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Morphological and genetic evidence for the synonymy of *Reticulitermes* species: *Reticulitermes dichrous* and *Reticulitermes guangzhouensis* (Isoptera: Rhinotermitidae)

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Abstract

The taxonomy of *Reticulitermes* Holmgren (Isoptera: Rhinotermitidae) in China is problematic and in need of revision. Most Chinese *Reticulitermes* species were described in the 1980s and 1990s, and have never been studied since then. In this study, morphological characteristics including coloration, pilosity, shape, and morphometric characteristics of *Reticulitermes dichrous* Ping and *Reticulitermes guangzhouensis* Ping were compared. In addition, a portion of the ribosomal RNA large subunit 16S (*16S rRNA*) and the mitochondrial DNA cytochrome oxidase subunit II (*COII*) genes from different populations of the 2 species were sequenced and analyzed. Morphological comparisons revealed the similarities between the 2 species in both discrete and morphometric characteristics. In the molecular phylogenetic trees inferred from *COII* and *16S rRNA* genes, all of the examined populations of the 2 species clustered into a common clade with a high bootstrap value. Based on the morphological comparisons and the molecular analyses, it is proposed that *R. dichrous* is a junior synonym of *R. guangzhouensis*.

Key Words: morphometric characteristic; 16S rRNA; COII

Resumen

La taxonomía del género *Reticulitermes* Holmgren (Isoptera: Rhinotermitidae) en China es problemática y necesita revisión. La mayoría de las especies chinas de *Reticulitermes* fueron descritas en los años ochenta y noventa, y nunca se han estudiado desde entonces. En este estudio se compararon las características morfológicas incluyendo coloración, pilosidad, forma y características morfométricas de *Reticulitermes dichrous* Ping y *Reticulitermes guangzhouensis* Ping. Además, se secuenció y analizó una parte de la subunidad 16S (*165 rRNA*) del ARN ribosómico y los genes de la subunidad II de la citocromo oxidasa (*COII*) del ADN mitocondrial de diferentes poblaciones de las 2 especies. Las comparaciones morfológicas revelaron las similitudes entre las 2 especies tanto en características discretas como morfométricas. En los árboles filogenéticos moleculares inferidos de los genes *COII* y *165 rRNA*, todas las poblaciones examinadas de las 2 especies se agruparon en un clado común con un alto valor de bootstrap. Basándose en las comparaciones morfológicas y en los análisis moleculares, se propone que *R. dichrous* es un sinónimo menor de *R. guangzhouensis*.

Palabras Clave: característica morfométrica; 16S rRNA; COII

Subterranean termites of the genus *Reticulitermes* Holmgren (Isoptera: Rhinotermitidae) are economically important termites, and taxonomic research on the genus is necessary. However, *Reticulitermes* species identification by morphology alone has been complicated due to interspecific overlap and intraspecific geographic variations in size (Ye et al. 2004; Austin et al. 2007; Lim & Forschler 2012). A combination of molecular and morphological taxonomy has solved some problems in termite identification, such as revealing synonymous relationships between *Reticulitermes flavipes* (Kollar) and *Reticulitermes santonensis* Feytaud (Isoptera: Rhinotermitidae) (Austin et al. 2005), between *Nasutitermes corniger* (Motschulsky) and *Nasutitermes costalis* (Holmgren) (Isoptera: Termitidae) (Scheffrahn et al. 2005); as well as between *Coptotermes gestroi* (Wasmann) and *Coptotermes vastator* Light (Isoptera: Rhinotermitidae) (Yeap et al. 2007). Termites of

the genus *Reticulitermes* are very diverse in China, where 111 species have been reported (Huang et al. 2000). In just a decade (1980–1989), 72 new *Reticulitermes* species were described based on morphological characters of the soldier and the alates. According to Krishna et al. (2013), 138 species of *Reticulitermes* have been described in subtropical and temperate regions worldwide. Therefore, the number of Chinese species seems to be out of proportion and some species may be proven to be invalid. *Reticulitermes* species from China are in need of careful monographic revisions (Eggleton 1999; Vargo & Husseneder 2009).

