Molecular Systematics of *Coptotermes* (Isoptera: Rhinotermitidae) From East Asia and Australia

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ABSTRACT Analyses of mitochondrial DNA sequences (12S, 16S, and COII) were conducted to determine the phylogenetic relationships among the following 11 putative subterranean termites of *Coptotermes*: *Coptotermes cochlearus* Xia & He, *Coptotermes curvignathus* Holmgren, *Coptotermes dimorphus* Xia & He, *Coptotermes formosanus* Shiraki, *Coptotermes gestroi* (Wasmann), *Coptotermes kalshoveni* Kemner, *Coptotermes sepangensis* Krishna, and *Coptotermes travians* (Haviland) from East Asia, and *Coptotermes acinaciformis* Froggatt, *Coptotermes frenchi* Hill, and *Coptotermes lacteus* (Froggatt) from Australia. Available sequences for these species and those of *Coptotermes guangzhouensis* Ping from GenBank also were included in the analyses. Maximum parsimony and maximum likelihood of the combined nucleotide matrices of the 12S, 16S, and COII genes resulted in two major clades with six subclades: I (*C. acinaciformis*), II (*C. lacteus* and *C. frenchi*), III (*C. curvignathus*), IV (*C. kalshoveni*, *C. sepangensis* and *C. travians*), V (*C. gestroi*) and VI (*C. formosanus*, *C. cochlearus*, *C. dimorphus* and *C. guangzhouensis*). *C. cochlearus* and *C. dimorphus* are possibly junior synonyms of *C. formosanus* with nucleotide differences of up to 1.0%.

KEY WORDS subterranean termites, *Coptotermes*, phylogenetics, Asia, mitochondrial DNA.

Coptotermes is a genus of the family Rhinotermitidae that is widely distributed in pantropical and subtropical regions. There is a growing concern about the economic impact of subterranean termites, especially those from genus *Coptotermes*, on urban structures, in forestry, and in agricultural crops in most subtropical and tropical countries of the world (Su and Scheffrahn 2000). These termites represent the major pest species in the Americas, Asia, and Australia (Lo et al. 2006, Takematsu et al. 2006). In Malaysia, *Coptotermes* spp. cause >90% of all infestations in buildings and structures (Lee 2002, 2007).

In addition, several invasive *Coptotermes* have been transported from their native range in the Orient to other parts of the world (Takematsu et al. 2006). These subterranean termites can extend themselves well beyond their normal habitation range. For example, *Coptotermes gestroi* (Wasmann) have established themselves as serious structural pests in Florida, West Indies, Mexico, Brazil, and Taiwan (Scheffrahn and Su 2000, Kirton and Brown 2003, Tsai and Chen 2003, Ferraz and Mendez-Montiel 2004, Yeap et al. 2007, Li et al. 2009).

The international termite taxonomy is in severe decline, especially due to the irregular taxonomic practice in China and Africa (Eggleton 1999). Description rates had risen enormously in China since the mid-1970s, but they decreased greatly in Africa over the same period. In China, between 1946 and 1996, 24 new species of *Coptotermes* were described based on morphological characteristics that overlapped greatly with sympatric and allopatric specimens in China (Crosland 1995, Eggleton 1999). Two *Coptotermes* spp. evaluated in this study, namely *Coptotermes cochlearus* Xia & He and *Coptotermes dimorphus* Xia & He were described by Xia and He (1986) in a controversial taxonomic compilation of Chinese termites. Li (2000) synonymized several *Coptotermes* spp., but his efforts have been limited as most of the earlier species were included without further scrutinization. The lack of robust morphological characters and the limited number of available specimens, especially the imago caste, have made identification of termite at the species level difficult and unreliable (Tho 1992).

On the contrary, outside China, several synonymities were described among the *Coptotermes* spp. Yeap et al. (2007) synonymized *Coptotermes vastator* Light with *C. gestroi.* Kirton and Brown (2003) reported taxonomic inconsistencies on the pest status of *Coptotermes havilandi* Holmgren in different regions within its geographic range and concluded that it is a junior synonym of *C. gestroi.* The authors also proposed *Coptotermes javanicus* Kemner to be a junior synonym to the latter species. Proper identification of species is imperative for generating accurate a good termite taxonomy that provides the baseline for all comparative biology. Moreover, accurate identifica-

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Table 1. Information about the termite specimens collected and used in this study

Sample code[a]	Species	Collecting sites	GenBank accession no.		
			12S	16S	COII
CG001TW	*C. gestroi*	Taiwan, Tainan 1	FJ235115	FJ376672	FJ384649
CG002TW	*C. gestroi*	Taiwan, Chang Jung Christian University	FJ235116	FJ376673	FJ384650
CG003TW	*C. gestroi*	Taiwan, Tainan 2	FJ235117	FJ376674	FJ384651
CF004JP	*C. formosanus*	Japan, Kagoshima, colony A	FJ235103	FJ376661	FJ384640
CF005JP	*C. formosanus*	Japan, Kagoshima, colony B	FJ235104	FJ376662	FJ384641
CF006JP	*C. formosanus*	Japan, Kagoshima, colony C	FJ235105	FJ376663	FJ384642
CF007JP	*C. formosanus*	Japan, Kagoshima, colony 1	FJ235106	FJ376664	FJ384643
CF008JP	*C. formosanus*	Japan, Kagoshima, colony 3	FJ235107	FJ376665	FJ384644
CF009JP	*C. formosanus*	Japan, Kagoshima, colony 5	FJ235108	FJ376666	FJ384645
CF010JP	*C. formosanus*	Japan, Kagoshima, colony 6	FJ235109	FJ376667	FJ384646
CF001TW	*C. formosanus*	Taiwan, Taichung 1	FJ235096	FJ376659	FJ384647
CF002TW	*C. formosanus*	Taiwan, Taichung 2	FJ235097	FJ376660	FJ384648
CF001CN	*C. formosanus*	China, Guangzhou, Guangdong Entomological Institute	FJ235098	FJ376668	FJ384636
CF002CN	*C. formosanus*	China, Guangzhou, Sun Yat-sen University, Pu Garden	FJ235099	FJ376669	FJ384637
CF003CN	*C. formosanus*	China, Guangzhou, AVON Co.	FJ235100	FJ376670	FJ384638
CF004CN	*C. formosanus*	China, Guangzhou, Guangdong Entomological Institute	FJ235101	FJ376671	FJ384639
CK001MY	*C. kalshoveni*	Malaysia, Penang, USM	FJ235118	FJ376684	N.A
CK002MY	*C. kalshoveni*	Malaysia, Penang, Pantai Keracut	FJ235119	FJ376685	FJ384652
CC001MY	*C. curvignathus*	Malaysia, Penang, USM	FJ235110	FJ376686	FJ384634
CC002MY	*C. curvignathus*	Malaysia, Penang	FJ235111	FJ376680	FJ384635
CC001SG	*C. curvignathus*	Singapore, Nim Road	FJ235112	FJ376681	N.A
CT001MY	*C. travians*	Malaysia, Perak, Teluk Intan	FJ915823	FJ915824	N.A
CS001MY	*C. sepangensis*	Malaysia, Perak, Bagan Datoh estate	FJ235120	FJ376679	N.A
CCO001CN	*C. cochlearus*	China	FJ235113	FJ376682	N.A
CD001CN	*C. dimorphus*	China	FJ235114	FJ376683	N.A
CFR001AU	*C. frenchi*	Australia, Canberra, ACT	FJ235094	FJ376677	FJ384632
CL001AU	*C. lacteus*	Australia, Canberra, ACT	FJ235095	FJ376678	FJ384633
CA001AU	*C. acinaciformis*	Australia, Darwin, NT	FJ235092	FJ376675	FJ384630
CA002AU	*C. acinaciformis*	Australia, Griffith, NSW	FJ235093	FJ376676	FJ384631

[a] Sample code XX001YY: XX is species; 001 is sample vial number, and YY is country of collection.

tion of species is needed for the effective management of these insects in urban settings and for establishing an environmentally sound management strategy (Copren et al. 2005, Takematsu et al. 2006).

Many recent studies on Rhinotermitidae have demonstrated the potential of using DNA sequence analysis in species identification, which would enable better understanding of phylogenies (Jenkins et al. 2000, 2007; Austin et al. 2004; Lo et al. 2004; Ohkuma et al. 2004; Takematsu et al. 2006). Mitochondrial genes have been widely used as reliable genetic markers for molecular phylogenetic studies of termites. However, the taxa analyzed so far include few *Coptotermes* spp.

