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Taxonomic assessment of the termite genus *Neotermes* (Isoptera: Kalotermitidae) in the Ryukyu-Taiwan Island arc, with description of a new species

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Abstract

The Ryukyu Archipelago of Japan is a recognized global biodiversity hotspot with many endemic species. However, our knowledge of the termite fauna of the Ryukyu Archipelago is insufficient. Here, we report a new species of endemic drywood termite (Kalotermitidae) from the Ryukyu Archipelago. Our systematic study of the genus *Neotermes* (Isoptera: Kalotermitidae) from the Ryukyu–Taiwan Island arc using molecular and morphological methods found that *N. koshunensis* had also been composed of a second cryptic species, *N. sugioi* sp. nov. These two species are distributed allopatrically in the Ryukyu–Taiwan Island arc, with the former only in Taiwan and the latter only in the Ryukyu Archipelago. Our discovery of a new drywood termite species from the Ryukyu Archipelago suggests that both morphological and molecular assessments of the species now considered to be distributed widely in the Ryukyu–Taiwan Island arc are needed to clarify the termite fauna of the Ryukyu Archipelago. [Species Zoobank registration: http://zoobank.org/urn:lsid:zoobank.org:pub:E8C9693A-E24F-445D-8445-320564565964]

Key words: taxonomy, endemic species, *Neotermes sugioi*, *Neotermes koshunensis*, Asian drywood termite

Introduction

The Ryukyu Archipelago of Japan, located in the subtropical zone of the western Pacific Ocean between mainland Japan and Taiwan, is characterized by many endemic species (Hatusima 1980; Motokawa 2000; Ota 2000). Numerous surveys have examined the insect fauna of the Archipelago and have recorded more than 7,500 insect species, of which about 27% are endemic to the Archipelago (Azuma & Kinjo 1987; Azuma *et al.* 2002; Sakamaki 2013). Unfortunately, current knowledge of the total insect fauna of the Ryukyu Archipelago is insufficient because of bias in the taxa targeted in the surveys (Sakamaki 2013).

Although termites are widely distributed throughout the world, they are predominantly tropical and subtropical (Krishna 1970; Eggleton 2000; Krishna *et al.* 2013). Mirroring the global pattern of termite diversity, the termite species diversity in the subtropical Ryukyu Archipelago is much higher than that in temperate mainland Japan (Morimoto 1980; Takematsu 2006). Nevertheless, no endemic termite species has been recorded from the Ryukyu Archipelago, except for those of the genus *Reticulitermes* (Rhinotermitidae), which is the best-studied termite genus in terms of its taxonomy and geographic distribution in Japan (Morimoto 1968; Takematsu 1999). Termites of genus *Neotermes* Holmgren, 1911 (Kalotermitidae) damages trees in tropical and subtropical regions, including the Ryukyu Archipelago (Krishna *et al.* 2013; Sugio *et al.* 2018). In the Ryukyu–Taiwan Island arc, only one member of the genus, *Neotermes koshunensis* (Shiraki, 1909), has been recognized (Ikehara 1966; Li *et al.* 2008), but this species was suspected of being a complex composed of multiple cryptic species (Yasuda *et al.* 2000).

In this study, we conducted molecular and morphological assessments of *Neotermes* in the Ryukyu–Taiwan Island arc to examine whether *N. koshunensis* is a species complex. We first collected colonies of *Neotermes* ter-

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mites in the Ryukyu Archipelago and Taiwan and conducted phylogenetic analyses to elucidate the evolutionary relationships among Ryukyu and Taiwanese populations of the termites. Then, based on phylogenetic evidence of the separate status of the Ryukyu and Taiwanese populations of the termites, we explored the morphological differences between them. Finally, based on morphological evidence of the separate status of the Ryukyu and Taiwanese forms of the termites and the type locality data of *N. koshunensis*, we describe a new *Neotermes* species from the Ryukyu Archipelago.

Materials and methods

Termites. We collected 37 mature *Neotermes* colonies from different logs: 30 in the Ryukyu Archipelago, Japan (Okinawa, Miyako, Ishigaki, Iriomote, and Yonaguni Islands) and seven on the main island of Taiwan (two of seven in the type locality of *N. koshunensis*, Hengchun, Pingtung County). We dismantled the nest carefully and extracted all of the colony members using an aspirator and forceps. The pseudergates from each colony were immediately preserved in 99.5% (vol/vol) ethanol in a vial for DNA extraction. Some imagos and soldiers from each colony were placed in a moist unwoven cloth in a 90-mm Petri dish, which was sealed with plastic tape after placing termites to avoid desiccation, and freeze-killed at −25°C (normally within 12 h) for morphological study. The sex of the imagos and soldiers was determined from the size of the 7th sternite under a stereomicroscope (SZX7; Olympus, Tokyo, Japan) (Weesner 1969).

Phylogenetic analyses. One pseudergate randomly chosen from each colony of the *Neotermes* termites was used for phylogenetic analyses based on the mitochondrial cytochrome *c* oxidase subunit II (*COII*) sequences and nuclear internal transcribed spacer 2 (*ITS2*) sequences. The head or legs of individual termites were ground in Chelex-100 resin solution (Bio-Rad, Richmond, CA, USA), and DNA was extracted and purified following standard Chelex-based protocols (Yashiro *et al.* 2018). The primer sequences, PCR conditions, and sequencing methods are detailed elsewhere (*COII*, Yashiro *et al.* 2011; *ITS2*, Maryańska-Nadachowska *et al.* 2010). We obtained *COII* (671-bp) and *ITS2* (381-bp) sequences. Consensus sequences were aligned using ClustalX (Thompson *et al.* 1997) with the BioEdit 7.0.4.1 sequence editor (Hall 1999) and were corrected manually. Bayesian inference was performed based on the combined data set (*COII* + *ITS2*) using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with the K80 + gamma model for the first codon position of *COII*, the HKY85 + Homogeneous model for the second codon position of *COII*, the HKY85 + Gamma model for the third codon position of *COII*, and the HKY85 + Homogeneous model for *ITS2* selected by the Bayesian Information Criterion (BIC) in Kakusan4 (Tanabe 2011). The *COII* and *ITS2* sequences from *Kalotermes flavicollis* (Fabricius, 1793) (*COII*, GenBank accession number MG333653; *ITS2*, GenBank accession number MG333692) and those from *Cryptotermes domesticus* (Haviland, 1898) (*COII*, GenBank accession number MG333652; *ITS2*, GenBank accession number MG333691) were used as first and second outgroups for the phylogenetic analysis, respectively. Two runs with four chains of Markov chain Monte Carlo iterations were performed for 2,000,000 generations, when the average standard deviations of split frequencies were below 0.01 (the first 25% of the generations as burn-in). Trees were kept for every 100 generations, and the latest 75% of the trees were used to calculate 50% majority-rule trees and to determine the posterior probabilities. Bayesian inference was also performed based on the *COII* sequences of kalotermitid species obtained in this study and five *Neotermes* species deposited in GenBank, including all identified *Neotermes* species deposited in GenBank, using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with the GTR + I + G model selected by the hierarchical Likelihood Ratio Test (hLRT) in MrModeltest 2.3 (Nylander 2004). The phylogenetic analysis was performed as described above. The *COII* and *ITS2* gene sequences obtained in this study were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession numbers MG333615–MG333692.