Reticulitermes dichrous Ping (Isoptera: Rhinotermitidae) and Reticulitermes guangzhouensis Ping (Isoptera: Rhinotermitidae) are indigenous to Guangdong Province, China, and many morphological character dimensions of both species overlap, suggesting that they may be

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conspecific. In this paper, the genetic relationship between *R. dichrous* and *R. guangzhouensis* was investigated based on 2 mitochondrial genes (*COII* and *16S rRNA*). The coloration, pilosity, shape, and 11 morphometric measurements were compared between the 2 species. Our results show that *R. dichrous* is a junior synonym of *R. guangzhouensis*.

Materials and Methods

TERMITE SAMPLES

Eight populations of *R. dichrous* and 12 populations of *R. guangzhouensis* were collected from 5 localities of Zhaoqing (23.1655833– 23.1737778°N, 112.5394917°–112.5573944°E), Guangdong Province and 4 localities of Huizhou (23.2487639°–23.3840917°N,

Table 1. Termite species and populations included in the present study.

114.0220361°–114.4395194°E), Guangdong Province, China, from 2011 to 2015 and were used in the morphological examinations. Some of the samples were chosen for molecular analyses according to the availability of worker termites and the success of DNA sequencing. A population of *R. tricholabralis* Ping and Li (Isoptera: Rhinotermitidae) collected from Wuhan (30.4795806°N, 114.3495361°E), Hubei Province, in Aug 2013 also was included in the molecular analysis. The specimens were preserved in 70% ethanol for morphological studies and in 100% ethanol for molecular analysis. Voucher specimens were deposited in Guangdong Institute of Applied Biological Resources, Guangzhou, China.

MORPHOLOGICAL EXAMINATIONS

Specimens were identified applying keys by Huang et al. (2000). The head and the pronotum of both species were photographed un-

	_	GenBank acc	cession no.		
Species	Collecting sites	16S rRNA	COII	References	Sequence codes
Reticulitermes guangzhouensis	Luofu Mountain, Huizhou, China	KX374680	KX374676	This study	Rg01-HZ
R. guangzhouensis	Xiangtou Mountain, Huizhou, China	KX374681	KX374678	This study	Rg04-HZ
R. guangzhouensis	Xiangtou Mountain, Huizhou, China	KX374683	KX374675	This study	Rg08-HZ
R. guangzhouensis	Guangzhou, China	KM245673	NS	Unpublished	Rg13-GZ
Reticulitermes dichrous	Dinghu Mountain, Zhaoqing, China	KX374687	KX374673	This study	Rd01-ZQ
R. dichrous	Dinghu Mountain, Zhaoqing, China	KX374684	KX374674	This study	Rd04-ZQ
R. dichrous	Dinghu Mountain, Zhaoqing, China	KX374686	NA	This study	Rd05-ZQ
R. dichrous	Dinghu Mountain, Zhaoqing, China	KX374685	KX374671	This study	Rd07-ZQ
R. dichrous	Dinghu Mountain, Zhaoqing, China	NA	KX374672	This study	Rd08-ZQ
Reticulitermes tricholabralis	Wuhan, Hubei, China	NA	KX374679	This study	Rt01-China
Reticulitermes chinensis	Beijing, China	NS	JX142147	Unpublished	Rc01-China
R. chinensis	China	NS	HQ012033	Huang et al. (2011)	Rc02-China
Reticulitermes flaviceps	Zhejiang, China	GU116564	NS	Unpublished	Rfc01-China
Reticulitermes speratus	Japan	NS	AB005463	Unpublished	Rs01-Japan
R. speratus	China	NS	KU061246	Unpublished	Rs02-China
R. speratus	China	NS	KU061240	Unpublished	Rs03-China
Reticulitermes flavipes	Georgia, USA	NS	EU689005	Unpublished	Rf01-USA
R. flavipes	Florida, USA	NS	AY808085	Su et al. (2006)	Rf02-USA
R. flavipes	Jalisco, Mexico	DQ001967	NS	Austin et al. (2005a)	Rfp01-Mexico
R. flavipes	Hamburg, Germany	AY257226	NS	Szalanski et al. (2003)	Rfp02-Germany
R. flavipes	Toronto, Canada	AY257229	NS	Szalanski et al. (2003)	Rfp03-Canada
Reticulitermes labralis	Beijing, China	NS	JX142151	Unpublished	Rla01-China
Reticulitermes balkanensis	NeaArtaki, Greece	NS	AY954667	Unpublished	Rb01-Greece
R. balkanensis	Areopolis, Greece	NS	AY267867	Luchetti et al. (2004)	Rb02-Greece
R. balkanensis	Dionissos, Greece	DQ431060	NS	Austin et al. (2006)	Rb03-Greece
Reticulitermes lucifugus	Policoro, Italy	NS	JQ231195	Ghesini & Marini (2012)	Rlu01-Italy
R. lucifugus	Komotini, Greece	NS	AY954665	Unpublished	Rlu02-Greece
R. lucifugus	Ankara, Turkey	DQ431052	NS	Austin et al. (2006)	RI01-Turkey
R. lucifugus	Kharman Maras, Turkey	DQ431057	NS	Austin et al. (2006)	RI02-Turkey
Reticulitermes malletei	Georgia, USA	NS	JF796227	Lim & Forschler (2012)	Rm01-USA
R. malletei	Georgia, USA	NS	GU550074	Unpublished	Rm02-USA
R. malletei	USA	DQ422137	NS	King et al. (2007)	Rm03-USA
R. malletei	USA	DQ422138	NS	King et al. (2007)	Rm04-USA
Reticulitermes hageni	North Carolina, USA	NS	DQ493729	Yashiro & Matsuura (2007)	Rh01-USA
R. hageni	Georgia, USA	NS	JF796225	Lim & Forschler (2012)	Rh02-USA
Reticulitermes hesperus	Oregon, USA	DQ004948	NS	Austin et al. (2005a)	Rh01-USA
Reticulitermes hesperus	California, USA	DQ389194	NS	Tripodi et al. (2006)	Rh02-USA
Coptotermes formosanus	East China	NS	FJ423459	Long et al. (2009)	Cf01-China
C. formosanus	Hainan, China	EU805742	NS	Li et al. (2009)	Cf02-China
Coptotermes gestroi	China	NS	KC515412	Unpublished	Cg01-China
C. gestroi	Taiwan, China	EU805727	NS	Li et al. (2009)	Cg02-China

