

Genetic Relationship Between *Coptotermes gestroi* and *Coptotermes vastator* (Isoptera: Rhinotermitidae)

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ABSTRACT The phylogenetic relationship of *Coptotermes gestroi* (Wasmann) and *Coptotermes vastator* Light (Isoptera: Rhinotermitidae) was determined using DNA sequence comparisons of mitochondrial genes. Partial sequences of the ribosomal RNA small subunit 12S, ribosomal RNA large subunit 16S, and mitochondrial COII were obtained from nine populations of *C. gestroi* from South East Asia (Malaysia, Singapore, Thailand, and Indonesia) and four populations of *C. vastator* from the Philippines and Hawaii. In addition, four populations of *Coptotermes formosanus* Shiraki and *Globitermes sulphureus* (Haviland) were used as the outgroups. Consensus sequences were obtained and aligned. *C. vastator* and *C. gestroi* are synonymous, based on high sequence homology across the 12S, 16S, and COII genes. The interspecific pairwise sequence divergence, based on Kimura 2-parameter model between *C. gestroi* and *C. vastator*, varied only up to 0.80%. Morphometric measurements of 16 characteristics revealed numerous overlaps between the examined individuals of both species. Based on the molecular phylogenetics and morphometric data, it is proposed that *C. vastator* is a junior synonym of *C. gestroi*.

KEY WORDS *Coptotermes gestroi*, *Coptotermes vastator*, ribosomal DNA, COII mitochondrial DNA, synonymy

Among the termite genera within Rhinotermitidae, *Coptotermes* is probably regarded as the most economically important genus worldwide (Lo et al. 2006). Several species of *Coptotermes*, including the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and *Coptotermes gestroi* (Wasmann) have been known for their destructive nature to buildings and structures in subtropical and tropical regions, respectively. Su (2002) reported that *C. formosanus* accounted for a considerable proportion of the total damage by termites worldwide.

In Malaysia, Thailand, and Singapore, *C. gestroi* contributes >85% of the total termite damage in buildings and structures in the urban area (Lee 2002, Lee et al. 2003). Geographic distribution of *C. gestroi* occurs from Assam through Burma and Thailand to Malaysia and the Indonesian archipelago (Kirton and Brown 2003). It is marked as the most destructive pest termite, damaging structural wood in the urban areas in Southeast Asia (Kirton 2005). More recently, *C. gestroi*, reported as *C. havilandi* in Gay (1969), has been brought into new geographical regions including the Turks and Caicos Islands in the Caribbean (Scheffrahn et al. 1990), and Florida in North America (Su et al. 1997) by “hitch-hiking” in the cargo onboard

ships and wooden components of sailing vessels (Kirton and Brown 2003).

Despite *C. gestroi* being widely distributed in the tropical South East Asia, *Coptotermes vastator* Light is the primary subterranean termite species in the urban environment in the Philippines (Yudin 2002), and it accounts for >90% of the termite damage to timber and wooden structures in metro Manila and other urban areas (Acda 2004). Damage costs nearly US\$1 million to residential and commercial properties of the Mariana Islands and between US\$8 and 10 million for the damage in and around Manila (Yudin 2002). This species was unintentionally introduced to Hawaii in 1918 during a shipment of banana stumps from Manila (Ehrhorn 1934, Gay 1969). *C. vastator* was found infesting a single structure in Honolulu at 1963, and it was not discovered again in Hawaii until 1999 (Woodrow et al. 2001). To date, *C. vastator* is recognized and known to cause major problems in the islands of Hawaii, Guam, and Saipan (Su and Scheffrahn 1998, Woodrow et al. 2001, Yudin 2002).

Both *C. gestroi* and *C. vastator* are very similar based on analysis of morphological characteristics, and it was suspected that *C. vastator* is a junior synonym of *C. gestroi* (Kirton 2005). However, no attempt has been executed so far to address this issue from a molecular phylogenetic perspective. Molecular phylogenetic analyses are able to reveal the relationship among populations and to differentiate species regardless of the termite caste (Szalanski et al. 2003). Mitochondrial

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Table 1. Termite specimens and published GenBank sequences used in this study