Morphological study. Two imagos (one male and one female) and two soldiers (one male and one female) were chosen randomly from each colony and measured using a stereomicroscope (SZX7; Olympus, Tokyo, Japan) with a digital imaging system (FLVFS-LS; Flovel, Tokyo, Japan), so that 74 imagos and 74 soldiers of the *Neotermes* termites were measured. For imagos, the following biometric measurements were made: maximum head width (HW), including the compound eyes; head length (HL), measured from the lateral margin of the mandible base to the posterior margin of the head capsule; maximum left eye diameter (ED), excluding the ocular sclerite; maximum left ocellus diameter (OD); left mandible length (LML), cross-length of the left mandible, measured from the tip to most distant visible point of the ventral condyle; right mandible length (RML), cross-length of the right mandible, measured from the tip to most distant visible point of the ventral condyle; maximum labrum width (LW); labrum length (LL), measured in the midline between the anterior and posterior margins; maximum postmentum width (PmW1); postmentum length (PmL), measured in the midline from the anterior to posterior margin; maximum pronotum width (PnW); pronotum length (PnL), measured in the midline between the anterior and posterior margins; maximum left forewing length (FWL), including the scale; maximum left hindwing length (HWL), including the scale; and maximum left tibia length (TL), excluding the proximal part of the articulation that is received into the distal end of the femur. For soldiers, the following biometric measurements were made: HW; HL; maximum head height (HH), excluding the postmentum; maximum left eye spot diameter (ESD); LML; RML; LW; LL; maximum anterior lobe width of the postmentum (PmW2); minimum postmentum width (PmW3); PmL; PnW; PnL; and TL. To evaluate the multivariate difference in measurements between Ryukyu and Taiwanese individuals in imagos and soldiers, respectively, we used Hotelling's T^2 test (JMP 9; SAS Institute, Cary, NC, USA). To further illustrate the morphological difference between Ryukyu and Taiwanese individuals in imagos and soldiers, respectively, we used the principle components analysis (PCA). To determine accuracy of species identifications based on morphological proportions in imagos and soldiers, we used the discriminant analysis (JMP 9; SAS Institute, Cary, NC, USA). Moreover, some individuals the *Neotermes* termites were observed using a scanning electron microscope (SEM) (VE-8800; Keyence, Osaka, Japan).

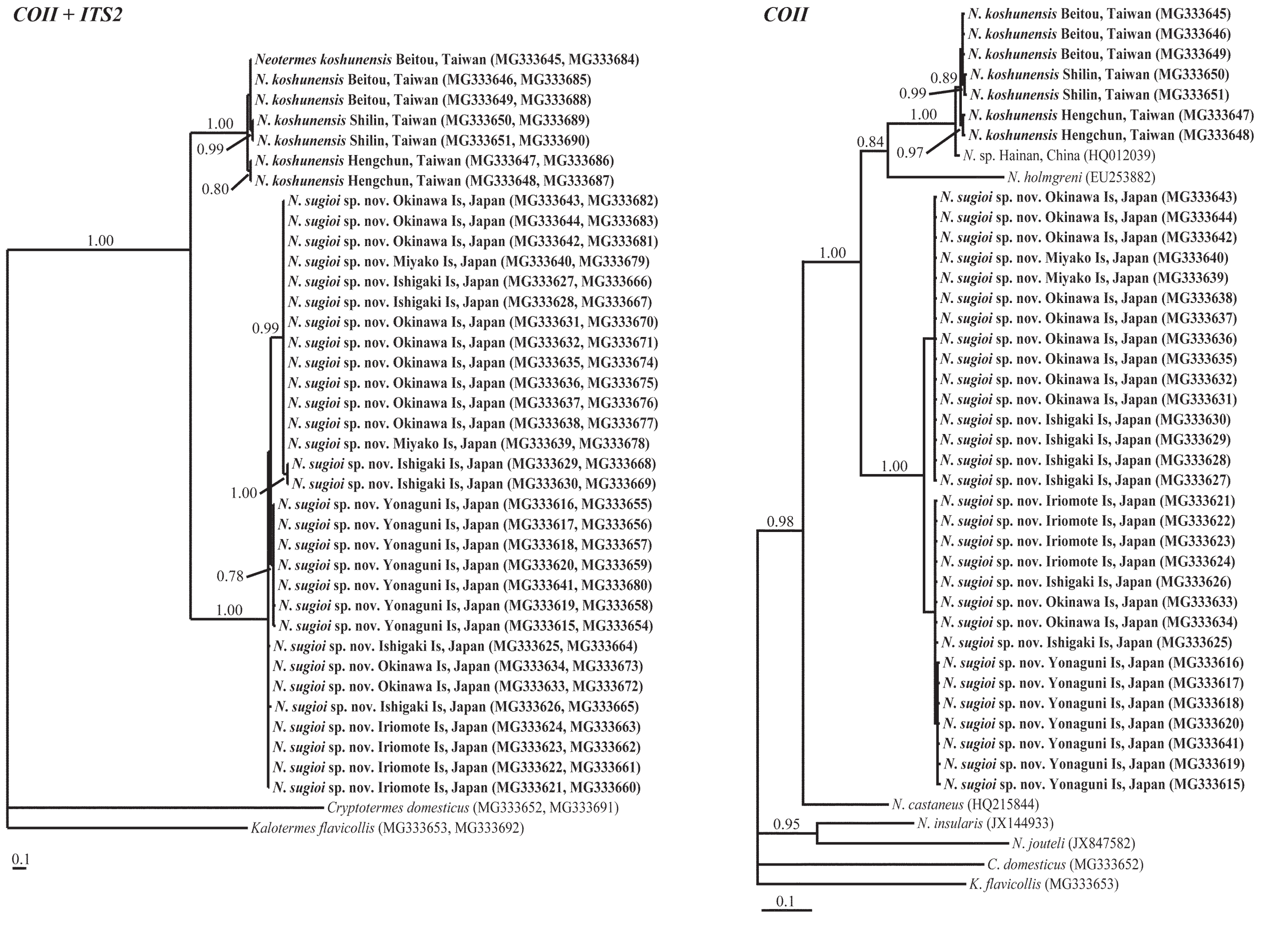


FIGURE 1. Bayesian phylogenetic trees of *Neotermes* termites in the Ryukyu Archipelago (*Neotermes sugioi* **sp. nov.**) and Taiwan (*N. koshunensis*) based on mitochondrial *COII* and nuclear *ITS2* sequences (left) and mitochondrial *COII* sequences (right). The corresponding posterior probabilities (≥ 0.70) are shown by the branches. GenBank accession numbers are shown in parentheses.

Results

Phylogenetic analyses. Phylogenetic tree based on combined mitochondrial *COII* and nuclear *ITS2* sequences, as well as that based on *COII* sequences, showed the separate status of Ryukyu and Taiwanese populations of the *Neotermes* termites. The monophyly of both Ryukyu [*COII* + *ITS2*, Bayesian posterior probability (BPP) = 1.00; *COII*, BPP = 1.00] and Taiwanese populations (*COII* + *ITS2*, BPP = 1.00; *COII*, BPP = 0.89) were unequivocally supported (Fig. 1). The phylogenetic tree based on *COII* sequences showed that *Neotermes* sp. from Hainan, Southern China (GenBank accession number HQ012039) (Huang *et al.* 2011) formed a clade with Taiwanese populations (BPP = 1.00) (Fig. 1).