In this study, we used mitochondrial DNA (mtDNA) sequences (12S, 16S, and COII) to revise the taxonomy and reveal the relationships among 11 putative *Coptotermes* spp. from 29 populations collected from East Asia and Australia. We also included *Coptotermes* sequences from GenBank into our phylogenetic analysis. By integrating molecular systematic data with morphometric analysis, we present here the phylogenetic relationship between various *Coptotermes* spp. from East Asia and Australia.

Materials and Methods

Sample Collection. Twenty-nine populations of the 11 putative *Coptotermes* spp. were studied. *Coptotermes* samples were collected and preserved in 100% ethanol (Table 1). Preserved soldier specimens were identified up to species level based on morphometric characters using an Olympus SZ2-LGB stereomicroscope connected to a computer-assisted imaging camera. The following eight morphological characteristics of the soldier termites were measured: maximum head width, head width at base of mandibles, length of head from base of mandibles, maximum width of gula, minimum width of gula, gula length, pronotum length, and pronotum width. These measurement data along with original data from Yeap et al. (2007) were subjected to analysis of variance (ANOVA), and means were separated by Tukey's honestly significant difference (HSD). The sequences for the outgroup species, *Globitermes sulfureus* Haviland and *Reticulitermes flaviceps* Oshima, were procured from GenBank from the work of Yeap et al. (2007) and Li et al. (2009), respectively. Voucher specimens were preserved in 100% ethanol and kept at the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Published sequences of *Coptotermes* spp. from GenBank (www.ncbi.nlm.nih.gov) also were included in the phylogenetic analysis (Table 2).

DNA Extraction. Total genomic DNA was extracted from a single worker termite from the 29 populations. The preserved specimen was washed with distilled water and laid flat to dry on a piece of filter paper. The intact termite was then frozen with liquid nitrogen and ground in a 1.5-ml tube. After grinding, 800 μl of sterile STE buffer (50 mM sucrose, 25 mM Tris-HCl, pH 7.0, and 10 mM EDTA) was added. DNA was extracted by incubation with proteinase K at 55°C for

Table 2. Published GenBank sequences used in this study

Species	Collection sites	GenBank accession no.			References
		12S	16S	COII	
C. acinaciformis	Australia: Darwin, Northern Territory	AY536380	AY302701		Unpublished
C. acinaciformis	Australia, Griffin, Canberra, New South Wales	AY536381	AY302702		Unpublished
C. acinaciformis		DQ441668			Inward et al. (2007)
C. acinaciformis	Australia: Darwin			DQ367826	Lo et al. (2006)
C. acinaciformis	Australia: Townsville			DQ367827	Lo et al. (2006)
C. acinaciformis	Australia: Sydney			DQ367828	Lo et al. (2006)
C. acinaciformis	Australia: Olney State Forest, NSW			DQ367829	Lo et al. (2006)
C. acinaciformis	Australia: Canberra			DQ367831	Lo et al. (2006)
C. acinaciformis	Australia: Walpeup			DQ367833	Lo et al. (2006)
C. frenchi	Australia: Canberra			DQ367836	Lo et al. (2006)
C. frenchi	Australia: Melbourne			DQ367837	Lo et al. (2006)
C. lacteus	Australia: Canberra, New South Wales	AY536390	AY302710		Unpublished
C. lacteus	Australia: Olney State Forest, NSW			DQ367840	Lo et al. (2006)
C. lacteus	BYU IGC IS41			EU253886	Lo et al. (2006)
C. curvignathus	Malaysia		AY683210	AJ854177	Unpublished
C. curvignathus	Malaysia			AJ854178	Unpublished
C. curvignathus	Indonesia		AY575853		Unpublished
C. formosanus	Japan, Wakayama	EF379978	EF379959	EF379941	Yeap et al. (2007)
C. formosanus	Japan, Wakayama	EF379979	EF379960	EF379942	Yeap et al. (2007)
C. formosanus	Japan, Okayama	EF379980	EF379961	EF379943	Yeap et al. (2007)
C. formosanus	USA, Hawaii, Oahu	EF379976	EF379958	EF379940	Yeap et al. (2007)
C. formosanus	USA: GA	AY536385	AY302706		Unpublished
C. formosanus	USA: HI	AY536386			Unpublished
C. formosanus	USA: New Orleans, LA	AY536387	AY302707		Unpublished
C. formosanus	USA: Florida, Golden Beach	AY168212	AY168225		Ye et al. (2004)
C. formosanus	China: Guangzhou		AY558911		Scheffrahn et al. (2004)
C. formosanus	China: Guangzhou			AB262474	Noda et al. (2007)
C. formosanus	Japan: Kagoshima, Amami			AB262501	Unpublished
C. formosanus	USA: Hawaii, Oahu			AY453588	Austin et al. (2004)
C. formosanus	China			FJ423459	Unpublished
C. formosanus	Japan			AB109529	Ohkuma et al. (2004)
C. formosanus	Japan			DQ493744	Yashiro and Matsuura (2007)
C. guangzhouensis	Japan		AB036673		Unpublished
C. gestroi	Malaysia, Penang, USM	EF379982	EF379963	EF379945	Yeap et al. (2007)
C. gestroi	Malaysia, Kuala Lumpur, Bangsar	EF379987	EF379969	EF379951	Yeap et al. (2007)
C. gestroi	Malaysia, Johor, Muar	EF379988	EF379970	EF379952	Yeap et al. (2007)
C. gestroi	Singapore, Serenity Terrace	EF379983	EF379964	EF379946	Yeap et al. (2007)
C. gestroi	Singapore, Serangoon Avenue 3	EF379985	EF379967	EF379949	Yeap et al. (2007)
C. gestroi	Thailand, Bangkok 1	EF379977	EF379965	EF379947	Yeap et al. (2007)
C. gestroi	Thailand, Bangkok 2	EF379986	EF379968	EF379950	Yeap et al. (2007)
C. gestroi	Indonesia, Cibinong	EF379981	EF379962	EF379944	Yeap et al. (2007)
C. gestroi	Indonesia, Bogor.	EF379984	EF379966	EF379948	Yeap et al. (2007)
C. gestroi	Singapore: Sommerville Wak		DQ004476		Jenkins et al. (2007)
C. gestroi	Singapore: Tampines		DQ004477	DQ923419	Jenkins et al. (2007)
C. gestroi	Singapore: Sime Ave.		DQ004478		Jenkins et al. (2007)
C. gestroi	Puerto Rico: Las Mareas		DQ004485	DQ923418	Jenkins et al. (2007)
C. gestroi	Australia, Queensland, Hamilton		DQ004487		Jenkins et al. (2007)
C. gestroi	Thailand, Bangkok, Royal Forest Department		DQ004488	EF092290	Jenkins et al. (2007)
C. gestroi	USA: Cleveland, Ohio		DQ004495	DQ923420	Jenkins et al. (2007)
C. gestroi	USA: Florida, Key West		EF156760	EF092291	Jenkins et al. (2007)
C. gestroi	USA: Miama, FL		AY558907		Scheffrahn et al. (2004)
C. gestroi	Turks and Caicos Islands: Grand Turk		AY558906		Scheffrahn et al. (2004)
C. gestroi	Antigua and Barbuda		AY558905		Scheffrahn et al. (2004)
C. vastator	USA, Hawaii, Oahu	EF379990	EF379971	EF379953	Yeap et al. (2007)
C. vastator	Philippines, Laguna Philippines, Los Banos, colony 1	EF379989	EF379972	EF379954	Yeap et al. (2007)
C. vastator	Philippines, Laguna Philippines, Los Banos, colony 2	EF379991	EF379973	EF379955	Yeap et al. (2007)
C. vastator	Philippines, Laguna Philippines, Los Banos, colony 3	EF379992	EF379974	EF379956	Yeap et al. (2007)
C. vastator	USA: Kalaeloa, Oahu Island, Honolulu, HI	AY536391	AY302711		Unpublished
C. vastator	Philippines: Manila	AY536392	AY302712		Unpublished
C. vastator	Philippines: Wedgewood, Laguna, Manila	AY536393	AY302713		Unpublished
C. vastator	Philippines: Manila	AY536394			Unpublished
C. sepangensis	Malaysia			AJ854163	Unpublished
C. sepangensis	Malaysia			AJ854165	Unpublished
C. travians	Malaysia			AJ854169	Unpublished
C. travians	Malaysia			AJ854170	Unpublished
R. flaviceps	Taiwan, Taitung County, Lanyu Township	EU667782	EU627778	EU627780	Li et al. (2008)
G. sulphurues	Malaysia, Penang, USM	EF379993	EF379975	EF379957	Yeap et al. (2007)

Table 3. Primers used for PCR and sequencing

Gene	Name	Sequence (5′–3′)
16S	LR-J-13007	TTA CGC TGT TAT CCC TAA
	LR-N-13398	CGC CTG TTT ATC AAA AAC AT
12S	12SF	TAC TAT GTT ACG ACT TAT
	12SR	AAA CTA GGA TTA GAT ACC C
COII	C2F2	ATA CCT CGA CGW TAT TCA GA
	TKN3785	GTT TAA GAG ACC AGT ACT TG

30 min, followed by the addition of 10% SDS and incubation for 3 h. After a single extraction using phenol/chloroform, total DNA was precipitated with ethanol and then resuspended in 25 μl of distilled water.