Sample code	Species	Collecting site	GenBank accession no.		
			12S	16S	COII
Samples from this study					
CG001MY	<i>C. gestroi</i>	Malaysia, Penang, USM	EF379982	EF379963	EF379945
CG004MY	<i>C. gestroi</i>	Malaysia, Kuala Lumpur, Bangsar	EF379987	EF379969	EF379951
CG005MY ^a	<i>C. gestroi</i>	Malaysia, Muar	EF379988	EF379970	EF379952
CG001SG	<i>C. gestroi</i>	Singapore, Serenity Terr.	EF379983	EF379964	EF379946
CG002SG	<i>C. gestroi</i>	Singapore, Serangoon	EF379985	EF379967	EF379949
CG001TH	<i>C. gestroi</i>	Thailand, Bangkok1	EF379977	EF379965	EF379947
CG002TH	<i>C. gestroi</i>	Thailand, Bangkok2	EF379986	EF379968	EF379950
CG001IN	<i>C. gestroi</i>	Cibinong, Indonesia	EF379981	EF379962	EF379944
CG002IN	<i>C. gestroi</i>	Bogor, Indonesia	EF379984	EF379966	EF379948
CF001JP	<i>C. formosanus</i>	Japan, Wakayama	EF379978	EF379959	EF379941
CF002JP	<i>C. formosanus</i>	Japan, Wakayama	EF379979	EF379960	EF379942
CF003JP	<i>C. formosanus</i>	Japan, Okayama	EF379980	EF379961	EF379943
CF001HW	<i>C. formosanus</i>	USA, Hawaii, Oahu	EF379976	EF379958	EF379940
CV001HW	<i>C. vastator</i>	USA, Hawaii, Oahu	EF379990	EF379971	EF379953
CV001PH	<i>C. vastator</i>	Los Banos, Laguna Philippines, colony1	EF379989	EF379972	EF379954
CV002PH	<i>C. vastator</i>	Los Banos, Laguna Philippines, colony2	EF379991	EF379973	EF379955
CV003PH	<i>C. vastator</i>	Los Banos, Laguna Philippines, colony3	EF379992	EF379974	EF379956
GS001MY	<i>G. sulphureus</i>	Malaysia, Penang, USM	EF379993	EF379975	EF379957
Other studies					
	<i>C. gestroi</i>	Malaysia, Penang Island	AY536388		
	<i>C. gestroi</i>	Thailand, Bangkok		AY302709	
	<i>C. gestroi</i>	USA, Miami, FL		AY558907	
	<i>C. gestroi</i>	Turks and Caicos Islands: Grand Turk		AY558906	
	<i>C. gestroi</i>	Antigua and Barbuda		AY558905	
	<i>C. vastator</i>	Philippines, Manila	AY536394		
	<i>C. vastator</i>	Philippines, Wedgewood	AY536393		
	<i>C. vastator</i>	Philippines, Manila	AY536392		
	<i>C. vastator</i>	Philippines, Wedgewood		AY302713	
	<i>C. vastator</i>	Philippines, Manila		AY302712	
	<i>C. vastator</i>	USA, Honolulu, HI		AY302711	
	<i>C. acinaciformis</i>	Australia, Griffin.	AY536381		
	<i>C. acinaciformis</i>	Australia, Darwin, Northern Territory	AY536380		
	<i>C. acinaciformis</i>	Australia		AY558913	
	<i>C. acinaciformis</i>	Australia			AF262610
	<i>C. lacteus</i>	Australia, Canberra	AY536390		
	<i>C. lacteus</i>	Australia, Beerburum		AY558912	
	<i>C. lacteus</i>	Australia			AF220600
	<i>C. michaelsoni</i>	Australia		AY558914	
	<i>C. curvinagthus</i>	Malaysia		AY558909	
	<i>C. heimi</i>	India		AY558908	
	<i>C. intermedius</i>	Africa, Togo		AY558904	
	<i>C. sjostedti</i>	Africa, Guinea		AY558903	
	<i>C. crassus</i>	Belize		AY558901	
	<i>C. testaceus</i>	Trinidad and Tobago		AY558900	
	<i>C. testaceus</i>	Grenada		AY558899	

^a Dried sample.

genes are known to evolve more rapidly than nuclear genes and are therefore good markers to analyze relatively close relationships, such as the species relationships within a genus (Miura et al. 2000). In this study, we combined analysis of three mitochondrial genes (12S, 16S, and COII) to determine the genetic relationship between *C. gestroi* and *C. vastator*. Together with morphological information, these phylogenetic analyses lead us to propose that *C. gestroi* and *C. vastator* are synonymous.

Materials and Methods

Termite Samples. Termites collected from Malaysia, Thailand, Singapore, Indonesia, the Philippines, Japan, and the United States (Table 1) were preserved in absolute ethanol. The *Coptotermes* soldiers were ran-

domly selected from each of the 16 populations and the following characteristics were measured using a stereomicroscope (model SZ2-LGB, Olympus, Tokyo, Japan): total length, length without head, length of head at base of mandibles, head (length to fontanelle), maximum width of head, width of head at base of mandibles, segment I of antennae (length), segment I of antennae (width), segment II of antennae (length), segment II of antennae (width), labrum length, maximum width of labrum, minimum gula width, maximum gula width, gula length, pronotum length, and pronotum width. Sample CG005MY was excluded from the morphometric measurements because it was a dried sample. Voucher specimens preserved in 100% ethanol are maintained at the Insect Museum, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. Selected

published sequences from GenBank (www.ncbi.nlm.nih.gov) on *Coptotermes* spp. also were included in phylogenetic analyses.

DNA Extraction. Absolute ethanol-preserved specimens were washed with distilled water and dried on a filter paper. Specimens were then placed in a 1.5-ml tube and homogenized in STE buffer (50 mM sucrose, 25 mM Tris-HCl, pH 7, and 10 mM EDTA). DNA was extracted by incubating with proteinase K at 55°C for 30 min and with 10% SDS for further 3-h incubation. After a single extraction using phenol/chloroform, total DNA was precipitated with absolute ethanol and then resuspended in 25 μ l of distilled H₂O.

