Journal of Insect Science, (2018) 18(2): 26; 1–10
doi: 10.1093/jisesa/iey018
Research

The Complete Mitochondrial Genome of *Coptotermes 'suzhouensis'* (syn. *Coptotermes formosanus*) (Isoptera: Rhinotermitidae) and Molecular Phylogeny Analysis

Juan Li,[1] Jin-long Zhu,[1] Shi-di Lou,[1] Ping Wang,[1] You-sen Zhang,[2] Lin Wang,[1] Ruo-chun Yin,[1] and Ping-ping Zhang[1]

[1]School of Life Sciences, Anhui University, Hefei 230601, Anhui, China, [2]Hefei Termite Control Institute, Hefei 230001, Anhui, China, and Corresponding author, e-mail: eveyin@163.com

Received 7 November 2017; Editorial decision 30 January 2018

Abstract

Coptotermes suzhouensis (Isoptera: Rhinotermitidae) is a significant subterranean termite pest of wooden structures and is widely distributed in southeastern China. The complete mitochondrial DNA sequence of *C. suzhouensis* was analyzed in this study. The mitogenome was a circular molecule of 15,764 bp in length, which contained 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNA genes, and an A+T-rich region with a gene arrangement typical of Isoptera mitogenomes. All PCGs were initiated by ATN codons and terminated by complete termination codons (TAA), except *COX2*, *ND5*, and *Cytb*, which ended with an incomplete termination codon T. All tRNAs displayed a typical clover-leaf structure, except for $tRNA^{Ser(AGN)}$, which did not contain the stem-loop structure in the DHU arm. The A+T content (69.23%) of the A+T-rich region (949 bp) was higher than that of the entire mitogenome (65.60%), and two different sets of repeat units (A+B) were distributed in this region. Comparison of complete mitogenome sequences with those of *Coptotermes formosanus* indicated that the two taxa have very high genetic similarity. Forty-one representative termite species were used to construct phylogenetic trees by maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogenetic analyses also strongly supported (BPP, MLBP, and MPBP = 100%) that all *C. suzhouensis* and *C. formosanus* samples gathered into one clade with genetic distances between 0.000 and 0.002. This study provides molecular evidence for a more robust phylogenetic position of *C. suzhouensis* and inferrs that *C. suzhouensis* was the synonymy of *C. formosanus*.

Key words: *Coptotermes suzhouensis*, mitochondrial genome, phylogenetic analysis, synonymy

Introduction

Termites (Isoptera) (Eggleton et al. 2007) comprise more than 3,000 species in approximately 283 genera (Inward et al. 2007a, Krishna et al. 2013, Cheng 2014). Of all termite species recognized globally, only 183 are significant pest species known to damage buildings (Krishna et al. 2013, Chouvenc et al. 2015), of which the genus *Coptotermes* contains the largest number (18) of pest species (Rust and Su 2012). Due to their destructive effect on wooden structures and their essential role in decomposition and nutrient recycling, research on the biological characteristics, classification, identification, and phylogenetic relationships of termites has received increasing attention (Wolstenholme 1992, Brody et al. 2010, Hausberger et al. 2011, Korb et al. 2015, Chouvenc et al. 2016, Bourguignon et al. 2016, Rocha et al. 2017). In China, 4 families, 44 genera, and 473 species of termites have been recorded (Huang et al. 2000, Cheng 2014). The Rhinotermitidae are widely distributed in China, with approximately 200 described species in seven genera, including representatives of major pest genera *Coptotermes*, *Reticulitermes*, and *Heterotermes* (Huang et al. 2000, Cheng 2012). The difficulty in identifying termites is recognized by many termite classification experts, especially in the genus *Coptotermes* (Emerson 1952, Watson and Gay 1991, Huang et al. 2000, Chouvenc et al. 2016). Taxonomic limits between species in genus *Coptotermes*, are poorly established, which has resulted in different names being used for the same species (Chouvenc et al. 2016). This has created additional difficulties in the identification of termites, which were already been complicated by the limited morphological features available for identifications. The number of species in the genus *Coptotermes* is frequently revised, as synonyms of other species are recognised (Engel and Krishna 2004, Krishna et al. 2013, Cameron 2014a). Most species of *Coptotermes* are considered to be similar to each other, and their identification is thus very difficult. Kambhampati (2000) also thought that many synonyms were found in the nomenclature of termites based on morphological classifications. The

classification and identification of *Coptotermes* have been revised based on molecular data (Cai and Chen 1964, Huang et al. 2000, Xu et al. 2009, Cheng 2012). To date, 22 species of *Coptotermes* have been recorded in China (Krishna et al. 2013). *Coptotermes suzhouensis* (Isoptera: Rhinotermitidae) is very similar to *Coptotermes formosanus* (Isoptera: Rhinotermitidae), a major invasive species, in morphology. *C. suzhouensis* is widely distributed in the regions of southeastern China and is a pest of wooden buildings (Xia and He 1986, Huang et al. 2000, Li 2002, Cheng 2012). There has always been controversy regarding the relationship between the two species (*C. suzhouensis* and *C. formosanus*).

In the study, we determined the complete mitogenome sequence of *C. suzhouensis* and compared it to available mitogenomes of other termites using phylogenetic analyses. In doing so, we provide robust molecular evidence for the taxonomic status of *C. suzhouensis* and reveal the evolutionary relationships of *C. suzhouensis* and *C. formosanus*.

Materials and Methods

Collection and Storage

Specimens of *C. suzhouensis* were collected from colonies of termites living in wooden buildings in Feixi County in Hefei, Anhui Province, China (31°42′31″N, 117°10′38″E). The specimens were preserved in 95% ethanol and stored at –20°C until DNA extraction.

Morphological Identification of Termites

Soldier specimens of all the populations collected were identified by the Hefei Termite Control Institute based on their morphological characteristics (Xia and He 1986, Huang et al. 2000).

DNA Isolation, Polymerase Chain Reaction, and Sequencing

Whole genomic DNA was extracted from the heads of 20 soldiers using One-tube General Sample DNAup Kit (Sangon Biotech, Shanghai, China) for polymerase chain reaction (PCR), following manufacturer's instructions. Mitochondrial genome sequences of the termites related to *C. suzhouensis* were downloaded from the NCBI database. ClustalX ver1.8 was used for alignment, and Premier Primer 5 software was applied to design five pairs of primers from conserved regions (Table 1). Fragments were amplified by long distance PCR using Takara LA Taq (Takara Bio, Japan) with the following cycling conditions: an initial denaturation for 3 min at 94°C, followed by 35 cycles of denaturation at 94°C (30 s), annealing at 52–58°C (30 s; Table 1), elongation at 72°C (10 min); and a final extension period 72°C (10 min). PCR products were checked using 1% agarose gels and were sequenced at MAP Biotech Company (Shanghai, China) with ABI3730 Genetic Analyzer.

Sequence Assembly, Annotation, and Analysis

Sequences were assembled and aligned with complete mitogenomes from *C. formosanus* in Sequencher 4.1.4. The location, size, and coding direction of each gene, including 13 protein coding genes (PCGs), 22 tRNA genes, and two rRNA genes were determined with DOGMA (Wyman et al. 2004). Secondary structures of tRNA were predicted using MITOS (Bernt et al. 2013) and tRNAscan-SE Search Server v.2.0 online (Lowe and Chan 2016). Amino acid composition and coding region for each PCG was identified with ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Nucleotide composition statistics (excluding stop codons) and relative synonymous codon usage (RSCU) of 13 PCGs were calculated with MEGA 5.1 (Tamura et al. 2014). Composition skew analysis was calculated according to formulas AT skew = [A–T]/[A+T] and GC skew = [G–C]/[G+C], respectively (Perna and Kocher 1995).

