

Systematic Position of Heterotermitinae and Coptotermitinae (Blattodea: Isoptera: Rhinotermitidae)¹

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Abstract Rhinotermitidae (Isoptera) is an economically important termite family globally. The family is not monophyletic, and the relationships among some of its taxa remain confusing. The taxonomic status of some subfamilies is likely questionable because of various morphological characters of the soldiers and imagoes. Twenty species in 10 genera, representing all six subfamilies of Rhinotermitidae were studied herein. Morphological phylogenetic trees based on 65 characters and molecular phylogenetic trees based on mitochondrial COII and 16SrRNA genes were reconstructed. The sequence composition and genetic distance were analyzed. Based on these results, Rhinotermitidae is polyphyletic. In the morphological trees, the family was divided into five clades. Heterotermitinae and Coptotermitinae were grouped into the most apical one of them and constituted the sister group to Termitidae in the Bayesian inference (BI) tree. In the combined molecular tree, Rhinotermitidae was divided into three clades. The position of the clade composing Heterotermitinae and Coptotermitinae was the same as in the morphological BI tree. The nucleotide sequence analyses also showed that the genetic distances between Heterotermitinae or Coptotermitinae with Termitidae might be closer than those between Heterotermitinae or Coptotermitinae with other subfamilies in Rhinotermitidae. It is postulated that the relationship of Heterotermitinae and Coptotermitinae with other subfamilies of Rhinotermitidae is not as close as that indicated in the current taxonomic system. They might be the real representative of the intermediate lineage from lower to higher termites. These findings further suggest that Heterotermitinae and Coptotermitinae should be separated from Rhinotermitidae and be upgraded as a whole to family rank.

Key Words Heterotermitinae, Coptotermitinae, polyphyly, morphological phylogeny, molecular data

The family Rhinotermitidae (Isoptera) comprises 335 species in 12 genera and 6 generally accepted subfamilies (Krishna et al. 2013). It is recognized as an economically important group and, thus, receives considerable attention because of the pest status of such genera as *Coptotermes* Wasmann, *Reticulitermes* Holmgren, and *Heterotermes* Froggatt. In addition, Rhinotermitidae is the most evolved family in taxonomically lower termites with variety of caste patterns and

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nesting behaviors. A number of studies have focused on the phylogenetic relationships within the family and with other families. Based on morphological and molecular data, the phylogenetic position of Rhinotermitidae in Blattodea has been clarified as being closely related to the families Serritermitidae and Termitidae (Engel et al. 2009, Lo et al. 2004). The heterogeneity of Rhinotermitidae also has been verified (Inward et al. 2007, Kambhampati and Eggleton 2000, Lo et al. 2004, Ohkuma et al. 2004, Thompson et al. 2000).

Due to the heterogeneity and the taxonomic confusion within the family, changes including members in Rhinotermitidae and their classification status often occur. The placement of *Prorhinotermes* Silvestri has always been debated. With its small fontanelle and narrow median groove forward from the fontanelle, *Prorhinotermes* was treated as a genus in Rhinotermitinae (Emerson 1971, Roonwal and Chhotani 1989); however, molecular phylogenetic analyses supported separating *Prorhinotermes* from Rhinotermitinae, whose members are characterized by the tip of the labrum possessing a fringe of hairs in soldier caste, and assigning it a subfamily rank (Lo et al. 2004, Ohkuma et al. 2004). This latter opinion has gradually been widely accepted in the scientific community. Similarly, *Glossotermes* Emerson was originally considered as a primitive rhinotermitid and was tentatively classified in the subfamily Psammotermitinae based on soldier morphology (Emerson 1950). According to the study on imagoes and workers, *Glossotermes* was reclassified to Serritermitidae (Cancello and DeSouza 2005). Furthermore, *Stylotermes* Holmgren and Holmgren has historically been classified as a subfamily in Rhinotermitidae along with the fossil genus *Parastylotermes* Snyder and Emerson. By virtue of lacking some of the derived features of Rhinotermitidae and having the unique feature of three tarsomeres, it has been upgraded to family rank (Engel et al. 2009, Krishna et al. 2013). After these revisions, the family Rhinotermitidae contains Coptotermitinae, Heterotermitinae, Prorhinotermitinae, Rhinotermitinae, Psammotermitinae, and Termitogetoninae. Nevertheless, the relationships among the six subfamilies remain ambiguous and contentious.

As an assemblage of subfamilies which might have no explicit synapomorphies, the morphological differences of different subfamilies in Rhinotermitidae are great. In the assemblage, soldier mandibles with prominent marginal teeth is a distinctive morphological character in Rhinotermitinae that also exists in the members of Kalotermitidae. Similarly, the same character of imago forewing vein M is shared not only by Heterotermitinae and Coptotermitinae but also by Termitidae. This indicates that some subfamilies in Rhinotermitidae might be evolutionarily distant from each other, and the current classification system might not reflect the real evolutionary relationships among subfamilies. Our objective in this study was to clarify the taxonomic status of selected subfamilies of Rhinotermitidae. We, thus, reexamined the phylogenetic relationships among 10 genera of all six subfamilies in Rhinotermitidae based on morphological characters and mitochondrial genes COII and 16S rRNA sequences.

Materials and Methods

Taxon sampling. Twenty termite species representing 10 genera within all six subfamilies of Rhinotermitidae and 11 species within the families Kalotermitidae,

Serritermitidae, and Termitidae were examined (Table 1). *Hodotermopsis sjostedti* Holmgren (Isoptera: Archotermopsidae) was treated as the outgroup taxon.

Morphological data. A suite of morphological characters (Table 2), including 30 soldier characters, 30 imago characters, 2 worker characters, along with 3 biological characters, were extracted from the characters described previously (Donovan et al. 2000, Engel et al. 2009). Characters were coded either by direct observations or by literature descriptions (Ahmad 1965; Chen et al. 2015; Chouvenc et al. 2015; Huang et al. 2000; King et al. 2007, Krishna et al. 2013; Li et al. 2011; Liu et al. 2012; Maiti 2006; Roonwal and Chhotani 1989; Scheffrahn et al. 2005, 2006; Takematsu and Vongkaluang 2012; Tsai and Chen 2003). Characters that could not be coded due to missing data and absence of homologous structures were denoted individually by a “?” and by a “-”. The data matrix is displayed in Tables 3 and 4.

Molecular data. Two mitochondrial genes, COII and 16S rRNA, were chosen for the study. Most sequence data were obtained from GenBank. Eleven sequences of six species collected from Guangxi, Yunnan, Hainan, and Guangdong provinces of China were generated from this study and from our previous studies using DNA extraction and polymerase chain reaction methods as previously described (Ke et al. 2017). Species and the GenBank accession numbers of their COII and 16S rRNA sequences are listed in Table 1. Primer sequences were as follows: TL2J3037 (alias AtLeu) (5'-ATGGCAGATTAGTGCAATGG-3') (Liu and Beckenbach 1992) and TKN3785 (alias BtLys) (5'-GTTTAAGAGACCAGTACTTG-3') (Simon et al. 1994) were for COII gene sequence, and LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati and Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al. 1994) were for 16S rRNA gene sequence.

Amplified sequences were screened using the BLAST (Basic Local Alignment Search Tool) implemented in NCBI to check for sequence homology with the known genes. Sequence alignments were performed in Clustal X (Thompson et al. 1997) using default parameters. Nucleotide composition, genetic distance, as well as saturation test in nucleotide substitutions (phylogenetic signal detection) following the Kimura 2-parameter model (Kimura 1980), were evaluated by MEGA 6.06 (Tamura et al. 2013).

