)", secondary primordia "A1(2nd)" and initiation of following staminal rings "A2", "A3". A, androecium; G, gynoecium; S, sepal; P, petal. Scale: 100µm (A, B).

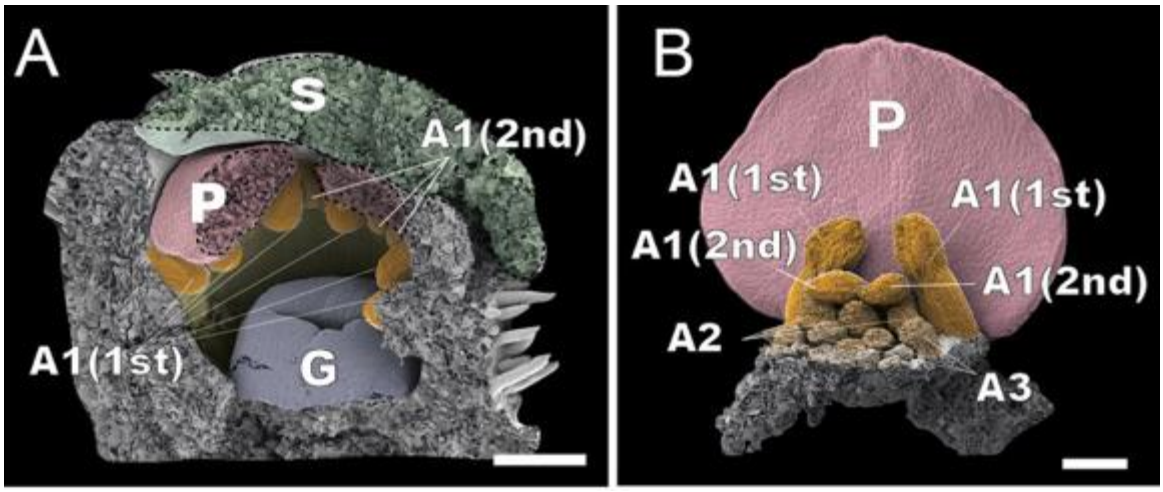


Plate 5: Proportion of reproductive organs in *Eugenia* flowers. Sect. *Umbellatae* appears to have larger styles and comparatively smaller ovules. (A) *Eugenia ligustrina* (sect. *Eugenia*), (B) *Eugenia citrifolia* (Sect. *Umbellatae*); (C) *Eugenia involucrata* (Sect. *Phyllocalyx*); (D) *Eugenia longiracemosa* (Sect. *Racemosae*); (E) *Eugenia dichroma* (Sect. *Speciosae*). All to scale.

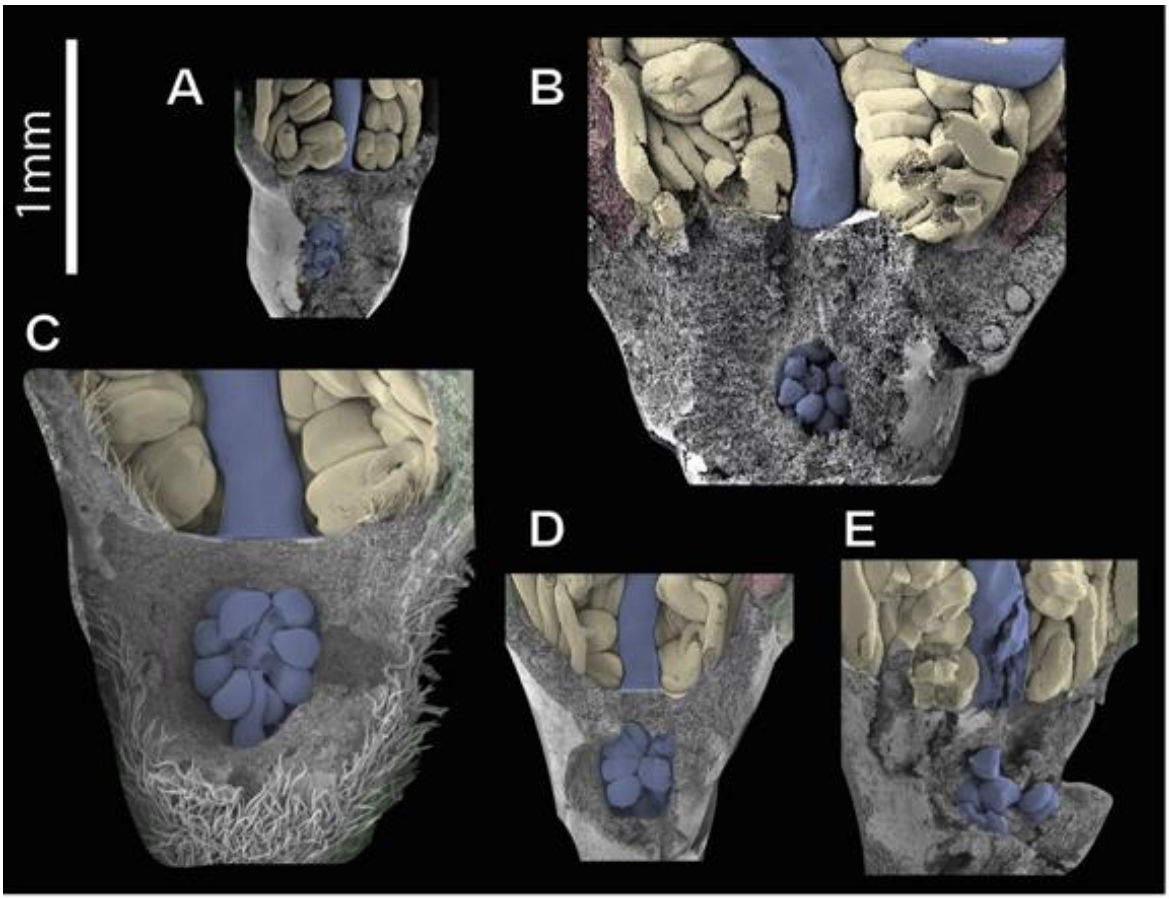
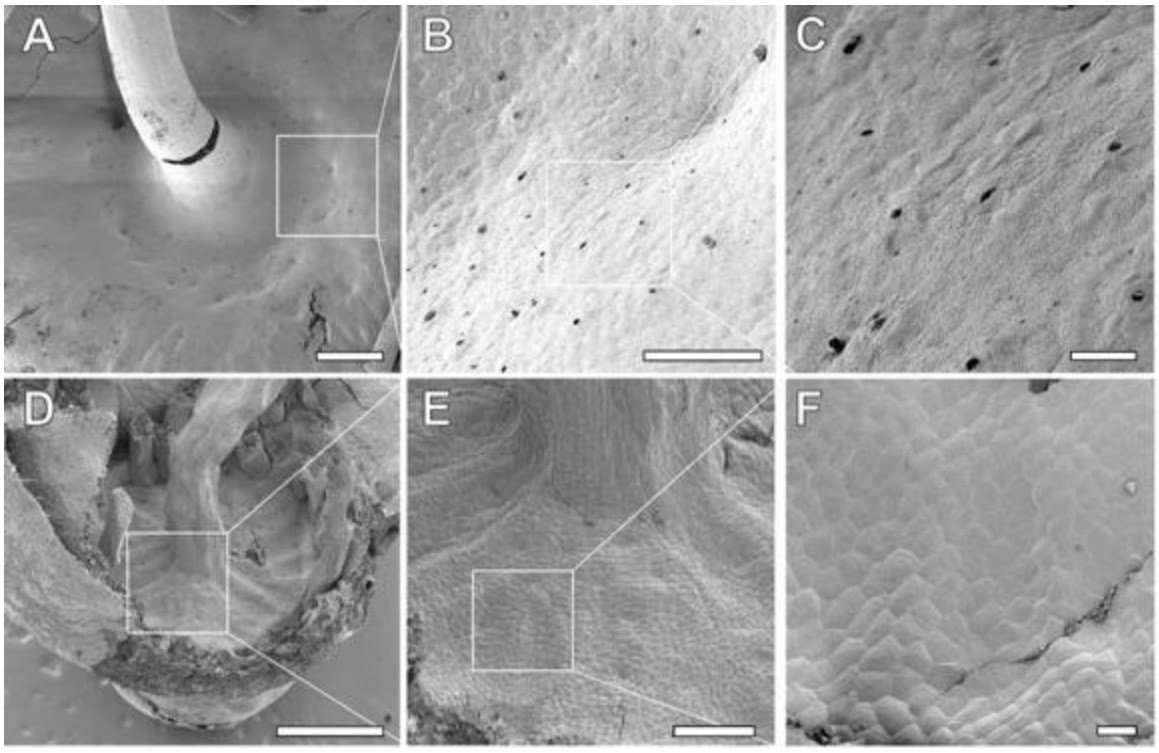


Plate 6: Nectaries in Myrtaceae and absence of nectaries in *Eugenia*. (A – C) Floral receptacle of *Syzygium paniculatum*, showing abundance of stomata in a nectary ring around the style (D – F) Same area in *Eugenia protenta*, showing absence of stomata or secretory structures. Scale: 25µm (C), 30µm (F), 100µm (B, E), 50 µm (A, D).



Chapter 6: Links between parallel evolution and systematic complexity in angiosperms — a case study of floral development in *Myrcia* s.l. (Myrtaceae)

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- T.N.C.Vasconcelos contributions: development of hypotheses, design of experiments, collection of samples, generation of SEM images, morphological analyses, phylogenetic analyses and writing of manuscript.

ABSTRACT

The greatest challenge to the modern plant systematist is the interpretation of molecular phylogenies that do not correspond to previous classifications based on morphological data. Characters that on first appearance seem highly diagnostic are brought into focus by phylogeny and frequently shown to have evolved multiple times independently. Parallelism is usually neglected in such systematic studies and the homoplastic distribution of a character in a phylogeny is commonly accredited to convergent evolution. The impact of parallel evolution on angiosperm systematics is examined here using a taxonomically complex and species-rich group of tropical tree genera (*Myrcia*, *Marlierea, Calyptranthes*; Myrtaceae) as a case study. These groups are traditionally distinguished by flower characters and have been shown to be polyphyletic by molecular data. Floral ontogeny of distinct lineages is examined using SEM and plotted on a five gene phylogenetic hypothesis to estimate ancestral states and phylogenetic signal for developmental variation. Results show that floral characters responsible for taxonomic confusion are a result of both parallel evolution and convergence. This is contrasted with other diverse and taxonomically complex angiosperm groups and problematic taxonomy appears linked to recent diversification events where the same genetic basis remains latent, demonstrating parallelism to be an important factor in problematic taxonomies. In this study, variations in early stage floral development produce the most labile characters. This is discussed in light of ontogenetic patterns in angiosperms with focus on the evolutionary consequences of homoplastic variation during early vs late floral development. The prevalence of parallelism must be appreciated by taxonomists of complex groups. Future classifications of groups affected by parallelism are likely to require data from detailed, multi-disciplinary studies of key characters to interpret phylogenies correctly.

Key words: convergence, floral evolution, morphology, Myrtaceae, taxonomy.

"[In the early 1800s] Martius, who had recently returned from Brazil with large collections, made his specimens available to De Candolle […] and in this way for the first time the size and complexity of the American Myrtaceous flora began to become apparent to European botanists. Berg, working only 30 years after De Candolle, recognized about 500 species of what De Cadolle would have called *Myrcia*"

(Mc Vaugh, 1968, p. 355)

INTRODUCTION

6.1 Morphology vs. molecular evidence in systematics of flowering plants

Since the 1990s, systematics has been revolutionized as morphological data was sidelined in favor of molecular surveys increasingly used to present evidence of relationships among taxa (e.g. Bruns et al*.,* 1991; Ragan et al*.*, 1994; Baldwin et al., 1995; Shaffer et al., 1997; Soltis et al., 1997; APG, 1998; Goodman et al., 1998). Currently, the advent of phylogenomics has further enhanced this process, rapidly sampling more DNA and more taxa in a quest for better resolved phylogenies (e.g. Delsuc et al*.,* 2005; Burki et al., 2007; McComarck et al*.,* 2012; LPWG, 2013; Nater et al., 2015). This molecular based progress produces topologies that, in conjunction with fossil, geological and ecological data, allow clarification of the environmental history in which taxa evolved (e.g. Hughes and Eastwood, 2006; Simon et al., 2009; Crisp et al*.,* 2011).

The explosion of such phylo-systematic techniques has resulted in the production of increasingly robust, complete and high statistically supported phylogenetic hypotheses. Contrary to expectation, however, systematic and taxonomic confusion has often increased. It is not uncommon to find classifications based on morphological characters that are highly incongruent with molecular data. Angiosperms, second only to beetles in species number (Wilson, 1999), have many such examples, particularly at lower taxonomic ranks (e.g. Soltis et al., 1996; Sweeney and Price, 2000; Plunkett et al., 2005; Kim et al., 2007; Goldenberg et al., 2008; Swenson et al., 2008; Lucas et al., 2011; Xue et al., 2011). Systematics of flowering plants is heavily based on reproductive characters (i.e. flower buds, flowers, fruits and seeds) as these present more morphological variation at higher taxonomic rank but are constrained within a species by the need for reproductive success. Nevertheless, since these structures are also susceptible to similar selective pressures in the long term, they often show a high degree of convergence (Tiffney, 1984; Fenster et al., 2004) resulting in common homoplastic patterns.

Recently, the search for morphological homologies that explain and support evolutionary relationships in systematically complex groups has intensified; a process of 'reciprocal illumination' (Hennig, 1966). Plant systematists are now focusing their attention on the development of these reproductive structures, where, in theory, convergences would be clarified by careful analysis of developmental patterns, allowing complex but fundamental characters to finally explain the phylogenetic hypothesis. Such studies, however, are often descriptive (Benhard, 1999; Buzgo and Endress, 2000; Koeyan and Endress, 2001) or focus on the discovery of single or few synapomorphies (e.g. Endress, 1986; Schonenberger and Endress, 1998; Benhard and Endress, 1999; Tucker, 2003; Prenner, 2004; Prenner and Rudall, 2007; Vasconcelos et al., 2015). Often ignored are cases where a homoplastic phenotype is found to be the result of similar developmental pathways; i.e. homoplasy at the developmental/structural level (e.g. Bess, 2005), suggesting parallel evolution instead of a convergence (*sensu* Patterson, 1982; reviewed by Hawkins, 2002).

6.2 Parallel evolution and deep homology

Parallel evolution (also referred to as “underlying synapomorphy” by Saether, 1983; or “latent homology” by Cronk, 2002) is the repetition of structural or developmental patterns as a result of the similar genetic basis of closely but not directly related lineages (e.g. nodules in legumes, Doyle, 1994, 1998; zygomorphy in angiosperms, Donoghue et al., 1998; Endress, 1999). This is equivalent to the concept of ‘deep homology’ and relates to the presence of a genotypic basis that is not always phenotypically expressed in one lineage (see also Endress, 2010). Parallel evolution, or parallelism, has often been discussed in terms of evolutionary patterns that repeat themselves in striking ways in unrelated taxa (e.g. evolution of C4 grasses, Giussani et al., 2011) and has been highlighted in gene-expression studies (e.g. Rodman et al., 1998). Parallelism is, however, rarely taken into consideration in systematic studies and taxonomic decisions. Following radical re-evaluation of relationships between major angiosperm groups in recent years, it is surprising that the implications of parallelism have been almost absent from the systematic debate. The theoretical importance of such evolutionary patterns in plants systematics is discussed by Hawkins (2002) and Scotland (2011), but there is still a lack of comprehensive case studies in flowering plants.

In the new era of systematics, morphology is experiencing a revival (Lee and Palci, 2015; Giribet, 2015), especially at the interface of morphology, development and evolution coined ‘MorphoEvoDevo’ by Wanninger (2015). To stimulate the discussion of the importance of parallel evolution in the context of systematics, we use a taxonomically complex and species rich tree clade traditionally distinguished by flower characters shown to be polyphyletic by molecular data. We characterize floral development in *Myrcia s.l.* and discuss observed variation in the context of the group’s evolution and systematics. The importance of considering parallel evolution as well as convergence when analyzing evolutionary and taxonomic problems in flowering plants is discussed here using floral development in *Myrcia s.l.* as a case study.

MATERIALS AND METHODS

6.3 Study group

An example of the conflict between molecular phylogeny and traditional classification can be found in *Myrcia sensu lato* (Lucas et al., 2011), one of the most species-rich Neotropical angiosperm genera (Wilson, 2011). *Myrcia s.l.* includes three genera: *Calyptranthes* Sw., *Marlierea* Cambess and *Myrcia* DC. (*sensu* WCSP, 2016; for detailed nomenclatural chronology see Lucas et al., 2011). These three genera are distinguished by morphological characteristics of the flower, particularly the degree of fusion in the calyx and its behaviour during anthesis (Fig. 6.1a; Berg, 1856-57; Mc Vaugh, 1968). Both *Calyptranthes* and *Marlierea* are recognized by having an almost or completely closed calyx in the bud, i.e. with indistinct calyx lobes or barely distinct in some *Marlierea.* The calyx opens as a cap-like structure (calyptra) during anthesis in *Calyptranthes* or by tearing regularly or irregularly in *Marlierea*. *Myrcia* on the other hand, is characterized by an open calyx in the bud, i.e. with distinct (usually variable between four or five), free sepals. Although McVaugh (1968) recognized that these generic boundaries were tenuous, this classification based on calyx characters was used until very recently (see also discussion in Staggemeier et al., 2015).

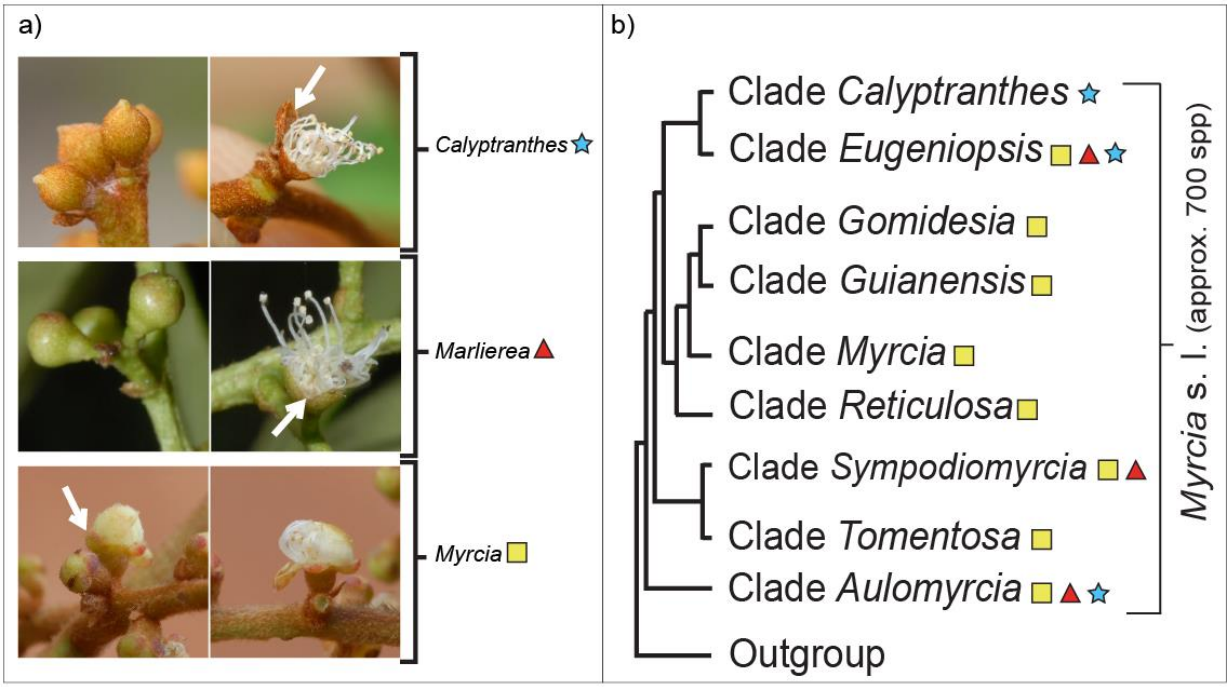


Figure 6.1: Simplified traditional classifications of *Myrcia s.l*. based on floral characters (a). Classification of *Myrcia s.l*. after molecular hypothesis (b). Phylogenetic position of species previously placed *Calyptranthes* (star), *Marlierea* (triangle) and *Myrcia* (square). Arrows indicate the calyptra in *Calyptranthes brasiliensis*, the line of rupture of *Marlierea excoriata* and the free calyx lobes of *Myrcia vittoriana*.

Previous molecular phylogenies of *Myrcia s.l.* (Lucas et al., 2011; Wilson et al., 2016) demonstrate that the nature of the calyx is not representative of natural lineages and found all of the previously recognized genera to be polyphyletic (Fig. 6.1b). Species originally described as *Calyptranthes* are found in clades *Calyptranthes, Eugeniopsis* and *Aulomyrcia*; species originally described as *Marlierea* are found in clades *Aulomyrcia, Sympodiomyrcia* and *Eugeniopsis*; species originally described as *Myrcia* are found in all clades except *Calyptranthes*. The phylogeny of *Myrcia s.l.* has been regularly revisited (Staggemeier et al., 2015; Wilson et al., 2016; Santos et al., 2016) and nine clades with high statistical support (bootstrap and posterior probability) are currently in preparation for formal publication as sections (Lucas et al., in revision; Santos et al., 2016). As most of these nine sections are still not yet formally published, results and discussion of analyses presented here (and in the following Chapter 7) refer to the informal names of the clades shown in Figure 6.1b. Species inclusion within informal groups of *Myrcia s.l.* follow the *Myrcia s.l.* scratchpad (*Myrcia s.l.* scratchpad, 2016) database and the suggested taxonomy of Lucas et al. (2011). A tendency of the last ten years has been to transfer published species of *Marlierea* and some *Calyptranthes* to *Myrcia*, and to publish new species that would previously been published as *Marlierea* in *Myrcia* (e.g. *Myrcia rupta* M.L.Kawas. & B.Holst and *Myrcia elevata* M.F.Santos). This is due to anticipated nomenclatural shifts that will be required as the new *Myrcia s.l.* classification is implemented.

While nomenclatural consensus at the rank of genus is stabilizing, the evolution of the floral characters that misled classical taxonomists for nearly two centuries are still poorly understood.

Meanwhile, floral characters previously considered of less taxonomic relevance such as anther morphology, pubescence and ovary locularity, have proved to be more systematically consistent (Lucas et al., 2011).

6.4 *Myrcia* s.l. flower structure (see also Chapter 3)

Myrcia s.l. flowers are small, radially symmetric, usually ca. 0.5 cm in diameter with calyx and corolla present. The androecium is polystemonous and organized in three whorls, with centripetal development (Werbeling, 1989; De Craene and Smets, 1991) and inner whorls shorter than outer. The ovary is inferior and usually bi- or tri-locular, with two ovules per locule. Calyx, corolla and stamens are inserted on the rim of the hypanthium, which is often extended above the ovary summit (see scheme in Fig. 6.2). While the number of some floral parts is always constant within a species (e.g. number of locules per ovary), other organ numbers are flexible, even within the same individual. These are, for instance, number of sepals and petals (which usually vary between four or five) and stamen number (usually between 50 and 100, likely related to flower size).

6.5 Sampling

Prior to sampling, a general literature survey (Lucas et al., 2011; Wilson et al.; 2016; Santos et al. 2016) was conducted to select species that would represent the highest possible diversity of flower morphology and phylogenetic lineages. A general survey of floral development was carried out to find developmental characters that appear to be fixed in a species. Flowering material representative of this variation was then gathered in Brazil, Jamaica, Costa Rica and the Dominican Republic. Buds were collected in all different stages and, where possible, more than one inflorescence per plant was collected. Inflorescences, buds and flowers were preserved in FAA or 70% alcohol immediately after collection. A few species critical for this study and not recently collected in the field were sampled from herbarium material and were rehydrated in boiling water for 10 minutes, left to cool overnight and then fixed in 70% alcohol. In total, 97 samples representing 64 species within *Myrcia s.l.* were sampled for comparative ontogenetic analyses. A list of all analysed samples is presented in the Appendix 6.1.

6.6 Ontogenetic analysis

Floral buds and flowers were dissected in 70% ethanol, dehydrated through an alcohol series to 100% ethanol, and critical-point dried using an Autosamdri-815B critical-point dryer (Tousimis Research, Rockville, Maryland, USA). Dried material was mounted onto specimen stubs, coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinsted, UK) and examined with a Hitachi cold field emission SEM S-4700-II (Hitachi High Technologies, Tokyo, Japan). Where necessary, different stages of development were viewed from different collections of the same species. Flower developmental stages are described from sepal initiation to anthesis. Images were processed using Adobe Illustrator CC 2015 (version 19.2.0). In total 642 images were taken and analyzed.

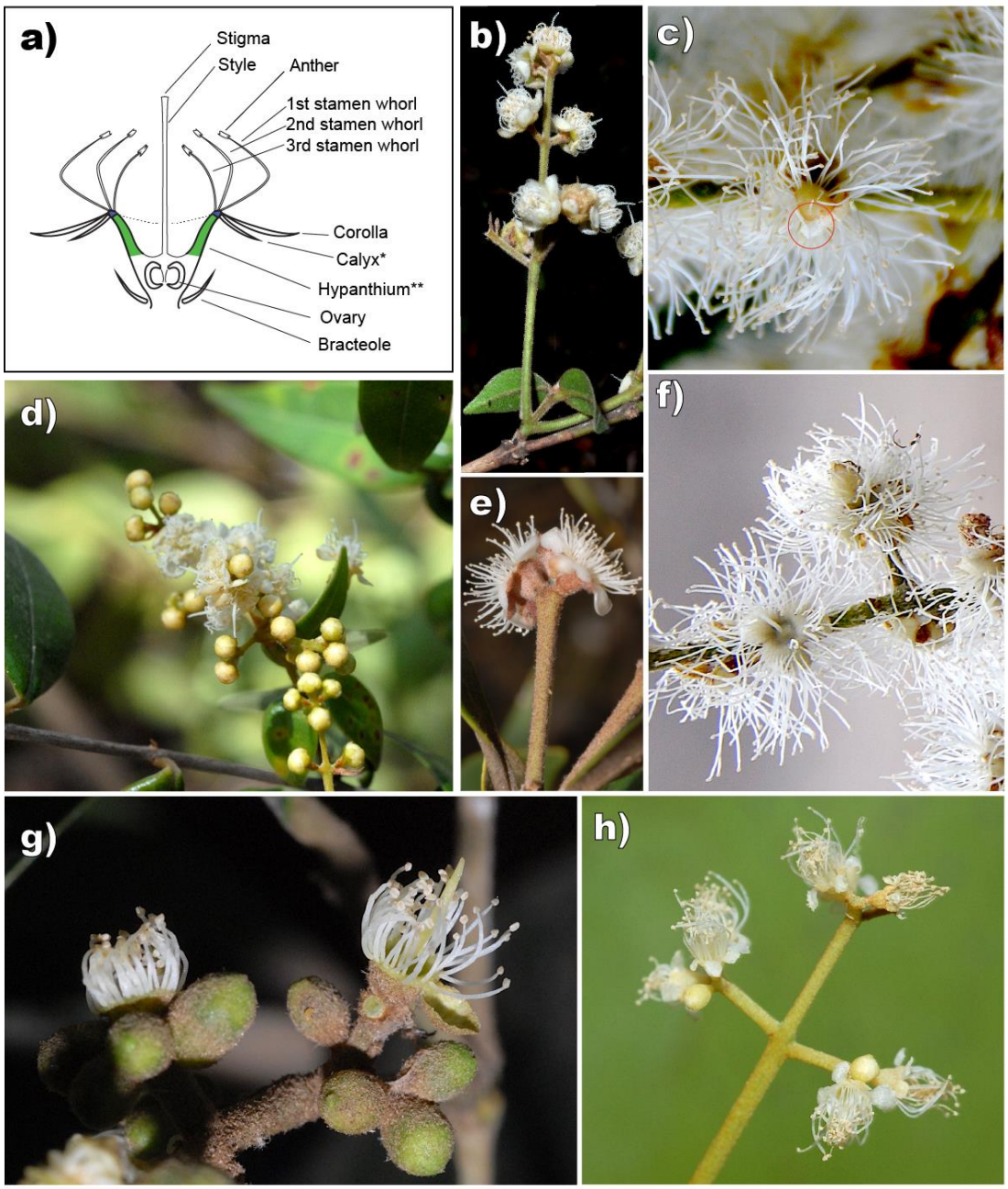


Figure 6.2: Floral diversity in *Myrcia* s.l. a) Simplified scheme showing longitudinal section of floral parts after anthesis. b-h) Pictures showing typical flowers of *Myrcia s.l.* in the field. b) *Myrcia splendens*. c e f) *Marlierea tomentosa.* Red circle highlights petal. e) *Myrcia sylvatica*. f) *Myrcia subavenia*. g) *Calyptranthes pallens*. h) *Myrcia multipunctata*. * Not applicable to *Calyptranthes.* **Hypanthium extension represented in green.

6.7 Phylogenetic reconstruction

The *Myrcia s.l.* phylogeny was reconstructed using DNA sequences of one nuclear (*ITS*) and four chloroplast (*psbA-trnH*, *trnL-trnF*, *trnQ-rps16*, *ndhF*) regions available from recent molecular studies (Staggemeier et al*.,* 2015; Wilson et al., 2016; Santos et al*.,* 2016). Published regions were sourced from GenBank; unpublished sequences from recently published works (Wilson et al., 2016, and Santos et al*.,* 2016) were contributed by the respective authors. Sixty-five species were included, representing all nine clades of *Myrcia s.l.* and four Myrteae taxa were used

as outgroups (list in Appendix 6.2). Evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-12337.8258) was used for analysis (Appendix 6.3). Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Phylogenetic analyses were conducted in MEGA 6 (Tamura et al., 2013). The resulting phylogeny represents c. 10% of the c. 700 species of *Myrcia s.l.*

6.8 Phylogenetic signal analysis

Varying morphological traits associated with different stages of floral development were selected for phylogenetic signal analysis to understand how trait modifications correlate to the phylogeny. Since all characters were analysed in simple binary perspective (presence or absence of a given variation), they were always coded as discrete, even if apparently continuous (e.g. number of locules per ovary). See Appendix 6.4 for matrix used in phylogenetic signal estimation.

Phylogenetic signal of variation in developmental characters was analysed using the *fitDiscrete* function in package *geiger* (Harmon et al., 2008) implemented in R (R Development Core Team, 2016). This function uses Markov models of trait evolution (see *Geiger* package documentation) developed by Pagel (1994) that estimate the potential for a trait to change between species based on a phylogeny scaled for substitutions per site (genetic distance) and uncorrected for molecular clock. The parameter model = Equal Rates (ER) was chosen, since no estimation of evolutionary rate was available, and thus no transformation in the tree (e.g. lambda, kappa) was applicable. The result of this function generates a value of *lnl* (an estimate of log-likelihood) that can be used to rank the phylogenetic signal of discrete traits: the lower the log likelihood, the less well the evolution of a character correlates with the phylogenetic hypothesis. To adjust the phylogeny for this analysis, the function *multi2di,* from package *ape* implemented in R (R Development Core Team, 2016) was used to remove polytomies from the tree. The function *drop.tip* from the same package was used to remove outgroups and species with NA values.

6.9 Ancestral reconstruction of characters

Reconstruction of ancestral characters was performed for the three developmental pathways within *Myrcia s.l.* phylogeny to ensure the characters in question share the same ancestral state, supporting the parallel evolution discussion (according to Scotland, 2011, characters obtained by parallel evolution have to share the same ancestral state). Reconstructions were executed using the *ace* function, with the “type=discrete” parameter, from the *ape* package implemented in R (R Development Core Team 2016). The function *drop.tip* from the same package was again used to remove species with no available information (see Appendix 6.4).

RESULTS

6.10 *Myrcia* s.l. floral development survey

Floral ontogeny in *Myrcia s.l.* can be divided into four stages. Stage 1 concerns the very early development of the flower, which comprises initiation and early development of the outmost whorls in the flower i.e. bracteole, sepals and petals. Stage 2 represents the initiation and early development of androecium and initiation of gynoecium. Stage 3 concerns the development of all

floral parts prior to the pre-anthetic stage. Stage 4 represents subsequent growth of the flower when the hypanthium elongates and the bud takes its final shape.

During the general ontogenetic survey, three significantly different floral developmental pathways were observed in different species of *Myrcia s.l.* and these are seen to be the main drivers of bud and flower shape. Differences between these pathways are observable from the first stage of development until anthesis. In all analysed samples, the first organs to develop are the two bracteoles at each side of the floral primordium. Even at this very early stage, distinction between the three developmental pathways are clear; these are most clearly characterised by differential rates of development of the calyx *vs* the hypanthium and gamosepalous (calyx tissue homogenous) *vs* aposepalous (free sepals) calyx development. Below, a description of the three developmental pathways is provided.

6.11 The "aposepalous" developmental pathway

The first pathway (Fig. 6.3) is here coined the "*aposepalous*" pathway. In this pathway, four or (more commonly) five sepals develop spirally, with the first initiating nearly opposite to the second bracteole and the second opposite to the first and so on (Fig. 6.3a). The corolla develops as the second whorl, with the first petal initiating between the first and third sepals (Fig. 6.3b,c) and the next ones following the same spiral sequence, intercalated with the sepals (Fig. 6.3d,e). In the observed material, the number of petals was always found to be the same as sepals; both whorls develop at a continuous rate and remain free throughout floral development. During the second developmental stage, the androecium is the third whorl of organs to develop. The first stamens initiate below the oldest petal (Fig. 6.3f) and tissue continues to differentiate under the remaining petals until the first complete ring of stamens is formed. The gynoecium is the last whorl to initiate; it begins as a depression on the floral apex during initiation of the first staminal whorls (Fig. 6.3f). By expansion of the surrounding tissue an apical pore is formed (Fig. 6.3g - arrow) as the surrounding tissue swells and extends to form the style (Fig. 6.3h). This is then followed by Stage 3, the extension of the floral parts during maturation of the bud (Fig.2 i-k). During this stage, a second and third whorl of stamens differentiates below the first following the same order (Fig. 6.3i). Pre-anthesis (Stage 4), anthers begin to differentiate at the tips of the filaments (Fig. 6.3l) and the bud takes its final shape. The development of the calyx and corolla continues at an even rate throughout all stages in the "*aposepalous*" pathway. During anthesis the sepals and the relatively showy petals are free and open to reveal the reproductive structures of the flower (Fig. 6.3m,n).

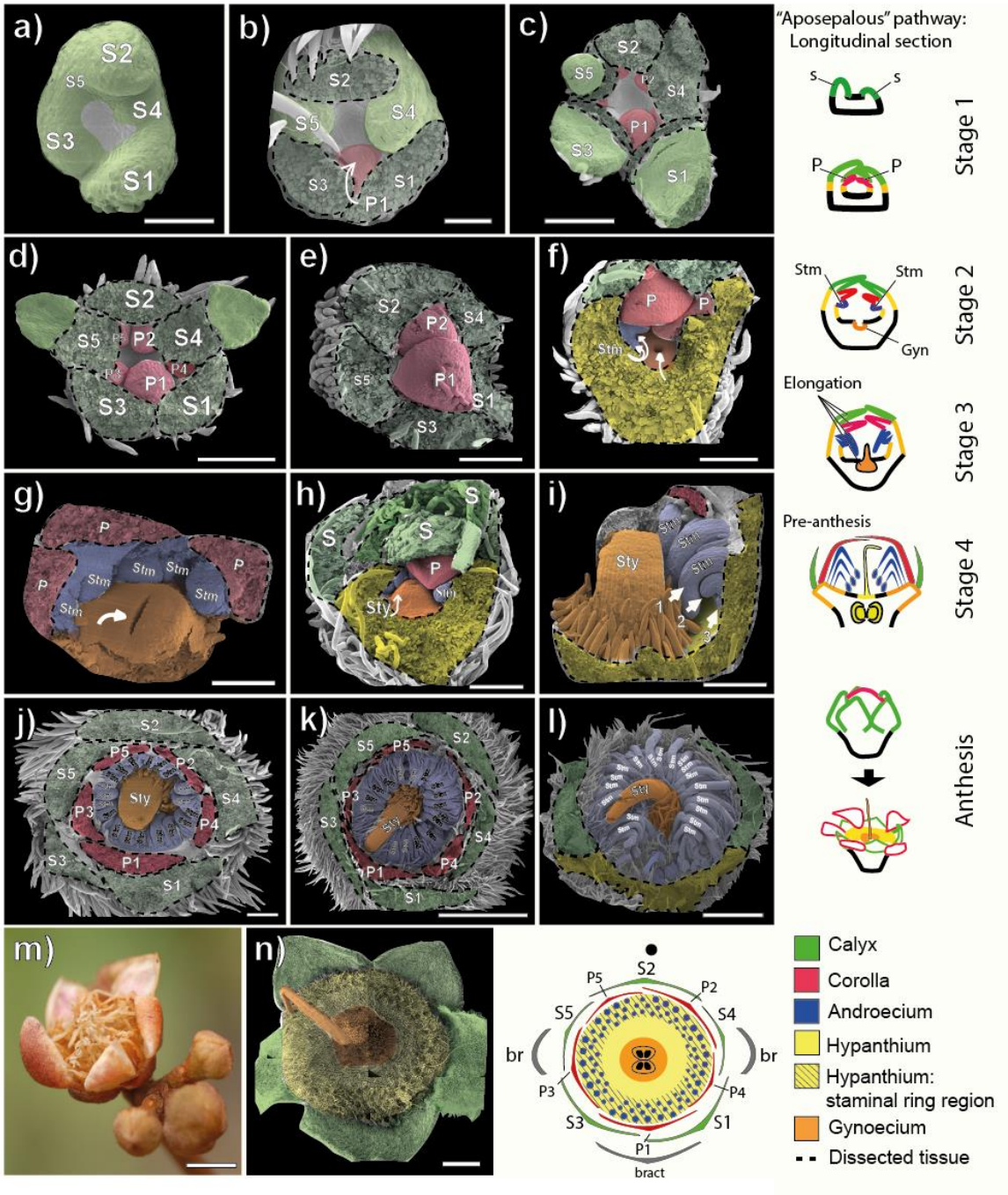
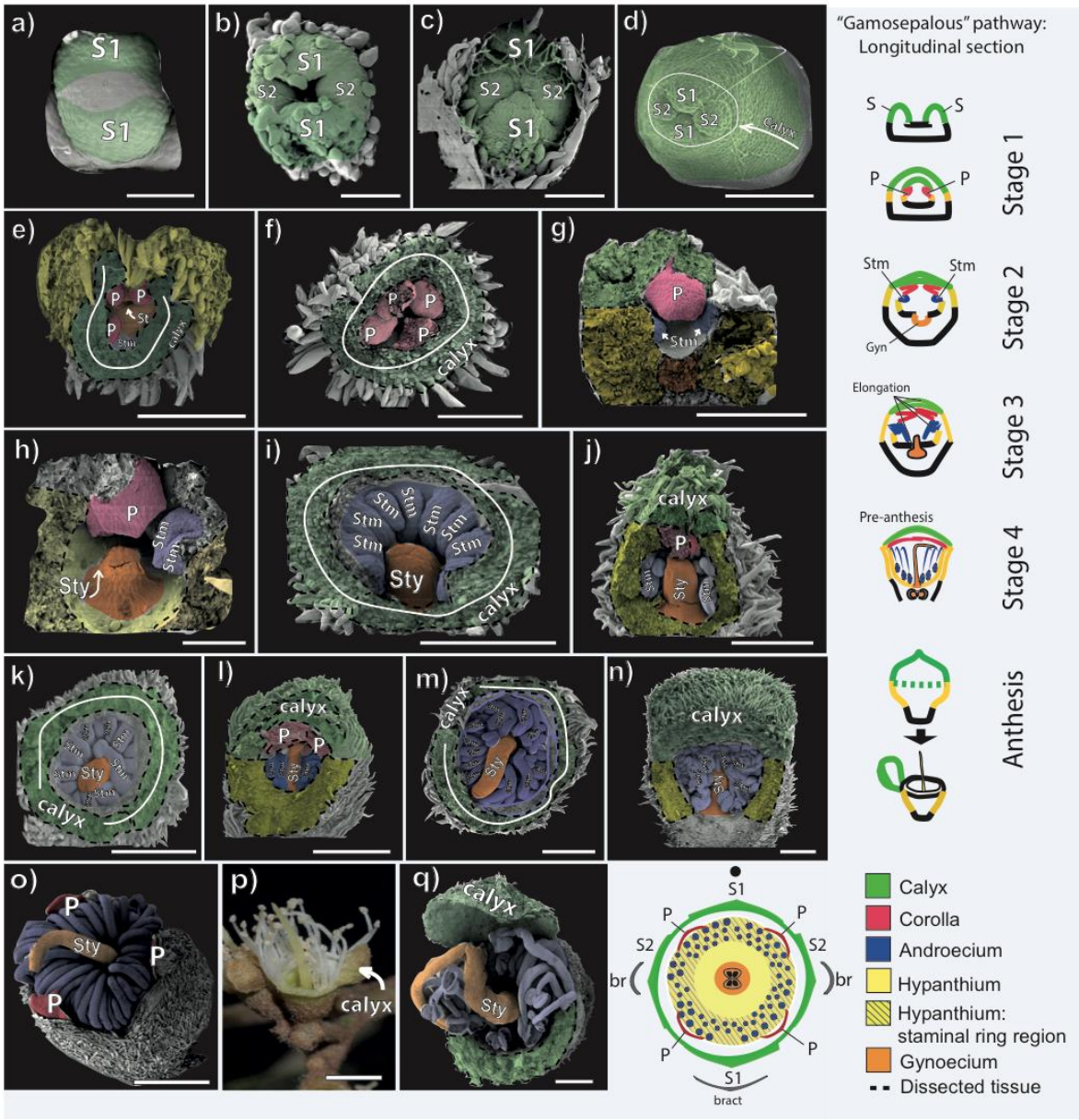


Figure 6.3: The "aposepalous" pathway as exemplified by *Myrcia fenzliana* (all images besides "m" and "n") and *Myrcia* sp. (T.Vasconcelos 439) ("m" and "n") (both clade *Gomidesia*). Removed structures are represented by a dashed line. The right hand side column summarizes stage by stage organ development; floral diagram is shown at the right bottom corner. a) Spiral sepal initiation. b) First petal initiation between first and third sepals. c,d,e) Spiral petal initiation and early petal development; petals alternating with the sepals. f,g) Initiation of first whorl of stamens and stigmatic depression. h) Upward development of style. i) Longitudinal section highlighting the development of first, second and third staminal whorls. j,k) Extension of floral parts prior to anthesis. l) Longitudinal section of pre-anthetic stage. White circles indicate anthers. m) Anthetic flower, showing free sepal lobes and developed petals. n) Flower after anthesis, stamens and petals removed. Br, bracteole; S, sepal; P, petal; Sty, style; Stm, stamen; Scale bars = 50 µm (a, b, g), 100 µm (c, d, e, f), 150µm (h, i, j), 500µm (j, k, l), 1mm (n), 3mm (m).