The Reconstruction of Phylogenetic Trees

Along with the *C. suzhouensis* mitochondrial genome, 41 termite mitogenomes and two outgroup species (*Shelfordella lateralis* and *Periplaneta australasiae*: Blattodea) were used in phylogenetic analysis. Sampling and sequence availability used in this study are summarized in Table 2. Concatenated amino acid sequences from the 13 PCGs was used in phylogenetic analysis, with maximum likelihood (RAxML7.03; Stamatakis et al. 2008), maximum parsimony (PAUP 4.0b10; Swofford 2002), and Bayesian inference methods (Mr Bayes v.3.1.2) (Huelsenbeck and Ronquist 2001). Modeltest ver3.06 (Posada and Crandall 1998) was used to infer best-fitting model for Bayesian inference (BI) and maximum likelihood (ML) analysis. The GTR + I + G model was selected based on the Akaike information criterion (Akaike 1974). Bayesian inference was performed with the following settings: four MCMC chains (one cold chain and three hot chains) for 10,000,000 generations until the average standard deviation of split frequencies reached a value less than 0.01. Bayesian posterior probabilities (BPP) were calculated from the sample points after the MCMC algorithm had started to converge. In ML and MP analyses, a heuristic search with 100 random addition replicates was applied. BPP values were mapped onto the tree, and nodal support for ML&MP was assessed using nonparametric bootstrapping (Felsenstein 1985) in PAUP for the MP analyses (MPBP) and RAxML (Stamatakis et al. 2008) for ML (MLBP) using 1,000 pseudoreplicates each.

Table 1. The primers for PCR in this study

Primers	Sequences (5′–3′)	Positions (5′–3′)	Size of PCR product (bp)	Annealing temperatures (°C)
Primer-1-F	TATCGCCATACCATCACTACGACTCCTA	3,340–3,367	5,154	55
Primer-1-R	TGCTCCCCCTTCTCTTAATCTTCTCGGT	8,493-8,466		
Primer-2-F	GAACCAAAGCAGACACAGGAGTAGGAGC	7,481–7,508	4,742	58
Primer-2-R	TGGGCTTCGTGCTTTGGCTCAGACTATC	12,222-12,195		
Primer-3-F	AGAAACCAACTCCGATTCCCCCTCAGCA	11,988–12,015	7,427	58
Primer-3-R	GTCGTCCTGGTGTGGCGTCTGTTTTTAC	3,650-3,623		
Primer-COX3-F	ATTCCACCAATACGACAACAGCCTA	4,872–4,896	582	52
Primer-COX3-R	GAGAAGTGTAGGGCTGCTTGTCGTA	5,453-5,429		
Primer-Cytb-F	GACATCAATACCGCATTTTCCAGAG	10,638–10,662	850	52
Primer-Cytb-R	GTCGTGCTCCGATTCAGGTAAGTAG	11,487-11,463		

Table 2. Detailed information of the termites species analyzed in this study

Family	Species	Size (bp)	Accession number	References
Rhinotermitidae	*Reticulitermes aculabialis*	16,475	KP334994.1	Wang et al. (2015)
	Reticulitermes chinensis	15,925	KM216388.1	Chen et al. (2016)
	Reticulitermes flavipes	16,565	EF206314.1	Cameron and Whiting (2007)
	Reticulitermes hageni	16,590	EF206320.1	Cameron and Whiting (2007)
	Reticulitermes santonensis	16,567	EF206315.1	Cameron and Whiting (2007)
	Reticulitermes virginicus	16,513	EF206318.1	Cameron and Whiting (2007)
	Reticulitermes labralis	15,914	KU877221.1	Wang et al. (2016)
	Reticulitermes speratus kyushuensis	15,898	KY484910.1	Lee et al. (2017)
	Reticulitermes sp.	14,907	KU925239.1	Bourguignon et al. (2017)
	Reticulitermes grassei	14,910	KU925237.1	Bourguignon et al. (2017)
	Reticulitermes flaviceps	16,485	KX712090.1	Chen et al. (2016)
	Heterotermes sp.	16,370	JX144936.1	Cameron et al. (2012)
	Heterotermes cf.occiduus	14,919	KU925230.1	Bourguignon et al. (2017)
	Heterotermes cf. *paradoxus*	14,904	KU925225.1	Bourguignon et al. (2017)
	Heterotermes nr. tenuis	14,944	KU925228.1	Bourguignon et al. (2017)
	Heterotermes platycephalus	14,919	KU925231.1	Bourguignon et al. (2017)
	Heterotermes tenuis	14,940	KU925233.1	Bourguignon et al. (2017)
	Heterotermes validus	14,922	KU925235.1	Bourguignon et al. (2017)
	Coptotermes acinaciformis raffrayi	14,897	KU925196.1	Bourguignon et al. 2017
	Coptotermes acinaciforis	14,896	KU925198.1	Bourguignon et al. (2017)
	Coptotermes amanii	14,894	KU925200.1	Bourguignon et al. (2017)
	Coptotermes lacteus	16,326	JX144934.1	Cameron et al. (2012)
	Coptotermes frenchi	14,912	KU925204.1	Bourguignon et al. (2017)
	Coptotermes gestroi	14,919	KU925205.1	Bourguignon et al. (2017)
	Coptotermes heimi	14,908	KU925206.1	Bourguignon et al. (2017)
	Coptotermes kalshoveni	14,889	KU925210.1	Bourguignon et al. (2017)
	Coptotermes michaelseni	14,900	KU925212.1	Bourguignon et al. 2017
	Coptotermes remotus	14,742	KU925213.1	Bourguignon et al. 2017
	Coptotermes sjoestedti	14,899	KU925217.1	Bourguignon et al. 2017
	Coptotermes sepangensis	14,905	KU925215.1	Bourguignon et al. (2017)
	Coptotermes travians	14,892	KU925222.1	Bourguignon et al. (2017)
	Coptotermes testaceus	15,752	KR872938.1	Li et al. (2016)
	Coptotermes formosanus	14,908	KU925203.1	Bourguignon et al. (2017)
	Coptotermes formosanus	16,324	AB626147.1	Tokuda et al. (2012)
	Coptotermes formosanus	16,326	AB626146.1	Tokuda et al. (2012)
	Coptotermes formosanus	1,326	AB626145.1	Tokuda et al. (2012)
	Coptotermes suzhouensis	15,764	MG000963	present study
Termitidae	*Macrotermes barneyi*	15,940	JX050221.1	Wei et al. (2012)
	Acanthotermes acanthothorax	15,231	KP026280.1	Bourguignon et al. (2015)
	Ancistrotermes pakistanicus	15,299	KP026267.1	Bourguignon et al. (2015)
	Macrotermes natalensis	16,325	KM405637.1	Meng et al. (2016)
	Macrotermes subhyalinus	16,351	JX144937.1	Cameron et al. (2012)
Serritermitidae	*Serritermes serrifer*	14,783	KP026264.1	Bourguignon et al. (2015)
	Glossotermes occulatus	14,791	KP026291.1	Bourguignon et al. (2015)
Kalotermitidae	*Glyptotermes satsumensis*	15,611	KP026257.1	Bourguignon et al. (2015)
	Cryptotermes secundus	15,695	KP026283.1	Bourguignon et al. (2015)
Mastotermitidae	*Mastotermes darwiniensis*	15,487	JX144929.1	Cameron et al. (2012)
Outgroup	*Shelfordella lateralis*	15,601	KU684413.1	Cheng et al. (2016)
	Periplaneta australasiae	15,605	KX640825.1	Ma et al. (2017)

Results

Sequencing and Organization of Mitochondrial Genome

The mitochondrial genome of *C. suzhouensis* is a typical circular DNA molecule of 15,764 bp in length (GenBank: MG000963). The mitochondrial DNA (mtDNA) consisted of 13 PCGs (*ATP6*, *ATP8*, *COX1-3*, *ND1-6*, *ND4L*, and *Cytb*), 2 rRNA genes (srRNA and lrRNA), 22 tRNA genes, and a noncoding A+T-rich region (Fig. 1, Table 4). Nucleotide composition was A/T (65.60%) biased and composed as follows: A = 6,891 (43.71%), T = 3,450 (21.89%), G = 1,854 (11.76%), C = 3,569 (22.64%), which is commonly observed in termites' mitogenomes (Bourguignon et al. 2015; Table 3). In total, 23 genes (9 PCGs and 14 tRNAs) were located on the majority strand (H-strand) and the others (4 PCGs, 8 tRNAs, and 2 rRNAs) were located on the minority strand (L-strand; Fig. 1). The order and the orientation of the genes were identical to those previously reported from other *Coptotermes* species, which retain the ancestral insect arrangement.

