



## SHORT NOTE

### First Record of the Soil-feeding Higher Termites (Isoptera: Termitidae) from Okinawa Island, the Central Ryukyus, Japan

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#### Abstract

Some termites have been introduced outside their native ranges, and it has been suggested that both wood-eating and wood-nesting lifestyles are critical for the transportation of propagules. Here, we present the first record of the soil-feeding and soil-nesting termite *Pericapritermes nitobei* (Isoptera: Termitidae) in the Motobu Peninsula on the northern part of Okinawa Island in the Central Ryukyus. The fact that previous extensive surveys of termites in the Ryukyu Archipelago did not detect *P. nitobei* in the Central Ryukyus, including the Motobu Peninsula, and its discontinuous distribution, suggest that *P. nitobei* has been recently introduced into the Motobu Peninsula of Okinawa Island from the Yaeyama Islands, similar to the fungus-growing and soil-nesting termite *Odontotermes formosanus* (Isoptera: Termitidae). Together with *O. formosanus* in the southern part of Okinawa Island, our findings raise the possibility that non-wood-feeding and non-wood-nesting termites can be introduced outside their native ranges by human activities.

Termites play important roles in ecosystems, especially as decomposers and soil engineers (Bignell & Eggleton, 2000; Holt & Lepage, 2000). Termites can also cause serious structural damage to buildings, crops, and plantation forests (Su & Scheffrahn, 2000; Rouland-Lefèvre, 2010). Some termite species have been introduced outside their native ranges, where they have become invasive, and caused substantial economic, and possible ecological impacts (Evans, 2010; Evans et al., 2013). Evans et al. (2013) recognized 28 invasive termite species and found that they all shared three characteristics: they eat wood, nest in wood, and easily generate secondary reproductives. To date, seven termite species, which are considered to have been introduced, are established in Japan: *Zootermopsis nevadensis* (Hagen), *Incisitermes immigrans* (Snyder), *I. minor* (Hagen), *I.*

*schwarzi* (Banks), *Coptotermes formosanus* Shiraki, *C. gestroi* (Wasmann), and *Reticulitermes kanmonensis* Takematsu (Morimoto & Ishii, 2000; Kitade & Hayashi, 2002; Ohmura & Tokoro, 2003; Ide et al., 2016; Yashiro et al., 2018). Although two of these termite species (*I. schwarzi* and *R. kanmonensis*) have not been listed as invasive by Evans et al. (2013), they also have the above-mentioned three characteristics of invasive termites (Luykx, 1986; Kitade et al., 2011).

The Ryukyu Archipelago of Japan is located in the subtropical zone of the western Pacific Ocean between mainland Japan and Taiwan. Faunistically, the Ryukyu Archipelago is classified into three regions: the Northern, Central, and Southern Ryukyus, based on their relation to the Tokara and Kerama gaps (Fig 1A, Kimoto, 1967; Ota, 1998; Motokawa, 2000). It has been known that the higher

