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Molecular Phylogeny among Local Populations of Weaver Ant *Oecophylla smaragdina*

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ABSTRACT—The molecular phylogeny of 24 *Oecophylla smaragdina* populations and two *O. longinoda* populations was studied using 647 bp of the mitochondrial cyt *b* gene. The phylogenetic analysis suggested that *O. smaragdina* and *O. longinoda* were separated from each other first, and after that the first within-species divergence of *O. smaragdina* occurred in early stage of their history, in which the Asian, Australian, and Sulawesian groups rose. This grouping was almost coincident with the distribution of landmass in glacial periods in Pleistocene. Thereafter, each group seemed to have independently diverged into present populations on each landmass.

Key words: biogeography, cytochrome *b* gene, molecular phylogeny, *Oecophylla,* weaver ant

INTRODUCTION

The weaver ant genus *Oecophylla* (Hymenoptera, Formicidae) is a relatively old genus in the subfamily Formicinae, and 11 fossil species of this genus have been found so far from the Oligocene and Miocene deposits. However, there are only two extant species, *Oecophylla longinoda* (Latreille) distributed in tropical Africa, and *O. smaragdina* (Fabricius) in southeastern Asia and Australia (Wheeler 1922, Bolton 1995). Both of them are arboreal and make peculiar global or elliptical nests of leaves spun with silk supplied by larvae. They form exceptionally aggressive and territorial colonies which sometimes dominate a wide range of forest canopies (Hölldobler 1979). In addition to these distinctive behaviors, they are morphologically unique in the loss of metapleural gland, which has been regarded as a diagnostic character discriminating Formicidae from other aculeate Hymenoptera, and this secondary loss of the gland in the genus is probably an adaptation to arboreal life (Hölldobler and Engel-Siegel 1978).

Oecophylla smaragdina is widespread from southern Asia to northern Australia, including many tropical western Pacific islands (Cole and Jones 1948, Lokkers 1986). Therefore, this species seems to be a valuable material for biogeographical study of Tropical Asia. *O. smaragdina* exhibits a high degree of morphological variation especially in the body color of workers: they are light to dark brown in Southeast Asia but are known as 'green tree ants' after astonishing green color of the abdomen in Australia. Although some authors have classified localized populations into several subspecies based on morphological variation, the phylogenetic relationships of these populations are still unknown.

The objective of this study was to investigate the phylogenetic relationship among local populations of *Oecophylla smaragdina* and between *O. smaragdina* and *O. longinoda*. The mitochondrial cytochrome *b* (cyt *b*) gene was used for this study, since this region seems to have a proper substitution rate for analyzing within-species phylogeny of Hymenoptera and some insect-specific oligonucleotide primers are already designed for this gene (Jermiin and Crozier 1994).

MATERIALS AND METHODS

DNA extraction and sequencing

We collected adult workers of *Oecophylla smaragdina* from a total of 110 colonies at 23 localities by sampling 1 to 9 (mean±SD: 4.6±2.6) colonies per locality (Table 1 and Fig. 1). From each colony, two or three workers were chosen to examine their DNA sequences (647 bp) from a part of the mitochondrial cyt *b* gene.

DNA was extracted from alcohol-preserved specimens using the reagent Chelex100 (BIO RAD) according to the manufacture's instructions. Total genomic DNA of each specimen was suspended in 20 μl distilled water after ethanol precipitation.

DNA amplification was performed using the polymerase chain reaction (PCR) with a PCR reagent kit (GibcoBRL) according to the manufactures' instructions in 50 μl reaction mixture containing 2 units of Taq polymerase, a pair of oligonucleotide primers (0.5 μM each), dNTP (0.2 mM), Tris-HCl ph 8.4 (20 mM), KCl (50 mM), $MgCl_2$ (2.5 mM), and the total genomic DNA as a template (5 μl) with an automated thermal cycler (PCR thermal cycler SP, Takara,

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Table 1. Locality codes, sampled localities, and country (island), of the sampled populations, of *Oecophylla longinoda* and *O. smaragdina* and number of colonies examined in this study.