The mitogenome of *C. suzhouensis* harbored a total of 139 bp of intergenic spacers, made up of 19 individual regions ranging in size from 1 to 21 bp. There were 35 base pairs of overlapping regions total, at 10 intergenic positions, which ranged in size from 1 to 8 bp (Table 4).

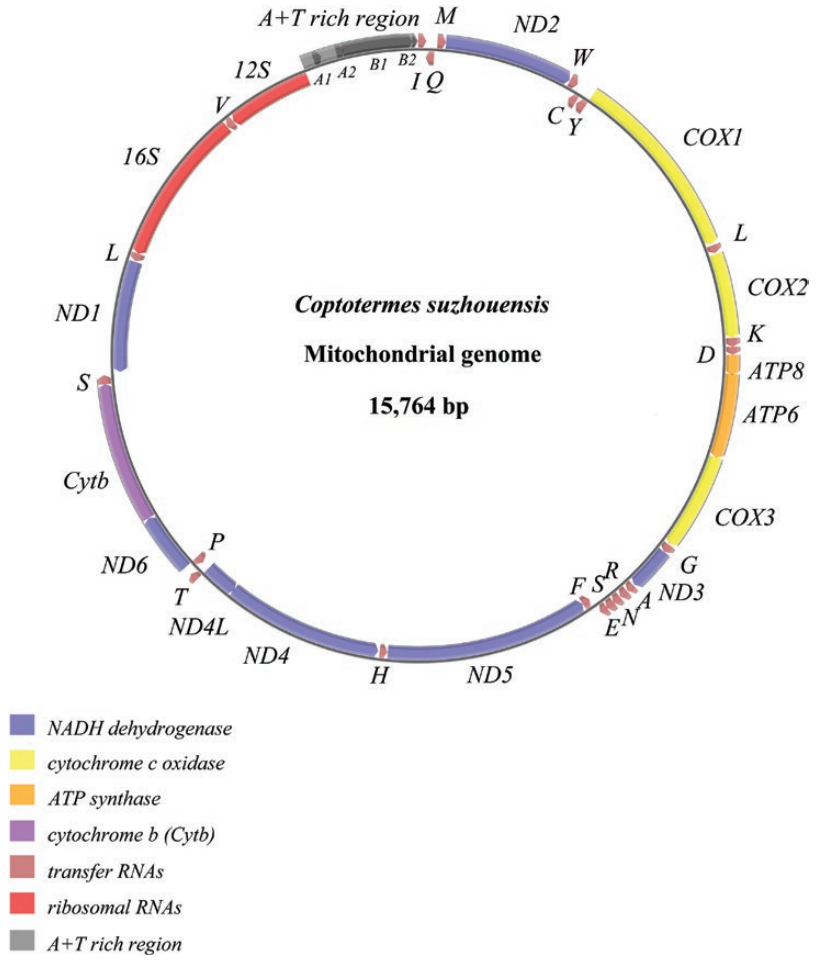

Fig. 1. Circular map of the mitogenome of *C. suzhouensis*. Genes encoded on the H-strand (clockwise orientation) are colored in the outside. Genes encoded on the L-strand (anticlockwise orientation) are colored in the inside.

Table 3. Nucleotides composition of the *C. suzhouensis* mitochondrion in different regions

Feature	Proportion of nucleotides							
	A%	T%	G%	C%	A+T%	AT-skew	GC-skew	Siz e(bp)
Whole genome	43.71	21.89	11.76	22.64	65.60	0.33	−0.32	15,764
PCGs	43.52	21.06	12.05	23.37	64.58	0.35	−0.32	11,166
tRNA genes	39.05	26.84	13.82	20.29	65.89	0.19	−0.19	1,498
srRNA genes	45.25	20.63	10.45	23.66	65.89	0.37	−0.39	727
lrRNA gene	48.56	22.05	8.56	20.83	70.61	0.38	−0.42	1,320
A+T-rich region	43.94	25.29	11.70	19.07	69.23	0.27	−0.24	949

Protein-Coding Genes

The total length of the 13 PCGs was 11,166 bp, representing 70.83% of the entire mitochondrial genome. PCGs used ATN as initiation codon, all had ATG as start codon, except for *ATP8*, *ND3*, *ND5*, *ND6* (ATA), and *COX1* (ATT). Ten PCGs used the standard stop codon TAA, whereas *COX2*, *ND5*, and *Cytb* genes used a single T nucleotide. Codon usage of the PCGs exhibited an AT bias with an A+T composition of 64.58% (Table 3). It was found that the relative synonymous codon usage values of NNU/NNA codons was essentially greater than of NNC/NNG, indicating higher U+A bias at third condons. Our analysis showed that UUU (Phe), CUA (Leu), AUA (Met), and AUU (Ile) were the most frequently used codons, accounting for 19.32% of all the codons (Table 5).

Transfer RNA and Ribosomal RNA Genes

The mtDNA contained 22 tRNA genes, ranging in size from 63 bp ($tRNA^{Ala}$) to 76 bp ($tRNA^{Tyr}$) in length, and were AT biased (65.89%). The secondary structures of tRNAs were predicted by MITOS online (Bernt et al. 2013) and tRNAscan-SE 2.0 online (Lowe and Chan 2016). The genes that could not be detected by the softwares were determined through comparison with published mitogenomes of *Coptotermes* (Cameron et al. 2012, Tokuda et al. 2012). All tRNAs could be folded into typical cloverleaf secondary structures, with the exception of the $tRNA^{Ser(AGN)}$, which was lacking a stable stem-loop structure in the DHU arm, as observed in most insects (Cameron 2014b; Fig. 2) .