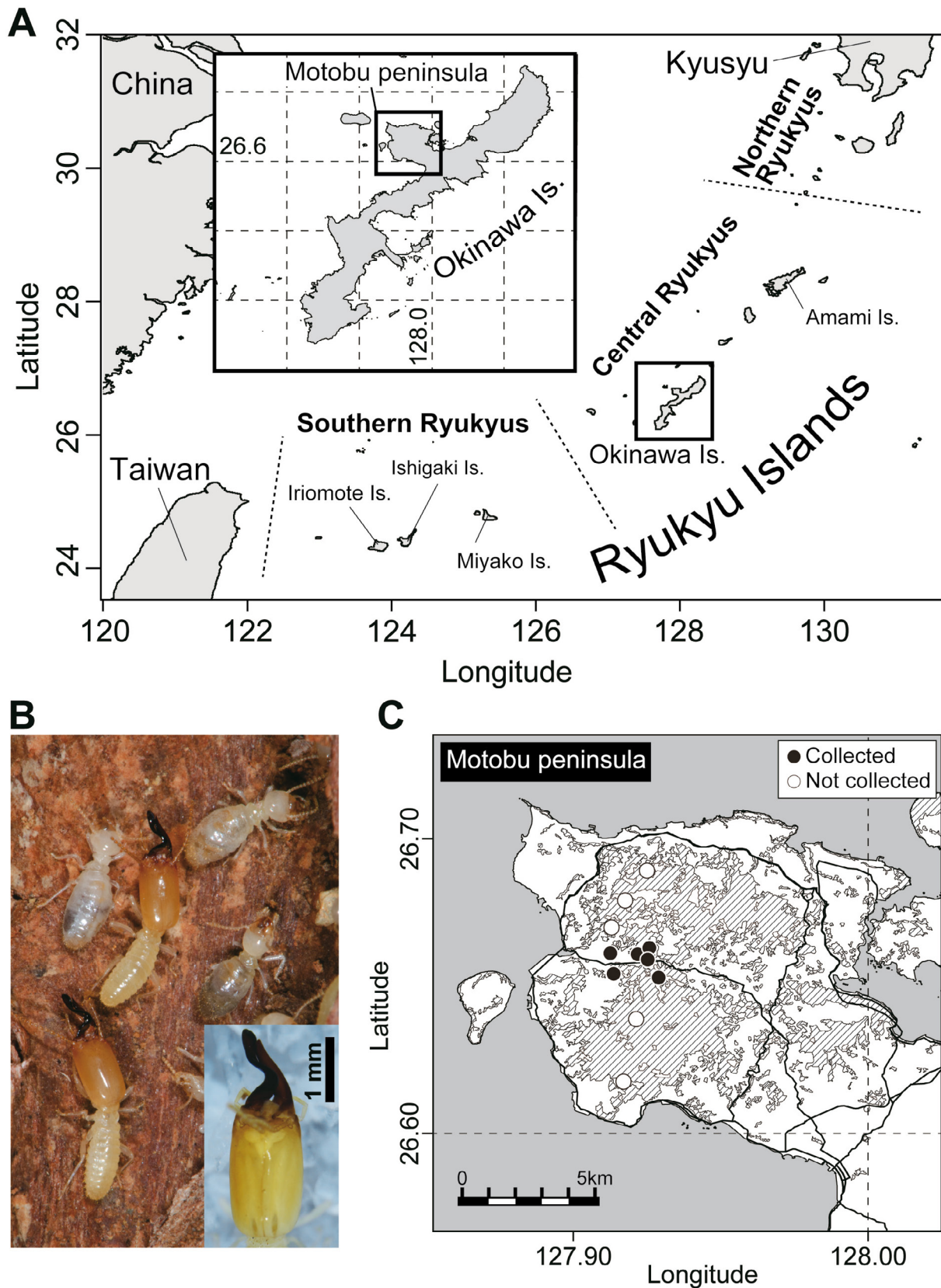
termites (family Termitidae) of the Ryukyu Archipelago are distributed only in the southern Ryukyus, including Ishigaki and Iriomote Islands, which are part of the Yaeyama Islands with one exception, the fungus-growing termite *Odontotermes formosanus* (Shiraki), which is distributed not only on the Yaeyama Islands, but also on the Okinawa Islands in the Central Ryukyus (Ikehara, 1966; Yasuda et al., 2000). It is suggested that the *O. formosanus* found on the Okinawa Islands has been introduced from the Yaeyama Islands (presumably from Iriomote Island), based on its discontinuous and restricted distribution on Okinawa Island (Hojo, 2019). In this study, we report the unusual findings of a population of the soil-feeding higher termite *Pericapritermes nitobei* (Shiraki) (Isoptera: Termitidae) on the northern part of Okinawa Island in the Central Ryukyus, as this species is native to the Yaeyama Islands and other regions (Taiwan, mainland China, and Southeast Asia) (Ikehara, 1966; Yasuda et al., 2000; Krishna et al., 2013).

