



Revision of *Neotermes* (Blattodea: Kalotermitidae) in Taiwan

Chia-Chien WU¹, Jing-Fu TSAI², Hou-Feng LI^{1,3,*}

1. Department of Entomology, National Chung Hsing University, 145 Xingda Rd., Taichung, Taiwan. Wu's e-mail: daisy100406@gmail.com. 2. Department of Biology, National Museum of Natural Science, 1 Kuan-Chien Rd., Taichung, Taiwan. E-mail: jingfu.tsai@gmail.com. 3. i-Center for Advanced Science and Technology, National Chung Hsing University, 145 Xingda Rd., Taichung, Taiwan. *Corresponding author's email: houfeng@nchu.edu.tw, Tel/Fax: +886-4-2287-5567

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ABSTRACT: *Neotermes koshunensis* (Shiraki, 1909) (Blattodea: Kalotermitidae) has been recorded in Taiwan, China, and Ryukyu Islands (Japan). However, Yashiro et al. clarified all *Neotermes* samples from Ryukyu Islands was a cryptic species in the *Neotermes koshunensis* species complex and hence further described it as a new species, *N. sugioi* Yashiro, 2019. To revise the identity and taxonomy of *Neotermes* spp. in Taiwan, this study examines 370 specimens which were collected from Taiwan Island and its offshore islands from 2006 to 2022. According to phylogenetic results reconstructed by using nucleotide sequences of two mitochondrial genes (16S rRNA and COI) and one nuclear gene (ITS2), three lineages were recognized. The three lineages represent *N. koshunensis*, *N. sugioi* as a new record species to Taiwan, and a new species *Neotermes ludaoensis* sp. nov. The winged imago and soldier castes of the three *Neotermes* species are described. A male winged imago of *N. koshunensis* collected from its type locality is designated as neotype.

KEY WORDS: Kalotermitidae, *Neotermes koshunensis*, *Neotermes sugioi*, *Neotermes ludaoensis*, new record, new species.

INTRODUCTION

The genus *Neotermes* Holmgren, 1911, including 120 extant species and 1 fossil species, is the second largest group in Kalotermitidae (Krishna et al., 2013; Scheffrahn, 2018; Yashiro et al., 2019; Ranjith et al., 2022). *Neotermes* is distributed in all zoogeographical regions except the Palaearctic and Antarctic Regions (Krishna et al., 2013). The taxonomy of termite in Taiwan was initiated in early twentieth century by Tokuichi Shiraki and Masamitsu Oshima (Li et al., 2010). Shiraki (1909) described the first *Neotermes* species in Far east Asia as *N. koshunensis* (Shiraki, 1909). No study of *Neotermes* of Taiwan and its neighboring islands has been revised and updated for over a hundred years.

Kalotermitids are difficult to be identified due to their highly interspecific morphological similarity and intraspecific morphological variations. Molecular methods were considered as effective tools to solve the taxonomic problems in recent years, for example, the *Neotermes* and *Incisitermes* research in Japan (Ide et al., 2016; Yashiro et al., 2019). Mitochondrial 16S ribosome RNA (16S rRNA) and cytochrome oxidase subunit I (COI) were used as efficient molecular characters in termites (Kambhampati et al., 1996; Ghesini et al., 2014; Scheffrahn and Carrijo, 2020).

Recently, Yashiro et al. (2019) describe a new *Neotermes* species, *N. sugioi*, from Ryukyu Islands, Japan. The species had been identified as *N. koshunensis* in Ryukyu Archipelago since 1966 (Ikehara, 1966). Although *N. sugioi* is morphologically similar to *N. koshunensis*, they present highly difference based on mitochondrial gene COII sequence and nuclear internal transcribed spacer 2 (ITS2) sequences. Hence, it turned

out the identity of *N. koshunensis* in Ryukyu Islands is a new species. Because of the geographic affinity between Taiwan and Ryukyu Islands, they share similar species component (Oshima, 1912; Takematsu and Yamaoka, 1997). Therefore, we supposed some unnoticed *Neotermes* species may occur in Taiwan. We examined the termite specimens obtained from long-term survey (2006 to 2022) all over the Taiwan Island and neighboring islands. We sorted and identified them into genus, and applied genetic markers (16S rRNA, COI and ITS2) to identify the species. In the present study, we used both molecular and morphology approaches to species identities. We redescribed all *Neotermes* from Taiwan and provided identification keys for both winged imago and soldier castes.

MATERIALS AND METHODS

A total of 370 samples of *Neotermes* species collected from Taiwan Island and its offshore islands including Green Island and Lanyu from 2006 to 2022 (Fig. 1A). Specimens were obtained from field collection by members of Laboratory of Urban Entomology, National Chung Hsing University, Taiwan. Soldier, worker and some winged imago samples were collected using hatchet, hand saw, or free hand. Winged imago samples were also collected by using flight intercepted trap and sweep net. All samples were preserved in 95% ethanol and deposited in National Chung Hsing University Termite Collection, Taichung, Taiwan (NCHU).

A total of 32 samples were using for DNA extraction (Fig. 1A). Genomic DNA was extracted from the muscle tissue of thorax and leg by using following the standard protocols suggested by QuickExtract DNA extraction kit

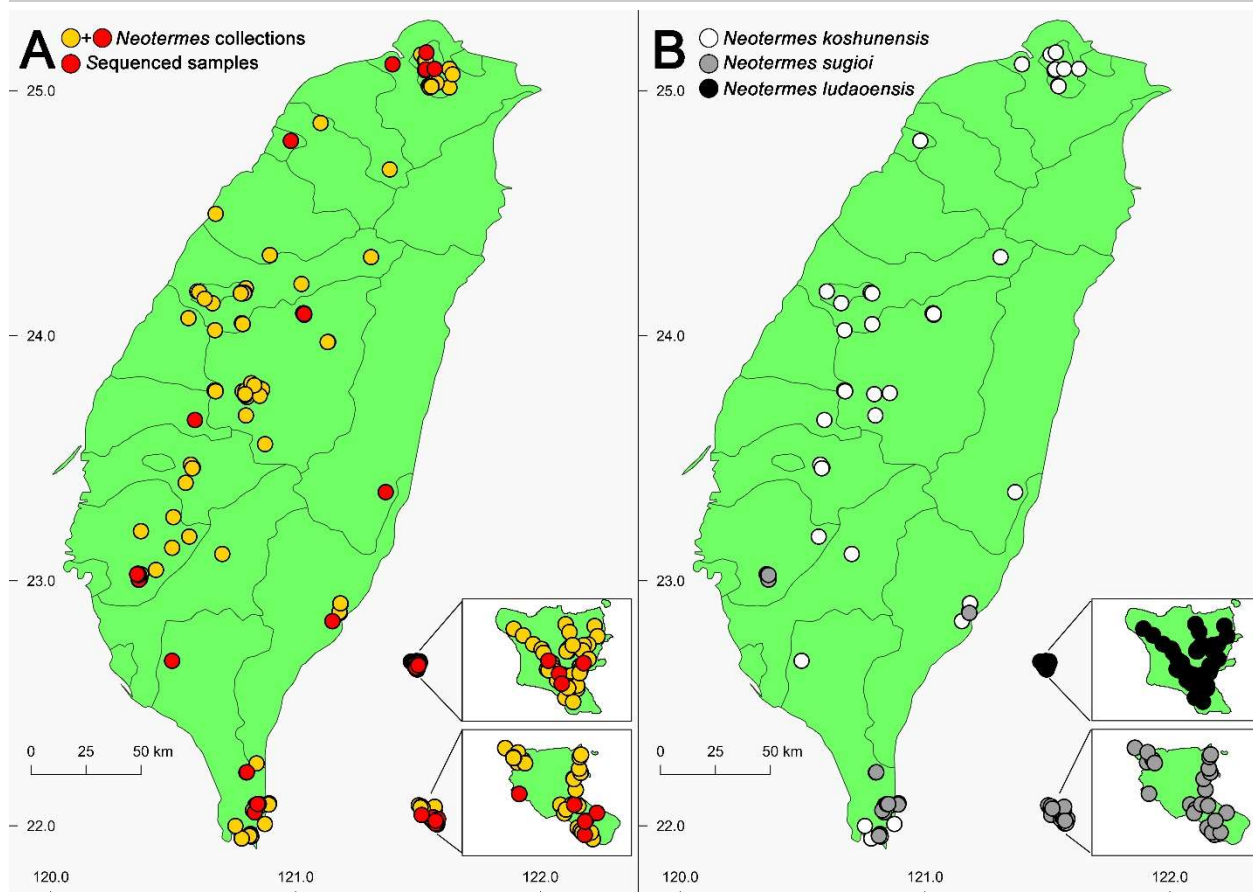


Fig. 1. Distribution of *Neotermes* termites in Taiwan. **A.** The 370 collection sites of *Neotermes* species across Taiwan, and 32 samples for sequencing; **B.** The 297 collection sites of *N. koshunensis*, *N. sugioi*, and *N. ludaensis* sp. nov.