Table 4. Annotation and gene organization of the *C. suzhouensis* mitogenome

Gene	Coding strand	Region	Size (bp)	Intergenic nucleotide	Overlapping nucleotide	Anticodon	Start codon	Stop codon
$tRNA^{Ile}$	H	1–66	66			GAT (30–32)		
$tRNA^{Gln}$	L	64–133	70		3	TTG (101–103)		
$tRNA^{Met}$	H	155–223	69	21		CAT (187–189)		
ND2	H	224–1,261	1,038				ATG	TAA
$tRNA^{Trp}$	H	1,267–1,334	68	5		TCA (1,298–1,300)		
$tRNA^{Cys}$	L	1,327–1,395	69		8	GCA (1,363–1,365)		
$tRNA^{Tyr}$	L	1,406–1,481	76	10		GTA (1,442–1,444)		
COX1	H	1,483–3,030	1,548	1			ATT	TAA
$tRNA^{Leu(UUR)}$	H	3,042–3,107	66	11		TAA (3,071–3,073)		
COX2	H	3,116–3,800	685	8			ATG	T
$tRNA^{Lys}$	H	3,801–3,871	71			CTT (3,831–3,833)		
$tRNA^{Asp}$	H	3,871–3,935	65		1	GTC (3,902–3,904)		
ATP8	H	3,936–4,094	159				ATA	TAA
ATP6	H	4,088–4,768	681		7		ATG	TAA
COX3	H	4,768–5,556	789		1		ATG	TAA
$tRNA^{Gly}$	H	5,563–5,630	68	6		TCC (5,597–5,599)		
ND3	H	5,631–5,984	354				ATA	TAA
$tRNA^{Ala}$	H	5,994–6,056	63	9		TGC (6,023–6,025)		
$tRNA^{Arg}$	H	6,062–6,130	69	5		TCG (6,091–6,093)		
$tRNA^{Asn}$	H	6,136–6,203	68	5		GTT (6,167–6,169)		
$tRNA^{Ser(AGN)}$	H	6,201–6,272	72		3	GCT (6,228–6,230)		
$tRNA^{Glu}$	H	6,270–6,333	64		3	TTC(6,299–6,301)		
$tRNA^{Phe}$	L	6,345–6,412	68	11		GAA (6,376–6,378)		
ND5	L	6,413–8,138	1,726				ATA	T
$tRNA^{His}$	L	8,142–8,206	65	3		GTG (8,173–8,175)		
ND4	L	8,220–9,554	1,335	13			ATG	TAA
ND4L	L	9,548–9,835	288		7		ATG	TAA
$tRNA^{Thr}$	H	9,839–9,903	65	3		TGT (9,869–9,871)		
$tRNA^{Pro}$	L	9,903–9,971	69		1	TGG (9,938–9,940)		
ND6	H	9,973–10,464	492	1			ATA	TAA
Cytb	H	10,464–11,595	1,132		1		ATG	T
$tRNA^{Ser(UCN)}$	H	11,596–11,668	73			TGA (11,629–11,631)		
ND1	L	11,688–12,626	939	19			ATG	TAA
$tRNA^{Leu(CUN)}$	L	12,633–12,699	67	6		TAG (12,668–12,670)		
lrRNA(16S)	L	12,700–14,019	1,320					
$tRNA^{Val}$	L	14,021–14,087	67	1		TAC (14,056–14,058)		
srRNA(12S)	L	14,089–14,815	727	1				
A+T-rich	nc	14,816–15,764	949					
Repeat A1	nc	14,913–14,978	66					
Repeat A2	nc	15,102–15,167	66					
Repeat B1	nc	15,156–15,717	562					
Repeat B2	nc	15,718–15,755	38					

Table 5. Codon usage in 13 PCGs of the *C. suzhouensis* mitochondrial genome

Codon (aa)	Count	%	RSCU	Codon (aa)	Count	%	RSCU	Codon (aa)	Count	%	RSCU	Codon (aa)	Count	%	RSCU
UUU(F)	201	5.42	1.24	UCU(S)	96	2.59	2.12	UAU(Y)	102	2.75	1.29	UGU(C)	38	1.02	1.55
UUC(F)	122	3.29	0.76	UCC(S)	19	0.51	0.42	UAC(Y)	56	1.51	0.71	UGC(C)	11	0.3	0.45
UUA(L)	103	2.78	1.16	UCA(S)	102	2.75	2.25	UAA(*)	0	0	0	UGA(W)	68	1.83	1.33
UUG(L)	146	3.93	1.64	UCG(S)	10	0.27	0.22	UAG(*)	0	0	0	UGG(W)	34	0.92	0.67
CUU(L)	61	1.64	0.68	CCU(P)	45	1.21	1.22	CAU(H)	22	0.59	0.61	CGU(R)	21	0.57	1.4
CUC(L)	17	0.46	0.19	CCC(P)	17	0.46	0.46	CAC(H)	50	1.35	1.39	CGC(R)	0	0	0
CUA(L)	189	5.09	2.12	CCA(P)	82	2.21	2.23	CAA(Q)	53	1.43	1.51	CGA(R)	35	0.94	2.33
CUG(L)	19	0.51	0.21	CCG(P)	3	0.08	0.08	CAG(Q)	17	0.46	0.49	CGG(R)	4	0.11	0.27
AUU(I)	155	4.18	1.11	ACU(T)	46	1.24	0.76	AAU(N)	65	1.75	0.87	AGU(S)	32	0.86	0.71
AUC(I)	124	3.34	0.89	ACC(T)	44	1.19	0.73	AAC(N)	85	2.29	1.13	AGC(S)	6	0.16	0.13
AUA(M)	172	4.63	1.42	ACA(T)	141	3.8	2.33	AAA(K)	60	1.62	1.52	AGA(S)	79	2.13	1.75
AUG(M)	70	1.89	0.58	ACG(T)	11	0.3	0.18	AAG(K)	19	0.51	0.48	AGG(S)	18	0.49	0.4
GUU(V)	114	3.07	1.82	GCU(A)	50	1.35	1.09	GAU(D)	36	0.97	1.04	GGU(G)	91	2.45	1.45
GUC(V)	18	0.49	0.29	GCC(A)	27	0.73	0.59	GAC(D)	33	0.89	0.96	GGC(G)	14	0.38	0.22
GUA(V)	96	2.59	1.53	GCA(A)	94	2.53	2.04	GAA(E)	63	1.7	1.47	GGA(G)	117	3.15	1.86
GUG(V)	23	0.62	0.37	GCG(A)	13	0.35	0.28	GAG(E)	23	0.62	0.53	GGG(G)	29	0.78	0.46