Species	Code	Locality	Country (island)	Colony number
Oecophylla longinoda	1	Campo Forest Reserve	Cameroon	1
	2	Ababuko-Sokoko Forest	Kenya	1
Oecophylla smaragdina	3	Nurbag	Bangladesh	3
	4	Hung Loc	Vietnam	2
	5	Hat Lot	Vietnam	2
	6	Ho chi Minh	Vietnam	3
	7	Bangkok	Thailand	5
	8	Kuala Lumpur 1	Malaysia	4
	9	Kuala Lumpur 2	Malaysia	2
	10	Palamgkaraya	Indonesia (Kalimantan)	6
	11	Poring	Malaysia (Kalimantan)	3
	12	Jakarta	Indonesia (Java)	4
	13	Mataram	Indonesia (Lombok)	4
	14	Bogor	Indonesia (Java)	8
	15	Surabaya	Indonesia (Java)	5
	16	Kuta	Indonesia (Bali)	5
	17	Solok	Indonesia (Sumatra)	4
	18	Ujung Pandang	Indonesia (Sulawesi)	5
	19	Soroako	Indonesia (Sulawesi)	2
	20	Lanowulu	Indonesia (Sulawesi)	2
	21	Mangkutana	Indonesia (Sulawesi)	2
	22	Biak	Indonesia (Biak)	5
	23	Port Moresby	Papua New Guinea	9
	24	Darwin	Australia	6
	25	Rifle Creek	Australia	8
	26	Cairns	Australia	9

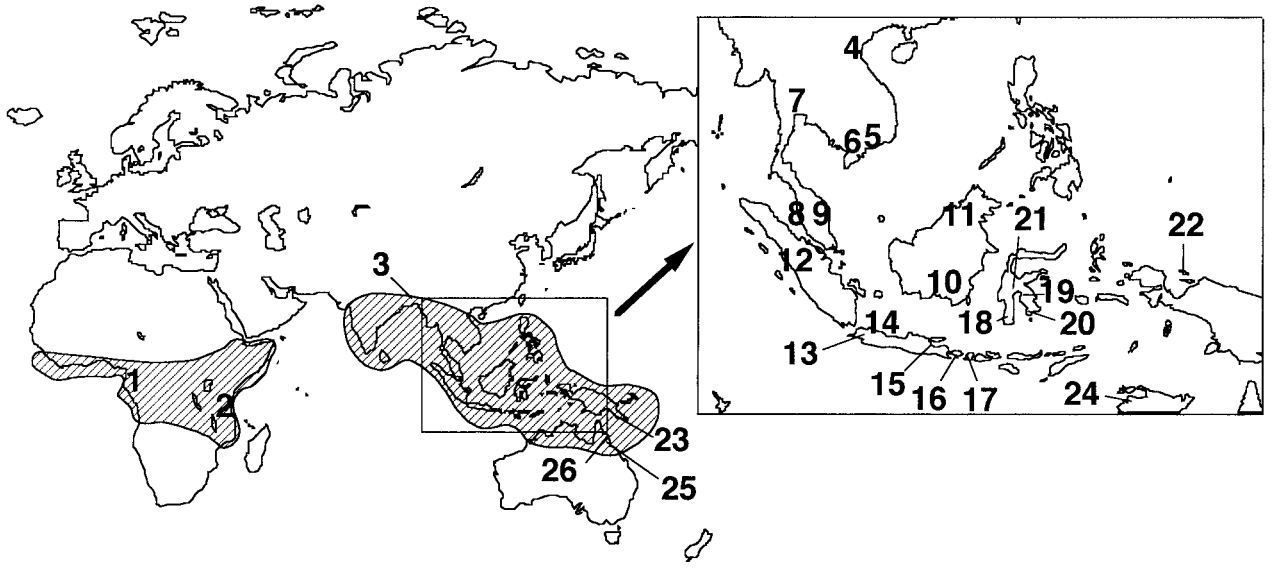

Fig. 1. Maps of the Old World and Southeast Asia showing collection sites. The numbers indicate the locality codes of Table 1. The shaded regions indicate present distribution of *Oecophylla smaragdina* (Asia and Oceania) and *O. longinoda* (Africa).

