

Morphological and Genetic Evidence for the Synonymy of Reticulitermes Species: Reticulitermes dichrous and Reticulitermes guangzhouensis (Isoptera: Rhinotermitidae)

Authors: Ke, Yunling, Wu, Wenjing, Zhang, Shijun, and Li, Zhiqiang

Source: Florida Entomologist, 100(1) : 101-108

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.100.0115>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

Yunling Ke^{1,2,3}, Wenjing Wu^{1,2,3}, Shijun Zhang^{1,2,3}, and Zhiqiang Li^{1,2,3,*}

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 (*16S 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 *16S 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. 2005b), 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 & Hussenader 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

¹Guangdong Institute of Applied Biological Resources, Guangzhou, 510260, China; E-mail: keyl@giabr.gd.cn (Y. K.), wuwj@giabr.gd.cn (W. W.), zhangsj@giabr.gd.cn (S. Z.), lizq@giabr.gd.cn (Z. L.)

²Guangdong Public Laboratory of Wild Animal Conservation and Utilization, Guangzhou, 510260, China

³Guangdong Key Laboratory of Integrated Pest Management in Agriculture, Guangzhou, 510260, China

*Corresponding author; E-mail: lizq@giabr.gd.cn (Z. L.)

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,

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-

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

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

NA, not available; NS, not submitted.

