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MORPHOMETRIC VARIATION AND GENETIC RELATIONSHIP OF *Coptotermes* spp. (BLATTODEA: RHINOTERMITIDAE) IN SARAWAK, MALAYSIA

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ABSTRACT

Amongst the termite genera within Rhinotermitidae, the genus *Coptotermes* is probably regarded as one of the most destructive pest in Malaysia as it contributes to more than 90% of the damages in buildings and structures. In this study, 17 parameters were used for morphometric analysis. Partial sequence of mitochondrial DNA cytochrome oxidase subunit II (COII) was obtained from 21 populations of *C. curvignathus*, seven populations of *C. sepangensis* and two populations of *C. kalshoveni* in Sarawak. In addition, 11 sequences of *Coptotermes* spp. were obtained from GenBank and included in the analysis. Based on Discriminant Function Analyses (DFA), the maximum width of postmentum (MxWPt) is identified as the best morphological character for differentiating the four species of *Coptotermes* in this study, proved by canonical discriminant function. Phylogenetic analyses of COII found that *C. sepangensis* and *C. kalshoveni* are nested within a single clade. Morphological comparison also showed these two species to be identical in terms of body measurements and diagnostic features. These evidence indicates that *C. sepangensis* and *C. kalshoveni* are the same species, thus we suggest *C. sepangensis* is a junior synonym of *C. kalshoveni.*

Key words: *Coptotermes*, synonym, morphometric variation, molecular phylogenetics

INTRODUCTION

The termite genus of *Coptotermes* from the family of Rhinotermitidae is an invasive pest in urban and suburban areas. The genus is very destructive to wood and wooden materials in the world (Takematsu *et al*., 2000) and has a wide distribution throughout Asia, Australia, Africa and the New World. In Peninsular Malaysia, widespread infestation of the termite was reported on rubber trees (*Hevea brasilliensis*) before it was considered as a serious pest in the early 1900s. Today, the species is highly regarded as a destructive pest as it is highly destructive to buildings and its furnishings. It is also an agricultural nuisance in places such as oil palm plantations, garden landscapes and dwellings (Lee, 2002a) and brings damage to any cellulosic materials such as books, papers, blankets, window frames and furniture (Chao *et al*., 1989).

A *Coptotermes* species is a global taxonomic challenge. The validity of each *Coptotermes* species name as a valid status differs tremendously in many level of support, from species synonymies (Yeap *et al*., 2009; Krishna *et al.*, 2013), discoveries of potentially new cryptic species (Lee *et al.*, 2015), an intraspecific soldier morphological variability (Chouvenc *et al.*, 2016), a limited caste samples for species identification (Li, 2000), and a little molecular evidence to provide robust recognition of a single species of termites (Chouvenc *et al.*, 2016).

The issues of species synonymies for *Coptotermes* spp. had risen enormously over the past centuries. Since 2000s, an extensive study and efforts has been made either by morphological comparison, morphometric, molecular, taxonomic and phylogenetic revision (Miura *et al*., 1998; Austin *et al*., 2004; Szalanski *et al*., 2004) to resolve many synonyms in *Coptotermes*. The advances in molecular tools and comprehensive taxonomic revision in *Coptotermes* spp. has provide a possible

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pathway to clarify the synonym status of certain *Coptotermes* spp. of more than 40 junior synonyms (Krishna *et al.*, 2013) such as a synonym between *C. gestroi* and *C. havilandi.*

A recent analysis using the molecular data (Yeap *et al*., 2009, 2011; Cheng *et al*., 2014) has provided some insights into *Coptotermes* phylogenetics and its radiation, especially in peninsular Malaysia. In light of the work by Krishna *et al.* (2013) and Chouvenc *et al.* (2016), it is clear that taxonomic revision of *Coptotermes* is urgently needed, as accurate species identification is importance for application for control practices as highlighted by Kirton (2005). In addition, recent surveys revealed more synonyms is expected to be found (Scheffrahn *et al.*, 2015), and thus resolving *Coptotermes* nomenclature is a work in progress (Krishna *et al.*, 2013).

The aim of this work is to determine the morphological variation of *Coptotermes* species from Sarawak, Malaysia using discriminant function analyses (DFA) and secondly to infer the genetic relationship based on partial mitochondrial gene of cytochrome oxidase II (COII) in order to complement the morphological identification. At present, to our best knowledge, no published molecular data of *Coptotermes* samples has been carried out from Sabah and Sarawak.

MATERIALS AND METHODS

Termites collection

Termites were collected from 39 colonies from selected areas in Sarawak (Figure 1) from September 2015 to December 2016. All specimens were collected from the field using a random sampling and standardized protocol by Jones *et al*. (2005) with a modification of sampling transects (i.e. An area of 100 m long and 2 m wide was divided into five small plots with dimension of 20 m × 2 m each and plot was sampled by one person for 30 minutes). Wherever possible, all castes were sampled. The samples were preserved in absolute ethanol and brought to the laboratory for identification. Additional samples were provided by NLC General Pest Company in Kuching, Sarawak. All specimens were stored in the Insect Reference Collection, Universiti Malaysia Sarawak (UNIMAS). The identification of termites to the species level was done using taxonomic keys and previous studies published by Thapa (1981), Tho (1992), Ahmad (1965), Krishna *et al*. (2013), Norsyarizan and Wan Nurainie (2016) and the species measurements data were also compared with the locotype and holotype series available for *Coptotermes* spp.. To establish the identity of *Coptotermes* spp., an

Fig. 1. Map of sample locations in Sarawak. Source: Modified from Australian National University CartoGIS CAP00-120.
Note: • indicated the sampling locations.

individual soldier belonging to each colony was used for morphometric analyses. Soldiers' morphological measurement (n > 5) was recorded for 24 colonies of *C. curvignathus*, 10 colonies of *C. sepangensis*, two colonies of *C. kalshoveni* and three colonies of *C. travians* (Table 1). The photographs of the soldiers were taken using a Motic SMZ-16B Series stereomicroscope attached to a Moticam 2000 camera. Calibrated measurements were done by using Motic Image Plus 2.0 software.