NA, not available; NS, not submitted.



Fig. 1. Photomicrographs of dorsal (A, D, E, F, I, and J), ventral (B and G), and lateral (C and H) views of *Reticulitermes dichrous* (A to E) and *Reticulitermes guang*zhouensis (F to J) soldier heads and pronotums.

der a stereomicroscope (SMZ1000, Nikon, Chiyoda-ku, Japan) connected to a computer-assisted imaging camera. Forty soldiers from the 8 populations of R. dichrous and from the 12 populations of R. guangzhouensis were examined with the stereomicroscope. The following 11 morphological characteristics were measured: head length to upper base of condyle of mandibles (HL), head maximum width (HW), left mandible length (upper base of condyle to tip, LML), labrum length (LL), labrum maximum width (LW), postmentum medium length (POL), postmentum maximum width (POW), postmentum waist width (PWW), pronotum maximum length (PRL), pronotum maximum width (PRW), and hind tibia length (HTL). These measurement data were subjected to analysis of variance (ANOVA), and the significance of the differences was compared by the Duncan Multiple Range Test at the level of 5% probability using SAS® software Version 9.0 (SAS 2002). The cluster dendrogram was constructed by using the minimum-variance method of Ward.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total genomic DNA of 3 worker termites from populations of *R. guangzhouensis* and *R. dichrous* was extracted (Table 1). To avoid contamination with gut contents, including polysaccharides, which act as PCR inhibitors (Schrader et al. 2012), the abdomen was removed from specimens before extraction, and just the head and thorax were extracted. The DNA extraction was performed by TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. Two mitochondrial genes, *COII* and *16S rRNA*, were amplified by polymerase chain reactions (PCRs) with primers TL2J3037 (alias AtLeu) (5'-ATGGCAGATTAGTGCAATGG-3') (Liu & Beckenbach 1992) and TKN3785 (alias BtLys) (5'-GTTTAAGAGACCAGTACTTG-3') (Simon et al. 1994) for *COII*, and with LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3')

(Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTAT-CAAAAACAT-3') (Simon et al. 1994) for *16S rRNA*. PCR amplification was performed in a 50 μ L reaction mixture containing 2 μ L DNA, 21 μ L dH₂O, 25 μ L Premix TaqTM (Ex Taq version, TaKaRa, Tokyo, Japan), 1 μ L forward primer at 10 μ M, and 1 μ L reverse primer at 10 μ M. Thermal cycling was conducted for 40 of the following cycles: predenaturation at 94 °C for 4 min, denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 40 s; followed by a final extension of 72 °C for 5 min after the cycles. Amplified products were checked on a 1% agarose gel, and sent to Generay Biotech Co., Ltd (Shanghai, China) for direct sequencing in both directions. GenBank accession numbers for termite DNA sequenced in this study are provided in Table 1, along with the accession numbers of some other species and populations.

