

Phylogeography of the *Coccus* scale insects inhabiting myrmecophytic *Macaranga* plants in Southeast Asia

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Abstract Comparative historical biogeography of multiple symbionts occurring on a common host taxa can shed light on the processes of symbiont diversification. Myrmecophytic *Macaranga* plants are associated with the obligate mutualistic symbionts: *Crematogaster* (subgenus *Decacrema*) ants and *Coccus* scale insects. We conduct phylogeographic analyses based on mitochondrial cytochrome oxidase I (COI) from 253 scale insects collected from 15 locations in Borneo, Malaya and Sumatra, to investigate the historical biogeography of the scales, and then to draw comparisons with that of the symbiotic, but independently dispersing, *Decacrema* ants which are not

specific to different *Coccus* lineages. Despite the different mode of ancient diversification, reconstruction of ancestral area and age estimation on the *Coccus* phylogeny showed that the scales repeatedly migrated between Borneo and Malaya from Pliocene to Pleistocene, which is consistent with the *Decacrema* ants. Just as with the ants, the highest number of lineages in the scale insects was found in northern northwest Borneo, suggesting that these regions were rainforest refugia during cool dry phases of the Pleistocene. Overall, general congruence between the Plio–Pleistocene diversification histories of the symbiotic scales and ants suggests that they experienced a common history of extinction/migration despite their independent mode of dispersal and host-colonization.

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Introduction

Phylogeography provides a good opportunity to identify the historical evolutionary processes, the glacial refugia and postglacial migration routes of organisms (Avice 2000). Several studies have compared the phylogeny between host and symbiont organisms (reviewed in Nieberding and Olivieri 2007), but there are currently few studies that compare phylogeography of co-occurring and distantly related symbiotic and parasitic taxa (e.g., Althoff et al. 2007; Whiteman et al. 2007). Comparative historical biogeography of multiple symbionts occurring on a common host taxa can shed light on the evolutionary processes of symbiont diversification (Whiteman et al. 2007).

Tropical rainforests in Southeast Asia harbor some of the greatest concentrations of biodiversity on earth. In

proportion to this biodiversity, however, there is a dearth of phylogeographic studies on the biota of the region. In Plio–Pleistocene, sea levels fluctuated considerably through the glacial and interglacial cycles, and repeatedly created connection and disconnection among the islands and mainland in vast areas of Southeast Asia. These land bridges may have enabled migration of many organisms across the Sunda region (Medway 1972). In the Pleistocene, the distribution of the rainforest was reduced by the influence of cool dry climates of the glacial cycles, and these changes might have contracted the rainforest to isolated refugia (Morley 2000). These geologic events would have influenced the current geographic distribution of genetic and taxonomic diversity in rainforest biota.

Obligate mutualisms between ants and myrmecophytes (ant–plants) are a phenomenon generally restricted to rainforests, and thus present an excellent system in which to study the generation of diversity in these ecosystems. In the Southeast Asian tropics, 26 species of myrmecophytic *Macaranga* trees engage in an obligate mutualism with *Crematogaster* (subgenus *Decacrema*) ants. The ants gain nest sites in hollow stems (domatia) and food bodies secreted by stipules and/or young leaves, while the plants gain protection against vines and herbivores from the ants (Fiala et al. 1989). In most cases, the ants also tend *Coccus* scale insects within the hollow stems, and these provide additional nutrients for the ants in the form of excreted plant sap, or honey-dew (Heckroth et al. 1998). The distribution of this tripartite symbiosis is strictly limited to the ever-wet rainforest in western Malesia (Sumatra, the Malay Peninsula, and Borneo; Fiala et al. 1999; Davies et al. 2001), and, thus, their evolutionary history might illuminate some of the history of the Southeast Asian rainforests.

Several authors suggest that the presence of scale insects (scales) plays an important role in the evolution of myrmecophytes (Benson 1985; McKey 1989; Ward 1991), and also in the successful establishment of an ant colony on a host plant (Moog et al. 2005). Heckroth et al. (1998) discovered 22 *Coccus* morphospecies from 19 species of myrmecophytic *Macaranga*; they are highly specific to the genus *Macaranga* while ranging from being monophagous to polyphagous towards individual host species of *Macaranga*. Ueda et al. (2008) further reported that the association of scales' mtDNA lineages with ants' was not specific but dictated by opportunity. Whereas the mode of host-colonization among the scales is not known, it is known that ants and scales colonize *Macaranga* independently of each other. Scales have not been observed to disperse in tandem with dispersing ant queens attempting to found new colonies, and the nymphs of the scales most probably disperse by wind drift, and, on their arrival, ant workers carry them into the interior of the stem (Fiala and

Maschwitz 1990; Gullan 1997). The *Coccus* scales inhabiting *Macaranga* appear to constitute a monophyletic group (P.J. Gullan and T. Kondo, personal communication), but molecular phylogenetic studies based on mtDNA are inconclusive (Ueda et al. 2008).

