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ORIGINAL ARTICLE

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Timing of butterfly parasitization of a plant–ant–scale symbiosis

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Abstract In the Southeast Asian tropics, *Arhopala* lycaenid butterflies feed on *Macaranga* ant-plants inhabited by *Crematogaster* (subgenus *Decacrema*) ants tending *Coccus*-scale insects. A recent phylogenetic study showed that (1) the plants and ants have been codiversifying for the past 20–16 million years (Myr), and that (2) the tripartite symbiosis was formed 9–7 Myr ago, when the scale insects became involved in the plant–ant mutualism. To determine when the lycaenids first parasitized the *Macaranga* tripartite symbiosis, we constructed a molecular phylogeny of the lycaenids that feed on *Macaranga* by using mitochondrial and nuclear DNA sequence data and estimated their divergence times based on the *cytochrome oxidase I* molecular clock. The minimum age of the lycaenids was estimated by the time-calibrated phylogeny to be 2.05 Myr, about one-tenth the age of the plant–ant association, suggesting that the lycaenids are latecomers that associated themselves with the pre-existing symbiosis of plant, ant, and scale insects.

Keywords *Arhopala* · Cytochrome oxidase · Molecular clock · Myrmecophytic *Macaranga* · Southeast Asian tropics

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Introduction

Diversifying coevolution between plants and insects is thought to be a major factor leading to species diversity in tropical rainforests (Ehrlich and Raven 1964), but before the development of modern molecular phylogenetic methods it was difficult to reconstruct plant–insect coevolutionary history owing to a dearth of fossil records of plant–insect interactions (Futuyma 2000). Now, by using molecular clocks and advanced molecular phylogenetic techniques, we can easily estimate when adaptive radiation and diversification of a taxonomic group occurred (Rambaut and Bromham 1998). For example, Becerra (2003) calibrated the molecular phylogenetic timelines of Neotropical *Bursera* hosts and the *Blepharida* leaf beetles that feed on them, and showed that the lineages of these beetles and their plant hosts diverged synchronously, suggesting that they reciprocally diversified (codiversified) during the past 112 million years (Myr).

Myrmecophytic *Macaranga* trees (Euphorbiaceae) have hollow stems, which *Crematogaster* (subgenus *Decacrema*) ants use as their nest sites while tending the *Coccus* scale insects that feed on the tree sap. The trees also provide food resources for the ants, both food-bodies secreted by stipules and young leaves, and honeydew excreted by the scale insects (Fiala et al. 1989; Heckroth et al. 1998). The ants, in turn, protect the trees that they inhabit from vines and herbivores (Itioka et al. 2000; Itino and Itioka 2001). Phylogenetic comparison

of *Macaranga*, *Decacrema*, and *Coccus* has shown that (1) the minimum age of the plant–ant mutualism is 20–16 Myr, and the two taxa have codiversified since then (Davies et al. 2001; Itino et al. 2001; Quek et al. 2004, 2007), and (2) the minimum age of the scale insects is 9–7 Myr; thus, the scales are relative latecomers in the evolutionary history of the tripartite symbiosis (Ueda et al. 2008, 2010).

In addition, *Arhopala* lycaenid butterflies (*amphimuta* subgroup) parasitize the protective plant–ant mutualism. *Arhopala* caterpillars eat *Macaranga* leaves, evading the ants' attacks by providing nectar to aggressive ants (Maschwitz et al. 1984). Okubo et al. (2009) surveyed the *Macaranga–Arhopala* interaction, and showed that each lycaenid feeds on one or two closely related *Macaranga* species in a species-specific manner (Table 1). Of approximately 200 species of *Arhopala*, only 5 species feed on *Macaranga*, which are categorized to *amphimuta* subgroup (Eliot 1963, 1972). Most of basal *Arhopala* species prefer to eat Fagaceae whereas the most recently diverged *amphimuta* subgroup has strict feeding habit to *Macaranga* (Maschwitz et al. 1984; Megens et al. 2005). The morphological and behavioral specialization of the *amphimuta* subgroup to the *Macaranga–Decacrema* protective mutualism suggests intimate coevolution between the lycaenids and the plants or ants, or both (Maschwitz et al. 1984; Megens et al. 2004a, b; Megens et al. 2005).