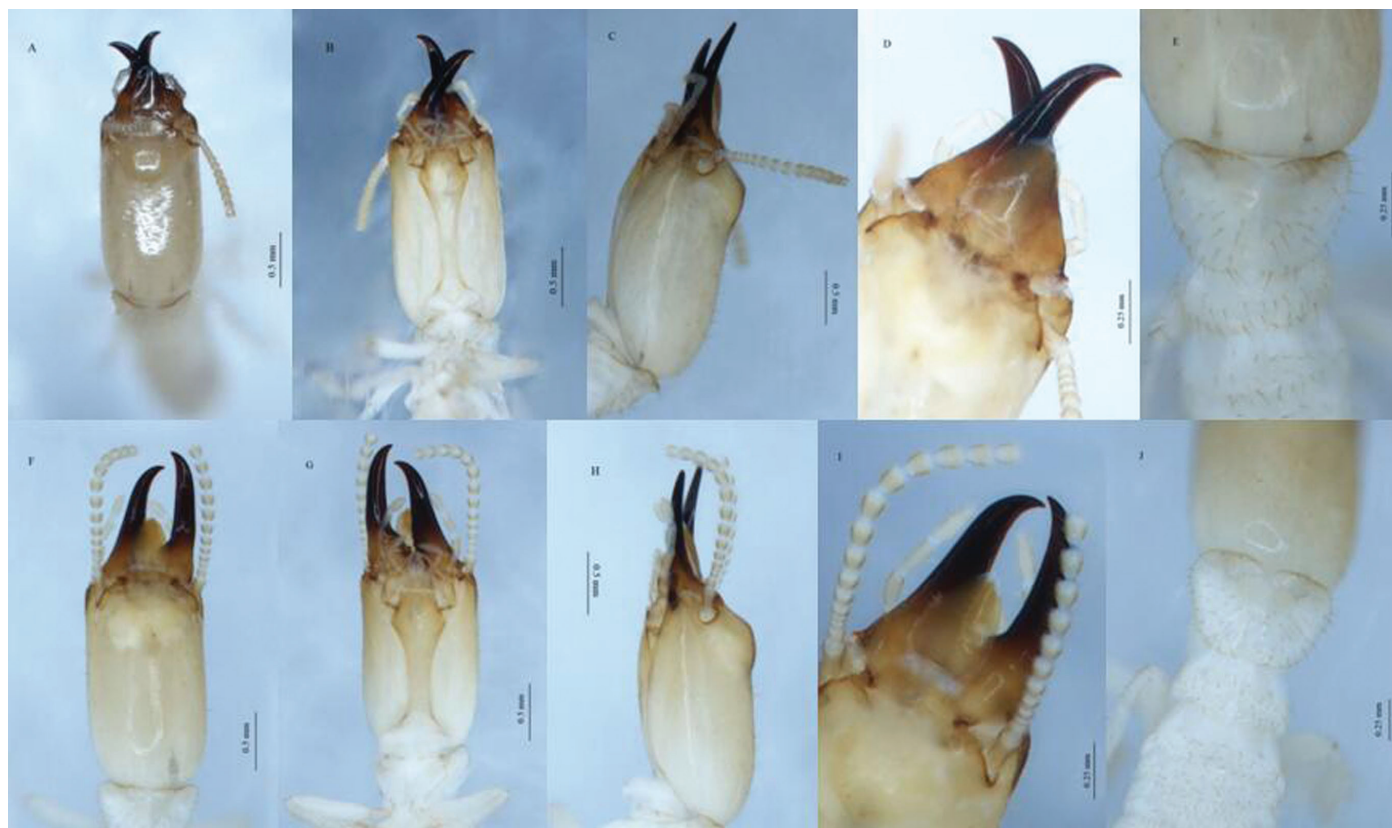


Fig. 1. Photomicrographs of dorsal (A, D, E, F, I, and J), ventral (B and G), and lateral (C and H) views of *Reticulitermes dichrous* (A to E) and *Reticulitermes guangzhouensis* (F to J) soldier heads and pronotums.

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'-TTACGCTGTATCCTAA-3')

(Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al. 1994) for *16S rRNA*. PCR amplification was performed in a 50 µL reaction mixture containing 2 µL DNA, 21 µL dH₂O, 25 µL Premix Taq™ (Ex Taq version, TaKaRa, Tokyo, Japan), 1 µL forward primer at 10 µM, and 1 µL reverse primer at 10 µM. 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-

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

Population	Measurements ^a (mean ± SE)										
	HL	HW	LML	LL	LW	POL	POW	PWW	PRL	PRW	HTL
<i>Reticulitermes guangzhouensis</i>											
Rg01	1.600 ± 0.029e-i	0.995 ± 0.010c	1.005 ± 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	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 ± 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 ± 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
<i>Reticulitermes dichrous</i>											
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	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 ± 0.010cd	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	0.478 ± 0.014d-g	0.675 ± 0.005gh	0.760 ± 0.006cd

^aMeans followed by different lowercase letters within the same column are significantly different by the Duncan multiple range test ($P \leq 0.05$).