Quek et al. (2007) investigated the phylogeography of *Decacrema* ants on *Macaranga*, and elucidated that the ants originated 20–16 Mya (million years ago), drastically diversified during the Pliocene, and migrated from Borneo (or Sumatra) to Malaya repeatedly through the Plio–Pleistocene. Ueda et al. (2008) subsequently reconstructed gene phylogeny of *Coccus* scale insects collected from the same trees of myrmecophytic *Macaranga* that were sampled by Quek et al. (2007) for the ants. The minimum age of the scales was estimated to be half that of the ants, at 9–7 Mya in the late Miocene, suggesting that they were latecomers in the evolutionary history of the symbiosis. However, the phylogeography of the scales has yet to be ascertained in comparison with the ants.

Have the scale insects experienced the common historical biogeography with the ants and plants? Because of the obligate symbiosis of *Macaranga*–*Decacrema*–*Coccus*, we may expect at its simplest that the distribution of the scales and ants are restricted to that of their host plants, and that they share common historical biogeographical processes. However, the fact that the scales from *Macaranga* are occasionally found with phytoecious (i.e., dwelling in live plant cavities) *Cladomyrma* ants (Moog et al. 2005), and further that a myrmecophytic *Macaranga* species (*M. puncticulata*) is not inhabited by any scale insects (Federle et al. 1998), both suggest that a common history (co-phylogeography) of the scales and ants is not necessarily to be expected. In these and other cases, regional extinctions of either scales or ants might have occurred, and this may cause disagreement of their phylogeography, location of glacial refugia, and/or migration routes.

The aim of the present study is to investigate the historical biogeography of the *Coccus* scale insects associated with myrmecophytic *Macaranga* plants in Southeast Asia, and then to compare it with the phylogeography of *Decacrema* ants, another obligate symbiont of *Macaranga* plants, as reported by Quek et al. (2007) who used the same DNA fragment as the scales in this study. We expand on the study by Ueda et al. (2008), in combination with new data and the data used therein, and set the focus to (1) infer the time frame of the migration/vicariance events on the *Coccus* phylogeography, (2) identify the glacial refugia of the *Coccus*, and (3) compare these results with those of *Decacrema* ants reported by Quek et al. (2007). Results were discussed in the context of the history of Sundaland's rainforest and the extant host plant distribution.

Materials and methods

Sampling of *Coccus* scale insects

A total of 253 scale insects were collected from 235 trees representing 22 *Macaranga* species [including the data from Ueda et al. (2008) and new data]. We sampled from 15 locations, of which 1 was in Sumatra, 7 were in Malaya, and 7 in Borneo. Most of the scales reported here were collected from ant colonies used in Quek et al. (2007). We concentrated the sampling on myrmecophytic *Macaranga* trees because the *Coccus* scales in *Macaranga* are known to be highly specific to the genus *Macaranga* (Heckroth et al. 1998). Usually, one individual scale per one *Macaranga* tree was analyzed. In some exceptional cases, when two morphologically different types of scale insects were detected on a tree (18 out of the 235 *Macaranga* trees sampled), each was individually analyzed. For the outgroups, we sequenced (1) four free-living *Coccus* species (*C. celatus*, *C. hesperidum*, *C. pseudomagnoliarum* and *C. viridis*) which are assumed to be close relatives of the *Coccus* on *Macaranga* (P.J. Gullan and T. Kondo, personal communication), and (2) two genera, *Eulecanium* and *Parthenolecanium*, in the subfamily Eulecaninae within Coccidae (Hodgson 1994). Host species, collection localities, elevation and GenBank accession numbers of the samples are presented in Table S1 in Electronic Supplementary Material (ESM).