On February 6th, 2017, we found a termite colony having soldiers with a twisted left mandible and curved right mandible (i.e., asymmetric snapping mandibles) from the soil in the Motobu Peninsula in the northern part of Okinawa Island (Fig 1B). Based on the soldier morphology, termites from that colony were suggested to belong to *Pericapritermes* (Bourguignon et al., 2008). This genus contains only one known species in the Ryukyu Archipelago, *P. nitobei* (Ikehara, 1966; Yasuda et al., 2000). During the second survey conducted on June 3rd and 4th, 2019, we successfully collected six colonies of *Pericapritermes* from the soil and observed egg piles in three of the six colonies (Table 1). These colonies were at least 100 m apart; the distribution was limited in the roadside forest (Fig 1C). The collected termites were fixed in absolute ethanol and brought back to the laboratory.

In order to confirm the identity of the termite species collected in the Motobu Peninsula and to clarify its relationship to other *Pericapritermes* species, we performed a phylogenetic analysis based on the sequences of the mitochondrial cytochrome *c* oxidase subunit II (*COII*) gene. One randomly chosen worker from the six colonies of the termite from the Motobu Peninsula was used for the phylogenetic analysis. Termite DNA was extracted from the head using the DNeasy Prep kit (QIAGEN) according to the manufacturer's protocol and used as a template for polymerase chain reaction (PCR). A fragment of *COII* was amplified by PCR using the primer set, modified A-tLeu (5'-CAGATAAGTGCATTGGATTT-3') (Miura et al., 1998) and B-tLys (5'- GTTTAAGAGACCAGTACTTG -3') (Liu & Beckenbach, 1992). The PCR amplification was performed in 20  $\mu$ L total reaction volumes containing 10  $\mu$ L of PrimeSTAR HS DNA Polymerase (Takara), 0.6  $\mu$ L of each primer (10  $\mu$ M), 1  $\mu$ L of template DNA, and 7.8  $\mu$ L of high-purity water. The PCR cycling conditions consisted of 35 cycles of denaturation (98°C, 10 s), annealing (50°C, 5 s), and extension (72°C, 1 min). Amplified double-stranded DNA was purified using a QIAquick PCR purification kit (Qiagen)

and then sent to an outside sequence service (FASMAC Inc., Kanagawa, Japan). We obtained 684 bp *COII* sequences. Consensus sequences were aligned using the ClustalX algorithm (Thompson et al., 1997) from the BioEdit 7.0.4.1 sequence editor (Hall, 1999) and corrected manually. The *COII* sequences of *P. nitobei* in its native range (including Ishigaki Island, Iriomote Island, Taiwan, and mainland China) (GenBank accession numbers (GB#) AB109517, MH194575, and MW073093–MW073099) and other termitid species (GB# AB109518, DQ442140, DQ442217, KP026278, and KY224616) were also used in the analysis. Bayesian inference was performed using MrBayes 3.1.2 (Ronquist et al., 2003) with the GTR + G model selected by the hierarchical likelihood ratio test (hLRT) in MrModeltest 2.3 (Nylander et al., 2004). *Homalotermes eleanorae* Emerson (GB# DQ442140) was used as an outgroup. The phylogenetic analysis methods have been described in a previous report (Yashiro et al., 2010). Additionally, we analyzed the obtained nuclear internal transcribed spacer 2 (*ITS2*) sequences (264 bp) of the termite from the Motobu Peninsula and the *ITS2* sequences of *P. nitobei* in its native range (GB# MW067661–MW067667). Primer sequences and PCR conditions are detailed in a previous report (Maryańska-Nadachowska et al., 2010), and PCR product purification and sequencing were conducted as described above. The *COII* and *ITS2* sequences obtained in this study were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases (GB# MW073087–MW073092 and MW067655–MW067660).