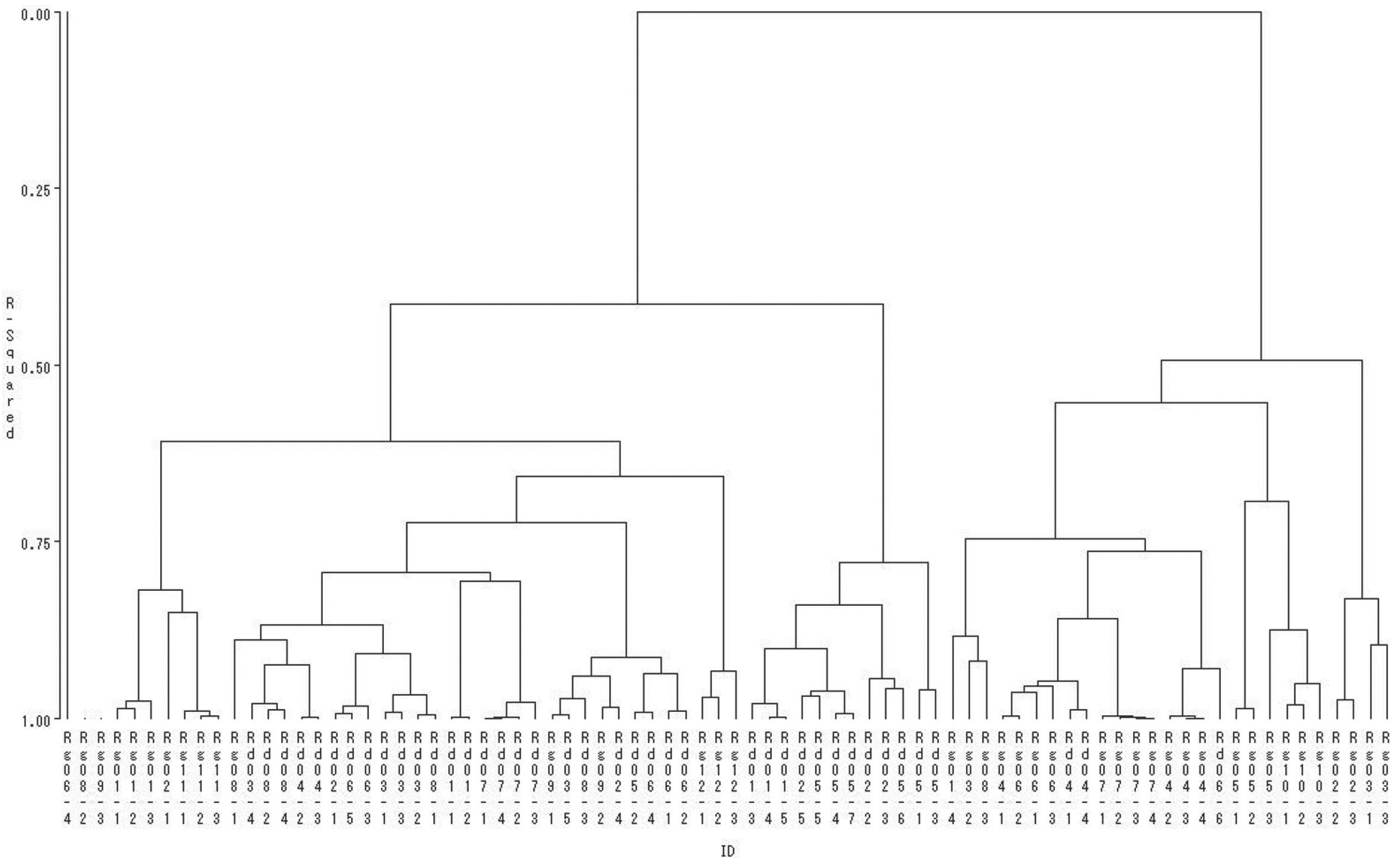


Fig. 2. Cluster dendrogram of 12 populations of *Reticulitermes guangzhouensis* and 8 populations of *Reticulitermes dichrous*.

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. guangzhouensis*, 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*.