Molecular datasets

The methods of DNA extraction and sequencing are reported in Ueda et al. (2008). We used two nucleotide sequence datasets in this study. Dataset 1 includes 521 bp of a mitochondrial cytochrome oxidase I (COI) for 253 ingroup samples, and was used for age estimation and migration-vicariance analysis. Dataset 2 includes 1,021 bp of COI for 20 ingroup exemplars representing the major clades from Dataset 1 phylogeny, and was used for confirming the monophyly of *Coccus* on *Macaranga*. The 20 samples represent the 7 lineages out of 8 from Dataset 1 phylogeny, and within each lineage, all the monophyletic clades from different geographic region (Sumatra, Malaya, or Borneo) were sampled, with one sample per clade being used for sequencing. The polymerase chain reaction (PCR) primers were shown in Table 1. The PCR temperature profile of Dataset 1 is reported in Ueda et al. (2008), and that of Dataset 2 was: 35 cycles of 95°C for 30 s, 45°C for 30 s and 72°C for 90 s.

Phylogenetic analysis

Maximum likelihood (ML) analysis was performed with PHYML version 2.4.4 (Guindon and Gascuel 2003). Best-fitted substitution model was selected for each dataset based on hierarchical likelihood ratio tests (hLRT; Huelsenbeck and Rannala 1997) using Modeltest version 3.7 (Posada and Crandall 1998). GTR + I + G substitution model was used as selected by hLRT in both datasets. Clade support was assessed with 1,000 bootstrap pseudoreplications. In addition, Bayesian posterior probabilities and maximum parsimony (MP) bootstrap support were obtained using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) and PAUP* 4.0b10 (Swofford 2002), respectively. The GTR + I + G substitution model was also used in Bayesian analysis, using the default run settings that perform two independent analyses with four chains each (one cold and three heated). The Bayesian analysis was run for 5 million generations, with a burn-in of 4 million generations, well after stationarity was reached. The parsimony bootstrap support was assessed with 1,000 bootstrap replicates, using 10 random addition replicates each.

To test whether the *Coccus* on *Macaranga* are the monophyletic group or not in ML tree of Dataset 2, the Shimodaira–Hasegawa (SH; Shimodaira and Hasegawa 1999) test was conducted with PAUP* using 1,000 bootstrap replicates and full optimization. In the SH test, the likelihood score of given topology was compared to the score of constrained topology in which the ingroup (L1–L8) is assumed to be monophyletic.

Operational lineages and age estimation

Operational lineages in the mtDNA tree were defined based on well-supported monophyly and on obvious phylogenetic breaks (i.e., large distances between clades) in order to infer the historical biogeography of the mtDNA lineages as in Ueda et al. (2008). To estimate the ages of divergence within the COI phylogeny of Dataset 1, we used 1.5% divergence per million years (uncorrected pairwise distance). As COI exhibits the least rate heterogeneity in arthropods (Gaunt and Miles 2002), it has been widely used for dating within arthropods (e.g., Degnan et al. 2004) with the substitution rate being about 1.5% per million years (Quek et al. 2004). Homogeneity of substitution rate on the ML phylogeny of Dataset 1 of the scale insects was tested using the LRT (Huelsenbeck and Rannala 1997) with the GTR + I + G model using PAUP* 4.0b10. All duplicate haplotypes were removed in the LRT test. Because the LRT showed significant deviations from rate constancy ($P < 0.01$), branch lengths were subjected to nonparametric

Table 1 The list of primers used in this study

Locus	Primer name	Sequence	Used for	Reference
COI	mtD-6	5'-GGATCACCTGATATAGCATTCCC-3'	Dataset 1 and 2	Simon et al. (1994)
COI	CI-N4	5'-CCTGGTAGGATTTAAAATATATAC-3'	Dataset 1	Ueda et al. (2008)
COI	CI-N5	5'-TTTTTADTATRITGTTGTGA-3'	Dataset 2	This paper
COI	a2761asp	5'-GGTATNCCATTTAATCC-3'	Dataset 2	Gwiazdowski et al. (2006)
tRNA-Leucine	Pat	5'-TCCAATGCACTAATCTGCCATATTA-3'	Dataset 2	Simon et al. (1994)

rate smoothing (Sanderson 1997) implemented in TreeEdit 1.0 (Rambaut and Charleston 2002). Three well-supported nodes of varying genetic divergences (5.9–13.9%) were used as calibration points to obtain the ages of the other nodes. This approach produces a range of ages, rather than a point estimate, and is thus more conservative. Mean uncorrected pairwise distances between sister taxa were calculated using MEGA 2.1 (Kumar et al. 2001).

