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Morphological and Molecular Identification of Fungus-growing Termites (Isoptera, Termitidae, Macrotermitinae) in Thailand

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Fungus-growing termites (FGTs) play ecologically important roles as both decomposers and producers of termite mushrooms. However, they are difficult to research due to a lack of an updated identification key and the inability to locate type specimens. Molecular identification may be helpful, but this requires database information that is lacking for many species found in Thailand. In addition, some researchers use the cytochrome oxidase subunit I (*COI*) gene as a barcoding gene, but others use cytochrome oxidase subunit II (*COII*). Thus, we offer detailed descriptions of nine FGT species commonly found in Thailand, together with the DNA sequences of both the *COI* and *COII* genes. The descriptions include those of both major and minor soldiers. The DNA sequences of the two genes confirm the morphological identifications. Our data will aid future FGT identification and facilitate research on the biodiversity, conservation, and sustainable use of FGTs and termite mushrooms.

Key words: Diversity, DNA barcoding, Morphology, *COI*, *COII*, *Odontotermes*

BACKGROUND

Termites are eusocial insects that live in colonies; the colony members are polymorphic and include reproductive members (kings, queens, virgin kings, and virgin queens) and sterile members (soldiers and workers) (Kambhampati and Eggleton 2000). Termites are abundant and very diverse in tropical and subtropical regions. A few species of termites are pests, but 99% of all species benefit their ecosystems. Their critical roles remain underestimated in the tropics (Abe 1979; Beaudrot et al. 2011; Ashton et al. 2019). The fungus-growing termites (FGTs) afford ecological benefits to humans by producing termite mushrooms, which serve as an important food source for local communities across Asia and Africa (Paloi et al. 2023). This group of termites cultivates fungus gardens of termite mushrooms and then degrades the fungal plant to yield food. The termites cultivate the fungus on combs of fecal material (Darlington 1994). There are approximately 330 extant species in 12 genera that have been described from the Old-World tropics and subtropics (Kambhampati and Eggleton 2000), and approximately 50 species in Thailand (Sornnuwat et al. 2004).

Research on termites has been difficult, as species identification is challenging. Termite taxonomy is usually based on soldier morphology; the characters of the soldier or pseudergate castes are consistently

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reliable (Kambhampati and Eggleton 2000; Sornnuwat et al. 2004; Bourguignon and Roisin 2011; Scheffrahn 2011), when studying termite diversity. However, identifying species below the genus level is difficult because many species have been described based on slight differences that may reflect intraspecific variation (Kambhampati and Eggleton 2000). Therefore, our limited understanding of the extents of variation within species compromises the reliability of identifications. Thus, in the absence of rigorous evaluation, morphological identifications alone can be ambiguous. In addition, many species of the genera *Odontotermes*, *Ancistrotermes*, and *Microtermes* remain essentially unidentified (Sornnuwat et al. 2004). Since 1965, Ahmad has surveyed and collected termite samples in Thailand. A dichotomous key was used to classify these species (Ahmad 1965); the key has never been revised. Inaccuracies in termite classification are attributable to long-standing confusion in the early literature, not only for Thailand but also for other Southeast Asian countries including Burma, India, and Malaysia (Kirton 2005). In addition, termite bodies are pliable; it is not easy to preserve specimens for the long term (Gibb and Oseto 2020). Thus, it is difficult to locate type specimens, which imposes limitations on species identification as well as termite taxonomy thus remains problematic, molecular markers and other species delimitation procedures are required to solve the current issues (Korb et al. 2019). Therefore, termitologists required taxonomy data by combined technique with morphology and molecular DNA analysis when seeking to identify termites into species (Kambhampati and Eggleton 2000; Casalla et al. 2016 2021; Castro et al. 2020).

Molecular identification has been used to assist standard taxonomic identifications when morphological characters alone are inadequate or uncertain, and when identifications are possible for only particular sexes, castes, or developmental stages. This is termed "DNA barcoding"; DNA sequences from defined regions are compared to known sequences of these regions lodged in DNA databases (*e.g.*, GenBank, EMBL, Ensembl, DDBJ, and others) (Hebert et al. 2003; Ratnasingham and Hebert 2007). The mitochondrial cytochrome oxidase subunit I (*COI* or *COX1*) gene has been widely used as a standard barcoding region that can be used to identify most animal species; the interspecific variation is high but the intraspecific variation low. However, it is not always optimal in terms of species identification. Thus, for some taxonomic groups, the cytochrome *b* or ribosomal RNA genes such as the 12S, 16S, 18S, or 28S genes have been used instead (Linacre 2012). For termites, *COII* analyses are optimal (Ohkuma et al. 2004; Inward et al. 2007; Legendre et al. 2008; Hausberger et al. 2011; Makonde et al. 2013) but many studies nonetheless used *COI* data (Aanen et al. 2002; Bourguignon et al. 2013). Molecular identification powerfully identifies species only if sequences from correctly identified species are deposited in databases. Many termitologists have extensively studied termite sequences but most have focused on higher-level taxa. Although Bourguignon et al. (2015 2017) sequenced several FGT mitochondrial sequences, most termite samples were from Africa. Thus, the sequences of Asian termites remain understudied. To assist species identification of Asian FGTs, particularly species that are common in Thailand, this study offers detailed descriptions of five genera and nine species of FGT together with the DNA sequences of both the *COI* and *COII* genes. We seek to assist researchers in terms of future molecular identification.

MATERIALS AND METHODS

Sample collection

Thirty-five FGT samples were collected during the rainy seasons of October 2018 and July–October 2023 in seven provinces of Thailand, including Chiang Mai, Chiang Rai, Suphan Buri, Phetchaburi, Loie, Khon Kaen, and Nakhon Ratchasima (Table S1). At each site, FGTs were collected from fallen logs and above- and belowground mounds in forests, crops, and residential areas. At least 30 soldiers and workers from each active termite assemblage in a mound or nest were gathered and placed in vials with 95% ethanol alcohol prior to morphometric analysis and DNA extraction. All samples were placed in a cool box and taken to our research laboratory, where they were kept in a refrigerator at 4°C before morphological and molecular classification.

Morphological examination

Collected specimens were examined using a stereo microscope (Carl Zeiss™, SteREO Discovery.V8 Microscopes) in the Laboratory of Entomology, Faculty of Agriculture, Kasetsart University. Identifications of termite external morphology were done on the soldier cases of each species. The terminology used for the external morphology follows (Sornnuwat et al. 2004, Krishna et al. 2013a). Synonymy or type data and distribution data of each species were checked from Ahmad (1965), Krishna et al. (2013b) and available reference data sources. Termite species-level were described by compare with specimen in insect museum at Faculty of Agriculture, Kasetsart University. The following morphometrics characters in measurements were in form of the length of the mandible, tooth length

to the apical tip, head length, lengths of the head and mandible, minimum head width, and pronotum length. All termite species were sorted and labeled by habitat. Voucher specimens were deposited in the Forest Entomology and Microbiology Section, Department of National Parks, Wildlife, and Plant Conservation, under the herbarium specimen numbers 15,381–15,415.