Phylogenetic analyses. Bayesian inference (BI) analyses were performed in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) on both morphological and molecular data sets. One million generations were performed, with four Markov chains (three hot and one cold chain) starting from random trees. Trees were sampled every 100 generations. The first 2,500 trees were considered as the “burn-in” and discarded. The 50% majority-rule consensus tree was calculated with supporting posterior probabilities on each clade. Before the molecular phylogenetic analysis, the appropriate nucleotide substitution model, GTR+I+G, for COII and 16S rRNA genes individually and for the tandem COII and 16S rRNA gene was selected using the program jModelTest 2.1.7 (Darriba et al. 2012) with the corrected Akaike Information Criterion (AIC).

The maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford 2002) on the morphological data set only. Heuristic searches were performed with 100 replicates of random addition sequences and tree-bisection-

Table 1. Taxa and the GenBank accession numbers included in this study.

Species	Family	Subfamily	GenBank Accession Numbers	
			COII	16S
<i>Cryptotermes domesticus</i> (Haviland)	Kalotermitidae	—	HQ012041	D89835
<i>Glyptotermes fuscus</i> Oshima	Kalotermitidae	—	AB109540	D89837
<i>Neotermes castaneus</i> (Burmeister)	Kalotermitidae	—	HQ215844	JN615267
<i>Incisitermes minor</i> (Hagen)	Kalotermitidae	—	GQ922441	HM542457
<i>Schedorhinotermes magnus</i> Tsai and Chen (YHK026)	Rhinotermitidae	Rhinotermitinae	MG781178 [†]	MG781185 [†]
<i>Schedorhinotermes magnus</i> (GDX008)	Rhinotermitidae	Rhinotermitinae	MG781179 [†]	MG781186 [†]
<i>Schedorhinotermes medioobscurus</i> (Holmgren)	Rhinotermitidae	Rhinotermitinae	DQ442240	AF262584
<i>Schedorhinotermes sarawakensis</i> Holmgren*	Rhinotermitidae	Rhinotermitinae	AY553143	DQ004486
<i>Parrhinotermes queenslandicus</i> Mjoberg	Rhinotermitidae	Rhinotermitinae	AB005585	AF262588
<i>Parrhinotermes aequalis</i> (Haviland)	Rhinotermitidae	Rhinotermitinae	AF262604	AF262586
<i>Parrhinotermes microdentiformis</i> Thapa	Rhinotermitidae	Rhinotermitinae	DQ442215	MK246851
<i>Dolichorhinotermes longilabius</i> (Emerson)	Rhinotermitidae	Rhinotermitinae	DQ442119	KP026258
<i>Rhinotermes marginalis</i> (L.)	Rhinotermitidae	Rhinotermitinae	EU253890	EU253754
<i>Prorhinotermes canalfrons</i> (Sjöstedt)	Rhinotermitidae	Prorhinotermitinae	EU253888	EU253752
<i>Prorhinotermes japonicus</i> (Holmgren) **	Rhinotermitidae	Prorhinotermitinae	AF262599	AF262580
<i>Prorhinotermes hainanensis</i> Ping and Xu	Rhinotermitidae	Prorhinotermitinae	NA [†]	MG781183 [†]
<i>Psammotermes allocerus</i> Silvestri	Rhinotermitidae	Psammotermitinae	AF262597	AF262578

Table 1. Continued.

Species	Family	Subfamily	GenBank Accession Numbers	
			COI	16S
<i>Termitogeton planus</i> (Haviland)	Rhinotermitidae	Termitogetoninae	AF262598	AF262579
<i>Coptotermes formosanus</i> Shiraki	Rhinotermitidae	Coptotermitinae	KU257993	KU257989
<i>Coptotermes curvignathus</i> Holmgren	Rhinotermitidae	Coptotermitinae	AY536402	KF853387
<i>Coptotermes gestroi</i> (Wasmann)	Rhinotermitidae	Coptotermitinae	HQ231233	MG781184 [†]
<i>Heterotermes tenuis</i> (Hagen)	Rhinotermitidae	Heterotermitinae	DQ442139	AY380238
<i>Heterotermes cardini</i> (Snyder)	Rhinotermitidae	Heterotermitinae	AY453590	AY380262
<i>Reticulitermes guangzhouensis</i> Ping	Rhinotermitidae	Heterotermitinae	KX374678	KX374681
<i>Reticulitermes flavipes</i> (Kollar)	Rhinotermitidae	Heterotermitinae	AY808087	EF363259
<i>Serritermes serrifer</i> (Hagen and Bates)	Serritermitidae	—	AF220598	AF262577
<i>Glossotermes oculatus</i> Emerson	Serritermitidae	—	AY553141	AY553181
<i>Odontotermes formosanus</i> (Shiraki)	Termitidae	Macrotermitinae	AB005581	JQ429119
<i>Ahmaditermes</i> sp.	Termitidae	Nasutitermitinae	MG781177 [†]	MG781182 [†]
<i>Nasutitermes corniger</i> (Motschulsky)	Termitidae	Nasutitermitinae	DQ442176	AB037344
<i>Pericapritermes nitobei</i> (Shiraki)	Termitidae	Termitinae	AB109517	JQ429134
<i>Globitermes sulphureus</i> (Haviland)	Termitidae	Termitinae	AB109509	JQ429127
<i>Hodotermopsis sjostedti</i> Holmgren	Archotermopsidae	—	EU253893	MG781189 [†]

* Synonym of *Schedorhinotermes malaccensis* (Holmgren).
** Synonym of *Prohinotermes flavus* (Bugnion and Popoff).
† GenBank accession numbers submitted in this study.
‡ NA, not available.

Table 2. Morphological characters and character states used in the phylogenetic analysis.

Characters	Character States
Soldier characters	
1. Length of head capsule to lateral insertion of mandibles, compared with greatest width of head	(0) distinctly longer than width; (1) slightly longer than width or about equal to width
2. Soldier heads	(0) normal; (1) flattened
3. Eyes	(0) distinct; (1) visible but vestigial, forming indistinct patch on surface; (2) absent
4. Frons and postclypeus with complete or at least partial conspicuous longitudinal groove	(0) no; (1) yes
5. Number of antennal segments	(0) 20 or more; (1) 19 or fewer
6. Frontal gland developed into distinct fontanelle	(0) absent; (1) present
7. Fontanelle pore	(0) absent; (1) present but minute, without an open pore, sometimes only distinct pit or depression visible; (2) small but clearly visible, with open pore; (3) medium to large open pore
8. Fontanelle position	(0) sunken in pit or groove; (1) more or less flush with surface of head; (2) extended above surface of head capsule on distinct tube; (3) located at end of nasus
9. Setulae surrounding fontanelle (directed toward fontanelle)	(0) absent; (1) present
10. Postmentum length	(0) distinctly greater than width; (1) more or less equal to or less than width
11. Labrum	(0) well developed; (1) vestigial
12. Anterior margin of labrum in dorsal view	(0) more or less straight to broadly rounded; (1) concave in middle, anterolateral corners extended into rounded, wide ends; (2) narrowly rounded to more or less sharp; (3) extended into long, thin, tapering points

Table 2. Continued.