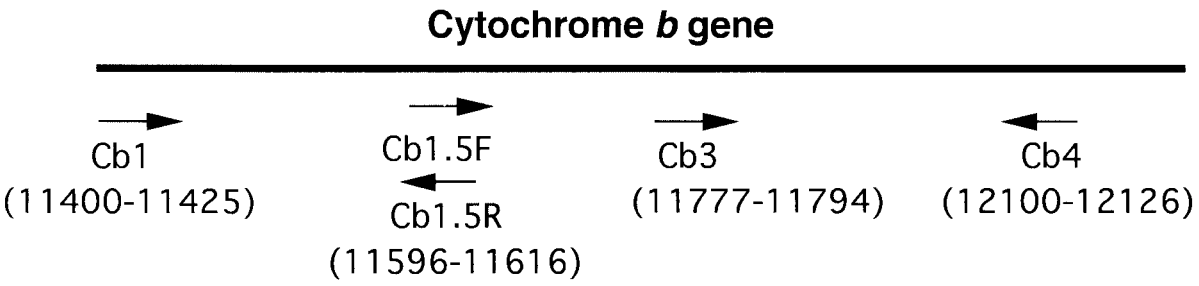

Fig. 2. PCR primer position. Number indicates the position, for Cb1 according to *Apis mellifera* mitochondrial genome (Crozier and Crozier, 1993) and for others calculated in this study.

Japan). The thermal cycling parameters were as follows: 95°C 5 min for hot start, 35 cycles of dissociation (92°C, 1 min), annealing (48°C, 1 min), and extention (70°C, 2 min). First we tried to amplify total cyt *b* gene with a set of primers, Cb1; 5'-TATGTACTACCATGAGGACAAATATC-3' (forward) and tRs; 5'-TATTTCTTTATTATGTTTTCAAAAC-3' (reverse) (Jermiin and Crozier 1994), but some of specimens could not be amplified enough, therefore we designated internal reverse primer Cb4; 5'-CTCATATTTTTATAATTAGAAATGAT-3', and succeeded to amplify a mitochondrial DNA fragment of a part of cyt *b* gene (ca. 680 bp). After electrophoresis with 1.5% agarose gels in TAE, the proper DNA fragments were cut from the gels and purified with a QIAquick Gel Extraction Kit (QIAGEN).

Purified PCR products were directly sequenced by an automated method using the Dye Terminator Cycle Sequence Kit (Perkin Elmer) and an automated sequencer (model 373A and 310, Division of Applied Biosystems, Perkin Elmer). For exact sequencing we used internal primers newly designated in this study, Cb1.5F; 5'-GAGATTTATATAAAATTCCT-3' (forward), Cb1.5R; 5'-AGGAATTTTATATAAATCTC-3' (reverse), and Cb3; 5'-CCAATTCATATTCAACC-3' (forward) in addition to Cb1 and Cb4. Primers' positions are indicated in Fig. 2.

Oecophylla longinoda was sampled by other researchers who sent us some specimens preserved in 100% ethanol. Only one colony was available from each of Kenya and Cameroon. Two or three workers of each colony were used for DNA sequence analysis in the same manner for *O. smaragdina*.

The sequence data of 26 populations are deposited in the DNA Data Bank of Japan database (DDBJ) with accession No. AB056064–056089.

Estimation of sequence diversity and construction of phylogenetic trees

Prior to the analysis of *Oecophylla smaragdina* haplotypes, the monophyly of *O. smaragdina* and *O. longinoda* was confirmed by constructing a molecular phylogenetic tree of nine formicine species, using neighbor-joining (NJ) method with Kimura's two-parameter method for estimating the genetic distance. Transversional nucleotide substitution in the beginning part of cyt *b* gene (405 bp) was available for *Formica lugbris* (accession No. AF191159), *Stigmacros* sp. (AF191162), *Notoncus* sp. (AF191161), *Lasius neglectus* (AF191160), *Liometopum occidentale* (AF146721), *Ochetellus* sp. (AF146722), and *Linepithema humile* (AF146720) cited from DDBJ/GenBank/EMBL databases.

After the separation of *Oecophylla smaragdina* and *O. longinoda* was confirmed, the latter species was used as an out-group for constructing molecular phylogenetic trees of haplotypes representing 24 localities. Both of transitional and transversional substitutions of cyt *b* gene (647 bp) were taken into account for constructing the trees by neighbor-joining (NJ) method with Kimura's two-parameter estimation and by maximum-likelihood (ML) method with HKY 85 model (PAUP*4.0 Beta Version 8).