Migration-vicariance analysis

A dispersal (migration)-vicariance analysis was conducted using DIVA version 1.1 (Ronquist 1997) to examine the historical biogeography of the *Coccus* scale insects and to generate hypothesis for migration or vicariance events in the given phylogeny. DIVA infers ancestral distributions based on a three-dimensional cost matrix that applies a cost of 1 to migration/extinction and no cost to vicariance, and that does not require a general hypothesis of area relationships (Ronquist 1997). Because DIVA analysis requires a bifurcating tree, we used summary topology of Dataset 1 containing geographically informative clades; each clade consists of OTUs sampled from the same distributional area. Each sample was coded as present or absent in each of two distributional areas: Borneo and/or Sumatra and Malaya. Because of the insufficiency of Sumatran samples ($n = 3$) and the sharing of the same haplotypes between Borneo and Sumatra, Borneo and Sumatra were tentatively pooled as a region in this study.

Results

Phylogeography and geographical distribution of lineages

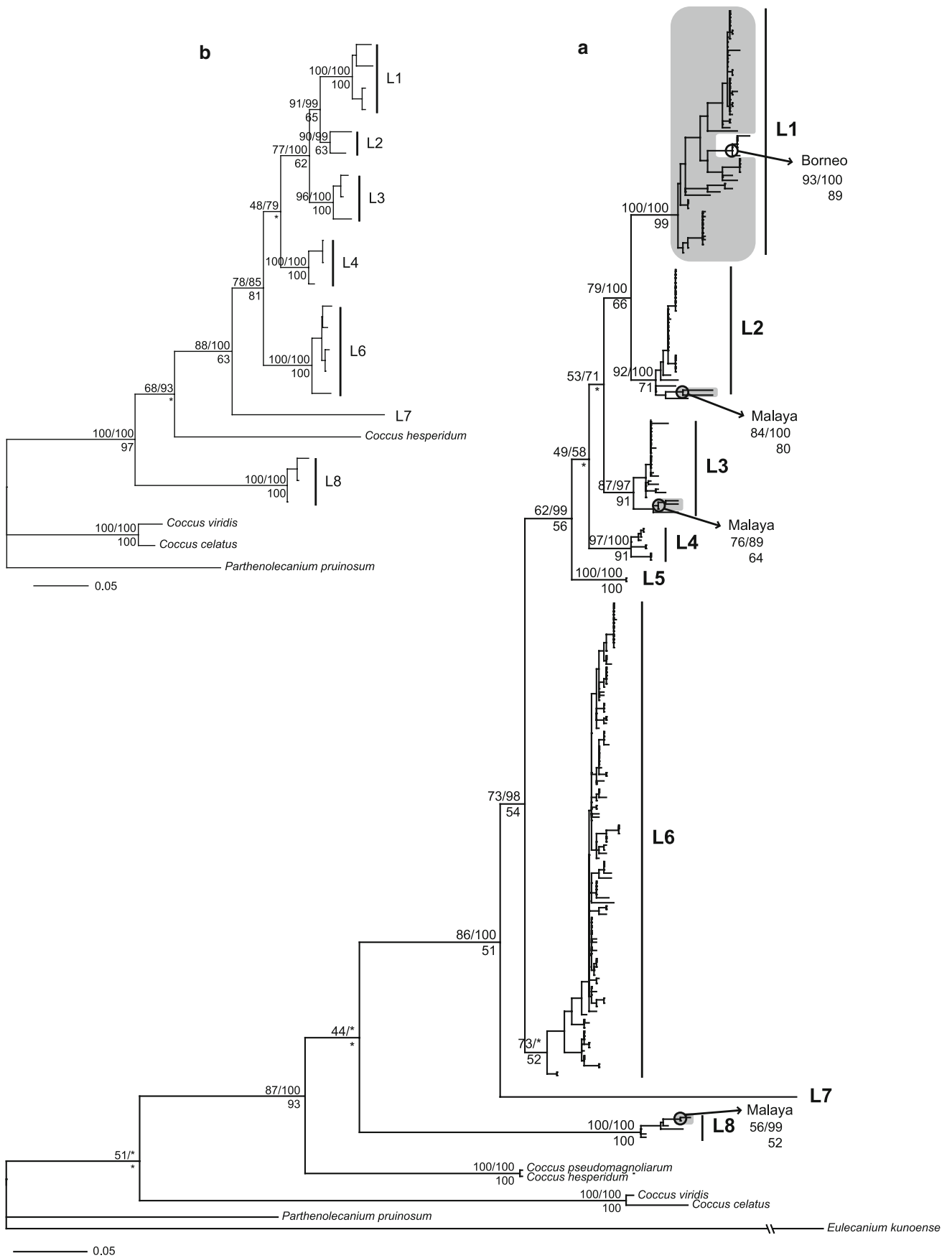
Eight operational lineages were detected in the mtDNA phylogeny from Dataset 1 (Fig. 1a). Here, we detected two new lineages that were not reported in Ueda et al. (2008): L5 ($n = 2$) from Lambir in Borneo and L7 ($n = 1$) from Berastagi in Sumatra. The phylogeny from Dataset 2 (Fig. 1b) provides a better support for each lineage than that from Dataset 1 (Fig. 1a), and does not support the