Two hypotheses, the codiversification model (Johnson and Stinchcombe 2007) and the latecomer model (Ueda et al. 2008), can potentially explain the community formation process between *Arhopala* and the *Macaranga* system. According to the codiversification model, the host–parasite interaction of the lycaenids with the *Macaranga* system began at the same time as the *Macaranga–Decacrema* symbiosis (20–16 Myr ago) and the lycaenids' diversification occurred synchronously with the *Macaranga–Decacrema* codiversification as a result of continuous community interaction over macroevolutionary time. In contrast, in the latecomer model, the lycaenids associated with the pre-existing *Macaranga* system, and the host (plant or ant) adaptation then led to the lycaenids' diversification. Megens et al. (2004a) inferred that the earliest radiation of genus *Arhopala* occurred between 7 and 11 Myr ago, but mentioned that these estimates are likely to be an underestimation because of the extreme compositional bias at third-codon. To test these hypotheses, in this study we reconstructed the molecular phylogeny of *Arhopala* lycaenid butterflies feeding on *Macaranga* trees by using mitochondrial and nuclear genes to estimate the timeline of the adaptive radiation and diversification of the lycaenids. We then inferred the minimum age of the *Arhopala–Macaranga* interaction and the timeline of the subsequent diversification of the lycaenids.

Table 1 Host plant associations of *Arhopala* species feeding on *Macaranga*

Arhopala species	Host plant species	References
A. amphimuta	*M. bancana*, *M. trachyphylla*	Maschwitz et al. (1984) and Okubo et al. (2009)
A. dajagaka	*M. hosei*	Maschwitz et al. (1984)
A. major	*M. gigantea*, *Macaranga* sp. A	Okubo et al. (2009)
A. moolaiana	*M. hulletti*	Maschwitz et al. (1984)
A. zylda	*M. beccariana*, *M. hypoleuca*	Maschwitz et al. (1984) and Okubo et al. (2009)

Macaranga sp. A would be the closest relatives to *M. gigantea* (both non-myrmecophytes)

Materials and methods

Sampling

More than 1,000 trees representing 16 *Macaranga* species were comprehensively surveyed for the presence of *Arhopala* species in three locations (Lambir Hills National Park and Kuching in Sarawak, Borneo, and Gunung Tebu in Terengganu, Peninsula Malaysia) from May 1999 to August 2007. Thirty-five lycaenids belonging to five *Arhopala* species (*A. amphimuta*, *A. dajagaka*, *A. major*, *A. moolaiana*, and *A. zylda*), all in the *amphimuta* subgroup, that feed on *Macaranga* trees (Megens et al. 2004b, 2005), were collected from seven *Macaranga* species. Most of the lycaenid specimens analyzed here were previously studied by Okubo et al. (2009), who studied ecological interaction between *Arhopala* and *Macaranga*. In addition, five sequences of the three *Arhopala* species studied by Megens et al. (2004b), who described genus-level phylogeny of *Arhopala*, were used in the phylogenetic analyses (one *A. amphimuta* sequence and two sequences each of *A. major* and *A. moolaiana*). For outgroups, we sequenced (1) *A. pseudocentaurus*, a member of the *centaurus* group, a sister clade of the *amphimuta* subgroup (Megens et al. 2004b), and (2) *A. kinabala*, a member of the *agesias* subgroup, a distant clade from the *amphimuta* subgroup (Megens et al. 2004b). Host plant species, collection locations, and GenBank accession numbers of the samples are listed in Table S1 in the Supplementary Material.

DNA extraction, PCR, and sequencing

DNA was extracted from a single, ethanol-preserved leg of each lycaenid with a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. One mitochondrial gene, *cytochrome oxidase I* (*COI*), and two nuclear genes, *wingless* (*WG*) and *elongation factor 1α* (*EF-1α*) were amplified by polymerase chain reaction (PCR) using Takara Ex *Taq* polymerase (Takara Bio, Shiga, Japan). The PCR primers are given in Table S2. The PCR temperature profile used for *COI* was 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 90 s, and that used for *WG* and *EF-1α* was 30 cycles of 95°C for 30 s, 55°C for 30 s, and

72°C for 30 s. After amplification, the PCR products were purified with ExoSap-IT (USB, Cleveland, OH, USA). Cycle sequencing reactions for both strands were performed with a BigDye Terminator v1.1 Cycle Sequencing Kit (ABI, Weiterstadt, Germany) on an ABI 3130 Genetic Analyzer.