RESULTS

Sequence diversity

DNA sequences of the two or three colony mates were always identical with each other. Moreover, the haplotypes were identical among different colonies within each locality except in Kuala Lumpur where six colonies were divided into two haplotypes. In this paper, population is defined as a group of colonies which are distributed in the same locality and show an identical haplotype of DNA sequence of 647 bp mitochondrial cyt *b* gene. Overall, the DNA sequence analysis detected a total of 24 *Oecophylla smaragdina* and two *O. longinoda* haplotypes from 25 localities, including two haplotypes in Kuala Lumpur, Kuala Lumpur 1 from Lake Garden and Kuala Lumpur 2 from the area of University of Malaya in Petaling Jaya. The haplotypes were grouped into four groups, i.e. African, Asian, Sulawesian and Australian groups (shown below), and the sequence divergences within and between groups are shown in Table 2. The sub-

Table 2. Range of sequence divergence (%) for the mitochondrial cytochrome *b* of *Oecophylla* estimated by Kimura's 2-parameter method for all codon positions and for first and second codon positions (in parentheses).

	O. longinoda	*O. smaragdina*		
	Africa	Asian group	Sulawesian group	Australian group
Africa	1.88 (0.15)	7.82–9.76 (1.25–2.20)	7.13–7.81 (1.88–2.52)	7.80–8.69 (1.56–2.36)
Asian group	–	0.15–2.05 (0.15–0.62)	3.99–5.50 (0.93–1.56)	4.64–6.53 (0.62–1.41)
Sulawesi group	–	–	0.15–1.25 (0.15)	3.33–4.48 (0.93–1.56)
Australian group	–	–	–	0.15–2.84 (0.15–0.78)

