J Mol Evol (2001) 53:651–659
DOI: 10.1007/s002390010252

JOURNAL OF MOLECULAR EVOLUTION

Phylogeny and Biogeography of Wood-Feeding Cockroaches, Genus *Salganea* Stål (Blaberidae: Panesthiinae), in Southeast Asia Based on Mitochondrial DNA Sequences

Kiyoto Maekawa,[1] Masahiro Kon,[2] Kunio Araya,[3] Tadao Matsumoto[1]

[1] Matsumoto Laboratory, Department of Biology, University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan
[2] Department of Environmental Science, University of the Shiga Prefecture, 2500 Hassaka-cho, Hikone, Shiga 522-8533, Japan
[3] Graduate School of Social and Cultural Studies, Kyushu University, 4-2-1 Ropponmatsu, Chuo-ku, Fukuoka City, Fukuoka 810-8560, Japan

Received: 4 April 2001 / Accepted: 20 April 2001

Abstract. Molecular phylogenetic relationships among 25 species of the wood-feeding cockroach belonging to the genus *Salganea* Stål (Panesthiinae; Blaberidae) in Southeast Asia were analyzed based on the DNA sequence of the complete mitochondrial cytochrome oxidase II (COII) gene. Most basal relationships among species of *Salganea* are poorly resolved by both neighbor-joining and nonweighted parsimony analyses, suggesting the possibility of a hard polytomy due to a rapid and potentially simultaneous radiation early in the history of the genus. For more apical relationships, however, some interesting phylogenetic relationships were recognized. The monophyly of the two species groups, *morio* and *foveolata*, the former of which is distributed mainly in the Sunda lands (containing the Malay Peninsula, Sumatra, Java, and Borneo), whereas the latter is Sulawesi endemic, was strongly supported. Based on the inferred phylogenetic patterns and recent palaeogeographic scenario for Southeast Asia, it is suggested that a radiation of *Salganea* species occurred in Southeast Asia presumably in the early Tertiary, and several barriers against dispersal and gene flow, such as the formation of straits or high mountains, have arisen from the middle Tertiary.

Correspondence to: Kiyoto Maekawa; *email:* cmae@mail.ecc.u-tokyo.ac.jp

Key words: Cockroach — *Salganea* — Molecular phylogeny — Biogeography — Mitochondrial COII gene — Southeast Asia

Introduction

The wood-feeding cockroach genus *Salganea* Stål (Blaberidae: Panesthiinae), including about 50 species, is distributed in the Indo-Malayan region and New Guinea of the Australian region (Roth 1979; Maekawa et al. 1999a). All *Salganea* species known from the field ecology live within galleries tunneled into rotten woods (Roth, 1979; Matsumoto, 1987; Asahina, 1991; Maekawa et al. 1999a). Based on their morphological characters, most *Salganea* species were categorized by Roth (1979) into five species groups, *papua, raggei, morio, foveolata,* and *nigrita,* and some species were not placed in any group. Of these species groups, two have restricted distributions. One is the *papua* species group (five described species), which is restricted to New Guinea; the other is the *foveolata* species group (six described species), which is restricted to Sulawesi. Three and five species in the *raggei* and *morio* species groups are distributed mainly in the Greater Sunda lands (including the Malay Peninsula, Sumatra, Java, and Borneo). The 14 species in the *nigrita* species group are widely distributed throughout the Asian mainland, the Sunda lands, various Pacific islands, and New Guinea. The remaining 12 species are not placed in any group;

some of them are distributed in Borneo or on the Malay Peninsula and others are found in India.

Southeast Asia is geologically one of the most intriguing areas on Earth (Holloway and Hall 1998), and the paleogeography of this region is becoming progressively better understood (Hall 1998). Phylogenetic analyses of fauna enable testing of palaeogeographical hypotheses (Avise 1994), and studies of terrestrial organisms ranging over this area based on molecular characters have recently been performed [e.g., on reptiles (Honda et al. 1999a, b, 2000)]. However, analyses of terrestrial insects in these regions using modern molecular methods are still lacking. Recently, Maekawa et al. (1999b) analyzed the molecular phylogeny of wood-feeding cockroaches belonging to the Panesthiinae on the Japan Archipelago and in Taiwan (five species of *Salganea* and *Panesthia*) and suggested that their speciation was influenced by some geological events such as land-bridge connection. Thus, analyses of these cockroach groups are also very useful for biogeographic discussions of Southeast Asia.

In this study, based on the DNA sequence of the complete mitochondrial cytochrome oxidase subunit II (COII) gene, we analyzed the phylogenetic relationships of 25 species of the genus *Salganea* belonging to four species groups of Roth (1979) collected from variable regions in Southeast Asia. No previous study has involved both a wide-ranging survey of *Salganea* species and phylogenetic analyses. Although we could not analyze species in the *papua* species group, using 685 bp of the COII gene, this study presents the first comprehensive phylogenetic relationships among species in these cockroach groups. We also discuss the historical biogeography of the genus *Salganea* on the basis of the resultant phylogenetic trees and genetic data.

Materials and Methods

Insect Material

The species investigated in this study are listed in Table 1. The inferred phylogeny among worldwide Panesthiinae suggests that *Miopanesthia* Saussure is the basal group in the Panesthiinae (Maekawa, in preparation). Thus the COII sequences of two individuals of *Miopanesthia deplanata* Hanitsch and the distantly related cockroach *Periplaneta fuliginosa* (Blattidae) (Accession Nos. AB036104, AB036105, and AB005461) were used as outgroups. *Salganea* new species (*S.* sp.), from Sulawesi, has fully developed tegmina and wings and apparently belongs to the *foveolata* species group based on its morphological characters.