monophyly of the *Coccus* on *Macaranga*: L1–L7 fall into a clade, whilst L8 falls outside the L1–L7 clade (Fig. 1b). The monophyly of the L1–L7 clade and the outgroup *C. hesperidum* was well supported by Bayesian posterior probability (93%), but was poorly supported by ML bootstrapping (68%) and not supported by MP bootstrapping (Fig. 1b). In addition, the SH test indicated that there is not a significant disagreement between the ML topology of Dataset 2 and the constrained topology in which the ingroup (L1–L8) is assumed to be monophyletic ($P > 0.05$).

In Fig. 1a, L1, L2, L3, and L8 distributed both in Malaya and Borneo; in L1, the well-supported Bornean clade is nested within Malayan samples; in L2, L3 and L8, well-supported Malayan clades are nested within Bornean samples. L4 and L5 are exclusively Bornean, and L7 is exclusively Sumatran lineage. L6 is distributed both in Borneo and Sumatra; Sumatran haplotypes in L6 do not form a monophyletic group.

The geographical distribution of each lineage differed quite substantially from that of the others (Fig. 2). Borneo harbored a higher number of *Coccus* lineages (7) than Malaya (4) and Sumatra (2). Malaya was dominated by L1 whilst Borneo and Sumatra were dominated by L6. L1, L2, and L3 are distributed from Malaya to western Borneo across the South China Sea; L4 was limited to Crocker Range and Kuching and L5 to Lambir; L6 is widespread from Borneo to Sumatra; L7 is endemic to Sumatra; L8 is patchily distributed in four locations (Johor, Crocker, Lambir, and Meratus) in both Malaya and Borneo (Fig. 2). The highest number of lineages was found in Lambir (five lineages) in northern northwest Borneo, and in Johor and Tioman (three lineages each) in Malaya.

Fig. 1 a Maximum-likelihood tree for *Macaranga*-inhabiting *Coccus* scale insects estimated from Dataset 1 (COI, 521 bp). **b** ML tree for exemplars from Dataset 2 estimated from Dataset 2 (COI, 1021 bp). The numbers above branches indicate ML bootstrap support (left of slash), Bayesian posterior probabilities (right of slash), and MP bootstrap support (below branch). An asterisk in the node support values indicates the node was not found recovered in MP bootstrap or Bayesian posterior probability analysis. Shaded clades indicate the Malayan scale insects



Biogeography

The reconstruction of ancestral area (Fig. 3) suggests that the scales' major axis of diversification (L4–L7) was in Borneo and/or Sumatra. The scales were inferred to have first diversified in Borneo and/or Sumatra 8.6–7.2 Mya in the late Miocene (node a in Fig. 3, Table 2). If we assume that L8 represents an independent colonization of *Macaranga* by *Coccus* (Fig. 1b), the minimum estimate of the first diversification of the scales is even younger at 6.8–5.7 Mya (node b in Fig. 3, Table 2). The DIVA analysis inferred no vicariance and five migration events: four migrations in Pliocene at node g (4.1–3.4 Mya), node j (2.2–1.8 Mya), node k (2.3–1.9 Mya), and node l (2.1–1.7 Mya), and one migration in Pleistocene at node i (1.3–1.1 Mya).

Discussion

Our aim in this paper is to infer the evolutionary and population history of the mtDNA of *Coccus* scale insects

associated with *Macaranga* trees in Southeast Asia in order to make comparisons with a similar study by Quek et al. (2007) of the *Decacrema* ants that tend them. Quek et al. (2007), based on COI phylogeography, showed that (1) the main diversification of the ants occurred in Borneo in the Miocene, (2) a number of lineages of ants dispersed to Malaya via Sumatra in the Plio–Pleistocene, and (3) historical rainforest refuges for the ants probably existed in northern northwest Borneo and the mountain ranges of Malaya and Sumatra (Quek et al. 2007).