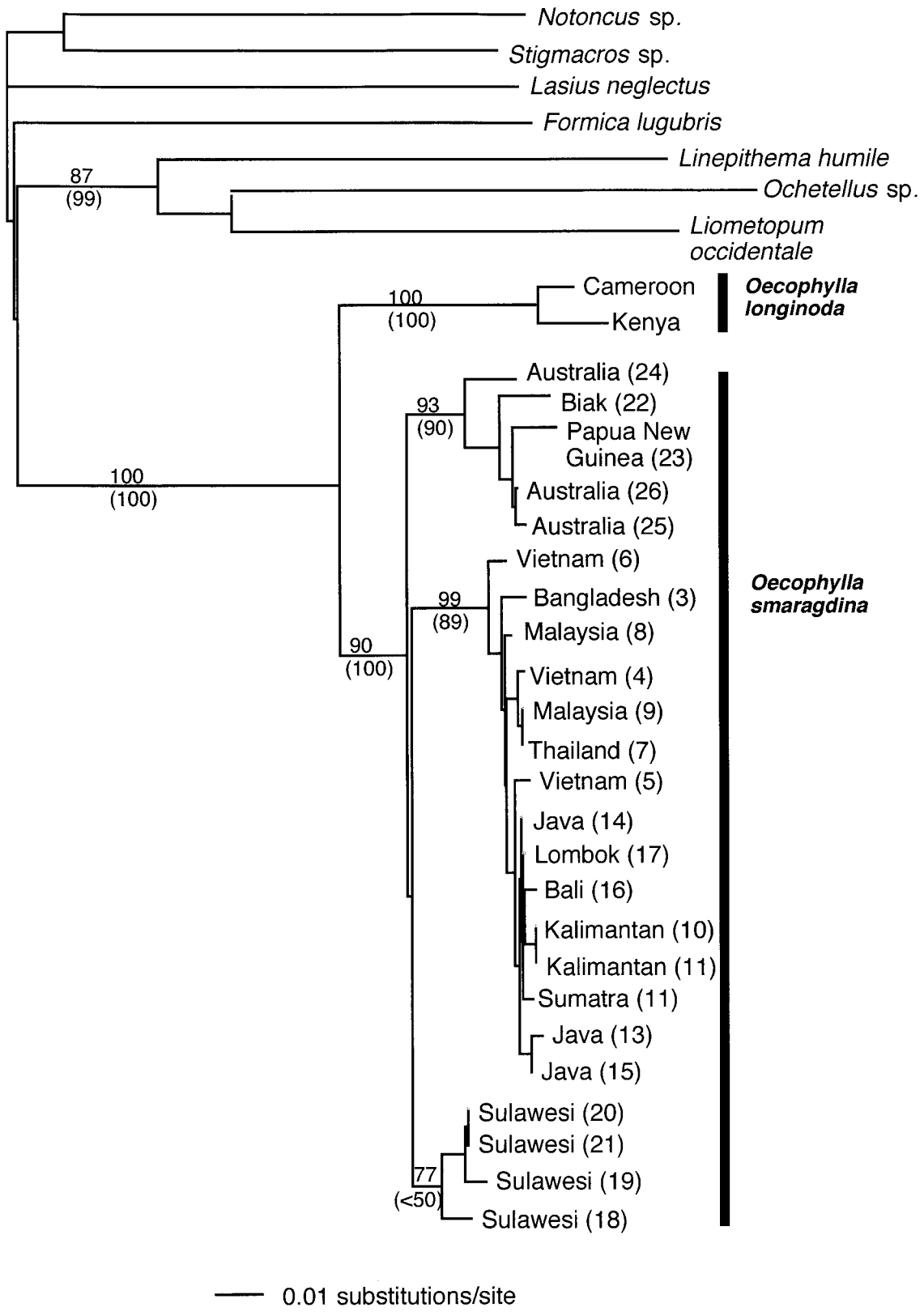

Fig. 3. Neighbor-joining tree inferred from the cyt *b* sequence (405 bp) of genus *Oecophylla* and some other ant species. The genetic distances were estimated by Kimura's 2-parameter method using all substitutions. The locality name at the end of each branch indicates the haplotype coincident in each place, and the figure in parentheses, locality code of Table 1 and Fig. 1. The figures above the branches indicate bootstrap values (%) derived from 1000 replications, and the figure below branches in parentheses indicate bootstrap values using only transversional substitutions.

stitution rates of first and second codon positions to all positions (mean±SD) were 0.232±0.049 among *smaragdina* groups and 0.229±0.045 between two species suggesting that probably the nucleotide substitutions are not saturated.

Molecular phylogenetic trees

Phylogenetic tree for 405 bp of cytochrome *b* gene by NJ method shows that genus *Oecophylla* was monophyletic, and the populations of *O. longinoda* were distinct from *O.*