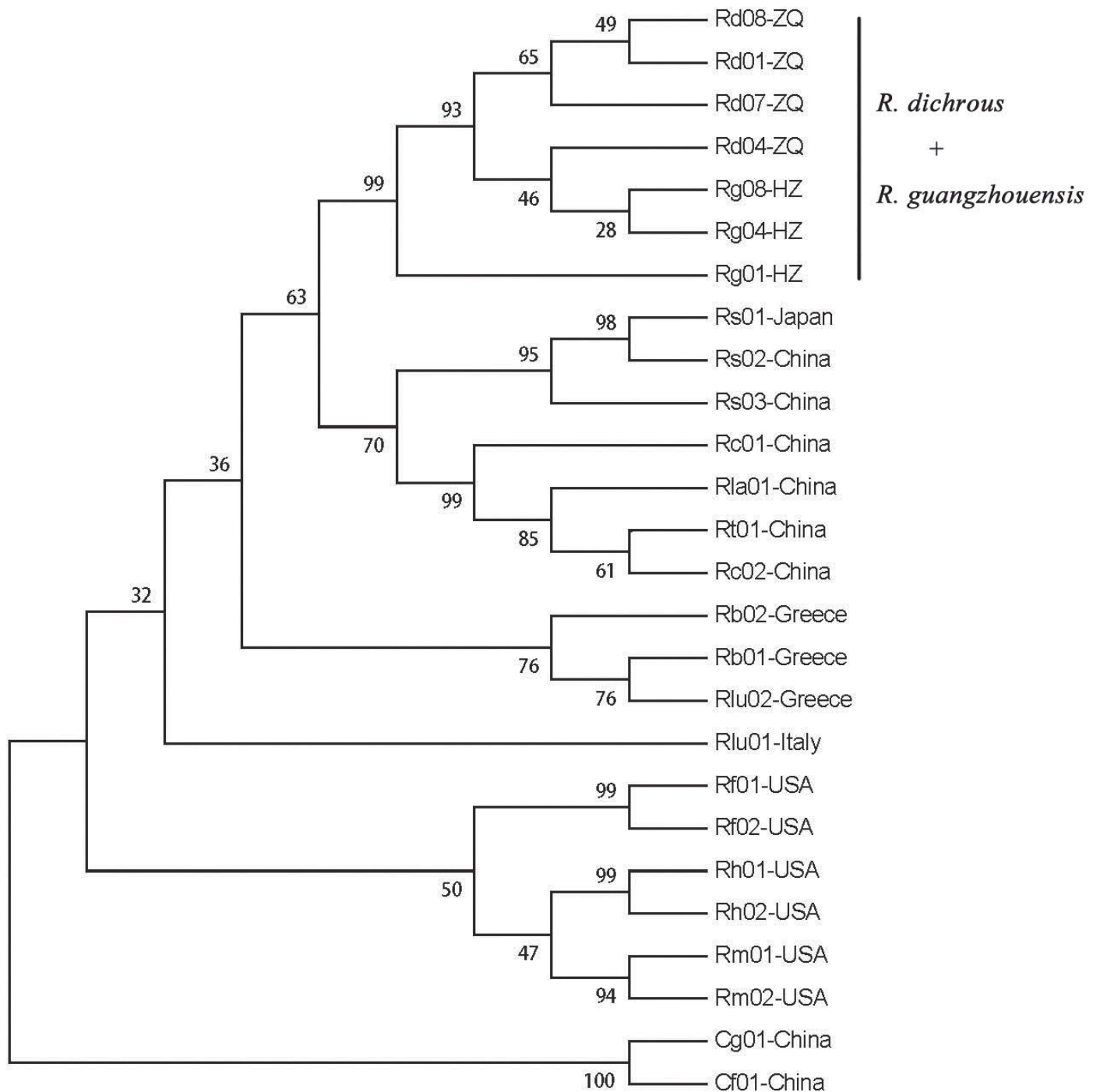


Fig. 3. Maximum likelihood phylogenetic tree of *Reticulitermes guangzhouensis* and *Reticulitermes dichrous* relative to other *Reticulitermes* species based on the *COII* gene. Bootstrap values are indicated at the nodes and represent 1,000 pseudoreplicates.

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. dichrous* and *R. guangzhouensis* from this study and a population of *R. guangzhouensis* 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 (Scheff-