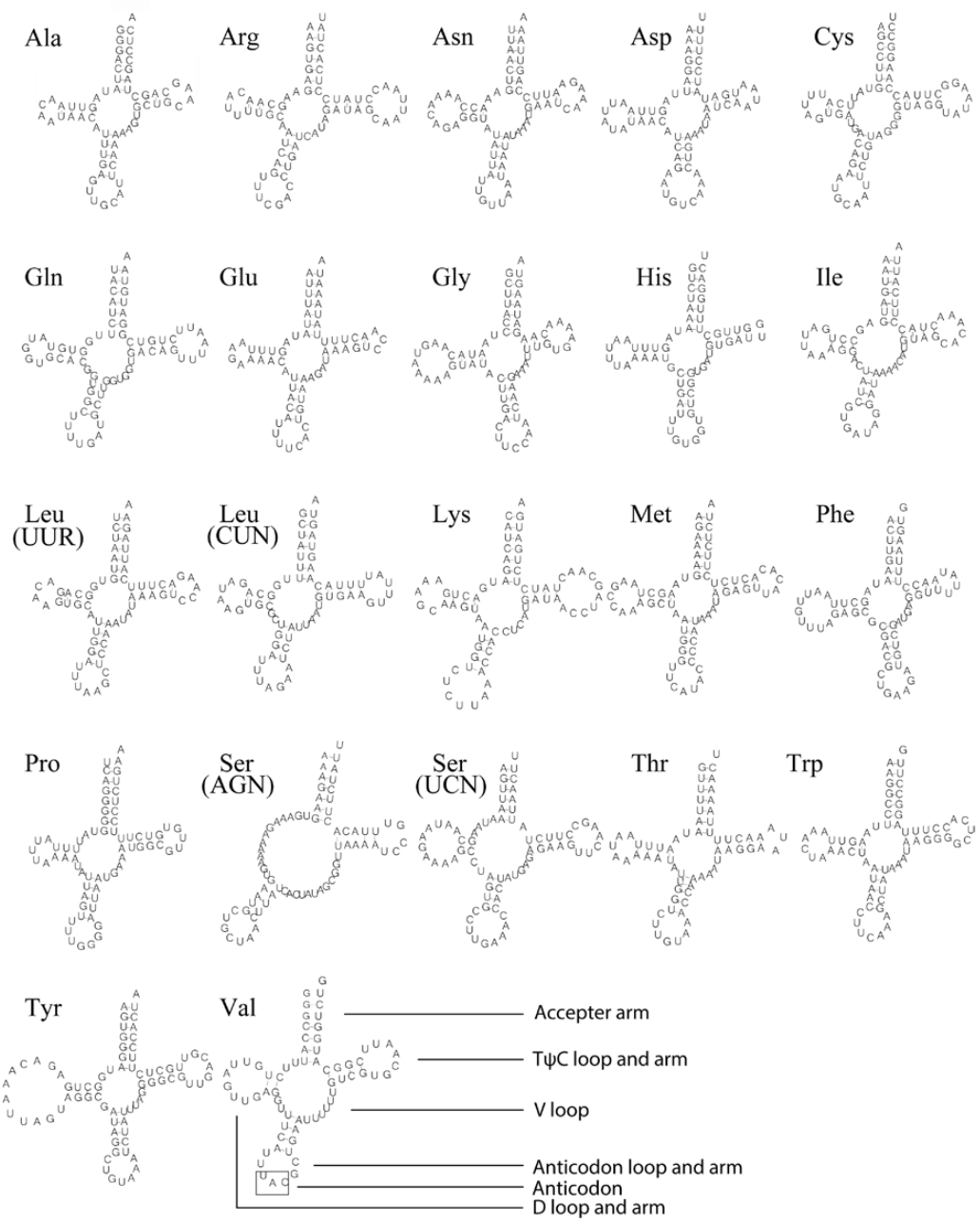

Fig. 2. Secondary structures for 22 tRNAs of *C. suzhouensis* mitogenome predicted by the the MITOS and tRNAscan-SE 2.0 online.

As typically observed in other insect mitogenomes, two rRNA genes (srRNA and lrRNA) were found on the L strand of the mitogenome, which were located between $tRNA^{Leu(CUN)}$ and $tRNA^{Val}$, $tRNA^{Val}$ and CR region, respectively. The rRNAs of *C. suzhouensis* were 1,320 bp for lrRNA and 727 bp for srRNA in length, and the A+T content of the two genes was 70.61 and 65.89%, respectively.

Control Region

The 949-bp control region of *C. suzhouensis* was located between srRNA and $tRNA^{Ile}$ with an A+T content of 69.23%, which was higher than that of the complete mitogenome (65.60%). There were two different sets of repeat units in the CR zone (A+B repeats). The A repeats contained two identical units A1 and A2 (66 bp). The B repeats consisted of one complete unit B1 (562 bp) and a partial unit B2 (38 bp).

Homology Analysis of Mitochondrial Sequences of *C. suzhouensis*

To explore the phylogenetic potential of the determined sequence, we performed multiple alignment of the mitogenomes determined for *C. suzhouensis* and Rhinotermitidae. The nucleic acid similarity rate between these taxa was found to rang from 85 to 99%. Further, *C. suzhouensis* shared the highest homology with *C. formosanus*, with nucleic acid similarity of more than 99%, whereas the deduced amino acids similarity of individual PCGs ranged from 99.72 to 100%. The base composition of Rhinotermitidae mitogenomes showed a high degree of similarity with A+T biased (61.77–66.33%).

Differences in mitochondrial sequences between *C. suzhouensis* and *C. formosanus* were shown in Table 6. Comparison of complete mitogenome sequences with the three populations of *C. formosanus Shiraki* (AB626145.1–AB626147.1) showed five to seven site

Table 6. Differences in the nucleotides of *C. suzhouensis* and *C. formosanus* mitochondrial genomes

Position	Gene	*C. suzhouensis* (MG000963) 15,764 bp	*C. formosanus* (AB626145.1) 16,326 bp	*C. formosanus* (AB626146.1) 16,326 bp	*C. formosanus* (AB626147.1) 16,324 bp	*C. formosanus* (KU925203.1) 14,908 bp
482	*ND2*	T(Phe)	T	T	T	**C(Leu)**
646		A(Met)	A	A	A	G(Met)
1,578	*COX1*	T(Leu)	T	T	T	C(Leu)
1,990		A(Met)	A	A	A	**G(Val)**
2,061		G(Leu)	G	G	G	A(Leu)
2,334		T(Phe)	C(Phe)	C(Phe)	C(Phe)	C(Phe)
3,056	$tRNA^{Leu(UUR)}$	C	C	C	C	T
3,063		A	A	A	A	G
3,337	*COX2*	A(Val)	A	A	A	G(Val)
3,397		G(Thr)	A(Thr)	A(Thr)	A(Thr)	A(Thr)
3,412		T(Ala)	T	T	T	A(Ala)
3,824	$tRNA^{Lys}$	A	A	C	A	A
4,223	*ATP6*	A(Met)	A	A	A	**T(Leu)**
4,348		C(Phe)	C	C	C	T(Phe)
4,465		T(His)	T	T	T	C(His)
5,580	$tRNA^{Gly}$	A	A	A	—	—
5,581		A	A	A	—	—
5,928	*ND3*	T(Leu)	T	T	T	C(Leu)
5,981		A(Lys)	A	A	A	**C(Asn)**
6,020	$tRNA^{Ala}$	A	A	A	A	G
7,598	*ND5*	T(Met)	T	T	T	**C(Val)**
7,719		C(Met)	C	C	C	T(Met)
7,866		C(Ser)	C	C	C	A(Ser)
8,523	*ND4*	C(Trp)	C	C	C	T(Trp)
10,722	*Cytb*	G(Gly)	G	**A(Ser)**	G	G
11,687–11,688	*intergenic region* ($tRNA^{Ser(UCN)}$—*ND1)*	—	—	—	—	TTAC
11,978	*ND1*	T(Ile)	**C(Val)**	**C(Val)**	T	T
13,040	*lrRNA(16S)*	C	T	T	T	T
13,660		C	C	C	T	T
14,514	*srRNA(12S)*	A	A	A	A	—
14,590		A	A	A	A	T
14,664		T	T	T	T	C
14,765		C	C	C	C	T
14,963	*CR*	T	C	C	T	—
14,816–15,764		(14,816–15,764) 949bp (A1,A2,B1,B3)	(14,816–16,326) 1,511bp (A1,A2.B1,B2,B3)	(14,816–16,326) 1,511bp (A1.,A2,B1,B2,B3)	(14,816–16,326) 1,511bp (A1,A2,B1,B2,B3)	— — —

Nonsynonymous substitutions are indicated with the bold font; corresponding amino acids are shown in parentheses. Positions are relevant to MG000963. Deletions are indicated as —.

differences and a long fragment deletion (B1 repeat region of 562 bp; 3.47% sequence divergence; Table 6). Variable sites between *C. suzhouensis* and *C. formosanus* were located in four PCGs (*COX1*, *COX2*, *Cytb*, and *ND1*), $tRNA^{Gly}$, lrRNA (16S), and Repeat A1 region; variation in *COX1* and *COX2* was synonymous. However, two sites in *Cytb* and *ND1* were nonsynomyous. The A to G (*C. suzhouensis*) at position 10,722 led to serine (AGA) to glycine (GGA) transformation, whereas the C to T at 11,978 led to valine (GTT) to isoleucine (ATT) transformation, respectively. In contrast, the differences between *C. suzhouensis* and *C. formosanus* (KU925203) were greater, with 31 differences, of which 13 were synonymous, and 5 were nonsynomyous. Comparative analyses suggested that the complete mtDNA of *C. suzhouensis* and *C. formosanus* are highly similar, consistant with them being the same species.

Phylogenetic Relationships

Phylogenetic trees were built from 13 PCGs using three different methods (ML, MP, and BI). The topological structure of the phylogenetic tree was largely consistent, and the classification of the family was clear. The analyzed species (41 termites) were divided into five major clades with the basic framework: (Mastotermitidae + (Kalotermitidae + (Serritermitidae + (Termitidae + Rhinotermitidae)))) (Fig. 3). In the phylogenetic tree, *Coptotermes*, *Heterotermes*, and *Reticulitermes* formed a monophyletic group, and the relationship between the three genera was *Reticulitermes* + (*Heterotermes* + *Coptotermes*), which is consistent with morphological data, and findings of previous molecular studies (Bourguignon et al. 2015, Bourguignon et al. 2017, Inward et al. 2007b, Lo et al. 2004).