Characters	Character States
13. Hyaline tip of labrum	(0) absent; (1) simple and narrow; (2) narrow, more or less triangular
14. Anterior margin of labrum	(0) without a fringe of hairs; (1) with a fringe of hairs like a brush
15. Labrum length compared to length of left mandible in plain view	(0) less than one-quarter length of mandible; (1) between one-quarter and one-half length of mandible; (2) between half and full length of mandible
16. Labrum length compared to head length in plain view	(0) distinctly less than one-quarter of the head length; (1) between one-quarter and half of the head length
17. Development of mandible blades	(0) well developed or well developed at least in major soldiers; (1) vestigial, bladeless lobes
18. Inner margin of left mandible blade	(0) with one or more distinct teeth; (1) with serrations on at least half of inner margin, but no distinct teeth; (2) almost smooth or with serrations only at base
19. Number of distinct marginal teeth on left mandible	(0) three or more; (1) two; (2) one; (3) zero
20. Position of point of principal marginal tooth on left mandible blade	(0) approximately three-quarters from base to apex; (1) approximately midway or within apical half
21. Inner margin of right mandible blade	(0) with one or more distinct teeth; (1) with serrations on at least half of inner margin, but no distinct teeth; (2) almost smooth or with serrations only at base
22. Number of distinct marginal teeth on right mandible	(0) two or more; (1) one; (2) zero
23. Position of point of principal marginal tooth on right mandible blade	(0) approximately three-quarters from base to apex; (1) approximately midway or within apical half
24. Width of pronotum in plain view	(0) approximately equal to or greater than head width; (1) less than head width but greater than half the head width; (2) approximately equal to or less than half the head width

Table 2. Continued.

Characters	Character States
25. Dorsal surface of pronotum in profile, ignoring any spines	(0) more or less flat; (1) with two distinct lobes, anterior lobe distinctly smaller and higher than posterior lobe
26. Number of fore tibial apical spurs	(0) four; (1) three; (2) two
27. Number of mid-tibial apical spurs	(0) three; (1) two
28. Number of hind tibial apical spurs	(0) four; (1) three; (2) two
29. Number of tarsal segments	(0) 5-segmented, or 4-segmented but second is clearly partially divided; (1) 4-segmented
30. Abdominal cerci	(0) with more than two segments; (1) with two segments
Imago characters	
31. Number of antennomeres	(0) 23–28; (1) 11–22
32. Eyes	(0) protruding well beyond lateral margin of head; (1) small, not protruding beyond lateral margin of head in frontal view
33. Anterior margin of postclypeus	(0) flat or concave; (1) convex
34. Postclypeal furrow	(0) absent; (1) present as shallow longitudinal furrow
35. Y-shaped coronal ecdysial cleavage line	(0) present; (1) absent or highly vestigial
36. Marginal teeth of left mandible	(0) greater than or equal to three; (1) two teeth; (2) one tooth
37. Subsidiary tooth of right mandible	(0) absent; (1) present
38. Pronotal lateral margins in dorsal view	(0) subparallel; (1) converging
39. Pronotal size	(0) width greater than or equal to head; (1) width significantly less than head width
40. Pronotal anterior margin in dorsal view	(0) concave; (1) relatively straight
41. Pretarsal arolium	(0) present; (1) absent

Table 2. Continued.

Characters	Character States
42. Wing membrane setae	(0) absent; (1) present, microsetulose
43. Wing membrane surface	(0) smooth; (1) nodulose or pimplate
44. Number of superior branches of Rs	(0) one or more; (1) zero
45. First Rs fork	(0) in basal half; (1) near midlength or beyond
46. Forewing vein M	(0) present; (1) absent
47. Position of forewing vein M	(0) close to Rs; (1) midway between Rs and CuA or closer to latter
48. Forewing CuP	(0) terminates in basal suture; (1) terminates prior to posterior tip of basal suture
49. CuP in forewing scale	(0) convex; (1) straight or concave
50. Forewing CuA	(0) short, to around wing midlength; (1) elongate and extensively developed, extending to apex or subapex
51. Costalization of forewing	(0) not costalized; (1) C+Sc+R and Rs extremely close and simple and parallel
52. Humeral margin of forewing scale	(0) flat; (1) swollen beyond level of costal margin
53. Distal margin of forewing scale	(0) evenly convex; (1) straight to slightly convex; (2) straight and diagonal
54. Hind wing basal cleavage suture	(0) present but rudimentary; (1) completely developed
55. Hind wing vein M	(0) present; (1) absent
56. Hind wing vein A1	(0) present; (1) absent
57. Forewing scale relative to hind wing scale	(0) apical margin of forewing scale meeting or overlapping hind scale; (1) scales well separated
58. Sternal gland on fourth sternum	(0) present; (1) absent
59. Sternal gland on fifth sternum	(0) present; (1) absent
60. Styli	(0) present in both sexes; (1) present in male only; (2) absent

Table 2. Continued.

Characters	Character States
Worker characters	
61. Clypeus in profile	(0) not keeled; (1) with keel
62. Number of Malpighian tubules	(0) eight or more; (1) four or fewer
Biological characters	
63. True worker caste	(0) absent; (1) present
64. Soldier dimorphism	(0) absent; (1) present
65. Symbiotic flagellates in gut	(0) present; (1) absent

Table 3. Data matrix for morphological characters 0–2 used in the analysis.

Taxon	Character*																												
	0								1								2												
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9
<i>Hodotermopsis sjostedti</i>	1	0	0	0	0	0	0	-	-	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cryptotermes domesticus</i>	1	0	1	0	1	0	0	-	-	1	0	0	0	0	1	0	0	0	0	1	0	0	?	0	0	1	0	1	1
<i>Glyptotermes fuscus</i>	0	0	1	0	1	0	0	-	-	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	1
<i>Neotermes castaneus</i>	?	0	1	0	1	0	0	-	-	?	0	0	0	?	?	0	0	0	?	0	0	?	0	0	1	0	1	1	
<i>Incisitermes minor</i>	0	0	1	0	1	0	0	-	-	0	0	0	0	0	0	0	0	0	?	0	0	1	0	0	1	0	1	1	
<i>Schedorhinotermes magnus</i> (YHK026)	1	0	?	1	1	1	2	0	0	0	0	1	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Schedorhinotermes magnus</i> (GXDX008)	1	0	?	1	1	1	2	0	0	0	0	1	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Schedorhinotermes medioobscurus</i>	1	0	1	1	1	1	2	0	0	0	0	1	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Schedorhinotermes sarawakensis</i>	1	0	1	1	1	1	2	0	0	0	0	1	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Parrhinotermes queenslandicus</i>	1	0	?	1	1	1	2	0	0	0	0	0	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Parrhinotermes aequalis</i>	1	0	?	1	1	1	2	0	0	0	0	0	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Parrhinotermes microdentiformis</i>	1	0	?	1	1	1	2	0	0	0	0	0	1	1	2	0	0	0	1	1	0	1	1	1	0	1	1	2	1
<i>Dolichorhinotermes longilabius</i>	0	0	?	1	1	1	2	0	0	0	0	1	1	1	2	1	0	0	1	1	0	1	1	1	0	1	1	2	1

Table 3. Continued.