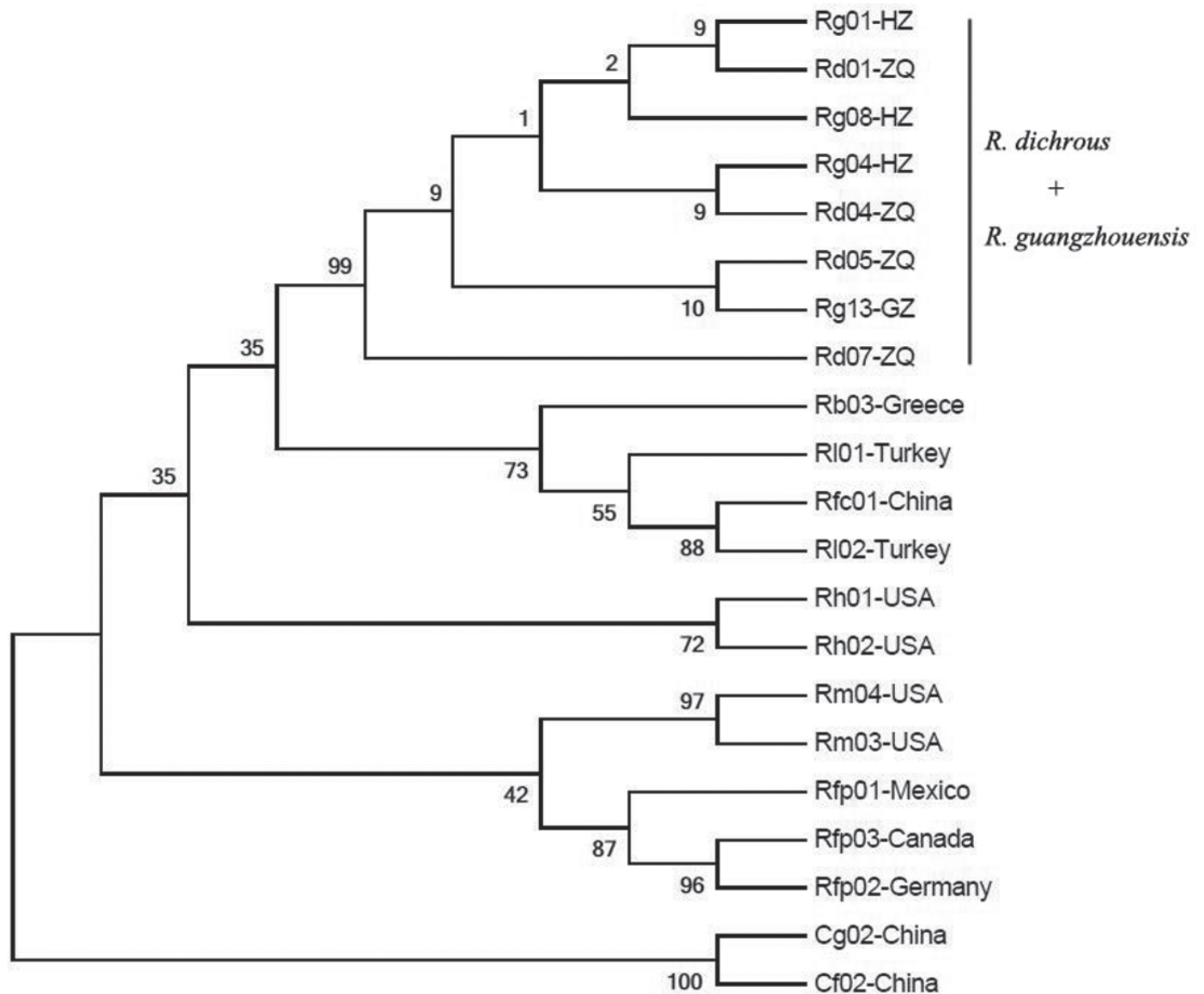


Fig. 4. Maximum likelihood phylogenetic tree of *Reticulitermes guangzhouensis* and *Reticulitermes dichrous* relative to other *Reticulitermes* species based on the 16S rRNA gene. Bootstrap values are indicated at the nodes and represent 1,000 pseudoreplicates.

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, Zhaoqing, 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. arenicola* 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. arenicola* 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. guangzhouensis* 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 varia-

tions 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.**

Acknowledgments

We are grateful to Jixing Guo (College of Life Sciences, Sun Yat-sen University) for photographing the specimens used in this study. This work was supported by the National Natural Science Foundation of China (31172140, 31172163) and Funds for Environment Construction and Capacity Building of GDAS' Research Platform (2016GDASPT-0107).