Polymerase Chain Reaction (PCR) Amplification and DNA Sequencing. PCR was conducted using the primers shown in Table 3 for amplification of 12S, 16S, and COII mtDNA genes (Kambhampati and Smith 1995, Miura et al. 1998). PCR amplification was performed in a standard 25- or 50-μl reaction volume with 2 μl of total genomic DNA, 1 pmol of each primer, 1.5 mM $MgCl_2$, 2 mM dNTPs, and 5 U/μl *Taq*DNA polymerase. Amplification was performed in a PTC-200 Peltier Thermol Cycle (MJ Research, Watertown, MA) with a profile consisting of precycle denaturation at 94°C for 2 min, a postcycle extension at 72°C for 10 min, and 35 cycles of a standard three-step PCR (51.3, 53.1, and 58.2°C annealing). Amplified DNA from individual termites was purified using a SpinClean gel extraction kit (Intron, Seongnam-Si, Gyeonggi-do, Korea). Samples were sent to Macrogen Inc. (Seoul, South Korea) for direct sequencing in both directions, which was conducted under BigDye terminator (Applied Biosystems, Foster City, CA) cycling conditions. The reacted products were then purified by using ethanol precipitation and ran for analysis using a DNA analyzer (Automatic Sequencer 3730xl, Applied Biosystems).

Phylogenetic Analysis. BioEdit version 7.0.5 software was used to edit individual electropherograms and form contigs (Hall 1999). Multiple consensus sequences were aligned using ClustalW (Thompson et al. 1994). Multiple alignment parameters for gap opening and extension penalties were 10 and 0.2, respectively. DNA sequences of other *Coptotermes* spp. obtained from GenBank were included in the alignments for phylogenetic comparisons.

The distance matrix option of PAUP* 4.0b10 (Swofford 2002) was used to calculate genetic distance according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Maximum parsimony (MP) analysis was performed with TBR branch swapping and 10 random taxon addition replicates under a heuristic search, saving no >100 equally parsimonious trees per replicate. To estimate branch support on the recovered topology, nonparametric bootstrap values were assessed with 1,000 bootstrap pseudo-replicates (Felsenstein 1985).

Before the maximum likelihood (ML) analysis, Modeltest 3.7 was used to find the optimal model of DNA substitution (Posada and Crandall 1998). According to Posada and Buckley (2004), the Akaike information criterion (AIC) (Akaike 1974) is more advantageous than the hierarchical likelihood ratio test. Therefore, our phylogenetic reconstruction for maximum likelihood was based on the best-fit model, which was selected by AIC. Heuristic ML searches using tree bisection-reconnection (TBR) branch swapping were performed in PAUP 4.0b10 (Swofford 2002). ML nodal support was estimated using the nonparametric bootstrap (Felsenstein 1985) and was restricted to 1,000 pseudo-replicates.

Results and Discussion

Morphology. The soldier heads in the genus *Coptotermes* are pear-shaped and rounded laterally. Milky exudate from the fontanelle was produced on the anterior part of the head when the soldier termite was disturbed. No teeth were apparent on the mandibles. Most standard morphological characters used for the identification of *Coptotermes* spp. have overlapping ranges; thus, identification can be based only on several key characters. Mandibles of all of the *Coptotermes* spp. in this study seemed to be parallel, except for those of *Coptotermes curvignathus* Holmgren, which strongly curved inwards. *C. curvignathus* was the largest species among all of the genera studied. *Coptotermes kalshoveni* Kemner is morphologically similar to *Coptotermes sepangensis* Krishna, but it is considerably smaller and the head capsule is narrow at the anterior end. *Coptotermes formosanus* Shiraki is readily distinguished from *C. gestroi;* the former has two pairs of setae that project dorsal laterally from the base of the fontanelle compared with only one pair of setae in the latter species. For the Australian *Coptotermes*, *Coptotermes frenchi* Hill is relatively small; the soldier's head is circular behind and has short mandibles. *Coptotermes lacteus* Froggatt is relatively larger and has a flat head and long mandibles. *Coptotermes acinaciformis* Froggatt is generally larger than all other Australian *Coptotermes* spp., and the soldier's head in this species is relatively long and has long mandibles.

Table 4 lists morphological measurements of the termite soldiers. It is clearly shown that the morphological characteristics of many *Coptotermes* spp. examined in this study were overlapped. Even within the same species, variations in the characters occur. For example, intraspecies comparisons of several populations of *C. formosanus* and *C. gestroi* revealed significant differences ($P < 0.05$) in morphological characteristics between the populations (Table 4). *C. gestroi* collected from the natural sites (secondary rain forests) were found to be larger than those collected from urban areas (data not shown). Dissimilarity in size between two populations of *C. curvignathus* from Malaysia also has been observed in this study (CC001MY and CC002MY). Light (1929) and Kirton and Brown (2003) both reported a continuous variation in size and shape within a single *Coptotermes* spp. In addition, termite morphology is influenced by the age and state of the colony and by habitat factors (Scheffrahn et al. 2005). Grace et al. (1995) noted that

Table 4. Measurement (in millimeters) of the soldier termite specimens used in this study and those of Yeap et al. (2007)