DNA Extraction

Total genomic DNA was extracted from leg tissue preserved in acetone or 100% ethanol. The extraction procedure was modified from Laird et al. (1991). Tissue was homogenized with a pair of dissecting scissors in a 1.5-ml microcentrifuge tube containing 500 μl of lysis buffer (100 m*M* Tris–HCl, 5 m*M* EDTA, 0.2% SDS, 200 m*M* NaCl) and 50 μg of proteinase K (Wako Chemicals). The mixture was incubated overnight at 56°C. Following the addition of 10 μg of RNase A (Boehringer Mannheim), the mixture was incubated at 37°C for 30 min. Following a series of phenol–chloroform and chloroform extractions, DNA was precipitated with an equal volume of isopropanol. Pellets were rinsed with 70% ethanol, dried under vacuum, and dissolved in 40 μl of TE buffer (10 m*M* Tris–HCl, 1 m*M* EDTA).

DNA Amplification, Purification, and Sequencing

The COII regions of mitochondrial DNA were amplified using PCR. Two pairs of primers were used for amplification. The primer sequences were as follows: A-tLEU, 5′-ATG GCA GAT TAG TGC AAT GG-3′ (forward); and B-tLYS, 5′-GTT TAA GAG ACC AGT ACT TG-3′ (reverse) (Liu and Beckenbach 1992). An internal primer, A-COII, 5′-TGA AGT TAT GAA TAT TCA GA-3′ (forward) (Maekawa et al. 1999b, c), was also used in combination with the aforementioned reverse primer. PCR was performed in a GeneAmp 2400 thermal cycler (Perkin–Elmer) under the following conditions: 35 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 70°C for 2 min (Liu and Beckenbach 1992). The reaction was performed in a 40-μl final volume of the following solution: 30 μl of distilled water, 4 μl of 10× PCR buffer [Takara; 100 m*M* Tris–HCl (pH 8.3), 500 m*M* KCl, 15 m*M* $MgCl_2$, and 0.01% (w/v) gelatin], 4 μl of dNTP mix (a 1 m*M* concentration of each dNTP), 0.2 μl of each primer (100 p*M*), 0.7 U of Taq polymerase (Takara), and 2 μl of template DNA. PCR products were electrophoresed in a 1% agarose gel and purified using a Prep-A-Gene DNA Purification Kit (Bio-Rad). Purified products were used as templates for sequencing. Sequencing reactions were performed using a Dye-Terminator Cycle Sequencing Kit (Perkin–Elmer) and a GeneAmp 2400 thermal cycler. Electrophoresis and data collection were performed using an automatic DNA sequencer (Perkin–Elmer Model 373S) with a 6% polyacrylamide gel (Toyobo; Super Reading DNA Sequence Solution), following the recommended procedure. Both strands of the amplified PCR product were sequenced.

Phylogenetic Analysis

Sequences were aligned using the Clustal W program package (Thompson et al. 1994) and confirmed with the aligned DNA and protein sequences of some insect orders (Liu and Beckenbach 1992; Maekawa et al. 1999c). The numbers of nucleotide substitutions were estimated according to Kimura's (1980) two-parameter method. Distance matrices were analyzed by the neighbor-joining method (Saitou and Nei 1987) to construct trees using Clustal W. Gap positions were excluded because gaps of 6 bp (*S. cavagnaroi,* positions 391–396) and 3 bp (*S. nalepae* and *M. deplanata,* positions 394–396) were probably a single deletion event. PAUP 3.1.1 (Swofford 1993) was used to carry out nonweighted parsimony analysis. Gaps were treated as missing data for the reason outlined above. For each analysis, the bootstrap confidence intervals on each branching pattern were calculated from 1000 replications of resampling.

Results

Sequence Variation

The COII genes examined in this study were 679–685 bp. Aligned sequences are available from the authors on request. Analysis of the aligned sequences from the 45 taxa yielded 339 variable sites, of which 315 were phylogenetically informative. Of the latter type of sites, 78