6.12 The "gamosepalous" developmental pathway

The second developmental pathway is here referred to as the "*gamosepalous*" pathway; the pair of bracteoles initiate on each side of the floral primordium. The first two sepals then also initiate simultaneously (Fig. 6.4a), followed by two further sepals that produce a decussate pattern relative to the bracteoles (Fig. 6.4b,c). All calyx lobes are free up to this point but now develop as a gamosepalous structure, completely fused and homogeneous at the base. The tips of the calyx remain free; the calyx is closed at the top of the bud, leaving a discreet mark (Fig. 6.4d). Petal initiation is simultaneous or nearly simultaneous (Fig. 6.4e). Unlike the sepals, petals in the "*gamosepalous*" pathway are free throughout floral development (Fig. 6.4f). The corolla usually does not develop at the same rate as the calyx, remaining poorly developed until anthesis (Fig. 6.4o). The initiation of the androecium and the gynoecium during Stage 2 and the extension of floral parts to the pre-anthetic stage during Stages 3 and 4 are very similar to the "*aposepalous*" pathway. The first staminal whorl develops under the petals while the stigmatic depression appears, shrinks and extends upwards to form stigma and style (Fig. 6.4g-m). Staminal whorls develop downwards and the anthers differentiate at the tips of the filaments in Stage 4 (Fig. 6.4n). This is followed by the pre-anthetic stage (Fig. 6.4o) and anthesis. In the mature bud, anthesis occurs by tearing of the homogeneous calyx tissue in several ways (see *Parallelism in Myrcia s.l.* in Discussion section). Most commonly the weakest point is at the base of the calyx, leading to a cap like structure that dehisces (Fig. 6.4p,q).

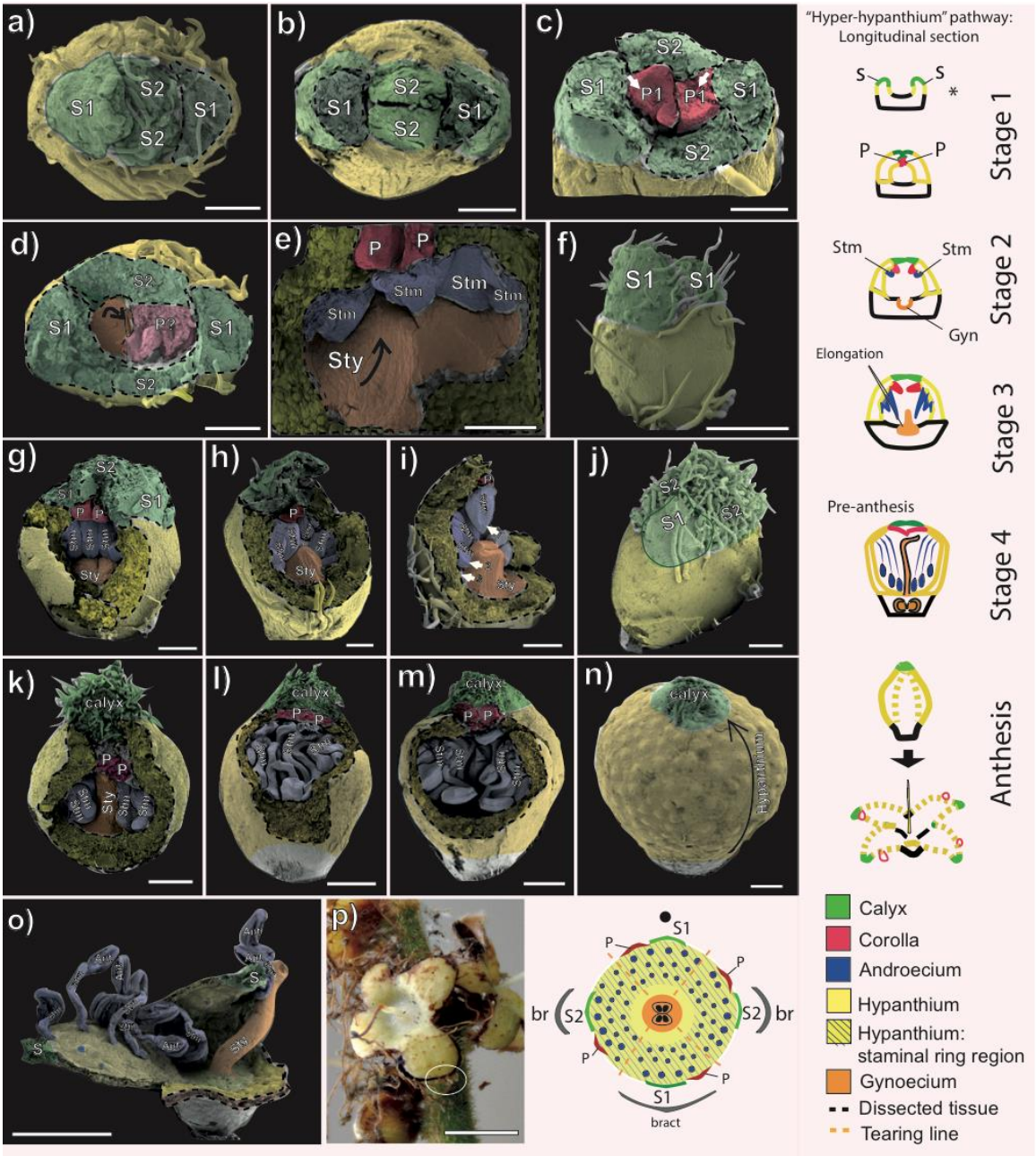
Figure 6.4 (next page): The "gamosepalous" pathway, as exemplified by *Calyptranthes pallens* (Clade *Calyptranthes*, all images besides "a" and "d") and *Calyptranthes multiflora* (Clade *Aulomyrcia*, "a" and "d"). Removed structures are represented by a dashed line. The right hand side column summarizes stage by stage organ development; floral diagrams are shown at the right bottom corner. a) Initiation of the two first sepals in median position. b,c) Initiation and early development of the four decussate sepals. d) Calyx with the free distal sepal lobes and fused base. e) Simultaneous or near simultaneous petal initiation. Depression in the young gynoecium becomes visible. f) Early development of the four petals. g) Longitudinal section showing initiation of first stamens under a petal. h) Early development of stamens and style. i-n) Sequence of floral part elongation prior to anthesis. o) Pre-anthetic phase, calyx removed. p,q) Anthesis highlighting the cap-like structure of the calyx. Br, bracteole; S, sepal; P, petal; Sty, style; Stm, stamen. Scale bars = 50 µm (a, b, c) 100 µm (d, e, f, g, h), 300µm (h, i, j, k, l, n), 1mm (o, q), 3mm (p).



6.13 The "hyper-hypanthium" developmental pathway

The third developmental pathway is here called the "*hyper-hypanthium*" pathway. Similar to the "*gamosepalous*" pathway, four or five sepals initiate either in a decussate or slightly sequential pattern relative to the bracteoles (Fig. 6.5a,b). The same number of petals then initiate in an intercalated position relative to the sepals (Fig. 6.5c,d). Sepals and petals remain free and their rate of growth becomes imperceptible. In contrast, hypanthium growth accelerates, extending massively and giving the appearance that in stage 2 stamen are formed on the inside of the bud apex, growing upside down (Fig. 6.5e). Gynoecium initiation and early development is similar to the other pathways (Fig. 6.5d,e). Most of what appears to be the outside of the bud at this point is actually extended hypanthium with very reduced calyx lobes remaining at the apex (Fig. 6.5f). During Stage 3, the hypanthium continues its extreme extension, "carrying" the staminal whorls upwards (Fig. 6.5g-j). At Stage 4 anthers differentiate at the tips of the filaments (Fig. 6.5k-m). The pre-anthetic bud from the outside resembles the pre-anthetic bud from the "gamosepalous" pathway. However, most of what is seen from the outside represents the long hypanthium extension, with very reduced calyx lobes on the top of the bud (Fig. 6.5n). At anthesis the reduced

calyx lobes of the mature bud move apart; the subsequent opening is too small to reveal the floral display and the pressure inside the bud increases. The hyper-extended hypanthium then tears along fissures below the sepals to expose the stamens (Fig. 6.5o,p). This anthesis behavior produces a display where the showiest parts of the flower are the hypanthia slices that hold the stamens. Calyx and corolla don't seem to play an important role as attractive to pollinators (see red



circle in Fig. 6.2c).

Figure 6.5: The "hyper-hypanthium" pathway, as exemplified by *Marlierea umbraticola* (clade *Aulomyrcia*). Removed structures are represented by a dashed line. The right hand side column summarizes stage by stage organ development; floral diagram is shown at the right bottom corner. * the earliest initiation of sepals was not observed. a,b) Early development of the four decussate sepals. Apical view. c) Simultaneous or nearly simultaneous initiation of petals alternating with sepals. Lateral view. d) Same bud as in 'a'-'c'; sepals and petals were partially or completely removed to show depression on the young gynoecium (highlighted by black arrow). Apical view. e)

Initiation of stamens below petals and on top of style. Longitudinal section. f) External view of bud in Stage 2. g-i) Longitudinal sections showing early extension of floral parts. j) External view of bud in Stage 3. k-m) Longitudinal sections showing floral parts continuous elongation prior to anthesis. n) External view of pre-anthetic bud in Stage 4. o) Anthesis, showing deep tearing lines up to the top layer of the ovary. Note the stamen insertion on the hypanthium and the tiny sepals on the tip of the hypanthium p) Old buds with the position of sepals encircled. Br, bracteole; S, sepal; P, petal; Sty, style; Stm, stamen; St, stigma. Scale bars = 50µm (e), 100µm (a, b, c, d, g, h, i), 200 µm (f, k, l, m, n), 1mm (o), 4mm (p).

6.14 Specific stage character variation

In addition to the three distinct pathways, further variation in floral characters was observed. These variations are independent of the developmental pathway and occur during developmental stages 2 to 4. In all material analysed of clades *Myrcia*, *Gomidesia* and *Reticulosa*, single-celled hairs were observed growing at the base of the staminal ring, usually appearing during early development of the first stamens or the initiation of the second staminal ring in Stage 2 (Fig. 6.6a). The staminal rings are glabrous in all other clades. During the same developmental stage, variation in the shape of the stigmatic depression was observed. This corresponds to the number of locules in the ovary, with a triangular form observed in species with three locules and an “H-shaped” form in species with two locules (Fig. 6.6b). During Stage 3, the base of the style was observed to be glabrous in most samples, however, in all material studied of clades *Myrcia*, *Gomidesia* and *Reticulosa* epithelial cells develop into single-celled hairs during style elongation (Fig. 6.6c).

In species that follow the “*aposepalous*” or “*gamosepalous*” pathways, the final shape of the hypanthium in the bud varies depending on developmental differences during late bud development (Stage 4). In flowers of the *Myrcia*- and *Gomidesia*-clades, vertical extension of the hypanthium is limited, growth then stops and hypanthial tissue expands horizontally, leaving the mature bud and opened flower without a tube or hypanthial cup as in all other clades (Fig. 6.6d). In the *Myrcia*- and *Gomidesia*- clades the inner surface of the short hypanthium wall is covered in hairs, while it is glabrous in all other clades of *Myrcia s.l*. In all sections, anther development is similar until Stage 4, when differential growth of the connective dislocates anther thecae in the *Gomidesia* clade (Fig. 6.6e), forming a structure that resembles a pore during anthesis. In all other clades, anthers open via longitudinal slits, forming an angle of nearly 180 degrees between the thecae (Fig. 6.6e).

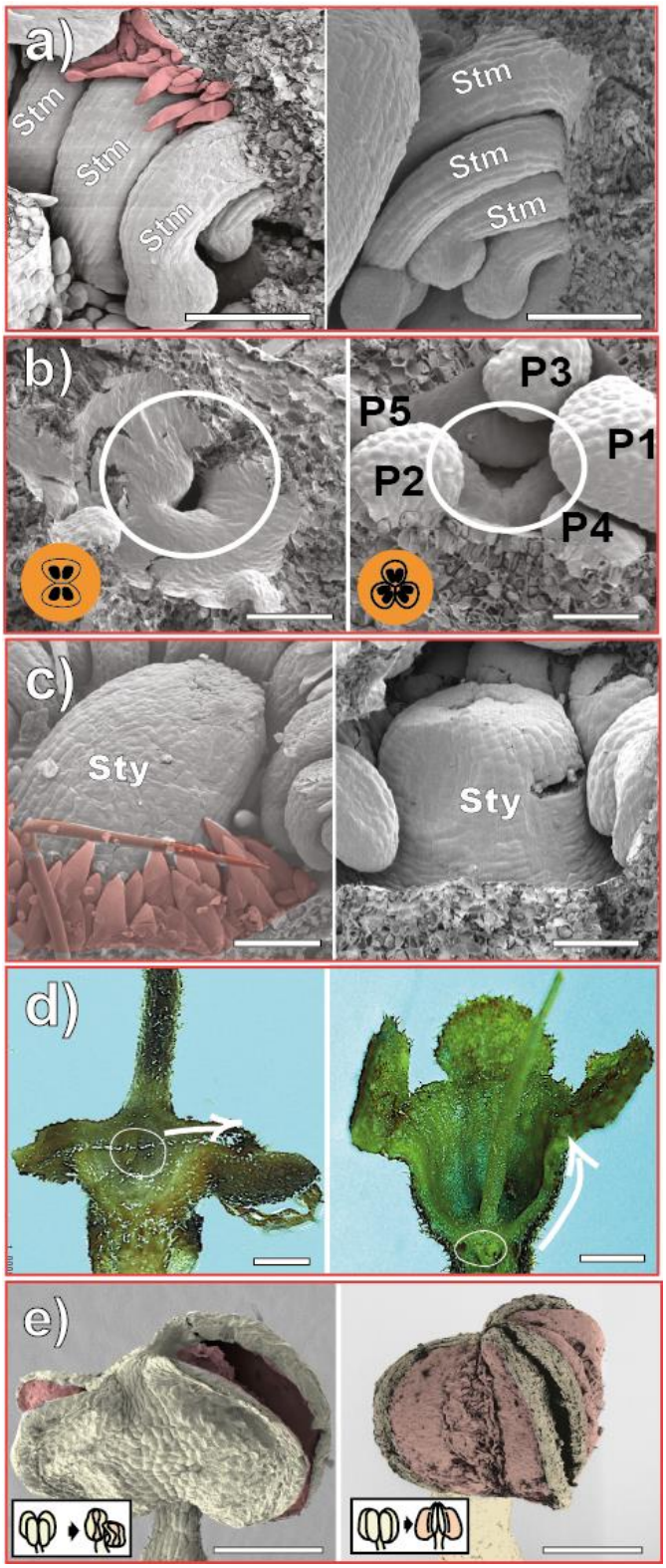


Figure 6.6: Stage specific variation in *Myrcia s.l.* floral organ development. a) Hairy or glabrous base of staminal ring (initiates in Stage 2). b) Definition of bi or tri ovary locularity (Stage 2). c) Hairy or glabrous base of style (initiates in Stage 3). d) Flat or vertically extended hypanthium in longitudinal section (Stage 4). Ovary position is circled. e) Uneven or even growth of anther connective leading to different anther openings (Stage 4) (inside of thecae highlighted in red). Stm, stamen; Sty, style.

6.15 Phylogenetic signal of developmental variation

Results presented here describe three developmental pathways that initiate in the first stage of development and other trait variation that appears in later stages of *Myrcia s.l.* floral development. Key differences were observed during the described developmental stages and can be summarised as follows: Stage 1 – Pathway determination; Stage 2 - Growth of hairs at the base of the staminal ring during androecium development (observed in all analysed samples of clades *Myrcia*, *Gomidesia* and *Reticulosa*), and determination of locule number (trilocular in samples of clades *Guianensis* and *Reticulosa*, bilocular in the other sections); Stage 3 - Growth of hairs at the base of the style (observed in all analysed samples of clades *Myrcia*, *Gomidesia* and *Reticulosa*), Stage 4 - fixation of hypanthium shape (flat and pubescent in all samples of clades *Myrcia* and *Gomidesia*, extended and glabrous in the rest), unequal growth of the anther connective (only in clade *Gomidesia*). Even though other small variations of floral development could be observed (e.g. four to five sepals), these were not considered here due to inconsistency at the intraspecific level. The non-independence of traits such as hairs at the base of stamen and style was considered as they are always found in the same species. They were not merged however, as the hairs appear at distinct development stages.

Correlation of key developmental characters with available phylogenetic hypotheses are shown (Fig. 6.7) estimated by means of log likelihood from the correlation of each character with the phylogeny. Results indicate that during floral development in *Myrcia s.l.*, the earlier the development of a structure varies, the lower the phylogenetic signal of this variation is. Consequently, early developing characters are more homoplastic and less congruent with the phylogeny. The most striking example of this is the developmental pathway determination in Stage 1. These show a clearly homoplastic pattern when correlated with the phylogeny and return the lowest log likelihood values (e.g. "*aposepalous*" pathway: -18.73 "*gamosepalous*" pathway: -16.58) in comparison to variation in later developed characters. The "*hyper-hypanthium"* pathway is exceptional in this case, scoring a slightly higher value of log likelihood (-13.38) as it occurs exclusively within the clade *Aulomyrcia*. The homoplastic pattern of similar developmental pathways increases evidences for parallelism in *Myrcia s.l.*

In contrast, characters that undergo later stage developmental variation return higher values of log likelihood and seem to be more phylogenetically congruent. For example, hairy staminal ring and style base, always observed to occur in the same species, return a moderately high (-13.07) log likelihoods; the same is true for the characters of hypanthium elongation and pubescence (-11.55). Uneven growth of the connective, a variation that occur in the last stages of development, is exclusive found Gomidesia and thus score the highest phylogenetic signal (-5.73). Locule number, although consistent in the lineage where it is found, scores the lowest phylogenetic signal (-13.78) among late developed characters.

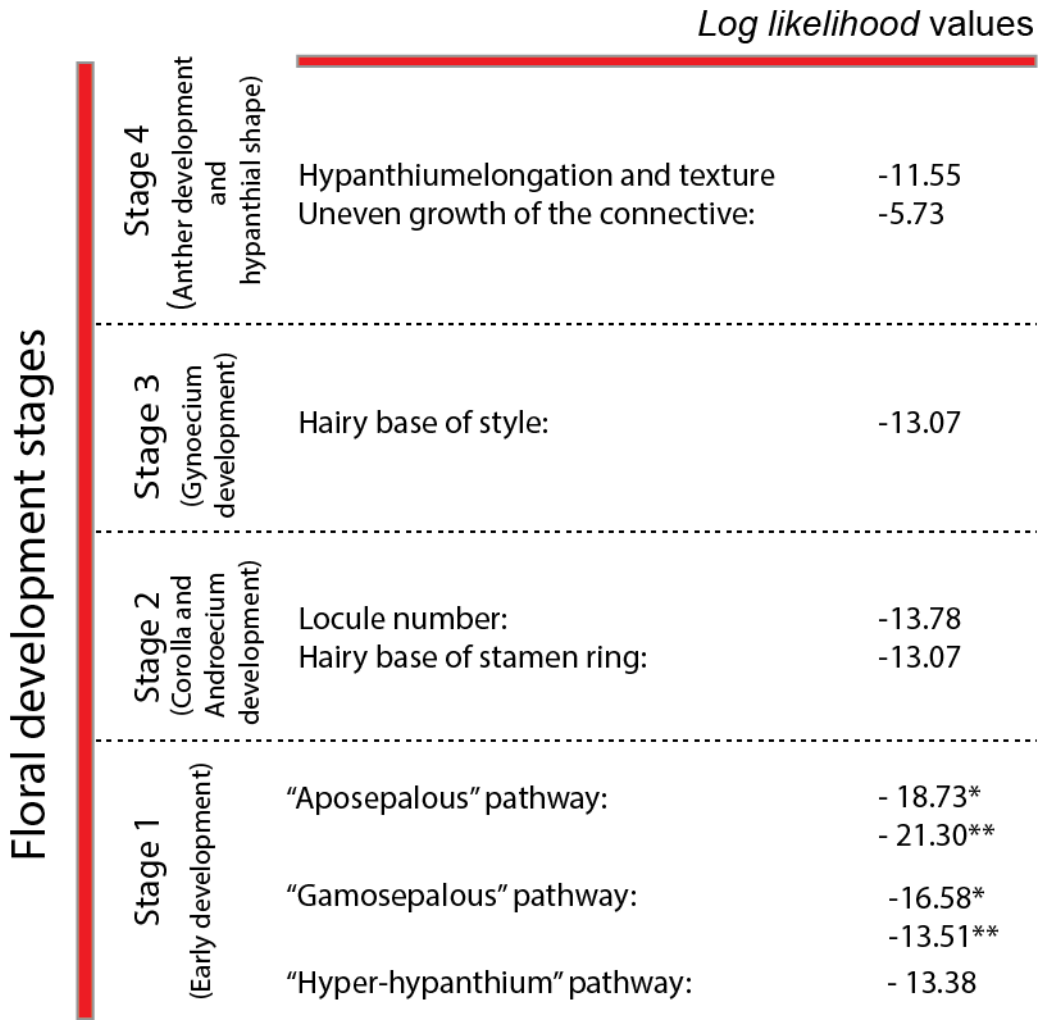


Figure 6.7: Comparison between phylogenetic signal based on *log likelihood* of character variation in distinct developmental stages. * *Marlierea glazioviana* is considered "*Gamosepalous*"; ** *Marlierea glazioviana* is considered "*Aposepalous*".

6.16 Ancestral reconstruction of developmental pathways

Ancestral reconstruction of the three developmental pathways indicate that the "*aposepalous*" pathway is the ancestral state for *Myrcia s.l.* (Fig. 6.8). The "*hyper-hypanthium*" pathway appears to have evolved twice independently inside clade *Aulomyrcia*. The "*gamosepalous*" pathway evolved independently four times in four different clades. Reversal from "*hyper hypanthium*" or "*gamosepalous*" pathway to "*aposepalous*" was not observed. Results show that a given developmental pathway always arises from the same ancestral state regardless of its phylogenetic position. *Marlierea glazioviana* was observed to present both pathways in the same individual. The results show that similar developmental pathways appear independently but can still present the same ancestral state. This is another evidence of parallelism in the evolutionary history of *Myrcia s.l.*

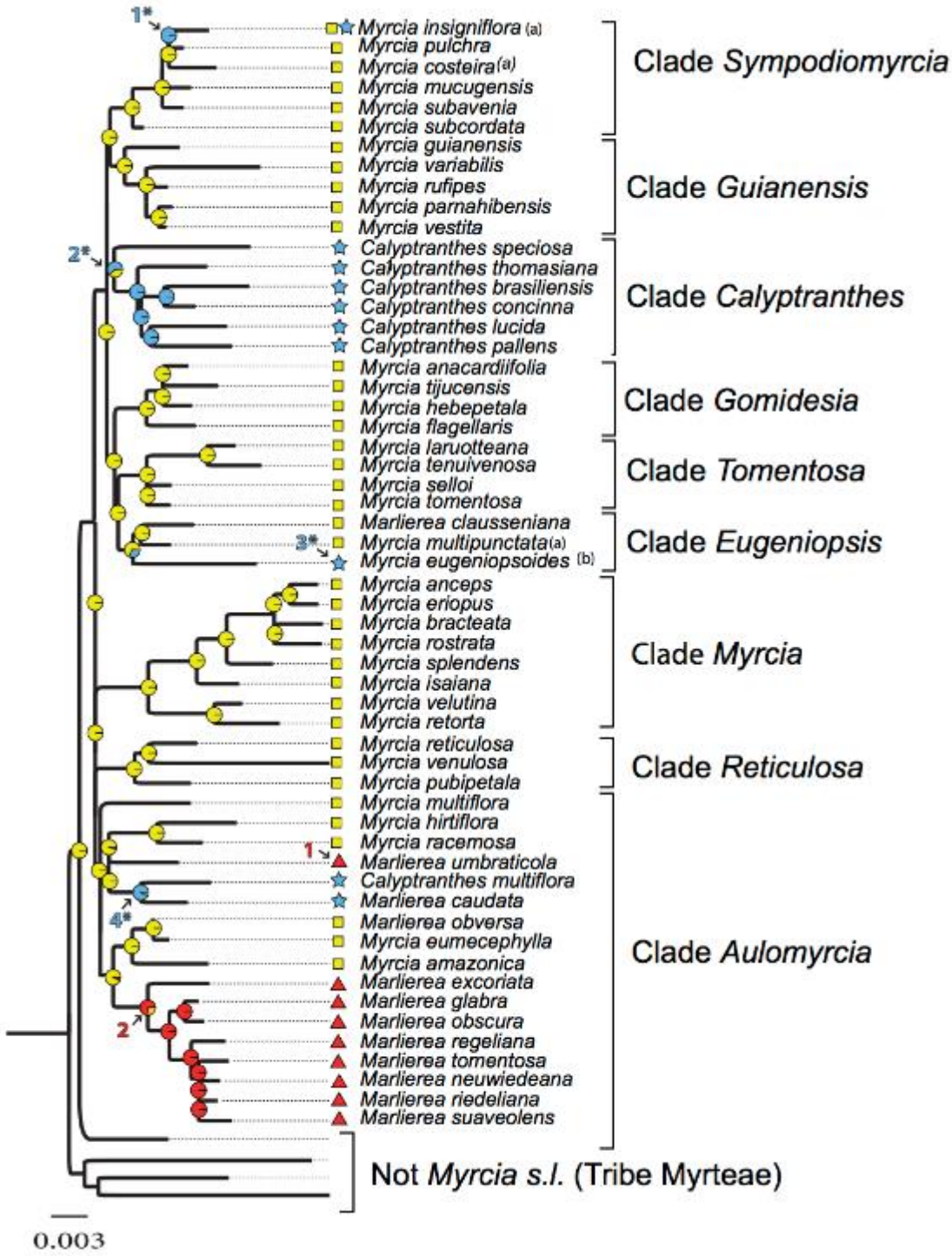


Figure 6.8: Distribution of the three developmental pathways in the *Myrcia s.l.* phylogeny. Yellow squares – *"aposepalous"* pathway; Red triangles – "*hyper-hypanthium"* pathway; Blue stars – *"gamosepalous"* pathway. Shifts from ancestral "*aposepalous"* to *"gamosepalous"* pathways are marked * (1–4); shifts from ancestral *"aposepalous"* to "*hyper-hypanthium"* are labelled (1–2). (a) Names first described in *Marlierea*. (b) Names first described in *Calyptranthes*.

DISCUSSION

6.17 Parallelism in *Myrcia* s.l.

Developmental variations in the calyx and hypanthium, which before were considered systematically consistent, can be categorized into three distinct developmental pathways that are

shown to be examples of parallel evolution (Fig. 6.9a-c). These pathways are polyphyletic and thus score low phylogenetic signal, reinforcing their ineffectiveness for classification of *Myrcia s.l.* The "*aposepalous*" pathway (Fig. 6.9a) has evolved just once (the ancestral state). However it has very low phylogenetic signal because it occurs in most lineages and therefore it is not a useful systematic character either. The "*gamosepalous*" pathway (Fig. 6.9b) is also of low systematic value because it has evolved independently at least four times and it scores a low phylogenetic signal. The "*hyper-hypanthium*" pathway (Fig. 6.9c) has more systematic relevance because it is found in a single lineage and scores the highest phylogenetic signal among the developmental pathways. It has, however, evolved at least twice independently within clade *Aulomyrcia* and still returns a value of phylogenetic signal lower than all later developed traits, with the exception of ovary locularity. Variation in floral characters acquired later in the development present a stronger correlation with the phylogeny and the combination of these characters can be more reliably used to accurately classify species.

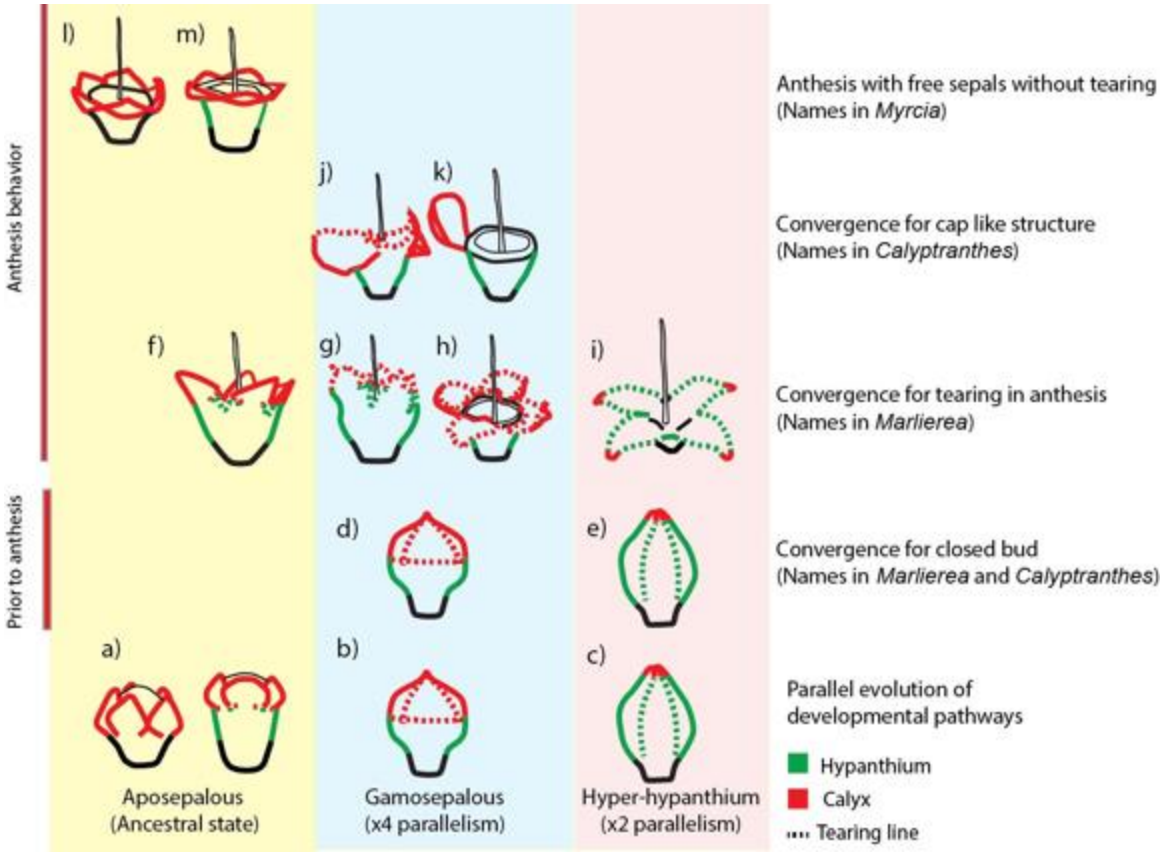


Figure 6.9: Historical taxonomic problems in *Myrcia s.l.* as a result of parallel (a-c) and convergent (d-m) evolution. Right hand column names show genera in which species are most commonly housed.

Systematic problems that arise from parallelisms in *Myrcia s.l.* might be further aggravated by errors of interpretation resulting from the three developmental pathways. These mistakes in interpretation are the root of the observed convergent bud types and behaviours at anthesis. Closed Myrtaceae buds have been associated with protection from dehydration and predation (Weberling, 1989) and prior to anthesis, buds resulting from the "*gamosepalous*" and "*hyper-hypanthium*" pathways can be indistinguishable. This has resulted in arbitrary assignments of species to

Marlierea and *Calyptranthes* (Fig. 6.9d,e). The lack of material of *Myrcia s.l.* in adequate phenological phases (i.e. anthetic flowers), in conjunction with very small flowers from which it is difficult to determine hypanthium length, added to the taxonomic confusion.

The nature of opening of the bud is also a taxonomically problematic, convergent character as all three developmental pathways have potential to tear at anthesis (Fig. 6.9f-k). This has given rise to nomenclatural confusion involving the genera *Marlierea* and *Calyptranthe*s. Despite the developmental pathway, most species with buds tearing at anthesis were described in *Marlierea* (Fig. 6.9f-i). In species of clade *Eugeniopsis*, however, tearing at anthesis can result in a greater portion of calyx tissue remaining on one side of the hypanthium (Fig. 6.9j) producing a structure reminiscent of, or convergent with, a calyptra (Fig. 6.9k). A systematic example is *Myrcia eugeniopsoides* that opens as described and was first described as *Calyptranthes* (1962; as *C. eugeniopsoides* D. Legrand & Kaussel) then transferred to *Marlierea* (1975; as *M. eugeniopsoides* D. Legrand & Kaussel (Legrand)) and finally placed in *Myrcia* (2014; as part of a current trend transferring all names to *Myrcia*; see Mazine et al., 2014). Species that follow the "*aposepalous*" pathway without tearing at anthesis (Fig. 6.9l,m) were always described in *Myrcia* regardless in which clade they are found.

This study shows that both parallelism and convergence are responsible for the two-century long problems with an accurate *Myrcia s.l.* taxonomy which has resulted from extreme emphasis on characters of the calyx, hypanthium and the nature of anthesis (see Lucas et al., 2011). In conclusion for the study group, characters related to the hypanthium and the mode of calyx dehiscence cannot be used alone to define a group of species at any systematic level. Characters with stronger phylogenetic signal, are the best for this purpose (e.g. presented here: hairs on the style base or staminal disk, locule number). The best approach to classification in such a group uses molecular data in combination with multiple characters from a broad range of parts of the plant. The floral characters investigated here can only imperfectly indicate broad species relationships and may only be used reliably for classification at the species level, and even then they must be used with care.

6.18 Parallelism in systematics of flowering plants

The persistent lack of consideration of parallelism in systematics is likely due to confusion between convergence and parallelism in the literature (Gould, 2002; Diogo, 2005). Saether (1983) described "shared common internal constraint of homologous genes or developmental pathways" as underlying synapomophy; this is here considered parallelism. Today homoplasy and homology are used as antagonistic terms. However homoplasy was first defined as a sub-category of homology (Lankester, 1870) as it is characteristic of a single lineage; today this is referred to as parallelism.

Parallelism is likely to be more common than previously considered and in some groups of angiosperms it is a significant source of systematic strife as shown in the present study for *Myrcia s.l.* Patterns of homoplasy that can be attributed to parallelism are frequently found in other angiosperms groups (e.g. inflorescences in *Panicum*, Bess et al., 2005; stamens in *Miconia*, Goldenberg et al., 2008). These incidents are not always clearly cited as parallelism and are often considered to "result from a shared developmental program (...) that is flexibly turned on and off

during evolution" (Hearn, 2006, p. 355, for growth form in *Adenia*). In other words, even if expression of these different developmental pathways remains latent for long periods of evolutionary history, they can occasionally be re-expressed and fixed following a specific selective pressure or genetic drift.

Nevertheless, such latent developmental pathways may be silenced through genetic mutation over time that would represent the end of potential for parallelism (Wagner, 1998; Hawkins, 2002). This may explain why systematic problems related to phenotypic polyphyly appear more prevalent in recently diversified groups where latent molecular signals for expression of similar structures can still be triggered, with abundant examples throughout flowering plants (e.g. *Adenia* – 26mya, Hearn, 2006; *Disa* – c. 18mya, Bytebier et al., 2007, 2010; *Miconia* – c. 10mya, Goldenberg et al., 2008; Berger et al., 2015; *Panicum* c. 15mya, Bouchenak-Khelladi et al., 2010; Giussani et al., 2011; *Mimosa* – c. 15mya, Simon et al., 2009, 2011) including the case study presented here (*Myrcia s.l.,* 22mya; Thornhill et al., 2015); whereas older groups are more likely to have matching morphology and phylogeny (e.g. *Piper* and *Peperomia* – Late cretaceous, Jaramillo and Manos, 2001; Quijano-Abril et al., 2006; Smith et al., 2008; Jaramillo et al., 2008).

Time-scale is therefore important when dealing with parallelisms in a systematic context. Estimates of a time limit for the re-expression of silenced genetic mechanisms that could lead to parallelism are c. 6 million years (Marshall et al., 1994, using empirical data of genes with different degrees of mutational rate). This can however, be much longer for traits that affect distantly related clades of a large group such as flowering plants. In the example of *Myrcia s.l.* the period between the occurrence of a calyptra in *Calyptranthes multiflora* (clade *Aulomyrcia*) and species within clade *Calyptranthes* is estimated at approximately 20 million years (Santos, 2014). When considering a single character for the whole Myrtaceae family it is possible to observe that a calyptra reoccurs in more distant lineages along similar developmental pathways (i.e. *Calyptranthes* and *Eucalyptus*, Weberling 1989; see Chapter 4) even though their last common ancestor was c. 65 mya (Thornhill et al., 2015).

6.19 The impact of parallelism on flower evolution

This study also provides insight into the evolution of floral development. Although recent floral evolution studies have mainly focused on classic evo-devo approaches (such as the ABC model, Erbar, 2005), macro-evolutionary and systematic aspects of flower evolution have also become more common. In such studies, stable early floral development within a lineage and homoplastic late floral development are considered the norm. Tucker (1992, 1997, 2003) found this arrangement in Fabaceae, another mega-diverse group of eudicot angiosperms. Re-expression of early developing characters in independent lineages of *Myrcia s.l.,* perhaps as a result of parallel evolution, makes floral development proceed in the exact reverse. In this case, characters that differentiate in later stages have higher phylogenetic signal than in earlier stages (Fig. 6.7). In contrast to the studies of Tucker, this pattern was also recently found in the Fabaceae tribe Cassinae (Marazzi and Endress, 2008); it is possible that these contrasting findings may be linked to extremely variable Fabaceae floral morphology. Such early stage changes might be important components of late flower display and have consequences for pollination. In *Myrcia s.l.* for example, different floral development pathways might bring discreet changes in post anthetic display, where

the calyptra or undeveloped petals of some pathways might play a role in flower presentation to pollinators (see Chapter 4).

These labile structural changes in early floral development that lead to morphological variation in the mature flower and that are responsible for flawed systematic interpretations may also play an important role in angiosperm evolution. The labile nature of these changes adds weight to suggestion that major changes in floral morphology evolve fast (Vasconcelos and Proença, 2015) rather than gradually, resulting in evolutionary jumps (Eldredge and Gould, 1972) and thereby increasing short-term adaptability and fitness of a lineage. Such flexibility is likely to have contributed to angiosperm success.

CONCLUSION

This study shows in-depth ontogenetic and anatomical research of apparently similar structures to be important in the detection of parallelism. Parallel evolution, as well as convergence, misleads taxonomists and evolutionists when searching for characters to define natural lineages (= morphological synapomorphies). Different developmental pathways can be labile and repeat themselves in non-related lineages of recently diversified groups, probably due to underlying homology in the genetic expression of these characters. It is therefore clear that morphological synapomorphy is particularly difficult to define in the presence of parallel evolution. The question then is: how to interpret homology when structural variation remains latent for long periods of evolutionary history, appearing just occasionally. This question is challenging, especially in the phylogenomic era where systematists have the benefit of robust hypotheses of species relationships that are often incongruent with morphology. Modern plant systematists must be comfortable to define and classify complex groups using combinations of characters rather than searching for or relying on a single homology. Furthermore, a better understanding of the development may clarify homoplasies as potential parallelisms instead of only convergences, additionally sharpening the focus on trait evolution in plants. Future studies are required that will investigate how genetic mechanisms are silenced and then re-expressed over time and the role of hybridization and introgression, known to be integral drivers of plant diversification (e.g. Gargiullio et al., 2015), in the context of maintaining such parallelisms.

APPENDIX

Appendix 6.1: List of analysed samples in the ontogenetic survey per clade of *Myrcia s.l.* Acronym of the herbarium in which the collection is deposited is shown between bracts after voucher details.