Data Collection. Amplification of the 12S, 16S, and COII genes was carried out by polymerase chain reaction (PCR) with primers 12SF (5'-TACTATGTTACGACTTAT-3') and 12SR (5'-AACTAGGATTAGATACCC-3') for 12S (Simon et al. 1994, Kambhampati 1995), LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3'), and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') for 16S (Simon et al. 1994, Kambhampati and Smith 1995), C2F2 (5'-ATACCTCGACGWTATTCAGA-3') and TKN3785 for COII (5'-GTTTAAAGACCACTACTTG-3') (Simon et al. 1994, Hayashi et al. 2003). PCR was performed with 2 μ l of extracted DNA and a profile consisting of a 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) by using a PTC-200, Peltier Thermol Cycle (MJ Research, Watertown, MA). Amplified DNA from individual termites was purified using a SpinClean Gel extraction kit (column). Samples were sent to Macrogen Inc. (Seoul, South Korea) for direct sequencing in both directions conducted under Big-DyeTM terminator cycling conditions. The reacted products were then purified using ethanol precipitation and run using Automatic Sequencer 3730xl (Applied Biosystems, Foster City, CA).

Nucleotide Data Analysis. BioEdit version 7.0.5 software was used to edit individual electropherograms and form contigs. Multiple consensus sequences were aligned using CLUSTAL X. The alignment results were adjusted manually for obvious alignment errors. DNA sequences of other *Coptotermes* species obtained from GenBank also were included into the alignments for phylogenetic comparisons. The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distance according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Maximum parsimony analyses on the alignments were conducted using Heuristic Search of PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing data. A bootstrap test was used to test the reliability of trees (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings was carried out with RANDOM addition sequence procedure, tree bisection-reconnection branch swapping in PAUP*. For maximum likelihood (ML) analysis, the default likelihood analysis parameter settings were used (HKY85-parameter model of nucleotide substitution, empirical base frequencies) with the transition/transversion ratio set to 2:1. These parameters were used to carry out a heuristic search with

PAUP*, by using the single most parsimonious tree as the starting tree.

Results and Discussion

Morphological Characteristics. Soldier of *C. formosanus* was readily distinguished from *C. vastator* and *C. gestroi* with two pairs of setae projecting dorso-laterally from the base of the fontanelle, compared with only a pair of setae in the latter two species (Roonwal and Chhotani 1962, 1989; Scheffrahn et al. 1990; Su et al. 1997). However, it is difficult to distinguish *C. vastator* from *C. gestroi*. Both species have a long tongue-shaped labrum extending beyond the middle of the mandibles; hyaline tip with two long hairs at its base. The pronotum, gula, and I and II antennal segments for both species are also congruent.

Variability among *C. vastator* samples from Manila and Culasi, Philippines, was originally addressed by Light (1929). Large variations were found among the conspecifics of *C. vastator* from similar and different geographical locations. Su and Scheffrahn (1998) reported a strong resemblance between the soldiers of *C. vastator* and *C. gestroi* (= *C. havilandi*), although the soldiers of the latter species were slightly larger than the former species, and had half-moon-shaped antennal spots. Other variabilities such as coloration can be influenced by the age and state of the colony or by environmental and storage conditions (Scheffrahn et al. 2005).

All these previous findings were evident in our current studies where large variations among the examined individuals were recorded. For example, it can vary up to 26% in the length of the soldiers examined, 43 and 31% in terms of length and width of head at base of mandibles, respectively, and 51 and 21% in terms of pronotum length and width, respectively (Table 2). The length of the *C. vastator* soldiers that ranged between 3.93 and 4.95 mm concurs well with that reported in Su and Scheffrahn (1998), but slightly shorter than that recorded in Light (1929). Most of the measured characteristics were found to overlap well with those recorded on the examined *C. gestroi* soldiers in this study. Our measurements on both *C. vastator* and *C. gestroi* also found that they fall within the range of the morphometric characteristics of *C. vastator* soldiers reported by Light (1929) (Table 2). Thus, it is likely that both *C. vastator* and *C. gestroi* are synonymous.

Nucleotide Analyses. DNA sequences of \approx 420, 428, and 680 base pairs were obtained for the genes 12S, 16S, and COII, respectively. The average base frequencies were A = 0.45, C = 0.22, G = 0.12, and T = 0.21 for the 12S gene; A = 0.42, C = 0.25, G = 0.11, and T = 0.22 for the 16S gene; and A = 0.39, C = 0.24, G = 0.14, and T = 0.23 in the COII gene.