Sequence alignments and character statistics

Mitochondrial *COI* and nuclear *EF-1α* and *WG* sequences were edited and aligned using SeqScape v. 2.5 (ABI, Weiterstadt, Germany). Of the 45 specimens used in this study, 38 represent unique haplo-genotypes. Base frequency homogeneity was tested separately for each dataset with Chi-square test using PAUP* 4.0b10 (Swofford 2002). Parsimony-uninformative sites were excluded from the test. The Chi-square test did not reject the hypothesis of homogeneity of nucleotide frequencies in every pair of taxa ($p > 0.69$; Table S3). To test conflicts in phylogenetic signal among each dataset, we conducted an incongruence length differences (ILD) test (Farris et al. 1994) in PAUP* 4.0b10 with heuristic searches with tree bisection and reconnection (TBR) and 100 random addition replicates for each. The ILD test revealed no conflict between each dataset (*COI* vs. *EF-1α* = 0.75; *COI* vs. *WG* = 0.47; *EF-1α* vs. *WG* = 0.65). The degree of substitution saturation in the third codon position was assessed by plotting the transitions (ti) and transversions (tv) ratio against genetic distance for each dataset using DAMBE with Xia's method (Fig. S1; Xia and Xie 2001). The substitution model of the third codon position for each dataset followed as the result of the model selections described in the next paragraph. The substitution saturation at the third codon position was not detected for every dataset ($p < 0.01$; Fig. S1). Following the results of the character statistics, we used all datasets and all positions for each dataset for phylogenetic analyses.

Phylogenetic analyses

Best-fitted substitution models were selected for each codon position of each gene, based on Bayesian information criterion 5 (BIC5) using KAKUSAN 3 software (Tanabe 2007). Maximum likelihood (ML) analysis was performed with TREEFINDER version October 2008 (Jobb et al. 2004) and the models selected by BIC5 (Table S4). Clade support was assessed with 1000 bootstrap replications in TREEFINDER. In addition, Bayesian posterior probabilities and maximum parsimony (MP) bootstrap support were obtained with MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) and PAUP* 4.0b10 (Swofford 2002), respectively. The models selected by BIC5 were also used in the Bayesian analysis, using the default run settings in which two independent analyses are performed with four chains each (one cold and three heated). The Bayesian analysis was run for 5 million generations, sampling every 1,000 generations. We assessed the log-likelihood for each sampling point against generation time to identify when the Markov chains reached a stationary distribution, and accordingly discarded the initial 1,000 trees as burn-in. The parsimony bootstrap support was assessed with 1,000 bootstrap replicates, using heuristic searches with TBR and ten random addition replicates for each.