Table 1. List of species used in this study

Groups and species	Location	Abbreviation of location	Accession no.
raggei species group			
S. raggei Roth	Santimon, Taiwan Is., China	SAN	AB007530
S. raggei Roth	Mt. Da Diao Luo, Hainan Prov., China	DDL	AB036206
S. perssoni Roth	Mt. Kinabalu, Sabah, Borneo Is., Malaysia	KIN	AB036207
S. perssoni Roth	Mt. Trus Madi, Sabah, Borneo Is., Malaysia	TRU	AB036208
S. aperturifera Roth	Genting Highland, Malay Peninsula, Malaysia	—	AB036209
morio species group			
S. duffelsi Roth	Mt. Sibayak, Sumatra Is., Indonesia	—	AB036210
S. aequaliterspinosa Princis	Danum Valley, Sabah, Borneo Is., Malaysia	DANU	AB036211
S. aequaliterspinosa Princis	Mt. Trus Madi, Sabah, Borneo Is., Malaysia	TRU	AB036212
S. aequaliterspinosa Princis	Lambir, Sarawak, Borneo Is., Malaysia	LAM	AB036213
S. aequaliterspinosa Princis	Tapah, Malay Peninsula, Malaysia	TAP	AB036214
S. aequaliterspinosa Princis	Fraser's Hill, Malay Peninsula, Malaysia	FRA	AB036215
S. aequaliterspinosa Princis	Bukit Soeharto, Kalimantan, Borneo Is., Indonesia	BUK	AB036216
S. guentheri Roth	Tapah, Malay Peninsula, Malaysia	TAP	AB036217
S. guentheri Roth	Fraser's Hill, Malay Peninsula, Malaysia	FRA	AB036218
S. guentheri Roth	Mt. Emas, Sabah, Borneo Is., Malaysia	EMA	AB036219
S. guentheri Roth	Sibolaugit, Sumatra Is., Indonesia	SIB	AB036220
S. sutteri Princis	Mt. Rinjani, Lombok Is., Indonesia	RIN	AB036221
foveolata species group			
S. foveolata Hanitsch	Lake Mooat, Sulawesi Is., Indonesia	—	AB036222
S. rufipes Hanitsch	Baruppu, Sulawesi Is., Indonesia	—	AB036223
S. fruhstorferi Hanitsch	Malino, Sulawesi Is., Indonesia	—	AB036224
S. sp.	Lake Mooat, Sulawesi Is., Indonesia	—	AB036225
nigrita species group			
S. ternatensis hirsuta Roth	Mt. Bosavi, Papua New Guinea	—	AB036226
S. amboinica Brunner	Dumoga Bone, Sulawesi Is., Indonesia	DUM	AB036227
S. amboinica Brunner	Lake Mooat, Sulawesi Is., Indonesia	MOO	AB036228
S. nigrita (Stoll)	Lake Danao, Leyte Is., Philippines	DANA	AB036229
S. nigrita (Stoll)	Gingoog, Mindanao Is., Philippines	GIN	AB036230
S. rugulata Saussure	Surat Thani, Thailand	—	AB036231
S. gressitti Roth	Mt. Habonsan, Taiwan Is., China	—	AB007528
S. taiwanensis ryukyuanus Asahina	Mt. Yonahadake, Okinawajima Is., Japan	—	AB007521
S. taiwanensis taiwanensis Roth	Mt. Omotodake, Ishigakijima Is., Japan	—	AB007523
S. esakii Roth	Mt. Mochomudake, Yakushima Is., Japan	—	AB007518
S. incerta Brunner	Doi Chang Dao, Thailand	—	AB036232
Species not placed in any group			
S. inaequaliterspinosa Hanitsch	Mt. Emas, Sabah, Borneo Is., Malaysia	EMA	AB036233
S. inaequaliterspinosa Hanitsch	Mt. Kinabalu, Sabah, Borneo Is., Malaysia	KIN	AB036234
S. obtusespinosa Hanitsch	Crocker Range, Sabah, Borneo Is., Malaysia	CRO	AB036235
S. obtusespinosa Hanitsch	Mt. Emas, Sabah, Borneo Is., Malaysia	EMA	AB036236
S. taylori Roth	Fraser's Hill, Malay Peninsula, Malaysia	FRA	AB036237
S. taylori Roth	Cameron Highland, Malay Peninsula, Malaysia	CAM	AB036238
S. taylori Roth	Genting Highland, Malay Peninsula, Malaysia	GEN	AB036239
S. rossi Roth	Mt. Brinchang, Malay Peninsula, Malaysia	—	AB036240
S. cavagnaroi Roth	Ooty, India	—	AB036241
S. nalepae Maekawa and Matsumoto	Mt. Trus Madi, Sabah, Borneo Is., Malaysia	—	AB036242

(24.8%), 36 (11.4%), and 201 (63.8%) were found at first, second, and third codon positions, respectively.

The nucleotide compositional bias (CB) in COII with respect to codon position observed in this analysis (Table 2) was a common property of insect mitochondrial DNA (Simon et al. 1994). The highest bias was observed in third codon positions (CB = 0.48); this position was rich in T (43.5%), followed by A (42.4%) and then C (10.9%), and was very low in G (3.2%). Second codon positions showed an intermediate bias (CB = 0.23), being rich in T (39.4%) and poor in G (13.3%), with intermediate percentages of A (27.8%) and C (19.6%). The smallest bias was observed in first codon positions (CB = 0.18). These sites were rich in A (38.3%) and poor in C (16.7%), and the proportions registered for T and G were 23.8 and 21.2%, respectively. Overall, low standard deviations are shown in Table 2; thus the COII genes examined in this study have very similar base compositions.

Phylogenetic Relationships

The tree based on the neighbor-joining method after 1000 bootstrap replicates is shown in Fig. 1. Monophyly

Table 2. Base composition at first, second, and third codon positions of *Salganea* species used in this study

	First codon				Second codon				Third codon				Total			
	A	C	G	T	A	C	G	T	A	C	G	T	A	C	G	T
Mean	38.3	16.7	21.2	23.8	27.8	19.6	13.3	39.4	42.4	10.9	3.2	43.5	36.1	15.7	12.6	35.5
SD	1.3	0.9	1.2	1.1	0.7	0.9	0.4	0.9	1.8	2.6	1.1	3.3	0.7	1.1	0.6	1.2
Bias[a]		0.18				0.23				0.48				0.29		

[a] Bias calculated using the fomula of Irwin et al. (1991).

of the *Salganea* clade was supported by 66% of the bootstrap values. Monophyly of the *morio* and *foveolata* species groups was supported by high bootstrap values (81 and 98%, respectively; thick lines in Fig. 1). The new species morphologically recognized as a member of the *foveolata* species group was settled in this clade. Monophyly of the other two species groups used in this study (*raggei* and *nigrita*) was not supported. All bootstrap values at the deep nodes of the *Salganea* clade were <50% (Fig. 1; shaded region). Some other monophyletic groups were also supported at the more apical nodes in this tree. The monophyletic clade containing species from the Japan Archipelago and Taiwan (*S. gressiti*, *S. taiwanensis*, and *S. esakii*) were found to be closely related to *S. incerta*, which is distributed in Myanmar, Thailand, and Assam of north India (the sample was collected in Thailand), with 77% of the bootstrap values. All four of these species belong to the *nigrita* species group. The *S. amboinica* clade, collected from north Sulawesi, which is widely distributed in Sulawesi, the Philippines, and Moluccas, was more closely related to *S. rugulata*, which is distributed in Java, Sumatra, Vietnam, and Thailand (the sample was collected in Thailand); both also belong to the *nigrita* species group (87% of bootstrap values). Two species distributed only in the Malay Peninsula (*S. taylori* and *S. rossi;* both species are not placed in any group) formed a monophyletic group (100% of bootstrap values).