(Epicentre Biotechnologies, Madison, WI). A total of 32, 22 and 20 samples were successfully amplified and used for analysis in mitochondrial 16S rRNA, COI sequence and nuclear ribosomal ITS2 regions, respectively. Mitochondrial genes 16S rRNA were amplified by using 16Sar (5'-CGCCTGTTTAACAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (Kerr *et al.*, 2005). COI sequence were amplified by using Kalo-COI-4F (5'-ACRTCTATTCCTACTGTRAA-3'), Kalo-COI-3R (5'-TTCAAAGCTATMAATAAAGA-3'), Kalo-COI-3F (5'-CCAATTGCTARTATDGCRAA-3'), Kalo-COI-1R (5'-AGAYTTACAATCTAHCACCT-3'). ITS2 were amplified by using CAS5p8sFc (5'-GCGAACATCGACAAGTCGAACGCACAT-3'), CAS28sB1d (5'-TTGTTTTCTCCGCTTATTAATATGCTTAA-3') (Kim and Lee, 2008). The polymerase chain reaction (PCR) assays were conducted in 25 μ l reagent mixtures, containing 1 μ l of termite DNA template, 0.5 μ l for each forward and reverse primers, 12.5 μ l of master mix (amaR OnePCR™). PCR programming of 16S rRNA were performed with an initial denaturing step at 94°C for 2 min, followed by 35 cycles of 94°C for 40 second, 55°C for 1.5 min and 72°C for 40 second, last cycle at 72°C for 10 min. PCR programming of COI sequence were

performed with an initial denaturing step at 94°C for 2 min, followed by 35 cycles of 94°C for 40 second, 45°C for 1.5 min, 72°C for 40 second, last cycle at 72°C for 10 min, using primer Kalo-COI-4F and Kalo-COI-3R; nested amplifications used 1 μ l of the primary PCR product as the template, performed with an initial denaturing step at 94°C for 2min, followed by 35 cycles of 94°C for 40 second, 45°C for 1.5 min, 72°C for 40 second, last cycle at 72°C for 10 min, using primer Kalo-COI-3F and Kalo-COI-1R. PCR programming of ITS2 were performed with an initial denaturing step at 94°C for 2 min, followed 35 cycles of 94 °C for 40 second, 50°C for 1.5min, 72°C for 40 second, last cycle at 72°C for 10 min. All DNA sequence are accessible in GenBank, accession numbers were LC774338–774370 (16S), LC773859–773880 (COI), and LC774318–774337 (ITS2).

All sequences were edited and verified in Bioedit (Hall, 1999) and aligned using the Muscle Alignment option in Mega7.0 (Kumar *et al.*, 2016). *Cryptotermes domesticus* (Haviland) was rooted as outgroup (accession numbers: LC774338 (16S rRNA), LC773859 (COI), MG333691 (ITS2)), and sequence of *N. sugioi* from Ryukyu Islands were analyzed. (accession numbers: MG333679–333683).

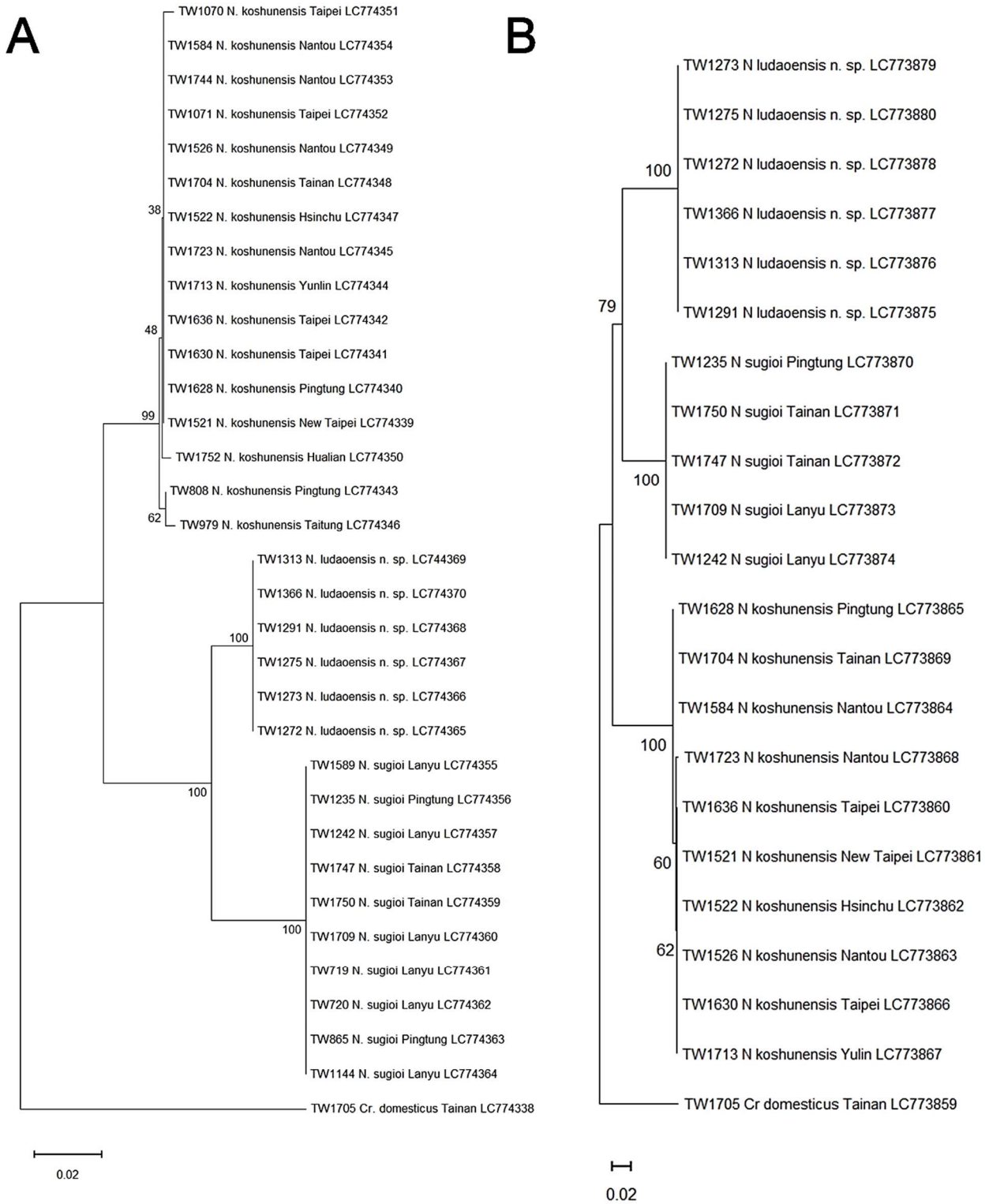


Fig. 2. Neighbor-joining tree of *Neotermes* species in Taiwan using mitochondrial gene sequences. **A.** Neighbor-joining tree of *Neotermes* based on 16S rDNA sequence of 33 samples, a concatenated alignment of 384 bp in the final dataset. **B.** Neighbor-joining tree of *Neotermes* based on COI sequence of 23 samples, a concatenated alignment of 500 bp in the final dataset.