Among the 13 populations of *C. gestroi/vastator* DNA sequences, the genetic diversity ranged from 0 to 0.54% in 12S gene, from 0 to 0.76% in COII gene, and from 0 to 0.80% in 16S gene. The genetic diversity recorded in this study were much lower than that reported by Scheffrahn et al. (2005) where 0–1.8% was

Table 2. Measurements (in millimeters) of termite soldiers of three *Coptotermes* species

Species	<i>n</i>	Length	Length without head	Length of head at base of mandibles	Head, length to fontanelle	Max width of head	Width of head at base of mandibles	Segment I of antennae, length	Segment I of antennae, width
<i>C. gestroi</i>									
CG001IN	10	4.92 (4.59-5.29)	3.01 (2.81-3.23)	1.36 (1.30-1.44)	1.30 (1.26-1.35)	1.15 (1.10-1.18)	0.54 (0.46-0.58)	0.15 (0.13-0.18)	0.08 (0.07-0.09)
CG002IN	10	4.86 (4.15-5.54)	2.92 (2.20-3.51)	1.32 (1.23-1.38)	1.21 (1.15-1.25)	1.14 (1.11-1.18)	0.58 (0.56-0.61)	0.16 (0.13-0.18)	0.08 (0.08-0.11)
CG001MY	10	4.13 (3.60-5.11)	2.31 (2.06-2.90)	1.27 (1.12-1.44)	1.27 (1.17-1.47)	1.15 (1.03-1.50)	0.58 (0.50-0.75)	0.16 (0.13-0.21)	0.08 (0.08-0.10)
CG004MY	5	3.73 (3.25-4.30)	2.03 (1.76-2.47)	1.24 (1.12-1.33)	1.19 (1.16-1.28)	1.02 (0.99-1.07)	0.52 (0.50-0.54)	0.15 (0.13-0.17)	0.08 (0.08-0.08)
CG001TH	10	3.83 (3.51-4.16)	2.07 (1.68-2.33)	1.21 (1.13-1.26)	1.16 (1.12-1.22)	1.07 (0.95-1.16)	0.52 (0.48-0.58)	0.15 (0.14-0.16)	0.08 (0.07-0.09)
CG002TH	10	3.94 (3.66-4.27)	2.19 (1.96-2.45)	1.22 (1.15-1.30)	1.15 (1.07-1.21)	1.11 (1.06-1.19)	0.53 (0.47-0.60)	0.15 (0.13-0.18)	0.08 (0.07-0.09)
CG001SG	1	4.41	2.58	1.34	1.25	1.09	0.6	0.16	0.08
CG002SG	1	5.65	3.71	1.3	1.28	1.05	0.58	0.16	0.08
<i>C. vastator</i>									
CV001HW	5	4.80 (4.60-4.95)	2.92 (2.65-3.19)	1.45 (1.42-1.49)	1.29 (1.26-1.32)	1.15 (1.09-1.21)	0.61 (0.57-0.68)	0.17 (0.16-0.18)	0.09 (0.08-0.10)
CV001PH	10	4.16 (4.05-4.30)	2.47 (2.30-2.63)	1.26 (1.13-1.33)	1.21 (1.19-1.23)	1.07 (1.04-1.10)	0.54 (0.52-0.56)	0.17 (0.15-0.19)	0.08 (0.07-0.08)
CV002PH	10	4.34 (4.03-4.52)	2.46 (2.05-2.73)	1.19 (0.99-1.43)	1.22 (1.17-1.25)	1.10 (1.06-1.16)	0.54 (0.52-0.57)	0.15 (0.15-0.17)	0.08 (0.07-0.09)
CV003PH	10	4.24 (3.93-4.50)	2.52 (2.15-2.81)	1.33 (1.16-1.42)	1.31 (1.30-1.32)	1.15 (1.11-1.19)	0.55 (0.53-0.56)	0.16 (0.14-0.19)	0.08 (0.07-0.09)
<i>C. formosanus</i>									
CF001HW	10	5.02 (4.10-5.49)	2.91 (2.04-3.25)	1.47 (1.42-1.54)	1.42 (1.38-1.48)	1.20 (1.17-1.24)	0.60 (0.58-0.64)	0.17 (0.16-0.19)	0.08 (0.08-0.10)
CF001JP	10	4.61 (4.41-4.84)	2.50 (2.26-2.65)	1.41 (1.30-1.54)	1.33 (1.30-1.34)	1.20 (1.10-1.66)	0.60 (0.58-0.62)	0.16 (0.14-0.18)	0.08 (0.08-0.08)
CF002JP	10	4.73 (4.34-5.02)	2.73 (2.19-2.93)	1.40 (1.24-1.48)	1.37 (1.34-1.41)	1.14 (1.11-1.21)	0.59 (0.56-0.61)	0.16 (0.14-0.18)	0.08 (0.08-0.09)