MOLECULAR PHYLOGENETIC ANALYSIS

Molecular phylogenetic analyses were carried out on a dataset comprising *COII* gene sequences of 4 populations of *R. dichrous* and 3 populations of *R. guangzhouensis*, as well as *16S rRNA* gene sequences of 4 populations of both species. DNA sequences of *R. tricholabralis* from this study and 10 other *Reticulitermes* species obtained from GenBank were included in the phylogenetic comparisons. Two species of *Coptotermes, C. formosanus* Shiraki (Isoptera: Rhinotermitidae), and *C. gestroi* were used as outgroup taxa.

Sequences were aligned with Clustal X (Larkin et al. 2007). Base compositional analyses were conducted with the computer program MEGA version 6.0 (Tamura et al. 2013). The distance estimation of MEGA 6.0 was used to calculate genetic distances according to the p-distance model. Molecular phylogenetic trees were constructed by the maximum likelihood method in MEGA 6.0. Consensus trees were determined using CONSENSE in the PHYLIP 3.6 software package (Uni-

					Meã	asurements ^a (mear	n ± SE)				
Population	HL	МН	TML	ΓĽ	ΓM	POL	POW	PWW	PRL	PRW	HTL
					Reticuliterm	ies guangzhouens.	is				
Rg01	1.600 ± 0.029e-i	0.995 ± 0.010c	$1.005 \pm 0.013b$	0.395 ± 0.013d-g	0.303 ± 0.003c-e	1.015 ± 0.036ef	0.435 ± 0.010a-c	0.170 ± 0.006a-d	0.488 ± 0.011c-f	0.763 ± 0.010a-d 0	.820 ± 0.029a-c
Rg02	1.767 ± 0.050ab	1.117±0.009a	0.963 ± 0.018bc	0.410±0.025b-e	0.347 ± 0.007a	1.120 ± 0.050cd	0.450 ± 0.012a	0.160 ± 0.000b-d 1	0.507 ± 0.012a-e	0.770 ± 0.029a-c 0	.820±0.015a-c
Rg03	1.800 ± 0.059a	1.120 ± 0.012a	1.070 ± 0.021a	0.357 ± 0.007hi	0.343 ± 0.007a	1.230 ± 0.012b	0.427 ± 0.007a-f	0.170 ± 0.010a-d (0.513 ± 0.020a-d	0.800 ± 0.032a 0	.863 ± 0.053ab
Rg04	1.748 ± 0.020a-c	0.990 ± 0.008cd	0.943 ± 0.008c-e	0.440 ± 0.008ab	0.325 ± 0.005a-c	1.235 ± 0.005b	0.430 ± 0.010a-e	0.158 ± 0.008b-d (0.540 ± 0.006a	0.765 ± 0.005a-d 0	.833 ± 0.008a-c
Rg05	1.710 ± 0.070a-d	$1.043 \pm 0.037b$	0.923 ± 0.026c-f	0.397 ± 0.023d-g	0.317 ± 0.013b-e	1.343 ± 0.033a	0.400 ± 0.010gh	0.167 ± 0.007a-d (0.487 ± 0.015d-f	0.750 ± 0.030a-e 0	.740 ± 0.071de
Rg06	1.678 ± 0.019b-e	0.960 ± 0.015c-f	0.920 ± 0.021c-g	0.403 ± 0.006d-f	0.330 ± 0.000ab	1.217 ± 0.007b	0.410 ± 0.021d-h	0.153 ± 0.003cd (0.500 ± 0.014b-e	0.723 ± 0.028b-g 0	798 ± 0.005b-d
Rg07	1.645 ± 0.005d-g	0.990 ± 0.008cd	0.928 ± 0.008c-f	0.420 ± 0.000b-d	0.335 ± 0.005ab	1.210 ± 0.000b	0.420 ± 0.000b-g	0.180 ± 0.000ab	0.510 ± 0.000a-e	0.745 ± 0.005a-f 0	.880 ± 0.000a