Molecular identification

For each sample, DNA was extracted from the heads of a pool of 1–5 worker using the Favorgen Tissue Genomic DNA Extraction Mini Kit (Taiwan). *COI* was amplified using the primers COIF_mtd6 (5'-GGAGGA TTTGGAAATTGATTAGTTCC-3') and COIR_calvin (5'-GGRAARAAWGTTAARTTWACTCC-3') (Simon et al. 1994). *COII* was amplified using the primers TerCOII_F (5'-ATGGCAGATTAGTGCAATGG-3') and TerCOII_R (5'-GTTTAAGAGACCAGTACTTG-3') (Lo et al. 2004). PCR was conducted in a total volume of 50 µL containing 1.25 U Taq polymerase (Apsalagen, Biotechrabbit, Hennigsdorf, Germany), PCR buffer, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM each primer, and ddH_2O. It was run on an Applied Biosystem Thermocycler with 3 min of pre-denaturation at 94°C; 40 cycles of denaturation at 94°C for 30 s, annealing at 45°C (both loci) for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. PCR products (5 µL volumes) were visualized after 1% agarose gel electrophoresis in TAE buffer. The amplified fragments were purified using a FavorPrep GEL/PCR Purification Mini Kit (Taiwan) before sequencing by Macrogen (Korea).

Sequences were edited using '4 peaks' (https://nucleobytes.com/) and 'BLASTed' (Camacho et al. 2009) software to delineate species based on the identity (> 97%) of the best-hit sequences from the GenBank database (http://blast.ncbi.nlm.nih.Gov/). Multiple sequence alignment (MAFFT) (Nakamura et al. 2018) was performed using AliView (Larsson 2014), and phylogenetic trees were constructed by employing the evolutionary model TN93+I for *COI* and TN93+G for *COII* (Tamura and Nei 1993) and the Maximum Likelihood phylogenetic trees were then constructed by MEGAX (Kumar et al. 2018) using 1000 iterations of bootstrap support. All *COI* and *COII* sequence data were deposited in GenBank under accession numbers PP313295–PP313329 and PP313120–PP313153 (Table S2), respectively.

RESULTS

Morphological identification and distribution of FGT species

Nine FGT species were found in this study (Fig. 1A–L, and external morphology, Type data and distribution of each species as follow:

Ancistrotermes pakistanicus (Ahmad, 1955)

Synonymy: *Microtermes pakistanicus* Ahmad, 1955a: and next available name for *Termes pallidus* Haviland, 1898, which is preoccupied by *Termes pallidus* Rambur, 1842, and *T. pallidus* Walker, 1853.

Material examined: The specimens of *A. pakistanicus* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi province and the deciduous dipterocarp forest at Nam Phong National Park in Khon Kaen province. Thirty soldier termites were used for describing.

Soldier: thorax, legs, and abdomen have light yellow in color (Fig. 1A). which serve as an important food source for local communities across Asia and Africa Head; yellow or brownish yellow, rectangularly oval to almost round with very sparsely hairy, length of head to side base of mandibles approximately 0.74 mm to 1.17 mm, width of head at side base of mandibles with 0.57 mm to 1.06 mm. Antennae; 15–16 segments, the second segment 1.5-fold longer than the third, antennae pale, in some specimens brownish yellow or light yellow. Mandibles; sickle-shaped with curved at tips and light to dark reddish brown in color, length of left mandible 0.51 mm to 0.67 mm, left mandible with a minute denticle above or in the middle region and right mandible with a similar denticle placed a little more posteriorly. Labrum; yellow to brownish yellow with long hairs on the anterior half, labrum convex-like (dome-shaped), lateral margins weakly convex; anterior margin broadly rounded, anterior margin of the post-clypeus convex. Anteclypeus; whitish, membranous, narrow, trapezoid, much wider than long. Pronotum; notched anteriorly in the middle, slightly emarginated posteriorly, a tinyhairs along the anterior margin and scattered hairs on the lateral margins and dorsal area, posterior margin not demarcated, pronotum length 0.35 mm to 0.53 mm, pronotum width in 0.52 mm to 0.89 mm. Postmentum; strongly curved, a few scattered hairs along the anterior half, indistinctly longer than broad, the fourth segment as long as or slightly shorter than the third.

Distribution: Malaysia, Indonesia, Singapore, India, Pakistan, Bangladesh, Myanmar, Vietnam, Thailand.

Fig. 1. Fungus-growing Termite species: (A) *Ancistrotermes pakistanicus*, (B) *Hypotermes makhamensis*, (C) *Microtermes obesi*, (D) *Macrotermes annandalei* (Major soldier), (E) *Macrotermes annandalei* (Minor soldier), (F) *Macrotermes carbonarius* (Major soldier), (G) *Macrotermes carbonarius* (Minor soldier), (H) *Macrotermes gilvus* (Minor soldier), (I) *Macrotermes gilvus* (Major soldier), (J) *Odontotermes feae*, (K) *Odontotermes takensis*, and (K) *Odontotermes longignathus*.

Hypotermes makhamensis Ahmad, 1965

Hypotermes was first introduced as a subgenus of *Odontotermes* Holmgren, 1910, and next available genus name for *Hypotermes* Holmgren, 1913.

Material examined: The specimens of *H. makhamensis* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi province and the deciduous dipterocarp forest at Nam Phong National Park in Khon Kaen province. Thirty soldier termites were used for describing.

Soldier: thorax, legs, and abdomen lighter than the head (Fig. 1B). Head; oval shape with lateral margins convex, broadest in the middle, dark brown slightly lighter anteriorly or yellowish brown with color varied by habitat, very scantily hairy, length of head to side base of mandibles approximately 1.09 mm to 1.51 mm, width of head at side base of mandibles with 0.56 mm to 0.80 mm. Antennae; 16 segments, the second segment half as long as the third and twice as long as the fourth and the last segment shortest. Mandibles; half as long as the head capsule, left mandible with three small crenulations at the base, right mandible more curved than the left mandible, finely serrated near the molar plate, length of left mandible with 0.53 mm to 0.71 mm. Labrum; brownish yellow with long hairs, parallel lateral margins and broadly rounded anteriorly. Pronotum sparsely hairy, pronotum length 0.39 mm to 0.54 mm, pronotum width 0.65 mm to 0.88 mm. Postmentum has two short hairs on the anterior margin, very broad, lateral sides convex, broadest in the middle.

Distribution: Cambodia, Vietnam, Thailand.

Microtermes obesi Holmgren, 1912

Type data: *Syntypes*: *Microtermes obesi* Holmgren, 1912: locality at Maharashtra, India.

