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Morphological and molecular characterization of *Termitomyces* (Lyophyllaceae, Agaricales) in Thailand

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Abstract. *Jannual N, Nipitwattanaphon M, Hasin S, Kaewgrajang T. 2020. Morphological and molecular characterization of* Termitomyces *(Lyophyllaceae, Agaricales) in Thailand. Biodiversitas 21: 2481-2491. Termitomyces* is considered to be a highly prized delicacy collected both for home consumption and for sale in local markets. Although the taxonomic information about this genus is well known in Africa, the identification of *Termitomyces* species in Thailand is unclear. Therefore, this study presented an assessment of phylogenetic relationships in the genus *Termitomyces* by means of sequencing of the ITS1-5.8S-ITS2 region and the species characterization by combination of morphological data and molecular data. Total of 61 *Termitomyces* specimens was collected from a variety of geographical localities of Thailand. They were classified into six species, including *T. clypeatus*, *T. cylindricus*, *T. fuliginosus*, *T. heimii*, *T. microcarpus*, and *T. striatus* based on morphological characteristics combining with molecular characteristics. This is the first diagnostic key of Thai *Termitomyces*.

Keywords: Diversity, morphology, termite mushroom, termitophilic Agaricales, taxonomy

Abbreviations: ACACIA: *Acacia* spp. plantation; DDF: dry dipterocarp forest; DEF: dry evergreen forest; EUCA: *Eucalyptus* plantation; HOPEA: *Hopea odorata* plantation; ITS: Internal transcribed spacer; MP: Maximum parsimony; TEAK: Teak plantation; TRF: tropical rain forest

INTRODUCTION

Thailand located in the tropical region, which has high diversity of flora and fauna, especially mushrooms (Tanticharoen 2004). Hyde et al. (2018) who reported that high species diversity of mushrooms are found in the northern part of Thailand and up to 93% of the mushrooms are novel species. Recently, Sangwanit et al. (2013) reported 2,575 species in the checklist of mushrooms in Thailand. Amongst these, species in the genus *Termitomyces* Heim are one of the popular wild edible mushrooms group in Thailand. The genus is one of highly prized delicacy collected both for home consumption and for sale in local markets or along roadside (Pegler and Vanhaecke 1994).

Termitomyces was firstly introduced by Roger Heim in 1942 to "termitophilic Agaricales" group associated with termite nests. Its various species are distributed throughout equatorial and Southern Africa (Otieno 1964; Mossebo et al. 2002), America (Tibuhwa et al. 2010), Europe (Olila et al. 2007), and Southeast Asia (Pegler and Vanhaecke 1994; Wei & Yao 2003; Wei et al. 2003, 2004, 2006; Tang et al. 2006a, 2006b). Since 1942, about 30 *Termitomyces* species have so far been represented worldwide (Kirk et al. 2008). Currently, 92 different names for species, subspecies, and varieties are available in literature (www.indexfungorum.org).

Termitomyces species in Thailand was first reported by Bel P.J and Pataragetvit S., which did the field survey from Kanchanaburi province western Thailand upward along with the Kwai river to Chiangmai province northern Thailand during 1978-1979. From this survey, four species, *T. clypeatus*, *T. fuliginosus*, *T. globulus*, *T. mammiforius* were recorded (Bels and Pataragetvit 1982). In 1994, three species, *T. aurantiacus*, *T. clypeatus*, and *T. globulus*, were described from specimens collected at Chiang Mai and Kanchanaburi provinces, Thailand (Pegler and Vanhaecke 1994). After that, several field surveys for *Termitomyces* had done by Thai mycologists and now 18 species of Thai *Termitomyces* had been reported (The Royal Society 1996; Pitchayangkul 1998; Sangwanit et al. 2013). However, most of reports were identified the mushrooms based on morphological characteristics according to constructing identification Key from Africa. Although molecular technique has been accepted as useful technique for mushroom classification, only a few studies used morphological characteristics together with molecular techniques for identification. Taprab et al. (2002) extracted DNA from nodules and classified into eight groups of *Termitomyces* species based on internal transcribed sequences (ITS) but no evidence of mushroom