Phylogenetic analyses suggested that *C. suzhouensis* and *C. formosanus* were clustered in one branch with strong support (BPP = 100%, MLBP = 100%, and MPBP = 100%), and these two groups formed a sister group to (*C. kalshoveni* + (*C. remotus* + *C. sepangensis*)). The genetic distances between *C. suzhouensis* and several *C. formosanus* samples was 0.000 (AB626145.1–AB626147.1), while the genetic distance between *C. suzhouensis*

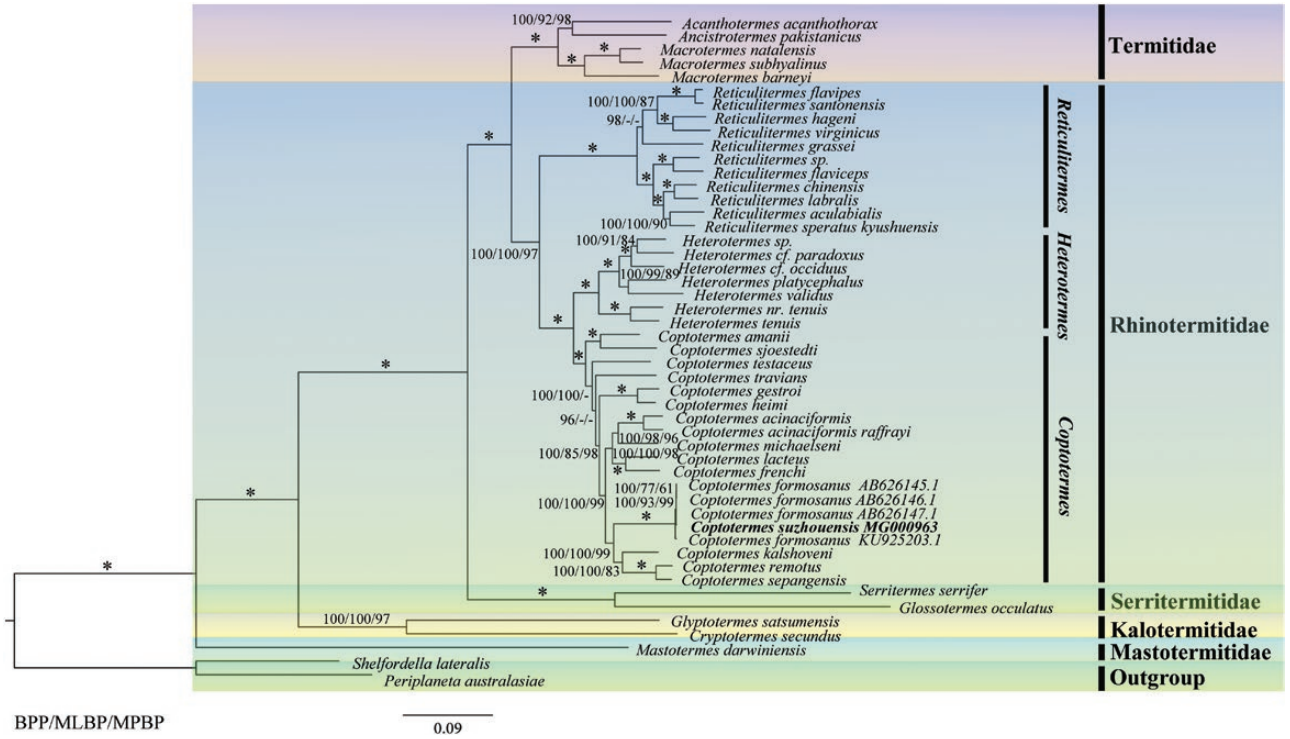

Fig. 3. Phylogenetic trees inferred with the amino acid sequences of 13 PCGs of the mitogenome of 41 termites species. *S. lateralis* (KU684413.1) and *P. australasiae* (KX640825.1) were used as outgroups. Numbers above the branches represent Bayesian posterior probabilities and bootstrap branch support for Maximum likelihood and Maximum parsimony, respectively. Nodes, which all support rates were 100%, were marked with an asterisk.

and *C. formosanus* was 0.002 (KU925203.1), which indicated that *C. suzhouensis* shared considerably close evolutionary relationships with *C. formosanus*.

Discussion

Termites play an important role in nutrient cycling and decomposition but are also often considered pest species as they may damage wooden buildings. Identifying termite species, especially those in the genus *Coptotermes*, is very difficult, and the taxonomic validity of many named *Coptotermes* species remains unclear (Chouvenc et al. 2015). mtDNA has been extensively used as an informative molecular marker for diverse evolutionary studies of animals, including in molecular evolution, phylogenetics, and population genetics (Gissi et al. 2008, Cameron 2014b, Qin et al. 2015). Thus, molecular tools may aid in the identification of termite species and resolves the relationships between *C. suzhouensis* and *C. formosanus*.

The present study analyzed the complete mtDNA sequence of *C. suzhouensis*. The gene arrangement of the mitogenome was identical to that of *Coptotermes*, as well as consistent with the ancestral arrangement of the insect mitogenome, indicating that it has been conserved during the evolution of these insects (Wei et al. 2010). Genetic analysis indicated interspecies genetic distance within Rhinotermitidae was 0.029–0.186. In *Coptotermes*, the difference among individuals within species (0.000–0.019) was lower than that among species (0.029–0.116), whereas the average genetic distance between *C. suzhouensis* and *C. formosanus* was 0.000–0.002, indicating that the two taxa share considerably close evolutionary relationships with each other (consistent with the findings of phylogenetic analyses; Fig. 3, Supp Table 1 [online only]). Our phylogenetic analyses support the viewpoint that *C. suzhouensis* was the synonymy of *C. formosanus*.

In Rhinotermitidae, the initiation and termination of 13 PCGs were essentially consistent. The majority of PCGs utilized canonical start codons (ATN) and stop codons (TAA, TAG, TA, or T). For *C. suzhouensis*, the stop codons of 13 PCGs were TAA, except for 3 PCGs (*COX2*, *ND5*, and *Cytb*), which were terminated with the partial stop codon T. Incomplete stop codons are common features of insect mitogenomes (Dietrich and Brune 2014, Hervé and Brune 2017), and it has been proposed that the complete termination codons are generated by the post-transcriptional polyadenylation (Ojala et al. 1981).

The A+T rich region, known for the initiation of replication, is located between srRNA and $tRNA^{Ile}$ in Rhinotermitidae. And, the A+T content (69.23%) of the control region (949 bp) in *C. suzhouensis* was higher than that of the entire mitogenome (65.60%), which is consistent with other Rhinotermitidae mitogenomes. The control region has the highly variability in the base composition and structure within Rhinotermitidae. In termites, the complicated double repeat units were first found in Reticulitermes, consisting of short (type-A) repeats adjacent to the srRNA end and long (type-B) repeats adjacent to the $tRNA^{Ile}$ end (Supp Table 2 [online only]). Cameron (2012) considered that the repeat units are involved in the replication-mediated processes and are a synapomorphic feature of Neotermitoidea, secondarily lost A-type repeats in higher Termitidae (non-macrotermitine termitids). In *C. suzhouensis*, the repeat units consist of two full A units, one full B, and one partial B unit (A-A-B-Bp). In addition, the second A and full B units overlap 12 bp. Compared to *C. formosanus* (A-A-B-B-Bp), a deletion of one complete B repeat was found in the CR zone of *C. suzhouensis*, and the phenomenon of repeat unit reduction was also observed in other termites [*Reticulitermes virginicus* (Isoptera: Rhinotermitidae), Cameron et al. 2007] . Comparative analysis of the control region within Rhinotermitidae revealed two conserved

sequences 'AATCCTAAACTTATCT' (located at B1 repeat region: from 15,223 to 15,238) and 'AGATAAGTTTAGGATT' (located at B1 repeat region: from 15,254 to 15,269) in the control regions of *C. suzhouensis* (Supp Fig. 1 [online only]) that formed the hairpin loop structure. The hairpin loop known as RGC (rare genomic change) is considered to have a high degree of sequence conservation in all termite species and speculated to be the origin of replication for the mitogenome (Saito et al. 2005). Further, a poly-T stretch (located at B1 repeat region: from 15,238 to 15,245) was found in the loop of hairpin structure, spanning 8-bp long, which also existed in other *Coptotermes* species. No typical microsatellite-like regions were found in the AT-rich region of *C. suzhouensis*, which are commonly found in other insect species, but absent from all reported Rhinotermitidae species identified thus far (Beier et al. 2017).