The parsimony analysis in which all characters were given equal weight resulted in 15 most-parsimonious trees, each of which required 1848 nucleotide changes. The strict consensus tree is shown in Fig. 2. In all 15 trees, *Salganea* formed a monophyletic clade (73% of bootstrap values). Although the resolution of the deep node was low, as the neighbor-joining tree showed, five monophyletic groups which were supported in Fig. 1 were also found in this consensus tree, with more than 66% of the bootstrap values.

Maekawa et al. (1999b) and Maekawa and Matsumoto (2000) suggested that, for the third codon position of the COII gene of cockroaches, at genetic distances greater than 10%, there might be saturation of transitions (TIs). Most genetic distances between species used in this study were >10% (data not shown), thus we employed weighted parsimony, excluding TIs at third codon positions entirely. This analysis resulted in seven most-parsimonious trees, with tree lengths of 999 (consistency index, 0.34; rescaled consistency index, 0.21; retention index, 0.62; trees not shown). Although the five monophyletic groups described above were strongly supported (>73% of bootstrap values), relationships among other species were not supported by 50% or more of the bootstrap values with the exception of that between *S. nigrita* and *S. obtusespinosa* (61%).

Discussion

Phylogenetic Conclusions

The results of the phylogenetic analyses presented here show that the *morio* and *foveolata* species groups, respectively, form unambiguous monophyletic groups. The monophyly of other species groups used in this study (*raggei* and *nigrita*), however, was not supported by either distance or parsimony analyses (Figs. 1 and 2). Although Roth (1979) divided these species groups based on the morphology of male genitalia, we suggest that reassignment of species to species groups is needed for *Salganea* taxonomy.

Relationships Among the morio *Species Group*

The inferred phylogenetic relationships among *Salganea* spp. belonging to the *morio* species group using both neighbor-joining and nonweighted parsimony analyses corresponded well to the geographic distributions of the taxa. Overall, populations of each species distributed in neighboring regions formed unambiguous clades. The cockroach members of this species group are distributed mainly in the Sunda lands. The close phylogenetic relationships among taxa distributed in these areas were also found in other animals [e.g., vertebrate, Reptilia (Honda et al. 1999a), and invertebrate, Lepidoptera (Kitching et al. 1987; Holloway 1998)]. In *S. aequaliterspinosa*, the populations from North Borneo (DANU, TRU, and LAM) are more closely related to those from the Malay Peninsula (TAP and FRA) than to that from South Borneo (BUK). The resultant trees indicate that the population of BUK first diverged within *S. aequaliterspinosa*, though no differences in general morphologies are evi-