Table 1. *Coptotermes* species and populations included in the present study

Sample Id#	Species Name	Locality
Samples from this study		
CC02UNS	*C. curvignathus* (Holmgren)	Sarawak, Kota Samarahan, UNIMAS
CC05UNS	*C. curvignathus* (Holmgren)	Sarawak, Kota Samarahan, UNIMAS
CC08UNS	*C. curvignathus* (Holmgren)	Sarawak, Kota Samarahan, UNIMAS
CC04SWS	*C. curvignathus* (Holmgren)	Sarawak, Lundu, Samunsam Wildlife Sanctuary
CC01TB	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman
CC01SKAG	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman
CC02SKAG	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman
CC01KL	*C. curvignathus* (Holmgren)	Sri Aman, Engkelili
CC02KL	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman, Engkelili
CC01KLKG	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman, Engkelili
CC02KLKG	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman, Engkelili
CC03KLKG	*C. curvignathus* (Holmgren)	Sarawak, Sri Aman, Engkelili
CC01BHR	*C. curvignathus* (Holmgren)	Sarawak, Kuching, Borneo Highland
CC02BHR	*C. curvignathus* (Holmgren)	Sarawak, Kuching, Borneo Highland
CC01SNR	*C. curvignathus* (Holmgren)	Sarawak, Kuching, Samajaya Nature Reserve
CC01MA	*C. curvignathus* (Holmgren)	Sarawak, Kuching, Matang
IK19R29P2	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IK19R31P4	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IK19R31P5	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IL19R16P1	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IL19R17P1	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IL19R18P1	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IL19R19P2	*C. curvignathus* (Holmgren)	Sarawak, Sibu
IL19R19P1	*C. curvignathus* (Holmgren)	Sarawak, Sibu
CS01UNS	*C. sepangensis* (Krishna)	Sarawak, Kota Samarahan, UNIMAS
CS06UNS	*C. sepangensis* (Krishna)	Sarawak, Kota Samarahan, UNIMAS
CS07UNS	*C. sepangensis* (Krishna)	Sarawak, Kota Samarahan, UNIMAS
CS02KNP	*C. sepangensis* (Krishna)	Sarawak, Kuching, Kubah National Park
CS01BHW	*C. sepangensis* (Krishna)	Sarawak, Kuching, Borneo Highland
CS02BHW	*C. sepangensis* (Krishna)	Sarawak, Kuching, Borneo Highland
CS03BHW	*C. sepangensis* (Krishna)	Sarawak, Kuching, Borneo Highland
CS04BHW	*C. sepangensis* (Krishna)	Sarawak, Kuching, Borneo Highland
CS03SWS	*C. sepangensis* (Krishna)	Sarawak, Lundu, Samunsam Wildlife Sanctuary
CS05SWS	*C. sepangensis* (Krishna)	Sarawak, Lundu, Samunsam Wildlife Sanctuary
CK03UNS	*C. kalshoveni* (Kemner)	Sarawak, Kota Samarahan, UNIMAS
CK04UNS	*C. kalshoveni* (Kemner)	Sarawak, Kota Samarahan, UNIMAS
CT01SNP	*C. travians* (Haviland)	Sarawak, Kuching, Santubong National Park
CT01KNP	*C. travians* (Haviland)	Sarawak, Kuching, Kubah National Park
CT01LHNP	*C. travians* (Haviland)	Sarawak, Miri, Lambir Hills National Park
CG01JBS	*C. gestroi* (Wasmann)	Johor, Skudai
Samples from previous study (data obtained from GenBank)		
FJ384634 (Yeap *et al.*, 2007)	*C. curvignathus* (Holmgren)	Penang, USM
FJ384635 (Yeap *et al.*, 2007)	*C. curvignathus* (Holmgren)	Penang
EF379945 (Yeap *et al.*, 2007)	*C. gestroi* (Wasmann)	Penang, USM
EF379951 (Yeap *et al.*, 2007)	*C. gestroi* (Wasmann)	Kuala Lumpur, Bangsar
EF379952 (Yeap *et al.*, 2007)	*C. gestroi* (Wasmann)	Johor, Muar
FJ384652 (Yeap *et al.*, 2007)	*C. kalshoveni* (Kemner)	Penang, USM
AJ854163 (unpublished)	*C. sepangensis* (Krishna)	Malaysia*
AJ854165 (unpublished)	*C. sepangensis* (Krishna)	Malaysia*
AJ854169 (unpublished)	*C. travians* (Haviland)	Malaysia*
AJ854170 (unpublished)	*C. travians* (Haviland)	Malaysia*
EF379957 (Yeap *et al.*, 2007)	*G. sulphurous* (Haviland)**	Penang, USM

*Collection site not stated; **outgroup.

Morphometric analysis

A minimum of 17 external characters was measured for each specimen: (a) total length (TL); (b) total length without head (TLH); (c) length of head at base of mandibles (TLM); (d) length to fontanelle (LF); (e) maximum width of head (WH); (f) width of head at base of mandibles (WHM); (g) length of labrum (LLb); (h) width of labrum (WLb); (i) length of antennae, segment 1 (AL1); (j) length of antennae, segment 2 (AL2); (k) width of antennae, segment 1 (WA1); (l) width of antennae, segment 2 (WA2); (m) length of pronotum (LPr); (n) width of pronotum (WPr); (o) length of postmentum (LPt); (p) maximum width of postmentum (MxWPt); and (q) minimum width of postmentum (MnWPt). The indices for body index (BI), head index (HI), pronotum index (PI) and postmentum index (GI) were calculated as follows: (1) Body index (WH/TL); (2) head index (WH/WHM); (3) pronotum index (WPr/LPr); and (4) postmentum index (MxWPt/MnWPt). These data were subjected to analysis of variance (ANOVA), and comparison of means was done based on Tukey's HSD (Honest Significant Difference) using STATISTIX Version 10.0.