The single gene analysis of mitochondrial COI in this study and Quek et al. (2004, 2007) may not reflect species (or population) tree due to introgression or incomplete lineage sorting (Avise 1994; Sota and Vogler 2001; Linnen and Farrell 2007). In fact, mtDNA phylogeny in this study disagreed with a preliminary nrDNA phylogeny using wingless gene and with morphology (S. Ueda, unpublished data). Therefore, in order to elucidate the species boundaries of *Coccus* on *Macaranga* in the future, several independently segregating loci should be used to infer the phylogeny of *Coccus* (e.g., Beltran et al. 2002). However, for the present study, the objective is to infer the historical

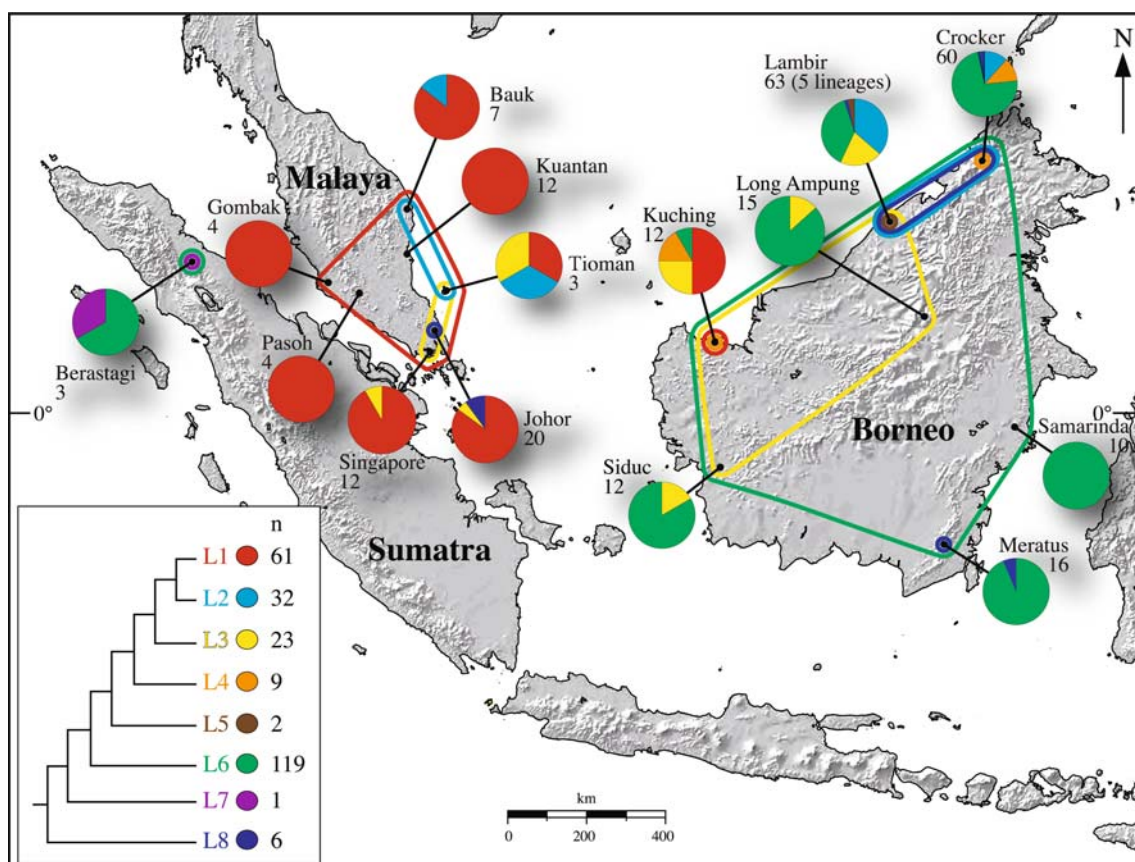


Fig. 2 Approximate distribution of the *Coccus* lineages as inferred by sampling location (colored lines). Pie chart shows the proportional abundance of lineages within each location with sample sizes

indicated. The *distributional lines* and *pie charts* are color-referenced to the *Coccus* phylogeny at the bottom left