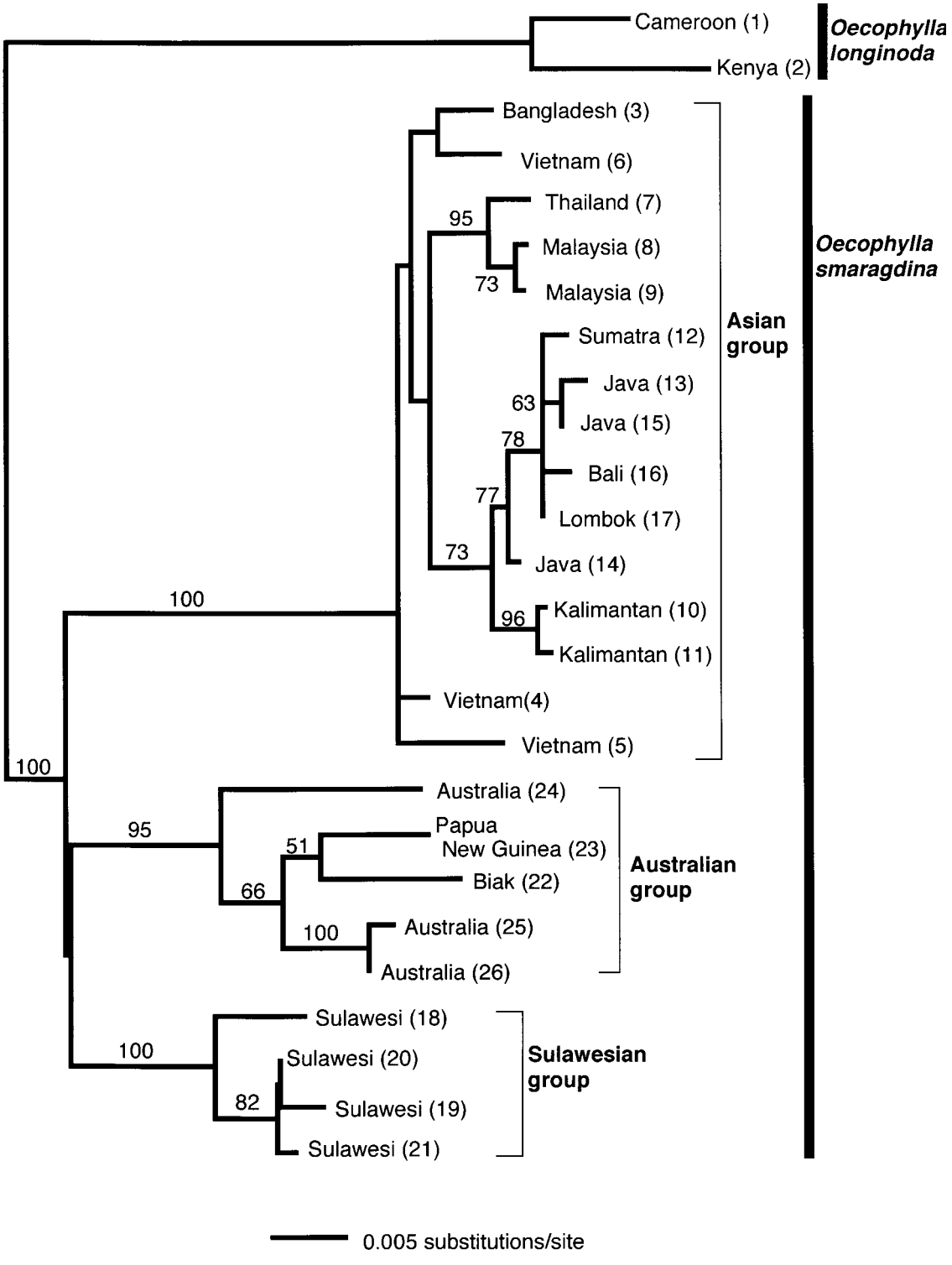

Fig. 4. Neighbor-joining tree inferred from the cyt *b* sequence (647 bp) of *Oecophylla smaragdina*, using *O. longinoda* as an out-group. The small figures above the branches indicate bootstrap values (%) derived from 1000 replications.

smaragdina, ratifying their classification as different species (Fig. 3). *Oecophylla smaragdina* and *O. longinoda* diverged from their common ancestor before *O. smaragdina* itself diversified into three lineages, i.e. Asian, Australian, and Sulawesi groups. After that, each lineage seemed to have further diverged into the present local populations independently. However, this tree comprises some branches with too low bootstrap values to determine the intraspecific phy-