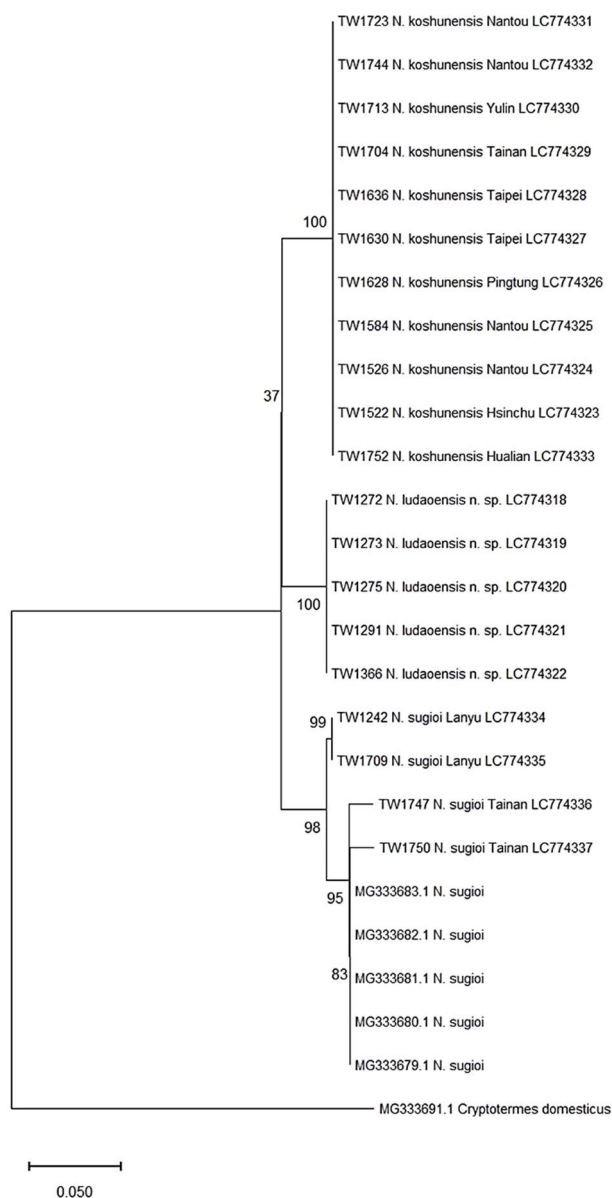


Fig. 3. Neighbor-joining tree of *Neotermes* species in Taiwan based on nuclear ribosomal ITS2 regions. A total of 20 *Neotermes* samples from Taiwan, compared with 5 Japanese *N. sugioi* samples and one *Cryptotermes domesticus* as outgroup, a concatenated alignment of 349 bp in the final dataset.

Phylogenetic inference of 16S rRNA, COI, and ITS2 were conducted using Neighbor-Joining method, and model select maximum composite likelihood, substitutions include transitions + transversions, each node was assessed by performing 1,000 bootstrap replications.

A total of 39 winged imagoes and 118 soldiers of *Neotermes* specimens collected from 61 locations were using for character measurement and photography. Samples were observed and measured by using Leica M205 C stereomicroscope, Leica MC170 HD digital camera and implemented with Leica Application Suite

(version 4.4.0, Wetzlar, Germany). All characters of imago and soldier castes were measured following Roonwal (1969). In imago caste, 7 quantitative characters were measured and compared, containing head length without mandible (Roonwal, 1969: fig. 3, line GG'), maximum head width (Roonwal: fig. 6, line SS'), head height (Roonwal: fig. 4, line VV'), compound eye diameter (Roonwal: fig. 11, line BB'), number of antenna articles, pronotum length (Roonwal: fig. 13, line SS'), pronotum width (Roonwal: fig. 13, line UU'). In soldier caste, 11 quantitative characters were measured and compared, containing head length without mandibles (Roonwal: fig. 4, line JJ'), maximum head width (Roonwal: fig. 6, line RR'), left mandible length (Roonwal: fig. 9, line NN'), labrum length (Roonwal: fig. 8, line FF'), labrum width (Roonwal: fig. 8, line EE'), head height (Roonwal: fig. 4, line VV'), number of antenna articles, postmentum maximum width (Roonwal: fig. 12, line QQ'), postmentum minimum width (Roonwal: fig. 12, line RR'), pronotum length (Roonwal: fig. 13, line SS'), and pronotum width (Roonwal: fig. 13, line UU'). All morphological characters were compared using analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). Specimens are photographed from ethanol-preserved specimens. The color of the samples was evaluated using the microscope and compared with the Munsell color system (Munsell, 1967). All photographs were conducted by a Canon EOS 760D camera with a MP-E 65 mm f/2.8 1-5X micro lens (Canon Inc., Tokyo, Japan) and combined using software Helicon Focus 7.6.4 Pro (Helicon Soft Ltd., 2000). Figures were contrast-enhanced and added scale bars using Adobe Photoshop CS6 (Adobe Inc., Mountain View, CA), and converted backgrounds into gray or black.

RESULTS

The neighbor-joining trees of 16S rRNA, partial COI, and ITS2 showed three *Neotermes* species (Figs. 2, 3). *N. koshunensis* is the recorded species. *N. sugioi* is a new record species, and *N. ludaensis* is a new species.

The result of aligned sequence of mitochondrial 16S rRNA including gaps is 384 bp. The actual sequence length is as follows: 326 bp for *N. koshunensis*, 334 bp for *N. sugioi*, and 329 bp for *N. ludaensis*. The aligned of partial COI sequence is 500 bp. The aligned sequence including gaps of nuclear ITS2 is 349 bp, 311 bp for *N. koshunensis*, 307–328 bp for *N. sugioi*, and 311 bp for *N. ludaensis*.

The base compositions range for T, C, A, and G in the 16S rRNA were 42.9–44.1%, 9.5–10.1%, 25.2–26%, and 20.4–21.8%; in the COI gene were 31.6–33%, 16.4–18.2%, 28–29.8%, and 20.4–21.8%; and in the ITS2 were 13.3–16%, 24.8–27.4%, 19.3–20.3%, and 38.1–40.3%. The range of genetic divergence of 16S rDNA intraspecies is 0–0.9%, interspecies genetic divergence



range is 4.1–9.3%; the range of genetic divergence of COI sequence intraspecies is 0–0.8%, interspecies genetic divergence range is 11.6–16%; and the average of genetic divergence of ITS2 showed that *N. koshunensis* to *N. sugioi*, *N. koshunensis* to *N. ludaensis*, and *N. sugioi* to *N. ludaensis* is 6.5%, 5.3%, and 5.9%, respectively.

The distribution map of the three *Neotermes* species was shown in Figure 1B (Supp. Table 1). *N. koshunensis* is common in Taiwan Island from lowland up to 1,100 m. *N. sugioi* occurs in southern Taiwan and Lanyu Island from lowland up to 500 m. All specimens collected from Lanyu Island were *N. sugioi*. All specimens collected in Green Island were *N. ludaensis*, which commonly found up to highest point of the island ~300 m. The dispersal flight of *N. koshunensis* occurs in June to November, and *N. sugioi* occurs from January to March in Taiwan Island.

In order to locate the type specimens and fix the identities of *N. koshunensis*, we examined all historical collections of Taiwan, Agricultural Research Institute, Wufeng, Taichung (TARI), Forestry Research Institute, Taipei (TFRI), Insect Museum of National Taiwan University, Taipei (NTU), National Museum of Natural Science, Taichung (NMNS), National Chung-Hsing University, Entomology Department, Taichung (NCHU), and National Pingtung University of Science and Technology, Plant Protection Department, Neipu, Pingtung (NPUST), where termites syntypes might be deposited. However, syntype was not found, and we considered they have been lost. Neotype of *N. koshunensis* in winged imago caste was designated.

TAXONOMIC TREATMENT

Neotermes koshunensis (Shiraki, 1909)

Calotermes koshunensis Shiraki, 1909: 241–242. **Syntype:** male imago, Insect Museum of National Taiwan University, Taipei (NTU) or Forestry Research Institute, Taipei (TFRI), lost! Type locality: Pingtung (Taiwan).

Calotermes (Neotermes) koshunensis Holmgren, 1912: 114–115. Synonymized by Hozawa, 1912.

Type material: **Neotype.** TW 808, Nanrenshan, Pingtung Co. (22°5'N, 120°50'E), 20-X-2008, leg. N. Kanzaki, Y.-C. Lan, H.-F. Li, and J.-F. Tsai, male alate, with 16S rRNA sequence, deposited in NMNS (008746-00001). Additional male and female alate, soldier, and worker castes collected with neotype are deposited in NMNS and NCHU.

Description: **Imago.** (Figs. 4A, 4D, 5A, 5D, 6A; Table 1) Head capsule reddish brown (10R 5/10, Fig. 4A, 4D). Head subcircular, longer than width, length without mandibles with 1.73 ± 0.09 mm (mean \pm standard deviation), maximum width with 1.52 ± 0.05 mm. Eye oval, maximum compound eye diameter with 0.53 ± 0.02 mm. Ocelli whitish, circular. Antenna with 14–18 articles; 1st article longest; 2nd article shorter than 1st article, as long as 3rd article; 4th article shortest; last article oblong.

Labrum reddish yellow (7.5YR 7/10, Fig. 4D), liguiform, anterior margin with 4–8 setae, tip of labrum overlapping the tip of mandibles. Left mandible with one apical tooth, two marginal teeth (1st + 2nd and 3rd marginal teeth, Fig. 5A); 1st + 2nd marginal tooth shorter than apical tooth; 1st + 2nd marginal tooth larger than 3rd marginal tooth; anterior margin of 3rd marginal tooth slightly wider than posterior margin of 1st + 2nd marginal tooth. Right mandible with one apical tooth, two marginal tooth and one molar tooth (Fig. 12); apical tooth longer than 1st marginal tooth; 1st marginal tooth longer than 2nd marginal tooth; posterior margin of 2nd marginal tooth slightly shorter than molar tooth.