CF003JP	10	4.93 (4.44-5.45)	2.84 (2.43-3.37)	1.44 (1.33-1.56)	1.40 (1.36-1.45)	1.16 (1.14-1.19)	0.60 (0.57-0.62)	0.17 (0.16-0.19)	0.08 (0.07-0.09)
<i>C. gestroi</i>									
CG001IN	0.07 (0.06-0.08)	0.06 (0.06-0.07)	0.32 (0.29-0.37)	0.28 (0.27-0.30)	0.24 (0.22-0.25)	0.40 (0.40-0.41)	0.95 (0.84-1.05)	0.40 (0.36-0.43)	0.80 (0.75-0.84)
CG002IN	0.06 (0.05-0.07)	0.37 (0.30-0.43)	0.28 (0.26-0.30)	0.22 (0.20-0.26)	0.38 (0.35-0.42)	0.38 (0.33-0.43)	1.04 (0.87-1.15)	0.41 (0.38-0.43)	0.79 (0.75-0.82)
CG001MY	0.07 (0.06-0.08)	0.34 (0.31-0.40)	0.29 (0.27-0.34)	0.23 (0.20-0.24)	0.39 (0.38-0.43)	0.39 (0.34-0.48)	0.86 (0.70-1.01)	0.39 (0.34-0.48)	0.78 (0.67-1.06)
CG004MY	0.07 (0.05-0.08)	0.34 (0.32-0.36)	0.26 (0.24-0.28)	0.21 (0.21-0.22)	0.35 (0.33-0.36)	0.35 (0.33-0.36)	0.79 (0.71-0.87)	0.32 (0.27-0.35)	0.66 (0.64-0.68)
CG001TH	0.07 (0.06-0.07)	0.33 (0.26-0.37)	0.26 (0.23-0.28)	0.21 (0.19-0.23)	0.32 (0.34-0.36)	0.34 (0.34-0.36)	0.77 (0.67-0.93)	0.34 (0.29-0.36)	0.72 (0.68-0.78)
CG002TH	0.06 (0.05-0.08)	0.33 (0.29-0.38)	0.27 (0.26-0.28)	0.21 (0.18-0.24)	0.36 (0.33-0.38)	0.36 (0.33-0.38)	0.79 (0.74-0.84)	0.36 (0.33-0.38)	0.75 (0.70-0.80)
CG001SG	0.07	0.06	0.38	0.25	0.21	0.39	0.87	0.35	0.75
CG002SG	0.07	0.06	0.35	0.27	0.23	0.37	0.82	0.42	0.77
<i>C. vastator</i>									
CV001HW	0.07 (0.06-0.08)	0.06 (0.06-0.07)	0.38 (0.34-0.41)	0.29 (0.29-0.30)	0.24 (0.23-0.25)	0.38 (0.37-0.38)	0.84 (0.79-0.89)	0.38 (0.37-0.40)	0.81 (0.78-0.82)
CV001PH	0.06 (0.05-0.08)	0.33 (0.30-0.36)	0.27 (0.26-0.28)	0.22 (0.20-0.25)	0.36 (0.32-0.38)	0.36 (0.32-0.38)	0.79 (0.75-0.84)	0.32 (0.28-0.35)	0.73 (0.69-0.76)
CV002PH	0.07 (0.06-0.08)	0.36 (0.34-0.38)	0.28 (0.27-0.29)	0.21 (0.19-0.23)	0.36 (0.35-0.37)	0.36 (0.35-0.37)	0.82 (0.77-0.85)	0.35 (0.30-0.39)	0.73 (0.69-0.75)
CV003PH	0.07 (0.06-0.08)	0.35 (0.33-0.36)	0.27 (0.25-0.29)	0.21 (0.19-0.23)	0.39 (0.37-0.40)	0.39 (0.37-0.40)	0.80 (0.74-0.90)	0.35 (0.31-0.40)	0.76 (0.72-0.79)
<i>C. formosanus</i>									
CF001HW	0.07 (0.06-0.08)	0.42 (0.34-0.53)	0.30 (0.29-0.32)	0.24 (0.23-0.25)	0.42 (0.40-0.44)	0.42 (0.40-0.44)	0.95 (0.87-1.03)	0.40 (0.35-0.47)	0.81 (0.76-0.87)
CF001JP	0.07 (0.05-0.08)	0.34 (0.32-0.37)	0.25 (0.25-0.27)	0.21 (0.20-0.23)	0.38 (0.36-0.39)	0.38 (0.36-0.39)	0.92 (0.84-0.99)	0.38 (0.36-0.41)	0.76 (0.74-0.79)
CF002JP	0.06 (0.06-0.07)	0.37 (0.30-0.42)	0.27 (0.25-0.28)	0.21 (0.20-0.23)	0.38 (0.36-0.40)	0.38 (0.36-0.40)	0.90 (0.85-0.96)	0.37 (0.31-0.41)	0.77 (0.75-0.79)
CF003JP	0.07 (0.04-0.09)	0.39 (0.33-0.44)	0.28 (0.27-0.29)	0.22 (0.21-0.23)	0.41 (0.40-0.42)	0.41 (0.40-0.42)	0.97 (0.86-1.06)	0.41 (0.37-0.43)	0.81 (0.75-0.84)