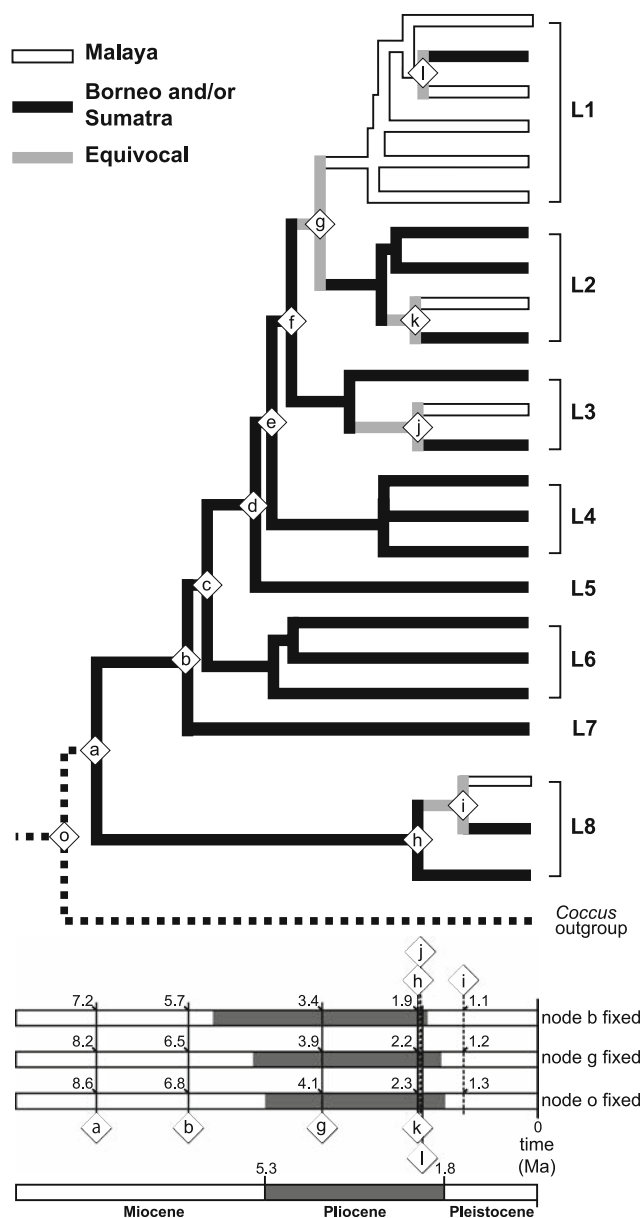


Fig. 3 Phylogeny of *Coccus* scale insects, showing the reconstruction of the ancestral area optimized by dispersal (migration)-vicariance analysis (DIVA). Branch lengths are proportional to time as inferred by nonparametric rate smoothing. Three timelines are inferred by fixing the age of nodes *o*, *b* or *e* using 1.5% divergence per million years in COI (Table 2). Branch color indicates the location where each sample inhabits. The exemplar samples containing geographic information are selected from Fig. 1a (based on Dataset 1, see text for more details), whilst, following Fig. 1b, monophyly of the *Coccus* on *Macaranga* (L1–L8) is denied

biogeography of the mtDNA of *Coccus* scales on *Macaranga*, and to draw comparisons of these results of scales with that of the co-habiting *Decacrema* ants.

The exemplar phylogeny from Dataset 2 indicated that the *Coccus* scale insects are non-monophyletic group (Fig. 1b). The non-monophyletic pattern of Dataset 2 may

support the hypothesis that multiple clades of the *Coccus* scales independently colonized to *Macaranga*, just as At-tine ants acquired fungal cultivars (Mueller et al. 1998, 2001). However, because the SH test did not reject the monophyly of the scales, it remains ambiguous whether the *Coccus* scales have colonized *Macaranga* multiple times. To resolve this issue, a further exhaustive sampling of free-living *Coccus* species and analyses of several additional neutral genes will be needed.

Ancestral area reconstruction on the *Coccus* phylogeny (Fig. 3) suggested that they originated in Borneo and/or Sumatra and subsequently dispersed to Malaya, which is consistent with the historical biogeographic picture emerging for their host *Macaranga* plants (Bänfer et al. 2006) and *Decacrema* ants based on DNA studies. However, the lack of enough samples of *Coccus* from Sumatra warrants caution for inferences of origination and dispersal in or between the regions. The timeline of the migration events (Fig. 3) also corresponds with that in the *Decacrema* ants reported in Quek et al. (2007); the four of five migration events in the scales (nodes *g*, *j*, *k*, and *l* in Fig. 3) occurred synchronously in the Pliocene and the other one occurred in the Pleistocene (node *i* in Fig. 3), whilst the *trans*-Sunda migration events in the *Decacrema* ants also occurred primarily in the Pliocene and less frequently in the Pleistocene (Quek et al. 2007). Migrations across the Sunda shelf in the Pliocene were also documented in Southeast Asian rainforest rodents and some frogs (Inger and Voris 2001; Gorog et al. 2004). These facts suggest that ancient land bridges of Sunda shelf, created during periods of low sea level (Hall 2001), permitted extensive migrations of rainforest elements across the South China Sea, and that the migrations primarily occurred in the Pliocene rather than in the Pleistocene. Whether the repeated and extensive exposures of the Pleistocene Sunda shelf facilitated the spread of tropical evergreen rainforest in the region is controversial (Kershaw et al. 2001; Quek et al. 2007).