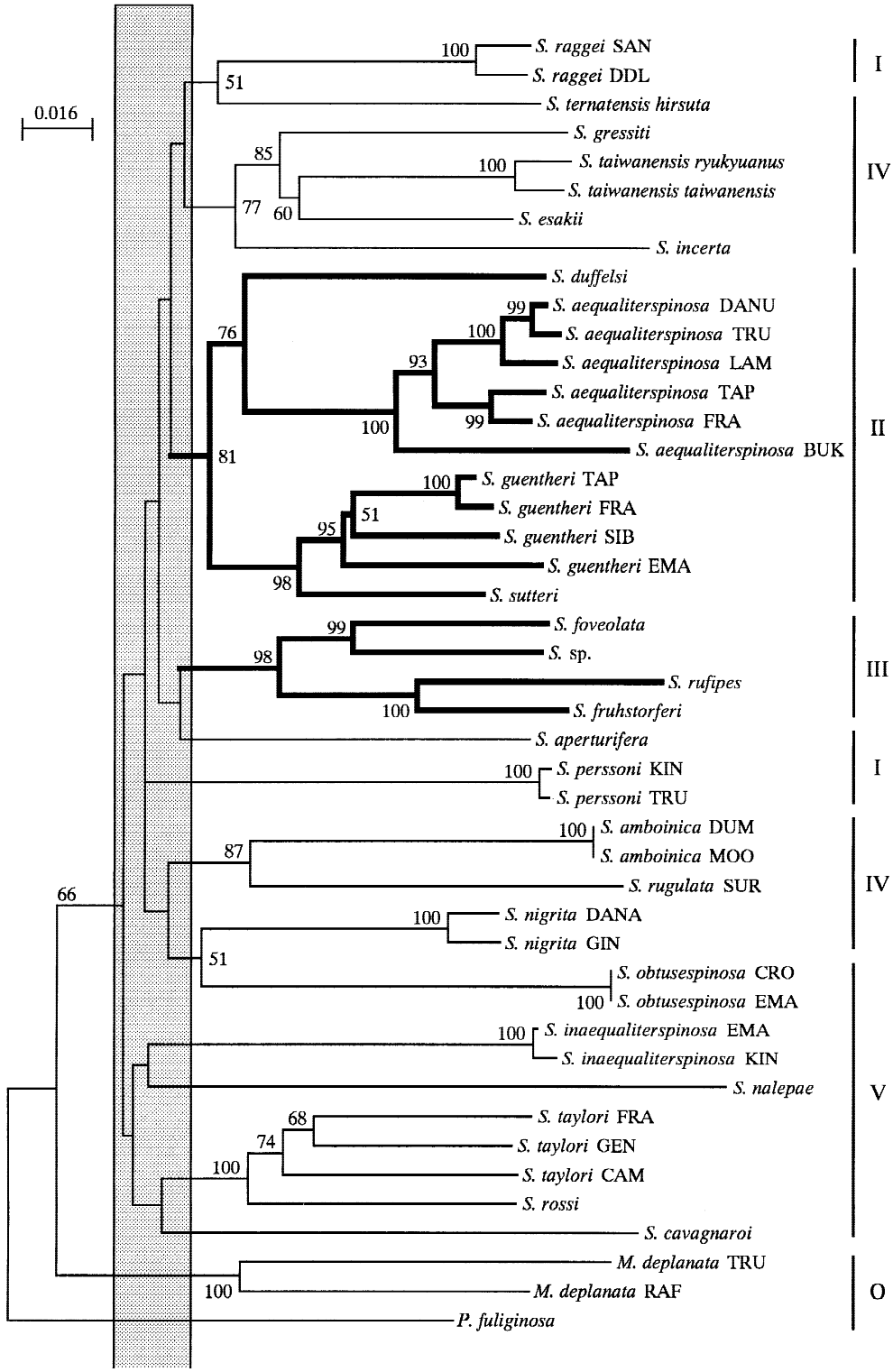

Fig. 1. Phylogenetic relationships of *Salganea* species based on the neighbor-joining method. The *numbers* above and below the branches correspond to the percentage of 1000 bootstrap replicates. All nodes in the shaded region are supported by 50% or less of the bootstrap values. Monophyletic relationships of two species groups (*morio* and *foveolata*) are shown as *thick lines.* See Table 1 for location abbreviations. I, *raggei* species group; II, *morio* species group; III, *foveolata* species group; IV, *nigrita* species group; V, species not placed in any group (Roth 1979). O, outgroups.

dent between the samples from BUK and those from the other localities. These relationships suggest that, even if this species intruded into Borneo more than once, the mountain chains running down the center of Borneo, from northeast to southwest (e.g., the Iran and Müller mountains), were a barrier to gene flow between these populations (see *Estimated Divergence Time and Biogeography of Salganea Species,* below). The close rela-