Taxon	Character*																															
	0								1								2															
	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9			
<i>Rhinotermes marginalis</i>	1	0	2	1	1	1	2	0	0	0	0	0	1	1	1	0	0	0	1	1	0	0	1	1	0	1	1	0	1	2	1	
<i>Prorhinotermes canalifrons</i>	0	0	?	1	1	1	2	0	0	0	0	0	1	0	0	0	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Prorhinotermes japonicus</i>	0	0	1	1	1	1	2	0	0	0	0	0	1	0	0	0	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Prorhinotermes hainanensis</i>	0	0	1	1	1	1	2	0	0	0	0	0	1	0	0	0	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Coptotermes formosanus</i>	0	0	2	0	1	1	3	2	1	0	0	2	2	0	1	1	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Coptotermes curvignathus</i>	0	0	2	0	1	1	3	2	1	0	0	2	2	0	1	1	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Coptotermes gestroi</i>	0	0	2	0	1	1	3	2	1	0	0	2	2	0	1	0	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Heterotermes tenuis</i>	0	0	2	0	1	1	1	1	1	0	0	2	2	0	?	?	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Heterotermes cardini</i>	0	0	2	0	1	1	1	1	1	0	0	2	2	0	?	?	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Reticulitermes guangzhouensis</i>	0	0	2	0	1	1	1	1	1	0	0	2	2	0	1	1	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Reticulitermes flavipes</i>	0	0	2	0	1	1	1	1	1	0	0	2	2	0	1	1	0	2	3	-	2	2	-	1	0	1	1	2	1			
<i>Odontotermes formosanus</i>	0	0	2	0	1	1	1	1	0	0	0	2	0	0	?	?	0	0	2	1	2	2	-	1	1	1	1	2	1			
<i>Ahmaditermes</i> sp.	1	0	2	0	1	1	2	3	0	1	1	0	0	0	2	0	1	-	-	-	-	-	-	2	1	2	1	2	1			
<i>Nasutitermes corniger</i>	1	0	2	0	1	1	2	3	0	1	1	0	0	0	2	0	1	-	-	-	-	-	-	2	1	2	1	2	1			
<i>Pericapritermes nitobei</i>	0	0	2	0	1	1	1	1	0	0	0	3	?	0	0	0	0	2	3	-	2	2	-	1	1	1	1	2	1			
<i>Globitermes sulphureus</i>	1	0	2	0	1	1	1	1	0	0	0	2	?	0	1	?	0	0	2	1	0	1	1	1	1	1	1	2	1			
<i>Psammotermes allocerus</i>	0	1	2	1	1	1	2	0	0	0	0	2	1	0	1	1	0	0	0	1	0	0	1	0	0	1	0	1	2	1		
<i>Termitogeton planus</i>	1	1	2	0	1	1	2	1	0	0	0	2	1	0	2	1	0	2	3	-	2	2	-	2	0	2	1	2	1			
<i>Serritermes serrifer</i>	1	0	1	0	1	1	2	1	0	?	0	2	1	0	?	1	0	1	3	-	1	2	-	1	0	2	1	2	1			
<i>Glossotermes oculatus</i>	0	0	?	0	1	1	?	1	0	0	0	0	1	0	?	1	0	1	3	-	2	2	-	1	0	2	1	2	1			

* -, inapplicable codings; ?, unknown states.

reconnection (TBR) option for branch swapping. Statistical support for each branch was evaluated by bootstrap analysis (Felsenstein 1985) with 1,000 replicates.

Maximum likelihood (ML) analyses were implemented in the application PhyML 3.1 (Guindon and Gascuel 2003) on molecular data sets only. BioNJ distance-based starting tree was used as well under the GTR+I+G model. Branch support for trees was estimated using nonparametric bootstrap sampling with 1,000 replicates.

Table 4. Data matrix for morphological characters 3–6 used in the analysis.

Taxon	Character*																																	
	3							4							5							6												
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9				
<i>Hodotermopsis sjostedti</i>	0	0	0	0	0	0	0	1	?	?	?	?	?	0	0	0	0	0	1	0	0	0	0	0	?	0	0	0	0	0	0			
<i>Cryptotermes domesticus</i>	1	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0		
<i>Glyptotermes fuscus</i>	1	1	0	0	0	1	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	0	0	0	1	0	?	0	0	0	0	0		
<i>Neotermes castaneus</i>	1	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	?	0	0	0	0	0		
<i>Incisitermes minor</i>	1	1	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	?	0	0	0	0	0		
<i>Schedorhinotermes magnus</i> (YHK026)	1	1	0	1	1	1	0	1	1	1	1	0	0	1	-	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0		
<i>Schedorhinotermes magnus</i> (GXDX008)	1	1	0	1	1	1	0	1	1	1	1	0	0	1	-	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0		
<i>Schedorhinotermes medioobscurus</i>	1	1	0	1	1	1	0	1	1	1	1	0	0	1	-	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0		
<i>Schedorhinotermes sarawakensis</i>	1	1	0	1	1	1	0	1	1	1	1	0	0	1	-	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0		
<i>Parrhinotermes queenslandicus</i>	1	1	?	?	1	1	0	1	?	1	?	1	?	?	?	?	?	0	?	1	1	1	1	0	?	?	0	1	0	1	0	0	?	
<i>Parrhinotermes aequalis</i>	1	1	?	?	1	1	0	1	?	1	?	1	?	?	?	?	?	0	?	1	1	1	1	0	?	?	0	1	0	1	0	0	?	
<i>Parrhinotermes microdentiformis</i>	1	1	?	?	1	1	0	1	?	?	?	?	1	?	?	?	?	?	0	?	1	1	1	1	0	?	?	0	1	0	1	0	0	?
<i>Dolichorhinotermes longilabius</i>	1	1	0	1	1	1	0	1	1	1	1	0	0	1	-	0	?	1	1	1	1	0	1	1	0	1	0	1	0	0	?	0	1	
<i>Rhinotermes marginalis</i>	1	1	0	1	1	1	0	1	1	1	1	0	0	1	-	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0	1	
<i>Prorhinotermes canalifrons</i>	1	1	0	0	1	?	0	1	1	1	1	0	0	1	-	1	-	1	1	1	0	1	1	0	1	0	1	0	1	1	0	0	0	
<i>Prorhinotermes japonicus</i>	1	1	0	0	1	?	0	1	1	1	1	0	0	1	-	1	-	1	1	1	0	1	1	0	1	0	1	0	1	1	0	0	0	
<i>Prorhinotermes hainanensis</i>	1	1	0	0	1	?	0	1	1	1	1	0	0	1	-	1	-	1	1	1	0	1	1	0	1	0	1	0	1	1	0	0	0	
<i>Coptotermes formosanus</i>	1	1	0	0	1	1	0	1	1	1	1	1	0	1	-	0	1	1	1	1	1	0	1	1	0	1	0	1	0	1	1	0	1	

Table 4. Continued.