Morphological studies. Each of all the morphological traits measured in imagos and soldiers overlapped between Ryukyu and Taiwanese individuals, respectively (Table 1), although PmW2 in male soldiers did not overlap between Ryukyu and Taiwanese individuals (Appendix 1). Hotelling's T^2 tests demonstrated significant multivariate differences between Ryukyu and Taiwanese individuals in imago morphology (Hotelling's $T^2 = 2.05$, $F_{15, 58} = 7.93$, $P < 0.0001$) and in soldier morphology (Hotelling's $T^2 = 8.31$, $F_{14, 59} = 35.03$, $P < 0.0001$). PCA for soldier morphometric data also showed distinct separation between Ryukyu and Taiwanese individuals, although PCA for imago morphometric data did not show clear separation between them (Fig. 2). Discriminant analysis based on HW and PmW2 in soldiers classified Ryukyu and Taiwanese individuals with 100% accuracy, whereas discriminant analyses based on all other combinations of two measurements in soldiers and those based on all combinations of two measurements in imagos could not perfectly classified them (Imagos, 85–54% accuracy; Soldiers, 99–50% accuracy). Furthermore, comparative morphological observations showed that overlapping wings of imagos are darker brown in Ryukyu individuals than in Taiwanese individuals, with only a few exceptions (Fig. 3).

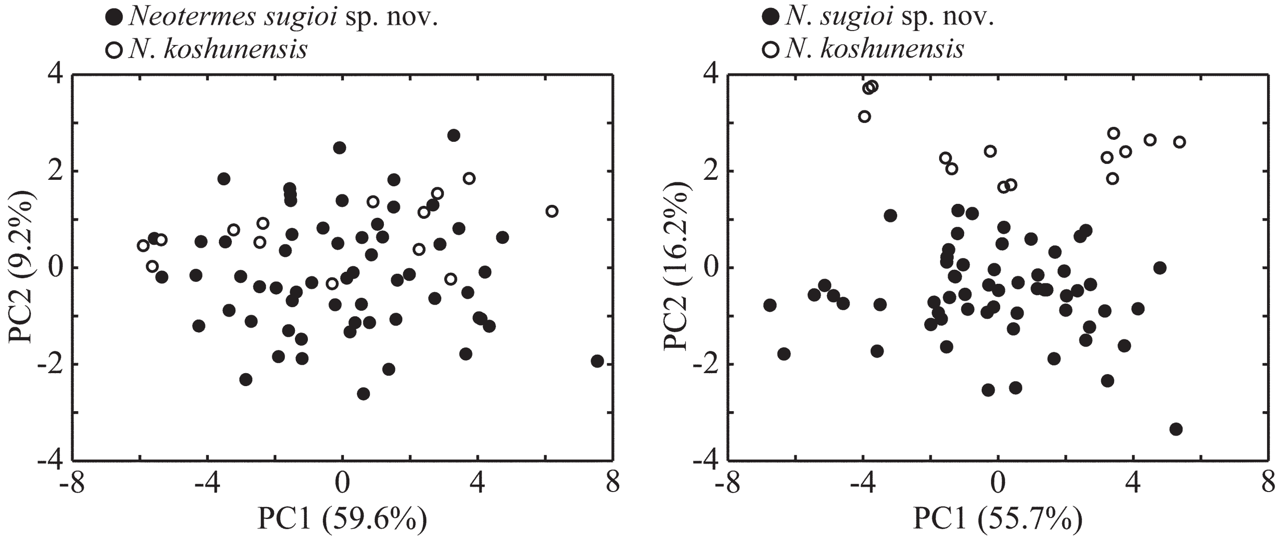


FIGURE 2. Principal components analyses for imago (left) and soldier (right) morphometric data of *Neotermes* termites in the Ryukyu Archipelago (*Neotermes sugioi* **sp. nov.**) and Taiwan (*N. koshunensis*).

Taxonomy

***Neotermes sugioi* Yashiro, sp. nov.**
Japanese name: Sugio-shiroari
(Figs 3–17)

Imago: Head capsule and pronotum reddish brown; abdominal tergites paler than head capsule; compound eyes black; ocelli translucent; mandibles blackish brown apically, paler posteriorly; antennae reddish brown basally, paler apically; labrum, postmentum, legs, and abdominal sternites pale yellowish brown; wing-scales and sclerotized veins brownish, remainder of wings brownish subhyaline.

Head capsule subquadrate, sides slightly broadening posteriorly, posterior margin convex in dorsal view; frons sloping gently anteriorly in lateral view; Y-suture distinct; fairly hairy with long and short setae on body. Compound eyes subcircular in lateral view; slightly protruding in dorsal view; situated very close to antennal bases. Ocelli oval in lateral view; slightly protruding in dorsal view; situated very close to compound eyes. Left mandible with 2 marginal teeth. Right mandible with 2 marginal teeth. Labrum subquadrate, sides convex, anterior margin substraight in dorsal view; with long setae anteriorly, long and short setae on body. Antennae with 17–20 articles, 1>2=3>4<5; fairly hairy with long and short setae. Postmentum suboctagonal, sides subparallel in ventral view; with long and short setae on margins and body.

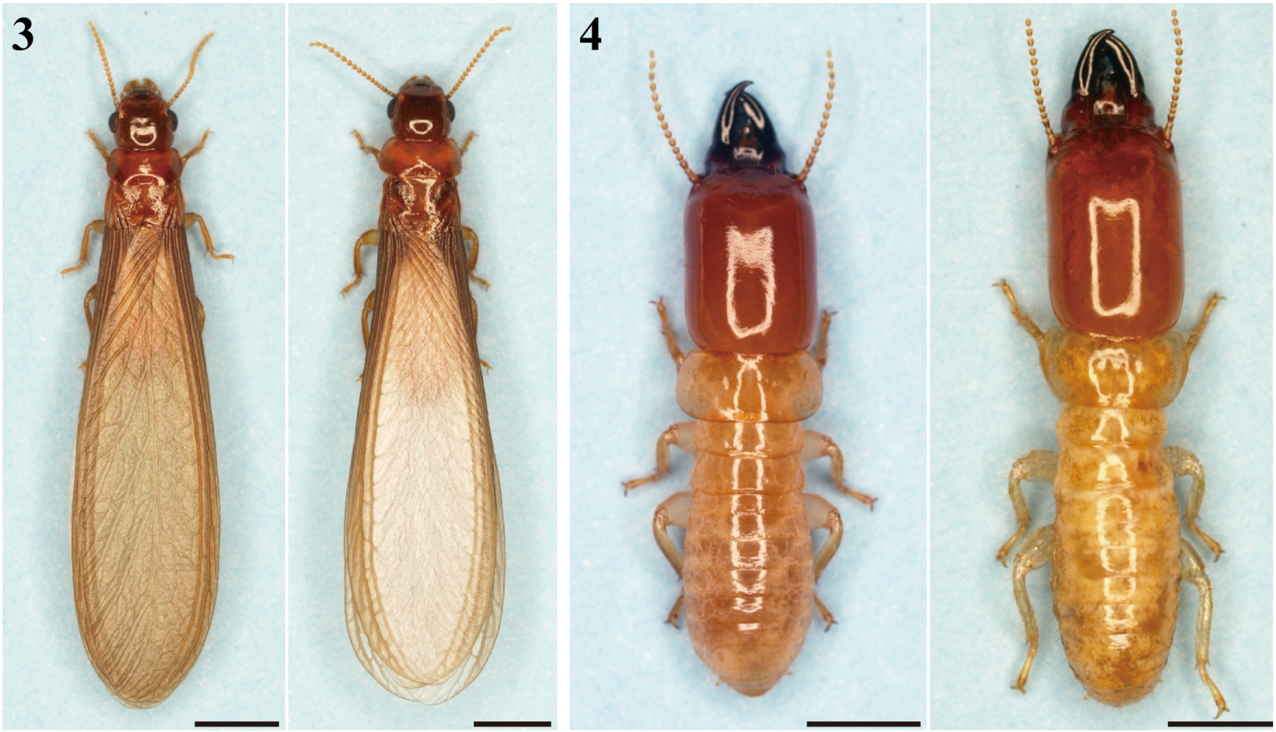
TABLE 1. Comparison of measurements of imagos and soldiers between Ryukyu and Taiwanese individuals of the *Neotermes* termites

