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Review

# A new record of *Reticulitermes kanmonensis* Takematsu, 1999 (Isoptera: Rhinotermitidae) from Korea

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ABSTRACT

In this study, we report the first record of *Reticulitermes kanmonensis* from Korea verified using soldier morphological characters and haplotypes obtained from two mitochondrial genes. *R. kanmonensis* Takematsu, 1999 (Isoptera: Rhinotermitidae) was collected from *Pinus densiflora* Siebold & Zucc. (Pinaceae) stumps in two provinces, Jeollabuk-do and Chungcheongnam-do, Korea. We compared *R. kanmonensis* with the more common *Reticulitermes speratus kyushuensis* Morimoto, 1968 using morphological and molecular characters. In the morphological comparison, *R. kanmonensis* was distinguished from *R. speratus kyushuensis* by a significantly higher number of hairs on the pronotum and ratio of the posterior postmentum width and length. In the molecular comparison, *R. kanmonensis* revealed genetic differences of 5.49% (range 5.20%–5.80%) and 8.11% (range 8.00%–8.80%) from *R. speratus kyushuensis* using the *cytochrome oxidase subunit I* (*COI*) and *subunit II* (*COII*) gene sequences, respectively.

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**Contents**



## Introduction

Members of the family Rhinotermitidae, commonly known as "subterranean termites", have a cryptic lifestyle, an ephemeral adult emergence, and a limited number of immature diagnostic castes (Miller, 1964; Thorne et al., 1996). There are 6 subfamilies, and 12 genera in the Rhinotermitidae with the genus *Reticulitermes* responsible for causing serious economic damages to wooden buildings worldwide (Su and Scheffrahn, 1990; Krishna et al., 2013). One species, *Reticulitermes speratus kyushuensis* Morimoto 1968, has been recorded in Korea (ESK and KSAE, 1994). Becker (1969) first reported this species from Korea based on morphology, but there have been questions about the number of termite species in Korea because of the lack of taxonomic studies

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(Park and Bae, 1997). In the last ten years, several reports using mitochondrial or nuclear gene sequences, have verified the prevalence of *R. speratus kyushuensis* (Park et al., 2006, 2013; Kim et al., 2012). However, recently, Kim et al. (2012) found another *Reticulitermes* sp. They identified as *Reticulitermes kanmonensis* Takematsu, 1999 based on a BLAST search (http://blast.ncbi.nlm.nih.gov/); however, due to the lack of morphological verification, it is still ambiguous whether *R. kanmonensis* is well distributed in Korea.

We surveyed, from April to November 2014, over 58 local regions of 6 provinces in Korea and collected a total of 1337 individuals, including workers and soldiers, from decomposed pine trees, *Pinus densiflora* Siebold & Zucc. (Pinaceae) (Figs. 1 and 2). Based on the taxonomic literature (Takematsu, 1999), we identified 45 samples as *R. speratus kyushuensis*; while, 13 were identified as *R. kanmonensis* and we describe the variation between these two species from Korea. Additionally we analyzed two mitochondrial genes, *cytochrome oxidase subunit I* (*COI*) and *cytochrome oxidase subunit II* (*COII*), to corroborate the morphometric identification of these two species, *R. kanmonensis* and *R. speratus kyushuensis*. Based on the results, *R. kanmonensis* is reported as a new species record for Korea.

## Materials and methods

### *Morphological comparison*

Termite samples containing both worker and soldier castes were collected from 58 local regions in 6 provinces, Jeollabuk-do (JB), Jeollanam-do (JN), Jeju-do (JJ), Gyeongsangnam-do (GN), Chungcheongnam-do (CN), and Gyeongsangbuk-do (GB), from April to November 2014 (Fig. 1). Termites were taken by examining standing and fallen dead pine trees and stumps using a chisel, hammer and ax to extract them from the infested wood. The termites collected from a single log, stump or limb were considered one sample, and a total of 58 samples were collected that contained at least 1 soldier and 2 worker samples, and these samples were used for this study. The samples were stored in 95% ethanol at −10 °C.

A total of 14 morphological characters were selected, which were generally used to discriminate termite species (Fig. 3): head length without mandibles (HL), maximum width of head (HW), left mandible length (LML), labrum length (LL), labrum width (LW), maximum height of head (HH), length of postmentum (PmL), maximum width of postmentum (PmW1), minimum width of postmentum (PmW2), length of posterior postmentum (PmPL), width of posterior postmentum (PmPW), maximum length of pronotum (PnL), maximum width of pronotum (PnW), and the number of hairs on pronotum. Digital images for illustrations were taken using a Leica 400B camera (Leica Microsystems, Germany) at a resolution of 600 dpi. Measurements were performed from the digital images of each specimen using analyzing software, i-Solution (IMT iSolutions, Inc., Chicago, IL, USA).

**Fig. 2.** *Reticulitermes kanmonensis* on *Pinus densiflora* in Wanju-si, JB, Korea (29.iv.2014).

### *Molecular comparison*

We sequenced and analyzed 583 bp and 579 bp fragments from the mitochondrial *COI* and *COII* from 13 individuals (one per collection) of *R. kanmonensis* and 45 individuals (one per collection) of *R. speratus kyushuensis* (Table 1). Genomic DNA was extracted from each specimen using a DNeasy Blood & Tissue kit (QIAGEN, Inc., Dusseldorf, Germany) following the manufacturer's protocol. Two primer sets, LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) for the *COI* gene (Folmer

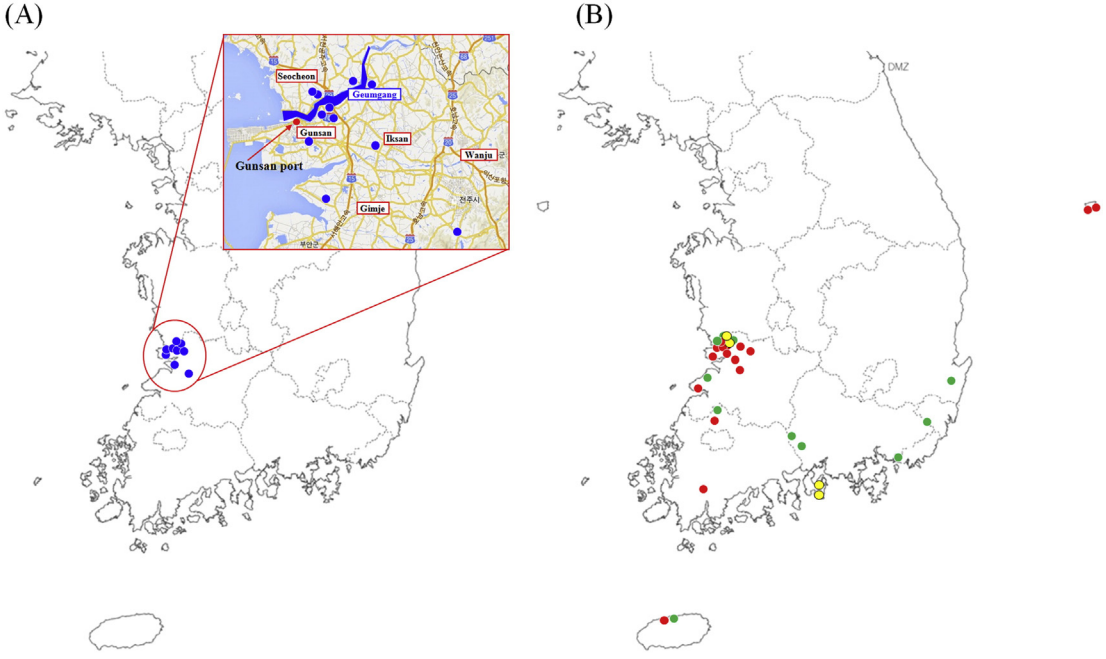


**Fig. 1.** Collection sites of (A) *Reticulitermes kanmonensis* and (B) *Reticulitermes speratus kyushuensis* (red circle, Hap-1; yellow circle, Hap-2; green circle, Hap-3) in Korea. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