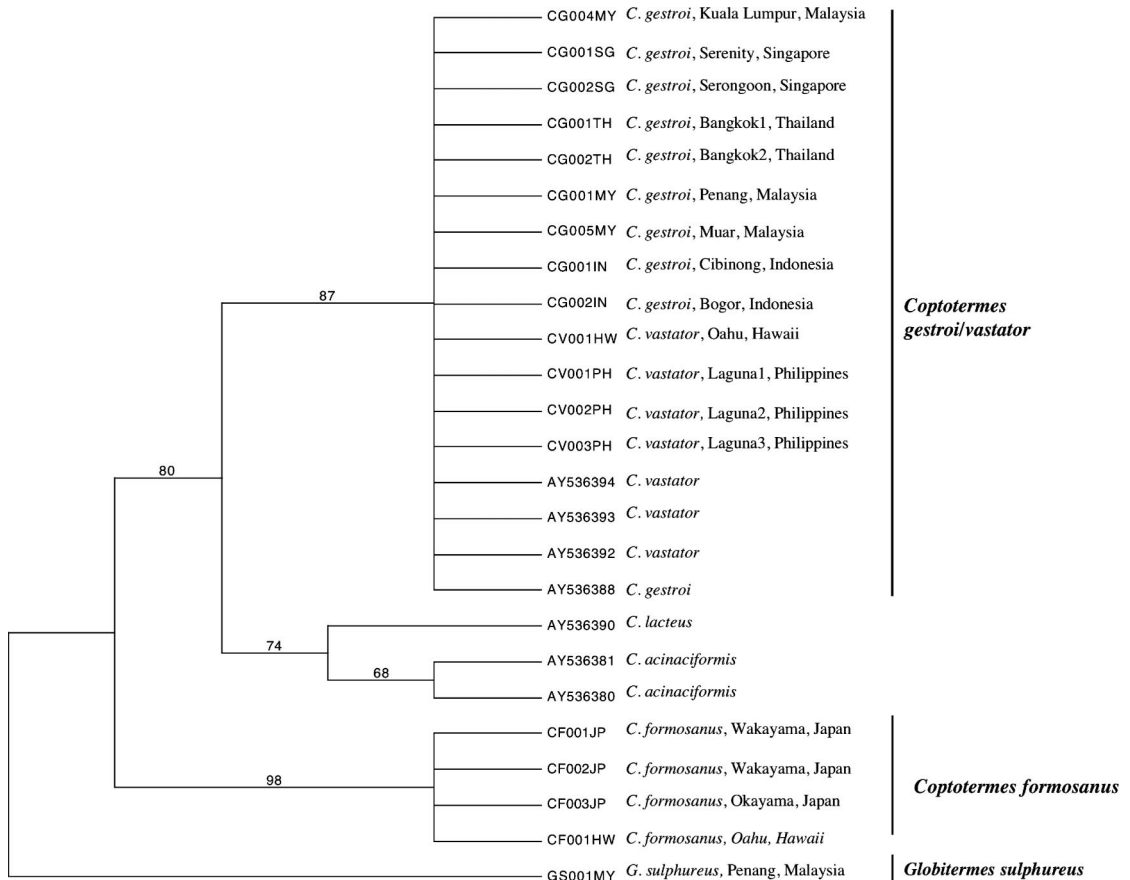


Fig. 1. A single most parsimonious tree obtained for 12S gene by using a heuristic search option in PAUP4.0b10 (Swofford 2001). Bootstrap values for 1,000 replicates are listed above the branches supported at $\geq 50\%$. 7084 GenBank accession numbers represent the samples that are pooled from the National Center for Biotechnology Information (NCBI) database.

found between *Nasutitermes corniger* (Motschulsky) and *Nasutitermes costalis* (Holmgren), which led the authors to propose that both species are synonymous.

Within the aligned sequences of *C. gestroi/vastator*, only two nucleotides (at nucleotide positions -61 and -170) in 12S gene, three nucleotides (at nucleotide positions -39, -103, and -135) in 16S gene, and five nucleotides (at nucleotide positions -174, -350, -490, -493, and -646) in COII gene were varied. At some of the above-mentioned positions, the two *C. gestroi* populations from Indonesia were sharing the same nucleotides as the *C. vastator*. Scheffrahn et al. (2005) who proposed the synonymy between *N. corniger* and *N. costalis* had recorded variability of 13 nucleotides on 16S gene.

Phylogenetic Relationships Inferred from 12S, 16S, and COII Genes. The multiple alignment of 12S gene sequences, including the outgroup taxon has 365 characters, of which 319 are constant and 15 parsimony informative. For the 16S gene, there are 385 characters, of which 326 are constant and 20 parsimony informative. There are 937 characters, of which 730 are constant and 77 parsimony informative in the alignment for COII gene. The data set had only one most

parsimonious tree for each of the genes (Fig. 1: length = 187, CI = 0.663, RI = 0.841; Fig. 2: length = 59, CI = 0.932, RI = 0.939; Fig. 3: length = 231, CI = 0.920, RI = 0.831), as documented using the heuristic search algorithm of PAUP*. The relationship of *C. gestroi* and *C. vastator* relative to the other *Coptotermes* taxa was the same for the ML analysis (-ln L 958.22732 for 12S, -ln L 1566.57300 for 16S, -ln L 2861.87516 for COII; trees not shown).