Measurement[a]	Ryukyu individuals (n = 60)		Taiwanese individuals (n = 14)	
	Range (mm)	Mean ± SD	Range (mm)	Mean ± SD
Imagos				
HW	1.55–1.89	1.73 ± 0.08	1.59–1.79	1.68 ± 0.08
HL	1.50–1.72	1.60 ± 0.05	1.48–1.68	1.59 ± 0.07
ED	0.51–0.64	0.57 ± 0.03	0.54–0.64	0.58 ± 0.03
OD	0.16–0.25	0.19 ± 0.02	0.19–0.25	0.22 ± 0.02
LML	0.67–0.80	0.72 ± 0.03	0.66–0.76	0.70 ± 0.03
RML	0.59–0.68	0.63 ± 0.02	0.58–0.69	0.62 ± 0.03
LW	0.58–0.66	0.62 ± 0.02	0.55–0.64	0.60 ± 0.03
LL	0.50–0.59	0.54 ± 0.02	0.48–0.57	0.53 ± 0.03
PmW1	0.50–0.61	0.56 ± 0.02	0.53–0.61	0.58 ± 0.03
PmL	0.83–0.97	0.89 ± 0.03	0.83–0.95	0.90 ± 0.04
PnW	1.74–2.07	1.92 ± 0.09	1.76–2.14	1.97 ± 0.11
PnL	0.77–1.04	0.94 ± 0.05	0.87–1.04	0.95 ± 0.06
FWL	11.30–13.79	12.49 ± 0.58	11.30–13.71	12.82 ± 0.75
HWL	10.53–12.80	11.59 ± 0.58	10.68–12.63	11.83 ± 0.68
TL	1.35–1.78	1.50 ± 0.07	1.35–1.63	1.48 ± 0.07
Soldiers				
HW	1.93–2.62	2.32 ± 0.13	2.06–2.41	2.23 ± 0.12
HL	2.88–3.80	3.35 ± 0.21	3.12–3.73	3.40 ± 0.23
HH	1.56–2.04	1.79 ± 0.11	1.62–1.95	1.79 ± 0.11
ESD	0.20–0.42	0.29 ± 0.04	0.11–0.34	0.25 ± 0.06
LML	1.70–2.13	1.95 ± 0.09	1.96–2.21	2.08 ± 0.08
RML	1.65–2.04	1.87 ± 0.09	1.90–2.14	2.00 ± 0.09
LW	0.50–0.60	0.54 ± 0.02	0.46–0.57	0.52 ± 0.03
LL	0.39–0.49	0.43 ± 0.02	0.40–0.50	0.45 ± 0.03
PmW2	0.65–0.82	0.75 ± 0.04	0.80–0.96	0.88 ± 0.05
PmW3	0.23–0.42	0.31 ± 0.04	0.31–0.42	0.37 ± 0.03
PmL	2.02–2.77	2.36 ± 0.16	2.21–2.88	2.46 ± 0.25
PnW	1.90–2.84	2.40 ± 0.19	2.11–2.76	2.36 ± 0.20
PnL	0.88–1.35	1.10 ± 0.10	0.90–1.21	1.07 ± 0.10
TL	1.46–1.88	1.65 ± 0.10	1.41–1.77	1.58 ± 0.12

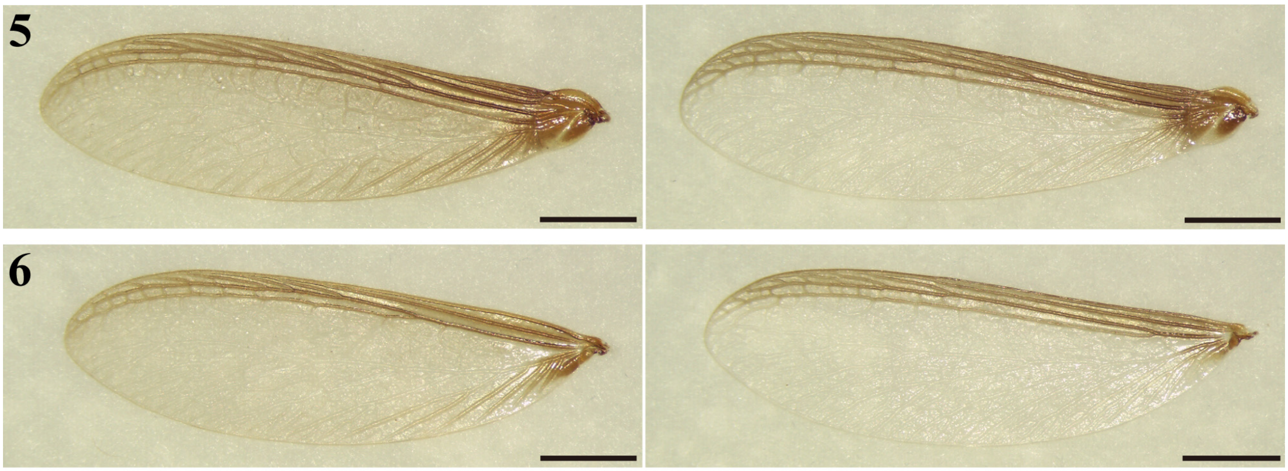
See also Appendix 1 for ranges of measurements within males and females.

[a] For abbreviations of measurements see Materials and methods.

Pronotum subrectangular, sides convex, anterior margin strongly concave, posterior margin faintly concave medially in dorsal view; fairly hairy with long and short setae on margins and body. Wing membrane non-tuberculate, with short setae on wing scales and along costal margins. Forewing subcosta meeting costa at about 2/5th of wing; radius meeting costa-subcosta at about middle; radial sector with 5 branches to costa-subcosta; media with 6–8 transverse veins to radial sector; area between media and cubitus reticulate; cubitus with 11–12 branches to posterior margin. Hindwing radius meeting costa-subcosta at about 2/3th of wing; radial sector with 4–5 branches to costa-subcosta; median originating from radial sector at about 1/3th of wing; cubitus with 10–11 branches. Legs with tibial spurs 3:3:3; tarsi 4-jointed; arolium present.