Age estimation

Because lycaenid butterflies lack a robust fossil record (Kandul et al. 2004), we estimated divergence times using relative divergence rate in the *COI* gene and set a calibration point on the ML phylogeny of the lycaenids. The *COI* gene has been reported to have the lowest rate heterogeneity in arthropods (Gaunt and Miles 2002), and the *COI* substitution rate converges to about 1.5% per Myr within the insects (Quek et al. 2004). This rate has been widely used for age estimation of several taxa of Lycaenidae (e.g., Als et al. 2004; Lohman et al. 2008), and also of *Crematogaster* ants and *Coccus* scale insects inhabiting *Macaranga* (Quek et al. 2004, 2007; Ueda et al. 2008, 2010). Thus, we used solely the *COI* divergence rate (uncorrected pairwise distance) to estimate the lycaenids' divergence times. First, the heterogeneity of the substitution rate of *COI* in the ML topology was tested by using the likelihood ratio test (LRT) with the models selected by BIC5, implemented in PAUP. The LRT results showed significant deviations from rate constancy ($p < 0.05$). Second, the range of divergence times of node A (Fig. 1) was estimated by non-parametric rate smoothing (NPRS, Sanderson 1997), implemented in TreeEdit v. 1.0 (Rambaut and Charleston 2002). In NPRS, mean uncorrected pairwise distances between sister taxa were calculated using MEGA v 2.1 (Kumar et al. 2001), and three well-supported nodes of varying genetic divergence (nodes A, B, and C; 2.1–5.8%) were chosen as calibration points for estimating the age of node A. The range of divergences was used for dating divergences in various arthropod groups (Table 4 in Quek et al. 2004). The age range at node A was estimated to be 3.87–1.95 Myr, which was chosen as the calibration time unit range in the Bayesian analysis. Finally, the lycaenids' divergence times were estimated by using a Bayesian approach, as implemented in the mcmctree program in the PAML v.4.2 package (Yang 2007). A Bayesian global-clock analysis was run using the HKY + Γ substitution model, sampling 1 million generations every two generations, after discarding the initial 100,000 steps as burn-in.

Results and discussion

Molecular phylogenies of the *Arhopala* lycaenids feeding on *Macaranga* were inferred from a total of 1,998 bp