Clade	Species	Collection Locality	Voucher (Herbarium code)
Outgroup	*Myrtus communis* L.	RBG Kew (cultivated)	*Lucas 211 (K)*
(Tribe Myrteae)	*Eugenia uniflora* L.	RBG Kew (cultivated)	*Chase 9077 (K)*
	Luma apiculata (DC.) Burret	RBG Kew (cultivated)	*Chase 17313 (K)*
Clade *Aulomyrcia*	*Calyptranthes multiflora* Poepp. Ex O.Berg.	RR - Brazil	*Vasconcelos 379 (K)*
(Clade 9 sensu Lucas et al. 2011)	*Calyptranthes multiflora* Poepp. Ex O.Berg.	RR - Brazil	*Giaretta 1429 (SPF)*
	Marlierea excoriata Mart.	MG - Brazil	*Faria 4270 (UB)*
	Marlierea excoriata Mart.	MG - Brazil	*Vasconcelos 493 (K)*
	Marlierea glabra Cambess.	ES - Brazil	*Faria. 4246 (UB)*
	Marlierea neuwiedeana (O.Berg) Nied.	ES - Brazil	*Vasconcelos 467 (K)*
	Marlierea obscura O.Berg	MG - Brazil	*Matsumoto 836 (UEC)*
	Marlierea obversa D.Legrand	ES - Brazil	*Matsumoto 820 (UEC)*
	Marlierea obversa D.Legrand	BA – Brazil	*Mori 14129 (K)*
	Marlierea regeliana O.Berg	ES - Brazil	*Matsumoto 814 (UEC)*
	Marlierea suaveolens Cambess.	SP - Brazil	*Lucas 85 (K)*
	Marlierea tomentosa Cambess.	SP - Brazil	*Matsumoto 798 (UEC)*
	Marlierea umbraticola (Kunth) O.Berg	AM - Brazil	*Vasconcelos 311 (K)*
	Myrcia amazonica DC.	SP - Brazil	*Lucas 59 (K)*
	Myrcia eumecephylla (O.Berg) Nied.	ES - Brazil	*Matsumoto 803 (UEC)*
	Myrcia hirtiflora DC.	BA – Brazil	*Vasconcelos 440 (K)*
	Myrcia multiflora (Lam.) DC.	ES - Brazil	*Faria 4235 (UB)*
	Myrcia racemosa (O.Berg) Kiaersk.	SP - Brazil	*Lucas 63 (K)*
	Myrcia spathulifolia Proença	BA - Brazil	*Faria 4214 (UB)*
	Myrcia spathulifolia Proença	MG - Brazil	*Vasconcelos 497 (K)*
	Myrcia thomasii B.S.Amorim & A.R.Lourenço	BA - Brazil	*Faria 4203 (UB)*
Clade *Calyptranthes*	*Calyptranthes blanchetiana* O.Berg	BA - Brazil	*Lucas 1208 (K)*
(Clade 1 sensu Lucas et al. 2011	*Calyptranthes brasiliensis* Spreng.	ES - Brazil	*Faria 4239 (UB)*
	Calyptranthes brasiliensis Spreng.	ES - Brazil	*Faria 4244 (UB)*
	Calyptranthes brasiliensis Spreng.	BA - Brazil	*Vasconcelos 449 (K)*
	Calyptranthes chytraculia (L.) Sw.	Jamaica	*Campbell 201548 (IJ)*
	Calyptranthes chytraculia (L.) Sw.	Jamaica	*Campbell 201554 (IJ)*
	Calyptranthes chytraculia (L.) Sw.	Jamaica	*Campbell 201559 (IJ)*
	Calyptranthes chytraculia (L.) Sw.	Costa Rica	*Vasconcelos 525 (K)*

	Calyptranthes concinna DC.	SP - Brazil	*Lucas 74 (K)*
	Calyptranthes lucida Mart. ex DC.	DF - Brazil	*Vasconcelos 259 (K)*
	Calyptranthes pallens Griseb.	Costa Rica	*Vasconcelos 534 (K)*
	Calyptranthes pallens Griseb.	Dom. Republic	*Vasconcelos 559 (K)*
	Calyptranthes thomasiana O.Berg	British Virgin Islands	*Polard 1195 (K)*
Clade *Eugeniopsis* (Clade 2 sensu Lucas et al. 2011)	*Marlierea clausseniana* (O.Berg) Kiaersk.	MG - Brazil	*Matsumoto 752*
	Marlierea clausseniana (O.Berg) Kiaersk.	SP - Brazil	*SPF 39728 (K)*
	Myrcia eugeniopsoides (D.Legrand & Kausel) Mazine	SP – Brazil	*Lucas 61 (K)*
	Myrcia eugeniopsoides (D.Legrand & Kausel) Mazine	SP - Brazil	*Lucas 81 (K)*
	Myrcia multipunctata Mazine	ES - Brazil	*Faria 4236 (UB)*
	Marlierea subacuminata Kiaersk.	Brazil	*Lucas 225 (K)*
	Myrcia tenuivenosa Kiaersk.	SP - Brazil	*Lucas 87 (K)*
Clade *Gomidesia* (Clade 3 sensu Lucas et al. 2011)	*Myrcia anacardiifolia* Gardner	RJ - Brazil	*Natruz 999*
	Myrcia eriocalyx DC.	MG - Brazil	*Vasconcelos 500 (K)*
	Myrcia fenzliana O.Berg	BA - Brazil	*Nic-Lughadha H50637 (K)*
	Myrcia flagellaris (D.Legrand) Sobral	SP - Brazil	*Lucas 83 (K)*
	Myrcia hebepetala DC.	SP - Brazil	*Lucas 64 (K)*
	Myrcia spectabilis DC.	BA - Brazil	*Lucas 1210 (K)*
	Myrcia spectabilis DC.	BA - Brazil	*Lucas 1214 (K)*
	Myrcia spectabilis DC.	ES - Brazil	*Vasconcelos 463 (K)*
	Myrcia tijucensis Kiaersk.	SP - Brazil	*Zappi 305 (K)*
	Myrcia vittoriana Kiaersk.	BA - Brazil	*Vasconcelos 439 (K)*
Clade *Guianensis* (Clade 4 sensu Lucas et al. 2011)	*Myrcia vestita* DC.	SP - Brazil	*Lucas 93 (K)*
	Myrcia guianensis (Aubl.) DC.	PE - Brazil	*Amorim 1912 (UFP)*
	Myrcia guianensis (Aubl.) DC.	DF - Brazil	*Vasconcelos 257 (K)*
	Myrcia guianensis (Aubl.) DC.	DF - Brazil	*Vasconcelos 258 (K)*
	Myrcia guianensis (Aubl.) DC.	BA - Brazil	*Vasconcelos 432 (K)*
	Myrcia littoralis DC.	BA - Brazil	*Vasconcelos 455 (K)*
	Myrcia littoralis DC.	BA - Brazil	*Vasconcelos 456 (K)*
	Myrcia nivea Cambess.	GO - Brazil	*Lima 492 (K)*
	Myrcia paracatuensis Kiaersk.	MG - Brazil	*Mello-Silva 1713 (K)*
	Myrcia rufipes DC.	MG - Brazil	*Vasconcelos 480 (K)*
	Myrcia subverticillaris (O.Berg) Nied.	MG - Brazil	*Lucas 251 (K)*
	Myrcia variabilis DC.	MG - Brazil	*Lucas 277 (K)*
Clade *Myrcia* (Clade 5 sensu Lucas et al. 2011)	*Myrcia anceps* (Spreng.) O.Berg	MG - Brazil	*Lucas E. 236 (K)*
	Myrcia retorta Cambess.	PR - Brazil	*Lucas 179 (K)*
	Myrcia bracteata (Rich.) DC.	French Guiana	*Prevost, 4212 (K)*
	Myrcia eriopus DC.	MG - Brazil	*Lucas 258 (K)*
	Myrcia isaiana G.M.Barroso & Peixoto	SP - Brazil	*Lucas 60 (K)*
	Myrcia splendens (Sw.) DC.	DF - Brazil	*Faria 4052 (UB)*

	Myrcia splendens (Sw.) DC.	GO - Brazil	*Rosa 1384 (UB)*
	Myrcia splendens (Sw.) DC.	DF - Brazil	*Vasconcelos 250 (K)*
	Myrcia splendens (Sw.) DC.	BA - Brazil	*Vasconcelos 407 (K)*
	Myrcia splendens (Sw.) DC.	MG - Brazil	*Vasconcelos 487 (K)*
	Myrcia splendens (Sw.) DC.	Dom. Republic	*Vasconcelos 587 (K)*
	Myrcia sylvatica (G.Mey.) DC.	BA – Brazil	*Lucas 1222 (K)*
	Myrcia sylvatica (G.Mey.) DC.	BA – Brazil	*Faria 4180 (UB)*
	Myrcia sylvatica (G.Mey.) DC.	GO – Brazil	*Vasconcelos 298 (K)*
	Myrcia sylvatica (G.Mey.) DC.	AM – Brazil	*Vasconcelos 336 (K)*
	Myrcia thyrsoidea O.Berg	BA – Brazil	*Vasconcelos 460 (K)*
Clade *Reticulosa*	*Myrcia pubipetala* Miq.	RJ – Brazil	*Lucas 477 (K)*
(Clade 6 sensu Lucas et al. 2011)	*Myrcia reticulosa* Miq.	MG – Brazil	*Savassi-Coutinho S.n. (K)*
	Myrcia venulosa DC.	PR – Brazil	*Cruz 195 (K)*
Clade *Sympodiomyrcia*	*Myrcia costeira* M.F. Santos	Brazil	*Lucas 71 (K)*
(Clade 7 sensu Lucas et al. 2011)	*Myrcia pulchra* (O.Berg) Kiaersk.	MG – Brazil	*Lucas 138 (K)*
	Myrcia mucugensis Sobral	BA - Brazil	*Vasconcelos 441 (K)*
	Myrcia subavenia (O.Berg) N.Silveira	MG - Brazil	*Vasconcelos 488 (K)*
	Myrcia subcordata DC.	MG - Brazil	*Faria 4257 (UB)*
		MG - Brazil	*Faria 4257 (UB)*
Clade *Tomentosa*	*Myrcia laruotteana* Cambess.	DF - Brazil	*Faria 4046 (UB)*
(Clade 8 sensu Lucas et al. 2011)	*Myrcia selloi* (Spreng.) N.Silveira	RJ - Brazil	*Lucas 110 (K)*
	Myrcia tomentosa (Aubl.) DC.	GO - Brazil	*Lima 491 (K)*
	Myrcia tomentosa (Aubl.) DC.	GO - Brazil	*Rosa 1379 (UB)*
	Myrcia tomentosa (Aubl.) DC.	DF - Brazil	*Vasconcelos 262 (K)*

Appendix 6.2: List of analysed species and GenBank accession numbers in the phylogenetic reconstruction of *Myrcia s.l.* Acronym of the herbarium in which the collection is deposited is shown between bracts after voucher details.

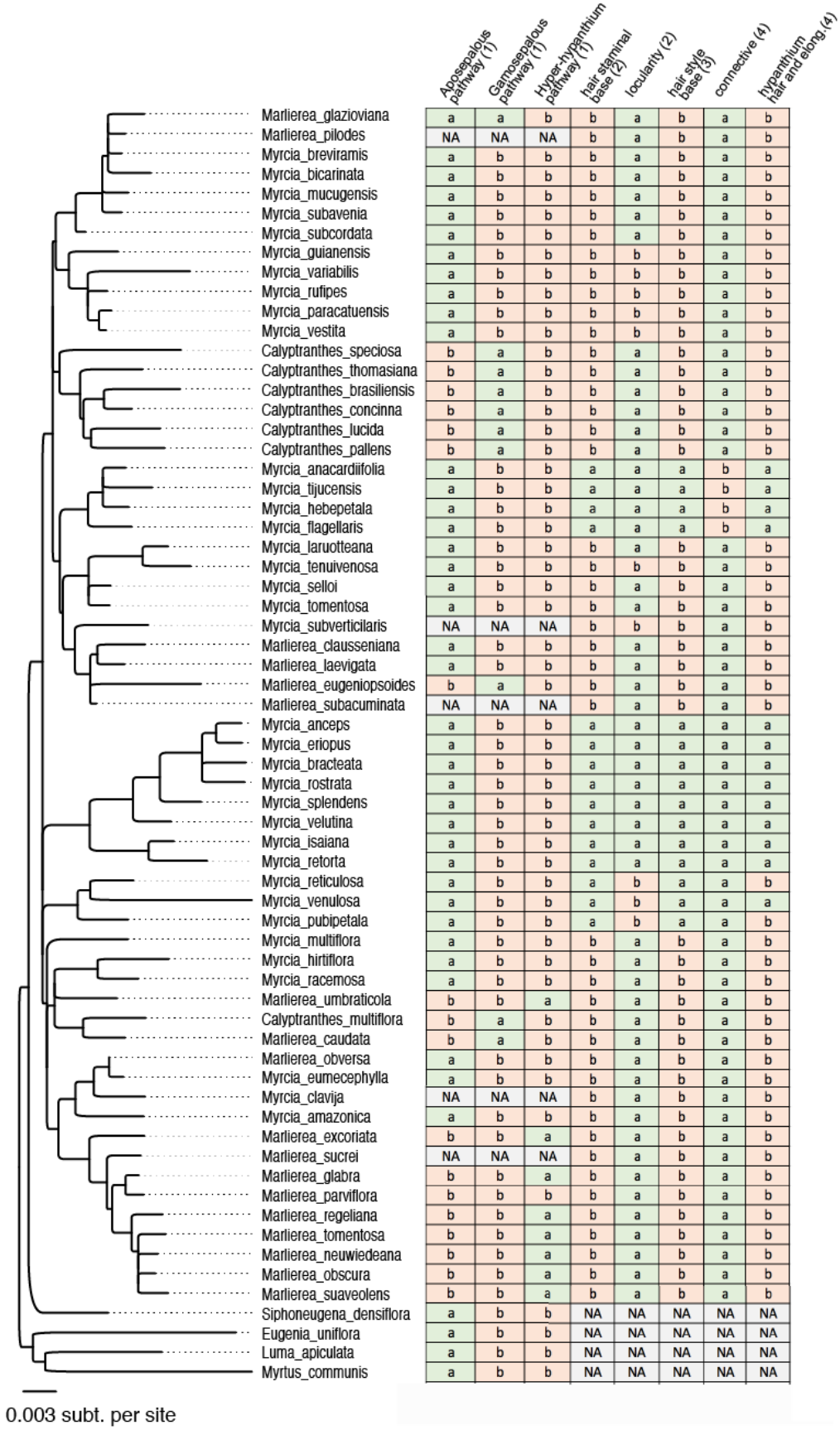
Clade	Species	Collection Locality	Voucher (Herbarium)	*ITS*	*psbA-trnH*	*trnL-trnF*	*rps16-trnQ*	*ndhF*
Outgroup	*Myrtus communis* L.	RBG Kew (cultivated)	*Lucas 211 (K)*	AM234149	AM489872	KP722327	KP722221	KP722420
(Tribe Myrteae)	*Siphoneugena densiflora* O.Berg	MG - Brazil	*Mazine. 1050 (K)*	AM489412	AM489571	JN091389	KP722220	KP722444
	Eugenia uniflora L.	RBG Kew (cultivated)	*Chase 9077 (K)*	AM234088	AM489828	KP722326	KP722202	KP722418
	Luma apiculata (DC.) Burret	RBG Kew (cultivated)	*Chase 17313 (K)*	AM234101	AM489843	KP722331	KP722209	KP722433
Clade *Aulomyrcia* (Clade 9 sensu Lucas et al. 2011)	*Calyptranthes multiflora* Poepp. Ex O.Berg.	RO - Brazil	*Araujo 1885 (K)*	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	-
	Marlierea excoriata Mart.	ES - Brazil	*Matsumoto 825 (UEC)*	JN091203	JN091394	JN091328	KP722226	KP722449
	Marlierea glabra Cambess.	RJ - Brazil	*Staggemeier 935 (K)*	KP722391	KP722299	KP722349	KP722245	KP722469
	Marlierea neuwiedeana (O.Berg) Nied.	SE - Brazil	*Staggemeier 793 (K)*	KP722402	KP722310	KP722360	KP698774	KP722480
	Marlierea obscura O.Berg	MG - Brazil	*Matsumoto 836 (UEC)*	JN091205	JN091396	JN091330	KP722228	KP722452
	Marlierea obversa D.Legrand	ES - Brazil	*Matsumoto 820 (UEC)*	JN091206	JN091397	JN091331	KP722227	KP722450
	Marlierea regeliana O.Berg	ES - Brazil	*Matsumoto 814 (UEC)*	JN091208	JN091399	JN091333	KP722225	KP722448
	Marlierea suaveolens Cambess.	SP - Brazil	*Lucas 85 (K)*	AM234108	AM489846	KP722329	KP722207	KP722431
	Marlierea sucrei G.M.Barroso & Peixoto	ES - Brazil	*Matsumoto 824 (UEC)*	JN091209	JN091400	JN091335	KP722222	KP722445

	Marlierea tomentosa Cambess.	SP - Brazil	*Matsumoto 798 (UEC)*	JN091210	JN091401	JN091336	KP722224	KP722447
	Marlierea umbraticola (Kunth) O.Berg	AM - Brazil	*Souza s.n. (INPA)*	KP722392	KP722300	KP722350	KP722246	KP722470
	Myrcia amazonica DC.	SP - Brazil	*Lucas 59 (K)*	JN091213	JN091404	JN091338	KP722240	KP722422
	Myrcia clavija Sobral	Brazil	*Lucas 244 (K)*	JN091220	JN091411	KP722332	KP722217	KP722442
	Myrcia eumecephylla (O.Berg) Nied.	ES - Brazil	*Matsumoto 803 (UEC)*	JN091223	JN091414	JN091349	KP722223	KP722446
	Myrcia racemosa (O.Berg) Kiaersk.	SP - Brazil	*Lucas 63 (K)*	AM234120	AM489861	JN091366	KP722259	KP722424
Clade *Calyptranthes* (Clade 1 sensu Lucas et al. 2011	*Calyptranthes brasiliensis* Spreng.	ES - Brazil	*Lucas 930 (K)*	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	NA
	Calyptranthes concinna DC.	SP - Brazil	*Lucas 74 (K)*	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	-
	Calyptranthes lucida Mart. ex DC.	MT - Brazil	*Sasaki 2448*	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	-
	Calyptranthes pallens Griseb.	Dom. Republic	*Araujo 1792*	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	-
	Calyptranthes speciosa Sagot	French Guiana	*Holst 9399 (K)*	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	Wilson et al. 2016	-
	Calyptranthes thomasiana O.Berg	British Virgin Islands	*Polard 1195 (K)*	AM234106	AM489820	JN091325	Wilson et al. 2016	-
Clade *Eugeniopsis* (Clade 2 sensu Lucas et al. 2011)	*Marlierea clausseniana* (O.Berg) Kiaersk.	MG - Brazil	*Matsumoto 752*	JN091202	JN091393	JN091326	-	-
	Myrcia eugeniopsoides (D.Legrand & Kausel) Mazine	SP – Brazil	*Lucas 61 (K)*	AM234107	AM489845	JN091327	KP722205	KP722429
	Myrcia multipunctata Mazine	Brazil	*Santos 836*	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016
	Marlierea subacuminata Kiaersk.	Brazil	*Lucas 225 (K)*	JN091207	JN091398	JN091332	KP722218	KP722443
	Myrcia tenuivenosa Kiaersk.	SP - Brazil	*Lucas 87 (K)*	JN091246	JN091437	JN091378	-	-

Clade *Gomidesia* (Clade 3 sensu Lucas et al. 2011)	*Myrcia anacardiifolia* Gardner	RJ - Brazil	*Natruz 999*	JN091216	JN091407	JN091341	KP722210	KP722419
	Myrcia flagellaris (D.Legrand) Sobral	SP - Brazil	*Lucas 83 (K)*	AM234113	AM489836	JN091350	KP722206	KP722430
	Myrcia hebepetala DC.	SP - Brazil	*Lucas 64 (K)*	AM234111	AM489834	JN091353	-	-
	Myrcia spectabilis DC.	SP - Brazil	*Lucas 75 (K)*	JN091241	JN091432	JN091372	-	-
	Myrcia tijucensis Kiaersk.	SP - Brazil	*Zappi 305 (K)*	AM234110	AM489833	JN091379	-	-
Clade *Guianensis* (Clade 4 sensu Lucas et al. 2011)	*Myrcia vestita* DC.	SP - Brazil	*Lucas 93 (K)*	JN091249	JN091440	JN091384	-	-
	Myrcia guianensis (Aubl.) DC.	BA - Brazil	*Harley 50307 (K)*	JN091225	JN091416	JN091351	-	-
	Myrcia paracatuensis Kiaersk.	MG - Brazil	*Mello-Silva 1713 (K)*	AM234118	AM489859	KP722328	KP722230	KP722421
	Myrcia rufipes DC.	MG - Brazil	*Lucas 280 (K)*	JN091239	JN091430	JN091369	-	-
	Myrcia subverticillaris (O.Berg) Nied.	MG - Brazil	*Lucas 251 (K)*	JN091244	JN091435	-	-	-
	Myrcia variabilis DC.	MG - Brazil	*Lucas 277 (K)*	JN091248	JN091439	JN091382	-	-
Clade *Myrcia* (Clade 5 sensu Lucas et al. 2011)	*Myrcia anceps* (Spreng.) O.Berg	MG - Brazil	*Lucas E. 236 (K)*	JN091217	JN091408	JN091342	-	-
	Myrcia retorta Cambess.	PR - Brazil	*Lucas 179 (K)*	JN091237	JN091428	-	-	-
	Myrcia bracteata (Rich.) DC.	French Guiana	*Prevost, 4212 (K)*	JN091218	JN091409	JN091344	-	-
	Myrcia eriopus DC.	MG - Brazil	*Lucas 258 (K)*	JN091222	JN091413	JN091348	-	-
	Myrcia isaiana G.M.Barroso & Peixoto	SP - Brazil	*Lucas 60 (K)*	JN091229	JN091420	JN091356	-	-
	Myrcia splendens (Sw.) DC.	SP - Brazil	*Lucas 73 (K)*	AM234122	AM489863	JN091374	-	-
Clade *Reticulosa* (Clade 6 sensu Lucas et al. 2011)	*Myrcia pubipetala* Miq.	RJ – Brazil	*Lucas 477 (K)*	AM234114	AM489855	JN091364	KP722273	KP722426
	Myrcia reticulosa Miq.	MG – Brazil	*Savassi-Coutinho S.n. (K)*	JN091236	JN091427	JN091367	-	-
	Myrcia venulosa DC.	PR – Brazil	*Cruz 195 (K)*	AM234125	AM489866	JN091383	-	-
Clade *Sympodiomyrcia*	*Myrcia insigniflora* M.F.Santos	Brazil	*Matsumoto 799 (UEC)*	JN091204	JN091395	JN091329	KP722275	KP722451

(Clade 7 sensu Lucas et al. 2011)	*Myrcia mutabilis* (O.Berg) N.Silveira	Brazil	*Mazine 1058 (ESA)*	JN091233	JN091424	JN091361	KP722241	KP722435
	Myrcia costeira M.F. Santos	Brazil	*Lucas 71 (K)*	AM234121	AM489862	JN091343	-	-
	Myrcia pulchra (O.Berg) Kiaersk.	MG – Brazil	*Lucas 138 (K)*	JN091235	JN091426	JN091365	-	-
	Myrcia mucugensis Sobral	Brazil	*Santos 823 (SPF)*	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016
	Myrcia subavenia (O.Berg) N.Silveira	Brazil	*Santos 715 (SPF)*	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016
	Myrcia subcordata DC.	Brazil	*Santos 586 (SPF)*	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016	Santos et al. 2016
Clade *Tomentosa*	*Myrcia laruotteana* Cambess.	SP - Brazil	*Lucas 198 (K)*	AM234115	AM489856	JN091357	KU171297	-
(Clade 8 sensu Lucas et al. 2011)	*Myrcia selloi* (Spreng.) N.Silveira	RJ - Brazil	*Lucas 110 (K)*	JN091240	JN091431	JN091371	KP722212	KP722436
	Myrcia tomentosa (Aubl.) DC.	PR - Brazil	*Lucas 160 (K)*	AM234116	AM489857	JN091380	-	-

Appendix 6.3: *Myrcia s.l.* morphological matrix against phylogeny used in phylogenetic signal estimation and character reconstruction. (a) character state present; (b) character state absent.



	Aposepalous pathway (1)	Gamosepalous pathway (1)	Hyper-hypanthium pathway (1)	hair staminal base (2)	locularity (2)	hair style base (3)	connective (4)	hypanthium hair and elong.(4)
Marlierea_glazioviana	a	a	b	b	a	b	a	b
Marlierea_pilodes	NA	NA	NA	b	a	b	a	b
Myrcia_breviramis	a	b	b	b	a	b	a	b
Myrcia_bicarinata	a	b	b	b	a	b	a	b
Myrcia_mucugensis	a	b	b	b	a	b	a	b
Myrcia_subavenia	a	b	b	b	a	b	a	b
Myrcia_subcordata	a	b	b	b	a	b	a	b
Myrcia_guianensis	a	b	b	b	b	b	a	b
Myrcia_variabilis	a	b	b	b	b	b	a	b
Myrcia_rufipes	a	b	b	b	b	b	a	b
Myrcia_paracatuensis	a	b	b	b	b	b	a	b
Myrcia_vestita	a	b	b	b	b	b	a	b
Calyptranthes_speciosa	b	a	b	b	a	b	a	b
Calyptranthes_thomasiana	b	a	b	b	a	b	a	b
Calyptranthes_brasiliensis	b	a	b	b	a	b	a	b
Calyptranthes_concinna	b	a	b	b	a	b	a	b
Calyptranthes_lucida	b	a	b	b	a	b	a	b
Calyptranthes_pallens	b	a	b	b	a	b	a	b
Myrcia_anacardiifolia	a	b	b	a	a	a	b	a
Myrcia_tijucensis	a	b	b	a	a	a	b	a
Myrcia_hebepetala	a	b	b	a	a	a	b	a
Myrcia_flagellaris	a	b	b	a	a	a	b	a
Myrcia_laruotteana	a	b	b	b	a	b	a	b
Myrcia_tenuivenosa	a	b	b	b	b	b	a	b
Myrcia_selloi	a	b	b	b	a	b	a	b
Myrcia_tomentosa	a	b	b	b	a	b	a	b
Myrcia_subverticilaris	NA	NA	NA	b	b	b	a	b
Marlierea_clausseniana	a	b	b	b	a	b	a	b
Marlierea_laevigata	a	b	b	b	a	b	a	b
Marlierea_eugeniopsoides	b	a	b	b	a	b	a	b
Marlierea_subacuminata	NA	NA	NA	b	a	b	a	b
Myrcia_anceps	a	b	b	a	a	a	a	a
Myrcia_eriopus	a	b	b	a	a	a	a	a
Myrcia_bracteata	a	b	b	a	a	a	a	a
Myrcia_rostrata	a	b	b	a	a	a	a	a
Myrcia_splendens	a	b	b	a	a	a	a	a
Myrcia_velutina	a	b	b	a	a	a	a	a
Myrcia_isaiana	a	b	b	a	a	a	a	a
Myrcia_retorta	a	b	b	a	a	a	a	a
Myrcia_reticulosa	a	b	b	a	b	a	a	b
Myrcia_venulosa	a	b	b	a	b	a	a	a
Myrcia_pubipetala	a	b	b	a	b	a	a	b
Myrcia_multiflora	a	b	b	b	a	b	a	b
Myrcia_hirtiflora	a	b	b	b	a	b	a	b
Myrcia_racemosa	a	b	b	b	a	b	a	b
Marlierea_umbraticola	b	b	a	b	a	b	a	b
Calyptranthes_multiflora	b	a	b	b	a	b	a	b
Marlierea_caudata	b	a	b	b	a	b	a	b
Marlierea_obversa	a	b	b	b	a	b	a	b
Myrcia_eumecephylla	a	b	b	b	a	b	a	b
Myrcia_clavija	NA	NA	NA	b	a	b	a	b
Myrcia_amazonica	a	b	b	b	a	b	a	b
Marlierea_excoriata	b	b	a	b	a	b	a	b
Marlierea_sucrei	NA	NA	NA	b	a	b	a	b
Marlierea_glabra	b	b	a	b	a	b	a	b
Marlierea_parviflora	b	b	b	b	a	b	a	b
Marlierea_regeliana	b	b	a	b	a	b	a	b
Marlierea_tomentosa	b	b	a	b	a	b	a	b
Marlierea_neuwiedeana	b	b	a	b	a	b	a	b
Marlierea_obscura	b	b	a	b	a	b	a	b
Marlierea_suaveolens	b	b	a	b	a	b	a	b
Siphoneugena_densiflora	a	b	b	NA	NA	NA	NA	NA
Eugenia_uniflora	a	b	b	NA	NA	NA	NA	NA
Luma_apiculata	a	b	b	NA	NA	NA	NA	NA
Myrtus_communis	a	b	b	NA	NA	NA	NA	NA

Chapter 7: *Innovation is not always the key: how one of the most diverse and abundant Neotropical tree genera achieved success by staying the same*

Manuscript – to be submitted to *Evolution*

- T.N.C.Vasconcelos contributions: development of hypotheses, design of experiments, collection of samples, morphometric data collection, morphological analyses, phylogenetic analyses, statistical analyses and writing of manuscript.

ABSTRACT

Due to their exceptional species richness, tropical forests provide environments particularly full of ecological opportunity for constant turnover of speciation, extinction and trait diversification. As a consequence, adaptive radiations are observed to arise repeatedly in these environments, usually associated with key phenotypic innovations. Here we test this premise by comparing trait diversification and macro-evolutionary dynamics in *Myrcia* s.l. (Myrtaceae), one of the most species rich and ecologically relevant tree genera in highly diverse rainforests of eastern South America. Correlation between 22 key phenotypic traits, the phylogenetic framework and environmental variables were tested using multi-trait analysis. Relationships between morphological disparity and clade age and correlation between phenotypic variation and shifts in diversification rates were also examined. Results show that macro-evolutionary dynamics and phenotypic diversification in *Myrcia* s.l. are amazingly conservative for a tropical group of its age and species richness. In spite of its exceptional morphological homogeneity, *Myrcia* s.l. species diversity does not result from explosive radiation but rather from gradual species accumulation over a long period of stable net diversification and homogenising phenotypic traits. Even though discreet floral specializations do occur, these present low phylogenetic signal and mostly no correlation with altitude, vegetation, inflorescence characters or plant size and do not significantly affect overall macro-evolutionary dynamics in the genus. Morphological disparity does increase with age but seems to stabilize, with older clades showing less disparity than younger ones, as long term centripetal selection tends to drive similarity over phenotypic extremes. These patterns of conservative net-diversification and phenotype are interpreted as consequences of a very stable adaptive peak related to the characteristic pollination system of *Myrcia* s.l. This highlights that particular eco-evolutionary systems can lead to arrangements that counter the expectations of environments full of opportunities for new ecological interactions such as tropical forests. Such systems produce little variation in macro-evolutionary regimes and low tendency to increase trait diversity, sometimes stable enough to last for tens of millions of years.

Key-words: rainforest, diversification, extinction, floral traits, *Myrcia* s.l., pollination.

7.1 Evolutionary theory behind tropical rainforest species and trait richness

Biologists have long been astonished by the remarkable latitudinal biodiversity gradient that ultimately culminates in highly species rich tropical rainforests (Grubb, 1977; Carlucci et al., 2016). In environments full of ecological opportunity such as these, lineages are constantly under strong selective pressure, responsible for accelerated processes of speciation and extinction that lead to high levels of species turnover over time (Pennington et al., 2015). This cycle of constant availability and filling of ecological niches is one of the fundamental processes expected to drive higher species and trait diversity in tropical biomes (e.g. Fine et al., 2014).

In flowering plants, tropical lineages show greater diversity of floral systems (Willmer, 2011) as in general, competition for pollinators leads to increasingly specialized floral phenotypes (e.g. Junker et al., 2012). Even though at the species level pollinator mediated interactions constrain floral resources and lead to stabilizing selection (Cresswell, 1998), at a macro-evolutionary scale this process leads to constantly diverging phenotypes over time (Ackerly, 2009). Highly diverse clades with homogeneous phenotypes do exist, but are usually associated with recent booms in diversification (e.g. Richardson et al., 2001) where phenotypic diversification by extinction of intermediate forms (Stebbins, 1974) has not yet occurred.

7.2 Considering mega-diversity in the lack of clear phenotypic innovations

Under these assumptions and at a macro-evolutionary scale, homogeneous phenotypes should not persist in tropical lineages for very long periods of time. Nevertheless, evolutionary concepts are never without exception and rarely explored counter-intuitive systems to understand the big picture of species and trait diversity in tropical rainforests (e.g. Vamosi and Vamosi, 2010; Lamanna et al., 2014) must not be overlooked.

In this study, a case of remarkable phenotypic conservation through time in a lineage of tropical rainforests trees is examined. With c. 700 species and an estimate of 30 million years old (Lucas et al., 2011; Santos et al., 2017), *Myrcia* s.l. (Myrtaceae, hereafter referred to simply as *Myrcia*) is one of the largest exclusively Neotropical genera of flowering plants (Fig. 7.1, see also *6.3* and *7.3 Study group*). It also represents the largest diversity of tree species and plays a central ecological role in threatened rainforest and savannah biodiversity hotspots of eastern South America, biomes more species rich than the Brazilian Amazon (Oliveira-Filho and Fontes, 2000; Murray-Smith et al., 2009; Lucas and Bunger, 2015; Staggemeier et al., 2017). *Myrcia* species are unevenly distributed throughout the phylogenetic reconstruction, with the largest clade (clade *Calyptranthes*) accommodating c. 300 species and the smallest one (clade *Tomentosa*) a diversity of only 8 species (see Fig.3.10, Chapter 3); for this reason it is surprising that morphological key-innovations cannot be easily identified. Floral evolution is here correlated with the phylogeny and with environmental variables in a multi-trait approach to infer the processes that shaped the macro-evolutionary regime of one of the most diverse tree lineages in the Neotropics.

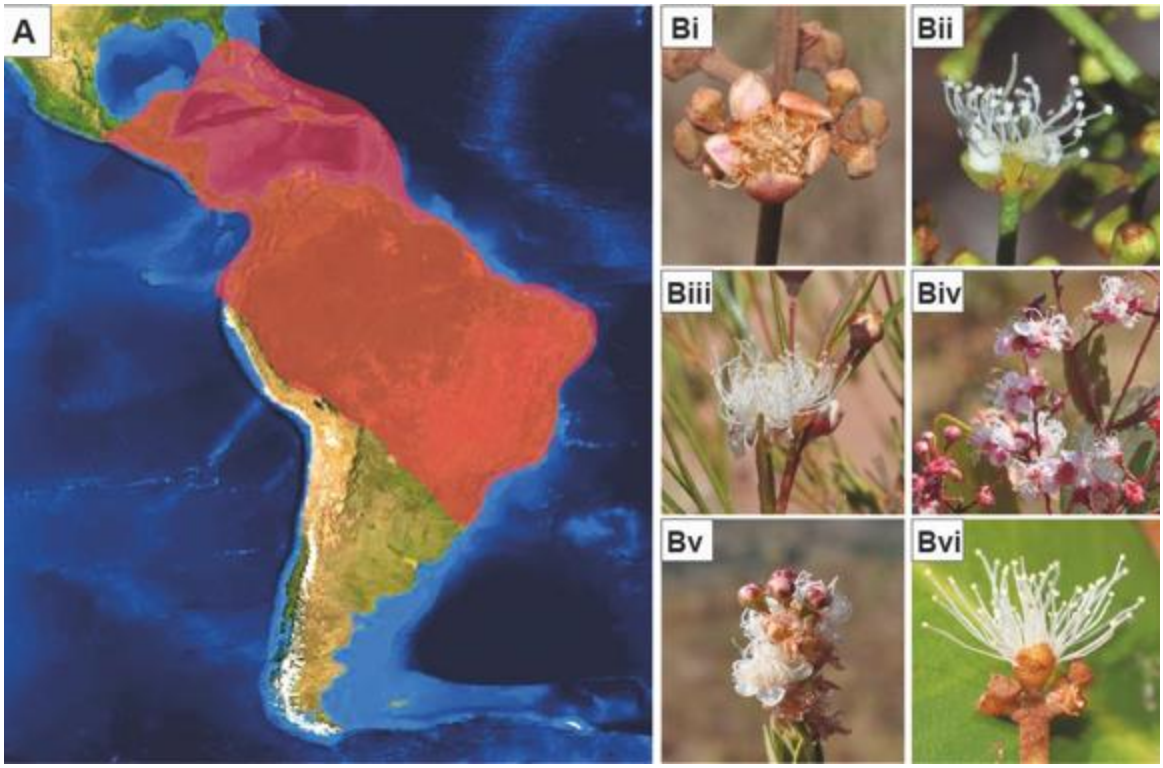


Figure 7.1: *Myrcia s.l.* distribution (A) and flower display in different clades (B). (Bi) *Myrcia* aff. *virgata* (clade *Gomidesia*); (Bii) *M. subcordata* (clade *Sympodiomyrcia*); (Biii) *M. linearifolia* (clade *Myrcia*); (Biv) *M. rubella* (clade *Aulomyrcia*); (Bv) *M. nivea* (clade *Guianensis*); (Bvi) *Calyptranthes brasiliensis* (clade *Calyptranthes*)

MATERIAL AND METHODS

7.3 Study group

A strong pattern of phenotypic homogeneity is not exclusive to *Myrcia* in Neotropical rainforest, but is common in other large sympatric tree genera, such as *Miconia* and *Eugenia* (McVaugh 1968; Renner 1989). However, using *Myrcia* as a model group to understand this pattern is advantageous due to its ecological relevance and the availability of a series of recent systematic revisions that have significantly increased taxonomic stability (e.g. Lucas et al. 2011, 2016; Stagemeier et al., 2015; Wilson et al., 2016; Santos et al., 2016). *Myrcia* is divided into nine infra-generic clades that recur with high statistical support in independent phylogenetic analyses (Lucas et al. 2011; see Chapter 6). From here on these after referred to as the nine infra-generic evolutionary units of *Myrcia* and are used for comparative analysis of morphological disparity, species diversity and age. Relatively reliable estimates of species diversity are available for these nine units (Lucas et al., 2011) and this information is necessary for evaluation of diversification rates in incomplete phylogenetic datasets. These nine evolutionary units are spread throughout the Neotropics, but the peak of species diversity and most likely ancestral diversification points are in the Atlantic Rainforest (Staggemeier et al., 2015; Santos et al. 2017).

7.4 Choosing a representative sample for phenotypic data

The selected sample for phenotypic examination of *Myrcia* flowers corresponds to the c. 130 species included in phylogenetic analysis of Santos et al. (2017). The species sampled in this study were intentionally selected to represent a broad phenotypic variability, geographical distribution and phylogenetic diversity in *Myrcia*, based on previous systematic revisions (Lucas et al., 2011; Staggemeier et al., 2015). As clades *Calyptranthes* and *Myrcia* are slightly underrepresented in the phylogeny, 18 additional species were included. This ensures all clades are represented by a minimum sample of 10% of their biodiversity for morphological disparity analysis (as suggested by Chartier et al., 2017). Additional samples of some widespread species complexes (*Myrcia guianensis*, *M. tomentosa*, *M. splendens*) were also included in the phenotypic analysis. These were not considered pseudo-replicates for the question addressed (phenotypic plasticity in these groups suggest that species delimitations are not clear in these complexes). The final list comprises 162 species, corresponding to 22% of *Myrcia* diversity (Table 7.1).

Table 7.1: Diversity per clade and sample size.

Section (Clade)	Estimated total diversity in number of species	Sample size (morphospace and phenotypic analysis)	Sample size – macro-evolutionary analysis and phylogenetic correlations
Aulomyrcia	147	37 (24%)	35 (24%)
Calyptranthes	292	32 (11%)	22 (7%)
Gomidesia	60	18 (18%)	11 (18%)
Guianensis	32	14 (37%)	12 (37%)
Myrcia	118	18 (15%)	10 (8%)
Eugeniopsis	22	14 (41%)	9 (41%)
Sympodiomyrcia	27	15 (52%)	14 (52%)
Reticulosa	21	6 (19%)	4 (19%)
Tomentosa	12	7 (3) (37%)	3 (37%)
Total	**731**	**162 (22%)**	**120 (16%)**

7.5 Phenotypic data – Floral display and additional information

Floral and inflorescence traits were chosen over other phenotypic characters as their variation in format and arrangement is greater and they are under strong selective pressure for reproduction. In addition, floral features can be used to reinforce adaptive radiations into discrete niches (see Endress 1996; Harder and Barret 2006; Willmer 2011). A preliminary survey established floral and inflorescence characters appropriate to address the aims of this study. Flower and inflorescence measurements (continuous data) were chosen according to the following criteria: 1) there is clear variation between species; 2) it is possible to record the character in question for every species (homologous structures are always present); 3) the character can be measured with a dissecting microscope; and 4) has or may have relevance in reproductive strategy (based on reproductive biology surveys such as NicLughadha and Proença 1996; Gressler et al., 2006; and field observations). A total of 16 measurements of the flower were taken (Fig. 7.2.; Table 7.2). Presence/absence of oil glands on the anthers was also noted (Fig. 7.3). Additional label data (altitude, plant height, vegetation; Fig 7.4B) and inflorescence traits (estimated number of flowers and length of main axis Fig 7.4A; position on the plant, Fig. 7.5; flowers clustered or not Fig.7.6)

were also recorded. Proportions were calculated as one variable divided by the other to estimate differential investment in one structure over the other. Total investment in the androecium is estimated by multiplying stamen number by anther length.

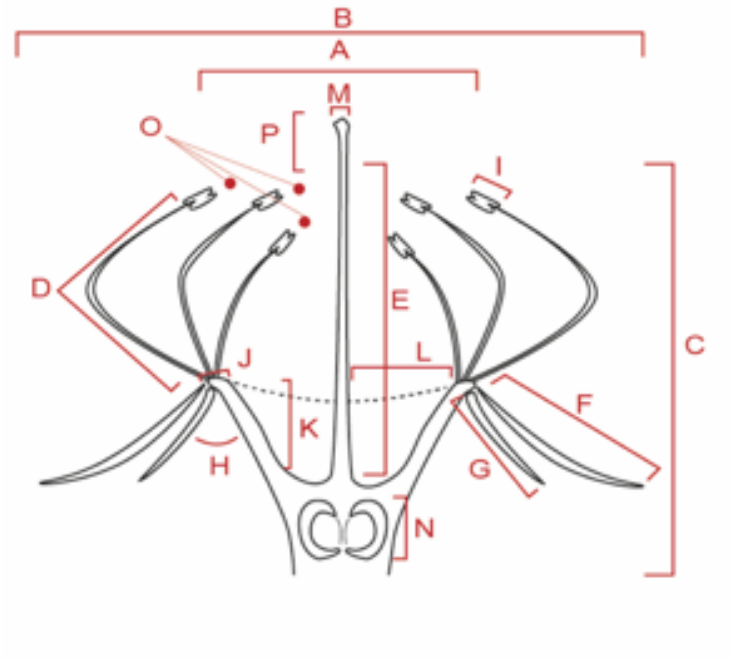


Figure 7.2. Schematic drawing of *Myrcia* flower in longitudinal section.

Table 7.2: Description of flower measurements for morphospace analysis in *Myrcia.* Letters refer to Figure 7.2.

Letter	Description of meassurement
A	Floral receptacle diameter (i.e. floral total diameter minus perianth)
B	Floral total diameter (i.e. floral receptacle diameter plus perianth)
C	Floral total length
D	Filament length
E	Style length
F	Petal length
G	Sepal length
H	Angle of staminal ring deflection at anthesis
I	Anther length
J	Thickness of staminal ring
K	Height of hypanthium elongation above the ovary
L	Distance between style base and staminal ring
M	Diameter of stigma
N	Ovule size
O	Number of stamens
P	Approximate height of stigma above anther line at anthesis (if negative, then stigma below anther line)

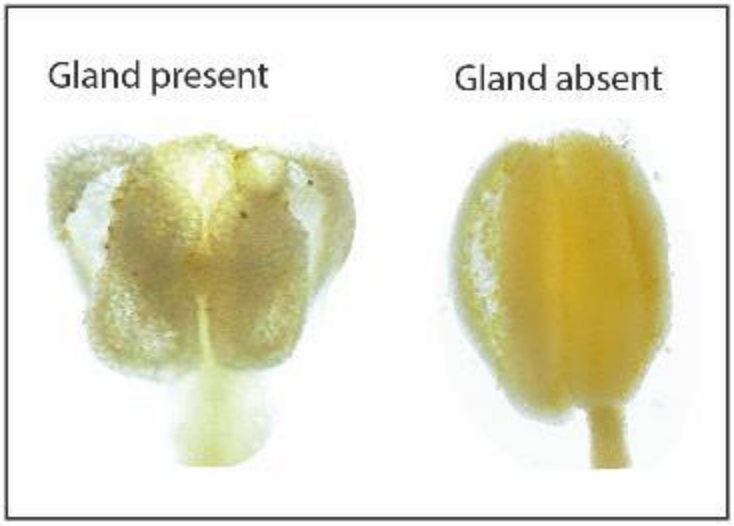


Figure 7.3: Presence and absence of anther oil gland.