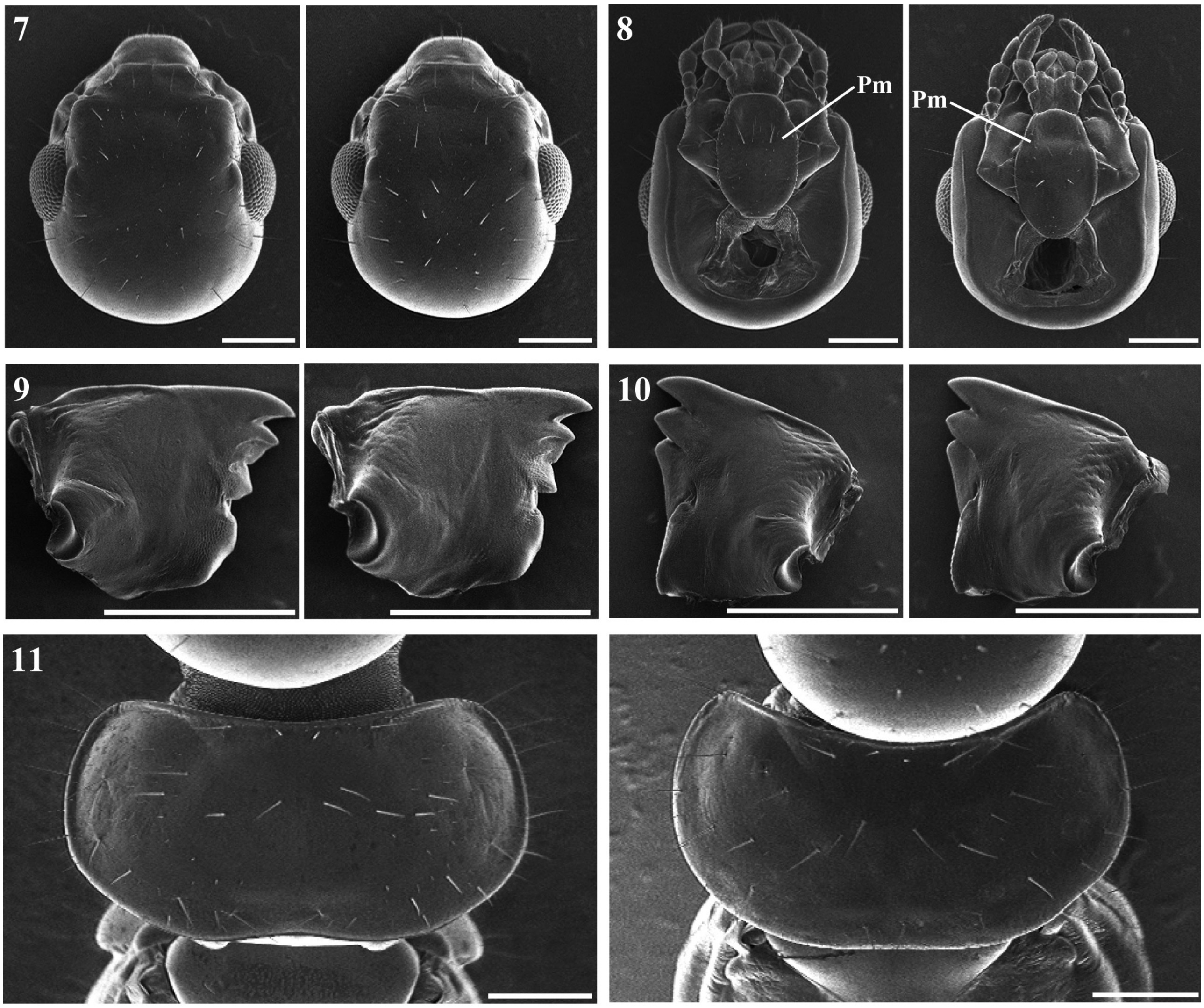
FIGURES 3–4. *Neotermes sugioi* **sp. nov.** (left) and *N. koshunensis* (right). 3, habitus of imagos, dorsal view; 4, habitus of soldiers, dorsal view. Scale bars, 2 mm.



FIGURES 5–6. *Neotermes sugioi* **sp. nov.** (left) and *N. koshunensis* (right). 5, left forewings of imagos, dorsal view; 6, left hindwings of imagos, dorsal view. Scale bars, 2 mm.

Soldier: Head capsule reddish brown, paler posteriorly; eye spots translucent; mandibles blackish brown apically, reddish brown basally; labrum yellowish to reddish brown; antennae reddish brown basally, paler apically; postmentum reddish brown, paler posteriorly; pronotum, legs, and abdomen yellowish white.

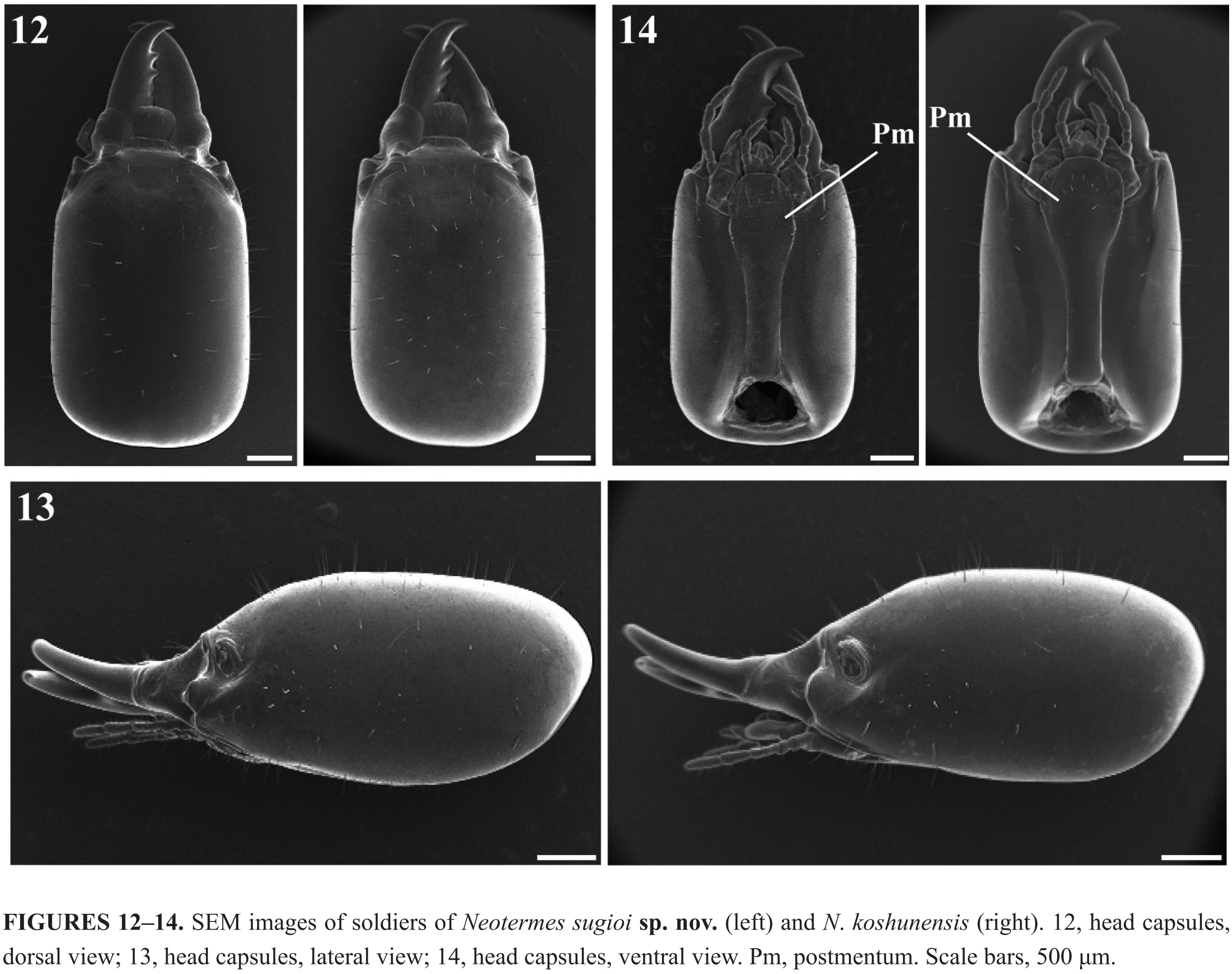
Head capsule subrectangular, sides subparallel, posterior margin weakly convex in dorsal view; frons sloping anteriorly toward clypeus at an angle of 25–40° in lateral view with shallow medial depression; Y-suture present, not distinct; sparsely hairy with long and short setae on body. Eye spots oval in lateral view; distinctly tuberculate; situated close to antennal bases. Left mandible robust; incurved apically; with 3 distinct marginal teeth; humped basally at outer margin. Right mandible robust; incurved apically; with 2 prominent marginal teeth; humped basally at outer margin. Labrum subquadrate, sides subparallel, anterior margin subparallel to posterior margin in dorsal view; with long setae anteriorly, long and short setae on body. Antennae with 13–17 articles, 1>2<3>4<5, 2>4; fairly hairy with long and short setae. Postmentum long, club-shaped in ventral view; with long and short setae on body.