Sample code	Species	*n*	Max head width	Head width at base of mandibles	Length of head from base of mandibles	Gula, min. width	Gula, max width	Gula, length	Pronotum, length	Pronotum, width
CFR001AU	*C. frenchi*	7	0.930 ± 0.010a	0.449 ± 0.006b	1.037 ± 0.016ab	0.213 ± 0.005c-i	0.319 ± 0.002ab	0.511 ± 0.040a	0.293 ± 0.016a	0.674 ± 0.008 abc
CL001AU	*C. lacteus*	10	1.134 ± 0.009f-k	0.567 ± 0.007d-k	1.333 ± 0.014e-k	0.249 ± 0.006mp	0.383 ± 0.005f-l	0.689 ± 0.017bc	0.350 ± 0.007c-f	0.792 ± 0.011f-j
CA001AU	*C. acinaciformis*	11	1.302 ± 0.017opq	0.703 ± 0.012m	1.524 ± 0.023l-q	0.242 ± 0.004j-p	0.438 ± 0.006rs	0.986 ± 0.026lm	0.438 ± 0.010lm	0.837 ± 0.010j-n
CA002AU	*C. acinaciformis*	11	1.235 ± 0.013k-p	0.707 ± 0.010m	1.571 ± 0.019pq	0.235 ± 0.011h-p	0.466 ± 0.009s	0.89 ± 0.061d-l	0.453 ± 0.011m	0.909 ± 0.016o
CF001CN	*C. formosanus*	10	1.073 ± 0.007c-h	0.563 ± 0.006d-j	1.306 ± 0.020d-j	0.201 ± 0.003cde	0.356 ± 0.002c-f	0.839 ± 0.023d-k	0.374 ± 0.006e-i	0.739 ± 0.005d-g
CF002CN	*C. formosanus*	10	1.323 ± 0.018pq	0.633 ± 0.010l	1.583 ± 0.020q	0.226 ± 0.006e-o	0.421 ± 0.006n-r	0.921 ± 0.027e-m	0.428 ± 0.006j-m	0.910 ± 0.007o
CF003CN	*C. formosanus*	10	1.149 ± 0.009f-l	0.591 ± 0.009g-l	1.447 ± 0.014i-q	0.199 ± 0.004cde	0.383 ± 0.003f-l	0.911 ± 0.017e-m	0.389 ± 0.008e-l	0.772 ± 0.009d-i
CF004CN	*C. formosanus*	10	1.165 ± 0.021f-l	0.608 ± 0.010j-l	1.496 ± 0.028k-q	0.204 ± 0.006c-f	0.340 ± 0.006i-o	0.948 ± 0.024h-m	0.409 ± 0.010h-m	0.867 ± 0.014no
CF001HW	*C. formosanus*	10	1.204 ± 0.008i-o	0.600 ± 0.006i-l	1.468 ± 0.013j-q	0.238 ± 0.003h-p	0.416 ± 0.003m-r	0.950 ± 0.017h-m	0.405 ± 0.012g-m	0.809 ± 0.012h-n
CF001JP	*C. formosanus*	10	1.193 ± 0.053i-n	0.597 ± 0.005h-l	1.409 ± 0.022f-q	0.213 ± 0.003c-i	0.377 ± 0.003d-k	0.924 ± 0.014f-m	0.380 ± 0.005e-j	0.764 ± 0.005d-h
CF002JP	*C. formosanus*	10	1.144 ± 0.011f-l	0.585 ± 0.004f-l	1.399 ± 0.025f-o	0.211 ± 0.002c-h	0.379 ± 0.004e-l	0.904 ± 0.012d-m	0.371 ± 0.011e-h	0.769 ± 0.005d-i
CF003JP	*C. formosanus*	10	1.163 ± 0.005f-l	0.600 ± 0.004h-l	1.441 ± 0.023h-q	0.218 ± 0.002d-k	0.407 ± 0.002k=q	0.968 ± 0.018klm	0.407 ± 0.007g-m	0.811 ± 0.009h-n
CF004JP	*C. formosanus*	9	1.286 ± 0.025nop	0.590 ± 0.008g-l	1.335 ± 0.023e-k	0.250 ± 0.005n-p	0.395 ± 0.007i-o	0.882 ± 0.040d-l	0.422 ± 0.010i-m	0.864 ± 0.010mno
CF005JP	*C. formosanus*	10	1.159 ± 0.010f-l	0.588 ± 0.002g-l	1.439 ± 0.014h-q	0.213 ± 0.004c-j	0.403 ± 0.003j-q	0.904 ± 0.020d-m	0.404 ± 0.008g-m	0.800 ± 0.009g-k
CF006JP	*C. formosanus*	10	1.211 ± 0.009j-o	0.620 ± 0.008kl	1.530 ± 0.029m-q	0.244 ± 0.003k-p	0.423 ± 0.003o-r	0.963 ± 0.017j-m	0.434 ± 0.007k-m	0.863 ± 0.005l-o
CF007JP	*C. formosanus*	10	1.165 ± 0.007f-l	0.608 ± 0.007j-l	1.390 ± 0.022f-n	0.223 ± 0.003e-n	0.402 ± 0.005i-p	0.957 ± 0.020i-m	0.386 ± 0.008e-k	0.797 ± 0.010g-j
CF008JP	*C. formosanus*	10	1.247 ± 0.006l-p	0.632 ± 0.008l	1.567 ± 0.022opq	0.255 ± 0.004p	0.430 ± 0.005pqr	0.992 ± 0.024lm	0.453 ± 0.006m	0.904 ± 0.007o
CF009JP	*C. formosanus*	10	1.160 ± 0.004f-l	0.598 ± 0.005h-l	1.472 ± 0.012j-q	0.221 ± 0.005e-l	0.390 ± 0.003g-m	0.897 ± 0.017d-l	0.421 ± 0.006h-m	0.797 ± 0.007g-j
CF010JP	*C. formosanus*	10	1.171 ± 0.009g-l	0.594 ± 0.006g-l	1.419 ± 0.024g-q	0.221 ± 0.005e-m	0.403 ± 0.002j-q	0.955 ± 0.018h-m	0.434 ± 0.007k-m	0.809 ± 0.009h-n
CF001TW	*C. formosanus*	5	1.064 ± 0.020b-f	0.524 ± 0.009cde	1.292 ± 0.019d-i	0.188 ± 0.007bc	0.372 ± 0.009c-i	0.822 ± 0.012c-i	0.304 ± 0.012abc	0.732 ± 0.022c-f
CF002TW	*C. formosanus*	8	1.184 ± 0.015i-m	0.569 ± 0.009d-k	1.421 ± 0.031g-q	0.221 ± 0.006e-m	0.405 ± 0.005j-q	0.925 ± 0.017f-m	0.373 ± 0.006e-i	0.829 ± 0.011i-n
CG001IN	*C. gestroi*	10	1.149 ± 0.009h-m	0.543 ± 0.011d-h	1.357 ± 0.016e-l	0.241 ± 0.003i-p	0.402 ± 0.001i-p	0.951 ± 0.020h-m	0.405 ± 0.008g-m	0.804 ± 0.009h-m
CG002IN	*C. gestroi*	10	1.139 ± 0.007f-k	0.581 ± 0.005e-l	1.322 ± 0.017e-k	0.221 ± 0.007e-l	0.376 ± 0.007d-j	1.038 ± 0.025m	0.403 ± 0.005g-m	0.793 ± 0.008f-j
CG001MY	*C. gestroi*	10	1.146 ± 0.042f-l	0.580 ± 0.023e-l	1.268 ± 0.027d-h	0.227 ± 0.004e-o	0.391 ± 0.005h-n	0.862 ± 0.028d-l	0.390 ± 0.012f-l	0.782 ± 0.033e-j
CG004MY	*C. gestroi*	5	1.020 ± 0.014a-d	0.520 ± 0.008cd	1.244 ± 0.037def	0.212 ± 0.002c-h	0.348 ± 0.005bcd	0.792 ± 0.033c-f	0.314 ± 0.016a-d	0.664 ± 0.007ab
CG001TH	*C. gestroi*	10	1.066 ± 0.019b-d	0.517 ± 0.009cd	1.205 ± 0.014b-e	0.212 ± 0.004c-h	0.351 ± 0.003cde	0.766 ± 0.028cd	0.339 ± 0.007a-e	0.717 ± 0.011bcd
CG002TH	*C. gestroi*	11	1.112 ± 0.012f-k	0.530 ± 0.011c-f	1.215 ± 0.014cde	0.212 ± 0.006c-h	0.357 ± 0.006c-f	0.789 ± 0.014c-f	0.357 ± 0.006d-g	0.753 ± 0.011d-h
CG001SG	*C. gestroi*	1	1.093	0.604	1.340	0.208	0.388	0.866	0.350	0.745
CG002SG	*C. gestroi*	1	1.048	0.583	1.299	0.234	0.369	0.822	0.421	0.769
CG001TW	*C. gestroi*	10	1.134 ± 0.016f-k	0.562 ± 0.014d-j	1.259 ± 0.020d-g	0.254 ± 0.004o-p	0.408 ± 0.002l-r	0.920 ± 0.026e-m	0.404 ± 0.011g-m	0.802 ± 0.005h-l
CG002TW	*C. gestroi*	10	1.136 ± 0.009f-k	0.564 ± 0.012d-j	1.137 ± 0.114a-d	0.248 ± 0.006l-p	0.400 ± 0.004i-p	0.914 ± 0.022e-m	0.409 ± 0.007h-m	0.807 ± 0.007h-n
CG003TW	*C. gestroi*	10	1.125 ± 0.009e-j	0.557 ± 0.011d-j	1.277 ± 0.023d-i	0.233 ± 0.003g-p	0.391 ± 0.004h-n	0.894 ± 0.016d-l	0.379 ± 0.008e-j	0.779 ± 0.006e-j
CV001HW	*C. gestroi*	9	1.152 ± 0.012b-h	0.612 ± 0.011j-l	1.450 ± 0.008i-q	0.244 ± 0.004k-p	0.376 ± 0.002d-j	0.846 ± 0.016d-k	0.380 ± 0.006e-j	0.806 ± 0.007h-n
CV001PH	*C. gestroi*	10	1.068 ± 0.006b-h	0.539 ± 0.004c-g	1.263 ± 0.018d-g	0.221 ± 0.005e-m	0.360 ± 0.005c-g	0.792 ± 0.009c-f	0.317 ± 0.007a-d	0.720 ± 0.008b-e
CV002PH	*C. gestroi*	11	1.100 ± 0.010d-i	0.539 ± 0.005c-g	1.187 ± 0.044b-e	0.210 ± 0.004c-h	0.358 ± 0.002c-f	0.817 ± 0.009c-h	0.347 ± 0.009b-f	0.732 ± 0.007c-f
CV003PH	*C. gestroi*	10	1.152 ± 0.008f-l	0.546 ± 0.004d-i	1.332 ± 0.026e-k	0.214 ± 0.004c-j	0.390 ± 0.004g-m	0.805 ± 0.016c-g	0.345 ± 0.010b-f	0.755 ± 0.007d-h
CK001MY	*C. kalshoveni*	10	1.010 ± 0.012a-d	0.373 ± 0.006a	1.066 ± 0.008abc	0.205 ± 0.007c-g	0.305 ± 0.005a	0.621 ± 0.012ab	0.297 ± 0.008ab	0.638 ± 0.007a
CK002MY	*C. kalshoveni*	10	0.965 ± 0.005ab	0.369 ± 0.009a	0.994 ± 0.020a	0.212 ± 0.003c-h	0.303 ± 0.007t	0.610 ± 0.013ab	0.300 ± 0.014abc	0.646 ± 0.006a
CS001MY	*C. sepangensis*	5	1.024 ± 0.021a-e	0.486 ± 0.016bc	1.188 ± 0.029b-e	0.170 ± 0.006ab	0.343 ± 0.007bc	0.783 ± 0.018cde		
CT001MY	*C. travians*	3	0.99 ± 0.011abc	0.560 ± 0.009d-j	1.280 ± 0.015d-i	0.142 ± 0.002a	0.361 ± 0.006c-h	0.833 ± 0.052d-k		
CC001MY	*C. curvignathus*	10	1.444 ± 0.010r	0.690 ± 0.009m	1.554 ± 0.026n-q	0.230 ± 0.003f-p	0.423 ± 0.004o-r	0.934 ± 0.028g-m	0.516 ± 0.006n	0.999 ± 0.012p
CC002MY	*C. curvignathus*	10	1.275 ± 0.018m-p	0.593 ± 0.015g-l	1.406 ± 0.027f-p	0.190 ± 0.006bcd	0.377 ± 0.004d-k	0.824 ± 0.024c-j	0.408 ± 0.009h-m	0.850 ± 0.011k-o
CC001SG	*C. curvignathus*	3	1.403 ± 00.007qr	0.627 ± 0.012l	1.357 ± 0.012e-m	0.243 ± 0.003k-p	0.433 ± 0.007qr	0.900 ± 0.012d-m	0.507 ± 0.007n	1.017 ± 0.028p
CCO001CN	*C. cochlearus*	1	1.268	0.617	1.185	0.200	0.410	0.950	0.480	0.960
CD001CN	*C. dimorphus*	1	1.227	0.640	1.442	0.228	0.391	0.916	0.440	0.910