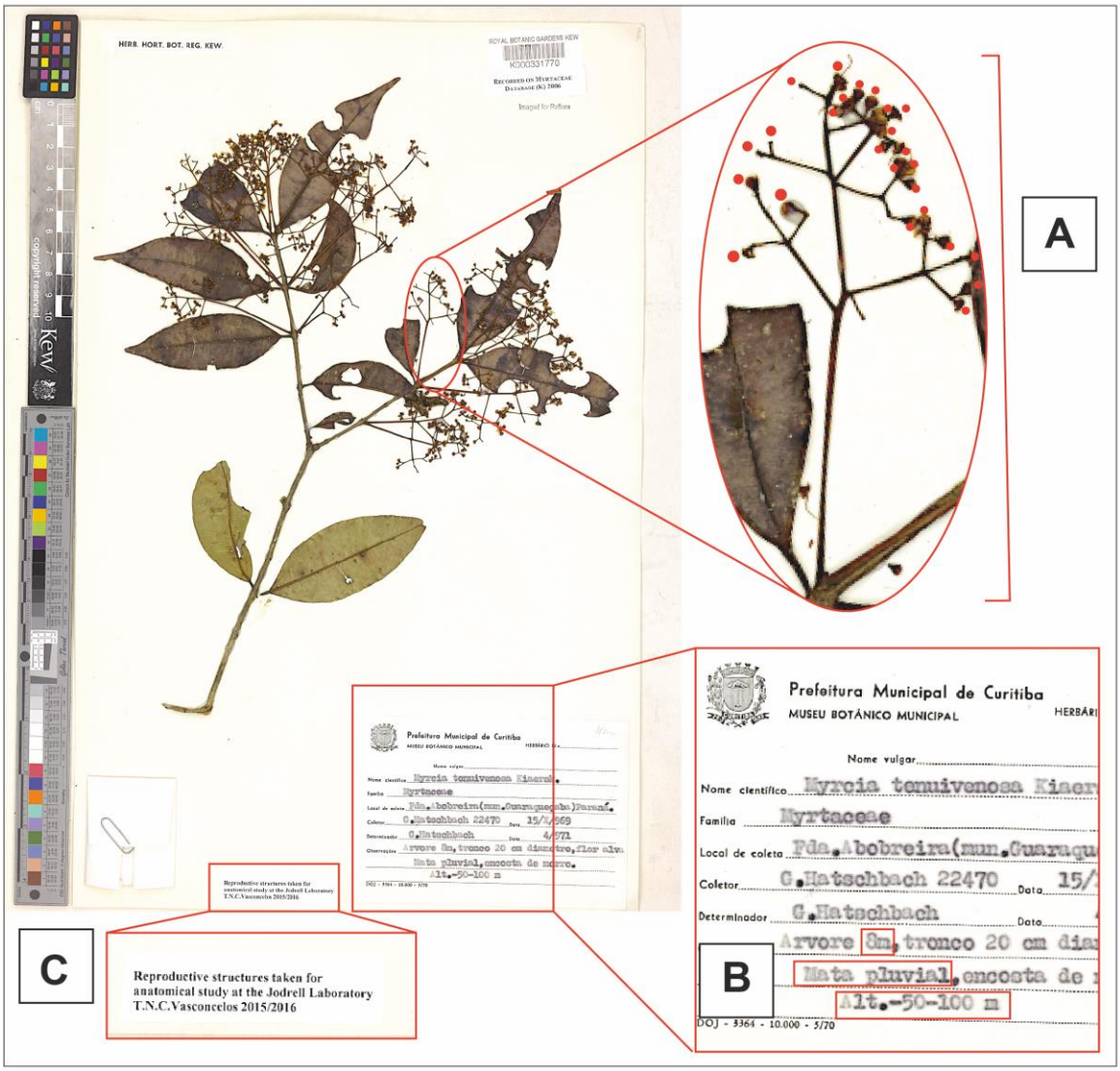


Figure 7.4: Data collection using herbarium specimen. (A) Measurement of main inflorescence axis and estimation of number of flowers; (B) Data from label (plant height, vegetation, altitude); (C) Specimens included in the analyses were labelled for future consultation.

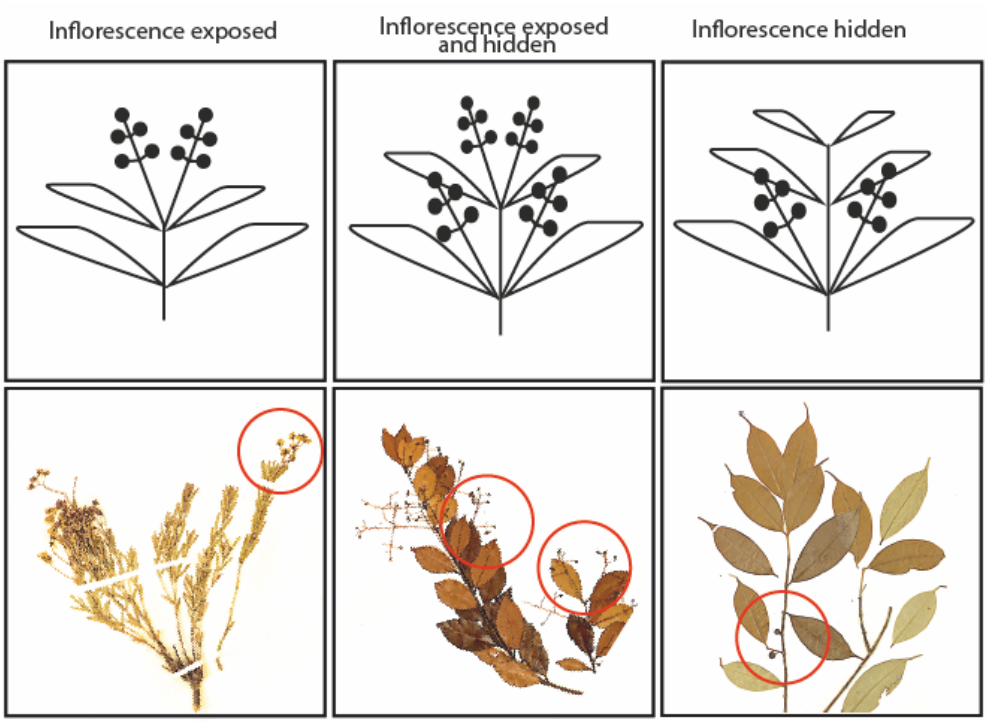


Figure 7.5: Inflorescence categories according to position in the plant.

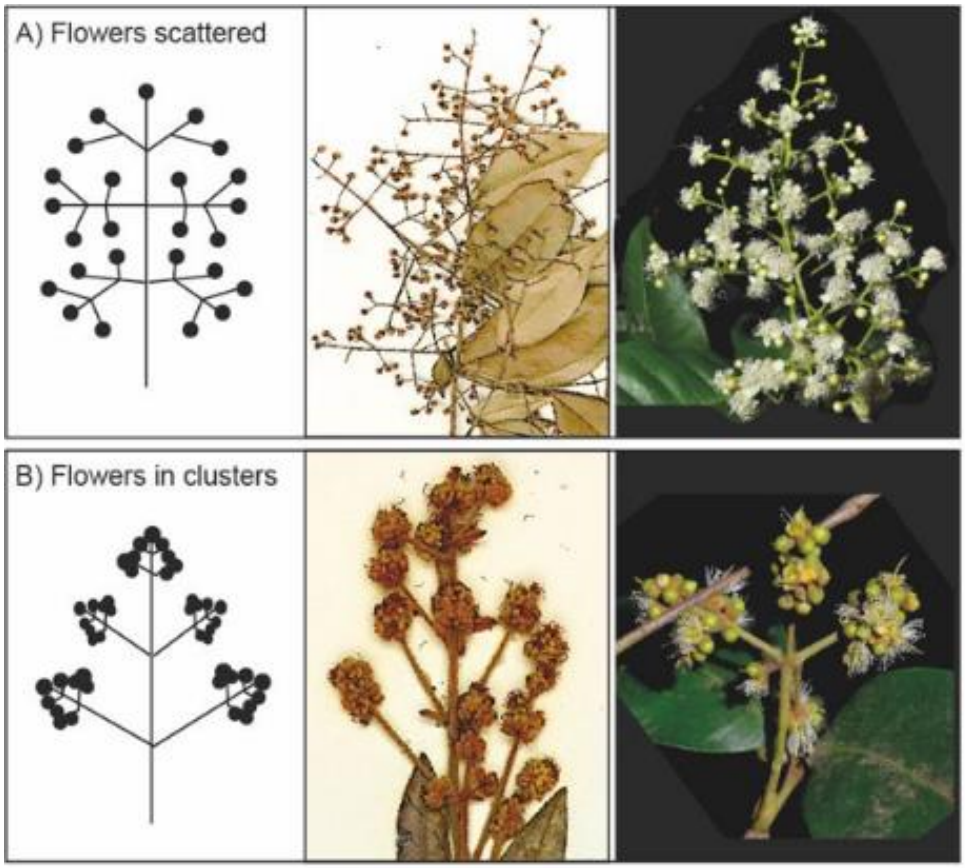


Figure 7.6: Inflorescence categories according to degree of floral clustering. (A) Flowers scattered in the inflorescence; (B) Flowers in clusters.

7.6 Data Annotation

Most data was taken from specimens available in the Royal Botanic Gardens Kew herbarium (K) using, where possible, the vouchers used in the phylogenetic analysis to avoid problems with species circumscription. Vouchers used in the phylogeny without flowers were substituted for flowering collections from similar geographical locations and with identifications by specialists. An average of three buds and three recently opened flowers were measured from each specimen. Buds and flowers from herbarium specimens were boiled for 10 minutes, left to cool overnight and then fixed in 70% ethanol for longer preservation. Material was also collected in the field directly into ethanol. Measurements and pictures were taken using a Nikon ShuttlePix model P-400R (Fig.7.7). ImageJ v.2 (Schindelin et al., 2015) was used to take measurements from species protologue illustrations when no suitable flowering material was available. Additional label data and inflorescence traits were annotated directly from herbarium material (Fig. 7.4). For a full list of selected samples and vouchers see Appendix 7.1. Most vouchers are from Brazil and available online at the Flora do Brasil website (floradobrasil.jbrj.gov.br). Some details in data collection include:

a) Number of flowers per inflorescence was estimated in five ordinate categories (1-5; 6-15; 16-50; 51-100 and more than 100 flowers) according to scars left in the inflorescence (Fig. 7.5A).
b) Plant height was considered an approximation of plant habit. When the raw height in meters was not specified, height estimation was annotated as: shrub = 2m, small tree = 5m, tree = 10m (Fig. 7.4B). If no plant habit or height was described in the label, then value was scored as NA.
c) Environmental variable (Vegetation and altitude): When not specified in the label, these values and categories were estimated by locality/coordinates plotted on Google Earth. Vegetation was scored in two categories: rainforest and savannah. All arboreous, humid vegetation was considered rainforest, including: “Restinga” coastal vegetation, Atlantic montane forests and Amazonian rainforests. All mostly shrubby seasonal vegetation was coded as savannah, including: “Cerrado” (in all variations) and “chaco” (dry vegetation from central South America) (see Fig. 7.4B).
d) Length of filament (D) was measured by choosing the longest, outermost filaments.
e) Anther gland present was considered when there was an obvious oil gland on the top of the connective and when the majority of anthers presented this gland.

In total, 3652 character states were recorded representing a significant amount of newly available trait data (see Appendix 7.1). 236 entries (c. 5%) were scored as “missing data” (NA), when no suitable material was available. Because most continuous trait analyses do not accept it, missing data was substituted by the mean of that measurement for the whole dataset. This was chosen over other imputation methods (e.g. means of closely related species; means based on similar morphotypes) because it was considered the most impartial.

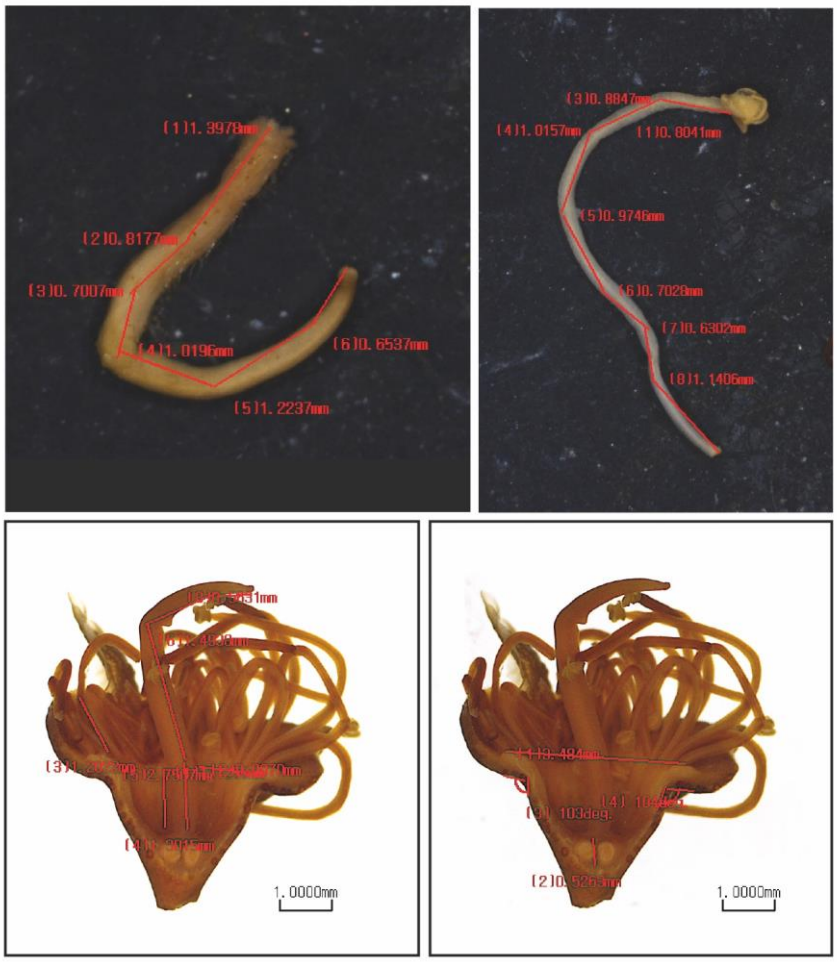


Figure 7.7: Examples of flower measurements using a Nikon ShuttlePix.Top left rand side: style measurement. Top right hand side: filament measurement. Bottom: longitudinal sections of *Myrcia rubella* bud and general measurements of structures.

7.7 Phylogenetic reconstruction

The Phylogenetic reconstruction used here is based on one nuclear (ITS) and four chloroplast markers (*psb*A-*trn*H, *trn*Q-*rps*16, *trn*L-*trn*F, *ndh*F) from previously published phylogenies. The molecular matrix is very similar to that of Santos et al. (2017), but duplicate species entries (e.g. *Myrcia mutabilis*) were removed leaving the voucher truest to the type. A dated phylogeny was reconstructed in BEAST (Drummond and Rambaut, 2007), using the pollen-fossil approach and secondary calibration points provided by Chapter 1. The final topology is similar to those of Lucas et al., (2011), Staggemeier et al., (2015) and Santos et al. (2017). The resulting tree contains 146 tips, including 133 ingroups and 13 outgroups and is available in Appendix 7.2. This is the topology used for phylogenetic signal estimation and overall macro-evolutionary dynamics analysis in this study.

7.8 Trait correlations and phylogenetic signal tests

The phenotypic dataset was first used to look for correlations among traits and for phylogenetic signal analyses. All analyses were done in R (R Core Team, 2017). Most flower measurements do not meet parametric criteria (normality and homoscedasticity), so a correlation matrix was built using the Spearman's rank correlation coefficient with function *cor*. For

phylogenetic signal analysis, the final tree was pruned (function *drop.tip*, in package *ape*; Paradis et al., 2004) to exclude outgroups. We used the function *fitContinuous* in the package *geiger* (Harmon et al., 2008) to estimate values of *lambda* for each surrogate. Values of *lambda* closer to 1 indicate stronger phylogenetic signal (Pagel, 1999), i.e. a strong dependence between trait and phylogeny.

7.9 Morphospace and morphological disparity

To understand evolution and disparity of the whole floral morphology of *Myrcia*, a morphospace analysis was conducted. This approach gives a visual interpretation of the morphological variability in the sample and also highlights specializations that can be visualized as clusters representing a similar combination of traits (e.g. Perret et al., 2007; Chartier et al., 2014, 2016). A first floral morphospace for *Myrcia* was built in form of a principal component analysis (PCA) using the function *PCA* in package *FactoMineR* (Lê et al. 2008) and included the 16 raw flower measurements (A – P; Table 7.2) for all 165 species in the phenotypic dataset. This analyses only supports continuous data and scores the effect of each measurement on the morphospace distribution. To visualize the distribution of the phylogeny over the PCA plot, the *phylomorphospace* function of the *phytools* package (Revell, 2012) was used.

A principal coordinate analysis (PCoA) was run to include all floral data (i.e. A – P plus presence of oil gland and inflorescence measurements) and provide a total picture of floral diversity in *Myrcia*. The PCoA produces a dissimilarity matrix (i.e. how distant are every pair of species in the morphospace) that can be used to interpret morphological disparity within discrete units. Clusters representing the nine *Myrcia* clades were tested for morphological disparity using a modified version of the function *adonis* from package *vegan* (see Chartier et al., 2017). Morphological disparity was further compared against age and total species diversity per clade. The hypothesis to be tested here is that morphological disparity increases with age and/or species diversity.

A Mantel test (function *mantel* in package *vegan*) was used to compare morphological and phylogenetic pairwise distance matrices between specimens to indicate phylogenetic signal in the total floral morphological evidence. The pairwise distance matrix from the morphological data was acquired using Euclidean distance and the phylogenetic dissimilarity matrix was estimated using the function *cophenetic.phylo* in package *ape* (Paradis et al., 2004).

7.10 Null hypothesis significance tests

A series of null hypothesis significance tests (NHSTs) were performed to test the relationship between floral traits and environmental variables. The hypothesis to be tested here is that extrinsic selective pressures produce floral phenotypes specialized for distinct habitats. T-tests (function *t.test*) and Kruskal-Wallis rank sum tests (function *kruskal.test*; alternative from one way anova for non-parametric datasets) was used to test nominal data against selected measurements. Manova (function *adonis*, package *vegan*; Oksanen et al., 2007) was used to test the morphospace distributon against the nominal data: vegetation (binary character), altitude (five discrete ordinate categories), floral clustering (binary), floral position (three categories) and anther gland (binary). Pairs of nominal data sources (e.g. presence of oil gland vs. vegetation) were tested using a simple chi2 (function *chisq.test*).

7.11 Analytical methods for interpreting phylogenetic heterogeneity

When analysing the phylogeny of a group of organisms over millions of years, variation in branching pattern and heterogeneity between clade diversity is expected (Rabosky, 2006). Macro-evolutionary dynamics fluctuate constantly over time as speciation accelerates (more speciation than extinction) or decelerates (more extinction than speciation). Therefore, analysis of phylogenetic branching patterns allows estimation of points in a phylogeny that have been subject to significant disparity of diversification or extinction rates. Increased availability of phylogenetic tree data has been accompanied by increased statistical power to analyze branch heterogeneity in ultra-metric trees (see summary in TESS vignette, Hohna et al. 2015), although not without controversy (e.g. Moore et al., 2016 to Rabosky's 2014 BAMM). Here three methods are contrasted; a BAMM analysis (v2.5, Rabosky, 2014, et al., 2017) was used to identify significant rate shifts in the tree that could be associated with cryptic key-innovative phenotypic characters highlighted by the multi-trait analysis. Empirical priors were generated based on the pruned *Myrcia* phylogeny and an estimated total diversity of 700 species (WCSP, 2017). Sampling estimates per clade are based on Lucas et al. (2011) and can be accessed in Table 7.2. TESS (Hohna et al., 2015) was used to estimate changes in diversification and extinction rates over time and to calculate possibility of rate shifts based on marginal likelihood and Bayes factors. For TESS, the original phylogeny had to be rescaled to minimize the effects of clade over representation; tips were randomly pruned from over-sampled clades prior to analysis (8 from clade *Sympodiomyrcia*, 5 from clade *Guianensis* and 4 from clade *Eugeniopsis*). RPANDA was used to identify the presence of different macro-evolutionary regimes (branching patterns) occurring across the phylogeny.

RESULTS

7.12 Descriptive statistics and phylogenetic signal

Descriptive data analysis shows correlation coefficients between flower measurements, based on a non-parametric Spearmen test (Fig. 7.8). All significant correlations between raw measurements (A - P) are positive, meaning that most structures have a positive relationship of dependence; i.e. an increase/decrease in size of a given flower structure leads to increase/decrease in the size of most other structures. Proportional analysis of structures against their equivalent raw-measurement (e.g. F against G/F) returned expected strong negative correlations. Unpredicted significant negative correlations include: (1) the proportion of receptacle diameter vs. hypanthium depth (A/K) is strongly anti-correlated with proportion of total length vs. total diameter (C/B) and (2) also strongly anti-correlated to relative investment in the staminal ring (L/J); and (3) the proportion between total length vs. total diameter (C/B) and the thickness of the staminal ring. This means that most species have two strategies: 1) long hypanthium tubes with thin staminal rings and stronger disparity between total flower length and total flower diameter or 2) short hypanthium tubes with thicker staminal rings and total flower length on average equal to total flower diameter (for confidence measurements see Appendix 7.3).

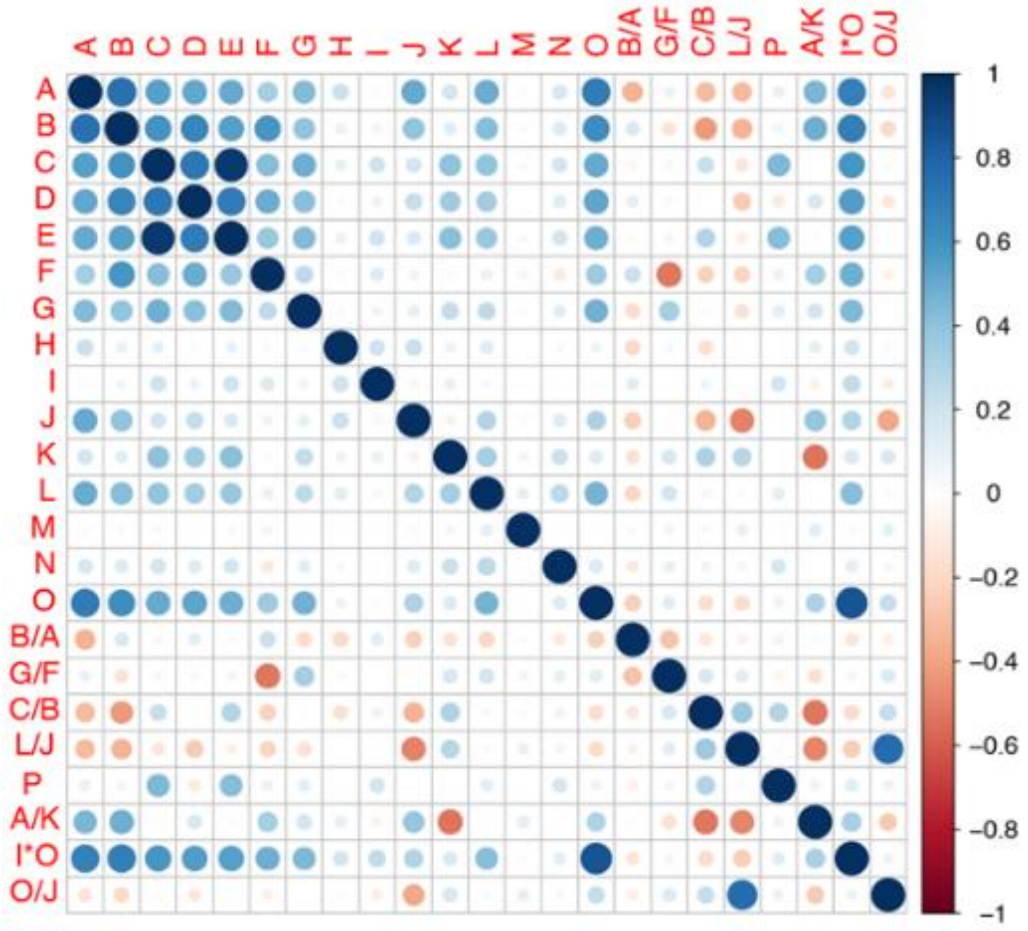
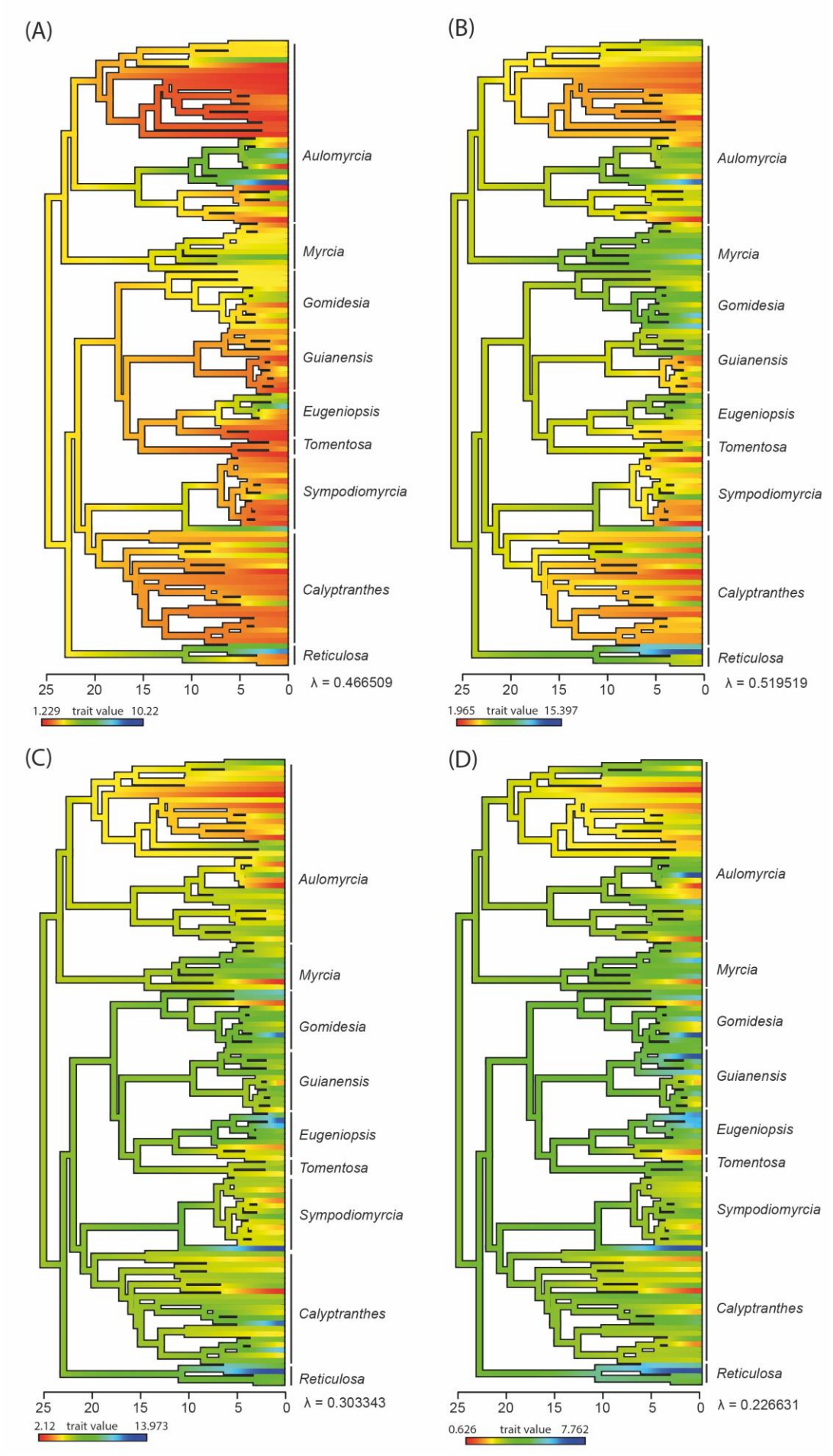
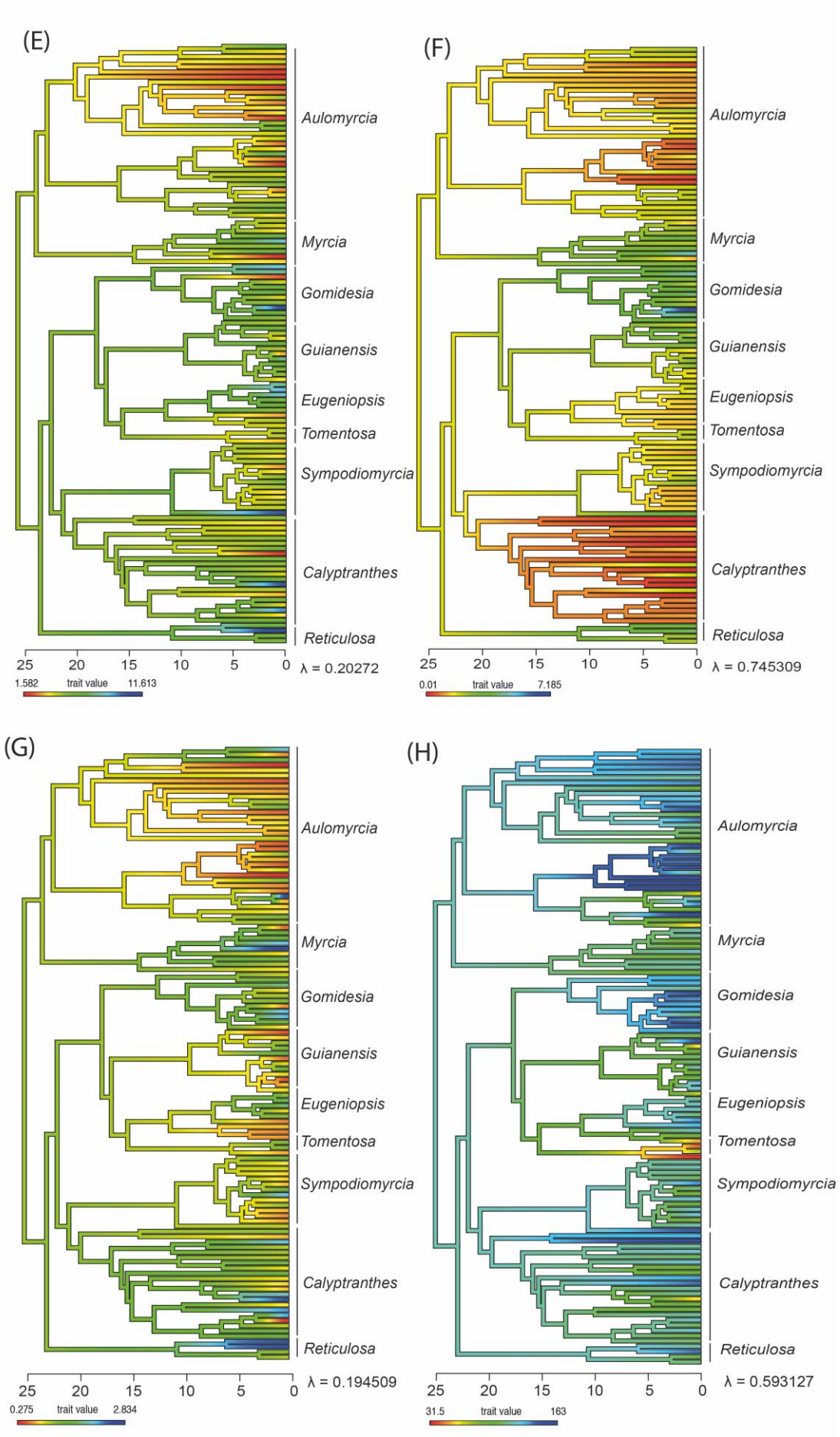


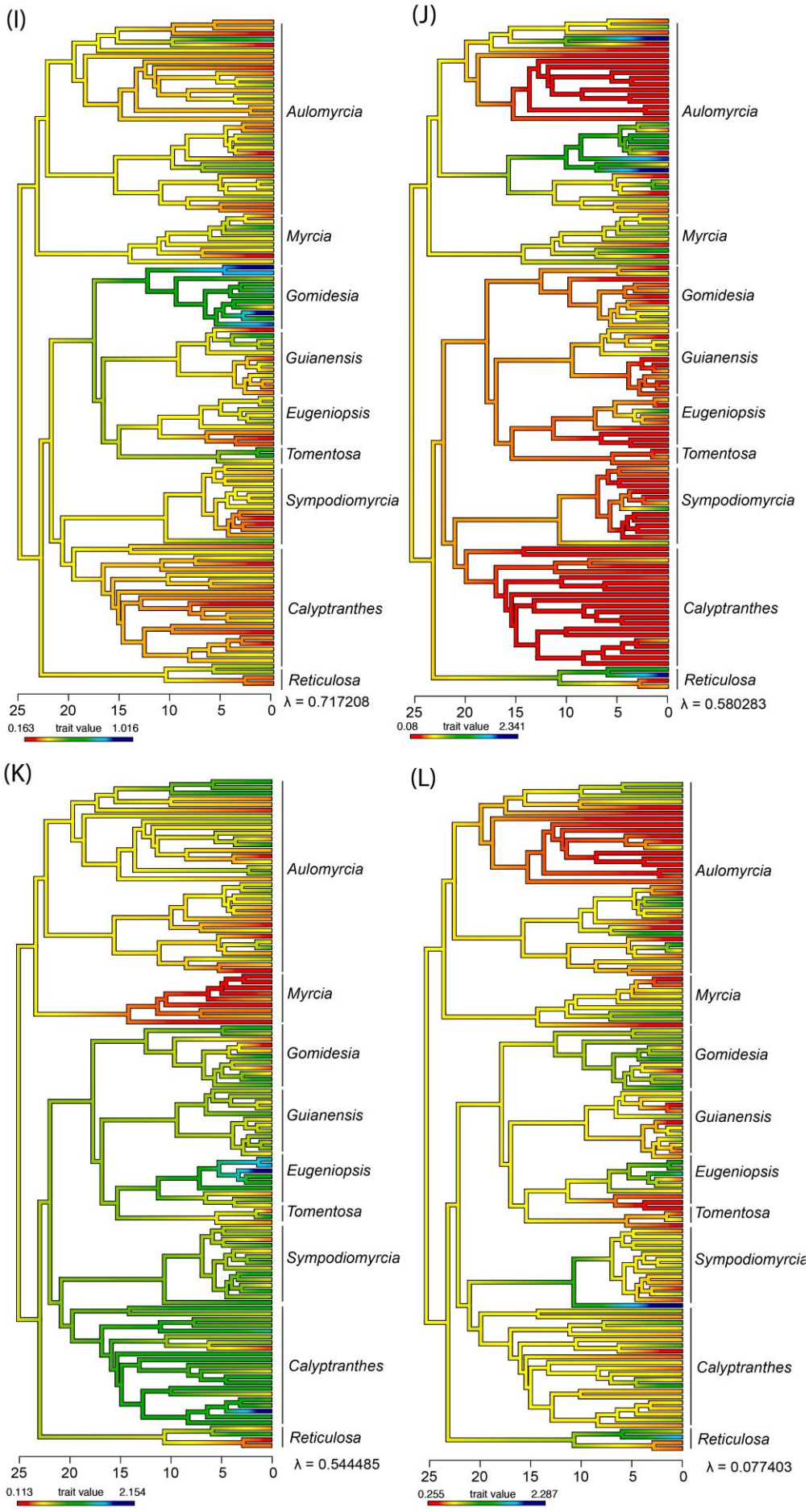
Figure 7.8: Correlations between floral measurements based on a non-parametric Spearmen correlation test. Larger and more colourful dots indicate stronger correlation. Blue: measurements are positively correlated, Red: measurements are anti-correlated. Clear dots or absent dots: measurements are not correlated.

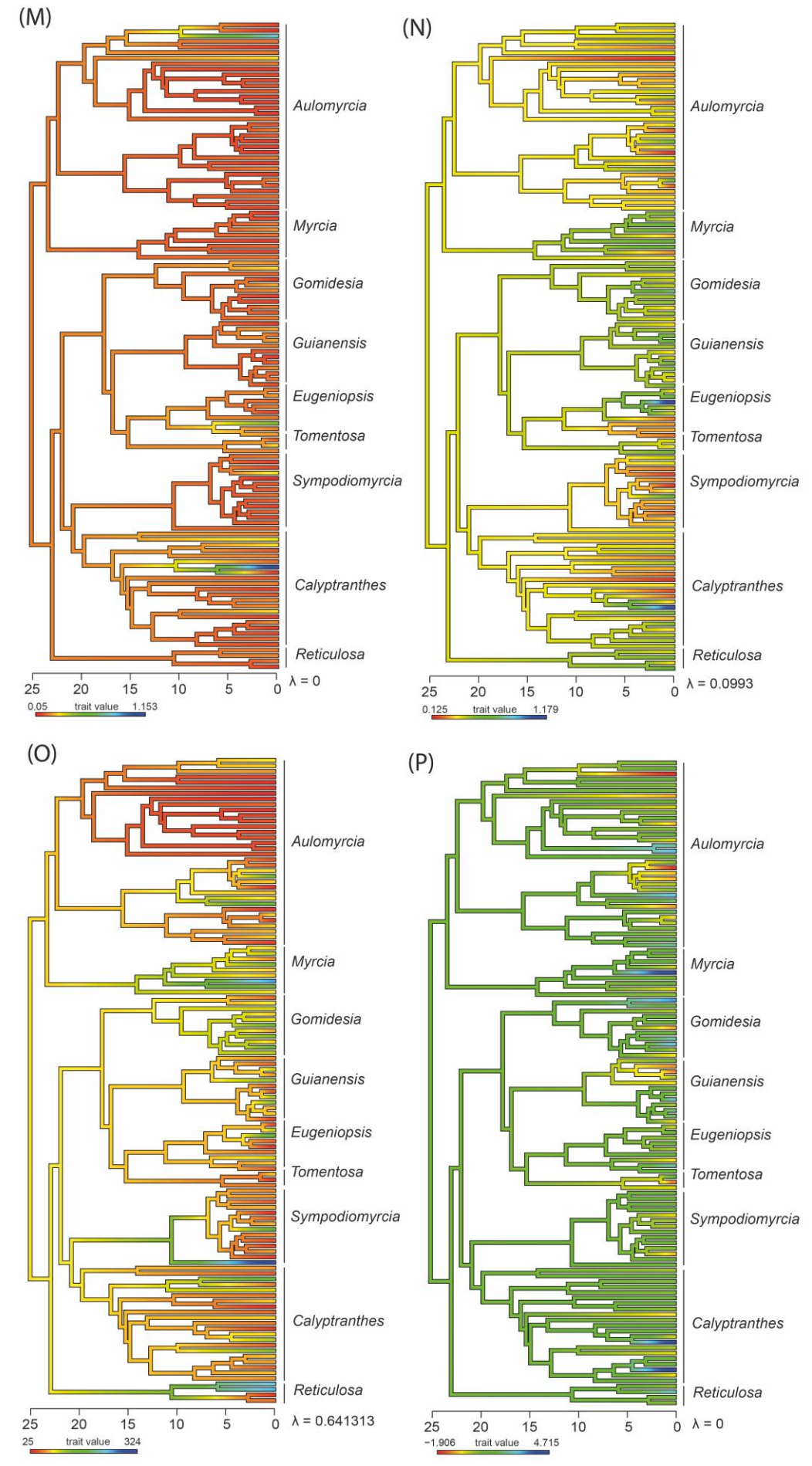
Exploratory analysis of morphometric data in light of the *Myrcia* phylogeny (Figure 7.9) and distribution of measurements per clade (Figure 7.10) show how investment in different floral parts are distributed over the phylogeny. Some tendencies are observed such as little petal investment in contrast with sepal size (G/F) in clade *Calyptranthes*, high investment in male reproductive structures (O*I) in clade *Gomidesia*, high floral total diameter in contrast to hypanthium depth (A/K) in clade *Myrcia* and highly reflexed staminal rings (H) in clade *Tomentosa*. These trends can be observed on boxplots (Figure 7.10) but phylogenetic signal is low for most measurements with the exceptions of F (λ = 0.74), I (λ = 0.71), G/F (λ = 1), I*O (λ = 0.74), A/K (λ = 0.86) (see λ values at the bottom right of each plot in Figure 7.8).

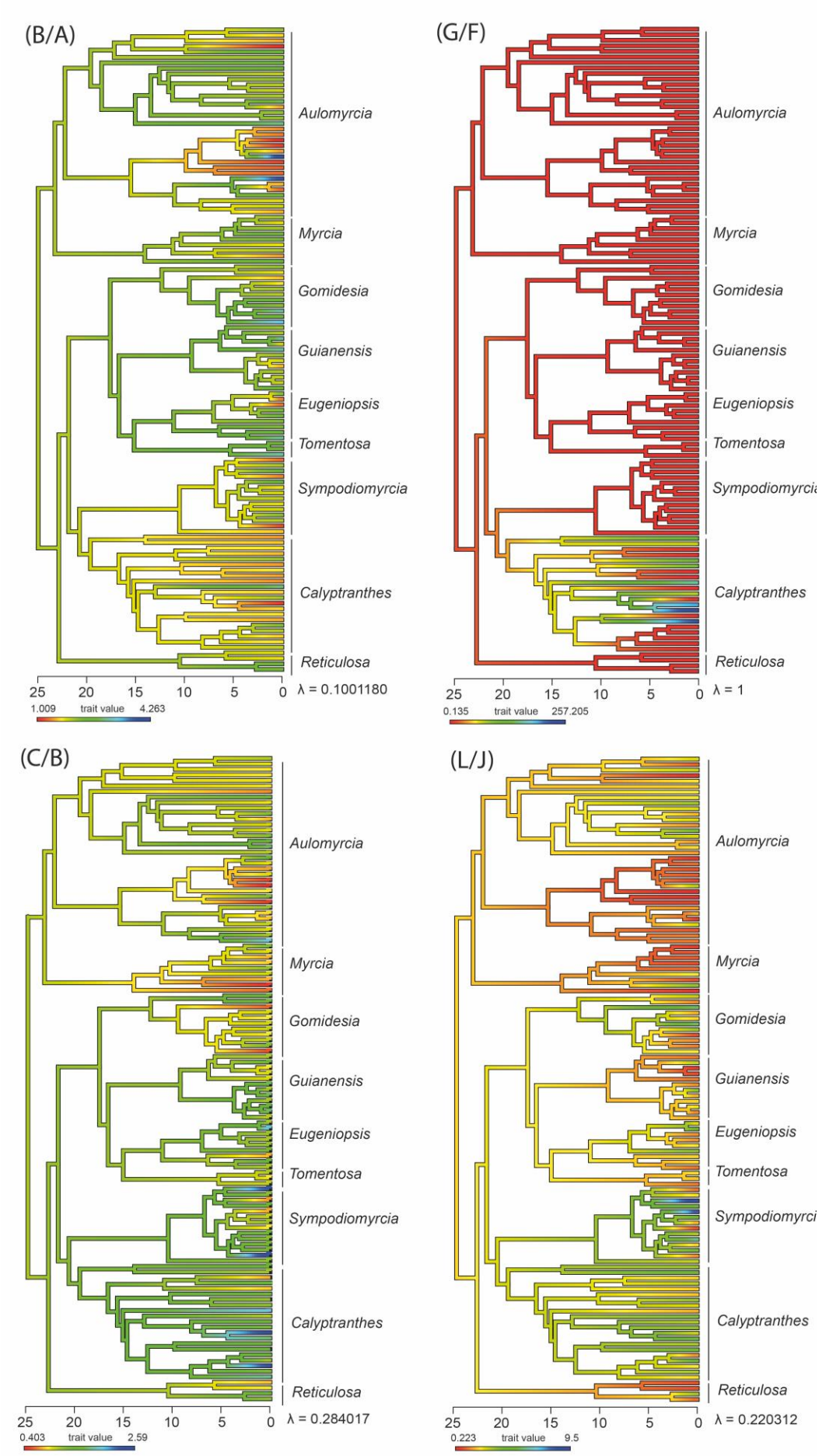
Figure 7.9 (next six pages): Map of continuous characters over *Myrcia* phylogeny (generated by function *contMap*() in package *phytools*). Phylogenetic signal (λ, generated by function *fitContinuous*() in package *geiger*) plotted on the bottom right of each plot. Letters refer to flower measurements and proportions (same as in Figs. 7.2 and 7.8).