FIGURES 7–11. SEM images of imagos of *Neotermes sugioi* **sp. nov**. (left) and *N. koshunensis* (right). 7, head capsules, dorsal view; 8, head capsules, ventral view; 9, left mandibles, dorsal view; 10, right mandibles, dorsal view; 11, pronota, dorsal view. Pm, postmentum. Scale bars, 500 µm.

Pronotum subrectangular, sides convex, anterior margin weakly concave, posterior margin substraight in dorsal view; fairly hairy with long and short setae on margins and body. Legs with tibial spurs 3:3:3; tarsi 4-jointed; arolium absent.

Type material. Holotype: ♂ soldier (in alcohol), Chibana, Okinawa, Okinawa Island, Japan, 26°21’50”N, 127°48’39”E, 93 m, 24. iv. 2013, collected by T. Yashiro. **Paratypes**: 1♂1♀ soldiers (in alcohol), same data as holotype; 2♂2♀ soldiers (dry-mounted), same data as holotype; 1♂1♀ imagos (dry-mounted), 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Chibana, Okinawa, Okinawa Island, Japan, 26°21’45”N, 127°48’38”E, 85 m, 24. iv. 2013, collected by T. Yashiro; 4♂4♀ imagos, 4♂4♀ soldiers, and 8 pseudergates (in alcohol), Katsurenhaebaru, Uruma, Okinawa Island, Japan, 26°19’57”N, 127°52’35”E, 38 m, 2. vii. 2014, collected by Y. Miyaguni; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Ogido, Kitanakagusuku, Nakagami, Okinawa Island, Japan, 26°17’35”N, 127°47’45”E, 134 m, 2. vii. 2014, collected by Y. Miyaguni; 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Okuma, Nakagusuku, Nakagami, Okinawa Island, Japan, 26°15’29”N, 127°47’03”E, 43 m, 1. vii. 2014, collected by Y. Miyaguni; 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Tsuha, Nakagusuku, Nakagami, Okinawa Island, Japan, 26°14’51”N, 127°46’50”E, 34 m, 2. vii. 2014, collected by Y. Miyaguni; 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Iso, Urasoe, Okinawa Island, Japan, 26°15’27”N, 127°43’22”E, 77 m, 25. iv. 2013, collected by T. Yashiro; 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Nakama, Urasoe, Okinawa Island, Japan, 26°15’09”N, 127°43’35”E, 116 m, 25. iv. 2013, collected by T. Yashiro; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Hirarahigashinakasonezoe, Miyakojima, Miyako Island, Japan, 24°47'57"N, 125°19'07"E, 52 m, 4. ii. 2014, collected by T. Yashiro; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Nosoko, Ishigaki, Ishigaki Island, Japan, 24°30'56"N, 124°15'26"E, 53 m, 28. vi. 2014, collected by Y. Miyaguni; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Fukai, Ishigaki, Ishigaki Island, Japan, 24°27'17"N, 124°11'28"E, 30 m, 28. vi. 2014, collected by Y. Miyaguni; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Tonoshiro, Ishigaki, Ishigaki Island, Japan, 24°22'50"N, 124°10'31"E, 99 m, 28. vi. 2014, collected by Y. Miyaguni; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Komi, Taketomi, Yaeyama, Iriomote Island, Japan, 24°19'11"N, 123°54'39"E, 5 m, 27. vi. 2014, collected by Y. Miyaguni; 2♂2♀ imagos, 2♂2♀ soldiers, and 4 pseudergates (in alcohol), Haimi, Taketomi, Yaeyama, Iriomote Island, Japan, 24°16'23"N, 123°49'51"E, 40 m, 26. vi. 2014, collected by Y. Miyaguni; 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Mt. Inbidake, Yonaguni, Yaeyama, Yonaguni Island, Japan, 24°26'58"N, 122°59'57"E, 164 m, 16. iv. 2013, collected by T. Yashiro; 5♂5♀ imagos, 5♂5♀ soldiers, and 10 pseudergates (in alcohol), Yonaguni, Yonaguni, Yaeyama, Yonaguni Island, Japan, 24°26'44"N, 122°58'26"E, 34 m, 24. vi. 2014, collected by Y. Miyaguni; 1♂1♀ imagos, 1♂1♀ soldiers, and 2 pseudergates (in alcohol), Yonaguni, Yonaguni, Yaeyama, Yonaguni Island, Japan, 24°27'23"N, 122°58'34"E, 102 m, 25. vi. 2014, collected by Y. Miyaguni.