Pronotum reddish yellow (5YR 6/14), broader than length, maximum length with 11.1 ± 0.05 mm, width with 1.78 ± 0.07 mm; anterior margin concave, without setae; sides circular, with 10–12 setae each side; posterior margin slightly concave, with about 20 setae (Fig. 5D). Leg whitish yellow. Arolium present. Tibia spur formula 3:3:3, tarsi 4-jointed. Abdomen oblong.

Forewing with radius run into costa at anterior one-third of costa (Fig. 6A); radial sector with 6–8 branches; median close to radial sector and parallel to each other; cubitus unsclerotized, with 12–15 branches. Hindwing shorter than forewing, radius run into costa at half of costa, radial sector and median meet before anterior one-fourth, split at anterior one-fourth, parallel till apex. Cubitus unsclerotized, with 12–15 branches.

Soldier. (Figs. 7A–C, 8A, 8D; Table 2) Head capsule orange (2.5YR 6/12, Figs. 7A, 7B). Head oblong, longer than width, length without mandibles with 32.3 ± 0.37 mm, maximum width with 2.12 ± 0.23 mm. Eye spot whitish, oval. Antenna with 11–15 articles; 1st article longer than 2nd article; 3rd article longer than 2nd article, two times longer than 4th article; last article oblong. Postmentum club-shaped (Fig. 7C), anterior one-third broadest with maximum width 0.76 ± 0.11 mm, lateral margin slightly circular; posterior two-third sides parallel with minimum width 0.33 ± 0.08 mm. Labrum liguiform, broader than length, length with 0.29 ± 0.05 mm, width with 0.48 ± 0.06 mm; anterior margin with 4–6 long setae. Left mandible with one apical tooth, three marginal teeth (Fig. 8A); 1st marginal tooth largest; 2nd marginal tooth longer than 3rd marginal tooth; anterior margin of 3rd marginal tooth wider than posterior margin of 2nd marginal tooth. Right mandible with one apical tooth, and two marginal teeth; 1st marginal tooth larger than 2nd marginal tooth.

Pronotum whitish yellow (2.5Y 8.5/8); broader than length, maximum length with 1.22 ± 0.23 mm, width with 2.21 ± 0.35 mm; with about 20–30 setae; anterior margin concave, without setae; lateral margin slightly circular, with 12–15 setae each side; posterior margin slightly concave, without setae (Fig. 8D). Leg whitish, tibia whitish yellow. Tibia spur formula 3:3:3. Tarsi 4-jointed. Abdomen oblong.

**Table 1.** Measurements of winged imagos of three *Neotermes* spp. in Taiwan.

Measurement, mm	<i>N. koshunensis</i> ^a		<i>N. sugioi</i> ^b		<i>N. ludaensis</i> ^c	
	Range	Mean ± SD	Range	Mean ±SD	Range	Mean ±SD
Head length without mandibles	1.55 – 1.90	1.73 ± 0.09A	1.60 – 1.92	1.76 ± 0.11A	1.43 – 1.89	1.73 ± 0.14A
Maximum width of head	1.42 – 1.61	1.52 ± 0.05A	1.40 – 1.69	1.58 ± 0.09AB	1.48 – 1.74	1.61 ± 0.08B
Maximum length of pronotum	1.04 – 1.21	1.11 ± 0.05A	0.90 – 1.36	1.16 ± 0.10AB	1.12 – 1.33	1.20 ± 0.08B
Maximum width of pronotum	1.58 – 1.86	1.78 ± 0.07A	1.64 – 2.05	1.89 ± 0.10B	1.84 – 2.13	1.98 ± 0.09C
Maximum height of head	1.03 – 1.14	1.08 ± 0.04A	0.99 – 1.25	1.11 ± 0.08A	0.98 – 1.39	1.12 ± 0.10A
Number of antennal articles	14 – 18	16.5 ± 1.20	16 – 19	17.6 ± 0.73	14 – 18	16.6 ± 1.50
Compound eye diameter	0.50 – 0.57	0.53 ± 0.02A	0.50 – 0.59	0.54 ± 0.02A	0.40 – 0.46	0.44 ± 0.02B

^a *N. koshunensis*, n = 12, from 6 colonies. ^b *N. sugioi*, n = 15, from 5 colonies. ^c *N. ludaensis* sp. nov., n = 12, from 5 colonies.



Fig. 4. Winged imago caste of *Neotermes* species in Taiwan. **A.** Winged imago caste of *N. koshunensis* in dorsal view. **B.** Winged imago caste of *N. sugioi* in dorsal view. **C.** Winged imago caste of *N. ludaensis* in dorsal view. **D.** Head of *N. koshunensis* in dorsal view. **E.** Head of *N. sugioi* in dorsal view. **F.** Head of *N. ludaensis* in dorsal view.

**Table 2.** Measurements of soldiers of three *Neotermes* spp. in Taiwan.

Measurement, mm	<i>N. koshunensis</i> ^a		<i>N. sugioi</i> ^b		<i>N. ludaensis</i> ^c	
	Range	Mean ± SD	Range	Mean ±SD	Range	Mean ±SD
Head length without mandibles	2.55 – 3.74	3.23 ± 0.37A	2.78 – 4.19	3.51 ± 0.39A	2.43 – 3.41	3.00 ± 0.20A
Maximum width of head	1.72 – 2.42	2.12 ± 0.23A	1.86 – 2.48	2.21 ± 0.17AB	1.89 – 2.52	2.29 ± 0.14B
Labrum length	0.20 – 0.37	0.29 ± 0.05A	0.29 – 0.53	0.40 ± 0.06B	0.24 – 0.45	0.36 ± 0.04C
Labrum width	0.39 – 0.56	0.48 ± 0.06A	0.45 – 0.62	0.56 ± 0.04B	0.45 – 0.57	0.51 ± 0.03C
Maximum length of pronotum	0.77 – 1.46	1.22 ± 0.23A	0.90 – 1.59	1.28 ± 0.19A	0.83 – 1.41	1.18 ± 0.12A
Maximum width of pronotum	1.56 – 2.80	2.21 ± 0.35A	1.82 – 2.91	2.25 ± 0.34A	1.70 – 2.63	2.20 ± 0.19A
Maximum height of head	1.41 – 1.87	1.67 ± 0.15A	1.52 – 2.16	1.81 ± 0.17B	1.45 – 1.91	1.72 ± 0.11AB
Number of antennal articles	11 – 15	13.3 ± 1.25	13 – 17	14.5 ± 1.44	12 – 16	14.6 ± 1.03
Maximum width of postmentum	0.57 – 0.93	0.76 ± 0.11A	0.68 – 1.04	0.83 ± 0.10B	0.61 – 0.82	0.72 ± 0.05A
Minimum width of postmentum	0.21 – 0.47	0.33 ± 0.08A	0.19 – 0.41	0.30 ± 0.06A	0.21 – 0.33	0.26 ± 0.03B
Left mandible length	1.64 – 2.23	2.02 ± 0.20A	1.69 – 2.38	2.08 ± 0.20A	1.55 – 2.02	1.78 ± 0.09B

^a *N. koshunensis*, n = 13, from 9 colonies. ^b *N. sugioi*, n = 20, from 8 colonies. ^c *N. ludaensis* sp. nov., n = 85, from 37 colonies.