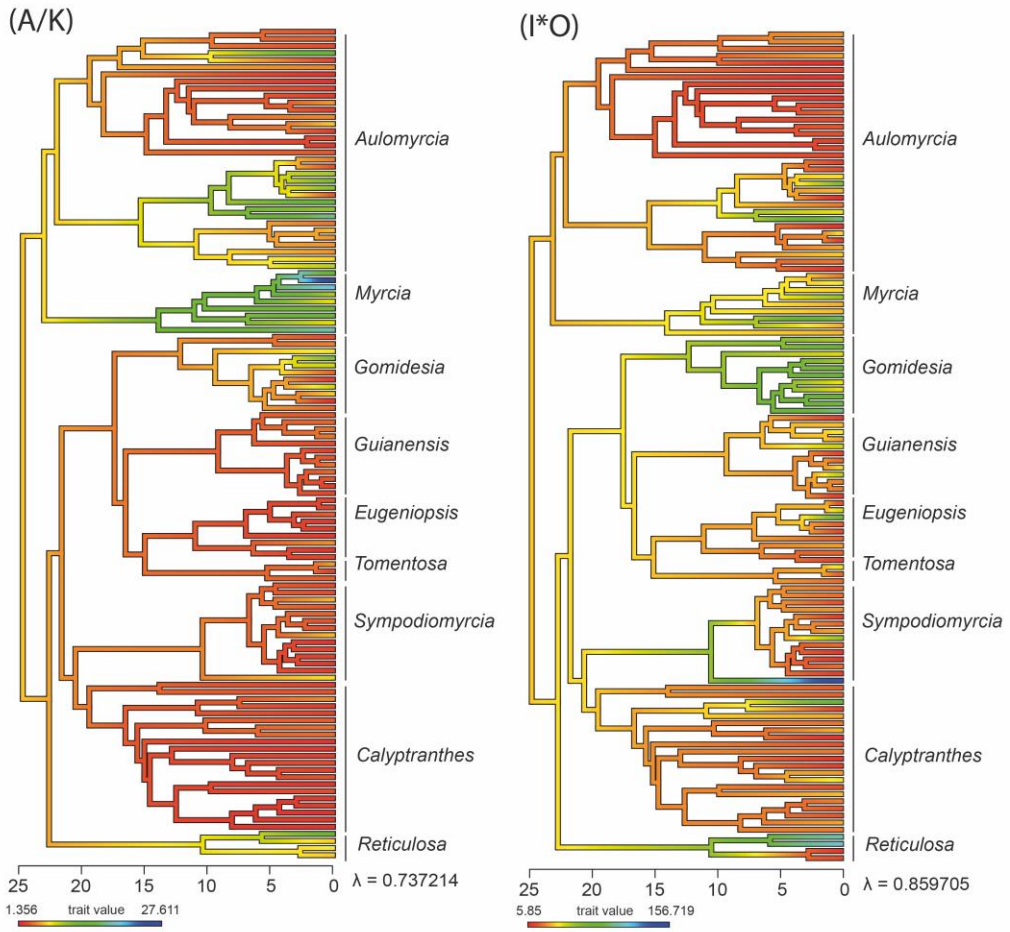

(A)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.466509
1.229 trait value 10.22
(B)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.519519
1.965 trait value 15.397
(C)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.303343
2.12 trait value 13.973
(D)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.226631
0.626 trait value 7.762


(E)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.20272
1.582 trait value 11.613
(F)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.745309
0.01 trait value 7.185
(G)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.194509
0.275 trait value 2.834
(H)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.593127
31.5 trait value 163


(I)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.717208
0.163 trait value 1.016
(J)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.580283
0.08 trait value 2.341
(K)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.544485
0.113 trait value 2.154
(L)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.077403
0.255 trait value 2.287


(M)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0
0.05 trait value 1.153
(N)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.0993
0.125 trait value 1.179
(O)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.641313
25 trait value 324
(P)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0
−1.906 trait value 4.715


(B/A)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.1001180
1.009 trait value 4.263
(G/F)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 1
0.135 trait value 257.205
(C/B)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.284017
0.403 trait value 2.59
(L/J)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.220312
0.223 trait value 9.5


(A/K)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.737214
1.356 trait value 27.611
(I*O)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.859705
5.85 trait value 156.719

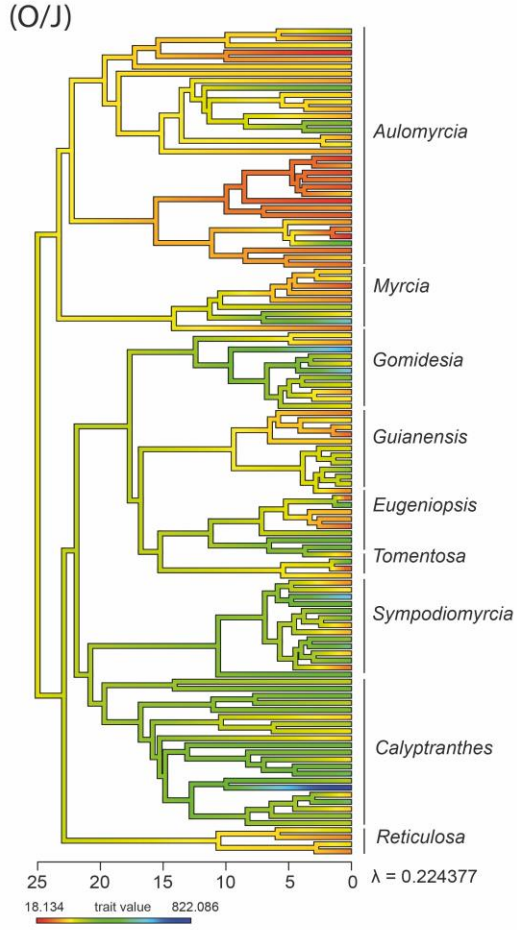
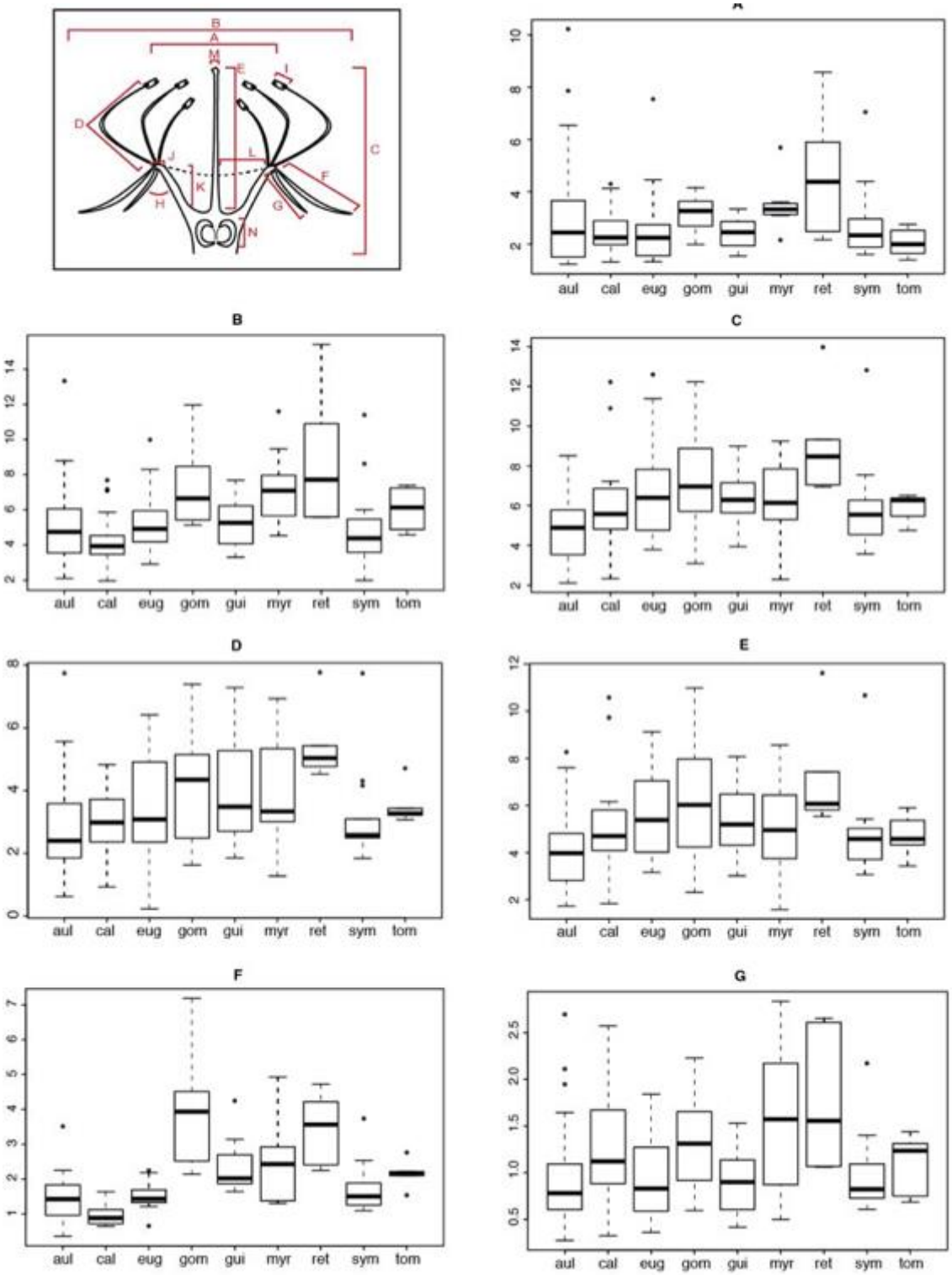
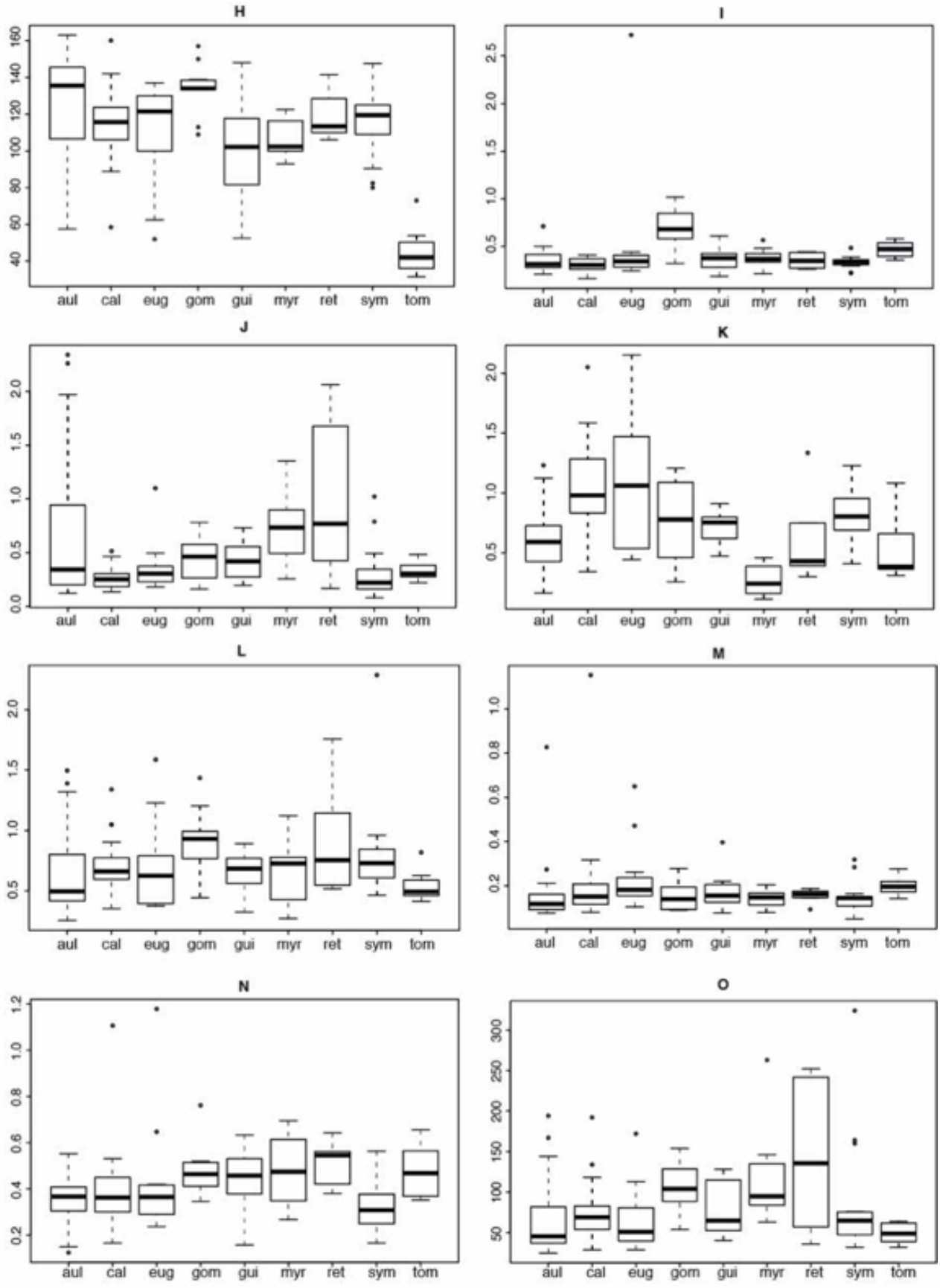
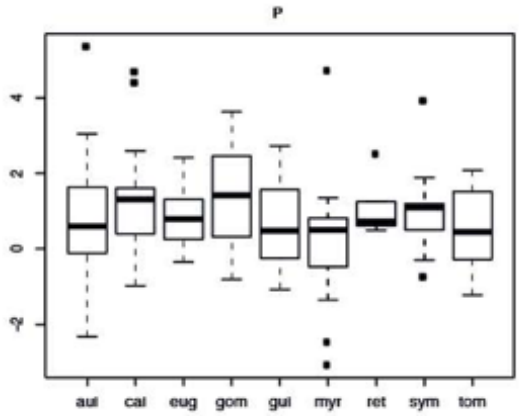
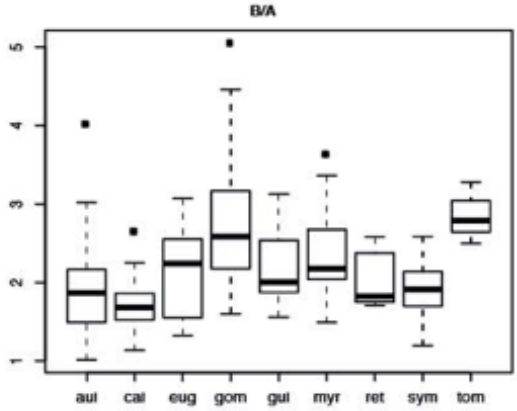

(O/J)
Aulomyrcia
Myrcia
Gomidesia
Guianensis
Eugeniopsis
Tomentosa
Sympodiomyrcia
Calyptranthes
Reticulosa
25 20 15 10 5 0
λ = 0.224377
18.134 trait value 822.086

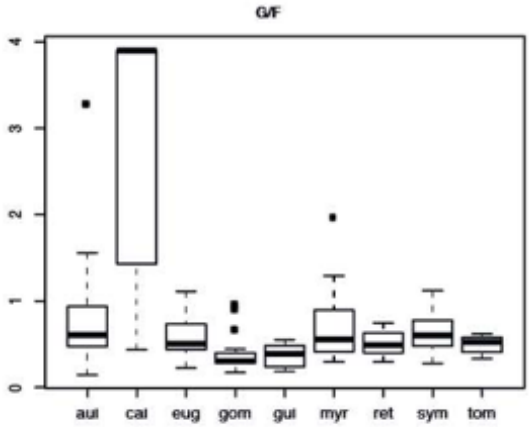
Figure 7.10 (below and next 2 pages): Profile of each floral measurement per clade of *Myrcia*. Letters refer to flower measurements according to flower diagram. aul, clade *Aulomyrcia*; cal, clade *Calyptranthes*; eug, clade *Eugeniopsis*; gom, clade *Gomidesia*; gui, clade *Guianensis*; myr, clade *Myrcia*; ret, clade *Reticulosae*; sym, clade *Sympodiomyrcia*; tom, clade *Tomentosa*.

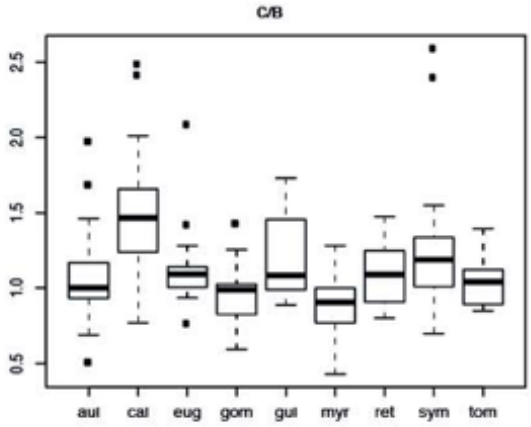


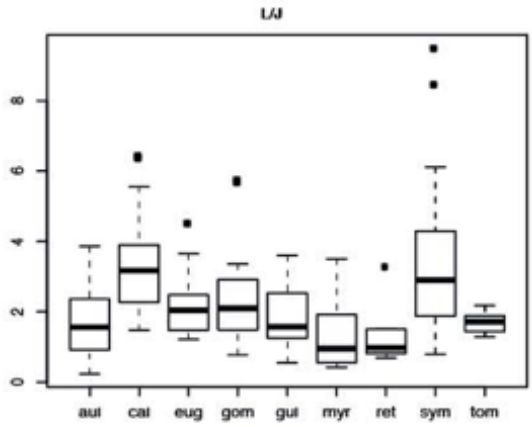

H
I
J
K
L
M
N
O
aul
cal
eug
gom
gui
myr
ret
sym
tom

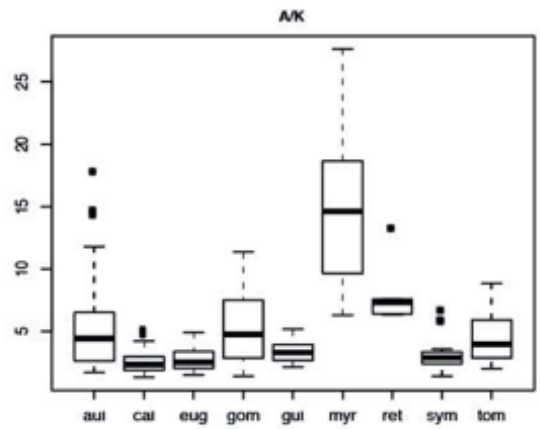

P
aul cal eug gom gui myr ret sym tom

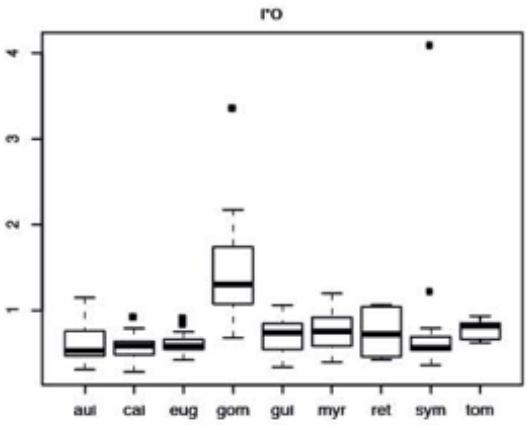

B/A
aul cal eug gom gui myr ret sym tom

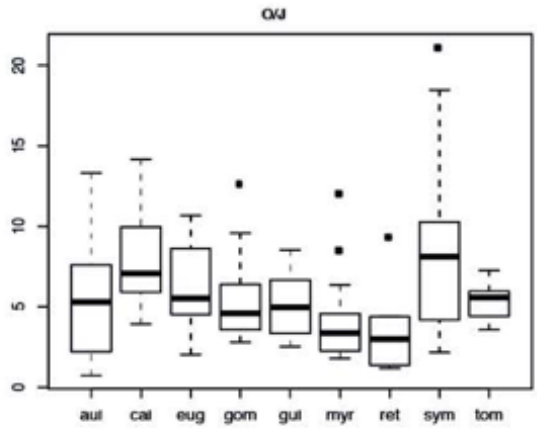

G/F
aul cal eug gom gui myr ret sym tom


C/B
aul cal eug gom gui myr ret sym tom


L/J
aul cal eug gom gui myr ret sym tom


A/K
aul cal eug gom gui myr ret sym tom


P*O
aul cal eug gom gui myr ret sym tom


O/J
aul cal eug gom gui myr ret sym tom

7.13 *Myrcia* floral morphospace

Floral disparity as measured by all 16 raw floral measurements (A – P) is distributed in principal component analysis (PCA) morphospace as a cloud of 165 points, each representing a measured specimen (Fig. 7.11; for a large sized plot see Appendix 7.5). Measurements with highest scores and affecting morphospace distribution most, in the first axis are: total flower diameter (B), total flower length (C), and style length (E); in the second and third axes, these are: petal length (F), staminal ring thickness (J), hypanthium tube length (K), distance between style and staminal ring (L) and stigma height relative to level of anthers (a proxy for herkogamy, P). The first three PCA axes explain 59.3% of variance and produce a combined eigen value of 9.4832 (see summary in Table 7.3). 90% of points fall near the center of the morphospace with just a few specimens scattered at the extremities.

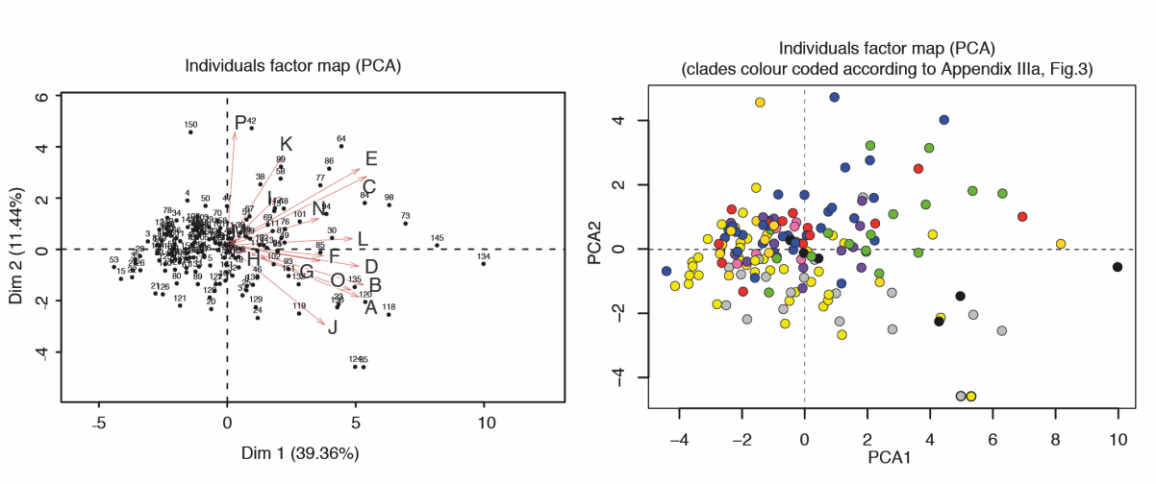


Figure 7.11: Myrcia floral morphospace inferred by a PCA analysis. Left hand side:165 specimens (numbers according to Appendix 7.1) and directionality of each flower measurement (red arrows). Right hand side: same PCA, but specimens color coded by clades. Yellow = Aulomyrcia, Blue = Calyptranthes; Gray = Myrcia; Pink = Black = Reticulosae; Pink = Tomentosa; Green = Gomidesia; Orange = Sympodiomyrcia; Purple = Aguava (=Guianensis); red = Eugeniopsis. See also Fig. 7.12.

The PCA, pruned for the 118 species with known phylogenetic position in *Myrcia*, with each clade represented by a different colour is shown in Figure 7.12 (clade by clade phylomorphospace). NPANOVA significance tests show that the area occupied by Section *Aulomyrcia* appears significantly different to that of Sections *Tomentosa*, *Gomidesia* and *Myrcia* and Section *Calyptranthes* is significantly different from Section *Gomidesia*. The remaining 32 clade cluster relationships return non-significant values of p and low F values (See Table 7.4 for values of significance between cluster overlap). These results highlight that floral morphological variance does not differ significantly for most *Myrcia,* a result empirically predicted by the tendency for specimen data points to fall intermixed, near to the centre of the floral morphospace (Figure 7.11).

Flower meassurement	Axis 1 (C1)	Axis 2 (C2)	Axis 3 (C3)
A	10.57883703	5.57009457	6.829751
B	**11.33027896**	3.27385322	2.805868
C	**11.83040254**	9.84677286	0.04966035
D	10.5365908	0.91266002	1.172219
E	**10.70011584**	12.17307578	0.06919969
F	5.65396034	0.23120645	**28.39405**
G	5.2197359	0.51128639	2.697771
H	0.91886802	0.37989053	8.211003
I	1.50002122	4.66510365	10.75651
J	5.7769172	**13.15372394**	1.526045
K	1.87996698	**16.73438388**	**22.44147**
L	9.54877882	0.09027983	**11.42822**
M	0.04938711	0.07352676	0.000549438
N	5.10793243	1.47906462	2.026834
O	9.33018407	4.19040826	1.239258
P	0.03802274	**26.71466924**	0.3515909
Eigen value	6.2983	1.830367	1.354694
Variance explained	39.36%	11.44%	8.4668%

Table 7.3: Scores of flower meassurements and eigen values per three first axes in the *Myrcia* morphospace PCA

	ret	cal	sym	myr	gom	tom	gui	eug	aul
ret	<NA>	3.542	2.115	0.86	1.572	4.376	2.433	1.577	4.957
cal	ns	<NA>	0.208	4.269	6.632	8.978	1.316	0.276	3.829
sym	ns	ns	<NA>	3.029	3.986	5.597	0.987	-0.072	1.223
myr	ns	ns	ns	<NA>	3.132	8.879	2.322	2.447	9.151
gom	ns	*	ns	ns	<NA>	18.253	5.485	3.615	8.627
tom	ns	ns	ns	ns	ns	<NA>	4.779	4.569	10.294
gui	ns	ns	ns	ns	ns	ns	<NA>	0.306	5.101
eug	ns	ns	ns	ns	ns	ns	ns	<NA>	1.767
aul	ns	ns	ns	*	*	*	ns	ns	NA

Table 7.4: Results from NPANOVA showing degree of dissimilarity between clades in the morphospace.Asterisks mark those with p<0.01 (significantly distinct clades).

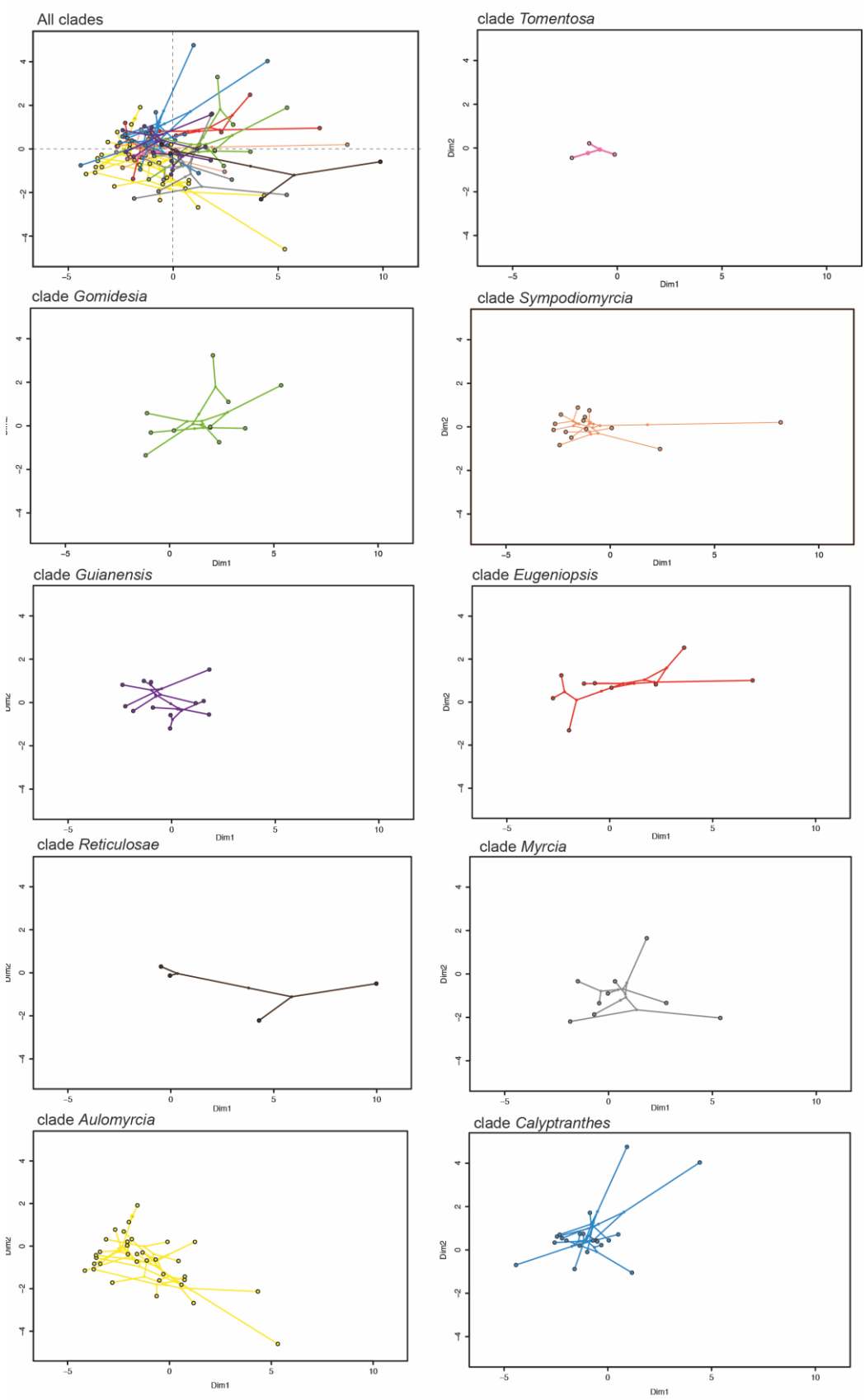


Figure 7.12: *Myrcia* phylomorphospace showing 118 data points that correspond to the phylogeny terminals. Plots show how each individual clade is distributed in the morphospace. Colors indicate distinct clades: Yellow = *Aulomyrcia*; Blue = *Calyptranthes*; Gray = *Myrcia*; Purple = *Guianensis*; Red = *Eugeniopsis*; Orange = *Sympodiomyrcia*; Black = *Reticulosae*; Pink = *Tomentosa*.

7.14 Phenotypic disparity and species diversity

Disparity analysis estimates mean pairwise distances between specimen points in the morphospace of a given clade (Fig.7.13A, orange bar). These distances are plotted against total species diversity for that clade (Fig.7.13A, gray bar) and correlated with mean clade age. Results show that disparity is not significantly correlated with species diversity (Fig.7.13Bi) or age (Fig.7.13Bii). However, when the two oldest sectional clades (Sections *Aulomyrcia* and *Calyptranthes*) are excluded from the analysis, correlations between disparity and age are strongly significant ($p<0.001$). Clade diversity is strongly correlated with clade age, meaning that the older the clade, the more species-rich it is (Fig.7.13Biii) and finally, contrast between pairwise distance morphological and phylogenetic dissimilarity matrices shows no significant correlation (Mantel statistic r = 0.01496; Significance = 0.3249), implying that overall floral morphological disparity is not correlated with phylogenetic distance.

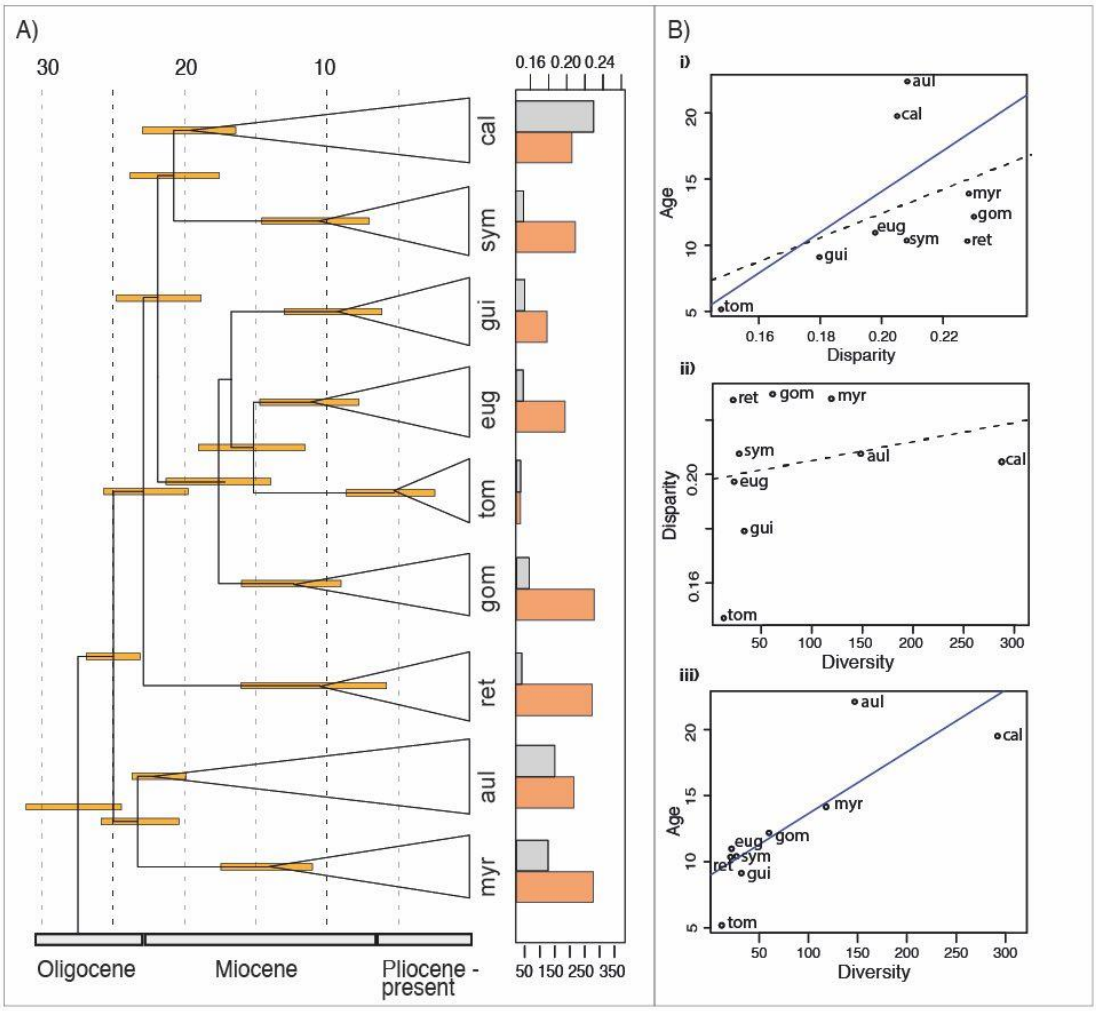


Figure 7.13: Relationships between clade diversity, age and morphological disparity in *Myrcia*. (A) *Myrcia* dated phylogeny plotted against estimates of disparity (orange bars) and species richness (gray bars). (B) Linear regressions contrasting (i) morphological disparity and species diversity (r2=-0.07906, p=0.541), (ii) Morphological disparity and age (p= 0.207119, r2 = 0.1044 for all dataset (dashed line); p<0.001 when Sections *Calyptranthes* and *Aulomyrcia* are excluded (blue line)), and (iii) Clade age and species diversity (p<0.01, r2=0.63).

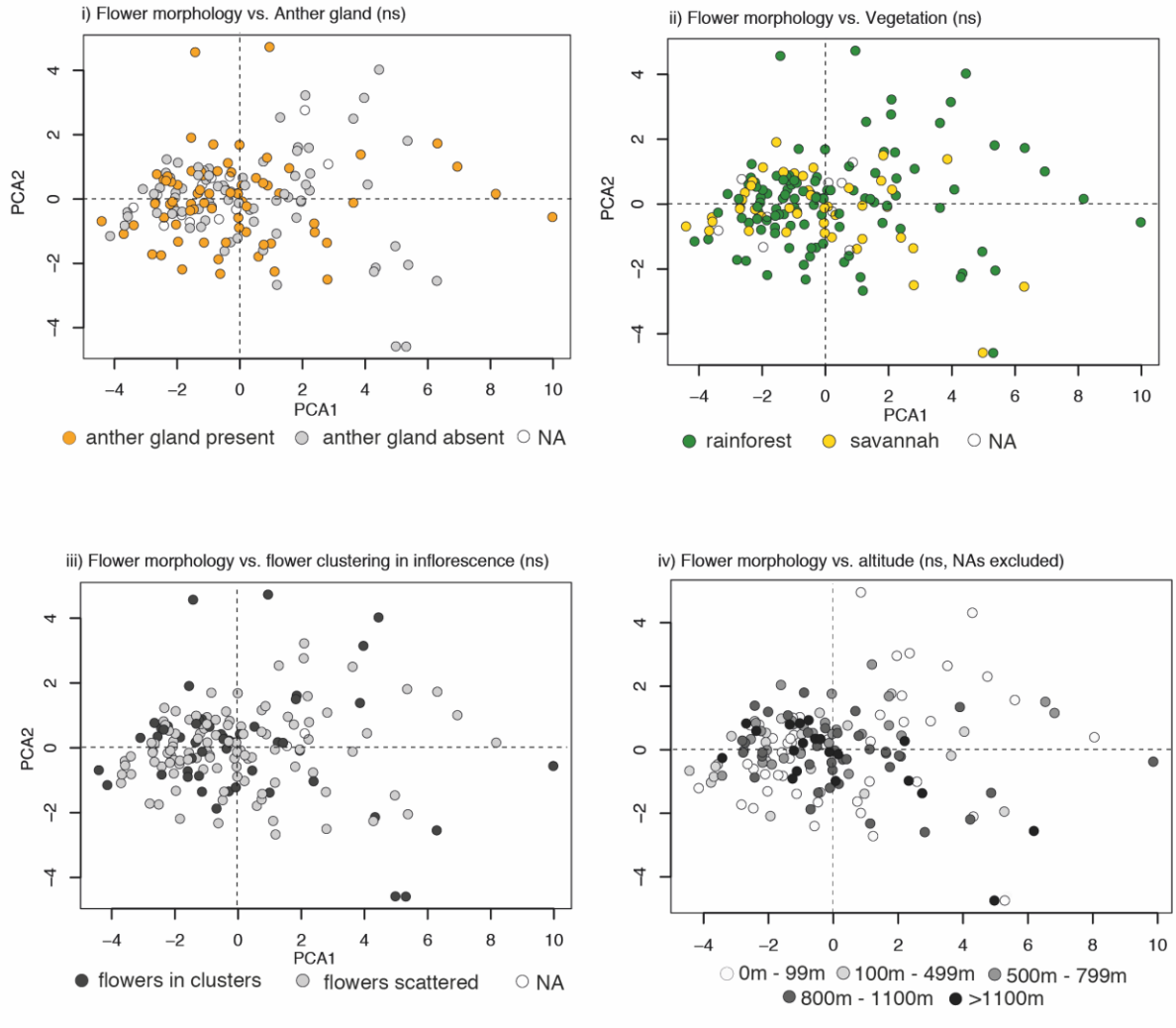
7.15 Floral specializations and environmental variables (NHSTs)

Results of NHSTs correlating overall floral traits, inflorescence measurements and environmental variables are almost all statistically unsupported (Table 7.5 and Fig. 7.14). The only significant correlations are those between flower size and inflorescence length (flower size increases with mean length of main inflorescence axis), flower size and proportion of inflorescence investment (flower size increases with mean inflorescence length divided by mean plant height) and proportion of inflorescence investment and vegetation type (three times greater investment in savanna biomes relative to rainforest). Summary is given in Table 7.5 below and Figure 7.14 (following pages).

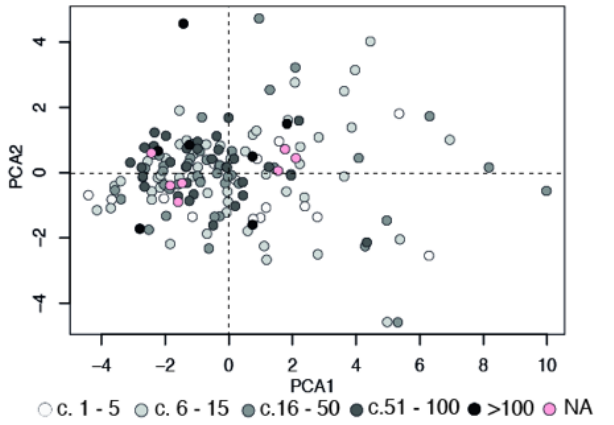
Table 7.5 (below): Relationships between selected traits, selected trait proportions and/or environmental variables based on NHSTs (NPMANOVA, Kruskal-Wallis tests, t tests and chi2 tests). Significant relationships are in bold. Roman numerals refer to plots in Figure 7.14.

	Correlation	F	p	chi2	r2	
i	floral morphology vs. anther gland	0.121	0.9171			ns
ii	floral morphology vs. vegetation	1.008	0.36			ns
iii	floral morphology vs. inflorescence clustering	0.577	0.5946			ns
iv	floral morphology vs. altitude	1.3874	0.31887			ns
v	floral morphology vs. flower no.	1.1938	0.44947			ns
vi	B vs. altitude		0.08219	8.2694		ns
vii	B vs. flower no.		0.1574	6.6189		ns
viii	B vs. inflo clustering		0.6156	0.25208		ns
ix	**B** vs. **inflo length**		**0.00327**		**0.04773**	*
x	B vs. vegetation		0.8889	0.019521		ns
xi	**B** vs. **inflo invest**		**0.00592**		**0.0558**	*
xii	I vs. anther gland		0.5118	0.43046		ns
xiii	O vs. anther gland		0.6053	0.26703		ns
xiv	anther gland vs. vegetation		0.4373	0.6034		ns
xv	anther gland vs. altitude		0.7406	0.10958		ns
xvi	flower no. vs. vegetation		0.1978	1.6586		ns
xvii	flower no. vs. altitude		0.659	-0.006987		ns
xviii	**Inflorescence investment - vegetation**		**0.000344**	**12.813**		**
	mean savannah = 0.04475419					
	mean forest = 0.01422814					
xix	plant height/inflo length - altitude		0.8525	1.3521		ns

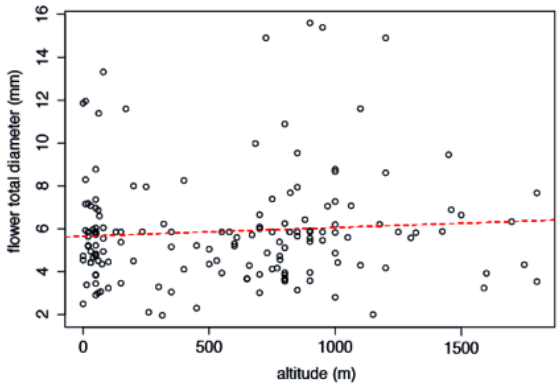
Figure 7.14 (below and next three pages): Null hypothesis significance test plots for correlations between *Myrcia* floral morphology and environmental variables. Numbers (i – xix) are according to Table 7.5. "ns" = correlation is non-significate.