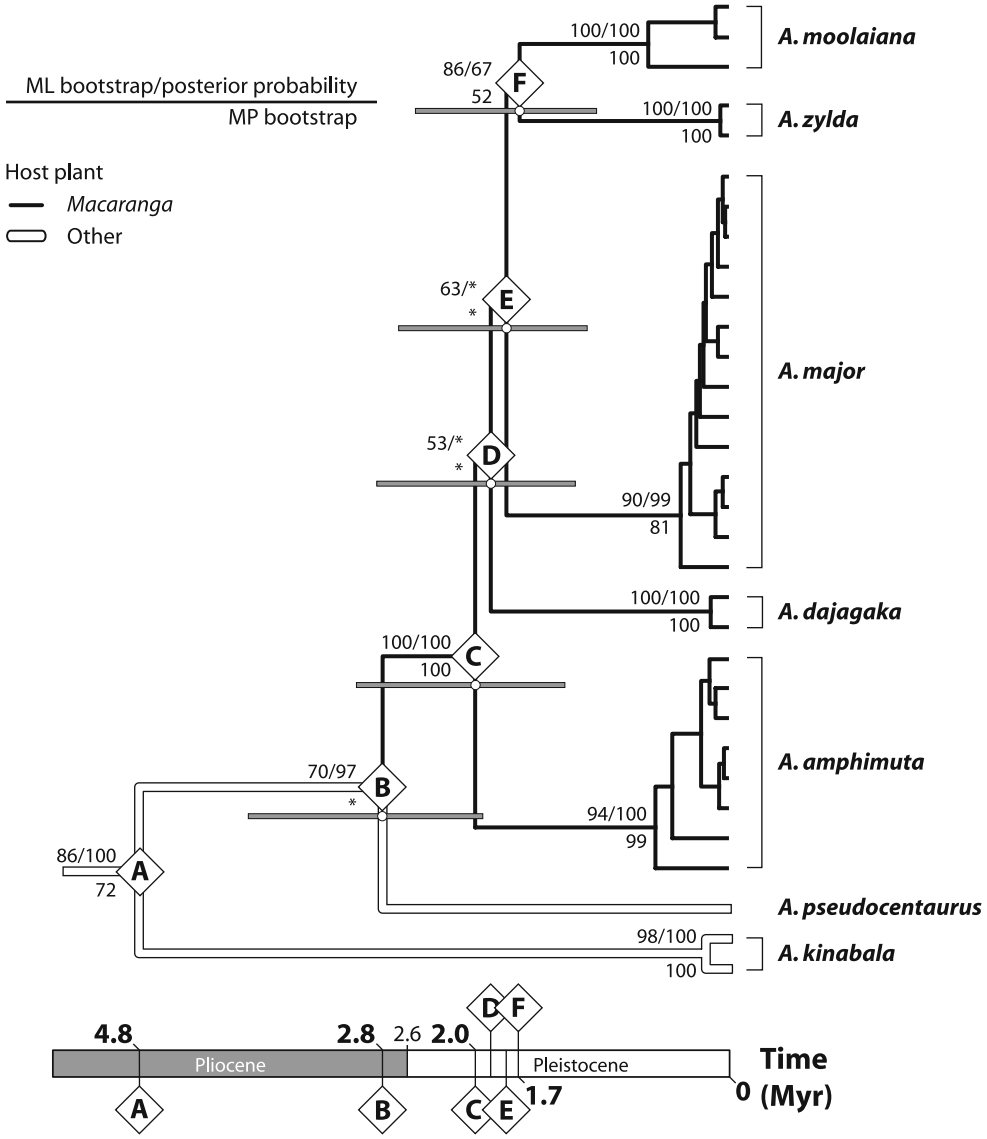

Fig. 1 ML phylogeny of *Arhopala* lycaenids feeding on *Macaranga* estimated from 1,010 bp of mitochondrial DNA sequences (*COI*) and 988 bp of nuclear DNA sequences (*WG* 393 bp and *EF-1α* 595 bp). The *numbers above the branches* indicate the ML bootstrap support (*left of slash*) and the Bayesian posterior probabilities (*right of slash*); the MP bootstrap support is shown below *each branch*. When a node was not recovered in the MP bootstrap or Bayesian posterior probability analyses, an *asterisk* is substituted for the node support values. Branch lengths are proportional to time inferred by Bayesian inference using the *COI* molecular clock and *gray bars* indicate 95% confidence intervals (Table 2)

from the three genes (mitochondrial *COI* and nuclear *WG* and *EF-1α*) by ML, MP, and Bayesian analyses. Monophyly of the *amphimuta* subgroup and of each species was recovered in the ML, MP, and Bayesian topologies (Fig. 1), but the relationships among the species were not well supported; node B was poorly supported by MP bootstrapping, and nodes D and E were poorly supported by both MP bootstrapping and Bayesian posterior probability analyses (Fig. 1).

The divergence time of the lycaenids was estimated from the *COI* molecular clock rate within the insects (1.5% per Myr; Quek et al. 2004). The minimum estimated age of the *amphimuta* subgroup that feed on *Macaranga* was 2.05 Myr, indicating a Pleistocene divergence (Table 2; Fig. 1, node C), whereas the minimum age of the *Macaranga–Crematogaster* association is 20–16 Myr (Quek et al. 2007) and that of *Macaranga–Coccus* is 9–7 Myr (Ueda et al. 2008, 2010). In addition, the divergence times of the two outgroups, *A. pseudocentaurus* and *A. kinabala*, were estimated to be 4.77 and 2.79 Myr ago, respectively (Table 2; Fig. 1, nodes A and B), which are also younger than the tripartite symbiosis (9–7 Myr). Thus, the lycaenids probably became involved in the *Macaranga* system after the origin of the tripartite symbiosis.

The time-calibrated phylogeny also showed that the species divergence of the *Arhopala* on *Macaranga* (2.05–1.68 Myr ago; Table 2; Fig. 1, nodes C–F)

Table 2 Mean age and 95% confidence interval (CI) of nodes in the *Arhopala* phylogeny obtained by Bayesian inference, based on the *COI* molecular clock (1.5% divergence per million years)

Node	Age (Myr)	
	Mean	95% CI
A	4.77	2.83–7.63
B	2.79	1.98–3.87
C	2.05	1.33–3.01
D	1.92	1.24–2.84
E	1.80	1.15–2.67
F	1.68	1.06–2.52

occurred during the Pleistocene, suggesting that a rapid radiation of the *amphimuta* subgroup occurred during the Pleistocene. Moreover, all *Macaranga* species and the mtDNA lineages of the *Decacrema* ants and *Coccus* scales were already in existence in the late Pliocene (Quek et al. 2007; Ueda et al. 2010), which also indicates that the species divergence of the lycaenids occurred after the origin of the tripartite symbiosis. Therefore, the lycaenids might have diversified to adapt to the pre-existing plant, ant, and scale insect symbiosis. Given the younger origin and later diversification of the lycaenids compared with the origin of the plant, ant, and scale symbiosis, the association between the *Arhopala* lycaenids and the *Macaranga* tripartite system is not consistent with the codiversification model of the coevolutionary history of the *Macaranga–Decacrema* mutualism but it is consistent with the latecomer model.