Bootstrap analysis of the aligned *Coptotermes* taxa for 12S, 16S, and COII genes with *Globitermes sulphureus* (Haviland) as the outgroup revealed that *C. gestroi* and *C. vastator* formed a common clade with strong bootstrap support. For 12S gene, we included *C. gestroi*, *C. vastator*, *Coptotermes acinaciformis* (Froggatt), and *Coptotermes lacteus* (Froggatt) sequences from the GenBank in the phylogenetic analysis. *C. gestroi* and *C. vastator* from existing GenBank fall within the same clade of *C. gestroi* and *C. vastator* in this study resulted in a sister group with *C. acinaciformis* and *C. lacteus* (Fig. 1). Another clade was made up of *C. formosanus* populations. For the COII gene, the relationship exhibited was similar to that reported

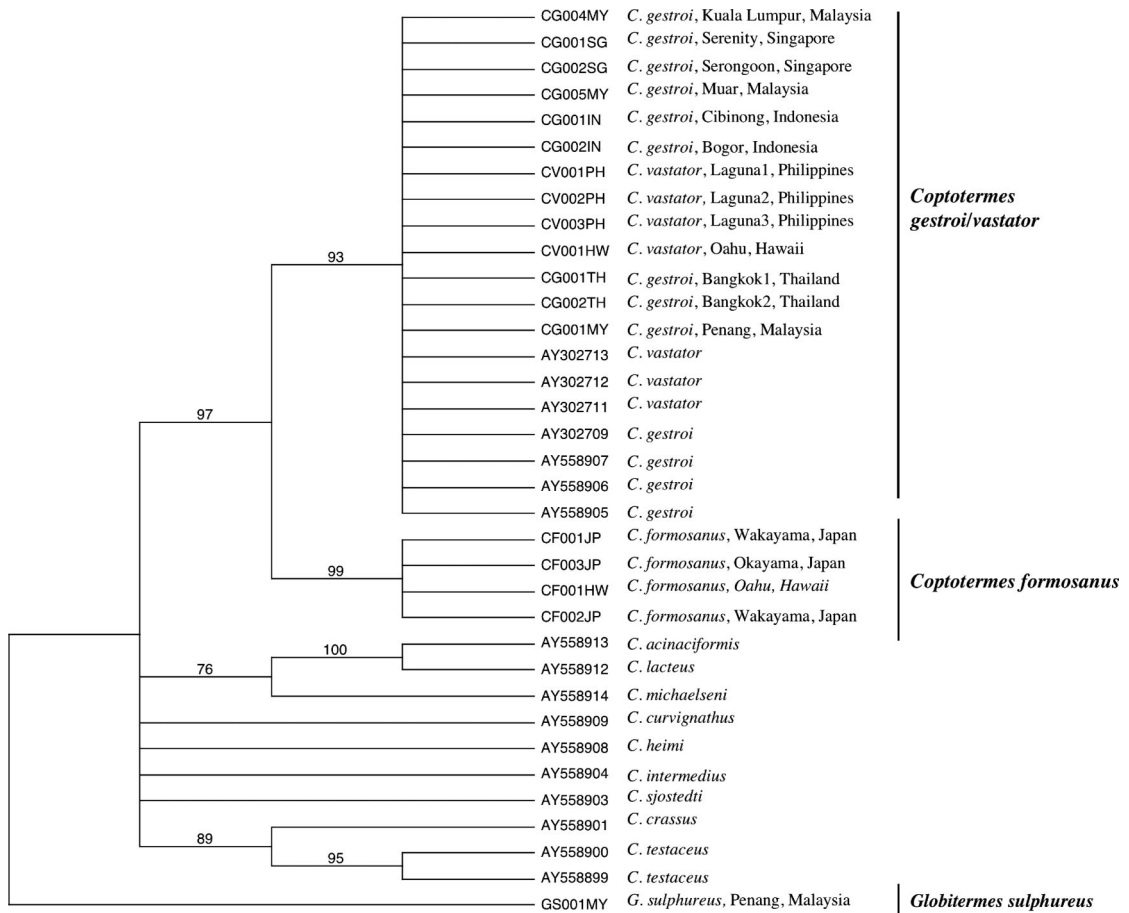


Fig. 2. A single most parsimonious tree obtained for 16S gene by using a heuristic search option in PAUP4.0b10 (Swofford 2000). Bootstrap values for 1,000 replicates are listed above the branches supported at $\geq 50\%$. GenBank accession numbers represent the samples that are pooled from the NCBI database.

for 12S, which is likely due to the same species of *Coptotermes* used in the analysis (Fig. 3).

For 16S gene, we included more *Coptotermes* species into the analysis. Regardless, *C. gestroi* and *C. vastator* remained in the same clade with *C. formosanus* as its sister group. *C. acinaciformis* and *C. lacteus* formed a common clade that resulted in a sister group with *Coptotermes michaelseni* Silvestri. *Coptotermes curvignathus* Holmgren, *Coptotermes heimi* (Wasmann), *Coptotermes intermedius* Silvestri, and *Coptotermes sjostedti* Holmgren branched out individually from the tree, whereas the last clade was made up of *Coptotermes crassus* Synder and *Coptotermes testaceus* (L.) (Fig. 2).

Across the three genes, the transition rate was 100%. Substitutions involving C and T occurred between *C. gestroi* and *C. vastator*. Comparable with the results in Ye et al. (2004), COII is considered as the fastest evolving gene of those examined, whereas 12S is the most conserved gene. The three markers provided comparable results in phylogenetic analysis. Different populations of *C. gestroi* and *C. vastator* showed high similarity in genetic distance and formed a single *C.*

gestroi/vastator clade in the three phylogenetic analyses. The DNA sequences and morphological data presented here clearly indicate that *C. gestroi* and *C. vastator* are synonymous.