Taxon	Character*																										
	3						4						5						6								
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	
<i>Coptotermes curvignathus</i>	1	1	0	0	1	1	0	1	1	1	1	1	1	0	1	-	0	1	1	1	1	0	1	1	0	1	0
<i>Coptotermes gestroi</i>	1	1	0	0	1	1	0	1	1	1	1	1	0	1	-	0	1	1	1	1	0	1	1	0	1	1	0
<i>Heterotermes tenuis</i>	1	1	1	0	1	?	0	1	?	1	?	1	1	0	1	-	0	1	1	1	1	0	1	1	0	1	0
<i>Heterotermes cardini</i>	1	1	1	0	1	?	0	1	?	1	?	1	1	0	1	-	0	1	1	1	1	0	1	1	0	1	0
<i>Reticulitermes guangzhouensis</i>	1	1	1	0	1	1	0	1	1	1	1	1	0	0	1	-	0	1	1	1	1	0	1	1	0	1	0
<i>Reticulitermes flavipes</i>	1	1	1	0	1	1	0	1	1	1	1	1	0	0	1	-	0	1	1	1	1	0	1	1	0	1	0
<i>Odontotermes formosanus</i>	1	1	0	?	1	?	1	0	1	1	1	?	?	?	?	-	0	1	1	1	1	0	2	?	0	1	1
<i>Ahmaditermes</i> sp.	1	1	?	?	?	1	1	0	1	1	1	?	?	?	?	-	0	1	1	1	1	0	2	?	0	1	1
<i>Nasutitermes corniger</i>	1	1	0	0	1	1	1	0	1	1	1	1	0	0	1	-	0	1	1	1	1	0	2	0	1	1	0
<i>Pericapritermes nitobei</i>	1	1	0	0	1	?	1	0	1	1	1	?	?	?	?	-	0	1	1	1	1	0	2	?	0	1	1
<i>Globitermes sulphureus</i>	1	1	0	0	1	1	1	0	1	1	1	?	?	?	?	-	0	1	1	1	1	0	2	?	0	1	1
<i>Psammotermes allocerus</i>	1	1	?	0	1	1	0	1	?	1	?	1	0	?	1	-	1	-	1	1	1	0	1	1	1	0	1
<i>Termitogeton planus</i>	1	1	0	0	1	1	0	1	?	1	?	1	?	?	?	1	-	1	-	1	1	1	0	1	1	1	0
<i>Serritermes serrifer</i>	1	1	?	0	1	0	2	0	1	0	1	1	0	?	1	-	1	-	0	0	1	1	?	1	1	0	2
<i>Glossotermes oculatus</i>	1	1	?	?	?	0	1	0	?	?	1	1	0	?	?	?	?	?	1	-	0	0	1	1	?	?	?

* -, inapplicable codings; ?, unknown states.

Molecular phylogenetic analyses were conducted in turn on data sets comprising isolate COII gene sequences of 31 ingroup taxa, comprising isolate 16S rRNA gene sequences of 32 ingroup taxa, as well as comprising combined COII and 16S rRNA gene sequences of 31 ingroup taxa. Each base was treated as a character, and gaps were treated as missing. All molecular and morphological characters were unordered and had equal weight.

Results

Morphological phylogenetic analyses. Both trees generated by MP analysis and by BI analysis revealed two major clades (Figs. 1, 2). One comprised the members of Kalotermitidae, which was the sister group to the remaining taxa. The

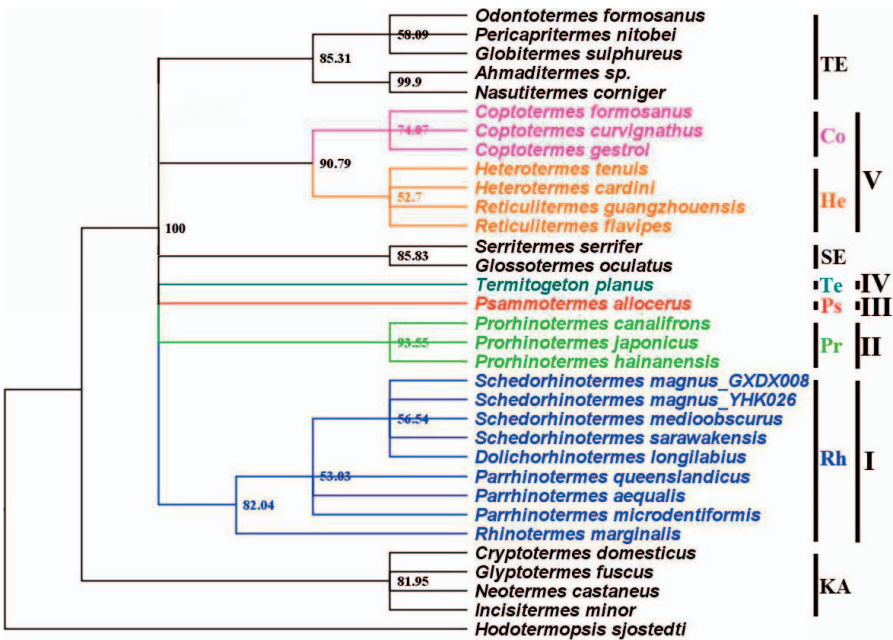


Fig. 1. The maximum parsimony (MP) tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on morphological characters. KA, Kalotermitidae; SE, Serritermitidae; TE, Termitidae; Co, Coptotermitinae (in pink font); He, Heterotermitinae (in orange font); Te, Termitogetoninae (in blue-green font); Pr, Prorhinotermitinae (in green font); Ps (in red font); Rh, Rhinotermitinae (in blue font). The same below. Numbers on the nodes indicate bootstrap (BP) values for 1,000 replicates.

other included the members of Rhinotermitidae, Serritermitidae, and Termitidae, in which Rhinotermitidae was divided into five clades. Subfamilies Heterotermitinae and Coptotermitinae were arranged into a stout monophyletic clade with high bootstrap value and posterior probability. The other four subfamilies clustered respectively into a clade. When interpreting the relationships among the five clades, MP tree made little contribution. The MP analysis produced 3,897 equally parsimonious trees (TL = 135, CI = 0.6296, RI = 0.8660). The bootstrap 50% majority-rule consensus tree is shown in Fig. 1. In the MP tree, the five clades Rh, Pr, He+Co, Ps, and Te, together with Serritermitidae and Termitidae, formed a polytomy, which made their relationship unresolved. In the BI tree, clade Rh was assigned as the most basal clade which was the sister to the rest of the members of Rhinotermitidae, Serritermitidae, and Termitidae. The clade Ps then branched, followed by clade Pr. Clade He+Co was assigned as the most apical clade of the five clades which showed a sister relationship with the members of Termitidae. Clade Te formed a trichotomy with the branch including He+Co and Termitidae as well as the branch including members of Serritermitidae. According to the BI tree, it

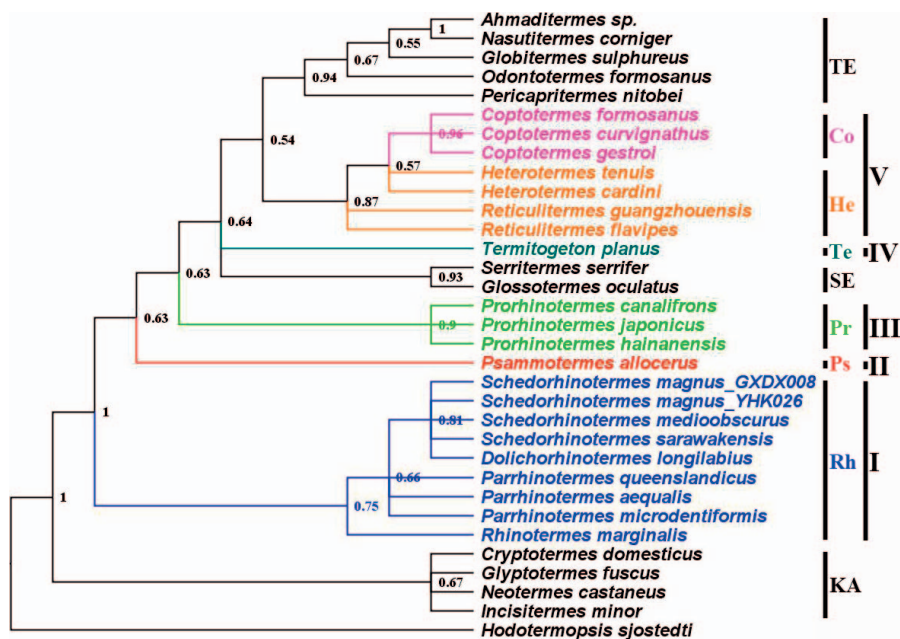


Fig. 2. The Bayesian inference (BI) tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on morphological characters. Numbers on the nodes indicate Bayesian posterior probabilities (PP).

is appears that Coptotermitinae and Heterotermitinae were more closely related to Termitidae than to other subfamilies in Rhinotermitidae.