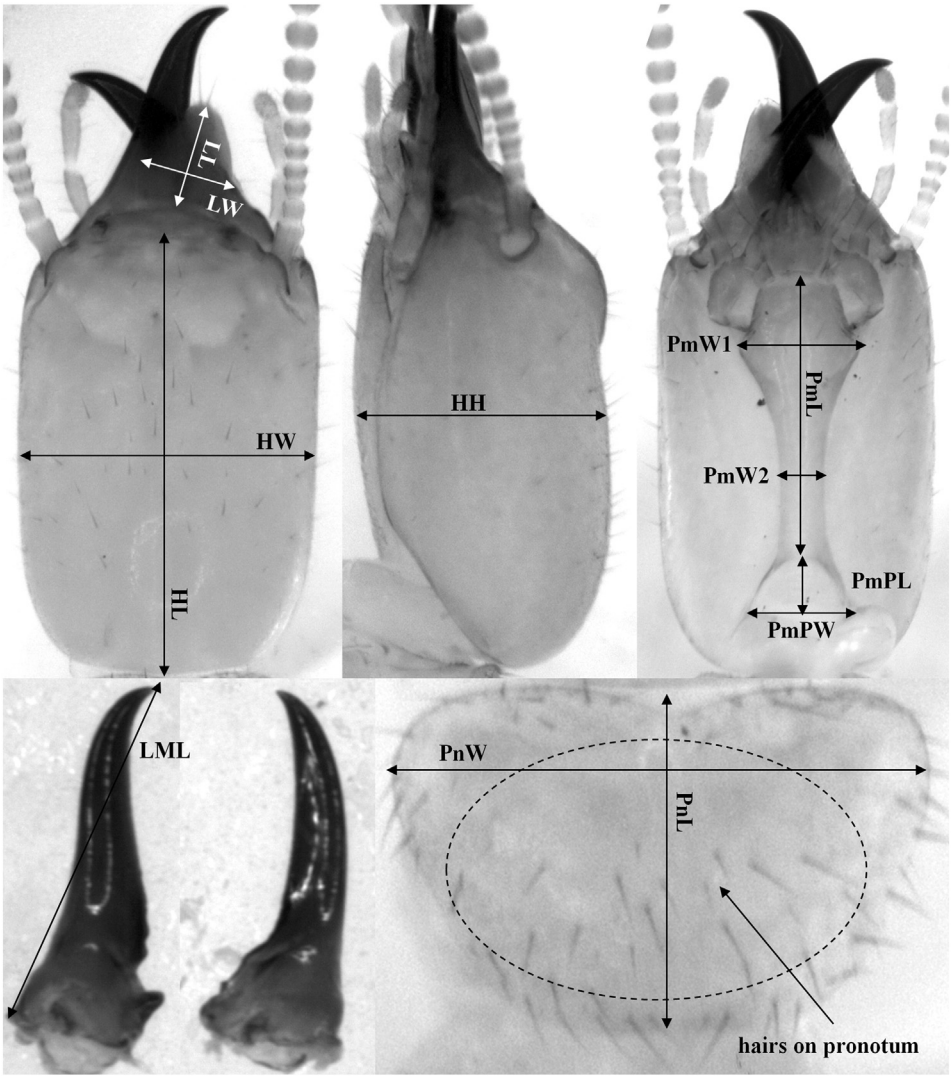


**Fig. 3.** A picture of 14 characters which were measured for morphological comparisons.

et al., 1994) and TL-J-3037 (5′-ATG GCA GA T AGT GCA ATG G-3′) (Liu and Beckenbach, 1992) and TK-N-3785 (5′-GTT TTA AGA GAC CAG TAC TTG-3′) for the *COII* gene (Simon et al., 1994), were used to amplify the *COI* and *COII* genes, respectively. The DNA fragments were amplified by using AccuPowerH PCR PreMix (BIONEER, Corp., Daejeon) in 20 μl reaction mixtures containing 0.4 μM of each primer, 20 μM of the dNTPs, 20 μM of the $MgCl_2$, and 0.05 μg of the genomic DNA template. PCR was performed using a GS1 thermo-cycler (Gene Technologies, Ltd., Essex) according to the following procedure: initial denaturation at 95 °C for 5 min, followed by 34 cycles at 95 °C for 30 s; an annealing temperature of 45–52 °C for 30 s; an extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. The PCR products were visualized by electrophoresis on a 1.5% agarose gel. A single band was observed, purified using a QIAquick PCR purification kit (QIAGEN, Inc.), and then sequenced directly using an automated sequencer (ABI PrismH 3730 XL DNA Analyzer) at ©MACROGEN LIC. Resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs in SeqMan™Pro (version 7.1.0, 2006, DNAstar Inc., Madison, WI, USA). The sequences were individually checked by eye, to avoid using pseudogenes from protein coding frame-shifts (Zhang and Hewitt, 1996). Consensus files were aligned using Clustal X 1.83 (Thompson et al., 1997). All sequences were deposited in GenBank (Table 1).

Pairwise sequence divergences among the 58 *COI* and 58 *COII* sequences were calculated, using Kimura's 2-parameter (K2P) distance model (Kimura, 1980) in MEGA 5.0 (Tamura et al., 2011). A neighbor-joining analysis was conducted using MEGA 5.0 (Tamura et al., 2011) based on three datasets, 58 *COI*, 58 *COII*, and combined 72 *COII* sequences (the 58 *COII* + previously reported 14 *COII*). The reported 14 *COII* sequences consist of *Coptotermes formosanus* (AF107488) and 11 *Reticulitermes* species (*R. amamianus* (DQ493721), *R. balkanesis* (AY954661), *R. banyulensis* (JQ431037), *R. chinensis* (FJ423458), *R. flaviceps* (EU627783), *R. flavipes* (JQ280689), *R. grassei* (JQ430999), *R. kanmonensis* (DQ493731, HM560009), *R. hesperus* (AY623441), *R. speratus* (DQ493740, DQ270499), *R. virginicus* (JF796223), downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

## Results

### *Morphological comparison*

Two species, *R. kanmonensis* and *R. speratus kyushuensis*, revealed similar morphologies. The lengths of 11 characteristics (HL, HW, LML, LL, LW, HH, PmL, PmW1, PmW2, PnL, and PnW) and four ratios (HL/HW, LML/HL, LL/LW, and PmW2/PmW1) showed overlapping ranges (Table 2). However, these species were clearly distinguished by two characteristics: i) the number of hairs on the pronotum and ii) the shape of the posterior margin of postmentum (Figs. 4 and 5; Table 2). *R. kanmonensis* had 22–33 hairs on the pronotum, distinguishable