To explain the diversification of genus *Arhopala,* two hypotheses have been proposed. Corbet (1946) suggested that vicariance in the Pleistocene accelerated speciation of the genus *Arhopala*. The Pleistocene glacial cycles caused sea levels to fluctuate, with the result that land bridges between islands and the mainland formed and resubmerged repeatedly (Medway 1972; Morley 2000). In contrast, Megens et al. (2004b), who inferred the minimum age of the genus *Arhopala* to be 11–7 Myr, hypothesized that large-scale climatic changes in the mid-Miocene accelerated *Arhopala* speciation. The South East Asian forest experienced a gradually cooling climate and intensified monsoons around 8 Myr ago (Zachos et al. 2001). Our age estimate for when the lycaenids began feeding on *Macaranga* suggests that the divergence (Table 2; Fig. 1, 2.05–1.68 Myr ago) occurred during the Pleistocene. Thus, Pleistocene vicariance may have played an important role in the diversification of the *amphimuta* subgroup, in accordance with Corbet's (1946) hypothesis. However, we did not investigate the geographical context of the lycaenid diversification because the lycaenid sampling locations were insufficiently widespread for phylogeographic inferences. Further sampling throughout the distribution area of myrmecophytic *Macaranga* (Sumatra, the Malay Peninsula, and Borneo; Fiala et al. 1999; Davies et al. 2001) would be necessary to clarify the postglacial biogeography of the lycaenids.

Cool, dry periods during the Pleistocene might have also helped the ancestor of the *amphimuta* subgroup to become established as a parasite on *Macaranga* by weakening the ants' ability to defend the trees. In rare instances, *A. kinabala* is known to feed on several species of *Macaranga* when the ants' defense ability is weakened by natural physical disturbances (Itioka, unpublished data). In addition, the feeding habit of *Arhopala* may have shifted from generalist to specialist. The *centaurus* group, a sister clade of *amphimuta* subgroup, is extremely polyphagous and feeds on several families of plants including Euphorbiaceae (Megens et al. 2005). If so, *Decacrema* ants, as well as *Macaranga* trees, may have played an important role in the origin and diversification of the *Arhopala* lycaenids. However, we have not investigated the specificity of the relationship between *Arhopala* and *Decacrema*. Phylogenetic comparison of *Macaranga*, *Decacrema*, and *Arhopala* might reveal whether the plants or the ants induced the lycaenid diversification and specialization.

The timeline inferred in this study, which indicates only the gross pattern of the adaptive radiation and diversification, is based on all known species of *Arhopala* lycaenids that feed on *Macaranga*. Further sampling throughout the Asian tropics is likely to reveal more lycaenid genotypes and result in some change in the estimated ages. However, our estimated minimum age of the lycaenids seems reasonable because it is consistent with the timing of the drastic climate changes caused by cyclical glaciation (Medway 1972) and the ages of migration events across the South China Sea in *Decacrema* ants and *Coccus* scale insects (Quek et al. 2007; Ueda et al. 2010). In addition, we should consider a possible deviation from the *COI* divergence rate of around 1.5% per Myr because rate constancy has been rejected. Therefore, we also estimated the age range using known *COI* rates (1.3–2.3%; Brower and DeSalle 1998; Quek et al. 2004). Using the range of *COI* rate, the minimum age of the *amphimuta* subgroup ranged from 1.34 to 2.36 Myr ago, which are not older than the Pleistocene and much younger than the tripartite symbiosis. Thus rate deviation may not introduce any substantial error on our conclusion. It is also plausible that there may have been extinctions of older taxa feeding on *Macaranga* trees, but the hypothesis is, unfortunately, untestable.