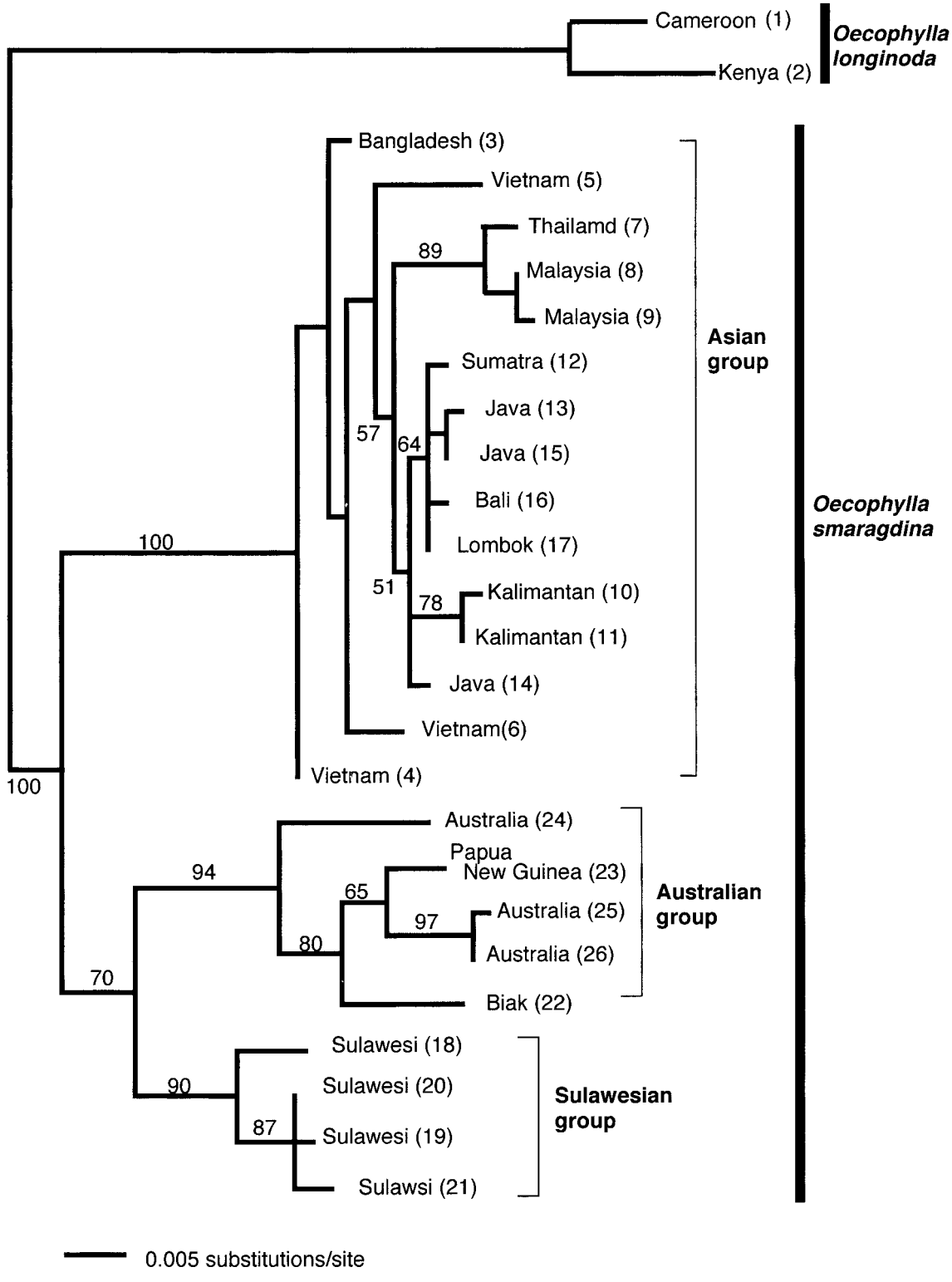

Fig. 5. Maximum likelihood tree by HKY 85 model inferred from the cyt *b* sequence (647 bp) of *Oecophylla smaragdina* populations, using *O. longinoda* as an out-group. The small figures above the branches indicate bootstrap values (%) derived from 500 replications.

logeny of *O. smaragdina*. For instance, Sulawesian group was ramified from Asian and Australian groups at low bootstrap. The uncertainty in this tree was partly due to the shortage of informative sites in short sequences of 405 bp.

NJ tree for 647 bp of cytochrome *b* gene of *Oecophylla smaragdina* with the out-group *O. longinoda* also shows that *O. smaragdina* diverged into three groups, including Sulawesian group which seems clearly independent of other two groups at present (Fig. 4). Compared with the deep separation in *O. smaragdina*, within-species divergence in *O. longinoda* seemed less extensive, because sequence divergence was as small as 1.88% even between Kenya and Cameroon which were located near the eastern and western margins of their distribution, respectively (see Table 2). The ML tree was almost consistent with NJ tree in outlines of divergence of three *O. smaragdina* groups (Fig. 5), and the difference between the two trees is attributed to some branches of low bootstrap values (<50%).

DISCUSSION

The sequence diversity of the mitochondrial cytochrome *b* gene suggests that *Oecophylla smaragdina* and *O. longinoda* are independent each other, and that *O. smaragdina* diverged into Asian, Australian, and Sulawesian groups each of which is clearly monophyletic.

Bolton (1995) listed 5 subspecies of *Oecophylla smaragdina* in his latest catalogue of ants, and some of them may reflect local populations or local groups which we found in this analysis. For example, one of the subspecies, *O. smaragdina selebensis* Emery may represent the present Sulawesian group. However, these subspecies names still remain merely following a nomenclatural rule without testing the compatibility with modern subspecies concept (Mayr and Ashlock, 1991). More detailed morphological comparisons are required for the classification of subspecies, and the present results will supply useful information to establish intraspecific taxonomy of *O. smaragdina* that is uncertain so far.

For ants, average evolutionary rate of mitochondrial cytochrome *b* was estimated as 0.165% substitutions per million years using the first and second codon positions (Crozier *et al.,* 1997). Using this rate, *Oecophylla smaragdina* and *O. longinoda* was estimated to have diverged at 7.5–15.3 Ma (million years ago), in Miocene, with following divergence among Asia, Sulawesi, and Australia groups of *O. smaragdina* at 3.8–9.5 Ma, in late Miocene or Early Pliocene. The internal diversifications of Asian, Sulawesi and Australian groups were estimated to have started at 3.8 Ma, 0.9 Ma and 4.7 Ma, respectively.