**Table 1**
Sample list of *Reticulitermes kanmonensis* and *Reticulitermes speratus kyushuensis* used in molecular analyses.

| ID | Species | Collection locality | The number of individuals | | Date | Voucher no. | Accession no. | |
|---|---|---|---|---|---|---|---|---|
| | | | Soldier | Worker | | | *COI* gene | *COII* gene |
| 1 | *R. kanmonensis* | Gui-myeon, Wanju-gun, JB | 15 | 60 | 29.iv.2014 | Coll#140428WH08 | KP727665 | KP727711 |
| 2 | *R. kanmonensis* | Oksan-myeon, Gunsan-si, JB | 1 | 2 | 16.v.2014 | Coll#140516SG01 | KP727667 | KP727713 |
| 3 | *R. kanmonensis* | Seongsan-myeon, Gunsan-si, JB | 1 | 2 | 28.v.2014 | Coll#140528SG01 | KP727669 | KP727715 |
| 4 | *R. kanmonensis* | Napo-myeon, Gunsan-si, JB | 1 | 2 | 28.v.2014 | Coll#140528SG02, | KP727671 | KP727717 |
| 5 | *R. kanmonensis* | Seosu-myeon, Gunsan-si, JB | 3 | 6 | 28.v.2014 | Coll#140528SG03 | KP727673 | KP727719 |
| 6 | *R. kanmonensis* | Ungpo-myeon, Iksan-si, JB | 3 | 6 | 3.vi.2014 | Coll#140603SG01 | KP727675 | KP727721 |
| 7 | *R. kanmonensis* | Seongdeok-myeon, Gimje-si, JB | 4 | 8 | 21.vi.2014 | Coll#140621SG01 | KP727677 | KP727723, |
| 8 | *R. kanmonensis* | Iksan-si, JB | 2 | 4 | 1.vii.2014 | Coll#140701SG01 | KP727679 | KP727725 |
| 9 | *R. kanmonensis* | Hansan-myeon, Seocheon-gun, CN | 3 | 20 | 4.ix.2014 | Coll#140904YH01 | – | – |
| 10 | *R. kanmonensis* | Hwayang-myeon, Seocheon-gun, CN | 3 | 6 | 5.ix.2014 | Coll#140905SG01 | KP727681 | KP727727 |
| 11 | *R. kanmonensis* | Hwayang-myeon, Seocheon-gun, CN | 7 | 14 | 22.ix.2014 | Coll#140922SG01 | KP727683 | KP727729 |
| 12 | *R. kanmonensis* | Gisan-myeon, Seocheon-gun, CN | 2 | 4 | 23.ix.2014 | Coll#140923SG01 | KP727685 | KP727731 |
| 13 | *R. kanmonensis* | Hansan-myeon, Seocheon-gun, CN | 1 | 2 | 24.ix.2014 | Coll#140924SG01 | KP727687 | KP727733 |
| 14 | *R. speratus kyushensis* | Dongsang-myeon, Wanju-gun, JB | 5 | 20 | 28.iv.2014 | Coll#140428WH02 | KP727689 | KP727735 |
| 15 | *R. speratus kyushensis* | Hwasan-myeon, Wanju-gun, JB | 11 | 50 | 28.iv.2014 | Coll#140428WH03 | – | – |
| 16 | *R. speratus kyushensis* | Jangseong-gun, Wanju-gun, JB | 30 | 75 | 29.iv.2014 | Coll#140428WH04 | – | – |
| 17 | *R. speratus kyushensis* | Bukha-myeon, Jangseong-gun, JN | 2 | 20 | 29.iv.2014 | Coll#140428WH05 | KP727690 | KP727736 |
| 18 | *R. speratus kyushensis* | Jinseo-myeon, Buan-gun, JB | 15 | 40 | 29.iv.2014 | Coll#140428WH06 | KP727691 | KP727737 |
| 19 | *R. speratus kyushensis* | Wanju-gun, JB | 10 | 25 | 29.iv.2014 | Coll#140428WH07 | – | – |
| 20 | *R. speratus kyushensis* | Gui-myeon, Wanju-gun, JB | 25 | 60 | 29.iv.2014 | Coll#140428WH09 | – | – |
| 21 | *R. speratus kyushensis* | Buan-gun, JB | 10 | 40 | 30.iv.2014 | Coll#140428WH10 | – | – |
| 22 | *R. speratus kyushensis* | Gui-myeon, Wanju-gun, JB | 30 | 60 | 30.iv.2014 | Coll#140428WH11, | KP727692 | KP727738 |
| 23 | *R. speratus kyushensis* | Dongseong-gil, Jeju-si, JJ | 10 | 23 | 14.v.2014 | Coll#140512WH01 | KP727693 | KP727739 |
| 24 | *R. speratus kyushensis* | Baekpodong-gil, Jeju-si, JJ | 2 | 13 | 14.v.2014 | Coll#140512WH02 | – | – |
| 25 | *R. speratus kyushensis* | Okgu-eup, Gunsan-si, JB | 2 | 4 | 27.v.2014 | Coll#140527SG02 | – | – |
| 26 | *R. speratus kyushensis* | Baekto-ro, Gunsan-si, JB | 2 | 5 | 27.v.2014 | Coll#140527SG03 | KP727700 | KP727746 |
| 27 | *R. speratus kyushensis* | Seongsan-myeon, Gunsan-si, JB | 3 | 6 | 28.v.2014 | Coll#140528SG04 | – | – |
| 28 | *R. speratus kyushensis* | Napo-myeon, Gunsan-si, JB | 3 | 6 | 28.v.2014 | Coll#140528SG05 | – | – |
| 29 | *R. speratus kyushensis* | Gunsanchang 2-gil, Gunsan-si, JB | 4 | 9 | 29.v.2014 | Coll#140528SG06 | KP727701 | KP727747 |
| 30 | *R. speratus kyushensis* | Ungpo-myeon, Iksan-si, JB | 4 | 10 | 3.vi.2014 | Coll#140603SG02 | KP727702 | KP727748 |
| 31 | *R. speratus kyushensis* | Okdo-myeon, Gunsan-si, JB | 4 | 9 | 9.vi.2014 | Coll#140609SG02 | – | – |
| 32 | *R. speratus kyushensis* | Gongdandae-ro, Gunsan-si, JB | 2 | 4 | 10.vi.2014 | Coll#140610SG02, | KP727703 | KP727749 |
| 33 | *R. speratus kyushensis* | Okdo-myeon, Gunsan-si, JB | 2 | 4 | 16.vi.2014 | Coll#140616SG02 | – | – |
| 34 | *R. speratus kyushensis* | Habuk-myeon, Yangsan-si, GN | 6 | 18 | 26.vi.2014 | Coll#140626WH01 | KP727694 | KP727740v |
| 35 | *R. speratus kyushensis* | Sangbuk-myeon, Ulju-gun, Ulsan | 10 | 20 | 26.vi.2014 | Coll#140626WH02 | KP727695 | KP727741 |
| 36 | *R. speratus kyushensis* | Seongbuk-dong, Gangseo-gu, Busan | 2 | 15 | 27.vi.2014 | Coll#140626WH03 | KP727696 | KP727742 |
| 37 | *R. speratus kyushensis* | Geumma-myeon, Iksan-si, JB | 1 | 2 | 2.vii.2014 | Coll#140702SG02 | – | – |
| 38 | *R. speratus kyushensis* | Sajeong-dong, Gunsan-si, JB | 2 | 4 | 2.vii.2014 | Coll#140702SG03 | – | – |
| 39 | *R. speratus kyushensis* | Samdong-myeon, Namhae-gun, GN | 10 | 20 | 17.vii.2014, | Coll#140717WH01 | KP727697 | KP727743 |
| 40 | *R. speratus kyushensis* | Sangju-myeon, Namhae-gun, GN, | 2 | 20 | 17.vii.2014 | Coll#140717WH02 | – | – |
| 41 | *R. speratus kyushensis* | Sicheon-myeon, Sancheong-gun, GN | 15 | 40 | 18.vii.2014 | Coll#140717WH03 | KP727698 | KP727744 |
| 42 | *R. speratus kyushensis* | Hwagae-myeon, Hadong-gun, GN | 6 | 15 | 18.vii.2014 | Coll#140717WH04 | KP727699 | KP727745 |
| 43 | *R. speratus kyushensis* | Yanghwa-myeon, Buyeo-gun, CN | 2 | 20 | 3.ix.2014 | Coll#140903YH01 | KP727706 | KP727752 |
| 44 | *R. speratus kyushensis* | Yanghwa-myeon, Buyeo-gun, CN | 3 | 15 | 3.ix.2014 | Coll#140903YH02 | – | – |
| 45 | *R. speratus kyushensis* | Yanghwa-myeon, Buyeo-gun, CN | 5 | 20 | 4.ix.2014 | Coll#140903YH04 | – | – |
| 46 | *R. speratus kyushensis* | Yanghwa-myeon, Buyeo-gun, CN | 1 | 30 | 4.ix.2014 | Coll#140904YH03 | – | – |
| 47 | *R. speratus kyushensis* | Hwayang-myeon, Seocheon-gun, CN | 2 | 4 | 5.ix.2014 | Coll#140905SG02 | KP727704 | KP727750 |
| 48 | *R. speratus kyushensis* | Seongjeon-myeon, Gangjin-gun, JN | 1 | 2 | 15.ix.2014 | Coll#140915SG02 | KP727707 | KP727753 |
| 49 | *R. speratus kyushensis* | Hwayang-myeon, Seocheon-gun, CN | 3 | 6 | 22.ix.2014 | Coll#140922SG02 | – | – |
| 50 | *R. speratus kyushensis* | Gisan-myeon, Seocheon-gun, CN | 5 | 10 | 23.ix.2014 | Coll#140922SG03 | – | – |
| 51 | *R. speratus kyushensis* | Hansan-myeon, Seocheon-gun, CN | 3 | 6 | 24.ix.2014 | Coll#140922SG04, | KP727705 | KP727751 |
| 52 | *R. speratus kyushensis* | Janghang-eup, Seocheon-gun, CN | 3 | 6 | 25.ix.2014 | Coll#140922SG05 | – | – |
| 53 | *R. speratus kyushensis* | Maseo-myeon, Seocheon-gun, CN | 5 | 10 | 25.ix.2014 | Coll#140922SG06 | – | – |
| 54 | *R. speratus kyushensis* | Jinhyeon-dong, Gyeongju-si, GB | 5 | 20 | 5.xi.2014 | Coll#141105WH01 | KP727708 | KP727754 |
| 55 | *R. speratus kyushensis* | Jeodong-ri, Ulleung-eup, Ulleung-gun, GB | 10 | 20 | 6.xi.2014 | Coll#141105WH02 | KP727709 | KP727755 |
| 56 | *R. speratus kyushensis* | Sadong-ri, Ulleung-eup, Ulleung-gun, GB | 1 | 15 | 6.xi.2014 | Coll#141105WH03 | – | – |
| 57 | *R. speratus kyushensis* | Buk-myeon, Ulleung-gun, GB | 5 | 10 | 7.xi.2014 | Coll#141105WH04 | KP727710 | KP727756 |
| 58 | *R. speratus kyushensis* | Sadong-ri, Ulleung-eup, Ulleung-gun, GB | 4 | 11 | 7.xi.2014 | Coll#141105WH05 | – | – |