Nucleotide analyses. For the COII gene, the multiple sequence alignment including outgroup had 684 characters, of which 295 were constant and 338 were parsimony-informative. For 16S rRNA, multiple sequence alignment including outgroup had 392 characters, of which 181 were constant and 177 were parsimony-informative. For the combination of COII and 16S rRNA genes, the multiple sequence alignment had 1,076 characters, of which 476 were constant and 513 were parsimony-informative. The average nucleotide composition was A = 0.38, T = 0.24, G = 0.14, C = 0.23 in COII gene, and was A = 0.23, T = 0.43, G = 0.23, C = 0.11 in 16S rRNA gene. Both genes showed an AT bias, which is common in insects.

In the morphological phylogenetic analyses, the relationship among subfamilies in Rhinotermitidae did not seem to be closer than their relationship with members of other families. Subfamilies Heterotermitinae and Coptotermitinae showed a closer relationship to members of Termitidae. Pairwise Kimura 2-parameter genetic distances among them were calculated. The distances of combined COII and 16S rRNA genes between Heterotermitinae and Coptotermitinae were from 0.107 (*Coptotermes formosanus* Shiraki and *Heterotermes cardini* (Snyder)) to 0.189 (*Coptotermes gestroi* (Wasmann) and *Reticulitermes flavipes* (Kollar)). The values

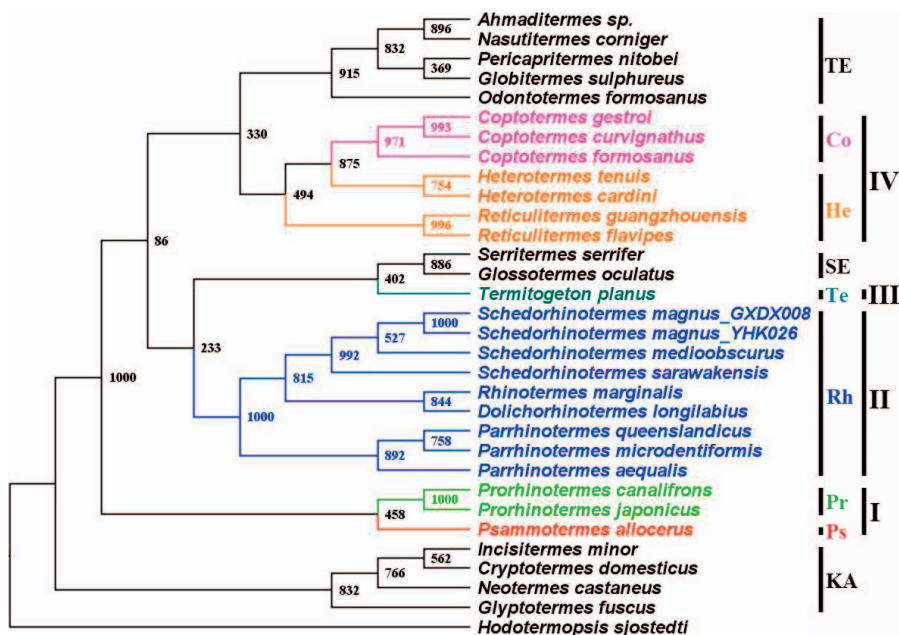


Fig. 3. The maximum likelihood (ML) tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on the mitochondrial COII sequence. Numbers on the nodes indicate bootstrap (BP) values for 1,000 replicates.

were from 0.199 (*C. formosanus* and *Prorhinotermes japonicus* (Holmgren)) to 0.240 (*Coptotermes curvignathus* Holmgren and *Parrhinotermes queenslandicus* Mjoberg) between Coptotermitinae and the other four subfamilies. However, the values between Coptotermitinae and Termitidae were smaller, whose range was from 0.178 (*C. formosanus* and *Globitermes sulphureus* (Haviland)) to 0.213 (*C. gestroi* and *Nasutitermes corniger* (Motschulsky)). Similarly, the genetic distances between Heterotermitinae and Termitidae were closer than those between Heterotermitinae and the other four subfamilies. Thus, it can be seen that the clade composing Heterotermitinae and Coptotermitinae might be closely related to Termitidae rather than to other members in Rhinotermitidae.

To analyze whether the divergences of COII, 16S rRNA, and combined sequences among species were saturated, the substitution saturation tests were performed by graphs. The distinctly linear correlation between the Ts/Tv values and the genetic distances indicated that saturations were not reached and then the data were suitable for phylogenetic analyses.

Molecular phylogenetic analyses. In both trees inferred from COII sequence, the monophyletic Kalotermitidae was the earliest branching lineage, the taxa of Rhinotermitidae, Serritermitidae, and Termitidae were collectively the sister group to the Kalotermitidae. The trees were inconsistent in the branching pattern of Rhinotermitidae (Figs. 3, 4). In the ML tree, the six subfamilies were assigned into

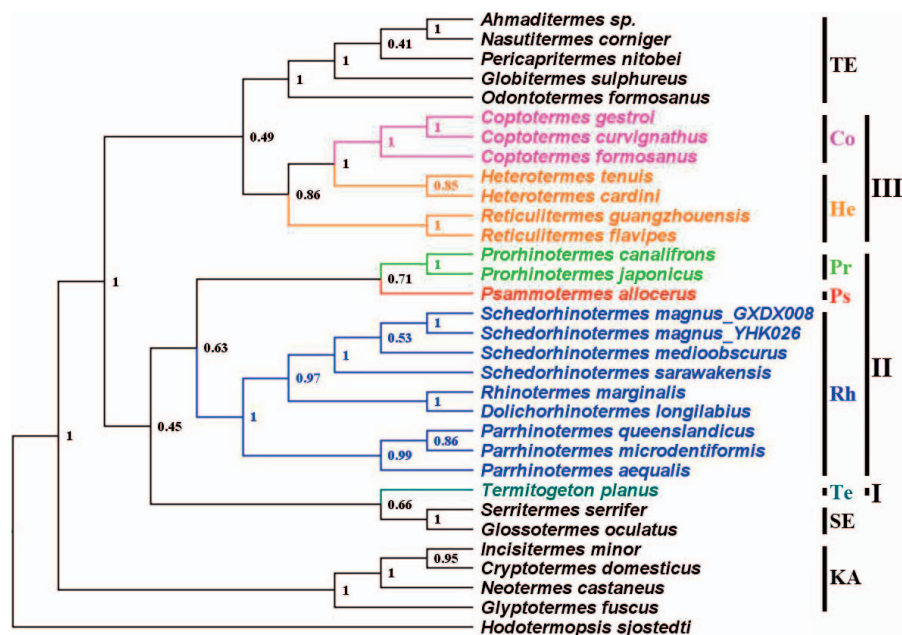


Fig. 4. The Bayesian inference (BI) tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on the mitochondrial COII sequence. Numbers on the nodes indicate Bayesian posterior probabilities (PP).

four clades: Ps+Pr, Rh, Te, and He+Co. Clade Ps+Pr was the basal lineage which was the sister to the remain taxa of Rhinotermitidae, Serritermitidae, and Termitidae. Clade Te was grouped first with the members of Serritermitidae, and then they showed a sister relationship with Clade Rh. Clade He+Co was not grouped with either of the other members in the same family, but grouped with the members of Termitidae. The relationships among them were ((Ps, Pr), ((Rh, (Te, SE)), (He+Co, TE))). In the BI tree, the six subfamilies were assigned into three clades: Te, Rh+(Ps+Pr), and He+Co. The apical Clade He+Co grouped also with the members of Termitidae. The relationships indicated in the BI tree was (((SE, Te), (Rh, (Ps, Pr))), (He+Co, TE)).