Paleontological evidence shows that several species of *Oecophylla,* perhaps including a common ancestor of *O. smaragdina* and *O. longinoda*, prospered in the Oligocene and Miocene (Bolton, 1995). Based on morphological and paleontological evidences Wilson and Taylor (1964) concluded that the common ancestor of the extant species was the fossil species *O. sicula* which had been discovered from a Miocene deposit in Sicily (Emery, 1891). This is not inconsistent with the present results suggesting that *O. longinoda* and *O. smaragdina* were established after the middle or late Miocene when molecular divergence occurred.

Fossil evidence suggests that *O. sicula* was distributed around Europe in Miocene, and this species might have expanded from Africa to Asia in this period. As temperatures started to fall at the beginning of the Pliocene, some populations probably persisted in tropics of Asia and Africa, while other populations became extinct in northern area. After that the expansion of desert over North Africa to the Middle East following the Pliocene cooling isolated Asian populations and African from each other completely, and gave rise of two species *O. longinoda* and *O. smaragdina.*

The present results further indicate that the first intraspecific divergence of *Oecophylla smaragdina* into Asian, Sulawesian, and Australian groups occurred shortly after speciation, and subsequent diversifications into local populations started at late Pliocene or at the beginning to middle of Pleistocene. This dating is consistent with the transgression and regression of land area in Asia. In the glacial periods in Pleistocene, the sea level was ca. 200 meters lower than today. Most of Greater Sunda Is. and Kalimantan were probably connected with the Asian Continent (Heaney, 1986), and the current continental shelves are considered to delineate the boundaries of landmasses. The Asian landmass was isolated from Australia–New Guinea landmass throughout Pleistcene, and Sulawesi was independent from the two landmasses even in the glacial periods. These geographic clusters are coincident to genetic clusters of *O. smaragdina* in this study. The biogeographical boundaries of Wallace's line and Lydekker's line also agree with the phylogenetic clustering, excepting Lombok. Although Wallace's Line lies between Bali and Lombok and the latter belongs to the Wallacea area consisting of Sulawesi and most of Lesser Sunda Is., the Lombok (Mataram) population is genetically closer to the Bali (Kuta) population than to Sulawesi populations. The Lombok population probably migrated from Bali or Java across the narrow Lombok Channel, by rafting or blow. Indeed, *O. smaragdina* has been found on isolated islands not connected with any other islands or continents, suggesting the occasional occurrence of accidental drifting.

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REFERENCES

Bolton B (1995) A New General Catalogue of the Ants of the World. Harvard University Press, London

Cole AC Jr. and Jones JW (1948) A study of the weaver ant, *Oecophylla smaragdina* (Fab.). Am Midl Nat 39: 641–651

Crozier RH and Crozier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics 113: 97–117

Crozier RH, Jermiin LS and Chiotis M (1997) Molecular Evidence for a Jurassic Origin of Ants. Naturwissenschaften 84: 22–23

Emery C (1891) Le formiche dell'ambra Siciliana nel Museo Mineralgico dell'Universita di Bologna. Memorie della R. Accademia delle Scienze dell' Istituto di Bologna (5) 1: 141–165

Heaney L (1986) Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. Biol J Linn Soc: 127–165

Hölldobler B (1979) Territoriality in ants. Proc Am Phil Soc 123: 211–218

Hölldobler B and Engel-Siegel H (1978) Tergal and sternal glands in male ants. Psyche 89: 113–132

Jermiin LS and Crozier RH (1994) The cytochrome *b* region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. J Mol Evol 38: 282–294

Lokkers C (1986) The Distribution of the Weaver Ant, *Oecophylla smaragdina* (Fabricius) (Hymenoptera: Formicidae) in Northern Australia. Aust J Zool 34**:** 683–687

Mayr E and Ashlock PD (1991) Principles of Systematic Zoology (2nd ed.) 475 McGraw-Hill Inc, New York

Wheeler WM (1922) Ants of the American Museum Congo Expedition. A Contribution to the Myrmecology of Africa. Bull Mus Nat Hist XLV, Article 1. 224–236

Wilson EO and Taylor RW (1964) A fossil ant colony: new evidence of social antiquity. Psyche 7182: 93–103

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