Material examined: The specimens of *M. obesi* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi province and the deciduous dipterocarp forest at Phu Toei National Park in Suphan Buri province. Forty soldier termites were used for describing.

Soldier: thorax, legs, and abdomen light yellow (Fig. 1C). Head; densely brownish with short hairs, head pale or light yellow, head capsule elongated oval, longer than broad, and widest slightly below the middle, length of head to side base of mandibles with 0.84 mm to 0.98 mm, width of head at side base of mandibles with 0.51 mm to 0.62 mm. Antennae; light yellowish brown with 13 segments; some specimens with 14 segments; the second segment longer than the third, fourth, and third (which were subequal). Mandibles slender, slightly curved apically, without denticles, mandible length with 0.46 mm to 0.57 mm. Labrum; light yellow, sparsely hairy, blade shaped but not pointed, longer than broad, and widest below the middle and lateral margins, basal third convex, converging anteriorly, Pronotum; saddle-shaped and extending to more than half the width, the anterior margin distinctly notched in the middle, the posterior margin weakly depressed in the middle, the lateral lobes prominent and broadly rounded, pronotum length with 0.28 mm to 0.36 mm, pronotum width with 0.49 mm to 0.59 mm. Postmentum; arched with scattered short hairs, slightly longer than wide, widest in the middle.

Distribution: India, Pakistan, Bangladesh, Bhutan, Sri Lanka, Vietnam, Myanmar Cambodia, Thailand.

Macrotermes annandalei (Silvestri, 1914)

Synonymy: *Termes annandalei* Silvestri, 1914.

Material examined: The specimens of *M. annandalei* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi province and the deciduous dipterocarp forest at Phu Toei National Park in Suphan Buri province and at Khun Chae National Park in Chiang Rai province. Sixty soldier termites (*i.e.*, major soldier 30 samples and minor soldier 30 samples) were used for describing.

Major soldier case: horax, legs, and abdomen brown (Fig. 1D). Head: reddish brown, margins slightly darker, a few scattered hairs, head capsule sub rectangular, slightly narrowed anteriorly, slightly less than 1.5-fold long as broad at the widest point; labrum brown; hyaline tip triangular, not elongated or sharp at the tip, length of head to side base of mandibles with 4.78 mm to 5.05 mm, width of head at side base of mandibles with 2.48 mm to 2.61 mm. Antennae; 17 segments, the third segment slightly less than twice as long as the second, the fourth segments shorter than the third and slightly longer than the second. Mandibles; dark brown, stoutly built, thick, tips curved, left mandible with a few distinct crenulations at the base, right mandible without crenulations, length of left mandible with 2.44 mm to 2.60 mm. Labrum brown; mandibles dark brown, bases slightly lighter, long hairs along the lateral sides. Pronotum; brownish, convex both anteriorly and posteriorly and concave in the middles of both margins, lateral lobes narrowly rounded; lateral margins of the mesonotum and metanotum somewhat angular, sparsely hairy, pronotum length with 1.35 mm to 1.98 mm; pronotum width with 2.65 mm to 2.86 mm. Postmentum; elongated or near-parallel-sided in the middle with two hairs near the tip, lateral margins near the posterior end slightly convex.

Minor soldier case: thorax, legs, and abdomen

brown (Fig. 1E). Head; reddish brown, scantily hairy, head capsule subrectangular, 1.5-fold as long as broad, broadest in the middle, length of head to side base of mandibles with 2.96 mm to 3.12 mm, width of head at side base of mandibles with 1.40 mm to 1.50 mm. Antennae with 17 segments; the third segment about 1.5-fold as long as the second and slightly longer than the fourth and fifth segment, and fourth which were subequal. Mandibles; reddish brown or dark brown, stoutly built, relatively slender, tips slightly curved, left and right mandibles without crenulations, length of left mandible with 1.82 mm to 1.92 mm. Labrum; more extended than broad, the lateral margins weakly convex, the tip triangular, the hyaline tip triangular, not elongated or sharpened at the tip, a few long hairs along the lateral sides. Pronotum; brownish, sparsely hairy, mesonotum and metanotum slightly lighter than the pronotum, indistinctly notched anteriorly in the middle over half the width, the posterior margin emarginate, the lateral lobes narrowly rounded, pronotum length with 0.98 mm to 1.06 mm, pronotum width with 1.71 mm to 1.87 mm. Postmentum; arched with a pair of hairs along each anterolateral margin, lateral margins almost parallel.

Distribution: China, Malaysia, Myanmar, Vietnam, Thailand.

Macrotermes carbonarius (Hagen, 1858)

Synonymy: *Termes* (*Termes*) *carbonarius* Hagen, 1858.

Material examined: The specimens of *M. carbonarius* were collected from the dry evergreen forest and deciduous dipterocarp forest at Sakaerat Environmental Research Station in Nakhon Ratchasima province. Sixty soldier termites (*i.e.*, major soldier 30 samples and minor soldier 30 samples) were used for describing.

Major soldier case: thorax and legs have brown or dark brown, abdomen sternites light brown, vertex reddish brown (Fig. 1E). Head; black in color, subrectangular, sides moderately convex, scantily hairy, post-clypeus brownish with a few short hairs, length of head to side base of mandibles with 4.77 mm to 5.00, width of head at side base of mandibles with 2.35 mm to 2.48 mm. Mandibles; stout, thick, and sward-shaped with dark brown, bases reddish brown, anteclypeus trapezoid, much broader than long, the inner margin of the left mandible crenulate, the right mandible without crenulations, length of left mandible with 2.74 mm to 2.86 mm. Antennae long and slender, with 17 segments; the third slightly more than twice as long as the second; the fourth segment shorter than the third and longer than the fifth. Labrum; dome-shaped or longer than broad, and broadest in the anterior half, brownish with few hairs along the lateral sides and at the base of hyaline tip, the hyaline tip triangular, not elongated or sharpened at the tip. Pronotum; crescent-shaped, twice as broad as long, anterior margin convex in the middle and slightly emarginate with a few tiny hairs on the margin, posterior margin slightly concave in the middle, lateral lobes rather broadly rounded, pronotum length with 1.40 mm to 1.56 mm, pronotum width with 2.80 mm to 3.01 mm. Mesonotum; slightly narrower than the pronotum, lateral sides angularly rounded, the metanotum nearly as wide as the mesonotum, the posterior margin almost straight. Postmentum; elongated and rectangular, the sides nearly parallel and slightly convex near the posterior margin.