Means followed by different letters within the same column are significantly different ($P < 0.05$; Tukey's HSD).

worker size is correlated with colony population size in aging colonies. Husseneder et al. (2008) reported a negative correlation between worker size and the number of reproductives in extended family colonies of the Formosan subterranean termite. These observations explain the variation we observed in soldier head morphology in different populations of a given species. They also illustrate that morphological identification of *Coptotermes* samples at the species level is difficult and has limited reliability.

DNA Sequence Results. DNA sequencing of 12S, 16S, and COII mtDNA amplicons from *Coptotermes* spp. revealed an average size of 420, 428, and 680 bp, respectively. The level of sequence variation based on the Kimura two-parameter differed among the three genes. Sequence divergence ranged from 0.0% between *C. formosanus* and *C. dimorphus* to 6.5% between *C. sepangensis* and *C. curvignathus* in the 12S gene, from 0.73% between *C. dimorphus* and *C. cochlearus* to 7.6% between *C. formosanus* and *C. travians* in the 16S gene, and from 4.4% between *C. lacteus* and *C. frenchi* to 11.5% between *C. gestroi* and *C. kalshoveni* in the COII gene.

Phylogenetic Analysis. The mtDNA sequences of *Coptotermes* spp. obtained in this study together with sequences from GenBank were aligned by using *G. sulfureus* and *R. flaviceps* as the outgroup taxa. The aligned DNA matrices are available at TreeBASE (http://www.treebase.org, submission SN4594). Before the analysis, 31 characters, 29 characters, and 71 characters were removed from the alignments of 12S, 16S, and COII, respectively. The multiple sequence alignment for the 12S gene, including the outgroup taxa, has 389 characters, of which 299 are constant and 39 are parsimony-informative. For the 16S gene, there are 399 characters, of which 297 are constant and 61 are parsimony-informative. For the COII gene, there are 609 characters, of which 401 are constant and 144 are parsimony-informative.

Maximum parsimony analysis of 12S, 16S, and COII nucleotide matrices resulted in a total of 53, 100, 100 (initial MaxTrees setting = 100) equally most parsimonious trees, respectively (Fig. 1: length = 257, consistency index [CI] = 0.805, retention index [RI] = 0.878; Fig. 2: length = 219, CI = 0.932, RI = 0.939; Fig. 3: length = 372, CI = 0.616, RI = 0.838). The best model for the maximum likelihood analysis of the 12S gene was "GTR+I+G" with the following parameter settings: base = (0.4526, 0.2227, 0.1242, 0.2005), Nst = 6, Rmat = (553073.9375, 831360.3125, 286817.6250, 98618.3984, 1777170.8750), rates = gamma shape = 0.4675, and pinvar = 0.3652. For the 16S gene, the selected model was "HKY+I+G" with the following parameter settings: base = (0.4426, 0.2410, 0.0834, 0.2330), Nst = 2, TRatio = 3.2315, rates = gamma shape = 0.2658, and pinvar = 0.4684. For the COII gene, the "GTR+I+G" model was selected with the following parameter settings: base = (0.3826, 0.2676, 0.1382, 0.2115), Nst = 6, Rmat = (2.6028, 14.4212, 2.1720, 0.5625, 45.7613), rates = gamma shape = 1.1930, and pinvar = 0.5309. A single tree was recovered for each of the genes (−ln L 1369.6742 for 12S, −ln L 1421.4773 for 16S, and −ln L 2792.4729 for COII; trees not shown). The most parsimonious trees with topology similar to that generated by the ML analysis were chose (Figs. 1–3).

A comparison of the three bootstrap trees revealed no cases in which a grouping with >50% bootstrap support in one tree conflicted with a grouping with >50% bootstrap support in another tree. Based on the overall low level of conflict between the three genes, the data were combined, and the most parsimonious tree was constructed. Two major clades within the *Coptotermes* genus with six subclades were apparent: I (*C. acinaciformis*), II (*C. lacteus* and *C. frenchi*), III (*C. curvignathus*), IV (*C. kalshoveni*, *C. sepangensis* and *C. travians*), V (*C. gestroi*) and VI (*C. formosanus*) (Fig. 4). Poor phylogenetic resolution (as indicated by a low bootstrap value) was found among most of these six subclades. However, the monophyletic *Coptotermes* clade demonstrated that genetic partitioning is strong for the studied species. The continuous overlapping nature of the data makes it difficult to code the morphological data in the molecular matrix (Table 4). Therefore, only a few distinct morphological characters were mapped on the combined tree (Fig. 4).

Australian *Coptotermes*. When other sequences of *C. acinaciformis* from GenBank were added into the analysis of the 12S, 16S, and COII genes, *C. acinaciformis* from Darwin (CA001AU) and *C. acinaciformis* from Griffith (CA002AU) branched out as a sister group with high bootstrap values in the 16S and COII trees (Figs. 2 and 3). The genetic diversity between these two populations of *C. acinaciformis* was low for the 12S gene (0.5%). However, for the 16S and COII genes, the genetic diversity between CA001AU and CA002AU was notably high (3.0% and 3.5%, respectively), despite the morphological similarity of the two populations. Samples from Darwin were more closely related to the samples from Townsville (Fig. 3). Samples from New South Wales, Griffith, Sydney, and Canberra were clustered into the same group, with the New South Wales and Griffith samples being closely related to each another. Previous cuticular hydrocarbon and molecular studies suggested that *C. acinaciformis* was genetically diverse and may even consist of several species (Brown et al. 2004, Lo et al. 2006). Besides nucleotide differences, termites of this species also are biologically distinct. In the north (Darwin), they are mound builders, whereas in southern Australia (e.g., Griffith) they generally nest inside trees, stumps, poles, or filled-in verandahs where timber has been buried. These habitat differences might explain why *C. acinaciformis* from Darwin and Griffith do not share the same lineage, as shown by differences in their 12S, 16S, and COII genes. Relative to the other species, *C. acinaciformis* exhibited a close relationship with the other two Australian *Coptotermes* (*C. frenchi* and *C. lacteus*). *C. lacteus* formed a sister group with *C. frenchi*, with genetic diversity of 1.1, 1.3, and 4.4% for the 12S, 16S, and COII genes, respectively.