The trees inferred from 16S rRNA sequence were in disagreement with those inferred from COII sequence (Figs. 5, 6). Serritermitidae was assigned solely as the most basal lineage of the three families. In the ML tree, the six subfamilies of Rhinotermitidae were arranged into four different clades: Rh, (Te+Ps)+Pr, Co+*Heterotermes* (one part of He), and *Reticulitermes* (the other part of He). Clade Rh was most primitive in the four clades. It was the sister to the remaining members of Rhinotermitidae and Termitidae. The member of Termitidae, *Odontotermes* Holmgren, along with He and Co constituted a monophyletic group which was assigned as the sister to Clade (Te+Ps)+Pr. In the BI tree, the six subfamilies were arranged into three clades. Besides Clade Rh and Clade

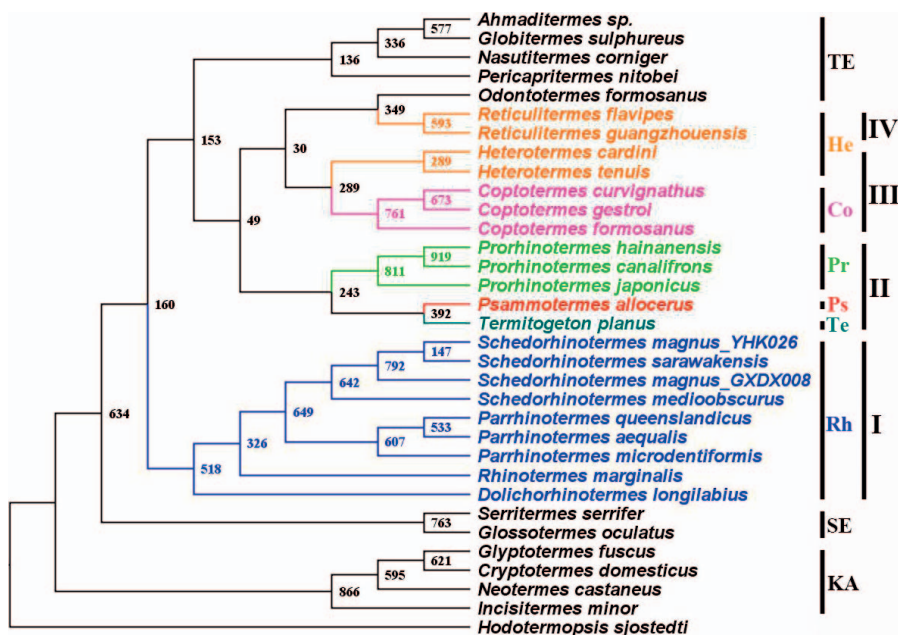


Fig. 5. The maximum likelihood (ML) tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on the mitochondrial 16S rRNA sequence. Numbers on the nodes indicate bootstrap (BP) values for 1,000 replicates.

Reticulitermes, which were the same as in the ML tree, Clade ((Te+Ps)+Pr)+(Co+*Heterotermes*) was the merger of Clade (Te+Ps)+Pr and Clade Co+*Heterotermes*. Of them, Clade Rh was still most primitive; Clade *Reticulitermes* fell into the clade including members of Termitidae.

The topologies of ML tree and BI tree inferred from the tandem COII and 16S rRNA gene sequences were completely consistent as shown in Fig. 7. And, the branching patterns were almost the same as that of the BI tree derived from COII sequence. Rhinotermitidae was not a monophyletic group, in which the members were assigned into three clades including Te, Rh+(Ps+Pr), and He+Co. Clade Te was the most basal clade; Clade Rh+(Ps+Pr) was the sister to the monophyletic group constituted by Serritermitidae and Clade Te. Clade He+Co was the most apical clade which was the sister to Termitidae.

Discussion

In this study, Rhinotermitidae is reconfirmed to be a heterogenetic group with its members not having any synapomorphic characters, which is in agreement with most previous studies (Cameron et al. 2012, Donovan et al. 2000, Inward et al. 2007, Legendre et al. 2008, Lo et al. 2004, Ohkuma et al. 2004). Rhinotermitidae is generally subdivided into six subfamilies which represent at least three clades as

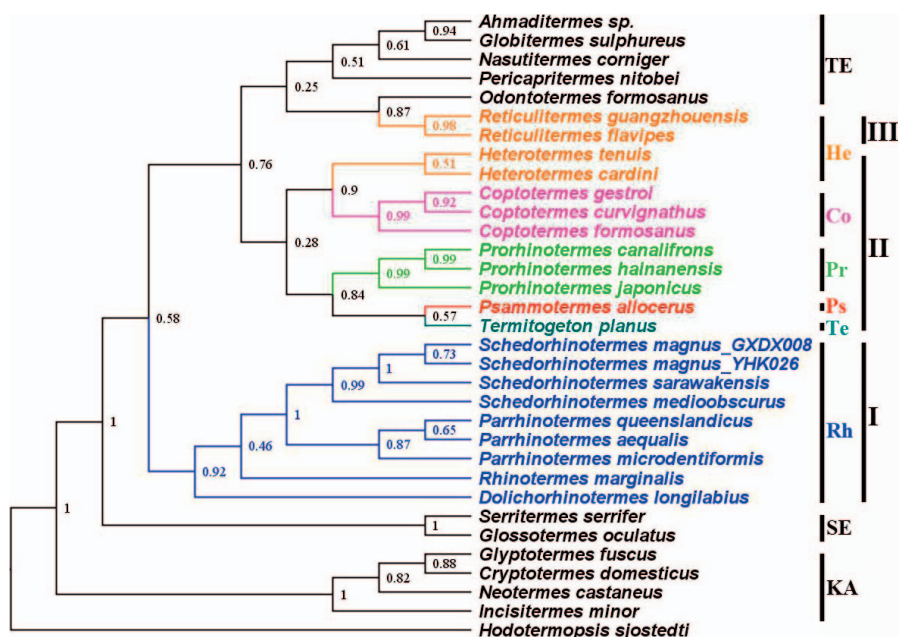


Fig. 6. The Bayesian inference (BI) tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on the mitochondrial 16S rRNA sequence. Numbers on the nodes indicate Bayesian posterior probabilities (PP).