Minor soldier case: thorax and legs have brown or dark brown (Fig. 1G). Head; dark brown, subrectangular or somewhat rectangular, broadest in the middle, length of head to side base of mandibles with 3.24 mm to 3.43 mm, width of head at side base of mandibles with 1.61 mm to 1.76 mm. Antennae; 17 segments, the third segment twice as long as the second, the fourth segment shorter than the third. Mandibles; long and sword-shaped, left mandible with crenulations at the base but the right mandible without crenulations, length of left mandible with 1.87 mm to 2.08 mm. Labrum; dome-shaped with long hairs along the lateral sides, broadest in the anterior half, the tip subtriangular, the hyaline tip triangular, not elongated or sharpened at the tip, a few hairs near the post-clypeus. Pronotum; twice as broad as long, a few short hairs along the anterior margin, the lateral angles distinct, the anterior margin convex and emarginate, the posterior margin moderately emarginate, pronotum length with 1.09 mm to 1.14 mm, pronotum width with 2.02 mm to 2.10 mm. Postmentum; rectangular, the sides almost parallel in the middle and slightly convex near the posterior margin. Mesonotum; narrower than the pronotum, the lateral lobes broadly rounded. Metanotum; slightly wider than the mesonotum.

Distribution: Cambodia, Indonesia, Malaysia, Mainland, Indonesia, Singapore. Vietnam, Thailand (Nakhon Ratchasima).

Macrotermes gilvus (Hagen, 1858)

Synonymy: *Termes* (*Termes*) *gilvus* Hagen, 1858, and next available name for *Termes azarelii* Wasmann, 1896 in Myanmar, which is preoccupied by *Termes malayanus* Haviland, 1898 in Singapore and Malaysia; *Termes* (*Macrotermes*) *luzonensis* Oshima, 1914 in Philippines; *Termes* (*Macrotermes*) *manilanus* Oshima, 1914 in Philippines; *Termes* (*Macrotermes*) *philippinae* Oshima, 1914 in Philippines; *Termes* (*Termes*)

copelandi Oshia, 1914 in Philippines; *Macrotermes gilvus padangensis* Kemner, 1930 in Indonesia; *Macrotermes gilvus borneensis* Kemner, 1933 in Indonesia; *Macrotermes gilvus angusticeps* Kemner, 1934 in Indonesia; *Macrotermes gilvus kalshoveni* Kemner, 1934 in Indonesia; *Macrotermes gilvus latinotum* Kemner, 1934 in Indonesia; *Macrotermes gilvus madurensis* Kemner, 1934 in Indonesia.

Material examined: The specimens of *M. gilvus* were collected from the deciduous dipterocarp forest at Nam Phong National Park in Khon Kaen province and at Phu Toei National Park in Suphan Buri province. Sixty soldier termites (*i.e.*, major soldier 30 samples and minor soldier 30 samples) were used for describing.

Major soldier case: thorax and legs have reddish brown in color, abdomen sternites light brown, vertex reddish brown (Fig. 1H). Head; light reddish brown or brown, subrectangular head capsule, lateral margins slightly convex, maximally wide in the middle, a few hairs in the anterior region, length of head to side base of mandibles with 3.23 mm to 4.01 mm; width of head at side base of mandibles with 1.44 mm to 2.09 mm. Antennae; 17 segments, the third segment 1.5-fold as long as the second, the fourth shorter than the third. Mandibles; stout, thick at bases, tips not curved, dark reddish brown, left mandible with a few crenulations at the base, right mandible without crenulations, length of left mandible with 1.42 mm to 2.02 mm. Labrum; reddish brown, slightly longer than broad, the hyaline tip triangular, not elongated and not sharpened at the tip, a row of hairs along the lateral sides and a few hairs at the base of the hyaline tip. The pronotum distinctly emarginated anteriorly and posteriorly; the lateral sides broadly rounded, a few tiny hairs along the anterior margin; dorsal area without hairs, pronotum length with 0.93 mm to 1.29 mm, pronotum width with 1.82 mm to 2.81 mm. Postmentum long, broadest in the apical fourth region; narrowest in the middle; lateral margins concave in the middle; convex in the basal fourth region. Mesonotum; broad and narrowly rounded lateral sides, the posterior margin broadly emarginate and concave. Metanotum; like the mesonotum, posterior margin broadly concave.

Minor soldier case: thorax and legs have brown or reddish brown in color, abdomen sternites light brown, vertex reddish brown (Fig. 1I). Head; reddish brown, scantily hairy, one pair of hairs in the posterior half, subrectangular head capsule, length of head to side base of mandibles with 1.82 mm to 2.28 mm, width of head at side base of mandibles with 0.88 mm to 1.35 mm. Antennae; 17 segments; the second segment shorter than the third; the fourth half as long as the third. Mandibles; long, rather slender, and slightly curved apically, dark reddish brown, bases lighter, the left mandible with a few crenulations at the base, and the right mandible without crenulations, length of left mandible with 1.14 mm to 1.50 mm. Labrum; more extended than broad; the sides moderately convex, and broadest slightly below the middle, the hyaline tip dome-shaped, not elongated, and sharp at the tip, a row of long hairs on each lateral side and a few hairs at the base of the hyaline tip. Pronotum; the lateral sides broadly rounded, a few tiny hairs along the anterior margin; dorsal area without hairs, pronotum length with 0.59 mm to 0.88 mm, pronotum width with 0.4 mm to 1.61 mm. Postmentum long, broadest in the apical fourth region; narrowest in the middle; lateral margins concave in the middle; convex in the basal fourth region. Mesonotum; broad and narrowly rounded lateral sides, the posterior margin broadly emarginate and concave. Metanotum; like the mesonotum, posterior margin broadly concave.

Distribution: Cambodia, East-West Timor, India, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Vietnam, Thailand.

Odontotermes feae (Wasmann, 1896)

Synonymy: *Termes feae* Wasmann, 1896, and next available name for *Odontotermes indicus* Takur, 1981 (imago, soldier, worker) in India.

Material examined: The specimens of *O. feae* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi, and the deciduous dipterocarp forest at Nam Phong National Park in Khon Kaen province and at Doi Inthanon National Park in Chiang Mai province. Thirty soldier termites were used for describing.

Soldier case: thorax and legs have reddish brown in color, abdomen sternites light brown, vertex reddish brown (Fig. 1J). Head; reddish brown, subrectangular, sides slightly convex gradually narrowing anteriorly, maximally wide below the middle, a few scattered hairs, length of head to side base of mandibles with 2.14 mm to 2.32 mm, width of head at side base of mandibles with 1.05 mm to 1.18 mm. Antennae; 17 segments, brownish, the second segment almost twice as long as the third and slightly longer than the fourth; the third segment the shortest. Mandibles; long and stout, dark brown and bases reddish brown, the left mandible with a large, sharply pointed, anteriorly directed tooth almost in the middle, the right mandible with or without a minute denticle in the middle, length of left mandible with 1.17 mm to 1.24 mm. Labrum; reddish brown, long hairs along the lateral sides and at the tip, The labrum tip narrowly rounded, broadest in the middle; lateral margins moderately convex. Pronotum; brownish yellow, twice as broad as long, weakly emarginate anteriorly and distinctly emarginate posteriorly, the

lateral lobes broadly rounded, sparsely hairy, pronotum length with 0.67 mm to 0.72 mm, pronotum width with 1.24 mm to 1.32 mm. Postmentum; two hairs near the anterior end. Postmentum; moderately curved, twice as broad as long in the basal half, deflated near the anterior margin.