Next, morphometric data were transformed to $\log_{10}x + 1$ by using Statistical Package for Social Sciences (SPSS) version 21 (SPSS Inc., 2006) in order to reduce the heterogeneity in variance (Smith *et al*., 2009). The data then was subjected to discriminant function analysis (DFA).

DNA extraction

The specimen preserved in absolute ethanol was washed with distilled water and dried on a filter paper. Total genomic DNA was extracted from single termite using DNeasy blood and tissue kit (QIAGEN, Valencia, CA) by following the manufacturer's protocol. The extracted DNA was re-suspended in 30 µl of buffer AE and stored at -20°C. Polymerase chain reaction (PCR) of 900 bp region of partial mitochondrial COII (mtCOII) gene was conducted using the primers C2F2: 5'- ATACCTCGACGWTA TTCAGA -3' and TKN3785 reverse: 5'- GTTTAA GAGACCAGTACTTG -3' (Simon *et al*., 1994). The PCR reactions were conducted with 2 µl of extracted DNA in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA), with a profile consisting of 35 cycles of 94°C for 1 min, 47.9°C for 1 min and 72°C for 1 min in a final reaction volume of 25 µl. Amplified PCR products were cleaned using QIAquick purification kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. The purified PCR products were sent to the First Base Laboratories (Selangor, Malaysia) and subjected for direct sequencing.

Genetic data analysis

CHROMAS program version 2.5.1 (McCarthy, 1998) was used to display fluorescence nucleotide bases of the DNA sequence results. Multiple alignments of the nucleotide sequences were generated in Clustal X program version 2.0 (Thompson *et al*., 1997). Base compositional analyses and pairwise genetic distance were used to calculate the genetic distance according to the Kimura 2-parameter model of sequence evolution (Kimura, 1980) using MEGA version 7 (Kumar *et al*., 2015). Phylogenetic analyses were performed on 32 sequences obtained in this study together with eleven sequences obtained from GenBank (Table 1). *Globitermes sulphureus* was used as an outgroup in order to generate a rooted phylogenetic tree.

Maximum parsimony (MP) analysis was performed with TBR branch swapping and ten random taxon addition replicates under a heuristic search, saving no > 100 equally parsimonious trees per replicate. All characters were unordered and weighted equally. Gaps were treated as missing data. To estimate branch support on the recovered topology, nonparametric bootstrap values were assessed with 1000 bootstrap pseudo-replicates (Felsenstein, 1985). Modeltest 3.7 was used to find the optimal model of DNA substitution (Posada and Crandall, 1998) prior to maximum likelihood (ML) analysis. The phylogenetic reconstruction for maximum likelihood was based on the best-fit model, which was selected by Akaike information criterion (AIC) (Akaike, 1974). Heuristic ML searches using tree bisection-reconnection (TBR) branch swapping were performed in PAUP 4.0b10 (Swofford, 2002). Phylogeny was assessed by 1000 bootstrap replication to test the reliability of inferred trees.

RESULTS

Morphometric variance

In this study, *C. curvignathus* and *C. sepangensis* were found to have strongly curved, slender mandibles with the curvature begins at the middle of its length. The soldier's mandibles of *C. curvignathus* are distinctive as in being large compared to the other mandibles of *Coptotermes* species. Based on the mandible features, it was difficult to distinguish between *C. kalshoveni* and *C. travians*. Both species have less curved mandibles and the curvature begins at the anterior third of its length (Figure 2). The only reliable diagnostic feature to differentiate between these two species was that the head of *C. travians* is ovoid in shape and much longer than wider compared to *C. kalshoveni*

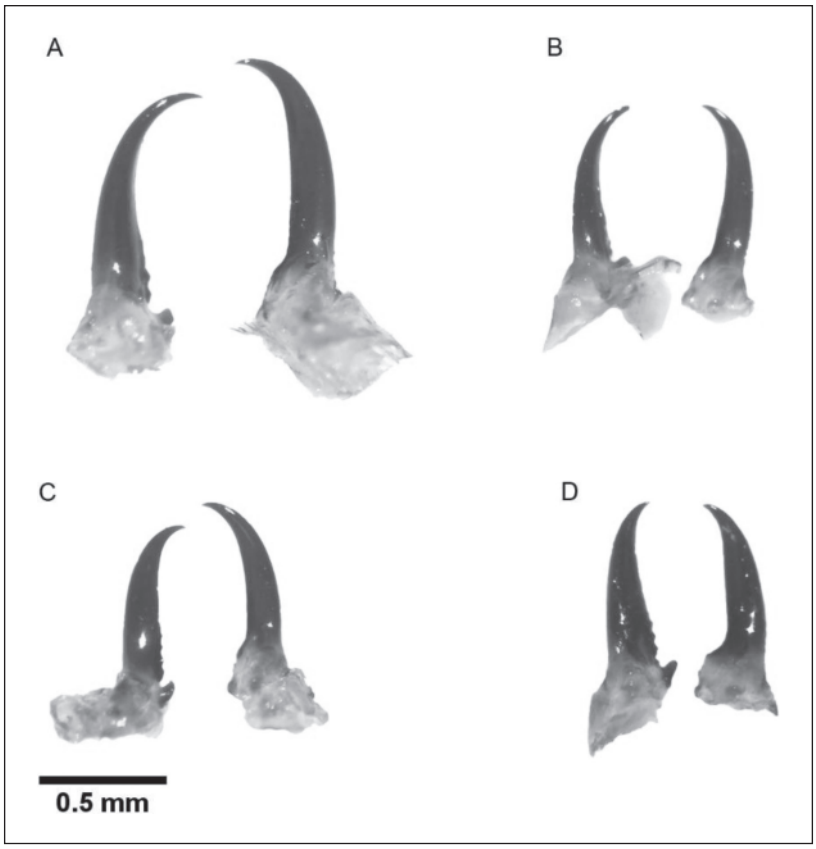

Fig. 2. The shape of mandibles of *Coptotermes* under 50x magnification.
A: *C. curvignathus;* **B:** *C. sepangensis;* **C:** *C. kalshoveni;* **D:** *C. travians.*