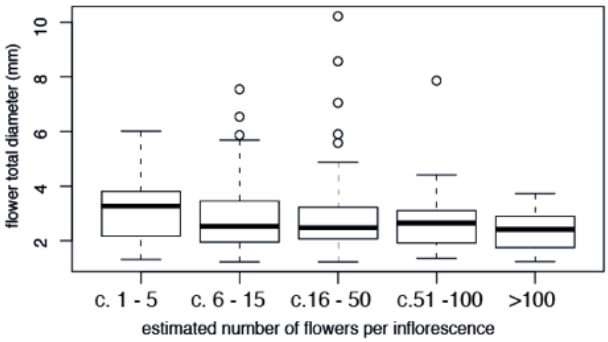
v) Flower morphology vs.Estimated number of flowers per inflorescence (ns)



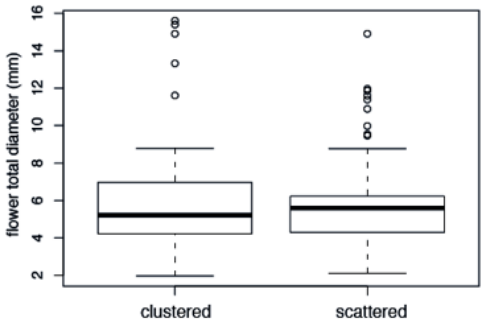
vi) Flower size vs.altitude (ns)



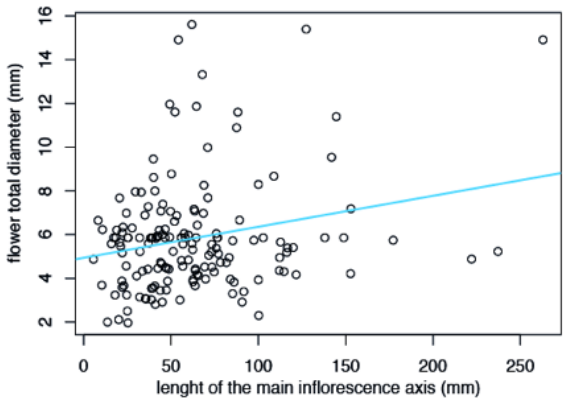
vii) Flower size vs.Estimated number of flowers per inflorescence (ns)



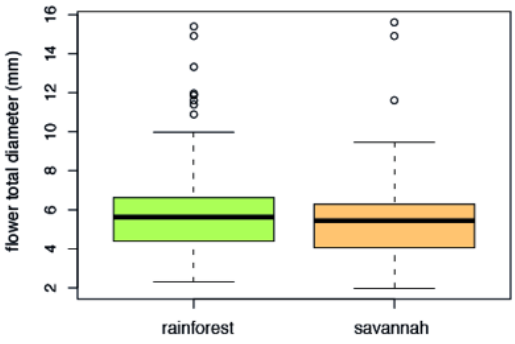
viii) Flower size vs. flower clustering in inflorescence (ns)



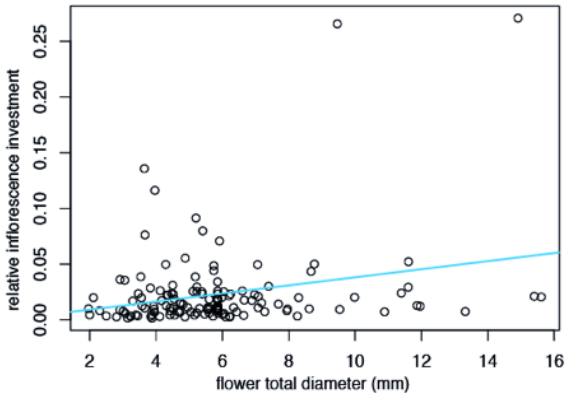
ix) Flower size vs. lenght of the main inflorescence axis (**p<0.01***)



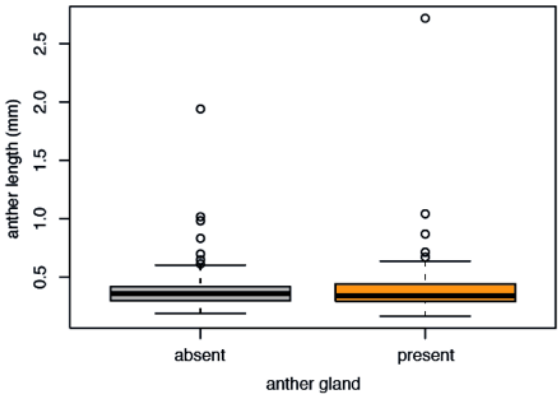
x) Flower size vs. vegetation (ns)



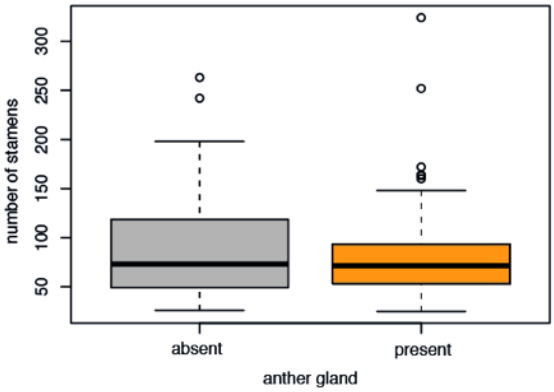
xi) Flower size vs. Relative inflorescence investment (**p<0.01***)



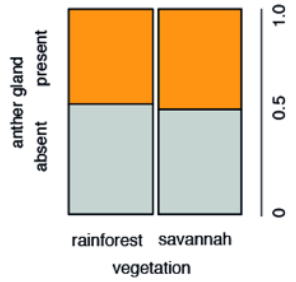
xii) Anther size vs. prensence/absence of anther gland (ns)



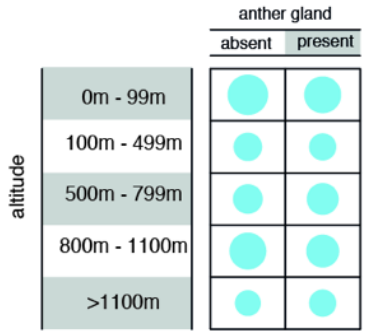
xiii) Number of stamens vs. prensence/absence of anther gland (ns)



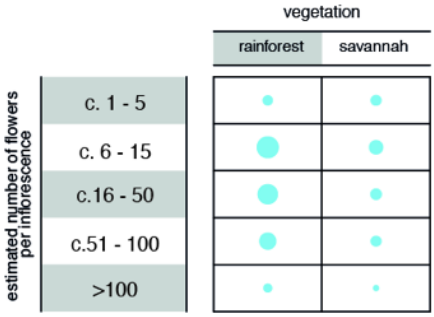
xiv) Anther gland vs. Vegetation (ns)

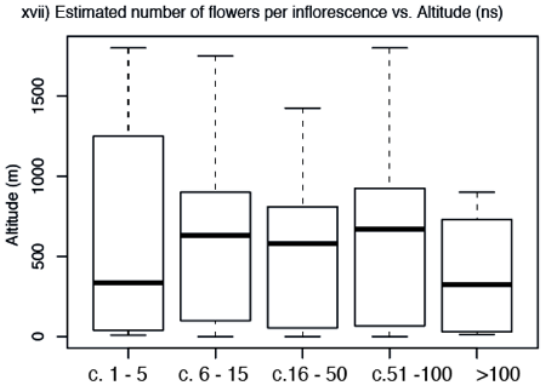


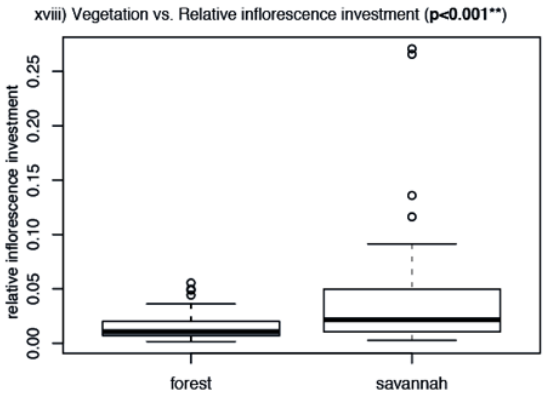
xv) Altitude vs. prensence/absence of anther gland (chi2 plot, ns)

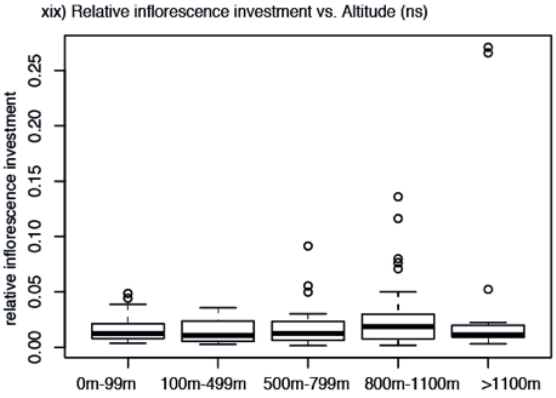


xvi) Estimated number of flowers per inflorescence vs. Vegetation (ns)









7.16 Macro-evolutionary dynamics

Despite apparent heterogeneity in diversity between infra-generic clades of *Myrcia*, all three macro-evolutionary dynamics analyses return similar results indicating no significant diversification rate shifts. BAMM estimates of diversification rate shifts in relation to priors report a strong probability that there are no shifts in the phylogeny (0 shifts, Fig.7.15A). This is corroborated by random shift configurations that show no consistent pattern of acceleration or deceleration of diversification rates (Appendix 7.4, Plot 1). A cohort plot comparing similarity of macro-evolutionary regimes between pairs of phylogeny tips, indicates the strongly homogeneous dynamics of the phylogeny (Appendix 7.4, Plot 2). Some heterogeneity is observed in extinction rate, however, these are not enough to change the general trend of net diversification (speciation minus extinction). RPANDA analysis reports no clear eigengap, providing no evidence of more than one macro-evolutionary regime in the dataset (Appendix 7.4, Plot 3). TESS results show no significant rate shifts (Fig. 7.15B-D); Episodic Birth-Death and Constant Birth-Death are the models that best fits the data, with very similar Bayes factors (49.41 and 44.67, respectively) (see more info in Appendix 7.4, Plot 4).

Results suggest constant and homogeneous accumulation of species diversity throughout the genus. Disparity in species diversity between sections/clades is likely due to the relative older age of some clades over others (based on crown node ages; Fig.7.13Biii). Older clades are therefore more diverse as they have had longer to accumulate species, not due to faster diversification rates. In this way, the highly conservative floral morphology of *Myrcia*, with overlapping clades in morphospace, no obvious environmental specialization and a remarkably

homogeneous phylogenetic framework, provide multiple sources of evidence of a stable and durable evolutionary process.

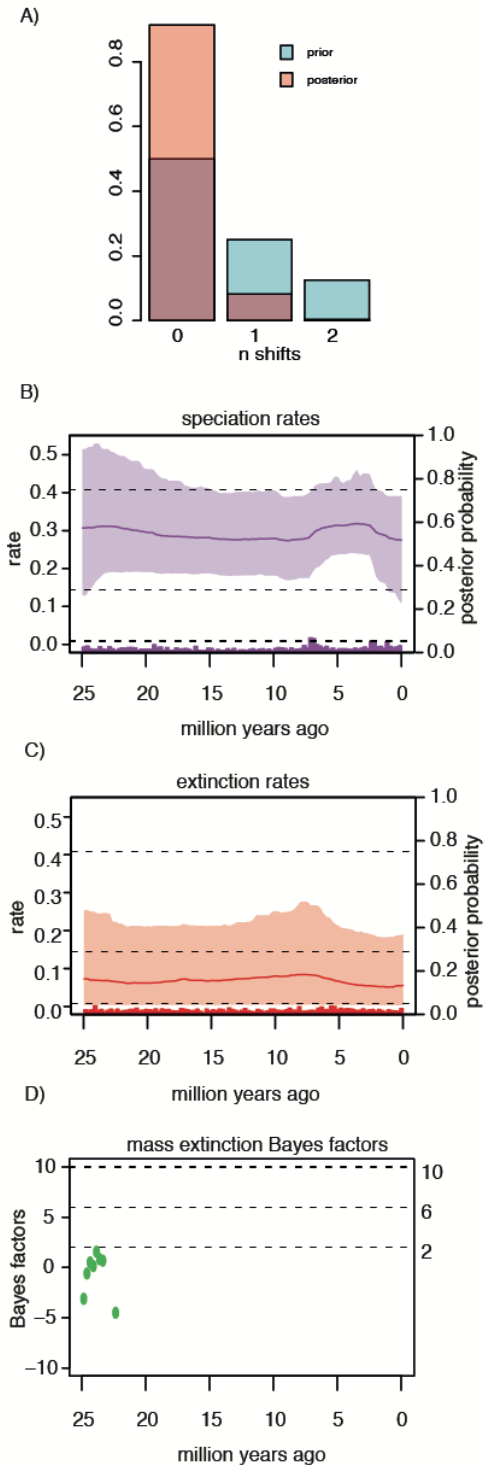


Figure 7.15 – Macroevolutionary homogeneity in *Myrcia*. (A) Posterior probabilities regarding number of shifts in diversification rates in relation to the analysis prior, showing high posterior probability for 0 shifts in BAMM. (B - D) TESS results showing constant (B) speciation and (C) extinction rates and (D) low probability of mass extinction, meaning low probability for species turnover.

7.17 Innovation is not (always) the key: centripetal selection of floral phenotypes

Innovative phenotypes are regarded as essential drivers in altering the diversification rates of a lineage through time. Identification of novelties that accelerate speciation, so called "key-innovations" has been central to evolutionary study in the last two decades (e.g. Hunter, 1998; Blount et al., 2008; Rabosky, 2014). In addition to recognition of key innovative traits, there is a tendency to assume that highly diverse groups with homogeneous phenotypes result from recent explosive speciation events but that there has not yet been time for clear phenotypic disparity (Stebbins, 1974). Both assumptions are particularly applied to tropical environments where excess ecological opportunity favours strong selective pressure for constant trait diversification and species turnover (Koleff et al., 2003).

The stable and durable evolutionary system described here in one of the most diverse and abundant tree genera in the Neotropics challenges both these assumptions. Phenotypic traits in *Myrcia* appear to have been homogeneous for almost 30 million years. The overall trend for less morphological disparity in younger clades is not respected by the two oldest clades suggesting eventual morphological stabilization. This pattern may be linked to the co-occurrence of the vast majority of species in the same area of the floral morphospace. Non-correlation of phylogenetic and morphological distances means that even distantly related species are selected towards a similar conservative, non-extreme phenotype with continuous morphological intermediates.

A lack of clustering in the morphospace, associated with floral specialization in other groups (e.g. Perret et al., 2007) combined with low levels of extreme scattering is interpreted as centripetal selection (Eldredge, 1984). Centripetal selection leads to extinction of very distinct floral morphologies favouring similarity over extremes under selective inertia (Stebbins, 1974). Further evidence that extreme phenotypes are selected against, is that some common and widespread *Myrcia* (e.g. *M. tomentosa, M. guianensis, M. splendens*; WCSP, 2017) emerge at the centre of the morphospace, whilst rarer or phylogenetically more isolated species (*Myrcia antonia, M. insigniflora*) are more frequently outliers. This may indicate that extreme phenotypes are more prone to extinction in the long term.

7.18 Walking in circles at the top of an advantageous adaptive peak

Such tendency to maintain highly stable floral phenotypes over long periods is observable in other large genera of Neotropical woody angiosperms (e.g. *Solanum,* Symon, 1979; Malpighiaceae, Anderson, 1979). It has been suggested that the overall homogeneous morphologies exhibited by these groups are examples of very established adaptive peaks (Renner, 1989; for *Miconia*). *Myrcia* flowers do not offer nectar but rely on bees as the sole functional pollinator (Willmer, 2011). In these cases, the link between homogenous flowers and pollen foraging bees is so advantageous that distinct strategies rarely appear (Renner, 1989). In fact, evidence from reproductive biology shows that the pollinators and pollination mode of *Myrcia* is similar throughout its geographic and phylogenetic range (see Fig.7.16 and Table 7.6). The bees responsible are mostly medium to large bodied and solitary with poliletic (generalist) female individuals that gather large quantities of pollen to feed their larvae at peak flowering time in *Myrcia* (Staggemeier et al., 2010).

This favourable bee-*Myrcia* relationship may have existed since the origin of both groups. *Myrcia* age and areas of early-diversification events on South American plateaus (Santos et al., 2017) correspond well to those of their most important bee pollinators (e.g. *Melipona*; Ramirez et al., 2009). This relationship may explain why, despite being a relatively old lineage, floral traits remain similar. Extreme phenotypes are selected against as they have lower fitness in a stable ecological system. Discreet specializations in floral morphology occur (See e.g. distribution of floral-organ size per clade in Figs. 7.9 and 7.10), but these do not destabilize the system or influence macro-evolutionary dynamics in *Myrcia*.

	Species	**Clade**	**Pollinator group****	**Biome**
i	*Myrcia racemosa*	*Aulomyrcia*	Apidae: Bombinae (Bombus morio) and Meliponinae (Melipona rufiventris, Melipona bicolor)	Atlantic Rainforest
ii	*Myrcia amazonica*	*Aulomyrcia*	Apidae: Meliponinae	NA
iii	*Myrcia brasiliensis*	*Gomidesia*	Apidae: Bombinae and Meliponinae; Anthophoridae: Xylocopinae	Atlantic Rainforest
iv	*Myrcia paivae*	*Myrcia*	Apidae: Meliponinae; Anthophoridae: Anthophorinae	Amazon Rainforest
v	*Myrcia tomentosa*	*Tomentosa*	Apidae: Bombinae; Anthophoridae: Xylocopinae Apidae: Meliponinae; Megachilidae: Megachilinae	Cerrado, Atlantic Rainforest
vi	*Myrcia multiflora*	*Aulomyrcia*	Apidae: Bombinae and Meliponinae; Anthophoridae: Xylocopinae; Halictidae: Halictinae	Atlantic Rainforest
vii	*Myrcia splendens*	*Myrcia*	Apidae: Meliponinae; Halictidae: Halictinae	Cerrado

Table 7.6: Effective pollinators of seven species of *Myrcia* and their biomes (according to review of Gressler et al., 2006). Roman numerals refer to the Figure 7.16.

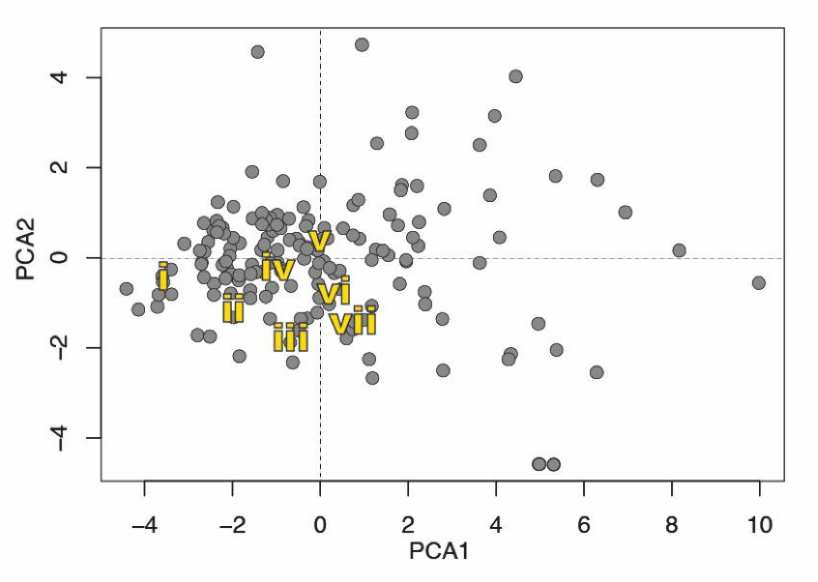


Figure 7.16: *Myrcia* floral morphospace with roman numeral indicating species with reproductive biology information available (see Table 7.6).

7.19 The dry diagonal did not destabilize the system

Evidence that *Myrcia* floral evolution is circular around a very stable adaptive peak is further supported by its lack of environmental specializations. Similar approaches showing positive correlations between phenotypic traits and abiotic factors are common (e.g. Galen, 1989). In *Myrcia* however, floral traits, possibly advantageous in a given ecosystem (e.g. number of stamens, anther oil gland, and flower size) do not correlate with environmental factors (e.g. altitude and vegetation). The only positive correlation returned, between relative inflorescence investment and vegetation type, may be linked to the origin of the dry-diagonal in South America (Werneck, 2011). As a widespread Neotropical group with a rainforest origin (Santos et al. 2017), *Myrcia* evolution was punctuated by the arrival of cerrado vegetation c. 10mya (Simon et al. 2009). Clear evidence of this is provided by the proportional increase in investment in the inflorescence visible during this period (Fig.7.17). This corroborates once again how floral and inflorescence phenotypes are constrained in *Myrcia* with the appearance of an entirely new and significantly different biome modifying plant habit but not floral phenotype.

7.20 Implications for macroevolution dynamics of tropical lineages

Richardson et al. (2001) link Neotropical rainforest tree biodiversity to recent explosive speciation events (i.e. the cradle hypothesis). The counter-argument is that high tropical rainforest diversity can be better explained by a long process of low extinction rates (i.e. the museum hypothesis). Since then, the relative influence of these hypothetical processes and the extent to which they explain high diversity of tropical rainforests has been hotly debated (e.g. Eiserhardt et al., 2017). In this context, the durable, stable phenotype and conservative macroevolutionary dynamics of *Myrcia* better support the museum hypothesis. This contrasts with the premature speculation Lucas and Bunger (2015) who assumed significant diversification rate shifts would be found in *Myrcia.*

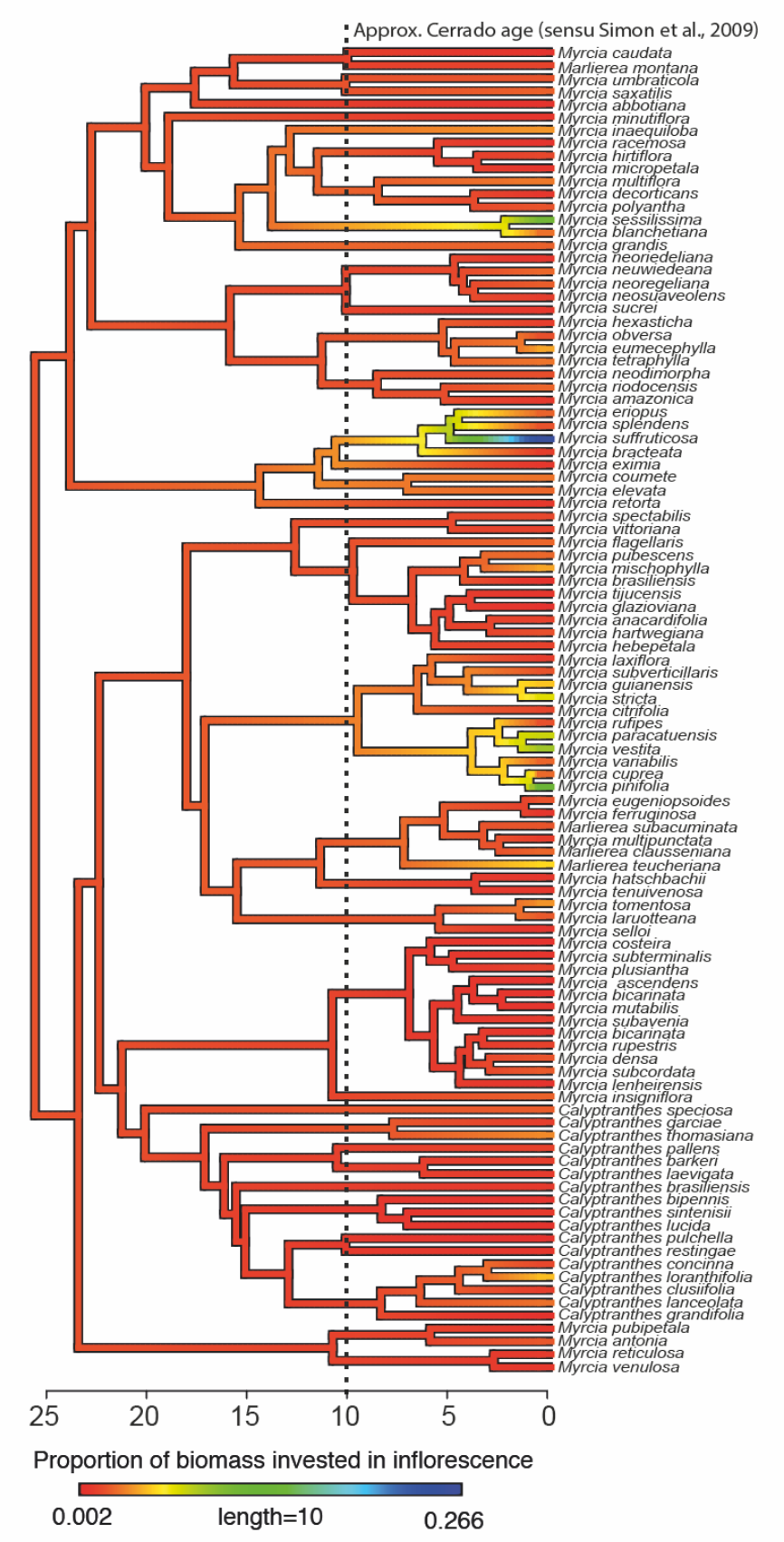


Figure 7.17: Proportional inflorescence investment in *Myrcia* phylogeny. Lineages with high proportional investment in inflorescence appear in the last 10 million years, matching the origin of the cerrado.

The tendency to polarise processes that have driven evolutionary biology in different biomes is problematic as these processes are affected by high levels of stochasticity (Lenormand et al., 2009). *Myrcia* co-occur and share a similar evolutionary history with lineages that are adhere variously with the cradle and museum hypotheses (Lucas and Bunger, 2015); results presented here emphasize this complex spectrum of eco-evolutionary systems present in tropical forests. Care must be taken before solely relating any given biome to cradle and museum patterns but should also take into account the eco-evolutionary systems of each lineage (Buckley and Jetz, 2008; for vertebrates). However, until the role of a lineage in its niche is completed known, analysing its ecological limits (phenotypic trends and functional traits) in conjunction with its evolutionary history (phylogenetic framework and branching pattern), assumptions regarding the evolutionary dynamics of a lineage are dangerous.

CONCLUSION

The origins of high species diversity in the absence of phenotypic change and maintenance of long lasting adaptive peaks are important elements in the evolution of tropical diversity. The conclusions of this study are 1) stable ecological-evolutionary systems may last for tens of millions of years even in environments full of ecological opportunities; 2) such stability keeps speciation rates constant and extinction rates low, leading to slow species accumulation over time; and 3) homogeneous morphology in largely diverse groups is not necessarily a result of explosive recent diversification events. In the case of *Myrcia,* the lack of phenotypic innovations may have been key to its success, building its remarkable species richness slowly but surely.

APPENDIX

Appendix 7.1: Voucher list and *Myrcia* floral morphological data. “phylo”: species is included in the phylogenetic analysis (y = yes, n = no); “nº”: refers to same numbers used in Appendix 7.5. Names according to WCSP (2017) and Lucas et al. 2016 (for clade *Aulomyrcia*).

phylo	voucher	locallity	nº	species	clade
y	D.H. Daris 186	Guiana	1	*Marlierea montana*	*Aulomyrcia*
y	E.J. Lucas 1108	Dominican Republic	2	*Myrcia abbotiana*	*Aulomyrcia*
y	J.J. de Granville 14303	French Guiana	3	*Myrcia amazonica*	*Aulomyrcia*
y	E. Melo 1362	Brazil (BA)	4	*Myrcia blanchetiana*	*Aulomyrcia*
y	W. Thomas 3864	Brazil (MT)	5	*Myrcia caudata*	*Aulomyrcia*
y	M.L.C. Neves 4	Brazil (BA)	6	*Myrcia decorticans*	*Aulomyrcia*
y	D.A. Folli 6414	Brazil (ES)	7	*Myrcia eumecephylla*	*Aulomyrcia*
y	C.F.P. von Martius 59	Brazil	8	*Myrcia excoriata*	*Aulomyrcia*
y	P. Acevedo-Rodriguez 8251	Brazil (AM)	9	*Myrcia grandis*	*Aulomyrcia*
y	S.V.A. Pessoa 1217	Brazil (RJ)	10	*Myrcia hexasticha*	*Aulomyrcia*
y	R.M. Harley 17943	Brazil (BA)	11	*Myrcia hirtiflora*	*Aulomyrcia*
y	Forest Department of British Guiana 2813	Guiana	12	*Myrcia inaequiloba*	*Aulomyrcia*
y	A. Ducke 291	Brazil (AM)	13	*Myrcia mcvaughii*	*Aulomyrcia*
y	J.L. da Paixão 289	Brazil (BA)	14	*Myrcia micropetala*	*Aulomyrcia*
y	P.A.C.I. Assunção 759	Brazil (AM)	15	*Myrcia minutiflora*	*Aulomyrcia*
y	M.F. Simon 219	Brazil (CE)	16	*Myrcia multiflora*	*Aulomyrcia*
y	R.M. Harley 27748	Brazil (BA)	17	*Myrcia neobscura*	*Aulomyrcia*
y	D.A. Folli 5747	Brazil (ES)	18	*Myrcia neodimorpha*	*Aulomyrcia*
y	L. Riedel 197	Brazil (RJ)	19	*Myrcia neograbla*	*Aulomyrcia*
y	K. Matsumoto 814	Brazil (ES)	20	*Myrcia neoregeliana*	*Aulomyrcia*
y	K. Matsumoto 770	Brazil (SP)	21	*Myrcia neoriedeliana*	*Aulomyrcia*
y	M.F. Santos 791	Brazil (RJ)	22	*Myrcia neosuaveolens*	*Aulomyrcia*
y	J.E.Q. Faria 6303	Brazil (SP)	23	*Myrcia neotomentosa*	*Aulomyrcia*
y	K. Matsumoto 815	Brazil (ES)	24	*Myrcia neuwiedeana*	*Aulomyrcia*
y	S.A. Mori 14129	Brazil (BA)	25	*Myrcia obversa*	*Aulomyrcia*
y	E. Ule. 8672	Brazil (RR)	26	*Myrcia platyclada*	*Aulomyrcia*
y	E. Melo 4260	Brazil (BA)	27	*Myrcia polyantha*	*Aulomyrcia*
y	Glaziou 11996	Brazil (RJ)	28	*Myrcia racemosa 1*	*Aulomyrcia*
n	H.C. de Lima 5868	Brazil (RJ)	29	*Myrcia racemosa 2*	*Aulomyrcia*
y	D. Sucre 7378	Brazil (MG)	30	*Myrcia racemosa 3*	*Aulomyrcia*
y	D.A. Folli 3197	Brazil (ES)	31	*Myrcia riodocensis*	*Aulomyrcia*
n	D.F. Lima 495	Brazil (GO)	32	*Myrcia rubella*	*Aulomyrcia*
y	J. Molino 2161	French Guiana	33	*Myrcia saxatilis*	*Aulomyrcia*
y	M.F. Santos 641	Brazil (MG)	34	*Myrcia sessilisima*	*Aulomyrcia*
y	S.A. Mori 13030	Brazil (BA)	35	*Myrcia sucrei*	*Aulomyrcia*
y	V.G. Staggemeier 926	Brazil (BA)	36	*Myrcia tetraphylla*	*Aulomyrcia*
y	T.N.C. Vasconcelos 311	Brazil (AM)	37	*Myrcia umbraticola*	*Aulomyrcia*
n	E.J.Lucas 221	Brazil (RJ)	38	*Calyptranthes aromatica*	*Calyptranthes*

y	T. Clase 7417	Dominican Republic	39	*Calyptranthes barkeri*	*Calyptranthes*
y	J.Y. Tamashiro 10537	Brazil (SP)	40	*Calyptranthes bipennis*	*Calyptranthes*
y	J.E.Q. Faria 4244	Brazil (ES)	41	*Calyptranthes brasiliensis*	*Calyptranthes*
y	S.A. Mori 9301	Brazil (BA)	42	*Calyptranthes clusiifolia*	*Calyptranthes*
y	O. Handro. sn	Brazil (SP)	43	*Calyptranthes concinna*	*Calyptranthes*
y	E.L. Ekman 13896	Dominican Republic	44	*Calyptranthes eriocephala*	*Calyptranthes*
y	T.A.W. Davis 2237	Guiana	45	*Calyptranthes fasciculata*	*Calyptranthes*
y	A.C. Araujo 1802	Dominican Republic	46	*Calyptranthes garciae*	*Calyptranthes*
n	G.G. Hatschbach 20899	Brazil (PR)	47	*Calyptranthes grandiflora 1*	*Calyptranthes*
n	P.S.S. Ferreira 2	Brazil (SP)	48	*Calyptranthes grandiflora 2*	*Calyptranthes*
y	R.M. Harley 2655	Brazil (BA)	49	*Calyptranthes grandiflora 3*	*Calyptranthes*
y	O. Handro s.n.	Brazil (SP)	50	*Calyptranthes grandifolia 4*	*Calyptranthes*
n	Hatschbachii 13142	Brazil (PR)	51	*Calyptranthes hatchbachii*	*Calyptranthes*
y	M. Hamilton 1	BVI Gorda Peak National Park	52	*Calyptranthes kiaerskovii*	*Calyptranthes*
y	T. Clase 7475	Dominican Republic	53	*Calyptranthes laevigata*	*Calyptranthes*
y	L.A. Mattos-Silva 492	Brazil (BA)	54	*Calyptranthes lanceolata*	*Calyptranthes*
n	L. Kollman 1631	Brazil (ES)	55	*Calyptranthes langsdrofii*	*Calyptranthes*
y	A.C. Araujo 1827	Brazil (MG)	56	*Calyptranthes loranthifolia*	*Calyptranthes*
y	B. Maguire 24300	Suriname	57	*Calyptranthes lucida*	*Calyptranthes*
n	D.S.Farias 120	Brazil (RJ)	58	*Calyptranthes martiusiana*	*Calyptranthes*
y	T.N.C. Vasconcelos 534	Costa Rica	59	*Calyptranthes pallens*	*Calyptranthes*
y	L. Kollmann 1823	Brazil (ES)	60	*Calyptranthes punchella*	*Calyptranthes*
y	E.J. Lucas 1087	Brazil (BA)	61	*Calyptranthes restigae*	*Calyptranthes*
y	A.C. Araujo 1785	Dominican Republic	62	*Calyptranthes sintenisii*	*Calyptranthes*
y	M.J. Jansen-Jacobs 6568	Suriname	63	*Calyptranthes speciosae*	*Calyptranthes*
y	R. Spruce 1551	Brazil (AM)	64	*Calyptranthes spruceana*	*Calyptranthes*
n	G. Hatschbachii 20886	Brazil (PR)	65	*Calyptranthes strigipes*	*Calyptranthes*
y	M. Hamilton 2	British Virgin islands	66	*Calyptranthes thomasiana*	*Calyptranthes*
n	Sellow sn	Brazil (SP)	67	*Calyptranthes variabilis*	*Calyptranthes*
n	E Nic Lughada 226	Brazil (MG)	68	*Calyptranthes wiedgreniana*	*Calyptranthes*
n	P. Wilson 8401	Bahamas	69	*Calyptranthes zusygium*	*Calyptranthes*
y	J.R. Pirani CFCR13269	Brazil (MG)	70	*Marlierea clausseniana 1*	*Eugeniopsis*
y	S.A. Mori 11027	Brazil (BA)	71	*Marlierea clausseniana 2*	*Eugeniopsis*
y	T.B. Cavalcante SCFCR 8428	Brazil (MG)	72	*Marlierea clausseniana 3*	*Eugeniopsis*
y	E.J. Lucas 225	Brazil (RJ)	73	*Marlierea subacuminata*	*Eugeniopsis*
y	J.M.A. Braga 2916	Brazil (RJ)	74	*Marlierea tenuivenosa*	*Eugeniopsis*
y	E.J. Lucas 673	Brazil (MG)	75	*Marlierea teuscheriana*	*Eugeniopsis*
y	G.G. Hatschbach 10169	Brazil (PR)	76	*Myrcia eugeniopsoides*	*Eugeniopsis*

y	P.R. Reitz 1601	Brazil (SC)	77	*Myrcia ferruginosa*	*Eugeniopsis*
y	G.G. Hatschbach 20475	Brazil (PR)	78	*Myrcia hatschbachii*	*Eugeniopsis*
y	W. Ganev 1209	Brazil (BA)	79	*Myrcia multipuncatata*	*Eugeniopsis*
y	K. Fiebrig 6318	Paraguay	80	*Myrcia oblongata*	*Eugeniopsis*
y	V.G. Sttagemeier 907	Brazil (ES)	81	*Myrcia sp.*	*Eugeniopsis*
y	E.J. Lucas 149	Brazil (PR)	82	*Myrcia tenuivenosa 1*	*Eugeniopsis*
y	G.G. Hatschbach 22470	Brazil (PR)	83	*Myrcia tenuivenosa 2*	*Eugeniopsis*
y	P.R. Reitz 6270	Brazil (SC)	84	*Myrcia anacardifolia*	*Gomidesia*
y	G.G. Hatschbach 23438	Brazil (PR)	85	*Myrcia brasiliensis*	*Gomidesia*
n	P. Fiaschi 3458	Brazil (ES)	86	*Myrcia cerqueira*	*Gomidesia*
n	H.S. Irwin 20640	Brazil (MG)	87	*Myrcia eriocalyx*	*Gomidesia*
n	E.P. Heringer 14897	Brazil (DF)	88	*Myrcia fenzliana*	*Gomidesia*
y	I.R. Costa 515	Brazil (SP)	89	*Myrcia flagellaris*	*Gomidesia*
y	M. Nadruz 1007	Brazil (RJ)	90	*Myrcia glazioviana*	*Gomidesia*
y	C.B. Costa 195	Brazil (SP)	91	*Myrcia hartwegiana 1*	*Gomidesia*
y	J.C. Lindeman 1970	Brazil (PR)	92	*Myrcia hartwegiana 2*	*Gomidesia*
y	E.J. Lucas 64	Brazil (SP)	93	*Myrcia hebepetala*	*Gomidesia*
n	R.M. Harley 19236	Brazil (BA)	94	*Myrcia ilheoensis*	*Gomidesia*
y	W. Ganev 3097	Brazil (BA)	95	*Myrcia mischophylla*	*Gomidesia*
n	J.R. Pirani 540	Brazil (SP)	96	*Myrcia palustris*	*Gomidesia*
y	M.F. Santos 632	Brazil (MG)	97	*Myrcia pubescens*	*Gomidesia*
n	W. Boone 1315	Brazil (ES)	98	*Myrcia ruschii*	*Gomidesia*
y	H.F. Leitão-Filho 34735	Brazil (SP)	99	*Myrcia spectabilis*	*Gomidesia*
y	J.C. Lindeman 13573	Brazil (PR)	100	*Myrcia tijucensis*	*Gomidesia*
y	D.A. Folli 1069	Brazil (ES)	101	*Myrcia vittoriana*	*Gomidesia*
y	Forest Department of British Guiana 2785	Guiana	102	*Myrcia citrifolia*	*Guianensis*
y	H.S. Irwin 5039	Brazil (PA)	103	*Myrcia cuprea*	*Guianensis*
y	B. Stannard CFCR6649	Brazil (MG)	104	*Myrcia guianensis 1*	*Guianensis*
y	E.J. Lucas 136	Brazil (PR)	105	*Myrcia guianensis 2*	*Guianensis*
y	A. Amorim 7130	Brazil (ES)	106	*Myrcia laxiflora*	*Guianensis*
y	L. Riedel 2493	Brazil (MG)	107	*Myrcia paracatuensis*	*Guianensis*
y	Glaziou 21129	Brazil (GO)	108	*Myrcia pinifolia*	*Guianensis*
y	Sandwith 1348	Guiana	109	*Myrcia rotundata*	*Guianensis*
y	G.G. Hatschbach 34709	Brazil (MT)	110	*Myrcia rufipes*	*Guianensis*
n	L.M.Borges 1060	GO Brazil	111	*Myrcia sp.*	*Guianensis*
y	E.P. Heringer 8459	Brazil (DF)	112	*Myrcia stricta*	*Guianensis*
y	E. NicLughada 225	Brazil (MG)	113	*Myrcia subverticillaris*	*Guianensis*
y	I.R. Costa 456	Brazil (MG)	114	*Myrcia variabilis*	*Guianensis*
y	S.A. Mori 16789	Brazil (MT)	115	*Myrcia vestita*	*Guianensis*
y	D.F. Lima 438	Brazil (BA)	116	*Myrcia anceps*	*Myrcia*
y	M.J. Jansen-Jocobs 1822	Guyana	117	*Myrcia bracteata*	*Myrcia*
n	E. P. Heringer 2539	Brazil (DF)	118	*Myrcia capitata*	*Myrcia*
n	T.N.C. Vasconcelos 274	Brazil (GO)	119	*Myrcia cardiaca*	*Myrcia*
y	E.J. Lucas 107	French Guiana	120	*Myrcia coumeta*	*Myrcia*