**Table 2**
Genetic divergences of *Reticulitermes kanmonensis* and *Reticulitermes speratus kyushuensis* according to two mitochondrial genes, *COI* and *COII*.

| Gene | Intraspecific genetic divergences of *R. kanmonensis* | Intraspecific genetic divergences of *R. speratus kyushuensis* | Interspecific genetic divergences between *R. kanmonensis* and *R. speratus kyushuensis* |
|---|---|---|---|
| *COI* | 0.00% | 0.27% (0.00%–0.50%) | 5.49% (5.20%–5.80%) |
| *COII* | 0.00% | 0.30% (0.00%–0.70%) | 8.11% (8.00%–8.80%) |

from 13 to 17 hairs of *R. speratus kyushuensis*, and a posterior margin of postmentum in *R. kanmonensis* was relatively concave than that of *R. speratus kyushuensis*. In particular, the ratio of PmPW/PmPL in *R. kanmonensis* was ranged from 0.40 to 0.71, comparing with a range from 0.29 to 0.37 in *R. speratus kyushuensis*.

### *Molecular analysis*

Totally, 58 *COI* and 58 *COII* sequences were analyzed from 13 individuals (one per collection) of *R. kanmonensis* and 45 individuals (one per collection) of *R. speratus kyushuensis*. In the definitive dataset, the number