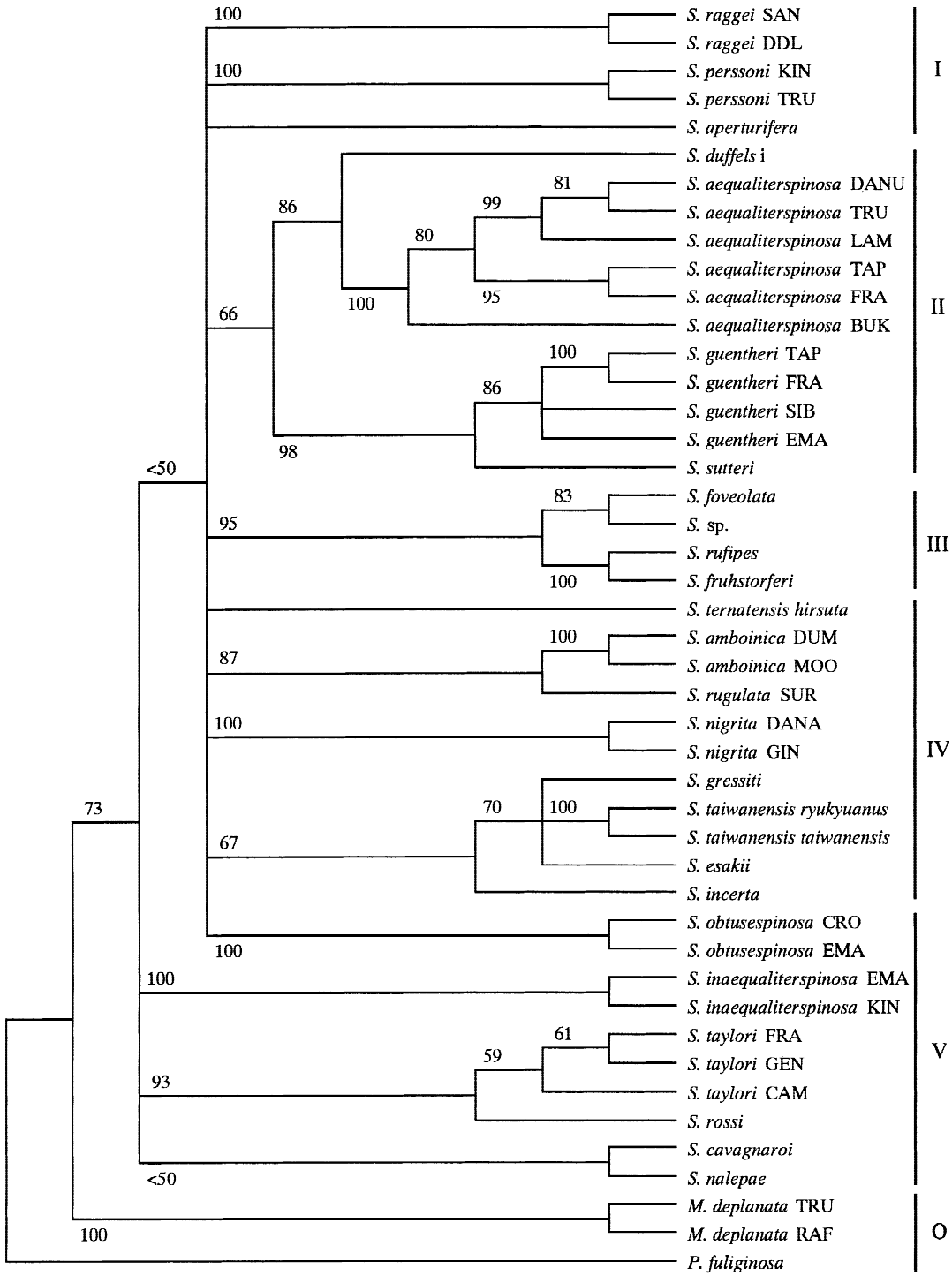

Fig. 2. Strict consensus of the 15 most-parsimonious trees resulting from nonweighted parsimony analysis. Tree length, 1848; consistency index, 0.32; rescaled consistency index, 0.17; retention index, 0.54. The *numbers* above and below each node indicate the level of bootstrap support (1000 replicates). See Table 1 for location abbreviations. I, *raggei* species group; II, *morio* species group; III, *foveolata* species group; IV, *nigrita* species group; V, species not placed in any group (Roth 1979). O, outgroups.

tionships between taxa distributed on the Malay Peninsula and in North Borneo were also shown in Reptilia (Honda et al. 1999b). If more samples of these taxa from different regions (south and west) of Borneo were analyzed, we could confirm the hypothesis of each population of this species remaining in neighboring areas and staying on their own phylogenetic trajectories.

Salganea sutteri from Lombok is shown to be most closely related to *S. guentheri* from the Malay Peninsula, Borneo, and Sumatra. *Salganea sutteri* probably diverged from a common ancestor with *S. guentheri* via the southern part of the Sunda lands, because the former successfully colonize only three neighboring islands around Lombok (Sumba and Sumbawa). Lombok and

Table 3. Mean transversion divergence of the COII gene and estimated times for *Salganea* species selected by degrees of relationship

Divergence	Transversion frequency (%)[a]	Estimated divergence time (mya)[b]	
		a	b
Interspecific			
Among five monophyletic groups and other species ($n = 765$)	7.26	24.2	55.86
S. amboinica vs *S. rugulata* ($n = 2$)	6.38	21.27	49.08
Within *foveolata* sepcies group ($n = 6$)	3.21	10.69	24.67
S. sutteri vs *S. guentheri* ($n = 4$)	1.9	6.32	14.58
Intraspecific			
S. aequaliterspinosa (BUK) vs other taxa ($n = 6$)	1.84	6.15	14.19
S. guentheri (SIB) vs other taxa ($n = 3$)	1.33	4.44	10.24

[a] Jukes–Cantor formula modified according to Beckenbach et al. (1993).
[b] Columns a and b are based on estimated divergence times of 0.3 and 0.13% transversion divergence/my (Beckenbach et al. 1993), respectively.

Sumbawa were originally formed by the growth of a volcanic arc in the Mio-Pliocene (~10 mya) (Monk et al. 1997). Because the sea barrier of the Bali–Lombok Strait has continued to exist since its formation, immigration of the ancestor of *S. sutteri* to Lombok could have been made possible by rafting across the strait.

Relationships Among Species Distributed in Sulawesi

Phylogenetic trees inferred here show the monophyly of *S. amboinica* from North Sulawesi and *S. rugulata* collected in Thailand. This suggests that the ancestor of *S. amboinica* diverged from that of *S. rugulata* in the Sunda lands, or vice versa. On the other hand, the speciation of the *foveolata* species group could have occurred after the formation of the Makassar Strait and the Sulawesi margin by the middle Oligocene (30 mya) (Hall 1998; Moss and Wilson 1998), because these species are endemic in this island (see *Estimated Divergence Time and Biogeography of* Salganea *Species*, below). Yet another less likely possibility is the extinction of species in this group in other regions. The origin of the eastern part of the eastern and southeastern arms of Sulawesi is suggested to be different from that of western regions of Sulawesi, and the former accreted to western Sulawesi during the Miocene (Moss and Wilson 1998). Further studies based on the samples collected in eastern Sulawesi will clarify the detailed scheme of the speciation of species distributed here from a paleogeographic point of view.

Other Monophyletic Relationships

Inferred trees strongly supported the monophyletic relationship of *S. taylori* and *S. rossi*, which are distributed only on the Malay Peninsula. Intriguingly, although the collection points are very close to one another (40–130 km), genetic distances among samples of *S. taylori* (Table 2) are relatively high: uncorrected distances, 8.5–9.6%; and Kimura's (1980) two-parameter distances, 9.1–10.4% (these values among the *foveolata* species group are 8.2–13.7 and 8.8–15.4%, respectively). *Salganea taylori* are micropterous and, perhaps except for a brief dispersal stage as late-instar nymphs or young adults, live their entire life cycle in rotten logs at high altitudes (>1000 m) isolated from each other (Maekawa and Matsumoto, unpublished). Thus gene flows between populations have probably been restricted, and consequently genetic distances among individuals, even among conspecifics, are very high. We can see the same situation in the genetic distance of two individuals of *M. deplanata*, the wingless cockroach, used as outgroups. The sampling points of each taxon are very close (~60 km), but genetic distances are very high (12.6 and 14.3%) (Table 2). Given the similar size, morphology, and life history of members of these genera, it is likely that substitution rates in the COII gene are very similar between and within these groups.