Rg08	1.567 ± 0.007f-i	0.993 ± 0.043c	0.930 ± 0.021c-f	0.373 ± 0.023f-h	0.317±0.007b-e	$1.113 \pm 0.013cd$	0.420 ± 0.000b-g	0.160 ± 0.010b-d (0.490 ± 0.021c-f	0.717 ± 0.017c-g 0	.835 ± 0.045a-c
Rg09	1.657 ± 0.007c-f	0.957 ± 0.007c-f	0.940 ± 0.021c-e	0.413 ± 0.007b-e	0.323 ± 0.007a-d	1.113 ± 0.007cd	0.427 ± 0.007a-f	0.173 ± 0.023a-d (0.497 ± 0.017b-e	0.743 ± 0.013b-f 0	770 ± 0.000cd
Rg10	1.750 ± 0.032a-c	0.977 ± 0.007cd	0.913 ± 0.007c-h	0.337 ± 0.007i	0.330±0.012ab	1.407 ± 0.026a	0.440 ± 0.000ab	0.170 ± 0.010a-d (0.523 ± 0.007a-c	0.777 ± 0.007ab 0.	797 ± 0.013b-d
Rg11	1.657 ± 0.018c-f	0.997 ± 0.007c	0.957 ± 0.007cd	0.370 ± 0.000gh	0.303 ± 0.007c-e	0.990 ± 0.000f	0.433 ± 0.007a-d	0.170 ± 0.010a-d (0.440 ± 0.000h	0.700 ± 0.000e-h 0.	687 ± 0.013ef
Rg12	1.587 ± 0.007e-i	0.947 ± 0.015c-f	0.733 ± 0.017j	0.357 ± 0.007hi	0.330 ± 0.012ab	1.097 ± 0.026c-e	0.407 ± 0.007e-h	0.187 ± 0.007a (0.530 ± 0.012ab	0.760 ± 0.032a-d 0.	740 ± 0.021de
					Reticulit	ermes dichrous					
Rd01	1.530 ± 0.015hi	0.914 ± 0.010f	0.872 ± 0.012g-i	0.402 ± 0.009d-f	0.302 ± 0.005c-e	1.054 ± 0.043d-f	0.412 ± 0.005c-g	0.150 ± 0.000d (0.440 ± 0.011h	0.660 ± 0.000h 0.	660 ± 0.000f
Rd02	1.550 ± 0.045g-i	0.926 ± 0.017ef	0.868 ± 0.023hi	0.406 ± 0.009c-e	0.296 ± 0.004ef	0.998 ± 0.043f	0.408 ± 0.005e-h	0.154 ± 0.009cd (0.456 ± 0.010f-h	0.712 ± 0.018d-h 0.	734 ± 0.021de
Rd03	1.608 ± 0.021d-h	0.924 ± 0.004ef	0.910 ± 0.008d-h	1 0.436 ± 0.002a-c	0.298 ± 0.005d-f	1.070 ± 0.015d-f	0.422 ± 0.002b-g	0.174 ± 0.004a-c (0.460 ± 0.009f-h	0.690 ± 0.009f-h 0.	742 ± 0.020de
Rd04	1.610 ± 0.030d-h	0.918±0.018ef	0.900 ± 0.000e-i	0.440 ± 0.000ab	0.335 ± 0.005ab	1.165 ± 0.032bc	0.440 ± 0.000ab	0.180 ± 0.000ab (0.475 ± 0.005e-h	0.735 ± 0.005b-f 0.	770 ± 0.000cd
Rd05	1.500 ± 0.027i	0.920±0.010ef	0.856 ± 0.016i	0.394 ± 0.007d-g	0.277 ± 0.006f	1.056 ± 0.021def	0.387 ± 0.006h	0.167 ± 0.005a-d (0.443 ± 0.005gh	0.700 ± 0.017e-h 0.	766 ± 0.017cd
Rd06	1.660 ± 0.029c-f	0.942 ± 0.010d-f	0.857 ± 0.009i	0.387 ± 0.003e-h	0.310±0.007b-e	1.100 ± 0.025c-e	0.413 ± 0.005c-g	0.157 ± 0.004b-d (0.485 ± 0.011d-f	0.725 ± 0.014b-g 0.	765 ± 0.013cd
Rd07	1.578 ± 0.019e-i	0.965 ± 0.005c-e	0.880 ± 0.000f-i	0.460 ± 0.000a	0.310 ± 0.000b-e	1.115 ± 0.010 cd	0.405 ± 0.005f-h	0.180 ± 0.000ab (0.400 ± 0.000i	0.660 ± 0.000h 0.	780 ± 0.006cd
Rd08	1.600 ± 0.012e-i	0.950 ± 0.018c-f	0.925 ± 0.017c-f	0.440 ± 0.008ab	0.313 ± 0.019b-e	1.065 ± 0.016d-f	0.420 ± 0.008b-g	0.180 ± 0.000ab ().478 ± 0.014d-g (0.675 ± 0.005gh 0.	760 ± 0.006cd
^a Means foll	owed by different lc	wercase letters with	in the same column	are significantly differ	ent by the Duncan m	nultiple range test (P	× ≤ 0.05).				