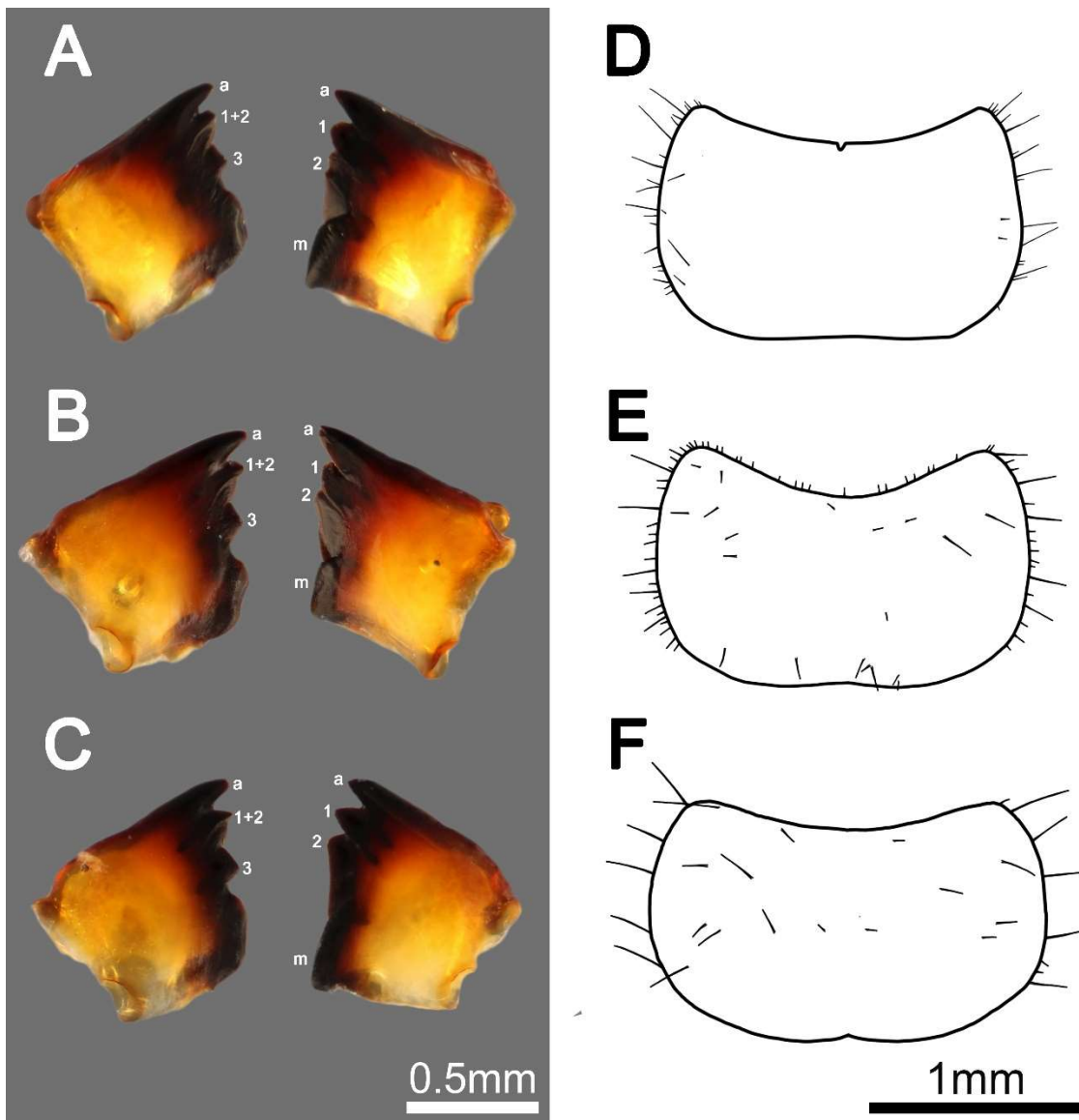


Fig. 5. Mandible and pronotum of winged imago caste of *Neotermes* species in Taiwan. **A.** Mandible of *N. koshunensis*. **B.** Mandible of *N. sugioi*. **C.** Mandible of *N. ludaensis*. **D.** Pronotum of *N. koshunensis*. **E.** Pronotum of *N. sugioi*. **F.** Pronotum of *N. ludaensis*. a: Apical tooth. 1: 1st marginal tooth. 2: 2nd marginal tooth. 3: 3rd marginal tooth. M: Molar tooth.

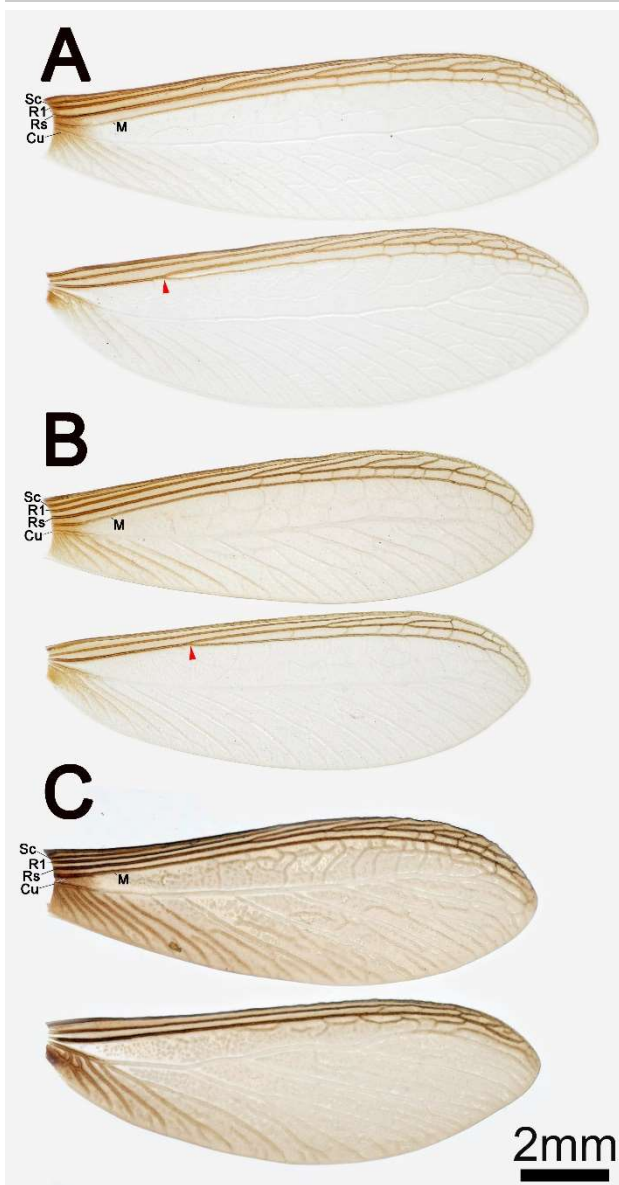


Fig. 6. Wings of winged imago of *Neotermes* species in Taiwan. **A.** Wings of *N. koshunensis*; **B.** Wings of *N. sugioi*; **C.** Wings of *N. ludaensis*. In each group of wings, up is forewing, down is hindwing. Sc: Subcostal. R1: Radius. Rs: Radial sector; M: Median. Cu: Cubitus. Red triangles: hind wing of *N. koshunensis* with radial sector and median meet before anterior one-fourth, and *N. sugioi* meet before anterior one-third.

Distribution: China (Tsai and Chen, 1964, Huang *et al.*, 2011, Krishna *et al.*, 2013), Taiwan.

Material examined. TAIWAN. TW808, Pingtung, 20-X-2008, leg. N. Kanzaki, Y.-C. Lan, H.-F. Li, and J.-F. Tsai; TW979, Taitung, 23-V-2010, leg. N. Kanzaki, Y.-C. Lan, H.-F. Li, and S.-H. Tzeng; TW1070, Taipei, 19-X-2012, leg. H.-F. Li; TW1071, Taipei, 19-X-2012, leg. H.-F. Li; TW1521, New Taipei, 8-III-2018, leg. S.-J. Su; TW1522, Hsinchu, 26-IV-2018, leg. Y.-T. Wang; TW1526, Nantou, 8-VI-2018, leg. C.-C. Wu, C.-I Chiu, and W.-R. Liang; TW1584, Tainan, 19-IV-2019, leg. C.-C. Wu, H.-F. Li, and C.-A. Zhou; TW1628, Taipei, 27-IX-2018, leg. R.-H. Wang; TW1630, Taipei, 18-II-2019, leg. H.-W. Yeh; TW1636, Taipei, 5-IX-2019, leg. C.-C. Wu, C.-I Chiu, and W.-R. Liang; TW1704, Tainan, 19-IV-2019, leg. J.-A. Jhou; TW1713, Yunlin,

21-XI-2020, leg. C.-I Chiu and R.-H. Liu; TW1723, Nantou, 11-I-1010, leg. R.-H. Liu; TW1744, Nantou, 15-II-2020, leg. C.-C. Wu, C.-I Chiu, and W.-R. Liang; TW1752, Hualien, 4-XI-2019, leg. G.-Y. Chen. The details refer to supplemental table S1.

Neotermes sugioi Yashiro, 2019

Neotermes sugioi Yashiro *et al.*, 2019: 549-561. **Holotype:** soldier male (B2324169), Museum of Nature and Human Activities, Hyogo. Type locality: Chibana, Okinawa (Japan).

Neotermes koshunensis (non Shiraki, 1909): Ikehara, 1966: 61. Misidentification.