Estimated Divergence Time and Biogeography of Salganea *Species*

If differences in DNA sequences accumulate at a roughly constant rate over time, levels of DNA sequence divergence can be used to date splitting events. Although using such a molecular clock is controversial, it provides a method for estimating approximate divergence times and formulating preliminary biogeographic hypotheses (Burns 1997; Foley et al. 1998). There might be saturation of the third codon position TIs of the COII gene of cockroaches at genetic distances greater than 10% (Maekawa et al. 1999b; Maekawa and Matsumoto 2000), thus we used transversion (TV) distances inferred from the COII gene of *Drosophila* species groups [0.13–0.30%/my (Beckenbach et al. 1993)] (Table 3).

Neither the neighbor-joining nor the nonweighted parsimony analyses show clear relationships among the species groups. In a report on the phylogenetic analyses among cockroach families, Maekawa and Matsumoto (2000) showed that saturation of COII gene substitutions other than third codon TIs did not occur in each family of cockroaches. Thus, it is conceivable that the early radiation of *Salganea* species involved the simultaneous or

nearly simultaneous diversification of numerous species. We suggest that this radiation disturbs the robust phylogenetic relationships among most species based on COII gene sequences and produces a hard polytomy (Fig. 2). Comparing COII genes among five well-supported monophyletic groups (*morio* and *foveolata* species groups, Japanese and Taiwanese *Salganea* + *S. incerta* clade, *S. amboinica* + *S. rugulata* clade, and *S. taylori* + *S. rossi* clade) and other species ($n = 765$) gives TV divergences of 7.26 ± 1.24% (average ± SE) [Jukes–Cantor formula modified according to Beckenbach et al. (1993)]. Using the mean TV frequency and a rate of 0.13–0.30%/my, the early radiation of *Salganea* species is suggested to have occurred during the first half of the Tertiary (60–25 mya) (Table 3). The paleogeographic map (Hall 1998) suggests that, during this period, the Sunda lands containing western Sulawesi existed and that Luzon and neighboring islands in the Philippines were located very close to the eastern edge of this land. These regions could have played an important part in the rapid speciation of this genus. The TV divergence data between *S. amboinica* and *S. rugulata* suggest that the ancestors of these species diverged from each other in the Eo-Oligocene (Table 3; 49–21 mya), a period similar to that determined by geological and tectonic studies for the formation of the Makassar Strait (Hall 1998; Moss and Wilson 1998). The former ancestor could colonize not only Sulawesi but also the Philippines and Moluccas (Roth 1979). The ancestor of the *foveolata* species group now endemic to Sulawesi is suggested to have diverged from other species in the early Tertiary, but their speciation occurred after the formation of the Makassar Strait and the complex of the Sulawesi margin (by 30 mya) (Hall 1998; Moss and Wilson 1998). The estimated divergence time within the *foveolata* species group (Table 3; 25–10 mya) supports this idea. The ancestor of *S. sutteri* is suggested to have diverged from that of *S. guentheri* during the Mio-Pliocene (15–6 mya; Table 3). It is interesting to note that both Lombok and Sumbawa islands were also formed during this period (Monk et al. 1997).

The routes of dispersal followed by New Guinean species are more difficult to discuss. Our DNA data suggest that *S. ternatensis hirsuta* collected from Papua New Guinea diverged from other species during the early to middle Tertiary. However, New Guinea was far from Southeast Asia and very close to Australia until the middle Oligocene (~30 mya) (Hall 1998). Moreover, this species is also found in Sarawak (northern Borneo) in Malaysia (Roth 1979). The most probable explanation is that the colonization of this species from the Sunda lands to New Guinea (or vice versa) and extinction at other regions occurred. Based on their molecular analyses of agamid lizards, Honda et al. (2000) showed that there was a taxon distributed in the Sunda lands that was phylogenetically clearly closely related to Australian (containing New Guinea) endemic fauna. Further sampling, of not only *S. ternatensis hirsuta* but also five species in the *papua* species group, and analyses are needed for profound discussion of the biogeography of New Guinean species.

Regarding intraspecific divergences, we focused on the *morio* species group (*S. aequaliterspinosa* and *S. guentheri*) from several localities. All analyses conducted here strongly suggest that *S. aequaliterspinosa* from southern Borneo (BUK) is the sister taxon to all the other populations (Figs. 1 and 2), and the genetic distances between BUK and other populations were the highest of those between the populations other than BUK. The central mountains on the Sarawak–Kalimantan border extending into Sabah became wider and higher from the middle Miocene (15 mya) to the early Pliocene (5 mya) (Mackinnon et al. 1996; Hall 1998). These palaeogeographic data, together with the estimated divergence time between BUK and the other populations (Table 3; 14–6 mya), suggest that these mountain ranges presumably formed a barrier to gene flow for this species. At that time (15–5 mya), mainland Asia, the Malay Peninsula, and Borneo formed a continuous land, and mountainous regions of the western edge parts of Sumatra existed as a land bridge connected to the above land at the southern part of Sumatra (Hall 1998). Consequently, the Malacca Strait (between Sumatra and the Malay Peninsula) was wider than it is today. The speciation of *S. guentheri* is suggested to have been influenced by these extents of land and sea; *S. guentheri* collected from northern Sumatra (SIB) is not as closely related to the populations from the Malay Peninsula (TAP and FRA) (Figs. 1 and 2), although the distance between the collection localities of these two populations is not very long (<500 km), compared with the relationships between SIB and the Bornean population (EMA) (~2000 km). It is suggested that gene flow between SIB and other populations probably occurred within 10 mya (Table 3).

In this study, we have shown the phylogenetic relationships among many species of *Salganea*. Their speciation and dispersal are suggested to have been influenced by some geological events. It is clear that phylogenetic analyses of other taxa, including species belonging to the *papua* species group and based on the sequence data of not only COII but also other slow-evolving genes, are needed to determine the precise relationships among species and to discuss their historical biogeography in more depth.