References Cited

- Austin JW, Szalanski AL, Scheffrahn RH, Messenger MT. 2005a. Genetic variation of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) in North America applying the mitochondrial *rRNA 16S* gene. *Annals of the Entomological Society of America* 98: 980–988.
- Austin JW, Szalanski AL, Scheffrahn RH, Messenger MT, Dronnet S, Bagnères A-G. 2005b. Genetic evidence for the synonymy of two *Reticulitermes* species: *Reticulitermes flavipes* and *Reticulitermes santonensis*. *Annals of the Entomological Society of America* 98: 395–401.
- Austin JW, Szalanski AL, Ghayourfar R, Kence A, Gold RE. 2006. Phylogeny and genetic variation of *Reticulitermes* (Isoptera: Rhinotermitidae) from the eastern Mediterranean and Middle East. *Sociobiology* 47: 873–890.
- Austin JW, Bagnères AG, Szalanski AL, Scheffrahn RH, Heintschel BP, Messenger MT, Clément JL, Gold RE. 2007. *Reticulitermes mallei* (Isoptera: Rhinotermitidae): a valid Nearctic subterranean termite from eastern North America. *Zootaxa* 1554: 1–26.
- Eggleton P. 1999. Termite species description rates and the state of termite taxonomy. *Insectes Sociaux* 46: 1–5.
- Ghesini S, Marini M. 2012. New data on *Reticulitermes urbis* and *Reticulitermes lucifugus* in Italy: Are they both native species? *Bulletin of Insectology* 65: 301–310.
- Huang FS, Zhu SM, Ping ZM, He XS, Li GX, Gao DR [eds.]. 2000. *Fauna Sinica, Insecta*, Volume 17: Isoptera. Science Press, Beijing, China.
- Huang Z, Chen X, Shi Y, Shen Z, Peng J, Yang H. 2011. Molecular analysis of some Chinese termites (Isoptera) based on the mitochondrial cytochrome oxidase (*ColI*) gene. *Sociobiology* 58: 107–117.
- Kambhampati S, Smith PT. 1995. PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Molecular Biology* 4: 233–236.
- King SW, Austin JW, Szalanski AL. 2007. Use of soldier pronotal width and mitochondrial DNA sequencing to distinguish the subterranean termites, *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae), on the Delmarva Peninsula: Delaware, Maryland, and Virginia, U.S.A. *Entomological News* 118: 41–48.
- Krishna K, Grimaldi DA, Krishna V, Engel MS. 2013. Termite evolution: diversity, distributions, phylogeny, fossil record, pp. 147–182 *In* *Treatise on the Isoptera of the World*, Volume 1, Bulletin of the American Museum of Natural History 377. American Museum of Natural History—Scientific Publications, New York, New York.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.
- Li H-F, Ye W, Su N-Y, Kanzaki N. 2009. Phylogeography of *Coptotermes gestroi* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Taiwan. *Annals of the Entomological Society of America* 102: 684–693.
- Lim SY, Forschler BT. 2012. *Reticulitermes nelsonae*, a new species of subterranean termite (Rhinotermitidae) from the southeastern United States. *Insects* 3: 62–90.
- Liu H, Beckenbach AT. 1992. Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Molecular Phylogenetics and Evolution* 1: 41–52.
- Long YH, Xiang H, Xie L, Yan X, Fan M, Wang Q. 2009. Intra- and interspecific analysis of genetic diversity and phylogeny of termites (Isoptera) in East China detected by *ISSR* and *COII* markers. *Sociobiology* 53: 411–430.
- Luchetti A, Trenta M, Mantovani B, Marini M. 2004. Taxonomy and phylogeny of north Mediterranean *Reticulitermes* termites (Isoptera, Rhinotermitidae): a new insight. *Insectes Sociaux* 51: 117–122.
- Ping ZM. 1985. Eight new species of the genus *Coptotermes* and *Reticulitermes* from Guangdong Province, China (Isoptera: Rhinotermitidae). *Entomotaxonomia* 7: 317–326.
- SAS (SAS Institute Inc.). 2002. SAS® software Version 9.0. SAS Institute Inc., Cary, North Carolina.
- Scheffrahn RH, Kreck J, Szalanski AL, Austin JW. 2005. Synonymy of Neotropical arboreal termites *Nasutitermes corniger* and *N. costalis* (Isoptera: Termitidae: Nasutitermitinae), with evidence from morphology, genetics, and biogeography. *Annals of the Entomological Society of America* 98: 273–281.
- Schrader C, Schielke A, Ellerbroek L, John R. 2012. PCR inhibitors – occurrence, properties and removal. *Journal of Applied Microbiology* 113: 1014–1026.
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651–701.
- Su N-Y, Ye W, Ripa R, Scheffrahn RH, Giblin-Davis RM. 2006. Identification of Chilean *Reticulitermes* (Isoptera: Rhinotermitidae) inferred from three mitochondrial gene DNA sequences and soldier morphology. *Annals of the Entomological Society of America* 99: 352–363.
- Szalanski AL, Austin JW, Owens CB. 2003. Identification of *Reticulitermes* spp. (Isoptera: Reticulitermitidae) from south central United States by PCR-RFLP. *Journal of Economic Entomology* 96: 1514–1519.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
- Tripodi AD, Austin JW, Szalanski AL, McKern J, Carroll MK, Saran RK, Messenger MT. 2006. Phylogeography of *Reticulitermes* termites (Isoptera: Rhinotermitidae) in California inferred from mitochondrial DNA sequences. *Annals of the Entomological Society of America* 99: 697–706.
- Vargo EL, Hussenader C. 2009. Biology of subterranean termites: insights from molecular studies of *Reticulitermes* and *Coptotermes*. *Annual Review of Entomology* 54: 379–403. doi: 10.1146/annurev.ento.54.110807.090443
- Yashiro T, Matsuura K. 2007. Distribution and phylogenetic analysis of termite egg-mimicking fungi “termite balls” in *Reticulitermes* termites. *Annals of the Entomological Society of America* 100: 532–538.
- Ye WM, Lee CY, Scheffrahn RH, Aleong JM, Su NY, Bennett GW, Scharfa ME. 2004. Phylogenetic relationships of Nearctic *Reticulitermes* species (Isoptera: Rhinotermitidae) with particular reference to *Reticulitermes arenicola* Goellner. *Molecular Phylogenetics and Evolution* 30: 815–822.
- Yeap BK, Othman AS, Lee VS, Lee CY. 2007. Genetic relationship between *Coptotermes gestroi* and *Coptotermes vastator* (Isoptera: Rhinotermitidae). *Journal of Economic Entomology* 100: 467–474.