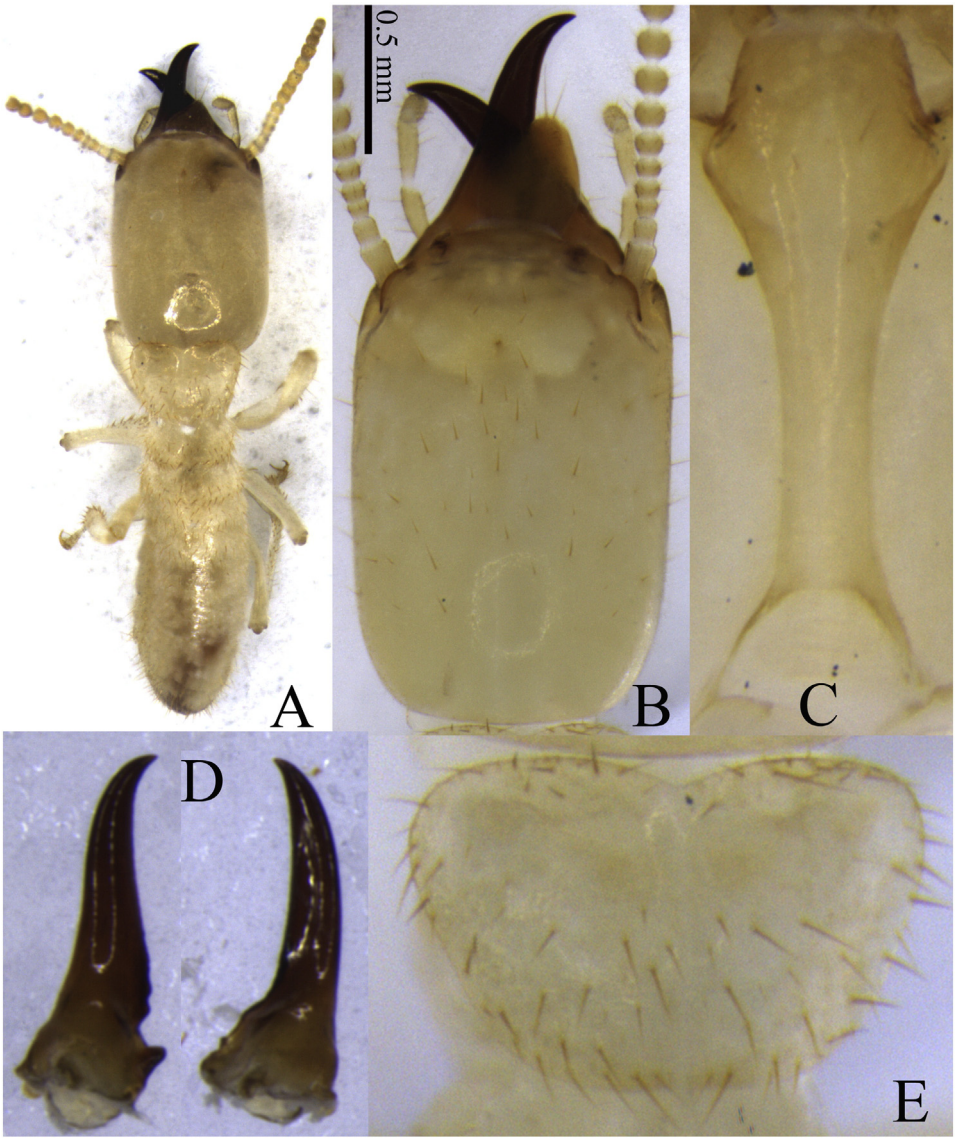


**Fig. 4.** Soldier of *Reticulitermes kanmonensis*: A, body; B, head; C, postmentum; D, mandibular; and E, pronotum.

of variable sites was 21 and parsimony informative sites were 21 in the *COI* gene, and 47 variable sites and 47 parsimony informative sites were in the *COII* gene. *R. kanmonensis* showed no intraspecific genetic divergences in the two genes, while intraspecific genetic divergences less than 1.00% were detected from *R. speratus kyushensis*. Interspecific genetic distances between these species were average 5.49% (range 5.20%–5.80%) and 8.11% (range 8.00%–8.80%) in the *COI* and *COII* genes, respectively (Table 3).

In NJ trees, the two Korean species formed separate clades, indicating that *R. kanmonensis* is a genetically distinct species from *R. speratus kyushuensis* (Fig. 6). In this study, we found that *R. kanmonensis* and *R. speratus kyushuensis* revealed one and three haplotypes (Hap-1, Hap-2, and Hap-3), respectively, in the two genes. In addition, *R. speratus kyushensis* revealed different topologies according to the *COI* and *COII* genes. For example, Hap-1 and Hap-2 were separated from Hap-C in the *COII* gene; whereas, there were no separations among the three haplotypes in the *COI* gene. In the combined *COII* dataset, all 13 *COII* sequences of *R. kanmonensis* were clustered to *R. kanmonensis* (DQ493731 and HM560009), and the Hap-2 and Hap-3 of *R. speratus kyushuensis* were clustered to *R. speratus* (DQ493740 and DQ270499), respectively. It indicated that the same types of the two Korean species, *R. kanmonensis* and *R. speratus kyushuensis*, have been distributed in other countries such as Japan. In this study, we also found that the Hap-1 is a new type in *R. speratus kyushuensis*, which have been not reported until now (Fig. 6).

## Discussion

In Korea, the only recorded species of termite, *R. speratus kyushuensis*, seems out of place, when compared with the numbers of species reported in Japan (ca. 27 species) and China (ca. 400 species) (Krishna et al., 2013). The dearth of taxonomic studies since Becker (1969) can explain, in part, the disparity in recorded termite species in Korea. Interestingly, Lee et al. (nd) reported detections of *C. formosanus* Shiraki, 1909 in three southern locations, Busan City, Jinju Island, and Geoge Island, in 1998. This report resulted in controversy about the distribution of *C. formosanus* in Korea (Paek et al., 2010). However, we surveyed those same locales in our study of 58 local regions and failed to observe *C. formosanus*.

*R. kanmonensis* has only been recorded in Japan (Nawa, 1911, 1912a, 1912b, 1917; Kitade and Matsumoto, 1993; Takematsu, 1999; Morimoto, 2000; Kitade and Hayashi, 2002). Nawa (1911) first reported *R. kanmonensis* in the area of the Kanmon Straits but it was identified as *R. flaviceps* distinguishable from the *R. speratus* in having an earlier alate