Distribution: Bangladesh, Bhutan, India, Myanmar, Nepal, Sri Lanka, Vietnam, Thailand.

Odontotermes takensis Ahmad, 1965

Type data: Holotype; locality at Tak, Thailand.

Material examined: The specimens of *O. takensis* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi, and the deciduous dipterocarp forest at Khun Chae National Park in Chiang Rai province, at Phu Ruea National Park in Loei province, and at Phu Toei National Park in Suphan Buri province. Thirty soldier termites were used for describing.

Soldier case: thorax and legs have reddish brown in color, abdomen sternites light brown, vertex reddish brown (Fig. 1K). Head; reddish brown, sparsely hairy, subrectangular head capsule, slightly longer than broad; the lateral margin weakly convex, length of head at side base of mandibles with 1.47 mm to 1.58 mm, width of head at side base of mandibles with 0.80 mm to 0.85 mm. Antennae; 16 segments, the second segment as long as the third and fourth combined, the fourth the shortest. Mandible; dark brown, short and stout, left mandible with a small, laterally directed tooth a little anterior to middle, the right mandible with two small denticles, one nearly in the middle and the other at the base, length of left mandible with 0.75 mm to 0.80 mm. Labrum; dome-shaped, longer than broad, sides convex, maximally wide in the middle, reddish brown and lighter at bases, long hairs along the lateral sides and at the tip. Pronotum; light brown, saddles-shaped, wider than long with anterior margins lightly notched, the posterior margin emarginate, the lateral lobes broadly rounded, pronotum length with 0.49 mm to 0.52 mm, pronotum width with 0.88 mm to 0.91 mm. Postmentum; moderately curved, about 1.5-fold as long as broad, broadest in the middle; lateral margins convex, a few hairs anteriorly; pronotum with scattered short hairs and medium hairs.

Distribution: Thailand.

Odontotermes longignathus Holmgren, 1914

Synonymy: *Odontotermes* (*Odontotermes*) *longignathus* Holmgren, 1914.

Material examined: The specimens of *O. longignathus* were collected from the dry evergreen forest at Kaeng Krachan National Park in Phetchaburi, and the deciduous dipterocarp forest at Doi Inthanon National Park in Chiang Mai province, and at Phu Toei National Park in Suphan Buri province. Thirty soldier termites were used for describing.

Soldier case: thorax and legs have reddish brown or brownish yellow, abdomen sternites light brown, vertex reddish brown (Fig. 1L). Head; subrectangular, scantily hairy, length of head to side base of mandibles with 2.70 mm 2.96 mm, width of head at side base of mandibles with 1.45 mm to 1.61 mm. Antennae; 17 segments, the second 1.5-fold as long as the third, the fourth slightly longer than the third and fifth. Mandibles; dark brown, long, thick, tips curved, left mandible broad-based with a slight tooth posteriorly directed in the middle, right mandible without any denticle; in some specimens, an indistinct denticle present, length of left mandible with 1.40 mm to 1.56 mm. Labrum; slightly longer than broad, the tip narrowly rounded, long hairs arranged in a row along the lateral sides. Pronotum; light yellow, the middle of the anterior region slightly indented, the posterior margin weakly concaved in the middle, curved upward, the lateral margins broadly rounded, length of pronotum with 0.83 mm to 0.88 mm, width of pronotum with 1.61 mm to 1.82 mm. Postmentum; elongated and slightly curved; the lateral margins shallowly concave, two hairs near the anterior region.

Distribution: Malaysia, Thailand.

Molecular identification of FGTs

The BLAST results for the *COI* and *COII* sequences of all samples showed %identity over than 97% and the e-value of 0. Most samples generally agreed with the morphological data except for samples identified as *O. feae* and *O. takensis* (Tables 1 and 2). Samples that were identified as *O. feae* (SH202317, SH202323 and IV202339) were best matched with *O. javanicus* in both *COI* and *COII* sequences with 97–98% identity. Samples that were identified as *O. takensis* (SH202305, SH202318, SH202329, IV202331, IV202342 and IV202343) were best matched with *O. hinanensis* and *O. takensis* in *COI* with equally comparable % identity (97–99.73%) while the *COII* sequences of these samples were best matched with *O. hinanensis* and *O. javanicus* with 98–99.56% identity.

We exclude three sequences (SH202314, SH202330 and IV202339) in the phylogenetic tree of the *COI* (Fig. 2) because these sequences were shorter than the other samples and caused alignment error. The phylogenetic trees for both *COI* (Fig. 2) and *COII* (Fig. 3) were generally congruent. Samples of the same species clearly clustered together. However, in

the *O. takensis* group, our samples clustered with *O. takensis* and *O. hinanensis* in terms of *COI* and with *O. hinanensis* and *O. formosanus* in terms of *COII*. This is consistent with the BLAST results; the sequences of *O. takensis* (*COII*) and *O. feae* (both *COI* and *COII*) were not available in the database.