Implication of Findings to Pest Management Industry. Kirton (2005) reviewed the importance of accurate termite identification to the pest management industry. With the recognition of a single pest species of *Coptotermes* in South East Asia, information concerning *C. gestroi* and *C. vastator* species from different geographical regions can be pooled.

In summary, morphometric and molecular phylogenetic analyses by using three mitochondrial genes in this study suggested that both *C. gestroi* and *C. vastator* are synonymous. In particular, 1) the morphological characters of both *C. gestroi* and *C. vastator* are highly parallel, 2) the genetic diversity between *C. gestroi* and *C. vastator* was very low (0–0.80%), and 3) there was only minimal nucleotide differences between *C. gestroi* and *C. vastator* compared with *C. gestroi/formosanus* and *C. vastator/formosanus*. There were only 10 nucleotides difference detected across the three genes between *C. gestroi* and *C. vastator*. Moreover,

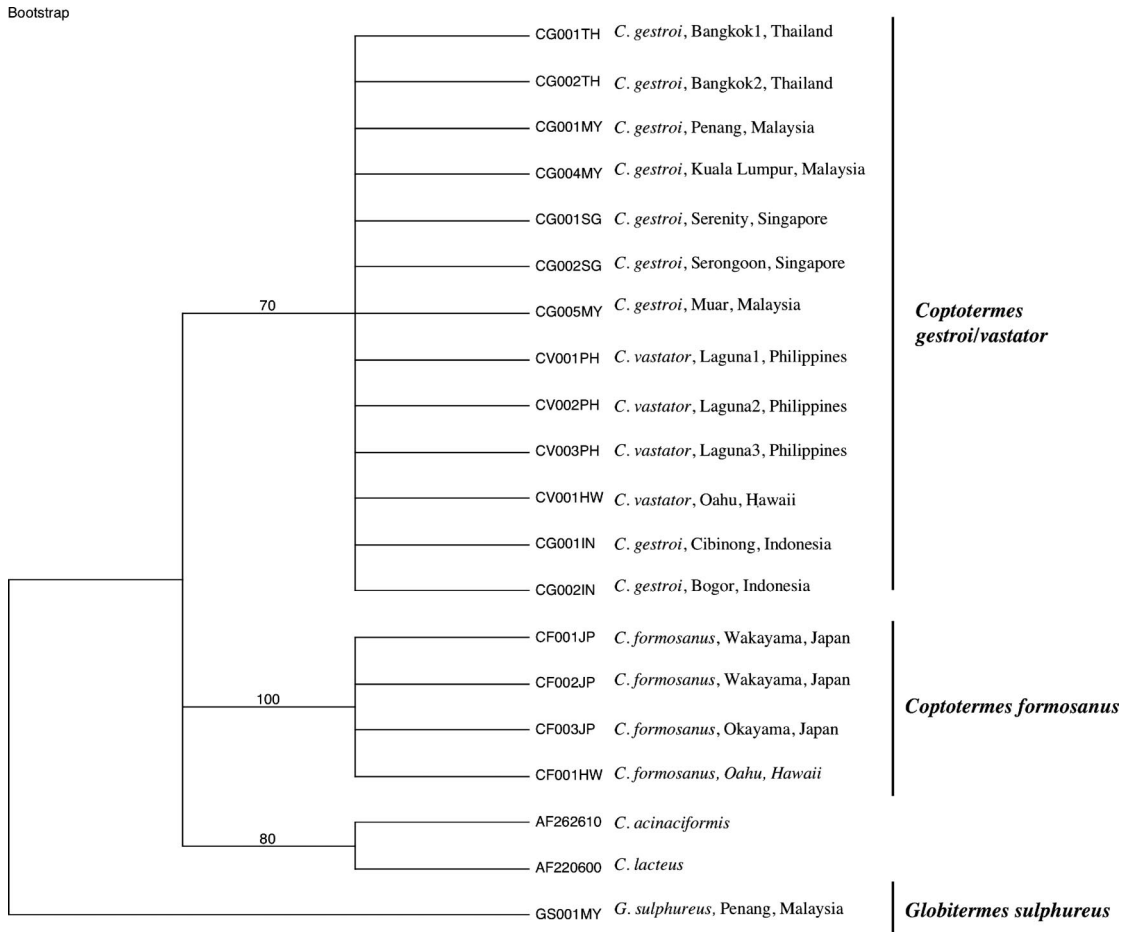


Fig. 3. A single most parsimonious tree obtained for COII gene using a heuristic search option in PAUP4.0b10 (Swofford 2000). Bootstrap values for 1,000 replicates are listed above the branches supported at $\geq 50\%$. GenBank accession numbers represent the samples that are pooled from the NCBI database.

out of the 10 nucleotides, only six nucleotides are species specific. In contrast, *C. gestroi/vastator* differ from *C. formosanus* at 166 nucleotide positions across the three genes. This suggests that nucleotide differences between *C. gestroi* and *C. vastator* might only be due to population variation. We envisage that if a higher number of populations were to be analyzed, the number of species-specific nucleotides might be reduced.

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