Table 2. Morphometric data (mm) of the soldier termites in different populations of Reticulitermes guangzhouensis and Reticulitermes dichrous.



Fig. 2. Cluster dendrogram of 12 populations of Reticulitermes guangzhouensis and 8 populations of Reticulitermes dichrous.

versity of Washington, Seattle, Washington) with the majority rule (extended) model. Poisson model and 1,000 bootstrap repetitions were applied for the analyses.

Results

MORPHOLOGICAL CHARACTERISTICS

Observations of R. dichrous and R. guangzhouensis soldiers from different populations indicated that the 2 species were similar in morphology. The head capsule of both species was elongated, broadly rounded at posterior margin, with a prominent hump at the front. In both species, the labrum was sharpened at the apex, the mandibles were stout with the tips slightly to moderately curved, the pronotum was covered by approximately 30 setae, and the anterior margin was concave with a shallow incised notch at the middle (Fig. 1). The morphological differences between the species mainly showed in the color of the head capsule and in the number of antennal segments. The head capsule color of R. dichrous was light brown, a little darker than that of R. guangzhouensis (Fig. 1A, F); the number of antennal segments of R. dichrous and R. guangzhouensis was 15 and 14 to 16, respectively. Furthermore, the maximum and minimum width of R. dichrous postmentum were generally narrower than those of R. guangzhouensis (Fig. 1B, G; Table 2), but the ratio of PWW to POW was similar in the 2 species. From the measurement data, large variations in significant differences in many characteristics were found among conspecific populations of both R. dichrous and R. guangzhouensi, but the differences were not significant between some populations of different species (Table 2).

The cluster dendrogram constructed with the measurement data of the 2 species also showed that some populations of different species, instead of absolutely conspecific populations, clustered into 1 branch (Fig. 2).

MOLECULAR PHYLOGENETIC ANALYSIS

The average sizes of PCR products of *COII* and *16S rRNA* genes for the 2 species examined were 763 and 432 base pairs (bp), respectively. For the *COII* gene, the multiple sequence alignment (including outgroups) resulted in 687 characters, of which 482 were constant and 148 were parsimony-informative. For the *16S rRNA* gene, the multiple sequence alignment (including outgroups) resulted in 432 characters, of which 356 were constant and 56 were parsimony-informative. Average nucleotide composition among ingroup taxa was as follows: A = 39.3%, T = 23.5%, G = 13.9%, and C = 23.3% in the *COII* gene; A = 23.1%, T = 40.8%, G = 22.7%, and C = 13.4% in the *16S rRNA* gene. Both mitochondrial genes were A and T rich.

The intraspecific genetic variations of *R. dichrous* ranged from 0.15 to 0.31% in the *COII* gene, with no variation in the *16S rRNA* gene. The intraspecific genetic variations of *R. guangzhouensis* ranged from 0.00 to 2.31% in the *COII* gene, with no variation in the *16S rRNA* gene. The genetic divergences between the 2 species ranged from 0.00 to 2.62% for *COII* and no variation was observed in the *16S rRNA* gene. This was slightly higher than, or equal to, the intraspecific genetic variations of *R. guangzhouensis*. By comparison, the ranges between *R. dichrous* and *R. guangzhouensis*, and other analyzed *Reticulitermes* species were 5.08 to 8.94% in the *COII* gene and 2.24 to 4.47% in the *16S rRNA* gene, which was higher than between *R. dichrous* and *R. guangzhouensis*.



Fig. 3. Maximum likelihood phylogenetic tree of *Reticulitermes guangzhouensis* and *Reticulitermes dichrous* relative to other *Reticulitermes* species based on the *COII* gene. Bootstrap values are indicated at the nodes and represent 1,000 pseudoreplicates.