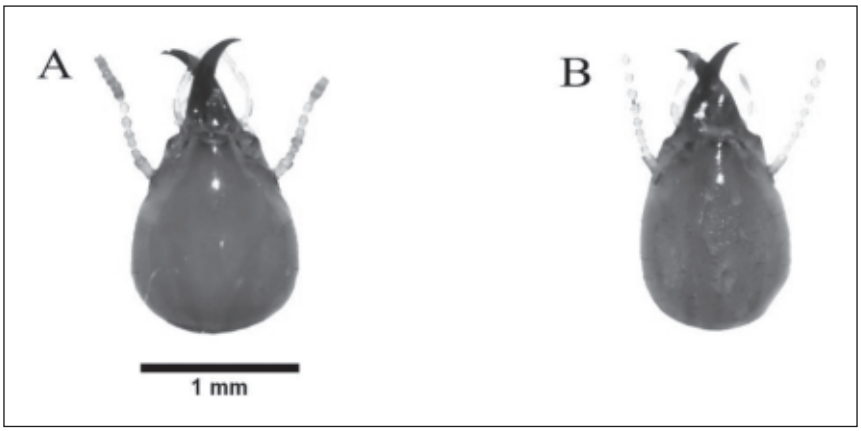

Fig. 3. Dorsal view of soldier head of *Coptotermes* under 30x magnification.
A: *C. kalshoveni;* **B:** *C. travians*

while *C. travians* was observed to be distinctively smaller in fontanelle size (Figure 3). The ANOVA F-statistics indicated the significant effect of the character types on *Coptotermes* species ($P < 0.05$; ANOVA test). Tukey-Kramer testing of individual characteristics provided groupings that indicated significant pairwise population differences. Descriptive statistics for the studied species are listed as in Table 2.

The current measurement data were compared with the several measurement character from Thapa (1981), Tho (1992), Ahmad (1965), locotype and holotype (Table 3 and Table 4). The current measurement of *C. curvignathus* for mean total length of head at base of mandibles (TLM) and mean length of pronotum (LPr) fell within the mean measurement from locotype (mean for TLM=1.48 and LPr=0.49). In addition, the current measurement

Table 2. Descriptive statistics of studied *Coptotermes* species

Species Character	*C. sepangensis* (n=67)			*C. curvignathus* (n=80)			*C. travians* (n=26)			*C. kalshoveni* (n=20)		
	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max
TL	4.02±0.55**a**	2.71	5.27	5.11±0.85**b**	2.91	6.48	4.76±0.79**b**	3.63	6.07	4.18±0.45**a**	3.61	5.35
TLH	2.36±0.39**a**	1.54	3.32	2.99±0.57**b**	1.57	4.1	2.72±0.59**b**	1.89	3.69	2.67±0.44**ab**	2.05	3.61
TLM	1.17±0.13**a**	0.91	1.56	1.46±0.18**b**	0.79	1.7	1.39±0.22**b**	1.09	1.96	1.06±0.12**c**	0.84	1.3
LF	1.17±0.10**a**	0.98	1.43	1.34±0.12**b**	1.01	1.56	1.36±0.21**b**	1.11	1.89	1.11±0.07**a**	0.96	1.21
WH	1.08±0.10**ac**	0.93	1.33	1.33±0.15**b**	0.93	1.53	1.13±0.17**c**	0.91	1.58	1.00±0.06**a**	0.93	1.11
WHM	0.39±0.10**a**	0.22	0.61	0.54±0.12**b**	0.25	0.81	0.56±0.10**b**	0.37	0.82	0.32±0.06**a**	0.22	0.42
AL1	0.15±0.03**ac**	0.07	0.22	0.18±0.04**b**	0.11	0.27	0.17±0.02**ab**	0.13	0.22	0.13±0.02**c**	0.1	0.18
WA1	0.08±0.01**ac**	0.05	0.12	0.10±0.01**b**	0.07	0.14	0.09±0.03**ab**	0.06	0.23	0.08±0.01**c**	0.06	0.09
AL2	0.07±0.01**a**	0.05	0.12	0.09±0.02**b**	0.05	0.14	0.08±0.02**b**	0.06	0.11	0.07±0.01**a**	0.04	0.09
WA2	0.06±0.01**a**	0.04	0.09	0.07±0.01**b**	0.05	0.1	0.07±0.02**b**	0.05	0.12	0.06±0.004**a**	0.05	0.06
LLb	0.22±0.04**a**	0.1	0.31	0.35±0.09**b**	0.1	0.5	0.29±0.05**c**	0.22	0.41	0.19±0.06**a**	0.11	0.33
WLb	0.22±0.04**a**	0.1	0.36	0.30±0.06**b**	0.14	0.48	0.27±0.10**bc**	0.08	0.6	0.22±0.07**ac**	0.12	0.33
MxWPt	0.33±0.03**a**	0.28	0.39	0.40±0.05**b**	0.28	0.46	0.40±0.05**b**	0.32	0.47	0.31±0.01**a**	0.29	0.34
MnWPt	0.23±0.03**a**	0.16	0.3	0.24±0.03**b**	0.19	0.3	0.22±0.02**a**	0.19	0.26	0.21±0.01**a**	0.19	0.25
LPt	0.67±0.11**a**	0.41	0.85	0.91±0.19**b**	0.39	1.5	0.88±0.13**b**	0.65	1.18	0.62±0.08**a**	0.48	0.75
LPr	0.37±0.04**a**	0.29	0.46	0.50±0.13**b**	0.1	1.09	0.36±0.05**c**	0.25	0.46	0.34±0.03**a**	0.3	0.41
WPr	0.73±0.08**a**	0.46	0.91	0.93±0.15**b**	0.6	1.73	0.81±0.11**a**	0.65	0.95	0.69±0.07**a**	0.62	0.91
Body Index	0.27±0.03**a**	0.23	0.37	0.26±0.02**a**	0.21	0.34	0.24±0.03**b**	0.21	0.37	0.24±0.02**b**	0.2	0.27
Head Index	2.96±0.73**a**	1.85	5.32	2.57±0.46**b**	1.84	4.24	2.02±0.22**c**	1.65	2.46	3.20±0.49**a**	2.44	4.5
Pronotum Index	1.96±1.32**a**	1.44	2.29	2.00±0.92**a**	0.55	9.8	2.24±0.18**a**	1.94	2.64	2.04±0.13**a**	1.79	2.33
Postmentum Index	1.45±0.10**a**	1.07	1.75	1.68±0.13**b**	1.34	2	1.83±0.12**c**	1.6	2.05	1.48±0.10**a**	1.28	1.63