previously described. It is found that not all the subfamilies are monophyletic groups. The monophyly of Rhinotermitinae is fully supported by the Bayesian posterior probabilities of 1.00 and bootstrap values of 100% in the tandem COII and 16S rRNA tree. Meanwhile, Rhinotermitinae is well supported by a series of synapomorphic characters including labral anterior margin with a fringe of hairs (character 14:1), left mandible with two distinct marginal teeth (character 19:1), and convex anterior margin of postclypeus in imagoes (character 33:1). Although the reduction in the number of marginal teeth of soldier mandibles is one of the synapomorphic characters that support Rhinotermitinae to be a monophyletic group, the presence of the teeth is still considered to be a primitive state in Rhinotermitidae. Therefore, Rhinotermitinae is supposed to be more primitive than the subfamilies with no teeth on mandibles, including Prorhinotermitinae, Termitogetoninae, Heterotermitinae, and Coptotermitinae. In our morphological BI tree, it was the most basal clade of Rhinotermitidae as many previous studies have shown (Bourguignon et al. 2015, Inward et al. 2007, Lo et al. 2004). Nevertheless, similar to the result from Ohkuma et al. (2004), the most basal clade was thought to be occupied by the monophyletic group composed of Termitogetoninae and Serritermitidae in our combined molecular tree. Prorhinotermitinae was not supported by synapomorphic characters in the present morphological phylogenetic analyses. It was found, however, to form a monophyletic group with a 1.00 posterior

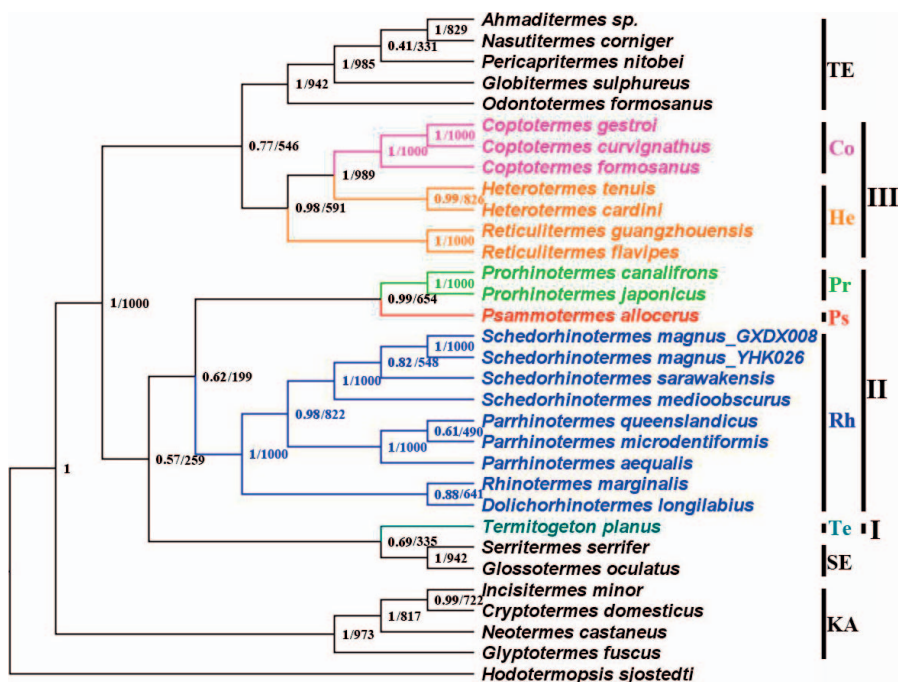


Fig. 7. The combined tree for Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae taxa based on the tandem COII and 16S rRNA sequences. Numbers on the left and right of the slash indicate, respectively, Bayesian posterior probabilities (PP) and bootstrap (BP) values for 1,000 replicates.

probability and 100% bootstrap support in the combined molecular tree. Prorhinotermitinae is the only group without true worker caste in Rhinotermitidae. The evolution of worker castes is known to have ecological relation to nesting strategy. As the most derived group in lower termites, members of most taxa in Rhinotermitidae construct intermediate nest or even separate-piece nest except for the members of Prorhinotermitinae. It appears that Prorhinotermitinae has undergone a reversal in nesting strategy to single-piece nest and, subsequently, lost its worker caste (Inward et al. 2007). As for the relationships between Prorhinotermitinae and other subfamilies, previous studies offered different opinions. It was found to be the sister to Psammotermitinae in Austin et al. (2004), Lo et al. (2004), and Inward et al. (2007). In Engel et al. (2009), the sister relationships between Prorhinotermitinae and Rhinotermitinae were supported. Moreover, other studies showed that Prorhinotermitinae was closely related to Termitogetoninae (Bourguignon et al. 2015, Legendre et al. 20080). Our present work indicated that Prorhinotermitinae was closely related to Psammotermitinae despite that their relationship was slightly different in the morphological tree and the combined molecular tree. In any case, Prorhinotermitinae is thought not to be evolutionarily lower than Rhinotermitinae.

Coptotermitinae is believed to be a definite monophyletic group. Its monophyly is supported by fontanelle with a medium to large open pore (character 7:3) and fontanelle extended above surface of head capsule on distinct tube (character 8:2) in the morphological phylogenetic analyses. This is also highly supported by the high Bayesian posterior probabilities and high bootstrap value in the tandem COII and 16S rRNA tree. Heterotermitinae is suspected to be nonmonophyletic by the morphological BI analyses, whose members, *Heterotermes* and *Reticulitermes*, were not clustered into a monophyletic group in spite that their imago eyes are uniformly small and not protruding beyond lateral margin of head (character 32:1). In both morphological BI tree and combined molecular tree, *Heterotermes* was grouped with *Coptotermes* first, *Reticulitermes* was the sister to them all. That is, *Heterotermes* and *Reticulitermes* are not closest relatives. The result agreed with that from most prior studies (Bourguignon et al. 2015, Cameron et al. 2012, Inward et al. 2007, Lo et al. 2004) except for the study of Engel et al. (2009). Although it may be problematic in the division of the subfamilies, the grouping of Coptotermitinae with Heterotermitinae is certain, which is supported not only by the presence of setulae surrounding fontanelle (character 9:1) and narrow, more or less triangular hyaline tip of labrum (character 13:2), but also by a posterior probability of 0.98 and a bootstrap value of 59.1% in the combined molecular tree. As the topmost clade in Rhinotermitidae, Heterotermitinae+Coptotermitinae appeared to be more closely related to Termitidae rather than to other subfamilies of Rhinotermitidae. In the morphological BI tree, Heterotermitinae+Coptotermitinae was assigned as the sister to Termitidae by the forewing vein M of imagoes being midway between Rs and CuA or closer to CuA (character 47:1). Their sister relationships were supported by a posterior probability of 0.77 and a bootstrap value of 54.6% in the combined molecular tree. Our nucleotide sequence analyses also showed that the genetic distances between Heterotermitinae or Coptotermitinae with Termitidae might be closer than those between Heterotermitinae or Coptotermitinae with other subfamilies in Rhinotermitidae. Thus, it is postulated that Heterotermitinae+Coptotermitinae should be the most evolved clade in Rhinotermitidae which is closer to higher termites.

In summary, our morphological and molecular phylogenetic analyses prove that Rhinotermitidae is a heterogenetic group which is composed of at least three separate phyletic clades. Although it remains controversial as to which clade is most primitive, Heterotermitinae+Coptotermitinae, as a definite clade, is believed to be most derived in Rhinotermitidae. It displayed a closer relationship with Termitidae rather than with other subfamilies of Rhinotermitidae. Based on the morphological phylogenetic analyses, it possesses synapomorphic characters that are not found in other clades of Rhinotermitidae. And, it shares a similar character with Termitidae. From the molecular phylogenetic analysis on the combined COII and 16S rRNA sequences, the grouping of Heterotermitinae+Coptotermitinae with Termitidae was supported by both BI method and ML method. Coptotermitinae and Heterotermitinae might be the real representative of the intermediate lineage from lower termites to higher termites. Based on these findings, we suggest that Heterotermitinae and Coptotermitinae should be separated from Rhinotermitidae and be upgraded entirely to the family rank.

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