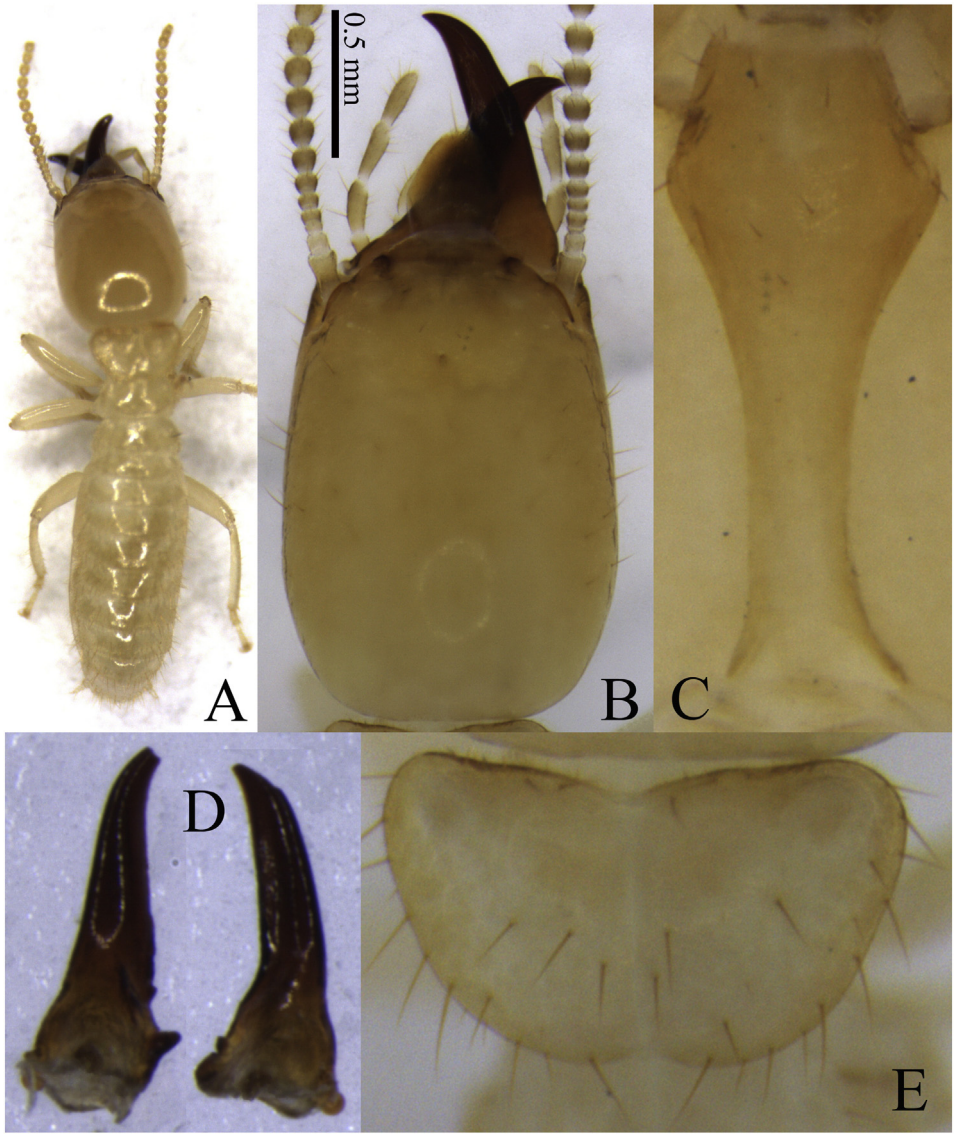


**Fig. 5.** Soldier of *Reticulitermes speratus kyushuensis*: A, body; B, head; C, postmentum; D, mandibular; and E, pronotum.

emergence and nuptial flight (Ito, 1994a, 1994b). Takematsu (1999) described *R. kanmonensis* while one year later Morimoto (2000) summarized the morphology, localities, ecology and research history of this species.

Kitade and Hayashi (2002) suggested three hypotheses to explain the localized distribution of *R. kanmonensis* in Japan: i) sympatric or parapatric speciation from *R. speratus*, ii) natural dispersion from the Korean Peninsula, and iii) an artificial introduction into Japan. There was, prior to this report, no record of *R. kanmonensis* on the Korean Peninsula, there is a difference in the symbiotic protist faunae between *R. kanmonensis* and *R. speratus* and the known distribution of *R. kanmonensis* includes Shimonoseki and Kitakyushu a base of international trading between Japan and China during the period from the 15th century to the early 18th century (Kirby, 1937; History of Shimonoseki City Editorial Committee, 1973; Yamaguchi Prefecture, 1991; Kitade and Matsumoto, 1993; Ren, 1998; Takematsu, 1999; Morimoto, 2000). In addition, the published *COII* sequences of *R. kanmonensis* are similar to those of *Reticulitermes* species distributed in Hong Kong and Guangzhou (southern China), suggesting *R. kanmonensis* (Kitade and Hayashi, 2002). Kitade and Hayashi (2002) therefore concluded that the most likely explanation was an artificial introduction to Japan from southern China a few hundred years ago.

In this study, we collected *R. kanmonensis* in the middle-western provinces of, Wanju, Gunsan, Iksan, Gimje, and Seocheon (Fig. 1a). These areas are located near a large river (Korean name: Geumgang), connected to the East Sea, and the port of Gunsan. The Gunsan port has been a base of international trade between Japan and Korea since the 18th century. Kitade and Hayashi (2002) concluded that their second hypothesis was not reasonable because there were no records of *R. kanmonensis* in the Korean Peninsula. Although we did find *R. kanmonensis* in this study, the second hypothesis still seems unreasonable. This is because the collection sites of *R. kanmonensis* were limited to the middle-western regions and we found only one haplotype at 11 disparate collection sites. We guess that *R. kanmonensis* has been introduced into the Gunsan port and transported through the Geumgang area by commercial ship and road traffic. It is necessary to study the distribution of *R. kanmonensis* in Korea further to illuminate the invasion and spread of this potentially damaging pest.

## Systematic accounts

***Reticulitermes kanmonensis* Takematsu, 1999** (Fig. 4 and Table 3)
*Reticulitermes kanmonensis* Takematsu, 1999b: 237–238.

**Table 3**
Biometrics of *Reticulitermes kanmonensis* and *Reticulitermes speratus kyushuensis*.

| Characters | | Species | | | |
|---|---|---|---|---|---|
| | | *R. kanmonensis* (n = 13) | | *R. speratus kyushuensis* (n = 45) | |
| Length | HL | 1.56 | 1.46–1.66 | 1.52 | 1.45–1.62 |
| | HW | 1.01 | 0.94–1.07 | 1.03 | 1.03–1.16 |
| | LML | 0.96 | 0.88–1.02 | 0.95 | 0.92–0.97 |
| | LL | 0.36 | 0.32–0.41 | 0.35 | 0.30–0.37 |
| | LW | 0.29 | 0.26–0.32 | 0.31 | 0.28–0.34 |
| | HH | 0.85 | 0.82–0.88 | 0.90 | 0.83–1.01 |
| | PmL | 1.03 | 0.96–1.08 | 0.99 | 0.95–1.03 |
| | PmW1 | 0.43 | 0.32–0.46 | 0.43 | 0.39–0.46 |
| | PmW2 | 0.16 | 0.14–0.18 | 0.16 | 0.13–0.18 |
| | PnL | 0.45 | 0.44–0.46 | 0.44 | 0.38–0.48 |
| | PnW | 0.74 | 0.66–0.89 | 0.72 | 0.68–0.81 |
| | PmPL | 3.66 | 3.08–3.96 | 2.73 | 2.52–2.94 |
| | PmPW | 1.82 | 1.23–2.64 | 0.88 | 0.84–0.92 |
| No. hairs on | Pronotum | 26.6 | 21–33 | 5.43 | 4–7 |
| Ratio | HL/HW | 1.54 | 1.39–1.68 | 1.44 | 1.39–1.52 |
| | LML/HL | 0.62 | 0.56–0.67 | 0.62 | 0.60–0.66 |
| | LL/LW | 1.25 | 1.11–1.47 | 1.17 | 0.97–1.31 |
| | PmW2/PmW1 | 0.38 | 0.35–0.43 | 0.36 | 0.33–0.40 |
| | PmPW/PmPL | 0.50 | 0.40–0.71 | 0.32 | 0.29–0.37 |

Abbreviations are explained in the Materials and methods section.

*Color in life*. (Soldier) Head capsule yellowish; anteclypeus whitish; postclypeus yellow, slightly darker than head; labrum yellow as postclypeus; antennae yellow as postclypeus; mandibles dark brown, paler basally. Thorax and abdomen yellowish white.

*Morphology*. (Soldier) Head capsule subrectangular, with sides slightly convex, widest at middle, with many long and short hairs; frontal area distinctly convex. Clypeus trapezoidal with clypeal setae absent or minute. Labrum triangularly at anterior margin, convex at sides, with two long hairs at apex and with a few minute hairs or bald on surface, without paraterminal setae. Right mandible with inner margin straight and curved inward at tip, Postmentum widest at anterior, narrowest at middle, with long and short hairs on anterior surface and with minute hairs on posterior surface. Pronotum flat, trapezoidal, slightly raised along anterior margin, faintly concave at anterior margin, rounded at anterior and posterior corners, with 21–33 hairs abounding on posterior half, with many long or short hairs at marginal portions. Abdominal terga and sterna densely haired on surface.