Description: Imago. (Figs. 4B, 4E, 5B, 5E, 6B; Table 1) Head capsule reddish brown (10R 6/14, Figs. 4B, 4E). Head subcircular, longer than width, length without mandibles with 1.76 ± 0.11 mm, maximum width with 1.58 ± 0.09 mm. Eye circular, maximum compound eye diameter with 0.54 ± 0.02 mm. Ocelli oval. Antenna with 16–19 articles; 1st article longest; 2nd article shorter than 1st article, as long as 3rd article; last article oblong. Labrum reddish yellow (2YR 7/12, Fig. 4E), linguiform, anterior margin with 4–6 setae, tip of labrum overlapping to middle or tip of mandibles. Left mandible with one apical tooth, and two marginal teeth (Fig. 5B); apical tooth longer than 1st + 2nd marginal tooth; 1st + 2nd marginal tooth as wide as 3rd marginal tooth. Right mandible with one apical tooth, two marginal tooth and one molar tooth; apical tooth larger than 1st marginal tooth; 1st marginal tooth longer than 2nd marginal tooth; posterior margin of 2nd marginal tooth slightly longer than molar tooth.

Pronotum reddish brown (10R 6/14), broader than length, maximum length with 1.16 ± 0.10 mm, width with 1.89 ± 0.10 mm; anterior margin concave; sides circular, with 15–20 setae each side; posterior margin slightly concave, without setae (Fig. 5E). Leg whitish yellow. Arolium present. Tibia spur formula 3:3:3; tarsi 4-jointed. Abdomen oblong.

Forewing with radius run into costa at anterior one-third of costa (Fig. 6B); radial sector with 6–8 branches; median close to radial sector and parallel to each other; cubitus unsclerotized, with 12–14 branches. Hindwing shorter than forewing, radius run into costa at two-third of costa, radial sector and median meet before anterior one-third, split at anterior one-third, parallel till apex. Cubitus unsclerotized, with 12–14 branches.

Soldier. (Figs. 7D–F, 8B, 8E; Table 2) Head capsule orange (5YR 7/14, Figs. 7D, 7E). Head oblong, longer than width, length without mandibles with 3.51 ± 0.39 mm, maximum width with 2.21 ± 0.17 mm. Eye whitish, oval. Antenna with 13–17 articles; 1st article about two times longer than 2nd article; 3rd article longer than 2nd article, two times longer than 4th article; last article oblong. Postmentum club-shaped (Figs. 7E, 7F), anterior one-third broadest with maximum width 0.83 ± 0.10 mm, sides of anterior one-fifth slightly notched; lateral margin slightly circular; posterior two-third sides slightly concave inward

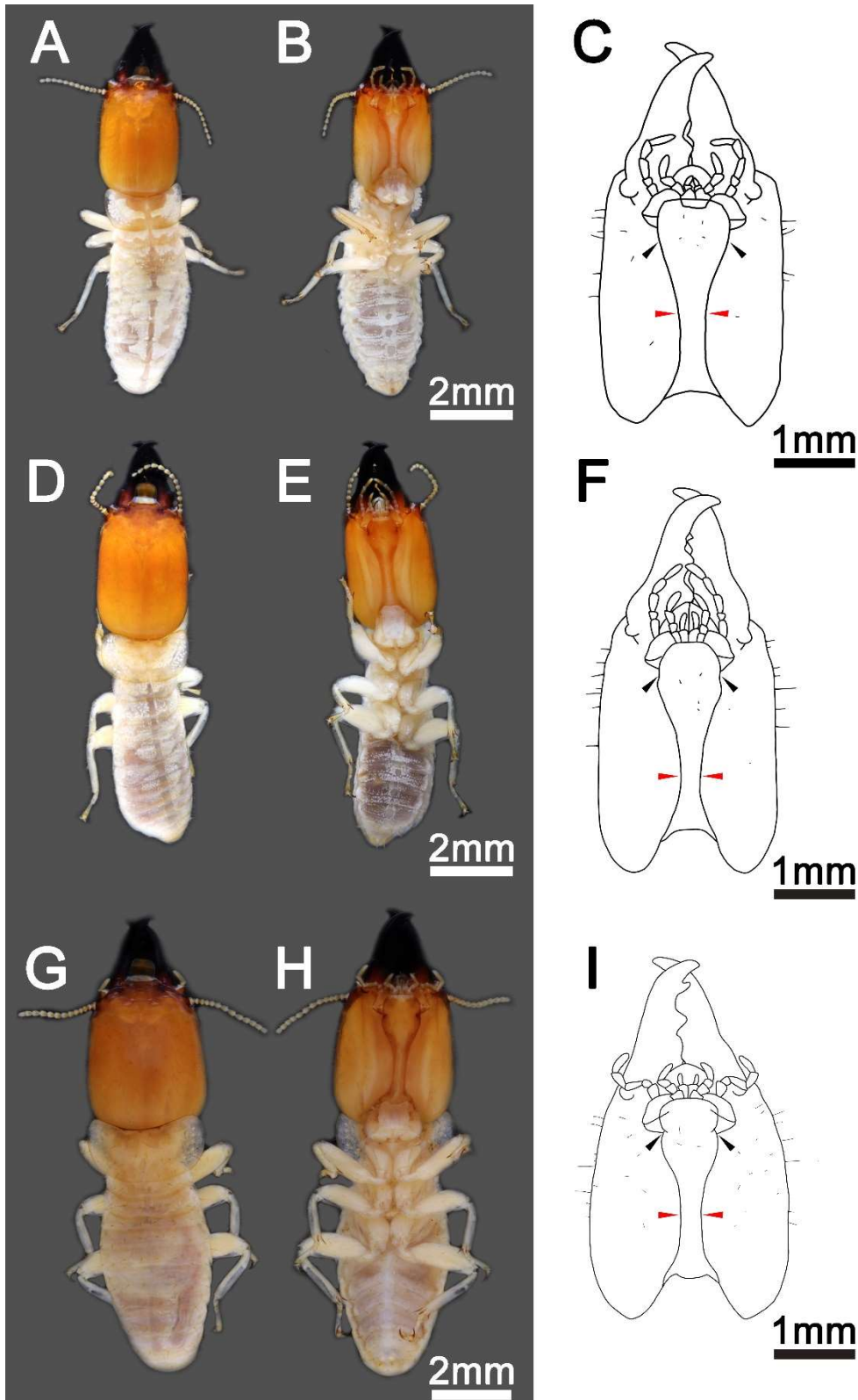


Fig. 7. Soldier caste of *Neoterme* species in Taiwan. **A.** Soldier of *N. koshunensis* in dorsal view. **B.** Soldier of *N. koshunensis* in ventral view. **C.** Head of *N. koshunensis* in ventral view. **D.** Soldier of *N. sugioi* in dorsal view. **E.** Soldier of *N. sugioi* in ventral view. **F.** Head of *N. sugioi* in ventral view. **G.** Soldier of *N. ludaoensis* in dorsal view. **H.** Soldier of *N. ludaoensis* in ventral view. **I.** Head of *N. ludaoensis* in ventral view. Black triangle: *N. sugioi* and *N. ludaoensis* with postmentum anterior part notched. Red triangle: *N. koshunensis* and *N. sugioi* with postmentum posterior part parallel.