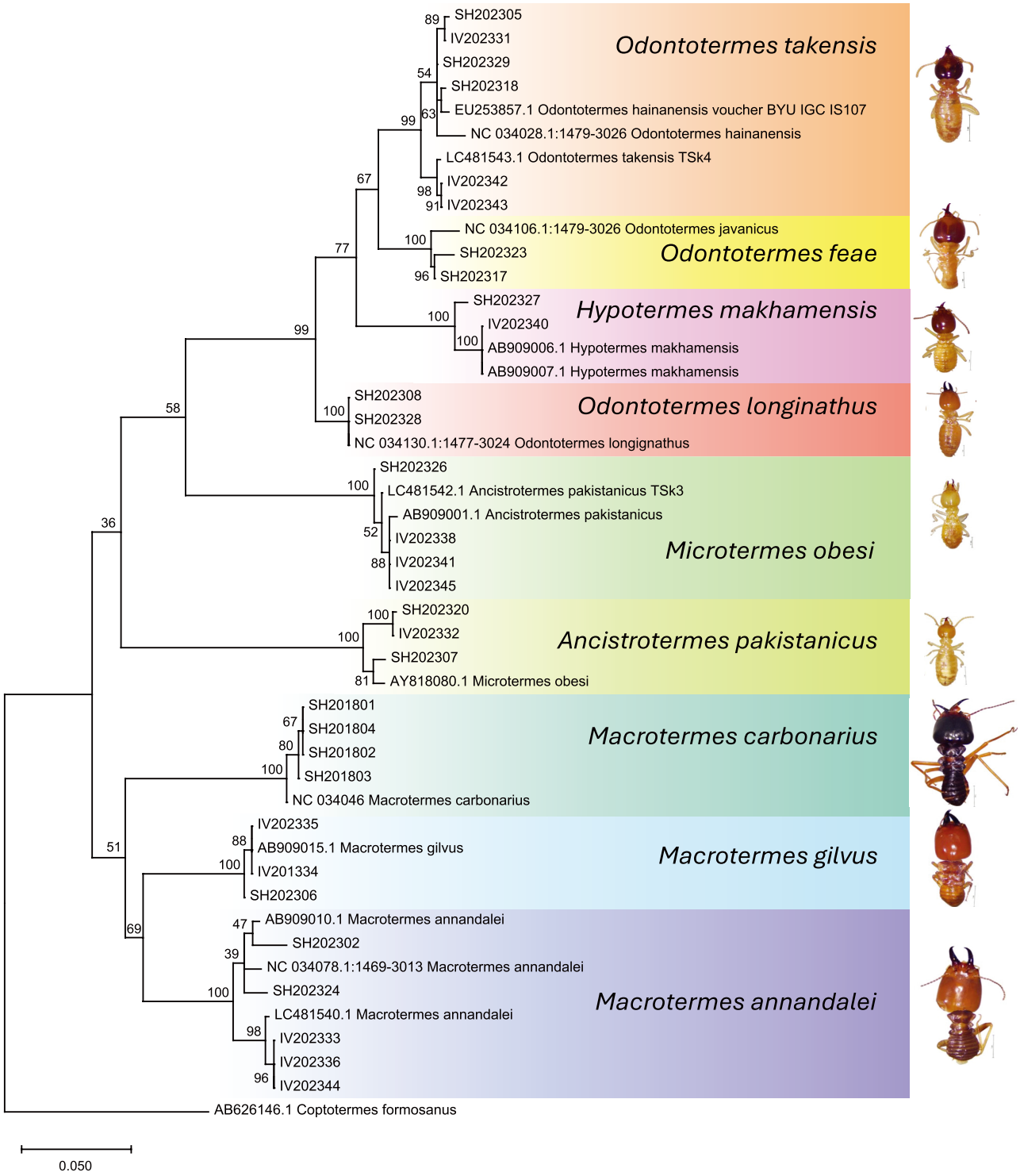

Fig. 2. Maximum Likelihood phylogenetic tree constructed by *COI* sequences.

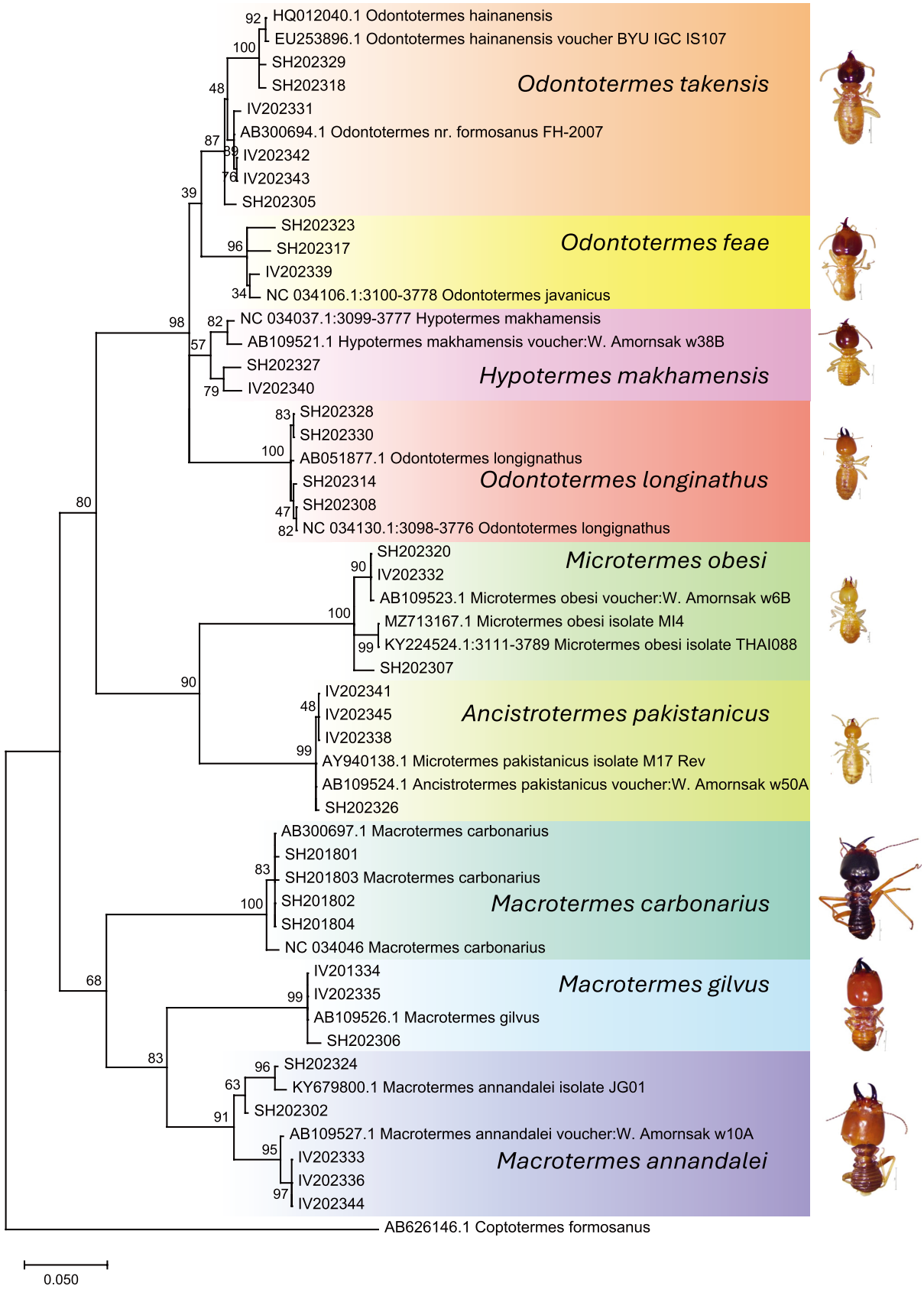

Fig. 3. Maximum Likelihood phylogenetic tree constructed by *COII* sequences.