Note: Mean, within rows, followed by the different letter are significantly different ($P < 0.05$; Turkey's HSD).

TL = total length; TLH = total length without head; TLM = length of head at base of mandibles; LF = length to fontanelle; WH = maximum width of head; WHM = width of head at base of mandibles; AL1 = length of antennae, segment 1; WA1 = width of antennae, segment ; AL2 = length of antennae, segment 2; WA2 = width of antennae, segment 2; WLb = width of labrum; LLb = length of labrum; MnWPt = minimum width of postmentum; MxWPt = maximum width of postmentum; LPt = length of postmentum; WPr = width of pronotum; and LPr = length of pronotum.

Indices: body index = WH/TL; head index WH/WHM; pronotum index WPr/LPr; and postmentum index = MxWPt/MnWPt.

Table 3. Measurements of *C. curvignathus* and *C. sepangensis* from type and previous studies

Measure (in mm) of:	*C. curvignathus*				*C. sepangensis*		
	(locotype)	(Thapa, 1981)	(Tho, 1992)	(Ahmad, 1965)	(holotype)	(Thapa, 1981)	(Tho, 1992)
Length of head to side base of mandibles	1.47-1.50 (1.48*)	1.45-1.65 (1.55*)	1.51-1.85 (1.68*)	1.65-1.76 (1.69*)	1.13-1.16 (1.14*)	1.12-1.25 (1.16*)	1.08-1.31 (1.23*)
Maximum width of head	1.27-1.30 (1.29*)	1.25-1.38 (1.33*)	1.28-1.57 (1.34*)	1.40-1.63 (1.53*)	0.98-1.01 (0.99*)	1.00-1.07 (1.03*)	0.85-1.08 (0.93*)
Width of head at side base of mandibles	0.76-0.77 (0.77*)	0.72-0.83 (0.78*)	0.65-0.82 (0.71*)	0.86-0.90 (0.87*)	–	0.52-0.60 (0.56*)	0.54-0.65 (0.61*)
Width of pronotum	0.85-0.91 (0.99*)	0.87-0.98 (0.93*)	–	1.04-1.18 (1.12*)	0.71-0.75 (0.72*)	0.68-0.75 (0.70*)	–
Length of pronotum	0.47-0.50 (0.49*)	0.52-0.55 (0.54*)	–	0.54-0.63 (0.57*)	0.38-0.41 (0.38*)	0.37-0.42 (0.39*)	–

Note: * Mean for each measurement.

Table 4. Measurements of *C. kalshoveni* and *C. travians* from previous studies

Measure (in mm) of:	*C. kalshoveni*			*C.travians*	
	(Thapa, 1981)	(Tho, 1992)	(Ahmad, 1965)	(Thapa, 1981)	(Tho, 1992)
Length of head to side base of mandibles	0.95-1.05 (1.01*)	1.02-1.20 (1.14*)	1.00-1.15 (1.07*)	1.25-1.32 (1.29*)	1.25-1.34 (1.29*)
Maximum width of head	0.85-0.93 (0.90*)	0.80-1.02 (0.91*)	0.90-0.99 (0.93*)	0.97-1.03 (1.01*)	0.68-0.77 (0.72*)
Width of head at side base of mandibles	0.47-0.51 (0.48*)	0.45-0.50 (0.45*)	0.46-0.52 (0.49*)	0.65-0.67 (0.65*)	0.63-0.68 (0.65*)
Width of pronotum	0.58-0.65 (0.60*)	–	0.61-0.72 (0.65*)	0.72-0.81 (0.77*)	–
Length of pronotum	0.31-0.37 (0.33*)	–	0.27-0.34 (0.30*)	0.37-0.42 (0.40)	–

Note: * Mean for each measurement.

character for total length of head at base of mandibles (TLM), width of head (WH), width of pronotum (WPr) and length of pronotum (LPr) of *C. sepangensis* also closed to measurement from holotype and Thapa (1981). However, no measurement characters for *C. travians* were closed to measurement from Thapa (1981) and Tho (1992) which indicates a size variation had occurred for *C. travians*'s soldier specimens.