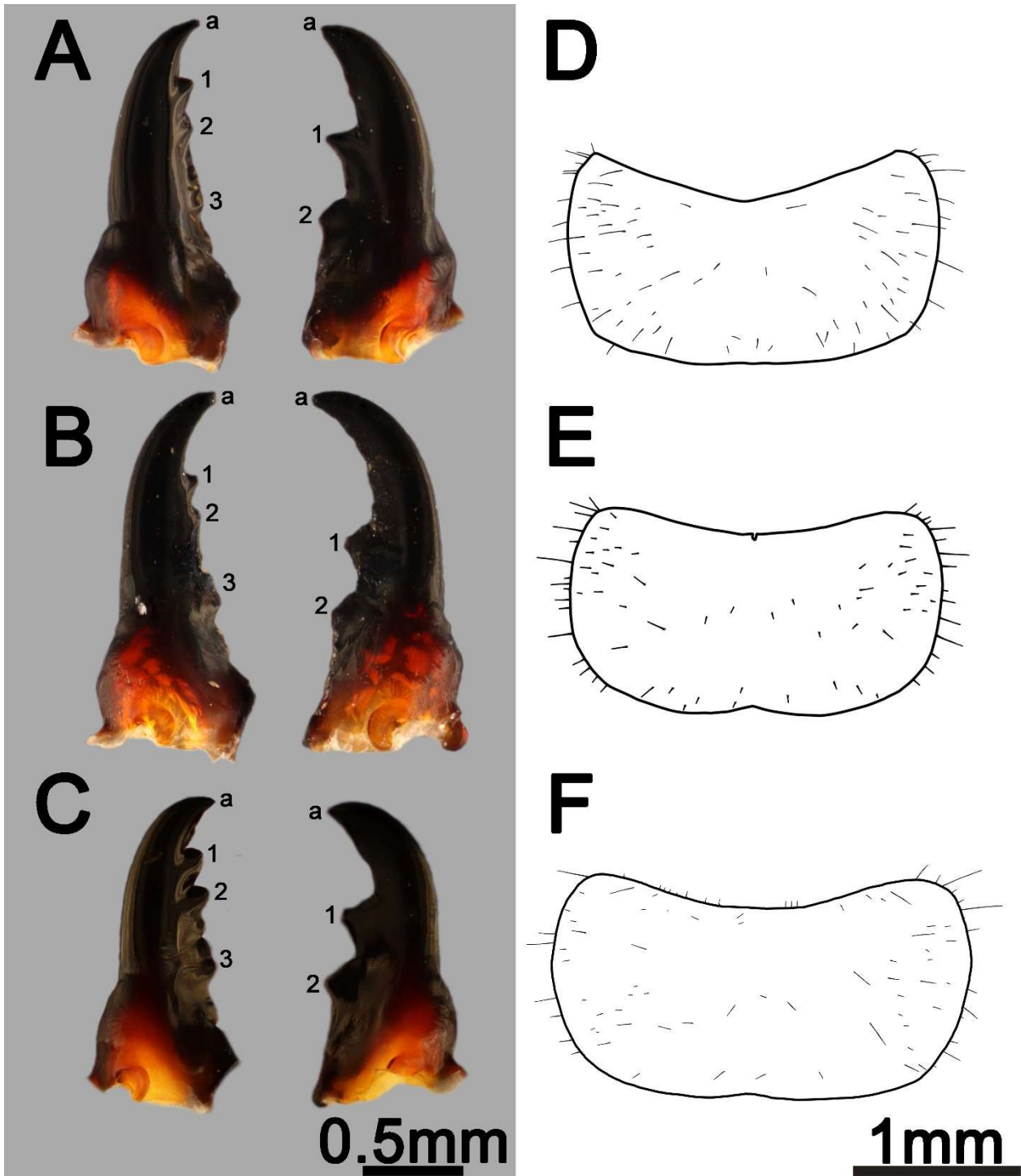


Fig. 8. Pronotum and mandible of soldier caste of *Neotermes* species in Taiwan. **A.** Mandible of *N. koshunensis*. **B.** Mandible of *N. sugioi*. **C.** Mandible of *N. ludaensis*. **D.** Pronotum of *N. koshunensis*. **E.** Pronotum of *N. sugioi*. **F.** Pronotum of *N. ludaensis*. a: Apical tooth. 1: 1st marginal tooth. 2: 2nd marginal tooth. 3: 3rd marginal tooth.

with minimum width 0.30 ± 0.06 mm. Labrum liguiform, broader than length, length with 0.40 ± 0.06 mm, width with 0.56 ± 0.04 mm; anterior margin with 6–8 setae. Left mandible with one apical tooth, three marginal teeth (Fig. 8B); 1st marginal tooth longer than 2nd marginal tooth;

posterior margin of 2nd marginal tooth wider than anterior margin of 3rd marginal tooth; 3rd marginal with slightly notched at tip. Right mandible with one apical tooth, and two marginal teeth; 1st marginal tooth as large as 2nd marginal tooth.



Pronotum whitish yellow (5Y 9/6); broader than length, maximum length with 1.28 ± 0.19 mm, width with 2.25 ± 0.34 mm; with about 20 long setae; anterior margin concave, without setae; lateral margin slightly circular, with 10–15 setae each side; posterior margin slightly concave (Fig. 8E). Leg whitish, tibia whitish yellow. Tibia spur formula 3:3:3. Tarsi 4-jointed. Abdomen oblong.

Distribution: Ryukyu Archipelago, Japan, Lanyu Island and Southern Taiwan (new record in present study).

Material examined: TAIWAN. TW719, Lanyu, 11-VI-2007, leg. H.-F. Li; TW720, Lanyu, 11-VI-2007, leg. H.-F. Li; TW865, Lanyu, 24-VI-2009, leg. C.-C. Chang, W.-M. Chung, Y.-C. Lan, H.-F. Li, and S.-H. Tzeng; TW1144, Lanyu, 24-I-2014, leg. C.-I Chiu, F.-S. Huang, B.-C. Lai, and W.-R. Liang, 16S; TW1146, Lanyu, 24-I-2014, leg. C.-I Chiu, F.-S. Huang, B.-C. Lai, and W.-R. Liang; TW1235, Pingtung, 25-VIII-2014, leg. W.-R. Liang; TW1242, Lanyu, 4-IV-2014, leg. Y.-Z. Jiang; TW1589, Lanyu, 9-IV-2019, leg. Y.-T. Chung; TW1709, Taichung, 30-III-2020, leg. Z.-T. Xu; TW1747, Tainan, 16-I-2020, leg. C.-C. Wu, C.-I Chiu, and W.-R. Liang; TW1750, Tainan, 16-I-2020, leg. C.-C. Wu, C.-I Chiu, and W.-R. Liang. The details refer to supplemental table S1.

Neotermes ludaensis C.-C. Wu, J.-F. Tsai, H.-F. Li, *sp. nov.* 綠島新白蟻

urn:lsid:zoobank.org:pub:6C16BC0E-1345-4F45-B9DC-88D336791846

Type material: **Holotype:** TW1291, Green Island (22°39'N, 121°29'E), 30-VI-2015, leg. H.-F. Li, C.-I Chiu, and W.-R. Liang, male alate, with 16S rRNA, COI, ITS2 sequences, deposited in NMNS (008746-00002).

Paratypes: TW1291, Green Island Co. (22°39'N, 121°29'E), 30-VI-2015, leg. H.-F. Li, C.-I Chiu, and W.-R. Liang, female alates and soldiers, deposited in NMNS (008746-00003) and NCHU.

Description: Imago. (Figs. 4C, 4F, 5C, 5F, 6C; Table 1) Head capsule reddish brown (10R 5/10, Figs. 4C, 4F). Head subcircular, longer than width, length without mandibles with 1.73 ± 0.14 mm, maximum width with 1.61 ± 0.08 mm. Eye circular, maximum compound eye diameter with 0.44 ± 0.02 mm. Ocelli whitish, oval. Antenna with 14–18 articles; 1st article longest; 2nd article as long as 3rd article; last article oblong. Labrum reddish yellow (5YR 6/10, Fig. 4F), liguiform, broader than length; anterior margin with 4–6 setae, tip of labrum overlapping the tip of mandibles. Left mandible with one apical tooth, two distinct marginal teeth (Fig. 5C); apical tooth longer than 1st + 2nd marginal tooth; 1st + 2nd marginal tooth as large as 3rd marginal tooth. Right mandible with one apical tooth, two marginal teeth, and molar tooth (Fig. 5C); apical tooth longer than 1st marginal tooth; 1st marginal tooth slightly longer than 2nd marginal tooth; 2nd marginal tooth with posterior margin longer than molar tooth.

Pronotum reddish yellow (2.5YR 5/12), broader than length, maximum length with 1.20 ± 0.08 mm, width with 1.98 ± 0.09 mm; anterior margin concave, without setae; lateral margin slightly circular, with 4–8 long setae and 15–20 short setae each side; posterior margin notched, without setae (Fig. 5F). Leg whitish yellow. Arolium

present. Tibia spur formula 3:3:3. Tarsi 4-jointed. Abdomen oblong.

Wing brownish (Fig. 6C). Forewing with radius run into costa at middle of wing; radial sector with 7–8 branches; median close to radial sector and parallel to each other, some individuals with median branched and meet with radial sector or cubitus; cubitus unsclerotized, with 14–15 branches. Hindwing shorter than forewing, radius run into costa at two-third of costa, radial sector and median meet till apex. Cubitus unsclerotized, with 14–15 branches.

Soldier. (Figs. 7G–I, 8C, 8F; Table 2) Head capsule orange (5YR 6/14, Figs. 7G, 7H). Head rectangular, longer than width, length without mandibles with 3 ± 0.2 mm, maximum width with 2.29 ± 0.14 mm. Eye whitish, small, oval. Antenna with 12–16 articles; 1st article longest; 2nd article as long as 3rd article; last article oblong. Postmentum club-shaped (Fig. 7H, 7I); anterior one-third two times broader than posterior two-third, with maximum width 0.72 ± 0.05 mm; sides of anterior one-fifth notched; sides of anterior one-third subcircular; posterior two-third sides parallel with minimum width 0.26 ± 0.03 mm. Labrum liguiform, broader than length, length with 0.36 ± 0.04 mm, width with 0.51 ± 0.03 mm; anterior margin with 4–6 long setae. Left mandible with one apical tooth, and three marginal teeth (Fig. 8C); apical tooth with a small bulge at posterior margin; 1st marginal tooth as long as 2nd marginal tooth; posterior margin of 2nd marginal tooth as wide as anterior margin of 3rd marginal tooth. Right mandible with one apical tooth, and two marginal teeth; 1st marginal tooth as large as 2nd marginal tooth.