y	G.T. Prance 3228	Brazil (AM)	121	*Myrcia elevata*	*Myrcia*
y	J. Ball s.n	Brazil (RJ)	122	*Myrcia eriopus*	*Myrcia*
y	L. P. Queiroz 4159	Brazil (BA)	123	*Myrcia eximia*	*Myrcia*
n	H.S.Irwin 8185	Brazil (DF)	124	*Myrcia federalis*	*Myrcia*
n	M.A.D.Souza 182	Brazil (AM)	125	*Myrcia fenestrata*	*Myrcia*
n	D Sucre 11361	Brazil (RJ)	126	*Myrcia ovata*	*Myrcia*
n	Prance 3668	Brazil (AM)	127	*Myrcia paivae*	*Myrcia*
y	J.M. Silva 3849	Brazil (PR)	128	*Myrcia retorta 1*	*Myrcia*
y	V. Nicolack 93	Brazil (PR)	129	*Myrcia retorta 2*	*Myrcia*
n	R Harler 10139	Brazil (MT)	130	*Myrcia schottiana*	*Myrcia*
y	T.N.C. Vasconcelos 591	Dominican Republic	131	*Myrcia splendens*	*Myrcia*
y	R. Mello-Silva 1690	Brazil (MG)	132	*Myrcia suffruticosa*	*Myrcia*
n	J.R.I. Wood. 15435	Bolivia	133	*Myrcia velutina*	*Myrcia*
y	G. Martinelli 9061	Brazil (RJ)	134	*Myrcia antonia*	*Reticulosae*
y	G.G. Hatchbachi 20955	Brazil (PR)	135	*Myrcia pubipetala 1*	*Reticulosae*
y	H.F. Leitão-filho 34701	Brazil (SP)	136	*Myrcia pubipetala 2*	*Reticulosae*
y	R.M. Harley 50309	Brazil (BA)	137	*Myrcia reticulosa*	*Reticulosae*
y	J.M. Cruz 195	Brazil (PR)	138	*Myrcia venulosa 1*	*Reticulosae*
y	R.M. Harley 27168	Brazil (BA)	139	*Myrcia venulosa 2*	*Reticulosae*
y	Lewis CFCR 7074	Brazil (BA)	140	*Myrcia ascendens*	*Sympodiomyrcia*
y	G.G. HatchsbachII 31837	Brazil (PR)	141	*Myrcia bicarinata*	*Sympodiomyrcia*
y	M.F. Santos 757	Brazil (BA)	142	*Myrcia bicolor*	*Sympodiomyrcia*
y	G.G. Hatschbach 31837	Brazil (PR)	143	*Myrcia costeira*	*Sympodiomyrcia*
y	N.P. Taylor 1590	Brazil (BA)	144	*Myrcia densa*	*Sympodiomyrcia*
y	M.F. Santos 682	Brazil (SP)	145	*Myrcia insigniflora*	*Sympodiomyrcia*
y	K. Matsumoto 793	Brazil (MG)	146	*Myrcia lenheirensis*	*Sympodiomyrcia*
y	F.F. Mazine 1052	Brazil (MG)	147	*Myrcia mutabilis*	*Sympodiomyrcia*
y	M. Peron 758	Brazil (RJ)	148	*Myrcia plusiantha*	*Sympodiomyrcia*
y	M.F. Santos 642	Brazil (MG)	149	*Myrcia rupestris*	*Sympodiomyrcia*
y	V.G. Sttagemeier 896	Brazil (ES)	150	*Myrcia sp.*	*Sympodiomyrcia*
y	T.N.C. Vasconcelos 488	Brazil (MG)	151	*Myrcia subavenia*	*Sympodiomyrcia*
y	G. Martinelli 13237	Brazil (RJ)	152	*Myrcia subcordata*	*Sympodiomyrcia*
y	H. Kollmann 4776	Brazil (ES)	153	*Myrcia subterminalis*	*Sympodiomyrcia*
y	J.S. Blanchet 2321	Brazil (BA)	154	*Myrcia tenuifolia*	*Sympodiomyrcia*
y	E.J. Lucas 196	Brazil (SP)	155	*Myrcia laruotteana*	*Tomentosa*
y	E.J. Lucas 110	Brazil (PR)	156	*Myrcia selloi 1*	*Tomentosa*
y	E.J. Lucas 204	Brazil (SP)	157	*Myrcia selloi 2*	*Tomentosa*
y	L.C. Giordano 2168	Brazil (RJ)	158	*Myrcia selloi 3*	*Tomentosa*
y	A.A. Arantes 476	Brazil (MG)	159	*Myrcia tomentosa 1*	*Tomentosa*
y	E.J. Lucas 160	Brazil (PR)	160	*Myrcia tomentosa 2*	*Tomentosa*
y	H.S. Irwin 48254	Brazil (AP)	161	*Myrcia tomentosa 3*	*Tomentosa*

no	species	A	B	C	D	E	F	G
1	*Marlierea montana*	3.162	4.813	4.235	4.239	3.458	1.756	1.643
2	*Myrcia abbotiana*	1.545	3.236	2.965	0.626	1.929	1.146	0.852
3	*Myrcia amazonica*	1.497	2.300	4.534	1.091	3.521	1.675	1.087
4	*Myrcia blanchetiana*	1.910	4.125	6.962	1.956	5.875	1.375	0.647
5	*Myrcia caudata*	3.103	5.160	4.916	2.078	3.973	NA	0.608
6	*Myrcia decorticans*	1.727	5.051	4.811	2.314	3.796	1.766	0.758
7	*Myrcia eumecephylla*	4.414	5.740	4.618	2.962	4.032	NA	0.650
8	*Myrcia excoriata*	6.014	6.979	NA	1.856	NA	NA	0.275
9	*Myrcia grandis*	1.478	4.459	4.854	2.331	4.238	1.865	1.002
10	*Myrcia hexasticha*	1.232	4.949	5.776	3.138	5.434	1.963	0.578
11	*Myrcia hirtiflora*	2.356	4.732	5.724	2.718	4.809	0.945	1.098
12	*Myrcia inaequiloba*	1.393	3.538	5.015	2.373	4.473	1.230	0.644
13	*Myrcia mcvaughii*	3.203	6.870	7.456	4.417	6.123	2.256	2.111
14	*Myrcia micropetala*	2.447	4.721	3.697	2.438	2.792	1.387	1.303
15	*Myrcia minutiflora*	1.230	3.072	2.120	1.822	1.727	1.001	0.447
16	*Myrcia multiflora*	2.215	4.500	4.469	2.890	3.895	2.215	0.492
17	*Myrcia neobscura*	2.953	4.174	6.065	3.249	4.492	0.666	0.461
18	*Myrcia neodimorpha*	3.606	6.658	6.454	3.992	5.274	1.803	0.852
19	*Myrcia neograbla*	3.290	4.211	5.176	1.880	4.254	0.357	1.173
20	*Myrcia neoregeliana*	4.690	6.969	3.531	2.231	2.844	1.115	0.455
21	*Myrcia neoriedeliana*	2.456	3.390	3.190	NA	2.641	0.404	0.514
22	*Myrcia neosuaveolens*	1.374	NA	2.359	0.932	1.881	0.644	0.941
23	*Myrcia neotomentosa*	7.862	8.779	8.509	7.736	7.603	1.689	0.692
24	*Myrcia neuwiedeana*	6.542	6.600	4.890	3.807	3.810	0.851	1.150
25	*Myrcia obversa*	3.725	NA	5.021	2.724	2.888	2.113	2.693
26	*Myrcia platyclada*	1.530	3.020	2.795	1.489	1.975	1.307	0.644
27	*Myrcia polyantha*	1.400	2.110	2.566	1.594	2.039	1.470	0.891
28	*Myrcia racemosa 1*	4.875	8.000	8.635	5.105	8.268	3.512	1.946
29	*Myrcia racemosa 2*	3.693	6.092	NA	2.536	NA	1.566	1.128
30	*Myrcia racemosa 3*	1.491	3.238	3.387	1.664	2.826	0.976	0.758
31	*Myrcia riodocensis*	3.116	6.040	6.762	3.687	6.126	2.221	1.284
32	*Myrcia rubella*	2.685	5.406	5.724	5.994	4.775	2.140	0.886
33	*Myrcia saxatilis*	1.229	3.463	3.231	1.467	2.522	1.596	0.836
34	*Myrcia sessilisima*	1.836	3.965	5.795	1.935	4.934	1.651	0.820
35	*Myrcia sucrei*	10.220	13.319	NA	5.569	NA	0.386	0.601
36	*Myrcia tetraphylla*	2.232	5.228	5.360	3.589	4.705	2.090	0.782
37	*Myrcia umbraticola*	5.870	5.974	5.427	2.496	4.425	0.392	0.287
38	*Calyptranthes aromatica*	3.014	5.388	9.042	3.903	7.992	NA	1.179
39	*Calyptranthes barkeri*	3.342	5.862	6.863	3.729	5.988	1.389	1.731
40	*Calyptranthes bipennis*	2.086	3.446	5.473	2.364	4.666	0.000	0.783
41	*Calyptranthes brasiliensis*	2.009	2.912	5.859	4.741	5.557	0.000	1.671
42	*Calyptranthes clusiifolia*	2.780	4.513	10.908	2.982	9.732	0.707	1.008
43	*Calyptranthes concinna*	2.100	4.727	6.681	3.750	5.572	0.991	2.298

44	*Calyptranthes eriocephala*	3.650	7.677	NA	3.223	NA	0.000	1.119
45	*Calyptranthes fasciculata*	2.274	3.684	4.188	1.505	3.592	0.000	0.737
46	*Calyptranthes garciae*	4.132	7.077	5.447	2.751	4.282	1.633	2.142
47	*Calyptranthes grandiflora 1*	3.689	5.553	8.491	3.980	7.205	1.253	2.571
48	*Calyptranthes grandiflora 2*	4.103	5.600	7.929	6.054	6.856	0.000	1.700
49	*Calyptranthes grandiflora 3*	2.893	4.166	7.229	3.152	6.165	1.124	1.156
50	*Calyptranthes grandifolia 4*	1.984	3.842	6.847	2.732	5.917	0.782	1.427
51	*Calyptranthes hatchbachii*	2.739	5.834	7.110	3.163	5.662	NA	1.919
52	*Calyptranthes kiaerskovii*	1.964	NA	NA	NA	NA	NA	0.880
53	*Calyptranthes laevigata*	1.316	1.965	2.338	0.933	1.830	0.650	0.893
54	*Calyptranthes lanceolata*	2.240	3.827	4.820	3.007	3.808	1.109	1.668
55	*Calyptranthes langsdrofii*	1.956	3.143	4.391	1.742	3.487	NA	1.456
56	*Calyptranthes loranthifolia*	2.129	4.290	4.818	1.498	4.116	0.749	0.324
57	*Calyptranthes lucida*	2.485	2.807	6.989	4.789	5.697	0.000	NA
58	*Calyptranthes martiusiana*	3.398	5.739	9.389	3.786	8.424	0.717	1.895
59	*Calyptranthes pallens*	2.648	3.928	6.162	2.485	4.796	0.000	1.047
60	*Calyptranthes punchella*	2.255	3.873	5.579	3.241	4.699	NA	0.826
61	*Calyptranthes restigae*	1.961	3.011	4.662	2.841	3.355	0.000	2.318
62	*Calyptranthes sintenisii*	1.911	3.941	5.130	1.861	4.173	NA	0.743
63	*Calyptranthes speciosae*	2.945	4.356	NA	3.726	NA	0.000	1.202
64	*Calyptranthes spruceana*	4.302	7.161	12.222	4.835	10.574	0.000	2.572
65	*Calyptranthes strigipes*	2.214	3.870	6.298	2.768	5.532	NA	1.071
66	*Calyptranthes thomasiana*	1.930	3.055	4.793	2.132	4.048	0.722	1.032
67	*Calyptranthes variabilis*	2.376	6.298	8.255	5.148	7.319	1.726	1.481
68	*Calyptranthes wiedgreniana*	3.028	NA	NA	3.354	NA	NA	1.733
69	*Calyptranthes zusygium*	2.994	5.928	7.780	4.422	6.586	NA	2.405
70	*Marlierea clausseniana 1*	2.313	NA	6.840	4.920	6.248	1.425	1.273
71	*Marlierea clausseniana 2*	2.743	5.716	6.410	3.924	5.393	2.246	1.397
72	*Marlierea clausseniana 3*	2.282	NA	6.415	NA	5.718	1.390	1.019
73	*Marlierea subacuminata*	7.548	9.982	11.384	6.145	8.758	1.915	1.840
74	*Marlierea tenuivenosa*	1.354	3.660	4.695	3.110	3.872	1.364	0.450
75	*Marlierea teuscheriana*	2.166	4.878	NA	3.004	NA	1.214	0.587
76	*Myrcia eugeniopsoides*	3.726	8.297	8.823	6.406	7.854	2.185	1.148
77	*Myrcia ferruginosa*	4.458	6.048	12.599	6.192	9.129	1.610	1.785
78	*Myrcia hatschbachii*	1.551	3.954	5.625	1.391	4.731	1.269	0.589
79	*Myrcia multipuncatata*	2.552	5.823	6.396	3.082	5.368	1.322	0.919
80	*Myrcia oblongata*	2.190	4.945	3.785	3.059	3.155	1.691	0.741
81	*Myrcia sp.*	3.147	NA	NA	2.489	NA	1.882	0.661
82	*Myrcia tenuivenosa 1*	1.646	4.354	4.064	2.274	3.275	1.607	0.362
83	*Myrcia tenuivenosa 2*	1.443	4.427	4.829	2.358	4.158	1.445	0.641
84	*Myrcia anacardifolia*	3.810	11.960	12.231	7.382	10.981	7.185	2.229
85	*Myrcia brasiliensis*	4.125	8.258	8.321	5.926	6.221	4.289	1.278
86	*Myrcia cerqueira*	2.978	NA	9.318	5.472	7.782	6.038	1.425
87	*Myrcia eriocalyx*	3.289	NA	8.061	4.793	7.192	4.373	3.949

88	*Myrcia fenzliana*	2.418	NA	7.142	4.568	6.591	3.859	1.079
89	*Myrcia flagellaris*	3.270	5.230	3.103	1.630	2.317	2.751	1.840
90	*Myrcia glazioviana*	3.051	6.643	6.071	2.273	5.095	2.286	2.203
91	*Myrcia hartwegiana 1*	2.443	6.200	6.200	4.043	NA	4.793	1.412
92	*Myrcia hartwegiana 2*	2.338	5.600	6.962	4.349	5.872	4.168	1.344
93	*Myrcia hebepetala*	3.472	11.867	NA	4.651	NA	4.158	0.833
94	*Myrcia ilheoensis*	3.089	15.608	9.623	5.050	9.097	5.723	1.664
95	*Myrcia mischophylla*	4.154	8.670	7.725	4.492	6.184	3.708	1.094
96	*Myrcia palustris*	1.939	NA	6.759	4.310	5.600	3.252	0.547
97	*Myrcia pubescens*	2.936	5.117	5.013	2.693	4.231	2.139	0.856
98	*Myrcia ruschii*	4.709	14.911	11.588	4.768	8.420	4.364	1.931
99	*Myrcia spectabilis*	3.307	7.180	10.286	4.342	8.906	2.945	0.976
100	*Myrcia tijucensis*	1.991	5.223	5.363	2.202	4.163	2.197	0.596
101	*Myrcia vittoriana*	3.262	NA	9.442	5.656	7.973	4.739	1.467
102	*Myrcia citrifolia*	2.474	7.685	7.442	5.827	6.437	2.694	1.491
103	*Myrcia cuprea*	2.321	4.546	6.297	2.704	5.482	1.755	0.417
104	*Myrcia guianensis 1*	2.260	7.062	6.278	5.271	4.771	2.150	1.136
105	*Myrcia guianensis 2*	2.448	6.208	6.625	3.892	5.495	2.024	0.605
106	*Myrcia laxiflora*	2.514	5.312	5.806	3.490	4.904	2.002	0.443
107	*Myrcia paracatuensis*	1.938	3.663	3.929	1.854	3.012	1.933	0.911
108	*Myrcia pinifolia*	1.888	3.644	NA	2.247	NA	1.709	0.757
109	*Myrcia rotundata*	1.610	4.456	4.709	3.354	3.705	1.868	0.898
110	*Myrcia rufipes*	1.545	3.296	5.511	2.271	4.744	1.640	0.523
111	*Myrcia sp.*	3.501	NA	8.204	4.987	6.753	4.207	0.770
112	*Myrcia stricta*	3.166	5.913	5.757	2.936	3.875	3.139	1.298
113	*Myrcia subverticillaris*	2.864	NA	8.124	7.282	7.083	4.245	0.769
114	*Myrcia variabilis*	3.062	6.246	6.868	5.385	6.520	2.550	0.920
115	*Myrcia vestita*	3.341	5.200	8.996	4.548	8.075	2.770	1.530
116	*Myrcia anceps*	2.157	4.519	5.801	3.217	4.711	1.372	0.500
117	*Myrcia bracteata*	3.437	7.963	9.251	3.416	8.574	2.194	2.834
118	*Myrcia capitata*	4.944	11.609	10.404	11.008	9.314	4.270	4.763
119	*Myrcia cardiaca*	4.558	8.773	7.636	5.878	5.526	3.854	2.001
120	*Myrcia coumeta*	5.693	11.601	9.017	5.341	6.817	4.927	1.743
121	*Myrcia elevata*	3.614	5.378	2.301	1.282	1.582	1.302	1.048
122	*Myrcia eriopus*	3.131	5.658	5.340	3.267	4.082	2.540	1.560
123	*Myrcia eximia*	3.550	6.429	6.492	4.113	5.205	2.925	0.863
124	*Myrcia federalis*	4.888	14.909	NA	7.741	NA	4.946	4.982
125	*Myrcia fenestrata*	1.285	4.666	4.417	3.096	3.741	1.767	0.790
126	*Myrcia ovata*	2.126	4.423	2.955	2.075	2.008	2.157	1.153
127	*Myrcia paivae*	1.677	5.639	5.267	3.205	3.953	1.704	0.703
128	*Myrcia retorta 1*	3.264	7.275	6.644	6.927	5.779	1.381	2.724
129	*Myrcia retorta 2*	3.092	7.941	4.894	3.008	3.660	2.836	0.872
130	*Myrcia schottiana*	2.937	6.229	6.690	6.152	5.883	2.380	2.131
131	*Myrcia splendens*	3.290	6.888	5.305	2.531	3.742	2.314	1.584

132	*Myrcia suffruticosa*	3.363	9.462	7.853	6.102	6.443	3.764	2.171
133	*Myrcia velutina*	2.369	6.335	4.627	2.495	3.277	2.154	1.046
134	*Myrcia antonia*	8.570	15.397	13.973	7.762	11.613	4.217	2.651
135	*Myrcia pubipetala 1*	5.584	9.537	9.337	5.435	7.435	4.723	1.884
136	*Myrcia pubipetala 2*	5.903	10.889	8.714	5.275	6.210	3.509	2.608
137	*Myrcia reticulosa*	2.168	5.581	8.230	4.778	5.813	2.407	1.226
138	*Myrcia venulosa 1*	3.183	5.564	6.943	4.802	5.930	3.617	1.059
139	*Myrcia venulosa 2*	2.478	5.880	7.052	4.529	5.535	2.245	1.066
140	*Myrcia ascendens*	1.884	4.870	3.565	2.840	3.065	1.545	0.772
141	*Myrcia bicarinata*	2.488	4.418	4.539	2.525	3.653	1.613	0.722
142	*Myrcia bicolor*	2.252	4.111	5.555	2.571	4.829	1.258	0.822
143	*Myrcia costeira*	1.890	2.500	6.000	3.100	5.000	1.800	1.400
144	*Myrcia densa*	1.606	3.559	4.696	2.459	3.753	1.171	0.731
145	*Myrcia insigniflora*	7.052	11.392	13.458	7.733	10.663	3.738	1.036
146	*Myrcia lenheirensis*	1.678	2.000	5.180	2.595	4.514	1.337	0.689
147	*Myrcia mutabilis*	2.865	5.466	6.277	4.162	5.322	2.299	1.384
148	*Myrcia plusiantha*	3.067	4.305	6.667	2.646	5.028	1.438	0.795
149	*Myrcia rupestris*	1.870	3.580	4.260	1.840	3.700	1.130	1.150
150	*Myrcia sp.*	1.325	2.915	NA	0.239	NA	0.656	0.733
151	*Myrcia subavenia*	4.392	8.617	7.553	NA	5.427	2.532	2.171
152	*Myrcia subcordata*	2.151	4.328	5.529	4.312	4.640	1.463	0.608
153	*Myrcia subterminalis*	2.380	6.000	4.190	2.440	3.650	NA	0.950
154	*Myrcia tenuifolia*	2.332	4.835	5.800	2.495	4.400	1.094	0.850
155	*Myrcia laruotteana*	2.542	7.078	6.383	4.713	4.571	2.187	1.235
156	*Myrcia selloi 1*	1.392	NA	5.198	3.272	4.070	2.105	0.770
157	*Myrcia selloi 2*	1.830	NA	6.365	NA	5.268	NA	0.728
158	*Myrcia selloi 3*	1.458	4.562	4.751	3.221	3.430	1.537	0.686
159	*Myrcia tomentosa 1*	1.994	5.181	5.738	3.075	4.579	2.111	1.251
160	*Myrcia tomentosa 2*	2.751	NA	6.514	NA	5.467	2.760	1.439
161	*Myrcia tomentosa 3*	2.506	7.393	6.282	3.434	5.896	2.217	1.373

no	species	H	I	J	K	L	M	N	O
1	*Marlierea montana*	140	0.236	0.341	1.125	0.969	0.827	0.416	68
2	*Myrcia abbotiana*	NA	0.382	0.196	0.333	0.398	0.164	0.392	36
3	*Myrcia amazonica*	58	0.262	0.377	0.164	0.476	0.112	0.366	37
4	*Myrcia blanchetiana*	107	0.295	0.228	0.876	0.499	0.086	0.372	48
5	*Myrcia caudata*	137	0.313	0.880	0.886	0.881	0.077	0.336	NA
6	*Myrcia decorticans*	140	0.407	0.120	0.282	0.464	0.100	0.464	40
7	*Myrcia eumecephylla*	139	0.339	1.337	0.891	0.715	0.125	0.210	36
8	*Myrcia excoriata*	161	0.278	1.971	0.420	0.439	NA	0.383	81
9	*Myrcia grandis*	104	0.317	0.313	0.448	0.495	0.161	0.389	45
10	*Myrcia hexasticha*	67	0.273	0.246	0.301	0.373	0.096	0.283	33
11	*Myrcia hirtiflora*	135	0.322	0.213	0.463	0.384	0.114	0.314	41

12	*Myrcia inaequiloba*	103	0.304	0.179	0.756	0.424	0.173	0.304	25
13	*Myrcia mcvaughii*	132	0.292	0.360	1.024	0.946	0.102	0.420	91
14	*Myrcia micropetala*	148	0.449	0.299	1.036	0.808	0.134	0.355	45
15	*Myrcia minutiflora*	139	0.290	0.151	0.708	0.355	0.274	0.125	26
16	*Myrcia multiflora*	107	0.312	0.295	0.472	0.443	0.141	0.408	44
17	*Myrcia neobscura*	157	0.283	1.008	0.665	0.483	0.124	0.394	45
18	*Myrcia neodimorpha*	161	0.443	0.837	0.728	0.742	0.165	0.349	78
19	*Myrcia neograbla*	161	0.493	0.642	0.309	0.255	0.078	0.323	86
20	*Myrcia neoregeliana*	163	0.396	1.129	0.429	0.745	0.094	0.380	82
21	*Myrcia neoriedeliana*	NA	0.275	0.399	NA	0.454	NA	0.210	53
22	*Myrcia neosuaveolens*	NA	0.209	0.181	0.414	0.547	0.078	0.150	31
23	*Myrcia neotomentosa*	143	0.435	1.308	0.667	1.195	0.086	0.531	144
24	*Myrcia neuwiedeana*	146	0.433	1.403	0.669	1.135	0.143	0.374	87
25	*Myrcia obversa*	NA	0.425	0.729	0.627	1.319	0.183	0.552	88
26	*Myrcia platyclada*	128	0.253	0.137	0.601	0.450	0.090	0.368	53
27	*Myrcia polyantha*	NA	0.316	0.148	0.581	0.385	0.091	0.267	49
28	*Myrcia racemosa 1*	95	0.469	1.008	1.232	1.390	0.212	0.476	167
29	*Myrcia racemosa 2*	111	0.475	0.507	1.404	1.082	NA	0.370	164
30	*Myrcia racemosa 3*	129	0.281	0.155	0.642	0.409	0.107	0.271	28
31	*Myrcia riodocensis*	92	0.269	0.491	0.478	0.631	0.127	0.334	83
32	*Myrcia rubella*	111	0.374	0.466	1.109	0.689	0.134	0.587	67
33	*Myrcia saxatilis*	128	0.225	0.161	0.464	0.360	0.081	0.251	26
34	*Myrcia sessilisima*	111	0.319	0.256	0.651	0.399	0.121	NA	37
35	*Myrcia sucrei*	156	0.500	2.341	0.573	1.496	NA	0.543	194
36	*Myrcia tetraphylla*	104	0.353	0.204	0.442	0.534	0.115	0.308	NA
37	*Myrcia umbraticola*	141	0.713	2.261	0.400	0.796	0.164	0.445	41
38	*Calyptranthes aromatica*	134	0.325	0.265	1.700	1.018	0.120	0.390	72
39	*Calyptranthes barkeri*	98	0.371	0.306	0.834	1.050	1.153	0.300	83
40	*Calyptranthes bipennis*	117	0.163	0.169	0.896	0.524	0.107	0.197	56
41	*Calyptranthes brasiliensis*	135	0.296	0.296	1.285	0.508	0.135	0.166	54
42	*Calyptranthes clusiifolia*	123	0.328	0.286	2.051	0.654	0.118	0.436	51
43	*Calyptranthes concinna*	122	0.293	0.465	0.946	0.684	0.192	0.531	80
44	*Calyptranthes eriocephala*	109	0.292	0.304	1.403	0.599	NA	0.255	103
45	*Calyptranthes fasciculata*	160	0.374	0.133	0.742	0.577	0.317	0.313	49
46	*Calyptranthes garciae*	NA	0.408	0.514	0.869	1.047	0.281	0.383	192
47	*Calyptranthes grandiflora 1*	130	0.377	0.443	1.760	0.895	0.169	0.617	126
48	*Calyptranthes grandiflora 2*	89	0.307	0.537	1.742	1.418	0.170	0.492	127
49	*Calyptranthes grandiflora 3*	119	0.394	0.305	1.586	0.774	0.225	0.428	77
50	*Calyptranthes grandifolia 4*	110	0.341	0.263	1.292	0.572	0.115	0.436	68
51	*Calyptranthes hatchbachii*	118	0.345	0.365	1.163	0.819	0.149	0.756	61
52	*Calyptranthes kiaerskovii*	142	0.267	0.195	1.035	0.735	NA	0.345	NA
53	*Calyptranthes laevigata*	103	0.262	0.137	0.343	0.353	0.081	0.238	29
54	*Calyptranthes lanceolata*	106	0.373	0.265	1.114	0.871	0.152	0.465	78
55	*Calyptranthes langsdrofii*	158	0.294	0.119	0.461	0.768	0.117	0.261	41

56	*Calyptranthes loranthifolia*	108	0.239	0.142	0.880	0.621	0.092	0.344	71
57	*Calyptranthes lucida*	59	0.321	0.238	1.017	0.661	0.189	0.337	86
58	*Calyptranthes martiusiana*	146	0.320	0.373	2.044	1.473	0.225	0.269	NA
59	*Calyptranthes pallens*	125	0.371	0.327	0.784	0.635	0.154	0.455	58
60	*Calyptranthes punchella*	89	0.304	0.184	1.211	0.596	0.273	0.527	50
61	*Calyptranthes restigae*	NA	0.241	0.163	0.672	0.667	0.103	0.313	134
62	*Calyptranthes sintenisii*	115	0.308	0.186	0.664	0.663	0.131	0.242	38
63	*Calyptranthes speciosae*	133	0.262	0.270	1.184	0.903	NA	0.380	69
64	*Calyptranthes spruceana*	106	0.351	0.241	1.337	1.339	0.190	1.107	118
65	*Calyptranthes strigipes*	126	0.239	0.165	0.934	0.599	0.108	0.237	NA
66	*Calyptranthes thomasiana*	123	0.224	0.226	0.874	0.666	0.138	0.449	59
67	*Calyptranthes variabilis*	129	0.323	0.132	1.188	0.841	0.153	0.391	74
68	*Calyptranthes wiedgreniana*	NA	0.195	0.265	0.589	0.822	NA	0.304	59
69	*Calyptranthes zusygium*	119	0.288	0.213	1.571	1.114	0.128	0.414	109
70	*Marlierea clausseniana 1*	137	0.441	0.331	1.072	0.790	0.105	0.420	31
71	*Marlierea clausseniana 2*	131	0.299	0.352	1.173	0.706	0.128	0.338	68
72	*Marlierea clausseniana 3*	100	0.374	0.464	1.488	0.730	0.650	0.389	29
73	*Marlierea subacuminata*	122	0.407	1.099	2.154	1.586	0.161	1.179	172
74	*Marlierea tenuivenosa*	63	0.265	0.269	0.533	0.394	0.262	0.380	40
75	*Marlierea teuscheriana*	132	0.334	0.199	1.050	0.726	NA	0.246	56
76	*Myrcia eugeniopsoides*	114	0.371	0.495	1.473	1.228	0.174	0.365	42
77	*Myrcia ferruginosa*	124	0.412	0.227	1.729	1.021	0.214	0.647	113
78	*Myrcia hatschbachii*	92	0.243	0.179	0.918	0.376	NA	0.290	81
79	*Myrcia multipuncatata*	110	0.337	0.372	1.243	0.545	0.182	0.417	53
80	*Myrcia oblongata*	122	0.283	0.232	0.444	0.483	0.472	0.275	100
81	*Myrcia sp.*	NA	NA	0.279	0.924	0.960	0.285	0.309	164
82	*Myrcia tenuivenosa 1*	NA	0.353	0.297	0.469	0.451	0.150	0.331	47
83	*Myrcia tenuivenosa 2*	52	0.263	0.313	0.537	0.379	0.212	0.237	49
84	*Myrcia anacardifolia*	139	1.017	0.575	0.595	0.853	NA	0.346	NA
85	*Myrcia brasiliensis*	134	0.584	0.250	1.107	1.435	0.195	0.761	148
86	*Myrcia cerqueira*	167	1.940	0.514	2.063	0.946	0.200	0.792	54
87	*Myrcia eriocalyx*	175	0.646	0.397	0.485	0.540	0.192	0.408	69
88	*Myrcia fenzliana*	134	0.518	0.307	0.306	0.680	0.214	0.508	92
89	*Myrcia flagellaris*	NA	0.436	0.160	0.435	0.909	0.142	0.371	104
90	*Myrcia glazioviana*	113	0.322	0.580	0.363	0.445	0.103	0.511	133
91	*Myrcia hartwegiana 1*	109	0.832	0.458	1.072	0.966	0.093	0.518	124
92	*Myrcia hartwegiana 2*	157	0.602	0.780	1.207	1.012	0.138	0.477	154
93	*Myrcia hebepetala*	135	0.869	0.524	0.951	1.205	NA	0.407	114
94	*Myrcia ilheoensis*	118	0.636	0.402	0.415	0.695	0.230	0.603	NA
95	*Myrcia mischophylla*	138	0.698	0.470	0.486	0.977	0.230	0.426	99
96	*Myrcia palustris*	134	0.435	0.195	0.388	0.581	0.139	0.471	74
97	*Myrcia pubescens*	150	0.670	0.337	0.258	0.599	0.092	0.416	84
98	*Myrcia ruschii*	152	1.041	0.643	1.029	1.695	0.211	0.861	122
99	*Myrcia spectabilis*	134	0.982	0.277	1.155	0.807	0.193	0.460	54

100	*Myrcia tijucensis*	134	0.580	0.216	0.835	0.725	0.090	0.467	72
101	*Myrcia vittoriana*	134	0.812	0.683	0.725	0.950	0.278	0.519	94
102	*Myrcia citrifolia*	108	0.376	0.731	0.912	0.890	0.170	0.475	128
103	*Myrcia cuprea*	127	0.323	0.271	0.709	0.684	0.145	0.415	74
104	*Myrcia guianensis 1*	59	0.382	0.606	0.571	0.328	0.162	0.539	106
105	*Myrcia guianensis 2*	53	0.498	0.454	0.474	0.648	0.397	0.157	56
106	*Myrcia laxiflora*	107	0.188	0.556	0.754	0.693	0.095	0.378	64
107	*Myrcia paracatuensis*	NA	0.425	0.195	0.620	0.702	0.078	0.413	46
108	*Myrcia pinifolia*	126	0.273	0.369	0.870	0.503	NA	0.371	NA
109	*Myrcia rotundata*	69	0.281	0.330	0.601	0.562	0.119	0.531	41
110	*Myrcia rufipes*	110	0.257	0.226	0.652	0.324	0.133	0.363	51
111	*Myrcia sp.*	111	0.413	0.336	1.336	0.941	0.171	0.447	120
112	*Myrcia stricta*	96	0.447	0.719	0.760	0.562	0.218	0.633	66
113	*Myrcia subverticillaris*	148	0.612	0.260	0.780	0.771	0.221	0.457	54
114	*Myrcia variabilis*	98	0.401	0.419	0.809	0.888	0.194	0.487	128
115	*Myrcia vestita*	95	0.300	0.471	0.799	0.811	0.150	0.600	124
116	*Myrcia anceps*	120	0.267	0.493	0.148	0.271	0.080	0.532	85
117	*Myrcia bracteata*	123	0.367	0.665	0.444	0.743	0.156	0.313	NA
118	*Myrcia capitata*	126	0.428	0.956	0.783	0.895	0.188	0.305	164
119	*Myrcia cardiaca*	126	0.501	1.206	0.241	0.559	0.202	0.503	163
120	*Myrcia coumeta*	116	0.359	1.353	0.388	1.122	0.153	0.694	263
121	*Myrcia elevata*	NA	0.214	0.254	0.460	0.891	0.113	0.267	146
122	*Myrcia eriopus*	117	0.367	0.557	0.113	0.505	0.116	0.415	112
123	*Myrcia eximia*	102	0.394	0.312	0.281	0.732	0.092	0.597	82
124	*Myrcia federalis*	129	0.528	1.125	0.357	0.563	NA	0.474	185
125	*Myrcia fenestrata*	136	0.315	0.139	0.073	0.410	1.099	0.236	47
126	*Myrcia ovata*	128	0.424	0.292	0.090	0.559	0.102	0.273	72
127	*Myrcia paivae*	135	0.238	0.363	0.244	0.366	0.106	0.288	46
128	*Myrcia retorta 1*	93	0.479	1.028	0.206	0.427	0.144	0.384	84
129	*Myrcia retorta 2*	100	0.339	0.870	0.166	0.383	0.167	0.348	95
130	*Myrcia schottiana*	129	0.386	0.496	0.206	0.991	0.121	0.289	89
131	*Myrcia splendens*	96	0.567	0.801	0.160	0.778	0.174	0.630	63
132	*Myrcia suffruticosa*	103	0.425	0.899	0.349	0.721	0.205	0.614	135
133	*Myrcia velutina*	134	0.339	0.380	0.150	0.484	0.088	0.660	54
134	*Myrcia antonia*	142	0.444	2.063	1.336	1.758	0.188	0.545	252
135	*Myrcia pubipetala 1*	110	0.325	1.678	0.750	1.145	0.156	0.561	198
136	*Myrcia pubipetala 2*	NA	0.437	1.070	0.444	0.875	0.177	NA	242
137	*Myrcia reticulosa*	106	0.273	0.167	0.301	0.547	0.169	0.380	36
138	*Myrcia venulosa 1*	129	0.373	0.425	0.419	0.635	0.147	0.421	73
139	*Myrcia venulosa 2*	114	0.262	0.467	0.391	0.517	0.094	0.642	57
140	*Myrcia ascendens*	138	0.354	0.085	0.522	0.719	0.050	0.253	32
141	*Myrcia bicarinata*	112	0.381	0.160	0.804	0.668	0.130	0.166	52
142	*Myrcia bicolor*	91	0.308	0.209	1.229	0.655	0.144	0.308	65
143	*Myrcia costeira*	NA	0.360	NA	0.680	0.560	NA	0.420	61

144	*Myrcia densa*	126	0.226	0.231	0.619	0.463	0.150	0.224	40
145	*Myrcia insigniflora*	148	0.484	0.788	1.046	2.287	0.151	0.383	324
146	*Myrcia lenheirensis*	120	0.318	0.345	0.751	0.492	0.147	0.325	37
147	*Myrcia mutabilis*	83	0.387	0.494	0.947	0.855	0.149	0.374	75
148	*Myrcia plusiantha*	109	0.352	0.192	0.965	0.845	0.319	0.178	76
149	*Myrcia rupestris*	123	0.220	0.090	0.700	0.550	0.100	0.250	46
150	*Myrcia sp.*	130	2.717	0.186	0.925	0.383	NA	NA	32
151	*Myrcia subavenia*	125	0.360	1.021	0.739	0.809	0.164	0.563	160
152	*Myrcia subcordata*	80	0.295	0.205	1.069	0.844	0.109	0.331	70
153	*Myrcia subterminalis*	115	0.320	0.080	0.410	0.760	0.110	0.250	49
154	*Myrcia tenuifolia*	120	0.311	0.341	0.807	0.731	0.097	0.381	66
155	*Myrcia laruotteana*	73	0.562	0.422	1.083	0.818	0.276	0.368	32
156	*Myrcia selloi 1*	35	0.369	0.304	0.350	0.553	0.196	0.565	62
157	*Myrcia selloi 2*	54	0.472	0.483	0.895	0.626	0.159	0.758	64
158	*Myrcia selloi 3*	32	0.356	0.345	0.426	0.442	0.186	0.559	58
159	*Myrcia tomentosa 1*	42	0.515	0.220	0.375	0.478	0.143	NA	NA
160	*Myrcia tomentosa 2*	47	0.584	0.287	0.311	0.494	0.245	0.655	40
161	*Myrcia tomentosa 3*	38	0.420	0.264	0.384	0.413	0.196	0.352	39

no	species	antGlad	infloLength	infloCat	infloDisplay	infloPosition
1	*Marlierea montana*	n	56	3	clustered	exposed
2	*Myrcia abbotiana*	n	17.97	2	spread	exposed
3	*Myrcia amazonica*	n	100.2	4	clustered	exposed
4	*Myrcia blanchetiana*	y	65.73	2	clustered	exposed
5	*Myrcia caudata*	NA	22.1	2	spread	exposedHidden
6	*Myrcia decorticans*	n	71.6	3	spread	exposed
7	*Myrcia eumecephylla*	n	177.2	4	spread	exposed
8	*Myrcia excoriata*	y	24.4	1	spread	hidden
9	*Myrcia grandis*	n	47.53	3	spread	exposed
10	*Myrcia hexasticha*	n	112.2	5	clustered	exposed
11	*Myrcia hirtiflora*	n	78.3	4	clustered	exposed
12	*Myrcia inaequiloba*	y	38.8	4	clustered	exposed
13	*Myrcia mcvaughii*	y	53.2	4	clustered	exposed
14	*Myrcia micropetala*	NA	81.7	4	clustered	hidden
15	*Myrcia minutiflora*	n	35.3	2	clustered	hidden
16	*Myrcia multiflora*	n	46.65	3	spread	hidden
17	*Myrcia neobscura*	n	64.86	4	spread	exposed
18	*Myrcia neodimorpha*	n	89.2	4	clustered	exposed
19	*Myrcia neograbla*	y	152.8	3	spread	hidden
20	*Myrcia neoregeliana*	y	68.5	3	spread	exposed
21	*Myrcia neoriedeliana*	y	91.6	5	spread	exposedHidden
22	*Myrcia neosuaveolens*	y	32	2	spread	hidden

23	*Myrcia neotomentosa*	n	NA	4	clustered	exposed
24	*Myrcia neuwiedeana*	n	52	2	spread	hidden
25	*Myrcia obversa*	n	138.1	5	spread	exposed
26	*Myrcia platyclada*	y	38.73	3	spread	exposed
27	*Myrcia polyantha*	n	20	1	spread	hidden
28	*Myrcia racemosa 1*	n	40.8	3	spread	exposedHidden
29	*Myrcia racemosa 2*	y	22.1	1	spread	exposed
30	*Myrcia racemosa 3*	NA	24.6	2	spread	exposedHidden
31	*Myrcia riodocensis*	y	75.9	3	clustered	exposed
32	*Myrcia rubella*	y	120	4	spread	exposed
33	*Myrcia saxatilis*	n	47.3	3	spread	exposed
34	*Myrcia sessilisima*	n	69.76	4	spread	exposed
35	*Myrcia sucrei*	n	67.9	3	clustered	exposed
36	*Myrcia tetraphylla*	n	237.2	4	spread	exposed
37	*Myrcia umbraticola*	y	60	2	spread	exposed
38	*Calyptranthes aromatica*	n	116.666667	3	spread	hidden
39	*Calyptranthes barkeri*	y	55.9	2	clustered	exposed
40	*Calyptranthes bipennis*	y	43.8	NA	clustered	exposed
41	*Calyptranthes brasiliensis*	y	45	3	clustered	exposed
42	*Calyptranthes clusiifolia*	y	73.4	3	clustered	exposed
43	*Calyptranthes concinna*	y	43.9	2	clustered	exposedHidden
44	*Calyptranthes eriocephala*	y	20.5	1	spread	exposed
45	*Calyptranthes fasciculata*	n	10.4	2	clustered	hidden
46	*Calyptranthes garciae*	n	44.2	1	spread	exposed
47	*Calyptranthes grandiflora 1*	n	73.3	4	spread	exposed
48	*Calyptranthes grandiflora 2*	n	62	2	spread	exposed
49	*Calyptranthes grandiflora 3*	y	121.6	4	spread	exposed
50	*Calyptranthes grandifolia 4*	y	62.6	3	spread	exposedHidden
51	*Calyptranthes hatchbachii*	n	38.1666667	2	spread	exposed
52	*Calyptranthes kiaerskovii*	y	17.13	1	clustered	hidden
53	*Calyptranthes laevigata*	y	25.3	1	clustered	exposed
54	*Calyptranthes lanceolata*	n	86.1	3	spread	exposed
55	*Calyptranthes langsdrofii*	y	32.0333333	2	spread	exposed
56	*Calyptranthes loranthifolia*	y	74.7	3	spread	exposed
57	*Calyptranthes lucida*	n	41	3	spread	exposedHidden
58	*Calyptranthes martiusiana*	NA	97.4333333	2	spread	exposed
59	*Calyptranthes pallens*	n	100	4	clustered	exposedHidden
60	*Calyptranthes punchella*	Y	21.87	2	spread	exposedHidden
61	*Calyptranthes restigae*	n	55.1	NA	clustered	exposed
62	*Calyptranthes sintenisii*	y	62.6	3	spread	exposedHidden
63	*Calyptranthes speciosae*	n	112.03	4	clustered	exposed
64	*Calyptranthes spruceana*	n	63.2	2	clustered	exposed
65	*Calyptranthes strigipes*	n	48.0666667	3	clustered	exposed
66	*Calyptranthes thomasiana*	y	35.7	2	spread	hidden

67	*Calyptranthes variabilis*	y	27.6666667	2	spread	hidden
68	*Calyptranthes wiedgreniana*	n	55.8666667	3	clustered	exposed
69	*Calyptranthes zusygium*	Y	41.9666667	1	spread	hidden
70	*Marlierea clausseniana 1*	n	38.8	3	spread	exposedHidden
71	*Marlierea clausseniana 2*	y	85.3	4	spread	exposed
72	*Marlierea clausseniana 3*	y	64.3	4	spread	exposedHidden
73	*Marlierea subacuminata*	y	71	2	spread	exposedHidden
74	*Marlierea tenuivenosa*	n	63.8	4	spread	exposedHidden
75	*Marlierea teuscheriana*	y	222.1	5	clustered	exposed
76	*Myrcia eugeniopsoides*	n	100*	2	spread	hidden
77	*Myrcia ferruginosa*	n	57.5	2	spread	exposedHidden
78	*Myrcia hatschbachii*	n	83.96	4	spread	exposed
79	*Myrcia multipuncatata*	y	41.3	2	spread	exposedHidden
80	*Myrcia oblongata*	y	83.25	3	spread	hidden
81	*Myrcia sp.*	n	76.5	4	spread	hidden
82	*Myrcia tenuivenosa 1*	n	63.87	4	spread	exposedHidden
83	*Myrcia tenuivenosa 2*	n	63.93	4	spread	exposedHidden
84	*Myrcia anacardifolia*	n	49.25	1	spread	hidden
85	*Myrcia brasiliensis*	y	68.8	2	spread	exposed
86	*Myrcia cerqueira*	n	18.4	2	clustered	hidden
87	*Myrcia eriocalyx*	n	46.5	NA	NA	exposed
88	*Myrcia fenzliana*	y	148.9	5	spread	exposed
89	*Myrcia flagellaris*	y	51.2	1	clustered	exposedHidden
90	*Myrcia glazioviana*	y	8.2	1	spread	hidden
91	*Myrcia hartwegiana 1*	n	43.2	3	clustered	exposed
92	*Myrcia hartwegiana 2*	n	37.1	2	spread	exposed
93	*Myrcia hebepetala*	y	64.53	2	spread	exposedHidden
94	*Myrcia ilheoensis*	y	61.9	2	clustered	exposed
95	*Myrcia mischophylla*	n	108.9	4	clustered	exposed
96	*Myrcia palustris*	NA	39.5	3	clustered	exposedHidden
97	*Myrcia pubescens*	y	77.1	3	spread	exposed
98	*Myrcia ruschii*	y	263	3	spreas	exposedHidden
99	*Myrcia spectabilis*	n	153	3	spread	exposed
100	*Myrcia tijucensis*	n	32.2	2	spread	exposedHidden
101	*Myrcia vittoriana*	NA	76.55	2	spread	exposed
102	*Myrcia citrifolia*	n	71.1	2	spread	exposed
103	*Myrcia cuprea*	n	56.7	4	spread	exposed
104	*Myrcia guianensis 1*	n	49.7	2	clustered	hidden
105	*Myrcia guianensis 2*	y	18.9	2	spread	exposedHidden
106	*Myrcia laxiflora*	n	62.4	2	spread	exposed
107	*Myrcia paracatuensis*	y	22.9	2	spread	exposed
108	*Myrcia pinifolia*	n	40.76	2	spread	exposed
109	*Myrcia rotundata*	n	42.4	NA	spread	exposedHidden
110	*Myrcia rufipes*	n	85.26	4	spread	exposed