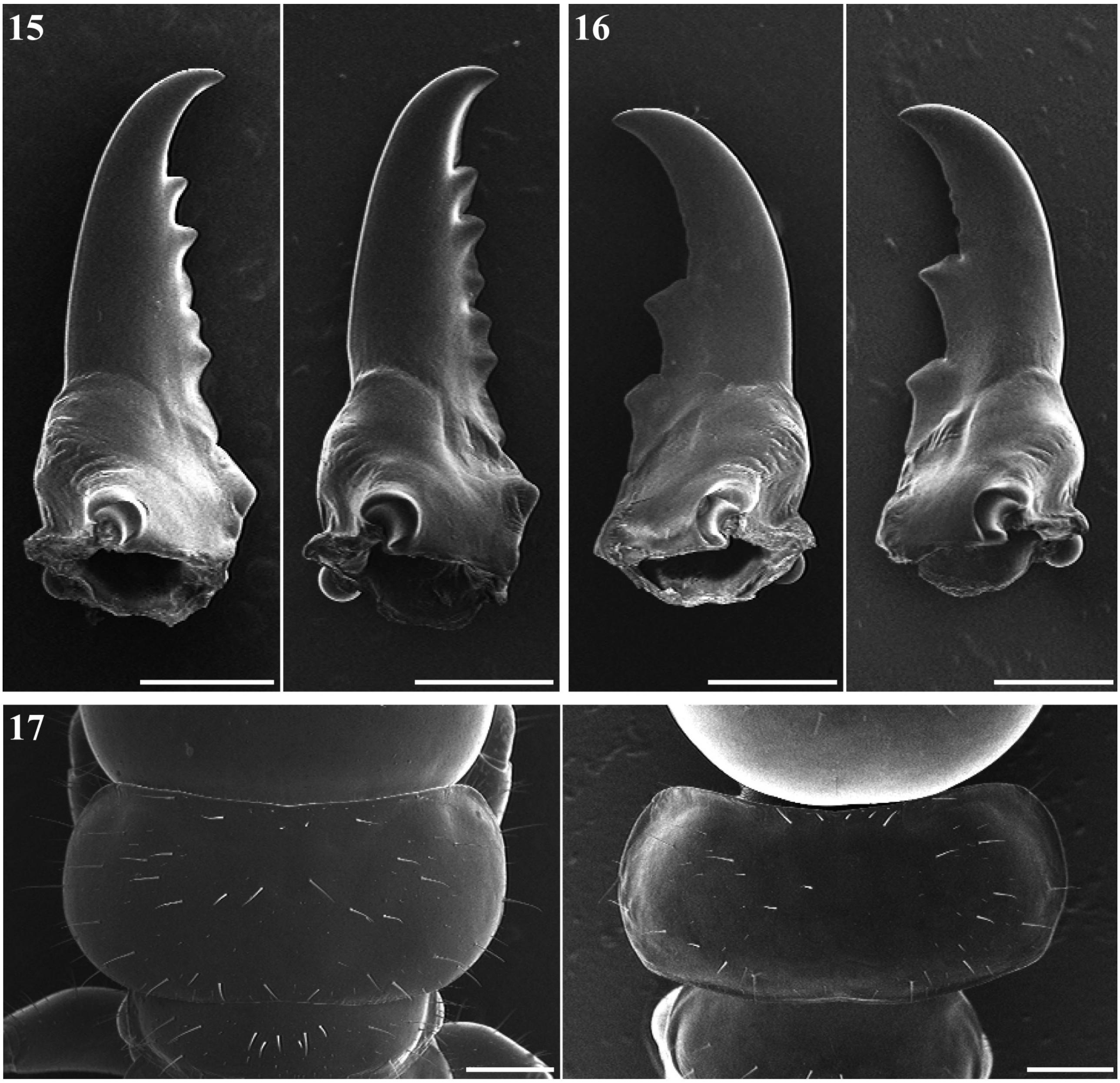


FIGURES 12–14. SEM images of soldiers of *Neotermes sugioi* **sp. nov.** (left) and *N. koshunensis* (right). 12, head capsules, dorsal view; 13, head capsules, lateral view; 14, head capsules, ventral view. Pm, postmentum. Scale bars, 500 μm.

Type-repository: The holotype (registration number B2324169) is deposited in the Museum of Nature and Human Activities, Hyogo, Japan. Paratypes are deposited in the Museum of Nature and Human Activities, Hyogo, Japan; the National Museum of Nature and Science, Tokyo, Japan; and the Laboratory of Insect Ecology, Kyoto University, Kyoto, Japan.

Etymology. This species is named in honor of Professor Koji Sugio, a Japanese entomologist and expert in the biology of this species.

Geographic distribution. Ryukyu Archipelago (Okinawa islands and Sakishima Islands), Japan (Fig. 18).



FIGURES 15–17. SEM images of soldiers of *Neotermes sugioi* **sp. nov.** (left) and *N. koshunensis* (right). 15, left mandibles, dorsal view; 16, right mandibles, dorsal view; 17, pronota, dorsal view. Scale bars, 500 µm.

Remarks. *Neotermes sugioi* forms a species complex with the allopatric species *N. koshunensis* but is distinguished from *N. koshunensis* in the soldier by having a proportionally narrower postmentum (PmW2/HW = 0.28–0.36 in *N. sugioi*, PmW2/HW = 0.38–0.40 in *N. koshunensis*) (Fig. 14). In imagos, overlapping wings of *N. sugioi* are almost always darker than that of *N. koshunensis* (Fig. 3). In addition, *N. sugioi* is distinguished from those of other 18 East Asian *Neotermes* species in the soldier by having antennae where the fourth article is the shortest, having a wider head capsule (HW/HL = 0.65–0.76), or measurements (Table 1) (Huang *et al.* 2000; Tan & Peng 2009).

Discussion

The genus *Neotermes* is the second largest kalotermitid genus, where 117 extant species have been recognized (Krishna *et al.* 2013). Of these, 19 species have been known from East Asia, including China, Taiwan, and Japan

(Huang *et al.* 2000; Tan & Peng 2009). Our molecular and morphological assessments of *Neotermes* in the Ryukyu–Taiwan Island arc demonstrated the existence of two cryptic species in the *Neotermes koshunensis* species complex (Figs 1–3 and 14). The type locality of *N. koshunensis* is Hengchun, Pingtung County, Taiwan (Shiraki 1909), and, therefore, the Taiwanese species is *N. koshunensis* and the Ryukyu species is a new species, *N. sugioi*. *Neotermes sugioi* and *N. koshunensis* can be definitely distinguished by soldier morphology and generally distinguished by imago morphology (see "Remarks" for *N. sugioi* above). *N. sugioi* can also be distinguished from all other East Asian *Neotermes* species by soldier morphology (see "Remarks" for *N. sugioi* above), but not by imago morphology. This is mainly due to the fact that detailed descriptions with measurements of imagos are still lacking in many East Asian *Neotermes* species, and a comprehensive taxonomic study, including detailed descriptions and measurements of imagos, of *Neotermes* species in the region is needed.