Pronotum whitish yellow (5Y 9/6), broader than length, maximum length with 1.18 ± 0.12 mm, width with 2.20 ± 0.19 mm; covered with about 10–15 setae; anterior margin concave, with about 15 setae; lateral margin slightly circular, with 8–10 setae each side; posterior margin slightly concave, without setae (Fig. 8F). Leg whitish yellow. Tibia spur formula 3:3:3, Tarsi 4-jointed. Abdomen oblong.

Etymology: This *Neotermes* species is named after “Ludao”, which is the name of Green Island in Mandarin.

Distribution: Green Island, Taiwan.

Material examined: TAIWAN. All *N. ludaensis* were collected by H.-F. Li, C.-I Chiu, and W.-R. Liang. TW1270, Green Island, 29-VI-2015; TW1271, Green Island, 29-VI-2015; TW1272, Green Island, 29-VI-2015; TW1273, Green Island, 29-VI-2015; TW1275, Green Island, 29-VI-2015; TW1278, Green Island, 29-VI-2015; TW1279, Green Island, 29-VI-2015; TW1283, Green Island, 29-VI-2015; TW1289, Green Island, 29-VI-2015; TW1290, Green Island, 30-VI-2015; TW1291, Green Island, 30-VI-2015; TW1296, Green Island, 30-VI-2015; TW1297, Green Island, 30-VI-2015; TW1302, Green Island, 30-VI-2015; TW1303, Green Island, 30-VI-2015; TW1304, Green Island, 30-VI-2015; TW1309, Green Island, 30-VI-2015; TW1313, Green Island, 30-VI-2015; TW1314, Green Island, 30-VI-2015; TW1315, Green Island, 30-VI-2015; TW1321, Green Island, 30-VI-2015; TW1323, Green Island, 1-VII-2015; TW1326, Green Island, 1-VII-2015; TW1328, Green Island, 1-VII-2015; TW1330,



Green Island, 1-VII-2015; TW1332, Green Island, 1-VII-2015; TW1334, Green Island, 1-VII-2015; TW1337, Green Island, 1-VII-2015; TW1338, Green Island, 1-VII-2015; TW1341, Green Island, 1-VII-2015; TW1342, Green Island, 1-VII-2015; TW1344, Green Island, 1-VII-2015; TW1350, Green Island, 2-VII-2015; TW1354, Green Island, 2-VII-2015; TW1357, Green Island, 2-VII-2015; TW1360, Green Island, 2-VII-2015; TW1362, Green Island, 2-VII-2015; TW1364, Green Island, 3-VII-2015; TW1365, Green Island, 3-VII-2015; TW1366, Green Island, 3-VII-2015; TW1369, Green Island, 1-VII-2015. The details refer to supplemental table S1.

Note: *N. ludaensis* was compared with the 24 species occurring in China, Japan, and Philippine. According to the original descriptions, seven characters of winged imago were listed: color of head, color of pronotum, color of wings, number of antenna articles, maximum width of head, head length without mandible, and maximum width of pronotum. The winged imago of *N. ludaensis* is similar to *N. microphthalmos* Light, 1930, *N. parviscutatus* Light, 1930, and *N. binovatus* Han, 1984. In winged imago caste, *N. ludaensis* can be distinguished from *N. microphthalmos* and *N. parviscutatus* by the head width of *N. ludaensis* (1.48–1.76 mm), which is smaller than the two species (*N. microphthalmos* 1.9 mm, *N. parviscutatus* 2.16 mm). Moreover, *N. binovatus* have 6–7 radial sector branches, 12 cubitus branches, while *N. ludaensis* has 7–8 radial sector branches, 14–15 cubitus branches.

In winged imago caste, *N. ludaensis* could be distinguished from *N. koshunensis* and *N. sugioi* by the brownish wing (Figs. 6), smaller compound eye of winged imago (Table 1). The compound eye of *N. ludaensis* significantly smaller than *N. koshunensis* and *N. sugioi*. The number of antenna articles of *N. sugioi* (16–19 articles) is different from *N. koshunensis* (14–18 articles) and *N. ludaensis* (14–18 articles). *N. ludaensis* has wider pronotum (mean = 1.98 mm) than *N. sugioi* (mean = 1.89 mm). The pronotum width of *N. koshunensis* is smaller than other two species (mean = 1.78 mm).

In soldier caste, the size of labrum of the three species are significantly different (Table 2), *N. sugioi* has larger labrum than *N. ludaensis*, labrum of *N. koshunensis* is smaller than other two species. The maximum head width of *N. koshunensis* (mean = 2.12 mm) is smaller than *N. ludaensis* (mean = 2.29 mm). The average head height of *N. sugioi* (mean = 1.81 mm) is larger than *N. koshunensis* (mean = 1.67 mm). Compare to *N. koshunensis* (mean = 2.02 mm) and *N. sugioi* (mean = 2.08), *N. ludaensis* (mean = 1.78) has smaller mandible. The difference between *N. koshunensis*, *N. ludaensis* and *N. sugioi* in soldier caste is the posterior sides of postmentum (Figs. 7C, 7F, 7I) and mandibles (Figs. 8A–C). The postmentum side of *N. koshunensis* is parallel, the 1st marginal of right mandible is sharper and longer than 2nd marginal tooth, and without a distinct cave between 2nd and 3rd marginal tooth of left mandible. The postmentum sides of *N. ludaensis* is parallel, the 1st marginal of right mandible is as large as 2nd marginal

tooth, and has a distinct gap between 2nd and 3rd marginal tooth of left mandible. *N. sugioi* with postmentum sides slightly concave inward, the 1st marginal of right mandible is sharper and longer than 2nd marginal tooth, left mandible has a small protrusion at the posterior margin of apical tooth, and has a clear cave between 2nd and 3rd marginal tooth of left mandible. The three species also have different labrum size, *N. sugioi* has larger labrum than *N. ludaensis*, and *N. koshunensis* has smaller labrum than *N. ludaensis*.

N. koshunensis exhibits a wide distribution in the mountainous regions of Taiwan, while *N. sugioi* is confined to the southern part of Taiwan and Lanyu Island. And *N. ludaensis* only distributed in Green Island. The altitude distribution of *N. koshunensis* ranges from 0 to 1,100 m, *N. sugioi* is found within the range of 0 to 500 m, and *N. ludaensis* occurs at altitudes from 0 to 300 m. The three *Neotermes* species are commonly found in the decaying sections of living trees, dead standing trees, or fallen wood. The dispersal flight season of the three *Neotermes* species are different. *N. koshunensis* occurs in June to November, *N. sugioi* occurs from January to March, and *N. ludaensis* only has the record in July in this study.

Key to the species of *Neotermes* occurring in Taiwan.

Winged imago:

1. Forewing median vein unsclerotized, running midway between radial sector and cubitus *Cryptotermes* or *Incisitermes*
- Forewing median vein running close and parallel to radial sector till tip of wing (Figs. 6). 2
2. Radial sector without branches *Glyptotermes*
- Radial sector with branches (Figs. 6). 3
3. Wings brownish. Cubitus with more than 6 branches sclerotized (Fig. 6C) *Neotermes ludaensis* sp. nov.
- Wings transparent. Cubitus with 4–5 sclerotized branches (Figs. 6A–B) 4
4. Radial sector and median of hindwing meet before anterior one-fourth, and split at anterior one-fourth (Fig. 6A, red triangle) *Neotermes koshunensis*
- Radial sector and median of hindwing meet before anterior one-third, and split at anterior one-third (Fig. 6B, red triangle) *Neotermes sugioi*

Soldier:

1. Mandibles shorter than half of head capsule *Glyptotermes* or *Cryptotermes*
- Mandible longer than half of head capsule 2
2. Anterior margin of pronotum with V-shaped caved *Incisitermes*
- Anterior margin of pronotum without V-shaped caved 3
3. Postmentum with posterior sides slightly concave inward (Fig. 7F, red triangle) *Neotermes sugioi*
- Postmentum with posterior sides parallel (Figs. 7C & I, red triangle) 4
4. 1st marginal tooth of right mandible sharper and larger than 2nd marginal tooth (Fig. 8A); anterior one-fifth of postmentum without notched (Fig. 7C, black triangle). *Neotermes koshunensis*
- 1st marginal tooth of right mandible as large as 2nd marginal tooth (Fig. 8C); anterior one-fifth of postmentum with notched (Fig. 7I, black triangle). *Neotermes ludaensis* sp. nov.



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