All of the mitochondrial *COII* sequences of individuals from the six colonies obtained from the Motobu Peninsula of Okinawa Island were identical to each other (GB# MW073087–MW073092), and to one of two haplotypes of *P. nitobei* on Ishigaki and Iriomote of the Yaeyama Islands (GB# MW073093–MW073096, and MW073098) (Fig 2). Therefore, the termite collected from the Motobu Peninsula were phylogenetically identified as *P. nitobei*. Phylogenetic trees based on mitochondrial *COII* sequences showed the monophyly of *P. nitobei*, including individuals from the Motobu Peninsula, the Yaeyama Islands, Taiwan, and mainland China populations (Bayesian posterior probability = 0.93), where individuals from the six colonies from the Motobu Peninsula formed a clade with those from the Yaeyama Islands (Bayesian posterior probability = 0.91) (Fig 2). In addition, all of the nuclear *ITS2* sequences of individuals from 12 colonies of *P. nitobei* in the Ryukyu Archipelago (six in the Motobu Peninsula, one on Ishigaki Island, and five on Iriomote Island) were identical to each other (GB# MW067655–MW067666), but these sequences were different from the *ITS2* sequence of *P. nitobei* in Taiwan (GB# MW067667) (98% sequence identity). Considering the fact that the previous extensive surveys of termites in the Ryukyu Archipelago (including the Motobu Peninsula) did not detect *P. nitobei* on Okinawa Island (Fig 1A, Ikehara 1966; Yasuda et al., 2000), and its discontinuous distribution, approximately 350 km east of the



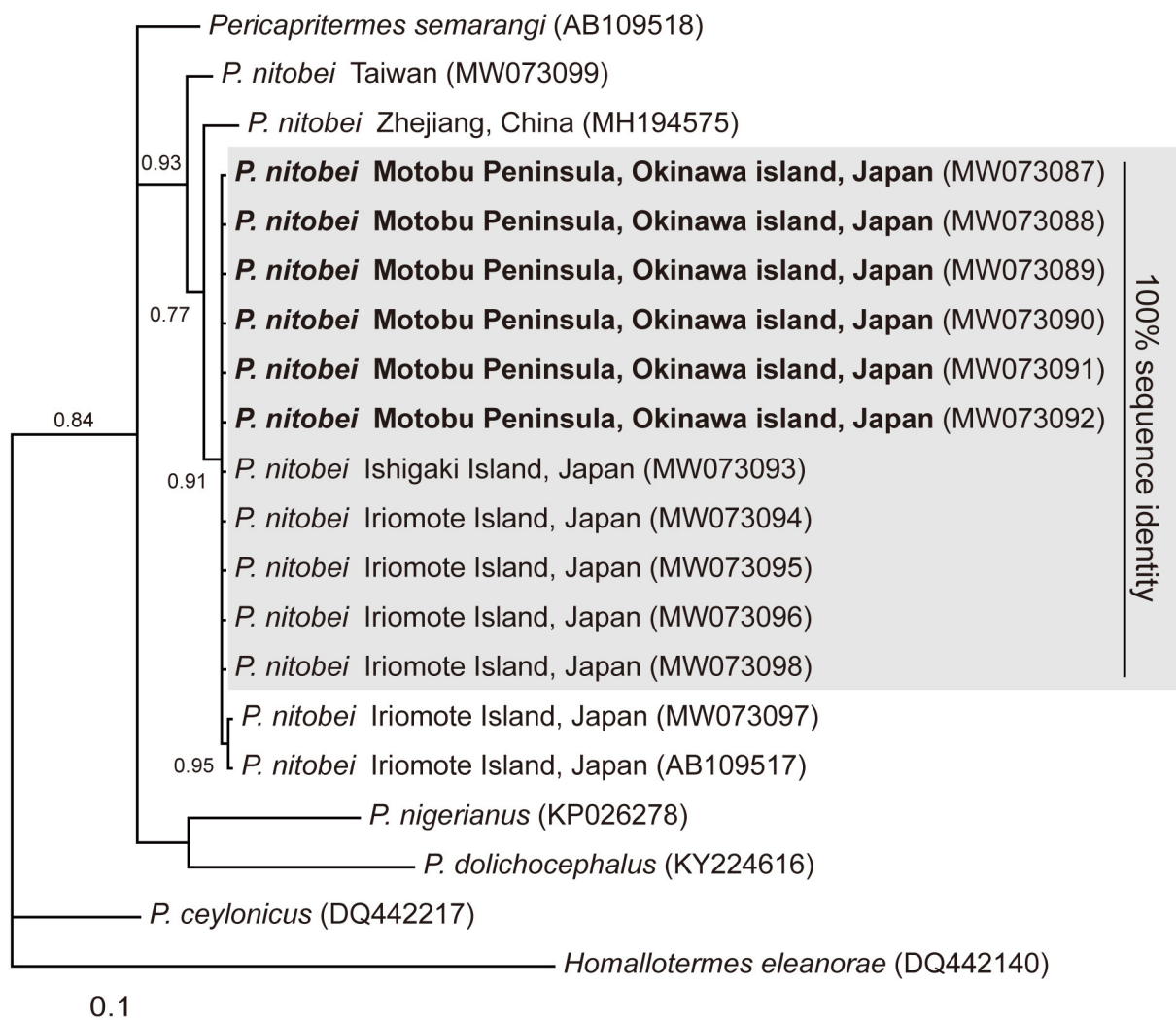
**Fig 1.** The soil feeding termite collected in the Motobu Peninsula of Okinawa Island. (A) The map of the Ryukyu Archipelago. (B) A photo of the termite, which was phylogenetically identified as *Pericapritermes nitobei*. The inset is an enlarged view of soldier head with a twisted left mandible and curved right mandible. (C) The map showing Motobu peninsula and the collection sites in the survey on June 2019. Filled and open circles indicate the presence and absence of the termite, respectively. Maps were drawn by the “mapproject” in the R software (version 3.6.2; <http://cran.r-project.org/>) with the data provided by National Land Numerical Information, Ministry of Land, Infrastructure, Transport and Tourism, Japan (<https://nlftp.mlit.go.jp/index.html>).