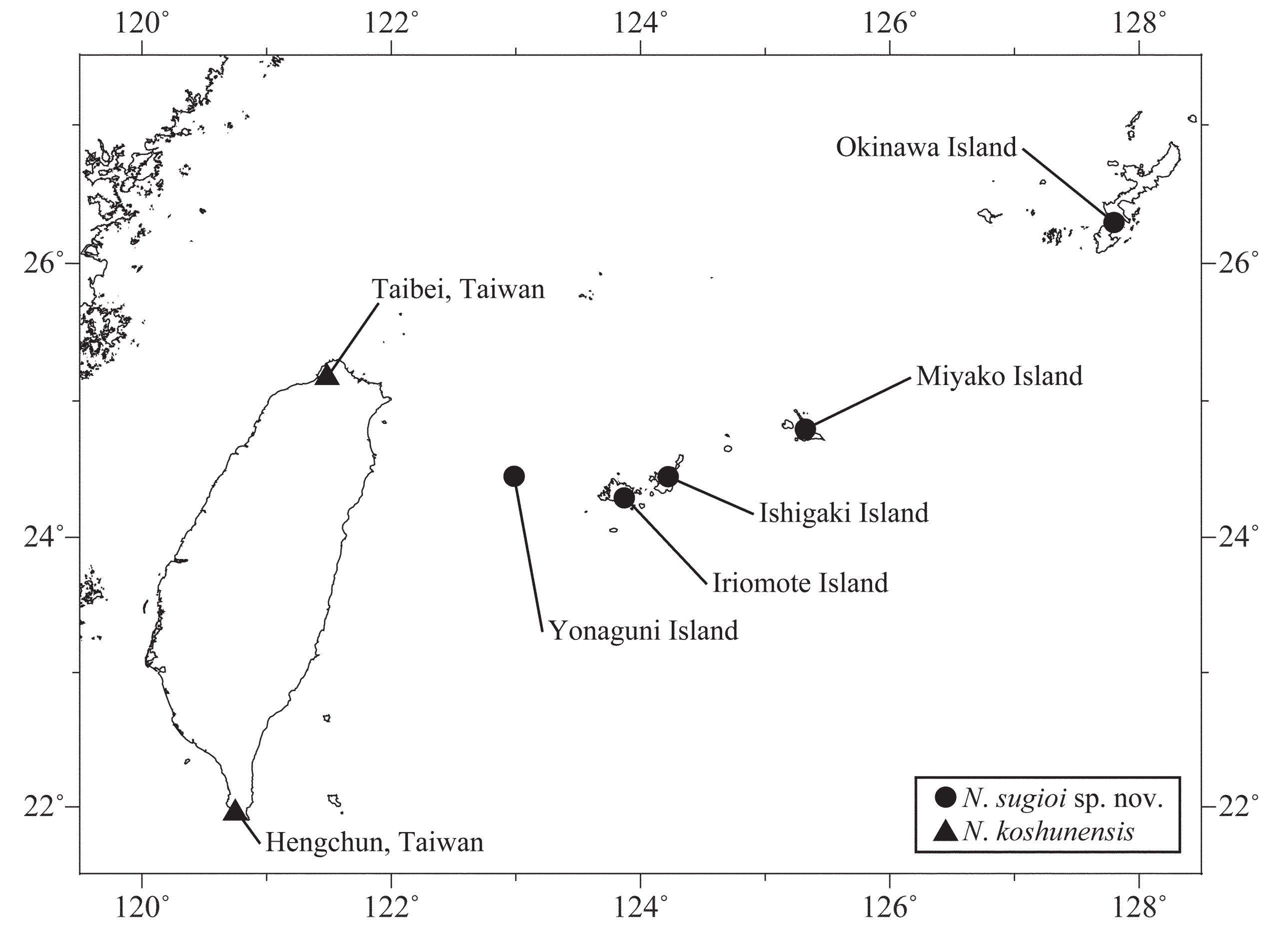


FIGURE 18. Map of collection sites for *Neotermes sugioi* **sp. nov.** and *N. koshunensis* in the Ryukyu–Taiwan Island arc. Each dot may indicate more than one site if they are located less than 20 km apart.

The *Neotermes koshunensis* species complex has also been recorded form southern China (Krishna *et al.* 2013), although detailed morphological examinations of southern Chinese populations are still lacking. A previous phylogenetic analysis based on *COII* sequences showed that *Neotermes* sp. from Hainan, Southern China and *N. sugioi* from the Ryukyu Archipelago might be a monophyletic group (Huang *et al.* 2011), although this relationship is supported only by a low bootstrap value and this analysis did not include *N. koshunensis* from Taiwan. Our phylogenetic analysis based on *COII* sequences showed that *Neotermes* sp. from Hainan, Southern China (Huang *et al.* 2011) and *N. koshunensis* from Taiwan form a well-supported monophyletic group against *N. sugioi* from the Ryukyu Archipelago (Fig. 1), suggesting that *N. koshunensis* is distributed in southern China as well as in Taiwan. Further phylogenetic analyses with additional genes and/or taxa are required to better understand the phylogenetic relationships within the specie complex.

The new species *N. sugioi* is the first endemic species of drywood termite identified in the Ryukyu Archipelago.

Our knowledge of the termite fauna of the Ryukyu Archipelago is insufficient, and several cryptic species complexes to be resolved have been proposed (Yasuda *et al.* 2000). Both morphological and molecular assessments of the species, which is currently thought to be distributed widely in the Ryukyu–Taiwan Island arc, are necessary for a better understanding of the termite fauna of the Ryukyu Archipelago.

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APPENDIX 1. Measurements of male and female imagos and soldiers of Ryukyu and Taiwanese *Neotermes* termites

Measurement[a]	Ryukyu individuals (Range [mm])		Taiwanese individuals (Range [mm])	
	Male (n = 30)	Female (n = 30)	Male (n = 7)	Female (n = 7)
Imagos				
HW	1.61–1.88	1.55–1.89	1.59–1.79	1.59–1.79
HL	1.50–1.72	1.51–1.69	1.48–1.65	1.49–1.68
ED	0.52–0.64	0.51–0.63	0.54–0.64	0.56–0.64
OD	0.17–0.25	0.16–0.24	0.20–0.24	0.19–0.25
LML	0.67–0.76	0.67–0.80	0.66–0.73	0.67–0.76
RML	0.59–0.67	0.60–0.68	0.59–0.65	0.58–0.69
LW	0.59–0.66	0.58–0.66	0.55–0.62	0.57–0.64
LL	0.50–0.57	0.50–0.59	0.48–0.53	0.50–0.57
PmW1	0.52–0.61	0.50–0.61	0.53–0.61	0.54–0.61
PmL	0.84–0.94	0.83–0.97	0.83–0.95	0.84–0.95
PnW	1.75–2.07	1.74–2.06	1.76–2.06	1.76–2.14
PnL	0.86–1.01	0.77–1.04	0.87–1.01	0.90–1.04
FWL	11.30–13.56	11.47–13.79	11.63–13.71	11.30–13.56
HWL	10.54–12.68	10.53–12.80	10.73–12.63	10.68–12.62
TL	1.35–1.62	1.38–1.78	1.35–1.54	1.38–1.63
Soldiers				
HW	2.00–2.62	1.93–2.49	2.07–2.34	2.06–2.41
HL	2.95–3.80	2.88–3.67	3.21–3.73	3.12–3.73
HH	1.57–2.04	1.56–1.97	1.62–1.95	1.68–1.94
ESD	0.24–0.42	0.20–0.36	0.17–0.34	0.11–0.34
LML	1.78–2.13	1.70–2.11	1.99–2.19	1.96–2.21
RML	1.72–2.04	1.65–2.00	1.91–2.11	1.90–2.14
LW	0.50–0.60	0.50–0.58	0.48–0.57	0.46–0.56
LL	0.39–0.49	0.39–0.47	0.40–0.50	0.41–0.47
PmW2	0.68–0.82	0.65–0.82	0.83–0.95	0.80–0.96
PmW3	0.23–0.42	0.24–0.38	0.34–0.42	0.31–0.39
PmL	2.08–2.77	2.02–2.74	2.21–2.88	2.25–2.80
PnW	2.08–2.84	1.90–2.76	2.11–2.76	2.14–2.64
PnL	0.93–1.35	0.88–1.31	0.90–1.21	0.93–1.17
TL	1.46–1.88	1.50–1.82	1.41–1.77	1.45–1.74

[a] For abbreviations of measurements see Materials and methods.