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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* (*COI*) gene sequences, respectively.

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# 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

\* Corresponding author. E-mail address: leesectp@gmail.com (Y.-H. Lee). (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

(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  $-10\,^{\circ}\text{C}$ .

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



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

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).

#### 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

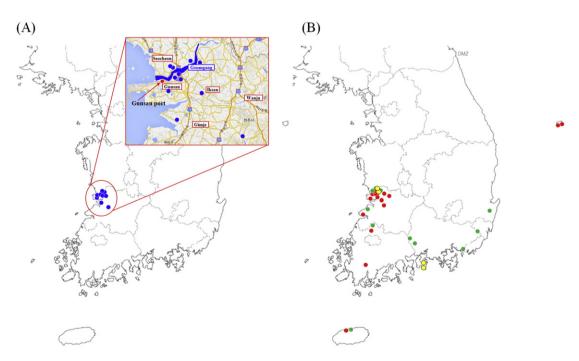


Fig. 1. Collection sites of (A) Reticulitermes kanmonensis and (B) Reticulitermes speratus kyushuensis (red circle, Hap-1; yellow circle, Hap-2; green circle, Hap-3) in Korea.

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