*Specimen examined*. 15 soldiers and 60 workers, Coll#140428WH08, Gui-myeon, Wanju-gun, JB, Korea, 29.iv.2014, Wonhoon Lee (W.Lee); 1 soldier and 2 workers, Coll#140516SG01, Oksan-myeon, Gunsan-si, JB, Korea, 16.v.2014, Seongjin Lee (S.Lee); 1 soldier and 2 workers, Coll#140528SG01, Seongsan-myeon, Gunsan-si, JB, Korea, 28.v.2014, S.Lee; 1 soldier and 2 workers, Coll#140528SG02, Napo-myeon, Gunsan-si, JB, Korea, 28.v.2014, S.Lee; 3 soldiers and 6 workers, Coll#140528SG03, Seosu-myeon, Gunsan-si, JB, Korea, 28.v.2014, S.Lee; 3 soldiers and 6 workers, Coll#140603SG01, Ungpo-myeon, Iksan-si, JB, Korea, 3.vi.2014, S.Lee; 4 soldiers and 8 workers, Coll#140621SG01, Seongdeok-myeon, Gimje-si, JB, Korea, 21.vi.2014, S.Lee; 2 soldiers and 4 workers, Coll#140701SG01, Iksan-si, JB, Korea, 1.vii.2014, S.Lee; 3 soldiers and 20 workers, Coll#140904YH01, Hansan-myeon, Seocheon-gun, CN, Korea, 4.ix.2014, Yong-Hyeon Lee (Y.Lee); 3 soldiers and 6 workers, Coll#140905SG01, Hwayang-myeon, Seocheon-gun, CN, Korea, 5.ix.2014, S.Lee; 7 soldiers and 14

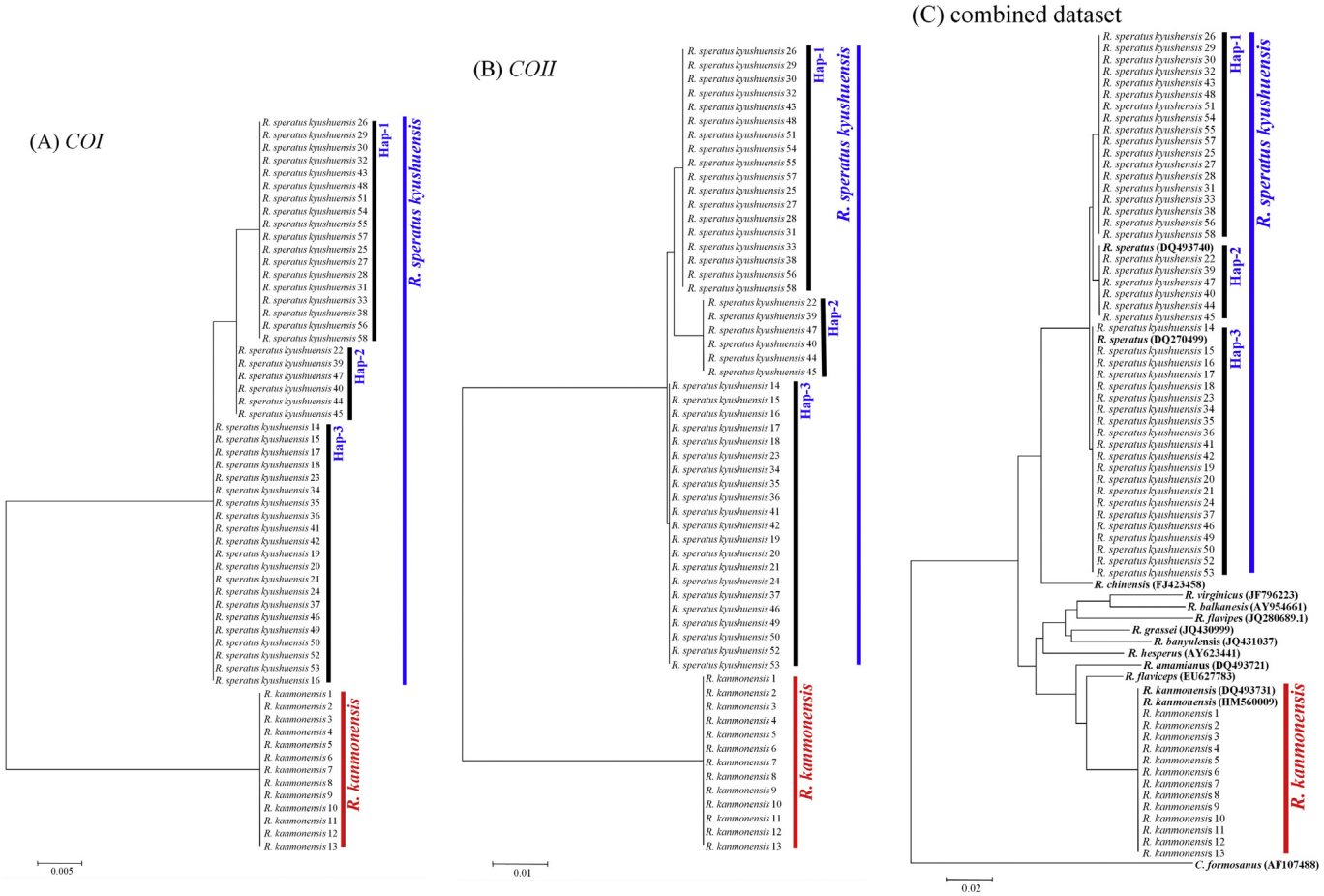


**Fig. 6.** Neighbor-joining trees analyzed based on three datasets, (A) 58 *COI* sequences, (B) 58 *COII* sequences, and (C) the combined 72 *COII* sequences.

workers, Coll#140922SG01, Hwayang-myeon, Seocheon-gun, CN, Korea, 22.ix.2014, S.Lee; 2 soldiers and 4 workers, Coll#140923SG01, Gisan-myeon, Seocheon-gun, CN, Korea, 23.ix.2014, S.Lee; 1 soldier and 2 workers, Coll#140924SG01, Hansan-myeon, Seocheon-gun, CN, Korea, 24.ix.2014, S.Lee.

*Host plant*. *Pinus densiflora* Siebold & Zucc. (Pinaceae)

*Distribution*. Japan and Korea (Wanju, Gunsan, Iksan, Gimje, and Seocheon)

***Reticulitermes speratus kyushuensis* Morimoto, 1968** (Fig. 5 and Table 3)

*Reticulitermes speratus kyushuensis* Morimoto, 1968: 70

*Color in life*. (Soldier) Head capsule yellowish; anteclypeus whitish; postclypeus yellow, slightly darker than head; labrum yellow as postclypeus; antennae yellow as postclypeus; mandibles dark brown, paler basally. Thorax and abdomen yellowish white.

*Morphology*. (Soldier) Head capsule subrectangular, with sides slightly convex, widest at middle, with long and short hairs sparsely; frontal area distinctly convex. Clypeus trapezoidal; clypeal setae absent or minute. Labrum triangularly at anterior margin, convex at sides, with two long hairs at apex and with a few long hairs on surface, without paraterminal setae. Right mandible with inner margin straight and curved inward at tip, Postmentum widest at anterior, narrowest at middle, with long and short hairs on anterior surface and with minute hairs on posterior surface. Pronotum flat, trapezoidal, slightly raised along anterior margin, faintly concave at anterior margin, rounded at anterior and posterior corners, with 4–7 hairs abounding on posterior half, with a few long hairs at marginal portions. Abdominal terga and sterna densely haired on surface.