Southeast Asian *Coptotermes*. *C. curvignathus* is widely distributed in Southeast Asia and has caused damage to wooden construction and tree plantations

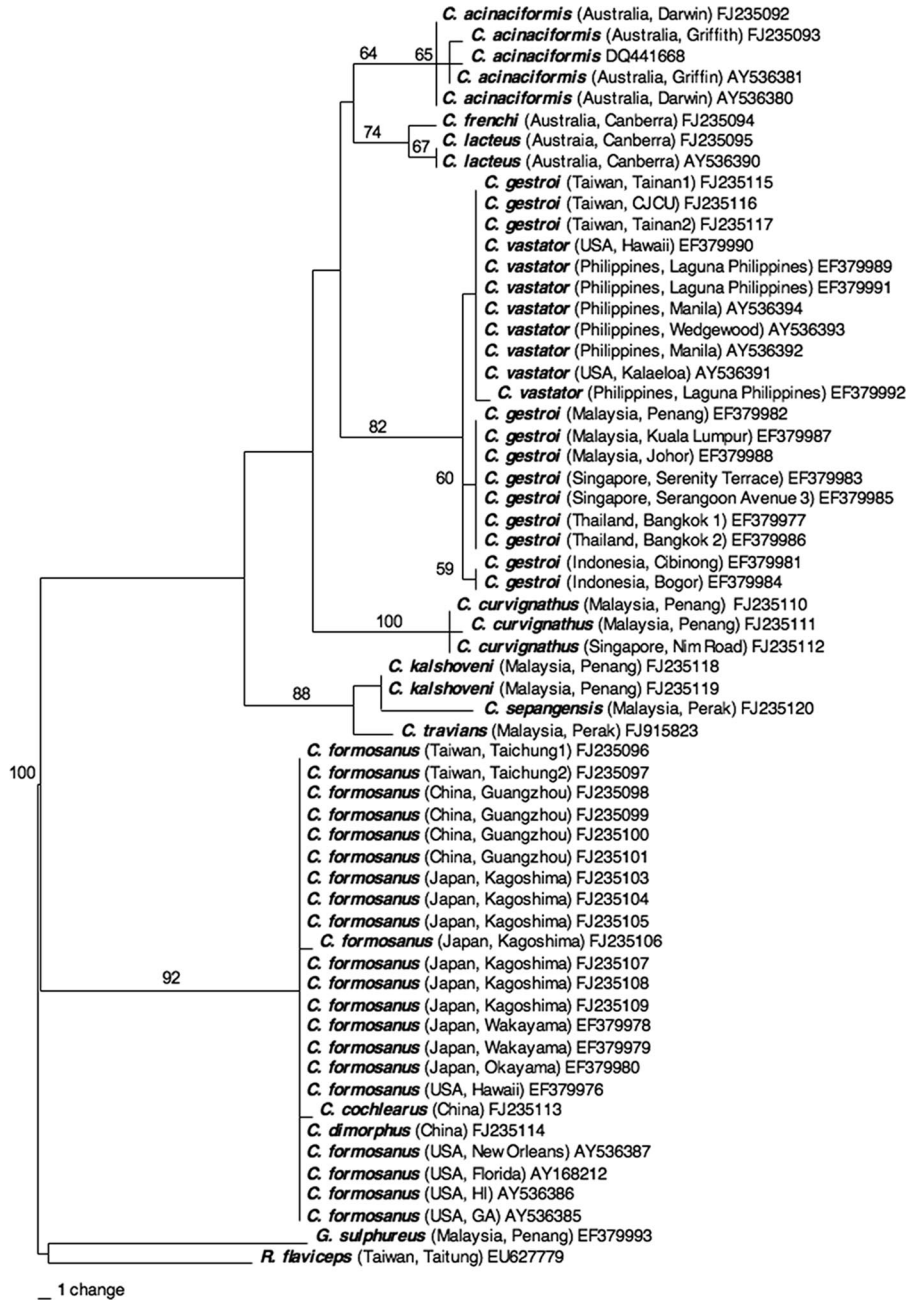

Fig. 1. The most parsimonious tree obtained for the 12S gene using a heuristic search option in PAUP4.0b10 (Swofford 2002). Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%.

(Roonwal 1970, Lee 2002, Takematsu et al. 2006). *C. curvignathus* from Malaysia and Singapore share identical sequences across the 12S, 16S, and COII genes. The soldier measurements of one *C. curvignathus* sample (CC002MY) collected in Penang, Malaysia, were considerably smaller compared with those from other *C. curvignathus* samples. However after phylogenetic analysis, CC002MY clustered together with the other *C. curvignathus* (Clade III) (Fig. 4). This finding clearly indicates that size variation affects the reliability of morphologically based taxonomy (Takematsu et al. 2006).

The phylogenetic trees constructed based on the 12S, 16S, COII, and the combination of the three genes showed that *C. kalshoveni*, *C. sepangensis*, and *C. travians* are closely related species. These three species clustered under the same clade in all of the trees, although *C. kalshoveni* had a closer relationship with

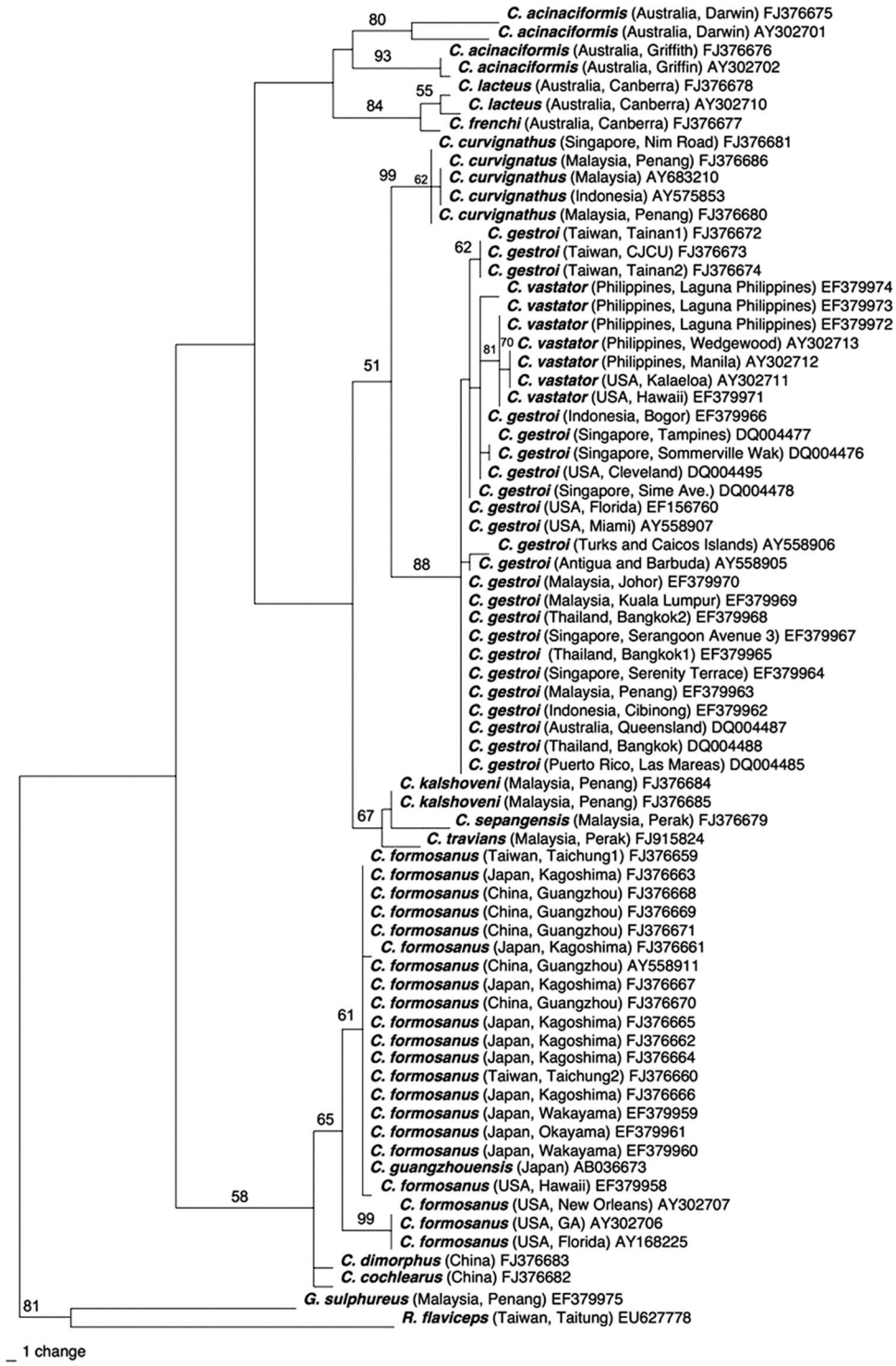

Fig. 2. The most parsimonious tree obtained for the 16S gene using a heuristic search option in PAUP4.0b10 (Swofford 2002). Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%.