In termites, species descriptions have historically relied upon morphological characters of the soldiers and alates, which has contributed to synonyms within *Coptotermes* (more than 40 junior synonyms) (Krishna et al. 2013). A recent analysis using molecular data has provided some insight into *Coptotermes* phylogenetics and radiation (Bourguignon et al. 2017). For example, *Coptotermes havilandi* (Isoptera: Rhinotermitidae) and *Coptotermes gestroi* (Isoptera: Rhinotermitidae), *Coptotermes elisae* (Desneux) (Isoptera: Rhinotermitidae), and *Coptotermes curvignathus* (Isoptera: Rhinotermitidae), as well as *C. gestroi* and *Coptotermes vastator* (Isoptera: Rhinotermitidae), were each revealed to be synonymous (Kirton and Brown 2003, Wang 2004, Kirton et al. 2005, Bengkeok et al. 2007, Lee et al. 2015) . In this study, we described the complete mitogenome sequence of *C. suzhouensis* and compared it to the available mitogenomes of other termites using phylogenetic analyses. Our results suggest that *C. suzhouensis* and *C. formosanus* are likely to be synonyms.

Acknowledgments

This work was support by Natural Science Foundation of Anhui Province (11040606M78), Key Research Program of the Education Department of Anhui Province (KJ2012A016) and Project of Hefei Termite Control Institute (Y06090504). We declare no competing interest. We express gratitude for support by Hefei Termite Control Institute.

References Cited

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Aut. Control 19: 716–723.

Beier, S., T. Thiel, T. Münch, U. Scholz, and M. Mascher. 2017. MISA-web: a web server for microsatellite prediction. Bioinformatics 33: 2583–2585.

Bengkeok, Y., A. S. Othman, V.S. Lee, and L. Chowyang. 2007. Genetic relationship between *Coptotermes gestroi* and *Coptotermes vastator* (Isoptera: Rhinotermitidae). J. Econ. Entomol 100: 467–474.

Bernt, M., A. Donath, F. Jühling, F. Externbrink, C. Florentz, G. Fritzsch, J. Pütz, M. Middendorf, and P. F. Stadler. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol 69: 313–319.

Bourguignon, T., N. Lo, S. L. Cameron, J. Šobotník, Y. Hayashi, S. Shigenobu, D. Watanabe, Y. Roisin, T. Miura, and T. A. Evans. 2015. The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol 32: 406–421.

Bourguignon, T., N. Lo, J. Šobotník, D. Sillam-Dussès, Y. Roisin, and T. A. Evans. 2016. Oceanic dispersal, vicariance and human introduction shaped the modern distribution of the termites *reticulitermes*, *heterotermes* and *coptotermes*. Proc. Biol Sci 283: 20160179.

Bourguignon, T., N. Lo, J. Šobotník, S. Y. Ho, N. Iqbal, E. Coissac, M. Lee, M. M. Jendryka, D. Sillam-Dussès, B. Krížková, et al. 2017. Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the tropics. Mol. Biol. Evol 34: 589–597.

Brody, A. K., T. M. Palmer, K. Fox-Dobbs, and D. F. Doak. 2010. Termites, vertebrate herbivores, and the fruiting success of Acacia drepanolobium. Ecology 91: 399–407.

Cai, B. H., and N. S. Chen. 1964. The classification and regional of termites in Chinese. Acta Entomol. Sinica 13: 25–37.

Cameron, S. L., and M. F. Whiting. 2007. Mitochondrial genomic comparisons of the subterranean termites from the Genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). Genome 50: 188–202.

Cameron, S. L., N. Lo, T. Bourguignon, G. J. Svenson, and T. A. Evans. 2012. A mitochondrial genome phylogeny of termites (Blattodea: Termitoidae): robust support for interfamilial relationships and molecular synapomorphies define major clades. Mol. Phylogenet. Evol 65: 163–173.

Cameron, S. L. 2014a. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. Syst. Entomol 39: 400–411.

Cameron, S. L. 2014b. Insect mitochondrial genomics: implications for evolution and phylogeny. Annu. Rev. Entomol 59: 95–117.

Chen, Q., K. Wang, Y. L. Tan, and L. X. Xing. 2016. The complete mitochondrial genome of the subterranean termite, *reticulitermes chinensis Snyder* (Isoptera: Rhinotermitidae). Mitochondrial DNA A. DNA Mapp. Seq. Anal 27: 1428–1429.

Cheng, D. B. 2012. Advances of researches on classification of termites (Isoptera). J. Entomol. Res. Central China 8:257–263.

Cheng, D. B. 2014. Termites. Science Press, Beijing, China.

Cheng, X. F., L. P. Zhang, D. N. Yu, K. B. Storey, and J. Y. Zhang. 2016. The complete mitochondrial genomes of four cockroaches (Insecta: Blattodea) and phylogenetic analyses within cockroaches. Gene 586: 115–122.

Chouvenc, T., E. E. Helmick, and N. Y. Su. 2015. Hybridization of two major termite invaders as a consequence of human activity. Plos One 10: e0120745.

Chouvenc, T., H. F. Li, J. Austin, C. Bordereau, T. Bourguignon, S. L. Cameron, E. M. Cancello, R. Constantino, A. M. Costa-Leonardo, and P. Eggleton 2016. Revisiting *Coptotermes* (Isoptera: Rhinotermitidae): a global taxonomic road map for species validity and distribution of an economically important subterranean termite genus. Syst. Entomol 41: 299–306.

Dietrich, C., and A. Brune. 2014. The complete mitogenomes of six higher termite species reconstructed from metagenomic datasets (Cornitermes sp., Cubitermes ugandensis, Microcerotermes parvus, Nasutitermes corniger, Neocapritermes taracua, and Termes hospes). Mitochondrial DNA 4: 1–2.

Eggleton, P., G. Beccaloni, and D. Inward 2007. Response to Lo *et al*. Biol Lett 3: 564–565.

Emerson, A. E. 1952. The biogeography of termites. Bull. Am. Mus. Nat. Hist. 99: 217–225.

Engel, M. S., and K. Krishna, 2004. Family-group names for termites (Isoptera). Am. Mus. Nov. 3432: 1–9.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Gissi, C., F. Iannelli, and G. Pesole. 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity (Edinb.) 101: 301–320.

Hausberger, B., D. Kimpel, A. van Neer, and J. Korb. 2011. Uncovering cryptic species diversity of a termite community in a West African savanna. Mol. Phylogenet. Evol 61: 964–969.

Hervé, V., and A. Brune 2017. The complete mitochondrial genomes of the higher termites *Labiotermes labralis* and *Embiratermes neotenicus* (Termitidae: Syntermitinae). Mitochondrial DNA 2: 109–110.

Huang, F. S., S. M. Zhu, Z. M. Ping, X. S. He, G. X. Li, and D. R. Gao 2000. Fauna Sinica Isecta. Vol. 17, Isoptera. Science Press, Beijing, China.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Inward, D., G. Beccaloni, and P. Eggleton. 2007a. Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. Biol. Lett 3: 331–335.