Discriminant function analysis using morphometric identification formed four groups as shown in Figure 4. *C. travians* and *C. curvignathus* were clearly distinguished from other groups; however, notable overlap of data points is evident between *C. sepangensis* and *C. kalshoveni*. In the DFA analysis, three significant functions (Functions 1, 2 and 3) were determined with their variance of 56.7%, 36.3% and 7.0%, respectively (Table 5). Function 1 has higher variability of characters in the analysis. The Wilk's lambda statistic (Table 6) for the tests of Function 1 through 3 functions (Wilk's lambda = 0.06), Function 2 through 3 functions (Wilk's lambda = 0.243) and Function 3 (Wilk's lambda = 0.724) has a probability of p = 0.000. It is suggested that MxPt (1.677) and WH (-2.261) as shown in Table 7 play a prominent role in separating *Coptotermes* spp. through Function 1 (56.7% of trace). Meanwhile, in Function 2 (36.3% of trace), separation of *Coptotermes* spp. was driven primarily by opposing contributions of WH (1.563) and TLH (-0.866).

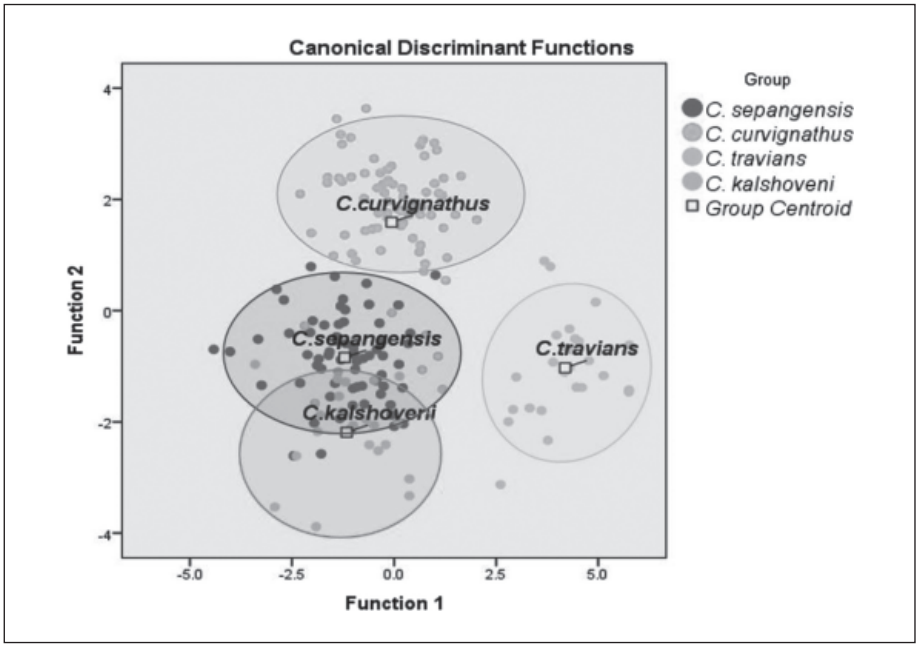

Fig. 4. CVA plot of Functions 1 and 2 of the four selected *Coptotermes* spp.

Table 5. Eigenvalues for DFA of four selected *Coptotermes* spp.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	3.078[a]	56.7	56.7	0.869
2	1.973[a]	36.3	93	0.815
3	.382[a]	7	100	0.526

Note: [a] = First 3 canonical discriminant functions were used in the analysis.

Table 6. Wilks' Lambda for DFA of four selected *Coptotermes* spp.

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	0.06	511.602	51	0
2 through 3	0.243	256.467	32	0
3	0.724	58.718	15	0

Table 7. Standardised Canonical Discriminant Function coefficients of four selected *Coptotermes* spp. Highest character loadings for each function were indicated with an arrow

Character	Function		
	1	2	3
TL	.524	.200	.095
TLH	-.619	-.866	-1.114
→ TLM	.662	-.207	.841*
LF	.986	-.744	.306
→ WH	-2.261	1.563*	-.295
WHM	.386	-.124	.695
AL1	.052	.175	.412
WA1	-.007	-.083	.142
AL2	.085	.163	-.266
WA2	.077	.029	-.169
WLb	-.147	.106	.021
LLb	.040	.419	-.049
MnWPt	-.835	-.543	.579
MxWPt	1.677*	.123	-.802
→ LPt	-.182	-.037	-.432
WPr	-.153	.153	.098
LPr	-.081	.375	.017

Note: * Diagnostic character in each function.

TL = total length; TLH = total length without head; TLM = length of head at base of mandibles; LF = length to fontanelle; WH = maximum width of head; WHM = width of head at base of mandibles; AL1 = length of antennae, segment 1; WA1 = width of antennae, segment; AL2 = length of antennae, segment 2; WA2 = width of antennae, segment 2; WLb = width of labrum; LLb = length of labrum; MnWPt = minimum width of postmentum; MxWPt = maximum width of postmentum; LPt = length of postmentum; WPr = width of pronotum; and LPr = length of pronotum.

Phylogenetic relationships inferred from mtCOII partial gene

Sequencing of the mtCOII partial gene generated 477 bp sequence fragment from all the individuals tested after a sequence trimming to align with sequence from GenBank. A pairwise sequence alignment of the partial COII gene sequences from the 34 specimens and ten specimens of *Coptotermes* spp. from NCBI containing 91 variable sites (19.1% of total sites) and 87 characters (95.6% of the variable sites) were parsimoniously informative. The mean frequency of nucleotides was biased towards Adenine (A) and Thymine (T). The A+T content at codon positions 3, 2, and 1 were 60.2%, 45.4% and 60.1%, respectively.