Coevolution between insects and their host plants has been proposed to be an important factor promoting global biodiversity, but few studies have demonstrated the historical process within a community context, including multipartite plant–insect interactions. Here, we showed that the *Arhopala* lycaenids were latecomers to the evolutionary history of the *Macaranga*-based community. The *Macaranga* community also includes other insect taxa that have evolved specific interactions, such as leaf-galling gall midges (Cecidomyiidae) and ant-predatory mirid bugs (Itino and Itioka 2001). Further phylogenetic analyses of these multipartite associations may provide insight into evolutionary dynamics of plant–insect communities in the South East Asian tropics.

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References

Als TD, Vila R, Kandul NP, Nash DR, Yen SH, Hsu YF, Mignault AA, Boomsma JJ, Pierce NE (2004) The evolution of alternative parasitic life histories in large blue butterflies. Nature 432:386–390. doi:10.1038/nature03020

Becerra JX (2003) Synchronous coadaptation in an ancient case of herbivory. Proc Natl Acad Sci USA 100:12804–12807. doi:10.1073/pnas.2133013100

Brower AVZ, DeSalle R (1998) Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of *wingless* as a source of characters for phylogenetic inference. Insect Mol Biol 7:73–82

Corbet SA (1946) Observations on the Indo-Malayan species of the genus *Arhopala* Boisduval (Lepidoptera: Lycaenidae). Trans R Entomol Soc Lond 96:73–88

Davies SJ, Lum SKY, Chan R, Wang LK (2001) Evolution of myrmecophytism in western Malesian *Macaranga* (Euphorbiaceae). Evolution 55:1542–1559. doi:10.1554/0014-3820(2001)055[1542:EOMIWM]2.0.CO;2

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

Eliot JN (1963) A key to the Malayan species of *Arhopala*. Malay Nat J 17:188–217

Eliot JN (1972) Some *Arhopala* from Borneo, with a revision of the *Arhopala cleander* group (Lepidoptera: Lycaenidae). J Nat Hist 6:1–15

Farris JS, Kallersjo M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics Int J Willi Hennig Soc 10:315–319

Fiala B, Maschwitz U, Tho YP, Helbig AJ (1989) Studies of a Southeast Asian ant–plant association: protection of *Macaranga* trees by *Crematogaster borneensis*. Oecologia 79:463–470. doi:10.1007/BF00378662

Fiala B, Jakob A, Maschwitz U (1999) Diversity, evolutionary specialization and geographic distribution of a mutualistic ant–plant complex:*Macaranga* and *Crematogaster* in South East Asia. Biol J Linn Soc Lond 66:305–331

Futuyma DJ (2000) Some current approaches to the evolution of plant–herbivore interactions. Plant Species Biol 15:1–9. doi:10.1046/j.1442-1984.2000.00029.x

Gaunt MW, Miles MA (2002) An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Mol Biol Evol 19:748–761

Heckroth HP, Fiala B, Gullan PJ, Idris AH, Maschwitz U (1998) The soft scale (Coccidae) associates of Malaysian ant–plants. J Trop Ecol 14:427–443. doi:10.1017/S0266467498000327

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Itino T, Itioka T (2001) Interspecific variation and ontogenetic change in antiherbivore defense in myrmecophytic *Macaranga* species. Ecol Res 16:765–774. doi:10.1046/j.1440-1703.2001.00432.x

Itino T, Davies SJ, Tada H, Hieda O, Inoguchi M, Itioka T, Yamane S, Inoue T (2001) Cospeciation of ants and plants. Ecol Res 16:787–793. doi:10.1046/j.1440-1703.2001.00442.x

Itioka T, Nomura M, Inui Y, Itino T, Inoue T (2000) Difference in intensity of ant defense among three species of *Macaranga* myrmecophytes in a Southeast Asian dipterocarp forest. Biotropica 32:318–326

Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol Biol 4:18. doi:10.1186/1471-2148-4-18

Johnson MTJ, Stinchcombe JR (2007) An emerging synthesis between community ecology and evolutionary biology. Trends Ecol Evol 22:250–257. doi:10.1016/j.tree.2007.01.014