*Specimen examined*. 5 soldiers and 20 workers, Coll#140428WH02, Dongsang-myeon, Wanju-gun, JB, Korea, 28.iv.2014, W.Lee; 11 soldiers and 50 workers, Coll#140428WH03, Hwasan-myeon, Wanju-gun, JB, Korea, 28.iv.2014, W.Lee; 30 soldiers and 75 workers, Coll#140428WH04, Jangseong-gun, JN, Korea, 29.iv.2014, W.Lee; 2 soldiers and 20 workers, Coll#140428WH05, Bukha-myeon, Jangseong-gun, JN, Korea, 29.iv.2014, W.Lee; 15 soldiers and 40 workers, Coll#140428WH06, Jinseo-myeon, Buan-gun, JB, Korea, 29.iv.2014, W.Lee; 10 soldiers and 25 workers, Coll#140428WH07, Wanju-gun, JB, Korea, 29.iv.2014, W.Lee; 25 soldiers and 60 workers Coll#140428WH09, Gui-myeon, Wanju-gun, JB, Korea, 29.iv.2014, W.Lee; 10 soldiers and 40 workers, Coll#140428WH10, Buan-gun, JB, Korea, 30.iv.2014, W.Lee; 30 soldiers and 60 workers Coll#140428WH11, Gui-myeon, Wanju-gun, JB, Korea, 30.iv.2014, W.Lee; 10 soldiers and 23 workers Coll#140512WH01, Dongseong-gil, Jeju-si, JJ, Korea, 14.v.2014, W.Lee; 2 soldiers and 13 workers Coll#140512WH02, Baekpodong-gil, Jeju-si, JJ, Korea, 14.v.2014, W.Lee; 2 soldiers and 4 workers, Coll#140527SG02, Okgu-eup, Gunsan-si, JB, Korea, 27.v.2014, S.Lee; 2 soldiers and 5 workers, Coll#140527SG03, Baekto-ro, Gunsan-si, JB, Korea, 27.v.2014, S.Lee; 3 soldiers and 6 workers, Coll#140528SG04, Seongsan-myeon, Gunsan-si, JB, Korea, 28.v.2014, S.Lee; 3 soldiers and 6 workers, Coll#140528SG05, Napo-myeon, Gunsan-si, JB, Korea, 28.v.2014, S.Lee; 4 soldiers and 9 workers, Coll#140528SG06, Gunsanchang 2-gil, Gunsan-si, JB, Korea, 29.v.2014, S.Lee; 4 soldiers and 10 workers, Coll#140603SG02, Ungpo-myeon, Iksan-si, JB, Korea, 3.vi.2014, S.Lee; 4 soldiers and 9 workers, Coll#140609SG02, Okdo-myeon, Gunsan-si, JB, Korea, 9.vi.2014, S.Lee; 2 soldiers and 4 workers, Coll#140610SG02, Gongdandae-ro, Gunsan-si, JB, Korea, 10.vi.2014, S.Lee; 2 soldiers and 4 workers, Coll#140616SG02, Okdo-myeon, Gunsan-si, JB, Korea, 16.vi.2014, S.Lee; 6 soldiers and 18 workers, Coll#140626WH01, Habuk-myeon, Yangsan-si, GN, Korea, 26.vi.2014, W.Lee; 10 soldiers and 20 workers, Coll#140626WH02, Sangbuk-myeon, Ulju-gun, Ulsan, Korea, 26.vi.2014, W.Lee; 2 soldiers and 15 workers, Coll#140626WH03, Seongbuk-dong, Gangseo-gu, Busan, Korea, 27.vi.2014, W.Lee; 1 soldier and 2 workers, Coll#140702SG02, Geumma-myeon, Iksan-si, JB, Korea, 2.vii.2014, S.Lee; 2 soldiers and 4 workers, Coll#140702SG03, Sajeong-dong, Gunsan-si, JB, Korea, 2.vii.2014, S.Lee; 10 soldiers and 20 workers, Coll#140717WH01, Samdong-myeon, Namhae-gun, GN, Korea, 17.vii.2014, W.Lee; 2 soldiers and 20 workers, Coll#140717WH02, Sangju-myeon, Namhae-gun, GN, Korea, 17.vii.2014, W.Lee; 15 soldiers and 40 workers, Coll#140717WH03, Sicheon-myeon, Sancheong-gun, GN, Korea, 18.vii.2014, W.Lee; 6 soldiers and 15 workers, Coll#140717WH04, Hwagae-myeon, Hadong-gun, GN, Korea, 18.vii.2014, W.Lee; 2 soldiers and 20 workers, Coll#140903YH01, Yanghwa-myeon, Buyeo-gun, CN, Korea, 3.ix.2014, Y.Lee; 3 soldiers and 15 workers, Coll#140903YH02, Yanghwa-myeon, Buyeo-gun, CN, Korea, 3.ix.2014, Y.Lee; 5 soldiers and 20 workers, Coll#140903YH04, Yanghwa-myeon, Buyeo-gun, CN, Korea, 4.ix.2014, Y.Lee; 1 soldier and 30 workers, Coll#140904YH03, Yanghwa-myeon, Buyeo-gun, CN, Korea, 4.ix.2014, Y.Lee; 2 soldiers and 4 workers, Coll#140905SG02, Hwayang-myeon, Seocheon-gun, CN, Korea, 5.ix.2014, S.Lee; 1 soldier and 2 workers, Coll#140915SG02, Seongjeon-myeon, Gangjin-gun, JN, Korea, 15.ix.2014, S.Lee; 3 soldiers and 6 workers, Coll#140922SG02, Hwayang-myeon, Seocheon-gun, CN, Korea, 22.ix.2014, S.Lee; 5 soldiers and 10 workers, Coll#140922SG03, Gisan-myeon, Seocheon-gun, CN, Korea, 23.ix.2014, S.Lee; 3 soldiers and 6 workers, Coll#140922SG04, Hansan-myeon, Seocheon-gun, CN, Korea, 24.ix.2014, S.Lee; 3 soldiers and 6 workers, Coll#140922SG05, Janghang-eup, Seocheon-gun, CN, Korea, 25.ix.2014, S.Lee; 5 soldiers and 10 workers, Coll#140922SG06, Maseo-myeon, Seocheon-gun, CN, Korea, 25.ix.2014, S.Lee; 5 soldiers and 20 workers, Coll#141105WH01, Jinhyeon-dong, Gyeongju-si, GB, Korea, 5.xi.2014, W.Lee; 10 soldiers and 20 workers, Coll#141105WH02, Jeodong-ri, Ulleung-eup, Ulleung-gun, GB, Korea, 6.xi.2014, W.Lee; 1 soldier and 15 workers, Coll#141105WH03, Sadong-ri, Ulleung-eup, Ulleung-gun, GB, Korea, 6.xi.2014, W.Lee; 5 soldiers and 10 workers, Coll#141105WH04, Buk-myeon, Ulleung-gun, GB, Korea, 7.xi.2014, W.Lee; 4 soldiers and 11 workers, Coll#141105WH05, Sadong-ri, Ulleung-eup, Ulleung-gun, GB, Korea, 7.xi.2014, W.Lee.

*Host plant*. *Pinus densiflora* Siebold & Zucc. (Pinaceae), *Picea abies* (L.) H.Karst. (Pinaceae), and *Acer pictum* subsp. *mono* (Maxim.) Ohashi (Aceraceae).

*Distribution*. Japan and Korea.

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