DISCUSSION

In most samples, morphological and molecular identification agreed. However, for samples identified as *O. feae*, molecular identification showed that these samples would be *O. javanicus*. This may be due to the fact that *COI* and *COII* sequences of *O. feae* are lacking in the database. It is possible that *O. javanicus* is closely related to *O. feae* and thus, the most similar species, *O. javanicus*, became the best hit in this result. We could not compare the morphologies of *O. feae* and *O. javanicus* because no description of *O. javanicus* was available. Our samples clearly varied in terms of head capsule size, which is a key identification feature of *O. feae* (Ahmad 1965). Therefore, this study is the first study submitted the sequences of *O. feae* together with the detailed description provided. Similarly, there were no *COII* sequences of *O. takensis* in the database; our best hits for the *COII* gene rather showed that the most similar sequences were those of either *O. hinanensis* or *O. formosanus*. Our study provided *COII* sequence of *O. takensis* that has not been previously deposited in the database. The best *COI* gene hits were those of *O. takensis* and *O. hinanensis*; the percentage identities

Table 1. BLAST result of *COI* sequences

Sample ID	Identified species	Best hit species	length (bp)	e-value	% Identity	Best hit acession	Seq. Accession
SH202302	*Macrotermes annandalei*	*Macrotermes annandalei*	907	0.00	97.89%	LC481540.1	PP313295
SH202305	*Odontotermes takensis*	*Odontotermes hainanensis*,	990	0.00	98.46%	EU253857.1	PP313297
		Odontotermes takensis			97.86%	LC481543.1	
SH202306	*Macrotermes gilvus*	*Macrotermes gilvus*	938	0.00	99.73%	AB909015.1	PP313298
SH202307	*Microtermes obesi*	*Microtermes obesi*	910	0.00	98.88%	AY818080.1	PP313299
SH202308	*Odontotermes longignathus*	*Odontotermes longignathus*	978	0.00	100.00%	NC_034130.1	PP313300
SH202314	*Odontotermes longignathus*	*Odontotermes longignathus*	657	0.00	99.67%	GU254073.1	PP313301
SH202317	*Odontotermes feae*	*Odontotermes javanicus*	919	0.00	98.19%	NC_034106	PP313302
SH202318	*Odontotermes takensis*	*Odontotermes hainanensis*,	899	0.00	98.99%	EU253857.1	PP313303
		Odontotermes takensis			98.11%	LC481543.1	
SH202320	*Microtermes obesi*	*Microtermes obesi*	947	0.00	97.60%	AY818080.1	PP313304
SH202323	*Odontotermes feae*	*Odontotermes javanicus*	991	0.00	97.74%	NC_034106.1	PP313305
SH202324	*Macrotermes annandalei*	*Macrotermes annandalei*	910	0.00	99.12%	NC_034078.1	PP313306
SH202326	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	990	0.00	99.60%	LC481542.1	PP313307
SH202327	*Hypotermes makhamensis*	*Hypotermes makhamensis*	898	0.00	98.57%	AB909006.1	PP313308
SH202328	*Odontotermes longignathus*	*Odontotermes longignathus*	990	0.00	99.80%	NC_034130.1	PP313309
SH202329	*Odontotermes takensis*	*Odontotermes hainanensis*,	823	0.00	98.89%	EU253857.1	PP313310
		Odontotermes takensis			98.53%	LC481543.1	
SH202330	*Odontotermes longignathus*	*Odontotermes longignathus*	657	0.00	99.84%	GU254073.1	PP313311
IV202331	*Odontotermes takensis*	*Odontotermes hainanensis*,	990	0.00	98.57%	EU253857.1	PP313312
		Odontotermes takensis			97.99%	LC481543.1	
IV202332	*Microtermes obesi*	*Microtermes obesi*	948	0.00	97.73%	AY818080.1	PP313313
IV202333	*Macrotermes annandalei*	*Macrotermes annandalei*	828	0.00	99.51%	LC481540.1	PP313314
IV201334	*Macrotermes gilvus*	*Macrotermes gilvus*	941	0.00	99.87%	AB909015.1	PP313315
IV202335	*Macrotermes gilvus*	*Macrotermes gilvus*	938	0.00	100.00%	AB909015.1	PP313316
IV202336	*Macrotermes annandalei*	*Macrotermes annandalei*	941	0.00	99.58%	LC481540.1	PP313317
IV202338	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	990	0.00	99.51%	AB909001.1	PP313318
IV202339	*Odontotermes feae*	*Odontotermes javanicus*	657	0.00	97.28%	NC_034106.1	PP313319
IV202340	*Hypotermes makhamensis*	*Hypotermes makhamensis*	823	0.00	100.00%	AB909007.1	PP313320
IV202341	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	990	0.00	99.46%	LC481542.1	PP313321
IV202342	*Odontotermes takensis*	*Odontotermes takensis*,	944	0.00	99.73%	LC481543.1	PP313322
		Odontotermes hainanensis			97.75%	EU253857.1	
IV202343	*Odontotermes takensis*	*Odontotermes takensis*,	990	0.00	99.73%	LC481543.1	PP313323
		Odontotermes hainanensis			97.85%	EU253857.1	
IV202344	*Macrotermes annandalei*	*Macrotermes annandalei*	941	0.00	99.45%	LC481540.1	PP313324
IV202345	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	990	0.00	99.51%	AB909001.1	PP313325
SH201803	*Macrotermes carbonarius*	*Macrotermes carbonarius*	902	0.00	99.56%	NC_034046.1	PP313326
SH201801	*Macrotermes carbonarius*	*Macrotermes carbonarius*	939	0.00	99.16%	NC_034046.1	PP313327
SH201802	*Macrotermes carbonarius*	*Macrotermes carbonarius*	951	0.00	99.04%	NC_034046.1	PP313328
SH201804	*Macrotermes carbonarius*	*Macrotermes carbonarius*	929	0.00	99.35%	NC_034046.1	PP313329

were comparable. As for *O. javanicus*, no description of *O. hinanensis* is readily accessible. Although the *COII* sequences of our *O. takensis* samples showed that they were similar to *O. formosanus*, it is unlikely that our samples were of this species. *O. takensis* differs from *O. formosanus* in terms of the head capsule shape (Ahmad 1965); our samples clearly showed the presence of this character (Fig. 1J).