Kandul NP, Lukhtanov VA, Dantchenko AV, Coleman JWS, Sekercioglu CH, Haig D, Pierce NE (2004) Phylogeny of *Agrodiaetus* Hubner 1822 (Lepidoptera: Lycaenidae) inferred from mtDNA sequences of *COI* and *COII* and nuclear sequences of *EF1-alpha*: karyotype diversification and species radiation. Syst Biol 53:278–298

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA 2.1: molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245

Lohman DJ, Peggie D, Pierce NE, Meier R (2008) Phylogeography and genetic diversity of a widespread Old World butterfly, *Lampides boeticus* (Lepidoptera: Lycaenidae). BMC Evol Biol 8:1–14. doi:10.1186/1471-2148-8-301

Maschwitz U, Schroth M, Hänel H, Pong TY (1984) Lycaenids parasitizing symbiotic plant–ant partnerships. Oecologia 64:78–80. doi:10.1007/BF00377547

Medway G (1972) The quaternary mammals of Malesia: a review. In: Ashton PS, Ashton M (eds) The quaternary era in Malesia transactions of the 2nd Aberdeen-Hull symposium on Malesian ecology. University of Hull Department of Geography Miscellaneous Series no. 13, Hull, pp 63–83

Megens HJ, van Moorsel CHM, Piel WH, Pierce NE, de Jong R (2004a) Tempo of speciation in a butterfly genus from the Southeast Asian tropics, inferred from mitochondrial and nuclear DNA sequence data. Mol Phylogenet Evol 31:1181–1196. doi:10.1016/j.ympev.2003.10.002

Megens HJ, Van Nes WJ, Van Moorsel CHM, Pierce NE, De Jong R (2004b) Molecular phylogeny of the Oriental butterfly genus *Arhopala* (Lycaenidae, Theclinae) inferred from mitochondrial and nuclear genes. Syst Entomol 29:115–131. doi:10.1111/j.1365-3113.2004.00228.x

Megens HJ, De Jong R, Fiedler K (2005) Phylogenetic patterns in larval host plant and ant association of Indo-Australian Arhopalini butterflies (Lycaenidae: Theclinae). Biol J Linn Soc 84:225–241. doi:10.1111/j.1095-8312.2005.00426.x

Morley RJ (2000) Origin and evolution of tropical rain forests. Wiley, Chichester

Okubo T, Yago M, Itioka T (2009) Immature stages and biology of Bornean *Arhopala* butterflies (Lepidoptera, Lycaenidae) feeding on myrmecophytic *Macaranga*. Trans Lepidopterol Soc Jpn 60:37–51

Quek SP, Davies SJ, Itino T, Pierce NE (2004) Codiversification in an ant–plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution 58:554–570. doi:10.1554/03-361

Quek SP, Davies SJ, Ashton PS, Itino T, Pierce NE (2007) The geography of diversification in mutualistic ants: a gene's-eye view into the Neogene history of Sundaland rain forests. Mol Ecol 16:2045–2062. doi:10.1111/j.1365-294X.2007.03294.x

Rambaut A, Bromham L (1998) Estimating divergence dates from molecular sequences. Mol Biol Evol 15:442–448

Rambaut A, Charleston M (2002) TreeEdit. Phylogenetic tree editor v1.0 alpha 10. Department of Zoology, University of Oxford, Oxford

Sanderson MJ (1997) A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol Biol Evol 14:1218–1231

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony. Ver. 4.0b10. Sinauer Associates, Sunderland

Tanabe AS (2007) KAKUSAN: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. Mol Ecol Notes 7:962–964. doi:10.1111/j.1471-8286.2007.01807.x

Ueda S, Quek SP, Itioka T, Inamori K, Sato Y, Murase K, Itino T (2008) An ancient tripartite symbiosis of plants, ants and scale insects. Proc R Soc B Biol Sci 275:2319–2326. doi:10.1098/rspb.2008.0573

Ueda S, Quek SP, Itioka T, Murase K, Itino T (2010) Phylogeography of the *Coccus* scale insects inhabiting myrmecophytic *Macaranga* plants in Southeast Asia. Popul Ecol 52:137–146. doi:10.1007/s10144-009-0162-4

Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. J Hered 92:371–373

Yang ZH (2007) PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol 24:1586–1591

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693