Just as with their host *Decacrema* ants, the higher number of lineages in Borneo was situated in northern northwest Borneo, the Crocker range and Lambir (Fig. 2), suggesting that, as for the ants, these locations represented rainforest refugia during cool dry Pleistocene phases in which ever-wet rainforests became fragmented, pending plentiful data accumulation from Sumatra. However, it is also possible that the high number of scale lineage in these two locations may be contributed to by high sampling intensities there; further sampling in the other locations is needed to confirm this trend.

A further parallel between ants and scales can be seen in Samarinda, which exhibits the lowest number (only one) of lineages) of scales among all the locations in Borneo

Table 2 Inferred ages of nodes on the *Coccus* chronogram (Fig. 3) obtained by nonparametric rate smoothing, based on a pairwise divergence rate of 1.5% per million years

Node	Mean Pairwise distance (%)	Age (Mya)		
		(Node o fixed)	(Node c fixed)	(Node g fixed)
o	13.9	9.27	7.75	8.85
a	15.0	8.57	7.17	8.18
b	13.7	6.76	5.65	6.45
c	8.0	6.38	5.33	6.08
d	6.9	5.46	4.56	5.21
e	7.6	5.08	4.24	4.85
f	6.2	4.42	3.95	4.51
g	5.9	4.12	3.44	3.93
h	1.7	2.26	1.89	2.15
i	1.5	1.27	1.06	1.21
j	2.1	2.19	1.83	2.09
k	3.3	2.25	1.88	2.15
l	2.2	2.12	1.77	2.02

Fixed ages are indicated in bold

(Fig. 2). This is also true for their tending ants, in which *Samarinda* ties with *Siduk* and *Kuching* for the bottom rank with two lineages. In contrast, an incongruence pattern between the scales and ants is found in *Kuching*, which harbors the second highest number (four) of *Coccus* lineages, but the lowest number (two) of ant lineages.

We were able to obtain only few samples from Sumatra (3 samples in one site), where myrmecophytic *Macaranga* and their *Decacrema* ants occur. Further sampling in Sumatra is desirable, and likely to reveal many more haplotypes, and also possibly more lineages. Further sampling in the weakly sampled localities in Borneo and Malaya are also needed to reduce the artifacts of uneven sampling intensities among locations. Nevertheless, the parallels seen between the ants and scales suggest sampling artifacts do not contribute worrisome errors to the patterns observed.

The overall congruence between the diversification histories of the symbiotic scales and ants suggests that they experienced the common history of extinction/migration despite their independent mode of dispersal and host-colonization. Common historical biogeography of multiple parasites occurring on a common host taxa is favored by a high level of specificity and vertical transmission of parasites among hosts (cf. Nieberding and Olivieri 2007; Nieberding et al. 2008). In contrast to this, Whiteman et al. (2007) reported a case where three parasites on a single host species differed in their population genetic, phylogeographical structure, and co-divergence with the host in ways that were predicted by the parasites' ecology such as

dispersal ability. In this context, the historical congruence of scales and ants suggest that they have not-so-different life history traits such as dispersal ability and survival. Then, what about *Macaranga* plants? Is their historical biogeography similar to that of the symbionts? Among section *Phachystemon* which contains the majority of *Macaranga* myrmecophytes, 15 species out of 25 are endemic to Borneo (Davies 2001) with others being Sumatran or Malayan species. This suggests their major axis of diversification in Borneo, but extensive phylogeographic study is needed to elucidate the congruence of the history of the three-partner symbiosis.

Because of the strict association of the scales, ants, and plants with ever-wet rainforests in Southeast Asia, this investigation contributes to the small but hopefully growing pool of studies utilizing molecular markers to piece together the historical ecology of this rich, understudied, and endangered ecosystem.

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