Inward, D. J., A. P. Vogler, and P. Eggleton. 2007b. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. Mol. Phylogenet. Evol 44: 953–967.

Kambhampati, S., and P. Eggleton 2000. Taxonomy and phylogeny of termites, pp. 1–23. *In* T. Abe, D.E. Bignell, M. Higashi (eds) Termites: evolution, sociality, symbiose, ecology. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Kim, I., S. Y. Cha, M. H. Yoon, J. S. Hwang, S. M. Lee, H. D. Sohn, and B. R. Jin. 2005. The complete nucleotide sequence and gene organization of the mitochondrial genome of the oriental mole cricket, *Gryllotalpa orientalis* (Orthoptera: Gryllotalpidae). Gene 353: 155–168.

Kirton, L. G., and V. K. Brown. 2003. The taxonomic status of pest species of Coptotermes in Southeast Asia: resolving the paradox in the pest status of the termites, *Coptotermes gestroi*, *C. havilandi* and *C. travians* (Isoptera: Rhinotermitidae). Sociobiology 42: 43–63.

Kirton, L. G., Lee, C. Y., and W. H. Robinson. 2005. The importance of accurate termite taxonomy in the broader perspective of termite management, pp. 1–7. P&Y Design Network. Penang, Malaysia.

Korb, J., M. Poulsen, H. Hu, C. Li, J. J. Boomsma, G. Zhang, and J. Liebig. 2015. A genomic comparison of two termites with different social complexity. Front. Genet. 6: 9.

Krishna, K., D. A. Grimaldi, V. Krishna, and M. S. Engel. 2013. Treatise on the isoptera of the world. Bull. Am. Mus. Nat. Hist. 377: 1–2704.

Lee, T. R., S. L. Cameron, T. A. Evans, S. Y. Ho, and N. Lo. 2015. The origins and radiation of Australian *Coptotermes termites*: from rainforest to desert dwellers. Mol. Phylogenet. Evol. 82 (Pt A): 234–244.

Lee, W., T. Han, J. H. Lee, K. J. Hong, and J. Park 2017. The complete mitochondrial genome of the subterranean termite, *Reticulitermes speratus kyushuensis* Morimoto, 1968 (Isoptera: Rhinotermitidae). Mitochondrial DNA Part B 2:178–179.

Legendre, F., M. F. Whiting, C. Bordereau, E. M. Cancello, T. A. Evans, and P. Grandcolas. 2008. The phylogeny of termites (Dictyoptera: Isoptera) based on mitochondrial and nuclear markers: implications for the evolution of the worker and pseudergate castes, and foraging behaviors. Mol. Phylogenet. Evol. 48: 615–627.

Li, G. X. 2002. Chinese termites and control. Science Press, Beijing, China.

Li, Y. X., X. G. Wang, J. Ou, F. J. Yao, Y. Yang, and Z. M. Wei. 2016. The complete mitochondrial genome of *Coptotermes testaceus* (Isoptera: Rhinotermitidae). Mitochondrial DNA A. DNA Mapp. Seq. Anal. 27: 3466–3468.

Lo, N., O. Kitade, T. Miura, R. Constantino, and T. Matsumoto. 2004. Molecular phylogeny of the Rhinotermitidae. Insectes. Soc. 51: 365–371.

Lowe, T. M., and P. P. Chan. 2016. tRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44: W54–W57.

Ma, J., C. Du, C. Zhou, Y. Sheng, Z. Fan, B. Yue, and X. Zhang. 2017. Complete mitochondrial genomes of two blattid cockroaches, *Periplaneta australasiae* and *Neostylopyga rhombifolia*, and phylogenetic relationships within the Blattaria. PLoS One 12: e0177162.

Meng, Z., S. Jiang, X. Chen, and C. Lei. 2016. The complete mitochondrial genome of fungus-growing termite, *Macrotermes natalensis* (Isoptera: Macrotermitinae). Mitochondrial DNA A. DNA Mapp. Seq. Anal. 27: 1728–1729.

Ojala, D., J. Montoya, and G. Attardi. 1981. tRNA punctuation model of RNA processing in human mitochondria. Nature 290: 470–474.

Perna, N. T., and T. D. Kocher. 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J. Mol. Evol. 41: 353–358.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Qin, J., Y. Zhang, X. Zhou, X. Kong, S. Wei, R. D. Ward and A. B. Zhang 2015. Mitochondrial phylogenomics and genetic relationships of closely related pine moth (Lasiocampidae: Dendrolimus) species in China, using whole mitochondrial genomes. BMC Genomics 16: 1–12.

Rocha, M. M., C. Cuezzo, and E. M. Cancello 2017. Phylogenetic reconstruction of Syntermitinae (Isoptera, Termitidae) based on morphological and molecular data. Plos One 12: e0174366.

Rust, M. K., and N. Y. Su. 2012. Managing social insects of urban importance. Annu. Rev. Entomol 57: 355–375.

Saito, S., K. Tamura, and T. Aotsuka. 2005. Replication origin of mitochondrial DNA in insects. Genetics 171: 1695–1705.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Syst. Biol. 57: 758–771.

Swofford, D. L. 2002. PAUP*. phylogenetic analysis using parsimony (*and other methods). version 4.0b10. Mccarthy, doi: 10.1111/j.0014-3820.2002.tb00191.x.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar 2014. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731–2739.

Tokuda, G., H. Isagawa, and K. Sugio 2012. The complete mitogenome of the Formosan termite, *Coptotermes formosanus Shiraki*. Insectes. Soc. 59: 17–24.

Wang, J. G. 2004. Phylognetic study of termites based on the morphological and molecular approache. Shouthen China Agricultral University, Guangzhou. pp. 1–142.

Wang, Y. L., Y. H. Chen, C. C. Xia, X. Q. Xia, R. S. Tao, and J. S. Hao. 2015. The complete mitochondrial genome of the Common Red Apollo, *Parnassius epaphus* (Lepidoptera: Papilionidae: Parnassiinae). J. Asia-Pac. Entomol. 18: 239–248.

Wang, P., J. Zhu, M. Wang, Y. Zhang, J. Wang, Y. Zhu, and P. Zhang. 2016. The complete mitochondrial genome of and implications for Rhinotermitidae taxonomy. Mitochondrial DNA Part B Res. 1: 392–393.

Watson, J., and F. Gay. 1991. Isoptera (Termites), pp. 330–347. *In*: CSIRO (ed.), Insects of Australia, Vol. 1. Cornell University Press, Ithaca, NY.

Wei, S. J., M. Shi, X. X. Chen, M. J. Sharkey, C. van Achterberg, G. Y. Ye, and J. H. He. 2010. New views on strand asymmetry in insect mitochondrial genomes. PLoS One 5: e12708.

Wei, S. J., J. F. Ni, M. L. Yu, and B. C. Shi. 2012. The complete mitochondrial genome of *Macrotermes barneyi* Light (Isoptera: Termitidae). Mitochondrial DNA 23: 426–428.

Wolstenholme, D. R. 1992. Animal mitochondrial DNA: structure and evolution. Int. Rev. Cytol. 141: 173–216.

Wyman, S. K., R. K. Jansen, and J. L. Boore. 2004. Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20: 3252–3255.

Xia, K. L., and X. S. He. 1986. The research on *Coptotermes* in Chinese Bulletin of Entomological Research. Shanghai Scientific & Technical Publishers, Shanghai. pp. 157–182.

Xiao, B., A. H. Chen, Y. Y. Zhang, G. F. Jiang, C. C. Hu, and C. D. Zhu. 2012. Complete mitochondrial genomes of two cockroaches, *Blattella germanica* and *Periplaneta americana*, and the phylogenetic position of termites. Curr. Genet. 58: 65–77.

Xu, L. P., X. S. Liang, Q. J. Zhou, and J. S. Xue 2009. Application of classification of termites by means of molecular biological techniques. J. Inspect. Quarantine 19: 70–74.