The molecular phylogenetic trees generated from the *COII* and *16S rRNA* genes by the maximum likelihood method revealed similar phylogenetic relationships between *R. dichrous* and *R. guangzhouensis* (Figs. 3 and 4). In the tree inferred from the *COII* gene, all the populations of *R. dichrous* and *R. guangzhouensis* formed a clade with strong bootstrap support (99%). The clade comprised 2 subclades: 1 with *R. dichrous* and 2 populations of *R. guangzhouensis* from Xiangtou Mountain, and the other was *R. guangzhouensis* from Luofu Mountain. In the tree inferred from the *16S rRNA* gene, populations of *R. guangzhouensis* from Singthouensis from GenBank also formed a common clade with a bootstrap value of 99%.

Discussion

According to the morphological comparison between *R. dichrous* and *R. guangzhouensis* in this study, they were alike in both discrete and morphometric characteristics. It was difficult to differentiate them by the shape and the pilosity of head capsule, labrum, mandibles, and pronotum. Although the head capsule coloration of the 2 species was a little different in the examined specimens, it was not reliable enough for species identification. Coloration can be influenced by the age and state of the colony or by environmental and storage conditions (Schef-



Fig. 4. Maximum likelihood phylogenetic tree of Reticulitermes quangzhouensis and Reticulitermes dichrous relative to other Reticulitermes species based on the 16S rRNA gene. Bootstrap values are indicated at the nodes and represent 1,000 pseudoreplicates.

frahn et al. 2005). For the morphometric characteristics, it was found that most measurements of the 11 characters of R. dichrous either fell within the range of those of R. guangzhouensis or overlapped extensively with them, as was observed in the original descriptions for type specimens of the 2 species. It was only in the length range of the left mandible that the inclusion or overlap did not occur (0.88-0.90 mm in R. dichrous; and 0.92-1.04 mm in R. guangzhouensis), based on the original descriptions (Ping 1985). In the samples of this study, however, the measurements of left mandible length of R. dichrous also fell within the range of R. guangzhouensis (Table 2). In addition, the populations of *R. dichrous* are collected only in Dinghu Mountain, Zhaoging, Guangdong Province. The distribution of R. guangzhouensis in Guangdong Province, by contrast, is much wider. It is likely that the samples of *R*. dichrous are actually the populations of R. guangzhouensis that are distributed in Dinghu Mountain.

The comparison of genetic divergences between different populations provided the evidence for clarifying the relationship between R. dichrous and R. guangzhouensis. There were no genetic divergences between analyzed populations of R. dichrous and R. guangzhouensis

in 16S rRNA. By contrast, R. arenincola Goellner (Isoptera: Rhinotermitidae) and R. flavipes, which were supposed to be conspecific, have a genetic divergence in 16S rRNA of 0.0 to 1.9% (Ye et al. 2004). Likewise, N. costalis has a genetic divergence of 0 to 1.8% in 16S rRNA with N. corniger, which is its junior synonym (Scheffrahn et al. 2005). The COII genetic divergence between R. dichrous and R. guangzhouensis (0.00-2.62%) was also lower than that between R. arenincola and R. flavipes (0-4%) (Ye et al. 2004), and was evidently lower than the least genetic divergence between R. dichrous and R. guangzhouensis, and other Reticulitermes species included in the study (5.08%). As the COII gene is faster evolved and larger in size than the 16S rRNA gene (Ye et al. 2004), the value of 2.62% was considered relatively low and should be regarded as intraspecific variation.

The high bootstrap values of the clade R. dichrous + R. guangzhouensis in the 16S rRNA and COII trees also showed that R. dichrous and *R. quangzhouensis* are closely related, although the population Rg01-HZ in the COII tree had a relatively distant relationship compared with other populations in the clade. The separation of Rg01-HZ from the subclade composed of other populations could be due to variations at the population level. Solving the relationship among the population Rg01-HZ and the other populations in the clade would require collecting more populations from the distribution localities and analyzing more molecular markers.

The morphological comparisons and the molecular phylogenetic analyses with mitochondrial *COII* and *16S rRNA* genes suggested that *R. dichrous* and *R. guangzhouensis* are conspecific. It is proposed that *R. dichrous* is a junior synonym of *R. guangzhouensis*.

Reticulitermes guangzhouensis Ping, 1985

Reticulitermes guangzhouensis Ping, 1985, Entomotaxonomia, 7: 321. Reticulitermes dichrous Ping, 1985, Entomotaxonomia, 7: 324. syn. nov.

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