C. sepangensis than with *C. travians*. *C. sepangensis* closely resembles *C. kalshoveni* based on the soldier caste (Kemner 1934); morphologically, they can only be separated by the shape and the curvature of the mandibles (Tho 1992). Strong quantitative support by bootstrap analysis also indicated that *C. kalshoveni* and

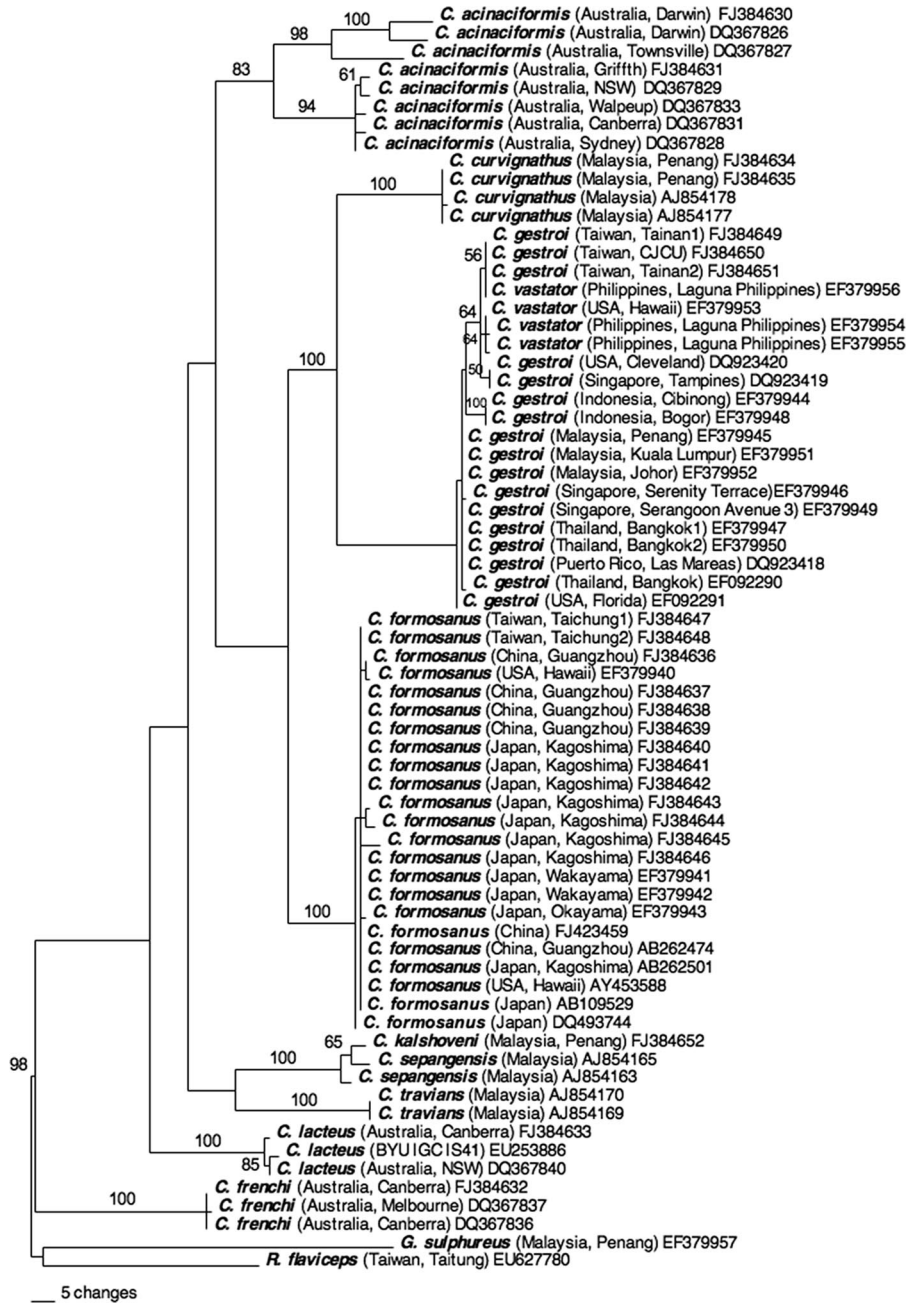

Fig. 3. The most parsimonious tree obtained for the COII gene using a heuristic search option in PAUP4.0b10 (Swofford 2002). Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%.

C. sepangensis are closely related. This result is supported by high degrees of similarity in the 12S (97%), 16S (99%), and COII (99%) genes. Further assessment of the morphological features of alate forms and type specimens would be required to resolve the relationship between these two species. The phylogenetic trees inferred from the three mitochondrial genes demonstrated that Australian *Coptotermes* spp. have a closer relationship with *C. curvignathus, C. kalshoveni, C. sepangensis,* and *C. travians* than with *C. gestroi.* However, this relationship was not supported by high bootstrap values.

C. gestroi. *C. gestroi* is believed to be native to Southeast Asia but has spread to the Marquesas Islands (Pacific Ocean), Mauritius and Reunion (Indian Ocean), the New World tropics (Brazil and Barbados), some islands of the West Indies, southern Mexico, the Southeastern United States (Scheffrahn and

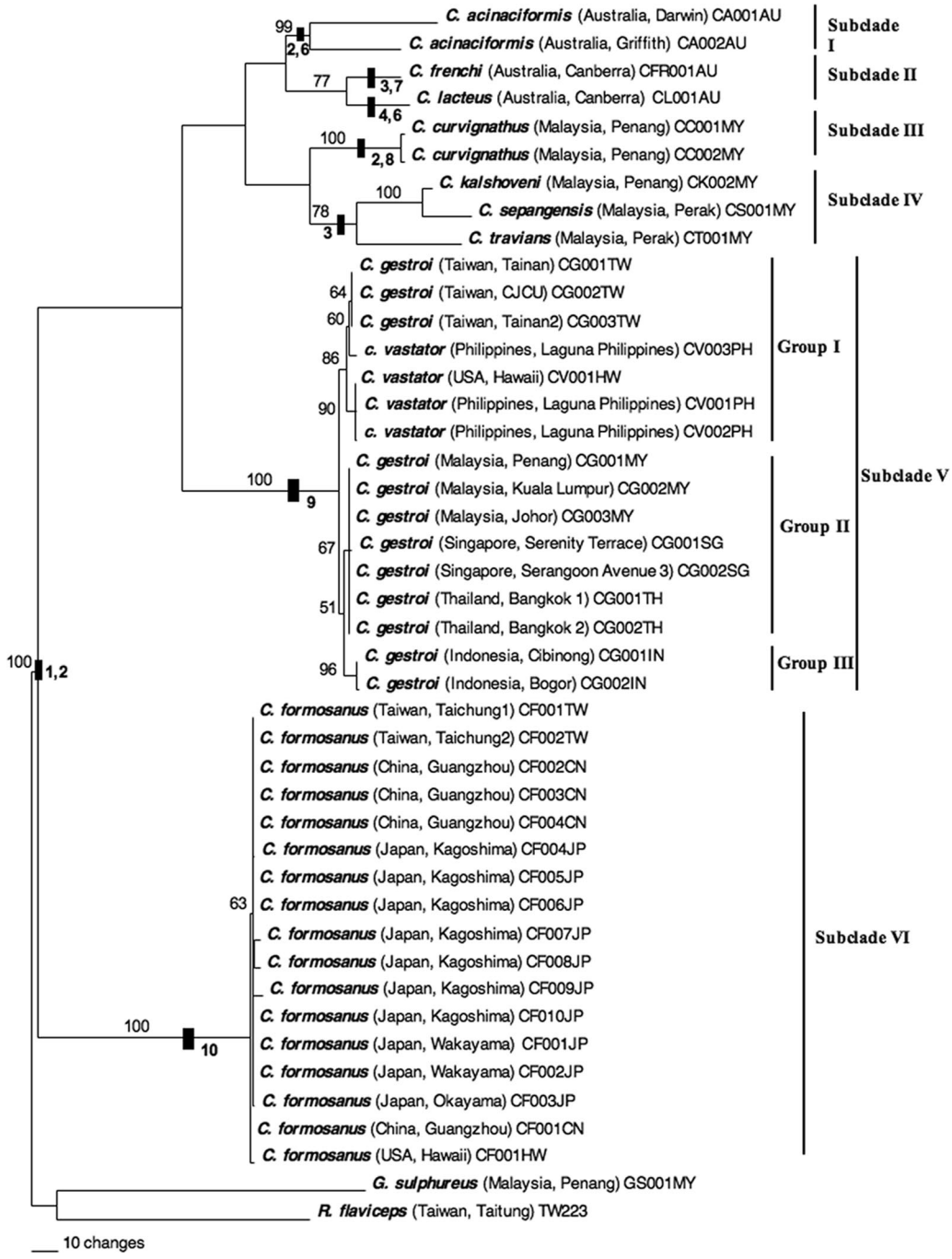

Fig. 4. The most parsimonious tree obtained for combined genes using a heuristic search option in PAUP4.0b10 (Swofford 2002) with morphological characters (bold number below branches). Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%. 1, pear-shaped head; 2, large size head; 3, small size head; 4, flat head; 5, number of teeth on mandibles; 6, long mandibles; 7, short mandibles; 8, mandibles strongly curve inward; 9, one pair of setae at fontanelle; and 10, two pairs of setae at fontanelle.