previous easternmost record of the species (Ishigaki Island, the Yaeyama Islands) (Fig 1A, Ikehara, 1966; Yasuda et al., 2000; Krishna et al., 2013), our results strongly suggest that *P. nitobei* in the Motobu Peninsula has been recently introduced from the Yaeyama Islands.

We found the soil feeding higher termite *P. nitobei* in the Motobu Peninsula in the Central Ryukyus in 2017 and 2019, and observed egg piles, indicating the establishment of a population. The termite is most likely to have been recently introduced from the Yaeyama Islands in the Southern Ryukyus, as is suspected for the fungus-growing higher termite *O. formosanus* on Okinawa Island (Hojo, 2019). Interestingly, both are soil-nesting species (Chiu et al., 2015; Chiu et al., 2018). Trade in live plants with soil is an important pathway facilitating long-distance invasions of soil-borne insects (Meurisse et al., 2019). So far, several tree and shrub species have been introduced from the Yaeyama

Islands into public parks on Okinawa Island, as well as in the Motobu Peninsula (Ocean Expo. Commemorative Park Management Foundation (1997)); therefore, soil transferred with live plants is one of the possible introduction pathways for these termites.

Our finding raised the possibility that not only wood-feeding and wood-nesting termites, but also soil-feeding termites can be introduced and established outside their native ranges by human activities. Although many aspects of its biology (including the capability of producing secondary reproductives) and its impact on other organisms in the ecosystem remains to be investigated, the population of *P. nitobei* on the Motobu Peninsula of Okinawa Island, together with the population of *O. formosanus* on Okinawa Island, would provide a promising model system for the study of biological invasion by non-wood-feeding and non-wood-nesting termites.



**Fig 2.** Bayesian phylogenetic tree of *Pericapritermes* termites based on mitochondrial *COII* sequences, including the soil feeding termite in the Motobu Peninsula, which was identified as *P. nitobei* (presented in bold). The corresponding posterior probabilities ( $\geq 0.70$ ) are shown by the branches. GenBank accession numbers are shown in parentheses.

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## Authors Contribution

TN: Conceptualization, Validation, Investigation, Resources, Data Curation, Writing – Original Draft, Writing - Review & Editing, Visualization, Supervision, Funding acquisition.

MH: Conceptualization, Investigation, Resources, Writing – Original Draft, Writing - Review & Editing.

TY: Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing.

KM: Conceptualization, Investigation, Writing - Review & Editing, Funding acquisition.

TN and MH were equally contributed.

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