Acknowledgments. We thank Drs. M.N. Dalimin, M. Maryati, M. Rosli, M. Matsui, and S. Panha for their advice during our field survey and Drs. C.A. Nalepa, T. Fukatsu, and T. Hikida and Messrs. H. Ota, T. Kikuta, and H. Kato for their help during field sampling. We are also grateful to Drs. Y. Johki, T. Miura, T. Suzuki, and E. Zhao for providing insects. Dr. L.M. Roth kindly identified some cockroach species. This study was supported by Research Fellowships from the Japan Society for the Promotion of Science for Young Scientists to K.M. and

partly by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan for the International Scientific Research Program (Nos. 08041144 and 11691174) and for Scientific Research (No. 10440231) and Research for the Future Program of the Japan Society for the Promotion of Science (JSPS).

References

Asahina S (1991) Blattaria of Japan. Nakayama-Shoten, Tokyo (in Japanese)

Avise JC (1994) Molecular markers, natural history and evolution. Chapman and Hall, New York

Beckenbach AT, Wei YW, Liu H (1993) Relationships in the *Drosophila obscura* species group, inferred from mitochondrial cytochrome oxidase II sequences. Mol Biol Evol 10:619–634

Burns KJ (1997) Molecular systematics of Tanagers (Thraupinae): Evolution and biogeography of a diverse radiation of neotropical birds. Mol Phylogenet Evol 8:334–348

Foley DH, Bryan JH, Yeates D, Saul A (1998) Evolution and systematics of *Anopheles:* Insights from a molecular phylogeny of Australasian mosquitoes. Mol Phylogenet Evol 9:262–275

Hall R (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall R, Holloway JD (eds) Biogeography and geological evolution of Southeast Asia. Backbuys, Leiden, pp 99–131

Holloway JD (1998) Geological signal and dispersal noise in two contrasting insect groups in the Indo-Australian tropics: R-mode analysis of pattern in Lepidoptera and cicadas. In: Hall R, Holloway JD (eds) Biogeography and geological evolution of Southeast Asia. Backbuys, Leiden, pp 291–314

Holloway JD, Hall R (1998) SE Asian geology and biogeography: An introduction. In: Hall R, Holloway JD (eds) Biogeography and geological evolution of Southeast Asia. Backbuys, Leiden, pp 1–23

Honda M, Ota H, Kobayashi M, Nabhitabhata J, Yong H-S, Hikida T (1999a) Phylogenetic relationships of the flying lizards, genus *Draco* (Reptilia, Agamidae). Zool Sci 16:535–549

Honda M, Kobayashi M, Yong H-S, Ota H, Hikida T (1999b) Taxonomic re-evaluation of the status *Draco cornutus* Günther, 1864 (Reptilia: Agamidae). Amphibia-Reptilia 20:195–210

Honda M, Ota H, Kobayashi M, Nabhitabhata J, Yong H-S, Sengoku S, Hikida T (2000) Phylogenetic relationships of the family Agamidae (Reptilia: Iguania) inferred from mitochondrial DNA sequences. Zool Sci 17:527–537

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome *b* gene of mammals. J Mol Evol 32:128–144

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kitching I, Vane-Wright RI, Ackery PR (1987) The cladistics of Ideas (Lepidoptera, Danainae). Cladistics 3:14–34

Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jeanisch R, Berns A (1991) Simplified mammalian DNA isolation procedure. Nucleic Acids Res 19:42–93

Liu H, Beckenbach AT (1992) Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. Mol Phylogenet Evol 1:41–52

Mackinnon K, Hatta G, Halim H, Mangalik A (1996) The ecology of Kalimantan. Periplus, Singapore

Maekawa K, Terayama M, Maryati M, Matsumoto T (1999a) The subsocial wood-feeding cockroach genus *Salganea* Stål from Borneo, with description of a new species (Blaberidae: Panesthiinae). Orient Insect 33:233–242

Maekawa K, Lo N, Kitade O, Miura T, Matsumoto T (1999b) Molecular phylogeny and geographic distribution of wood-feeding cockroaches in east Asian islands. Mol Phylogenet Evol 13:360–376

Maekawa K, Kitade O, Matsumoto T (1999c) Molecular phylogeny of orthopteroid insects based on the mitochondrial cytochrome oxidase II gene. Zool Sci 16:175–184

Maekawa K, Matsumoto T (2000) Molecular phylogeny of cockroaches based on mitochondrial COII gene sequences. Syst Entomol 25:511–519

Matsumoto T (1987) Colony compositions of the subsocial wood-feeding cockroaches *Salganea taiwanensis* Roth and *S. esakii* Roth (Blattaria: Panesthiinae). In: Eder J, Rembold H (eds) Chemistry and biology of social insects. Verlag J. Peperny, Munchen, p 394

Monk KA, Frates YD, Reksodiharjo-Lilley G (1997) The ecology of Nusa Tenggara and Maluku. Periplus, Singapore

Moss SJ, Wilson MEJ (1998) Biogeographic implications of the Tertiary palaeogeographic evolution of Sulawesi and Borneo. In: Hall R, Holloway JD (eds) Biogeography and geological evolution of Southeast Asia. Backbuys, Leiden, pp 133–163

Roth LM (1979) A taxonomic revision of the Panesthiinae of the world II. The genera *Salganea* Stål, *Microdina* Kirby and *Caeparia* Stål (Dictyoptera: Blattaria: Blaberidae). Aust J Zool Suppl 69:1–201

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequence and a composition of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Swofford DL (1993) PAUP: Phylogenetic analysis using parsimony, version 3.1.1. Smithsonian Institution, Washington, DC

Thompson JD, Higgins DG, Gibson TJ (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680