Su 2000, Ferraz and Mendez-Montiel 2004), and more recently to Taiwan (Tsai and Chen 2003). The phylogenetic tree generated from the three mitochondrial genes revealed that the populations of *C. gestroi* could be divided into three geographical groups: group I: Taiwan, the Philippines, and Hawaii populations (node support of 86%); group II: Thailand, Malaysia, and Singapore populations (node support of 67%); and

group III: Indonesia populations (node support of 96%) (Fig. 4). These results agree with the report of Li et al. (2009).

In this study, we found that the *C. gestroi* samples from southern Taiwan were more closely related to those from the Philippines and Hawaii than to those from other geographic locations. Luzon Strait is the passage between northern Luzon, the Philippines, and southern Taiwan. It is an important strait connecting the China Sea on the west with the Philippine Sea on the east, and it extends 200 miles (320 km) between the islands of Taiwan (north) and Luzon, Philippines (south) and is used for shipping and communication. Many ships from the Americas use this route to reach important East Asian ports. It is possible that *C. gestroi* was introduced to Taiwan via this maritime route.

Thailand, Malaysia, and Singapore share a well-developed transportation infrastructure, including some world-renowned port systems, railway network, and modern highway system that accommodate intra- and intercountry travel and commerce (Jenkins et al. 2007). Furthermore, a partnership between railway systems in Thailand and Malaysia allows minimum inspection of sealed railway containers. The numerous modes of transportation, many characterized by minimal inspection of cargo, may have enabled the easy spread of *C. gestroi* across the three countries. This may explain the close relationship between the *C. gestroi* samples collected from Thailand, Malaysia, and Singapore.

The two populations of *C. gestroi* from Indonesia (Cibinong and Bogor) branched out as a distinct group. This will certainly require more *C. gestroi* samples from Sumatra, Borneo, and Sulawesi to further substantiate current findings.

The high adaptability of *C. gestroi* allowed it to be transported and fortuitously introduced into many countries. *C. vastator* (=*C. gestroi;* see Yeap et al. 2007) was recently found in Minami Torishima (Marcus Island), Japan (Morimoto and Ishii 2000). This was the first record of *C. gestroi* in Japan. The island is located at 24° 18′ N 153° 58′ E, which shares a similar latitude with Miami, FL in the United States (25° 79′ N 80° 22′ W) where activities of *C. gestroi* have been recorded. According to Bess (1970), in 1963 a number of *C. vastator* (=*C. gestroi*) imagos and soldiers were found in several buildings in Honolulu, HI. This was the period during which Minami Torishima (Marcus Island) was occupied by the U.S. army (i.e., between 1952 and 1968). Thus, material transportation conducted by the U.S. Army might be related to the expansion of *Coptotermes* to various places within the Pacific Ocean (Morimoto and Ishii 2000).

Chinese *Coptotermes*. *C. formosanus* is believed to have originated from southern China and Taiwan because of the higher level of genetic variability (Li et al. 2009). It is highly adapted to the urban environment and has been dispersed by the railway and by ships (Jenkins et al. 2002, Scheffrahn and Su 2005, Austin et al. 2008) through 10 southeastern states in the United States over the past 50 yr (Su 2003). It accounts for a considerable amount of the total termite damages (Su 2002). In our study, among *C. formosanus* populations, samples form Taiwan, Japan, and China grouped together (node support of 63%) and separated out the Hawaii samples and one sample from China (CF001CN) (Fig. 4). This result agrees with data in Li et al. (2009). However, more samples of *C. formosanus* from Hawaii and Florida are required to generate a clearer picture of their population structures.

Table 5. Nucleotide and haplotype variations for 12S gene among *C. formosanus*, *C. dimorphus*, and *C. cochlearus*

Species	Haplotype	28	124	348
CF001JP	F1	A	–	A
CF002JP	F1	*	–	*
CF003JP	F1	*	–	*
CF004JP	F1	*	–	*
CF005JP	F1	*	–	*
CF006JP	F1	*	–	*
CF007JP	F2	*	–	G
CF008JP	F1	*	–	*
CF009JP	F1	*	–	*
CF010JP	F1	*	–	*
CF001CN	F1	*	–	*
CF002CN	F1	*	–	*
CF003CN	F1	*	–	*
CF004CN	F1	*	–	*
CF001TW	F1	*	–	*
CF002TW	F1	*	–	*
CF001HW	F1	*	–	*
CD001CN		*	A	*
CCO001CN		G	–	*

Note: – refers to a gap, * refers to the same nucleotide as the nucleotide on the first row.

In this study we only obtained sequences of the 12S and 16S genes for *C. dimorphus* and *C. cochlearus.* Therefore, these two species could not be included in the overall analysis of the combined genes. *C. dimorphus* and *C. cochlearus* fell within the subclade of *C. formosanus* in the 12S tree (Fig. 1); in fact, they showed 100% similar to *C. formosanus* for the 12S gene. They branched out as a sister group to *C. formosanus* in the 16S tree (Fig. 2). As the 16S gene is a faster evolving gene than the 12S gene (Ye et al. 2004), this may explain why greater genetic diversity found between these species when the 16S gene was used.

From the phylogenetic analysis, only two haplotypes were found in the 12S gene among 17 populations of *C. formosanus* from China, Taiwan, Japan, and Hawaii (Table 5). These two haplotypes differ only by a single nucleotide. Both *C. dimorphus* and *C. cochlearus* differed from *C. formosanus* by only a single nucleotide in the 12S sequence. For the 16S gene, four haplotypes were found among the 17 populations of *C. formosanus* (Table 6). These four haplotypes exhibited only one or two nucleotide differences among them. In total, *C. dimorphus* and *C. cochlearus* have eight and ten nucleotides that differ from those of *C. formosanus*, respectively. Thus, the molecular data strongly suggests that both *C. dimorphus* and *C. cochlearus* are junior synonyms of *C. formosanus.* However, because of the limited sample size was used in this study, it is important to have more samples examined, including their morphological characters, before the current findings can be confirmed. In addi-

Table 6. Nucleotide and haplotype variations for 16S gene among *C. formosanus*, *C. dimorphus*, and *C. cochlearus*

Species	Haploptye	60	82	96	107	131	144	145	163	169	185	212	240	277	374
CF001JP	Fa	A	T	T	C	T	–	–	A	C	T	C	A	T	–
CF002JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF003JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF004JP	Fb	*	*	C	*	*	–	–	*	*	*	*	*	*	–
CF005JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF006JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF007JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF008JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF009JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF010JP	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF001CN	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF002CN	Fc	*	*	*	*	*	T	A	*	*	*	*	*	*	–
CF003CN	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF004CN	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF001HW	Fd	*	*	*	*	*	–	–	*	*	*	*	*	*	T
CF001TW	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CF002TW	Fa	*	*	*	*	*	–	–	*	*	*	*	*	*	–
CD001CN		C	*	*	*	*	–	–	C	T	C	T	G	C	T
CCO001CN		C	C	*	T	C	–	–	C	G	C	*	G	C	T

Note: – refers to a gap, * refers to the same nucleotide as the nucleotide on the first row.

tion, *C. guangzhouensis* sequence from the GenBank also was grouped within the *C. formosanus* subclade (Fig. 2). A thorough molecular phylogenetic study of *Coptotermes* spp. in China is urgently warranted.

Accurate identification and information about phylogenetic diversity of *Coptotermes* are important for the improvement of termite management strategies. With the finding of synonymous species, information concerning those species from different geographical regions can now be pooled. Regulatory authorities may now be able to accept efficacy assessments of termite management strategies from any given region if the targeted species is the same, as compared with the past when they were thought to be different species. This will benefit all parties and will in the long run save time and resources (Kirton 2005).

In summary, the following six subclades with poor relationships among the *Coptotermes* spp. from East Asia and Australia were revealed based on phylogenetic analyses of three mitochondrial genes: (I) *C. acinaciformis;* (II) *C. frenchi* and *C. lacteus;* (III) *C. curvignathus;* (IV) *C. kalshoveni*, *C. sepangensis*, and *C. travians;* (V) *C. gestroi;* and (VI) *C. formosanus.* Based on the genetic evidence, it is likely that *C. dimorphus* and *C. cochlearus* are possibly junior synonyms of *C. formosanus;* however, more samples would be required to further substantiate this observation. Morphological characters are often ambiguous and the eight measurements on the soldier termites used in this study overlapped for the 11 *Coptotermes* spp. examined. These results suggest that the use of both molecular and morphological approaches is crucial in ensuring accurate species identification for this genus.

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