Genetic diversity of COII gene showed intraspecific variation of *C. curvignathus* ranging from 0% to 0.7% while *C. gestroi* showed no genetic diversity. The genetic diversity of *C. sepangensis* ranged from 0% to 1.3% in COII. *C. kalshoveni* has a genetic diversity ranging from 0.4% to 2.3%, while the genetic diversity of *C. travians* from Sarawak showed a high genetic distance with *C. travians* from Penang with 11.3%. All sequences were separated by an outgroup, *G. sulphureus* with a value range from 21.8% to 23.7%. Four major clades were observed, as shown in Figure 5. Clade I composed of all individuals that were identified morphologically as *C. curvignathus* in our study were clustered together and this cluster was consistent with the morphometric analyses conducted in the current study. A common Asian subterranean termite, *C. gestroi* in clade II formed a sister clade with the rubber tree termite, *C. curvignathus*. Clade III consists of individuals of *C. sepangensis* and *C. kalshoveni*, and the cluster was found inconsistent to DFA analyses result in which two different cluster with the overlapping measurement characters were formed for both species (Figure 4). *C. travians* in Clade IV formed a sister clade with Clade III. Close relationship was observed between *C. sepangensis* and *C. kalshoveni* in Clade III in MP tree with bootstrap value of 100%. Morphological identification of the seven colonies of *C. sepangensis* investigated in this study, showed a good agreement with their molecular phylogenetic results. *C. sepangensis* and *C. kalshoveni* were found to exhibit similar diagnostic features and closed genetic distance. Interestingly, under Clade III, two subclades of *C. sepangensis* and *C. kalshoveni* were generated, in which individuals from Sarawak and Peninsular Malaysia were grouped separately according to their locations. This may suggest geographical variation among them.

DISCUSSION

Identification of termite pest especially subterranean termite pest by using morphological character and molecular methods is believed to facilitate the development of termite control and may improve the termite pest response in addition to the currently available approaches. In this study, COII was used to infer the genetic relationship of *Coptotermes* at the species level. Through a combination of mitochondrial markers and morphological characters, we observed that the species cluster in DFA analyses corresponded well with the molecular phylogenetic tree constructed except for *C. sepangensis* and *C. kalshoveni*.

Generally, our DFA results suggest the feasibility of the size corrected morphometric data in distinguishing *Coptotermes* species. From the 17 sizes corrected characters that were found to contribute significantly to the group separation, three characters are identified as the best morphological character for differentiating the four species of *Coptotermes* in this study: the maximum width of postmentum (MxWPt); the maximum width of head (WH); and the length of head at base of mandibles (TLM). It is recommended that additional

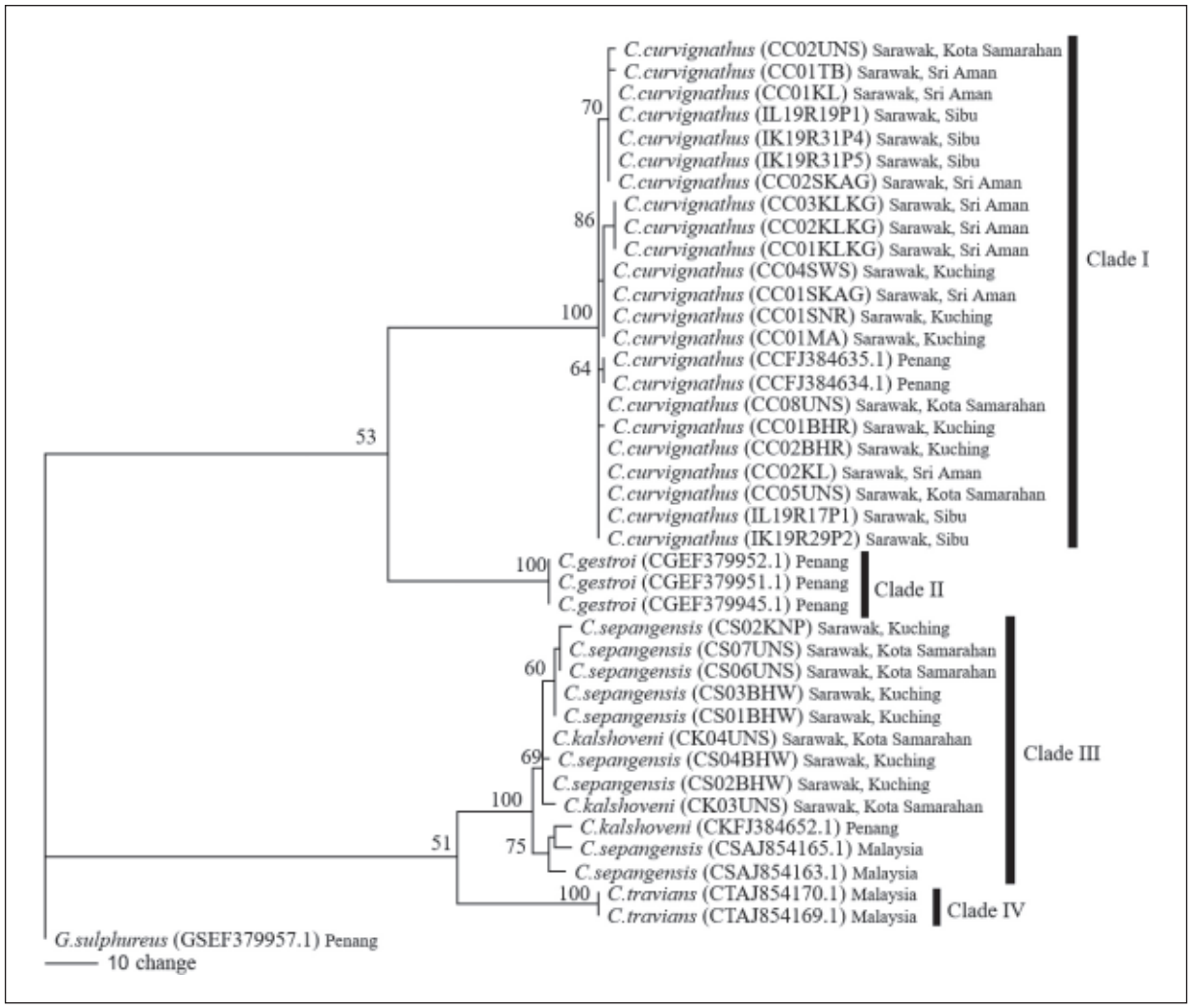

Fig. 5. Maximum parsimony (MP) analysis of COII nucleotide matrices resulted in a total of 100 (initial Max Trees setting = 100) equally most parsimonious tree (length = 205, consistency index (CI) = 0.7092, retention index (RI) = 0.9394). Bootstrap values for 1000 replicates are listed above the branches supported at e"50%.