111	*Myrcia sp.*	n	NA	NA	spread	exposed
112	*Myrcia stricta*	y	42.5	2	spread	exposed
113	*Myrcia subverticillaris*	n	58.1	NA	NA	hidden
114	*Myrcia variabilis*	n	46.6	2	spread	exposedHidden
115	*Myrcia vestita*	n	73.2	5	clustered	exposed
116	*Myrcia anceps*	y	69.2	NA	clustered	hidden
117	*Myrcia bracteata*	n	29.6	2	clustered	exposedHidden
118	*Myrcia capitata*	n	52.3	1	clustered	exposed
119	*Myrcia cardiaca*	y	50.2	2	spread	exposed
120	*Myrcia coumeta*	n	88.2	2	spread	exposedHidden
121	*Myrcia elevata*	y	75.8	2	spread	exposedHidden
122	*Myrcia eriopus*	y	112.7	3	spread	exposed
123	*Myrcia eximia*	n	65	3	spread	exposed
124	*Myrcia federalis*	n	54.1666667	2	clustered	exposed
125	*Myrcia fenestrata*	y	44.5666667	2	spread	exposedHidden
126	*Myrcia ovata*	y	48.8	3	spread	exposed
127	*Myrcia paivae*	y	20.6666667	1	spread	hidden
128	*Myrcia retorta 1*	y	36.95	2	spread	exposed
129	*Myrcia retorta 2*	y	32.95	2	clustered	exposed
130	*Myrcia schottiana*	y	10.7666667	1	clustered	exposed
131	*Myrcia splendens*	y	35	2	spread	exposed
132	*Myrcia suffruticosa*	y	39.86	1	spread	exposed
133	*Myrcia velutina*	n	22.5	4	clustered	exposed
134	*Myrcia antonia*	y	127.4	3	clustered	exposed
135	*Myrcia pubipetala 1*	n	141.95	3	spread	exposed
136	*Myrcia pubipetala 2*	n	87.5	3	spread	exposed
137	*Myrcia reticulosa*	n	15.8	1	clustered	hidden
138	*Myrcia venulosa 1*	n	56.8	4	spread	exposed
139	*Myrcia venulosa 2*	n	49.2	3	spread	exposed
140	*Myrcia ascendens*	NA	5.55	2	clustered	exposedHidden
141	*Myrcia bicarinata*	y	36.7	2	spread	exposed
142	*Myrcia bicolor*	y	30.3	2	spread	exposed
143	*Myrcia costeira*	NA	25	3	spread	exposed
144	*Myrcia densa*	y	39.7	3	spread	exposed
145	*Myrcia insigniflora*	y	144.6	3	spread	exposed
146	*Myrcia lenheirensis*	y	13.6	2	clustered	exposed
147	*Myrcia mutabilis*	y	21.1	3	spread	exposed
148	*Myrcia plusiantha*	n	114.6	4	spread	exposed
149	*Myrcia rupestris*	NA	22.12	3	spread	exposedHidden
150	*Myrcia sp.*	y	90.7	5	clustered	exposed
151	*Myrcia subavenia*	y	40	1	clustered	exposed
152	*Myrcia subcordata*	y	33.65	2	clustered	hidden
153	*Myrcia subterminalis*	NA	45	3	spread	exposedHidden
154	*Myrcia tenuifolia*	y	60	3	spread	exposed

155	*Myrcia laruotteana*	NA	63.6	3	spread	hidden
156	*Myrcia selloi 1*	y	25.2	2	spread	exposedHidden
157	*Myrcia selloi 2*	y	25.4	2	spread	exposedHidden
158	*Myrcia selloi 3*	n	24.3	2	spread	exposedHidden
159	*Myrcia tomentosa 1*	NA	116.3	4	clustered	exposedHidden
160	*Myrcia tomentosa 2*	NA	102.75	4	spread	exposedHidden
161	*Myrcia tomentosa 3*	NA	45.3	3	clustered	hidden

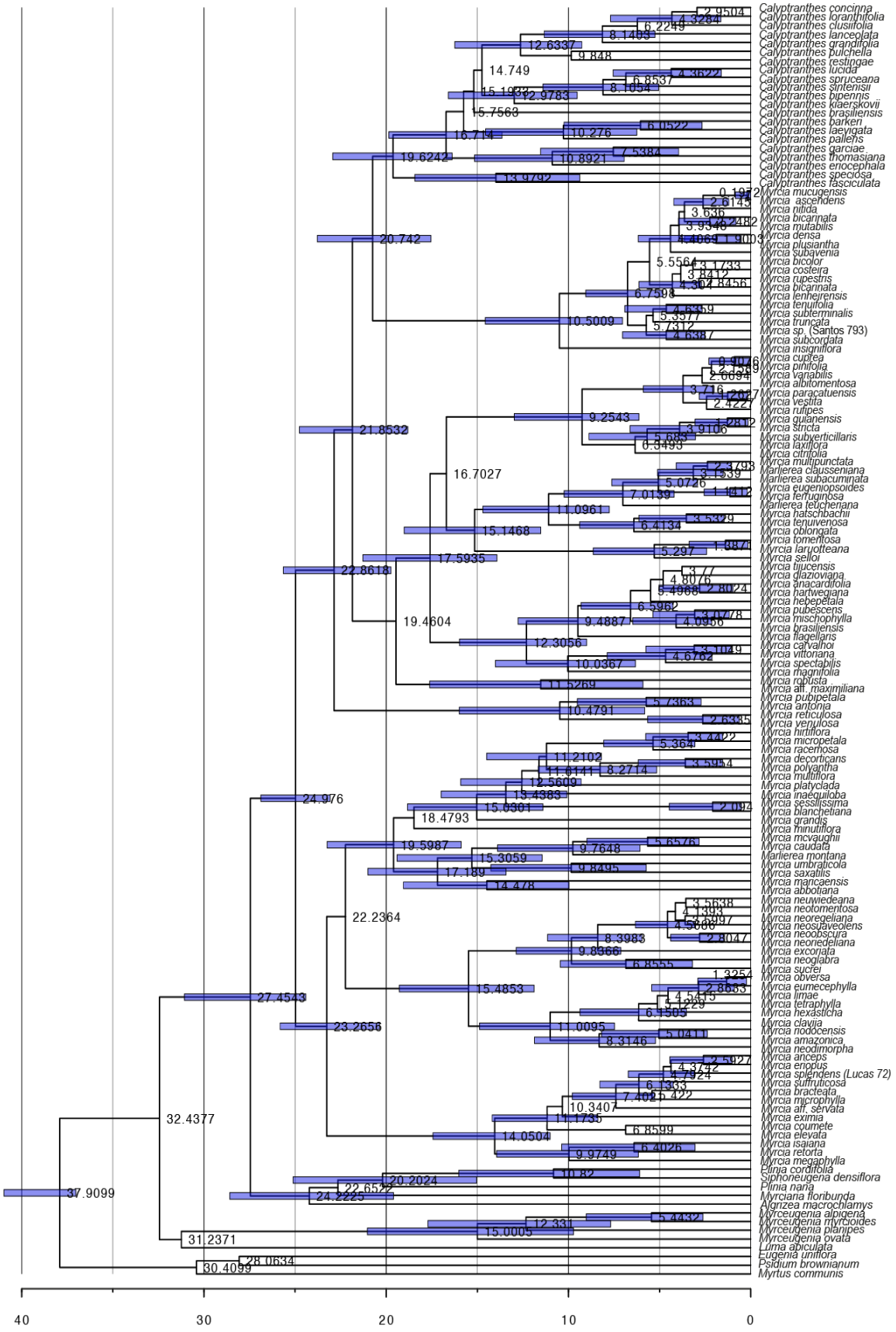
no	species	plantHigh	vegetation	altitude
1	*Marlierea montana*	4	forest	50
2	*Myrcia abbotiana*	6	savannah	100
3	*Myrcia amazonica*	12	forest	450
4	*Myrcia blanchetiana*	2.5	savannah	750
5	*Myrcia caudata*	5	forest	350
6	*Myrcia decorticans*	10	forest	500
7	*Myrcia eumecephylla*	4	forest	50
8	*Myrcia excoriata*	NA	NA	NA
9	*Myrcia grandis*	2	forest	100
10	*Myrcia hexasticha*	10	forest	30
11	*Myrcia hirtiflora*	5	forest	0
12	*Myrcia inaequiloba*	1	NA	1800
13	*Myrcia mcvaughii*	NA	forest	60
14	*Myrcia micropetala*	12	forest	50
15	*Myrcia minutiflora*	6	forest	70
16	*Myrcia multiflora*	1.5	savannah	200
17	*Myrcia neobscura*	NA	forest	1200
18	*Myrcia neodimorpha*	5	forest	700
19	*Myrcia neograbla*	NA	forest	NA
20	*Myrcia neoregeliana*	3	forest	50
21	*Myrcia neoriedeliana*	20	forest	14
22	*Myrcia neosuaveolens*	3	forest	150
23	*Myrcia neotomentosa*	7	forest	50
24	*Myrcia neuwiedeana*	2	forest	65
25	*Myrcia obversa*	8	forest	50
26	*Myrcia platyclada*	NA	NA	700
27	*Myrcia polyantha*	1	savannah	260
28	*Myrcia racemosa 1*	NA	forest	200
29	*Myrcia racemosa 2*	8	forest	700
30	*Myrcia racemosa 3*	7	forest	1590
31	*Myrcia riodocensis*	3	forest	50
32	*Myrcia rubella*	1.5	savannah	900
33	*Myrcia saxatilis*	2	savannah	150
34	*Myrcia sessilisima*	0.6	savannah	900

35	*Myrcia sucrei*	9	forest	80
36	*Myrcia tetraphylla*	8	forest	20
37	*Myrcia umbraticola*	3	forest	40
38	*Calyptranthes aromatica*	5	forest	779
39	*Calyptranthes barkeri*	5	NA	235
40	*Calyptranthes bipennis*	12	forest	50
41	*Calyptranthes brasiliensis*	5	forest	50
42	*Calyptranthes clusiifolia*	7	forest	60
43	*Calyptranthes concinna*	3	forest	780
44	*Calyptranthes eriocephala*	NA	forest	1800
45	*Calyptranthes fasciculata*	NA	savannah	650
46	*Calyptranthes garciae*	4	savannah	30
47	*Calyptranthes grandiflora 1*	8	forest	80
48	*Calyptranthes grandiflora 2*	10	forest	610
49	*Calyptranthes grandiflora 3*	7	forest	770
50	*Calyptranthes grandifolia 4*	20	forest	800
51	*Calyptranthes hatchbachii*	2	forest	1000
52	*Calyptranthes kiaerskovii*	NA	forest	350
53	*Calyptranthes laevigata*	2.5	savannah	314
54	*Calyptranthes lanceolata*	3	forest	50
55	*Calyptranthes langsdrofii*	18	forest	850
56	*Calyptranthes loranthifolia*	1.5	savannah	659
57	*Calyptranthes lucida*	15	forest	1000
58	*Calyptranthes martiusiana*	2	forest	50
59	*Calyptranthes pallens*	8	forest	1600
60	*Calyptranthes punchella*	14	forest	700
61	*Calyptranthes restigae*	7	forest	60
62	*Calyptranthes sintenisii*	6.5	forest	550
63	*Calyptranthes speciosae*	5	savannah	500
64	*Calyptranthes spruceana*	NA	forest	10
65	*Calyptranthes strigipes*	10	forest	50
66	*Calyptranthes thomasiana*	1	forest	350
67	*Calyptranthes variabilis*	NA	NA	NA
68	*Calyptranthes wiedgreniana*	3	forest	820
69	*Calyptranthes zusygium*	NA	forest	10
70	*Marlierea clausseniana 1*	1.5	NA	580
71	*Marlierea clausseniana 2*	25	forest	670
72	*Marlierea clausseniana 3*	5	savannah	130
73	*Marlierea subacuminata*	3.5	forest	683
74	*Marlierea tenuivenosa*	6	forest	650
75	*Marlierea teuscheriana*	4	forest	730
76	*Myrcia eugeniopsoides*	5	forest	10
77	*Myrcia ferruginosa*	10	forest	80
78	*Myrcia hatschbachii*	12	forest	800

79	*Myrcia multipuncatata*	4	savannah	1320
80	*Myrcia oblongata*	NA	NA	80
81	*Myrcia sp.*	8	forest	950
82	*Myrcia tenuivenosa 1*	NA	forest	75
83	*Myrcia tenuivenosa 2*	8	forest	1010
84	*Myrcia anacardifolia*	4	forest	10
85	*Myrcia brasiliensis*	20	forest	400
86	*Myrcia cerqueira*	3	forest	47
87	*Myrcia eriocalyx*	2	savannah	1250
88	*Myrcia fenzliana*	8	savannah	900
89	*Myrcia flagellaris*	2	forest	50
90	*Myrcia glazioviana*	2	NA	1500
91	*Myrcia hartwegiana 1*	2	forest	1000
92	*Myrcia hartwegiana 2*	2	forest	1050
93	*Myrcia hebepetala*	5	forest	0
94	*Myrcia ilheoensis*	3	savannah	900
95	*Myrcia mischophylla*	2.5	savannah	1000
96	*Myrcia palustris*	4	forest	20
97	*Myrcia pubescens*	3	savannah	800
98	*Myrcia ruschii*	NA	forest	725
99	*Myrcia spectabilis*	10	forest	20
100	*Myrcia tijucensis*	5	forest	450
101	*Myrcia vittoriana*	6	forest	50
102	*Myrcia citrifolia*	5	forest	822
103	*Myrcia cuprea*	3	savannah	0
104	*Myrcia guianensis 1*	1	forest	970
105	*Myrcia guianensis 2*	6	forest	1176
106	*Myrcia laxiflora*	6	forest	600
107	*Myrcia paracatuensis*	0.3	savannah	800
108	*Myrcia pinifolia*	0.3	savannah	800
109	*Myrcia rotundata*	NA	NA	NA
110	*Myrcia rufipes*	5	forest	300
111	*Myrcia sp.*	NA	savannah	750
112	*Myrcia stricta*	0.6	savannah	900
113	*Myrcia subverticillaris*	3	forest	820
114	*Myrcia variabilis*	2	savannah	800
115	*Myrcia vestita*	0.8	savannah	600
116	*Myrcia anceps*	3	forest	530
117	*Myrcia bracteata*	3	forest	250
118	*Myrcia capitata*	1	savannah	1100
119	*Myrcia cardiaca*	1	savannah	1000
120	*Myrcia coumeta*	3	forest	170
121	*Myrcia elevata*	3	forest	150
122	*Myrcia eriopus*	NA	forest	850

123	*Myrcia eximia*	9	savannah	880
124	*Myrcia federalis*	0.2	savannah	1200
125	*Myrcia fenestrata*	6	forest	40
126	*Myrcia ovata*	4.5	forest	30
127	*Myrcia paivae*	2	forest	20
128	*Myrcia retorta 1*	5	forest	1000
129	*Myrcia retorta 2*	4	forest	850
130	*Myrcia schottiana*	4	savannah	320
131	*Myrcia splendens*	2	savannah	1460
132	*Myrcia suffruticosa*	0.15	savannah	1450
133	*Myrcia velutina*	2	savannah	1700
134	*Myrcia antonia*	6	forest	950
135	*Myrcia pubipetala 1*	15	forest	850
136	*Myrcia pubipetala 2*	12	forest	800
137	*Myrcia reticulosa*	1.5	savannah	1300
138	*Myrcia venulosa 1*	5	forest	900
139	*Myrcia venulosa 2*	6	savannah	1425
140	*Myrcia ascendens*	2	savannah	1000
141	*Myrcia bicarinata*	8	forest	30
142	*Myrcia bicolor*	10	forest	400
143	*Myrcia costeira*	7	forest	0
144	*Myrcia densa*	2	savannah	800
145	*Myrcia insigniflora*	6	forest	62
146	*Myrcia lenheirensis*	3	savannah	1150
147	*Myrcia mutabilis*	4	savannah	950
148	*Myrcia plusiantha*	9	forest	1100
149	*Myrcia rupestris*	1.75	savannah	900
150	*Myrcia sp.*	2.5	forest	NA
151	*Myrcia subavenia*	4	savannah	1200
152	*Myrcia subcordata*	1.5	savannah	1750
153	*Myrcia subterminalis*	9	forest	700
154	*Myrcia tenuifolia*	NA	forest	50
155	*Myrcia laruotteana*	3	forest	1063
156	*Myrcia selloi 1*	3	forest	20
157	*Myrcia selloi 2*	3	forest	550
158	*Myrcia selloi 3*	5.5	forest	781
159	*Myrcia tomentosa 1*	3	savannah	20
160	*Myrcia tomentosa 2*	3	forest	850
161	*Myrcia tomentosa 3*	1.5	forest	750

Appendix 7.2: *Myrcia* dated phylogeny (modified from Santos et al., 2017).



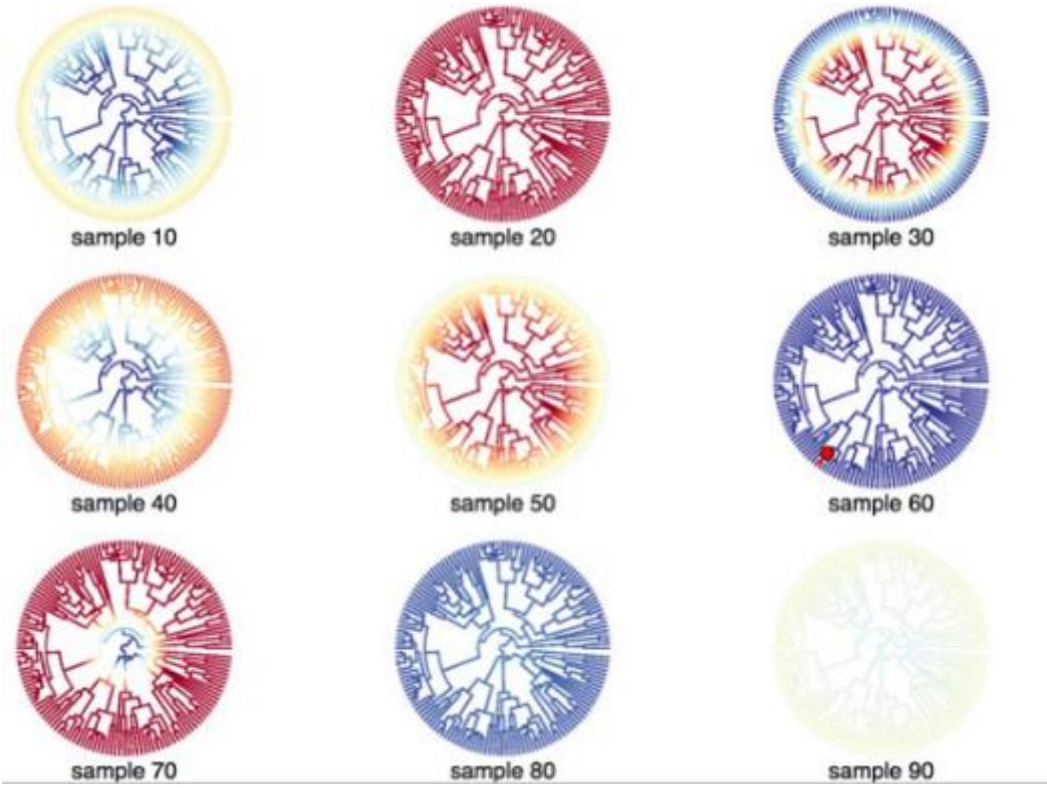
Appendix 7.3: Computed correlation used spearman-method with listwise-deletion.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	B.A	G.F	C.B	L.J	A.K	I.O	O.J
A		0.745	0.542	0.515	0.504	0.335	0.44	0.22	0.024	0.504	0.19	0.495	-0.029	0.174	0.69	0.02	-0.348	0.088	-0.315	-0.313	0.454	0.677	-0.15
B	0.745		0.598	0.657	0.549	0.582	0.398	0.094	0.069	0.394	0.124	0.424	-0.031	0.149	0.616	-0.072	0.157	-0.149	-0.428	-0.343	0.489	0.683	-0.191
C	0.542	0.598		0.716	0.956	0.428	0.483	0.1	0.203	0.185	0.407	0.392	-0.043	0.186	0.503	0.288	-0.054	0.049	0.222	-0.134	0.018	0.583	-0.038
D	0.515	0.657	0.716		0.696	0.493	0.414	0.033	0.08	0.239	0.351	0.344	0.01	0.132	0.524	-0.264	0.114	-0.044	0.004	-0.257	0.17	0.558	-0.123
E	0.504	0.549	0.956	0.696		0.379	0.436	0.089	0.196	0.154	0.418	0.371	-0.042	0.18	0.484	0.33	-0.033	0.058	0.296	-0.089	-0.034	0.55	0.015
F	0.335	0.582	0.428	0.493	0.379		0.258	0.03	0.143	0.072	-0.023	0.095	-0.064	-0.116	0.355	-0.05	0.21	-0.526	-0.222	-0.213	0.33	0.482	-0.083
G	0.44	0.398	0.483	0.414	0.436	0.258		0.037	0.068	0.106	0.245	0.251	-0.025	0.123	0.476	0.022	-0.181	0.34	-0.028	-0.159	0.187	0.443	-0.009
H	0.22	0.094	0.1	0.033	0.089	0.03	0.037		0.202	0.217	0.079	0.13	-0.006	-0.041	0.078	0.046	-0.193	0.049	-0.166	0.01	0.107	0.182	-0.032
I	0.024	0.069	0.203	0.08	0.196	0.143	0.068	0.202		-0.056	0.1	0.044	-0.018	-0.018	-0.028	0.174	0.122	0.004	0.077	-0.01	-0.075	0.248	-0.099
J	0.504	0.394	0.185	0.239	0.154	0.072	0.106	0.217	-0.056		-0.072	0.296	-0.039	0.135	0.304	-0.063	-0.234	-0.015	-0.345	-0.493	0.388	0.3	-0.382
K	0.19	0.124	0.407	0.351	0.418	-0.023	0.245	0.079	0.1	-0.072		0.338	-0.068	0.21	0.143	-0.028	-0.152	0.167	0.304	0.281	-0.534	0.157	0.163
L	0.495	0.424	0.392	0.344	0.371	0.095	0.251	0.13	0.044	0.296	0.338		0.11	0.266	0.461	0.077	-0.204	0.185	-0.046	0.035	0.013	0.424	0.037
M	-0.029	-0.031	-0.043	0.01	-0.042	-0.064	-0.025	-0.006	-0.018	-0.039	-0.068	0.11		-0.012	-0.02	0.001	0.038	0.063	-0.037	0.094	0.115	-0.035	0.103
N	0.174	0.149	0.186	0.132	0.18	-0.116	0.123	-0.041	-0.018	0.135	0.21	0.266	-0.012		0.147	0.061	-0.111	0.103	0.071	0.057	-0.004	0.117	0.049
O	0.69	0.616	0.503	0.524	0.484	0.355	0.476	0.078	-0.028	0.304	0.143	0.461	-0.02	0.147		-0.009	-0.23	0.128	-0.176	-0.184	0.3	0.852	0.233
P	0.02	-0.072	0.288	-0.264	0.33	-0.05	0.022	0.046	0.174	-0.063	-0.028	0.077	0.001	0.061	-0.009		-0.115	0.011	0.313	0.077	-0.032	0.048	0.08
B.A	-0.348	0.157	-0.054	0.114	-0.033	0.21	-0.181	-0.193	0.122	-0.234	-0.152	-0.204	0.038	-0.111	-0.23	-0.115		-0.281	-0.129	-0.071	0.025	-0.137	-0.09
G.F	0.088	-0.149	0.049	-0.044	0.058	-0.526	0.34	0.049	0.004	-0.015	0.167	0.185	0.063	0.103	0.128	0.011	-0.281		0.168	0.125	-0.166	0.052	0.146
C.B	-0.315	-0.428	0.222	0.004	0.296	-0.222	-0.028	-0.166	0.077	-0.345	0.304	-0.046	-0.037	0.071	-0.176	0.313	-0.129	0.168		0.369	-0.527	-0.18	0.234
L.J	-0.313	-0.343	-0.134	-0.257	-0.089	-0.213	-0.159	0.01	-0.01	-0.493	0.281	0.035	0.094	0.057	-0.184	0.077	-0.071	0.125	0.369		-0.483	-0.248	0.77

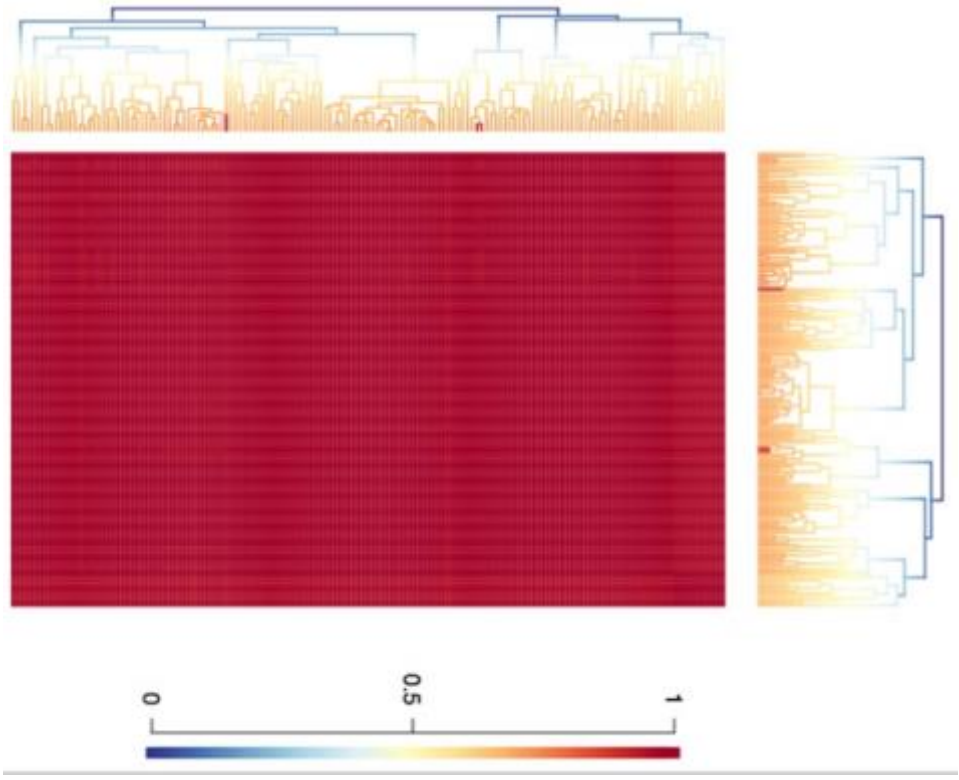
A.K	0.454	0.489	0.018	0.17	-0.034	0.33	0.187	0.107	-0.075	0.388	-0.534	0.013	0.115	-0.004	0.3	-0.032	0.025	-0.166	-0.527	-0.483		0.321	-0.256
I.O	0.677	0.683	0.583	0.558	0.55	0.482	0.443	0.182	0.248	0.3	0.157	0.424	-0.035	0.117	0.852	0.048	-0.137	0.052	-0.18	-0.248	0.321		0.083
O.J	-0.15	-0.191	-0.038	-0.123	0.015	-0.083	-0.009	-0.032	-0.099	-0.382	0.163	0.037	0.103	0.049	0.233	0.08	-0.09	0.146	0.234	0.77	-0.256	0.083	

Appendix 7.4: Plots from macro-evolutionary dynamics analysis in *Myrcia* using BAMM, TESS and RPANDA.

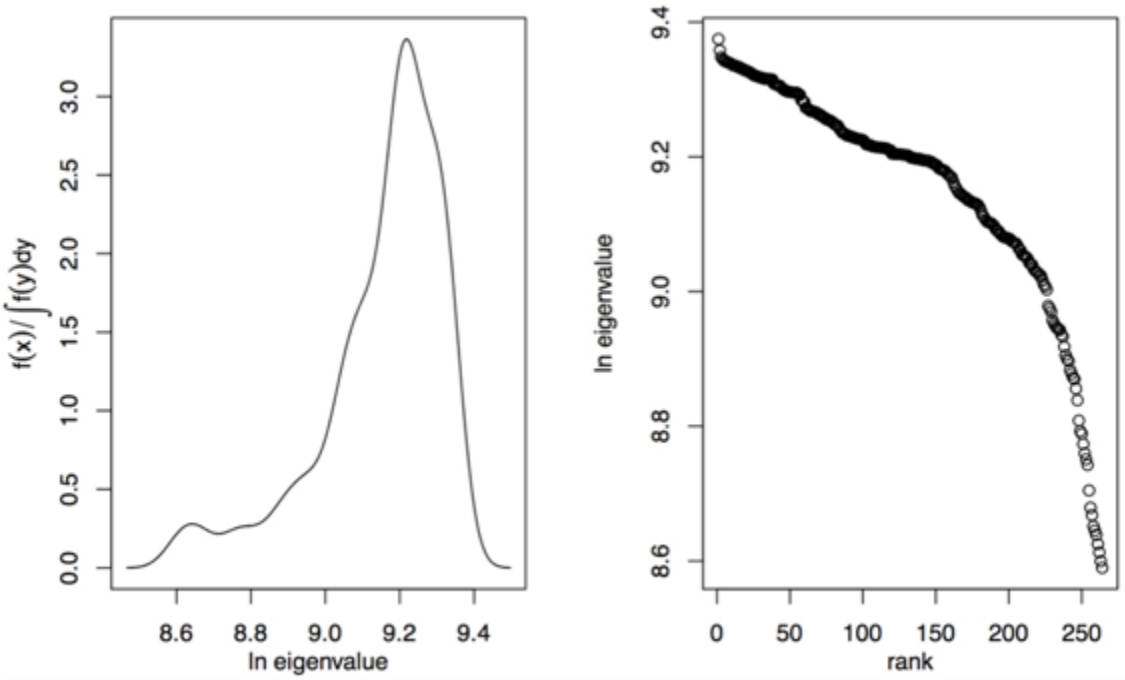
Plot 1: Nine random sampled shift configurations in BAMM, showing no clear pattern of phylogenetic heterogeneity or changes in diversification rate.



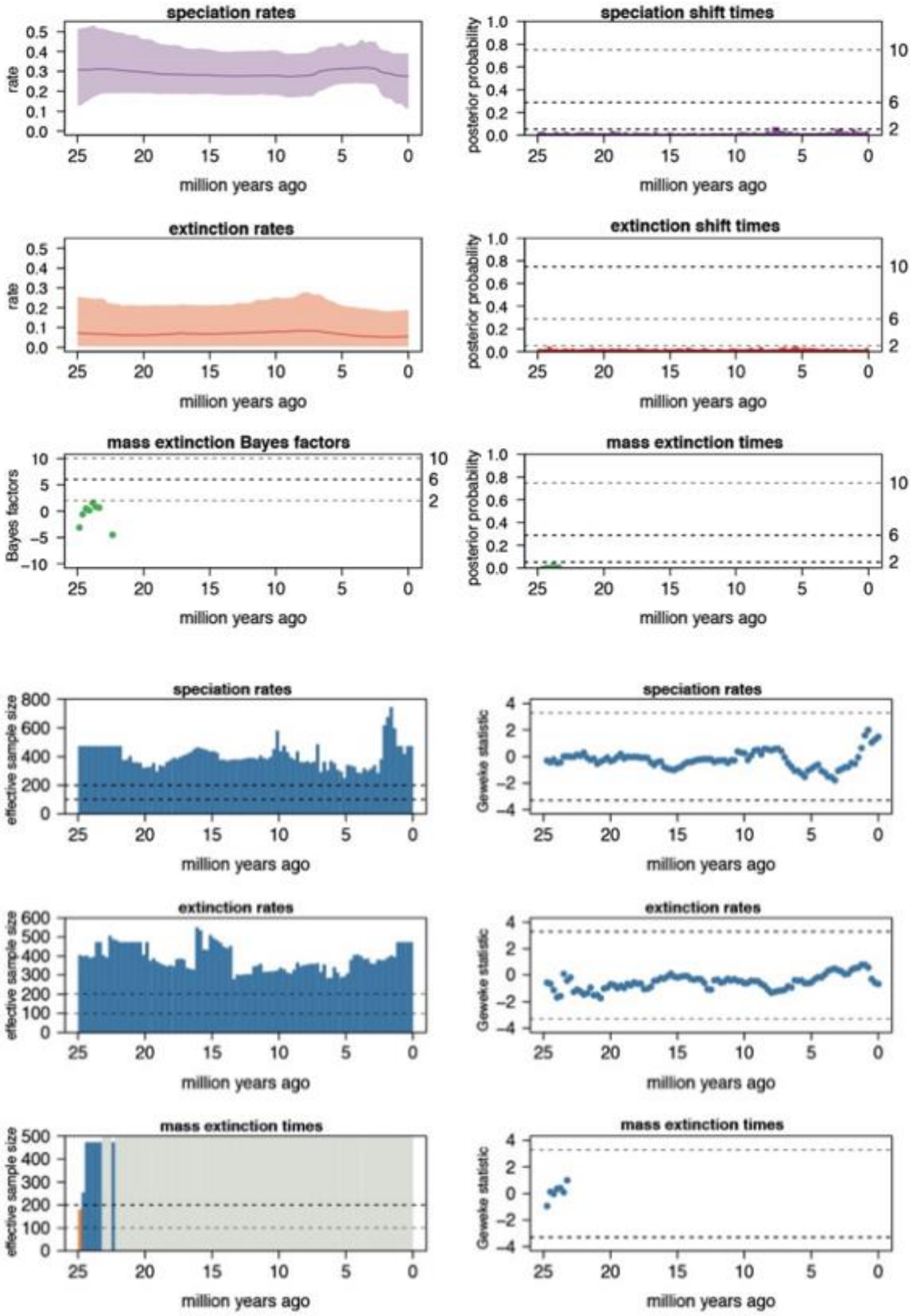
Plot 2: Cohot plot showing similar macroevolutionary regimes all pair of species in the *Myrcia* phylogeny (see Rabosky et al., 2014).



Plot 3: Spectral density plot and corresponding eigen values ranked in descending order for *Myrcia*, as generated by RPANDA. There is a lack of eigengaps which would indicate changes in macroevolutionary regimes (see Morlon et al., 2016)



Plot 4: Diversification rates estimated by TESS using empirical hyperpriors. No significant support for diversification rate shifts or mass extinction events was found. (see Hohna et al., 2016)



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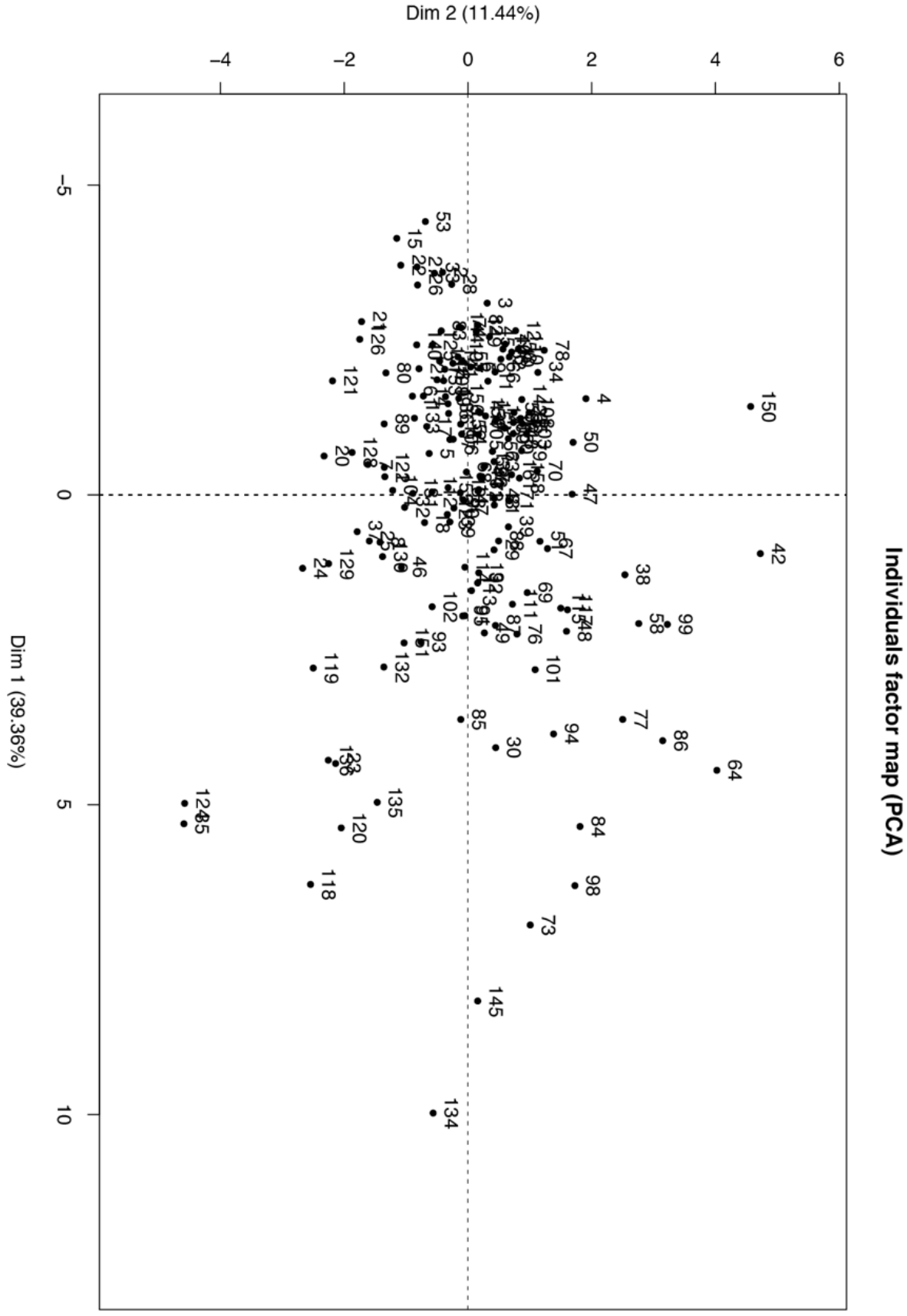
Appendix 7.5: Morphospace distribution of 165 *Myrcia* specimens (large size) (numbers according to Appendix 7.1.)



Chapter 8 - General Discussion and Concluding Remarks

8.1 Key findings

It is here demonstrated how even morphologically homogeneous groups can have heterogeneous evolutionary histories. Macro-evolutionary hypotheses such as this can only be drawn and tested when a robust phylogenetic tree is analysed in conjunction with careful descriptions of phenotypic data, as presented here. In this sense, this study moves forward considerably in the understanding of one of the most ecologically important and taxonomically complex tropical plant groups. An up to date phylogenetic tree and evolutionary context (Chapter 1) is provided alongside with detailed descriptions of floral diversity (Chapter 2 and 3), that is abundant despite its apparent lack of variation. This phylogenetic and phenotypic data can now be used for more precise ecological and evolutionary modelling in the Neotropical region and to improve identification and classification of Myrteae lineages.

Flowers of Myrteae are reasonably homogeneous for a group that is estimated to be at least 40 million years old, but variation exists and can be combined with other data for accurate diagnosis. In this sense, the lack of attention to flower traits by Myrteae systematists in light of the most up to date phylogenetic hypothesis shows that focus on “wrong” morphological characters was responsible for producing long lists of para- and polyphyletic genera. The historical focus on perianth traits for example – with their demonstrated high levels of parallelism and convergence, must be dropped. Androecium and gynoecium are considerably more systematically relevant.

Nevertheless, analyses of the calyx and other homoplastic traits in the framework of phylogenetic trees is important in the discussion of systematic complexity and macro-scale evolutionary patterns. Parallelism, especially, is rarely taken in consideration as a factor of taxonomic confusion in Angiosperms even though it is so recurrent (Chapters 4 and 6). Morphological homogeneity also underpins important evolutionary processes in mega diverse lineages and should be investigated more often with extreme care. This lack of phenotypic variation is demonstrated to keep extinction rates low and promote accumulation of species over time (Chapter 7) and highlight the role of heterochronic patterns in promoting flexibility in reproductive strategies (Chapter 5).

8.2 Taking this study forward

Results presented here clarify the evolutionary picture and take forward systematic understanding of complex taxonomic groups such as Myrteae. However, it also raises a number of questions for future studies. These could be tackled in combined lines of macro and microevolutionary research approaches.

Concerning macro-evolutionary approaches, progressive understanding of trait-function and generation of increasingly robust morphological and molecular data will be key to answer the most intriguing questions in Myrteae evolution. A concise species level phylogeny, for example, is necessary to use specific trait-dependant diversification analyses to correctly evaluate how changes in the floral structure discussed here affect macro-evolutionary dynamics. Furthermore, the association between certain poorly understood evolutionary trends such as ovule-oversupply and andromonoecy and environmental variables could be tested when such data is available. The

importance of Antarctica in early Myrteae diversification and trait-adaptations required to survive at such high latitudes should also be further investigated. Detailed morphological descriptions for other plant organs such as wood, leaves and fruits would also be interesting to improve the assignment of old macro-fossils with unclear placement, indispensable to clarify early diversification events in Myrteae.

In the micro-evolutionary context, reproductive biology alongside population genetics studies are required to evaluate how the variation in floral traits discussed here affect gene-flow and speciation in distinct lineages and niches. Hypotheses drawn in Chapter 3, such as the role of the herkogamic effects of some stamen-style configurations in reproductive success, should be tested in the field so the assertion of how strongly under selection such traits are could be evaluated. On the matter of parallelisms, understanding how genetic mechanisms promote re-expression of long silenced genes are of utmost importance not only for systematics but also to truly understand plant evolution as a whole.

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