Earlier, major problems in terms of *Odontotermes* identification in West Africa were reported (Korb et al. 2019). Thus, our data will assist molecular identification, particularly of these two species. At the genus level, samples of the same genus were grouped together as a monophyletic clade, except for samples in the genus *Hypotermes*, which were grouped with *Odontotermes* in both phylogenetic trees. This is not surprising; this genus has always been grouped within the *Odontotermes* clade in previous studies (Aanen et al. 2002; Ohkuma et al. 2004; Makonde et al. 2013). *O. feae* and *O. takensis* grouped as a sister clade in the phylogenetic tree but *O. longinathus* was more distinct from these two species. Similarly, *M. gilvus* and *M. annandalei* were grouped as a sister clade, whereas *M. carbonarius* was more distantly related. This is consistent with the study by Ohkuma et al. (2004). *Microtermes* and *Ancistrotermes* were more similar to *Odontotermes* than *Macrotermes* in both phylogenetic trees, but in the *COII* tree, they clustered together. This contrasts with the results of Aanen et al. (2002), who found that *Microtermes* and *Ancistrotermes* were both more similar to *Macrotermes* than *Odontotermes*. In addition, Hausberger et al. (2011) and Bourguignon

Table 2. BLAST result of *COII* sequences

Sample ID	Identified species	Best hit species	length (bp)	e-value	% Identity	Best hit acession	Seq. Accession
SH202302	*Macrotermes annandalei*	*Macrotermes annandalei*	609	0.00	97.82%	KY679800.1	PP313120
SH202305	*Odontotermes takensis*	*Odontotermes* nr. *formosanus* FH-2007, *Odontotermes hainanensis* isolate SY	677	0.00	98.82%, 98.27%	AB300694.1 JQ518439.1	PP313122
SH202306	*Macrotermes gilvus*	*Macrotermes gilvus*	618	0.00	99.35%	AB109526.1	PP313123
SH202307	*Microtermes obesi*	*Microtermes obesi*	677	0.00	97.64%	AB109523.1	PP313124
SH202308	*Odontotermes longignathus*	*Odontotermes longignathus*	677	0.00	99.72%	NC_034130.1	PP313125
SH202314	*Odontotermes longignathus*	*Odontotermes longignathus*	648	0.00	99.56%	NC_034130.1	PP313126
SH202317	*Odontotermes feae*	*Odontotermes javanicus*	677	0.00	97.78%	NC_034106.1	PP313127
SH202318	*Odontotermes takensis*	*Odontotermes hainanensis*	609	0.00	99.36%	HQ012040.1	PP313128
SH202320	*Microtermes obesi*	*Microtermes obesi*	677	0.00	99.71%	AB109523.1	PP313129
SH202323	*Odontotermes feae*	*Odontotermes javanicus*	677	0.00	97.01%	NC_034106.1	PP313130
SH202324	*Macrotermes annandalei*	*Macrotermes annandalei*	614	0.00	99.21%	KY679800.1	PP313131
SH202326	*Ancistrotermes pakistanicus*	*Microtermes pakistanicus*	677	0.00	99.70%	AB109524.1	PP313132
SH202327	*Hypotermes makhamensis*	*Hypotermes makhamensis*	677	0.00	96.95%	NC_034037.1	PP313133
SH202328	*Odontotermes longignathus*	*Odontotermes longignathus*	677	0.00	99.70%	AB051877.1	PP313134
SH202329	*Odontotermes takensis*	*Odontotermes hainanensis*	677	0.00	99.16%	EU253896.1	PP313135
SH202330	*Odontotermes longignathus*	*Odontotermes longignathus*	677	0.00	99.56%	AB051877.1	PP313136
IV202331	*Odontotermes takensis*	*Odontotermes* nr. *formosanus* FH-2007, *Odontotermes hainanensis*	677	0.00	99.41% 98.28%	AB300694.1 JQ518439.1	PP313137
IV202332	*Microtermes obesi*	*Microtermes obesi*	677	0.00	99.85%	AB109523.1	PP313138
IV202333	*Macrotermes annandalei*	*Macrotermes annandalei*	650	0.00	98.93%	AB109527.1	PP313139
IV201334	*Macrotermes gilvus*	*Macrotermes gilvus*	631	0.00	100.00%	AB109526.1	PP313140
IV202335	*Macrotermes gilvus*	*Macrotermes gilvus*	625	0.00	100.00%	AB109526.1	PP313141
IV202336	*Macrotermes annandalei*	*Macrotermes annandalei*	615	0.00	99.19%	AB109527.1	PP313142
IV202338	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	677	0.00	99.56%	AB109524.1	PP313143
IV202339	*Odontotermes feae*	*Odontotermes javanicus*	677	0.00	98.31%	NC_034106.1	PP313144
IV202340	*Hypotermes makhamensis*	*Hypotermes makhamensis*	677	0.00	97.34%	NC_034037.1	PP313145
IV202341	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	677	0.00	99.56%	AB109524.1	PP313146
IV202342	*Odontotermes takensis*	*Odontotermes* nr. *formosanus* FH-2007, *Odontotermes hainanensis* isolate SY	677	0.00	99.56% 98.36%	AB300694.1 JQ518439.1	PP313147
IV202343	*Odontotermes takensis*	*Odontotermes* nr. *formosanus* FH-2007, *Odontotermes hainanensis* isolate SY	677	0.00	99.56% 98.41%	AB300694.1 JQ518439.1	PP313148
IV202344	*Macrotermes annandalei*	*Macrotermes annandalei*	615	0.00	99.19%	AB109527.1	PP313149
IV202345	*Ancistrotermes pakistanicus*	*Ancistrotermes pakistanicus*	677	0.00	99.56%	AB109524.1	PP313150
SH201803	*Macrotermes carbonarius*	*Macrotermes carbonarius*	641	0.00	99.69%	AB300697.1	PP313151
SH201801	*Macrotermes carbonarius*	*Macrotermes carbonarius*	645	0.00	99.41%	AB300697.1	PP313152
SH201802	*Macrotermes carbonarius*	*Macrotermes carbonarius*	677	0.00	99.69%	AB300697.1	PP313153
SH201804	*Macrotermes carbonarius*	*Macrotermes carbonarius*	670	0.00	99.85%	AB300697.1	PP313120

et al. (2015) reported that *Macrotermes* clustered with *Odontotermes* but *Ancistrotermes* clustered with *Microtermes*. The branch lengths of the phylogenetic trees for both genes differed slightly. This may be because the evolutionary rates of the two genes differ, thus affecting both clustering and the phylogenetic trees. Thus, the phylogenetic trees of various studies that evaluated different gene regions indicated distinct evolutionary relationships. Target genes must be carefully selected when it is sought to accurately construct an evolutionary relationship. Our study focused on species identification; either *COI* or *COII* gene data allowed species identification. In addition, the morphological characteristics of the various species can be evaluated using our descriptions. Thus, this integrated study of both the morphological features and DNA sequences of the common genera *Ancistotermes*, *Microtermes*, and *Odontotermes* of Thailand and many other Asian countries will facilitate future research on FGTs.

CONCLUSIONS

This is the first study to provide detailed descriptions nine FGT species commonly found in Thailand together with the two most popular barcoding gene, *COI* and *COII* sequences, especially the sequences of *O. feae* and *O. takensis* that has not been reported before. Our data will decipher identification problem and facilitate other aspects of Asian FGT study in the future.

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Competing interests: All authors declare that they have no conflict of interest.

Availability of data and materials: DNA sequences generated in the study have been deposited in the GenBank database.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Supplementary materials

Table S1. Coordinate of fungus growing termite in sampling sites. (download)

Table S2. Acession number of COI and COII sequences of all samples in this study. (download)