features such as shape of mandibles and fontanelle are included in order to facilitate the field identification. Our result showed that predetermined grouping for individuals of *C. curvignathus* and *C. travians* were successfully discriminated based on the 17 characters that were used in DFA analyses. However, it was observed that there were overlapping morphological characters for *C. sepangensis* and *C. kalshoveni.* This might be due to the large size variation of the morphological characters which influenced by the age of the colony, seasonal change and degree of inbreeding (Waller, 1988; Grace *et al*., 1995; Hussenеder *et al*., 2005). Other than that, the variability of the observed distributions of canonical discriminant scores can be caused by measurement error, preservation and storage effects (e.g, shrinkage and deformation) (Smith *et al*., 2009).

C. curvignathus is relatively larger species compared to *C. sepangensis, C. kalshoveni* and *C. travians. C. curvignathus* can be easily distinguished from the three aforementioned species as it has the most incurved mandible, slender and saber shaped body and it was agreed with the descriptions from termites of Peninsular Malaysia (Thapa, 1981) and Sabah (Tho, 1992) as well as morphometric study of *Coptotermes* from Sarawak (Norsyarizan & Wan Nurainie, 2016). Apart from the mandibles, the notable diagnostic features that differentiate *C. curvignathus* from other *Coptotermes* spp. were the large and oval shaped head capsule, pronotum, postmentum and large fontanelle size. The morphological and morphometric analyses of *C. curvignathus* presented here were clearly indicated by the group separation of this species from other species and were supported by the molecular identification and phylogenetic relationship.

Our morphological variance and genetic analyses suggest that there is a potential synonym

between *C. sepangensis* and *C. kalshoveni*, where the former might have the potential to be a junior synonym to the latter. The morphology of *C. sepangensis* was found to be highly similar to *C. kalshoveni*. This is further supported by the genetic distances between these two species indicated by a low genetic diversity (0% to 3.2%). The close relationship between these two species as inferred from COII genes sequences is in agreement with the molecular phylogenetic results reported by Yeap *et al*. (2009) and Lee *et al*. (2015) that clustered *C. sepangensis* and *C. kalshoveni* together despite their different localities. However, further investigation should be performed in order to relegate the junior synonym status, such as by using alates samples instead of single soldier caste for morphology (Yeap *et al.,* 2009) along with additional molecular data using a range of genetic markers. Furthermore, the original description and type specimens (if available) should be included to provide evidence for a more robust identification of each species.

The samples of *C. sepangensis* and *C. kalshoveni* from West Malaysia (Penang) were found to be genetically related their namesake from Sarawak although they were observed to exhibit divergence and form separate subclade. It is worth noting that the current phylogenetic study was sampled from the island of Borneo, which separated from continental Asia 20,000 years ago. The South China Sea boundary, which exists between Borneo and Peninsular Malaysia, could be the factor for this group separation. Previous studies showed that *Coptotermes* spp. from Peninsular Malaysia were closely related to other *Coptotermes* spp. from Thailand and Singapore (Yeap *et al*., 2009). As Thailand, Malaysia and Singapore share a well-developed transportation system such as road highway, railway and port system (Jenkins *et al*., 2007), these have enabled the easy spread of *Coptotermes* spp. across the three countries. However, between Sarawak and the Peninsular Malaysia, the mode of transportation is restricted to sea and air access. The meticulous inspection of cargo in the ports may have parried the spread of the species across the two regions which may explain the group separation among *C. kalshoveni, C. sepangensis* and *C. travians* samples from Sarawak and Peninsular Malaysia. Futher in-depth studies will certainly require more samples of *C. sepangensis, C. kalshoveni* and *C. travians* from Peninsular Malaysia, Sabah and Sarawak to further substantiate current findings and require knowledge of population genetics of *Coptotermes* from mainland Asia.

The phylogenetic relationship as inferred from COII gene of *Coptotermes* spp. has facilitated a better understanding on the ecological differences of the species and this in turn, provides useful insights into their basic biology. *C. curvignathus* and *C. gestroi* represent structural and forestry pests in Southeast Asia and both species apparently have a wide distribution overlap in their native range. Our molecular data showed that *C. curvignathus* and *C. gestroi* have a close genetic relationship compared to the other *Coptotermes* spp. Other than that, our results showed that *C. curvignathus* was the most common species encountered (40% of the samples) in Sarawak and can be found in primary and secondary forests as well as affected houses in urban areas. It was contradictory to previous studies that reported the occasional infestation by *C. curvignathus* in West Malaysia (Lee, 2002a; Lee 2002b).

Over the past centuries, *Coptotermes* taxonomy faced major problems which involved into various subjective synonymies (Chouvenc *et al.*, 2015). Our results showed that morphological keys for the identification of *Coptotermes* species have certain limitations such as a lack of quantitative description of diagnostic characters and wide variation of intraspecific soldier morphology. Future phylogenetic studies of *Coptotermes* species need to be intensified by the addition of the description of alates along with soldiers and in the case of large sampling, quantitative morphometrics should be considered. Such tools will allow much greater certainty for the discovery of new cryptic species and clarification of synonymous species. The mtCOII genes implemented in our study for the phylogenetic relationship was found to be consistent with morphometric data. Our findings provide new insights into the infestation patterns of *C. curvignathus* between Peninsular Malaysia and Sarawak. Furthermore, we proved that molecular data is valuable in assisting the identification of termites. The results testify the overlapping of morphological characteristics of *C. sepangensis* and *C. kalshoveni,* which can be identified by molecular techniques and it suggested that *C. sepangensis* has potential to be a junior synonym to *C. kalshoveni*.

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