morphological support. Additionally, Kosakul et al. (2007) used isozyme markers to determine the genetic diversity of *T. auranticus*, *T. entolomoides*, *T. heimii*, *T. clypeatus* and *T. cylindricus* from 20 *Termitomyces* species collected from the central region of Thailand but also without description of morphological characteristics. Only the report of Sawhasan et al. (2010) classified nine species of *Termitomyces* collected in Kanchanaburi province based on morphological characteristics and ITS1-5.8S-ITS2 rDNA sequences. It was shown that although the taxonomic information about this genus is well known in Africa, the identification of *Termitomyces* species in Thailand is unclear because there are some morphological variations from the description were found, especially in *T. clypeatus*, such as acutely spiniform and color of perforatorium, depended on environmental factors (Sawhasan et al. 2011). Thus, it could lead to misidentification and most of the samples remained unidentified. To overcome the difficulty of identifying species of this genus with morphology, molecular techniques should be combined with morphological characteristics to provide accurate species identification (Siddiquee et al. 2012). ITS (Internal transcribed spacer) region of the ribosomal DNA gene (rDNA) has been widely used for fungal classification and phylogenic studies (Hajibabaei et al. 2007; Seifert 2009; Bellemain et al. 2010; Hibbett et al. 2016; Xu and Adamowicz 2016; Raja et al. 2017). ITS combined with macroscopic and microscopic features to identify *Termitomyces* mushrooms has been done in many studies (Hofstetter et al. 2002; Rouland-Lefèvre et al. 2002; Sawhasan et al. 2011; Hussain et al. 2015; Raja et al. 2017). However, currently, very few presumptive sequence data of *Termitomyces* from Thailand are available making it difficult to use identify by DNA sequences. Thus, it is important to have the diagnostic key specific to Thai *Termitomyces* together with molecular study to allow any comparison between studies and to facilitate future identification by using DNA barcoding method. Consequently, the focus of this study was to identify *Termitomyces* species collected from various geographical localities of Thailand based on macroscopic features, microscopic features, and DNA barcoding, and to provide description of *Termitomyces* species with a diagnostic key.

MATERIALS AND METHODS

Sampling sites and sample collections

Termitomyces specimens were collected from a variety of geographical localities of Thailand (Table 1). The collection of this genus was carried out from 2015 to 2018 during August - October (the best time for collecting *Termitomyces*). The fruiting bodies were photographed, carefully examined by digging the soil to get complete pseudorhiza, and then removed the soil on the surface of pseudorhiza. The specimens were kept in a wax paper and brought to laboratory for observing macroscopic and microscopic features. Fruiting bodies for each isolate are maintained in the herbarium at the Forest Pathology Laboratory, Department of Forest Biology, Faculty of Forestry, Kasetsart University (Table 1).

Macroscopic and microscopic identification

Before oven-drying (45ºC), the macroscopic features were recorded in detail and were then made the spore print following Largent (1973). All the dried specimens were kept in silica gel for microscopic features and molecular works. Thin sections of dried specimens, including lamellae, pileal context, and partial veils were done by a free-hand section. The observation of microscopic features including size of basidiospores, basidia, hymenophoral trama, pileal context, and cutis was done under the compound microscope (Zeiss Axioskop 40, magnification 1000×) following Largent et al. (1977). At least 30 basidiospores, 20 basidia, and 20 cystidia of each specimen were measured. The specimens were identified based on diagnostic macroscopic and microscopic features examination (Pegler and Vanhaecke 1994; Wei et al. 2004; 2006; 2009; Mossebo et al. 2009; Sawhasan et al. 2011; Karun et al. 2013).

Molecular identification

Samples for DNA extraction were excised from dry or fresh basidiomes. Genomic DNA was isolated using FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen Inc., Taiwan). The ITS regions were amplified with *Termitomyces* specific primer, ITS1FT (5´-GTTTTCAACCACCTGTGCAC-3´) (Nobre et al. 2010), ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) (White et al. 1990) and LR7 (5´-TACTACCACCAAGATCT-3´) (Vilgalys and Hester 1990).

PCR reaction was performed in 50 µL containing ~ 20 ng of DNA template, 1X PCR buffer, 0.5 mM dNTP, 5 mM $MgCl_2$, 0.625 µM of each primer and 1.25 U Taq DNA polymerase (Apsalagen, Thailand). PCR condition includes pre-denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 60-90 s, and final extension at 72°C for 5 min. PCR products were checked by 1% agarose gel electrophoresis and purified with FavorPrep™ GEL/PCR Purification Kit (Favorgen Inc., Taiwan). After that, purified PCR products were sent to Macrogen Inc. (Korea) for Sanger sequencing. All sequences data in this study were deposited in Genbank (MN160255- MN160316) (Table 1).

Other *Termitomyces* sequences were retrieved from GenBank for phylogenetic analysis together with sequences from this study. All sequences were aligned using MAFFT (Nakamura et al. 2018) and manually edited the alignment using Bioedit program, version 7.2.5 (Hall 1999). Phylogenetic trees were reconstructed by MEGA7 program (Kumar et al. 2016) using Maximum parsimony (MP) method. Number of bootstraps was set to 1000 replicates.

Table 1. List of *Termitomyces* specimens included in this study

Code	Herbarium no.	Vegetation/ Forest type	Sampling site	GenBank accession no.	Length (bp)
M02 css1	TERM001	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160255	1942
M02 css2	TERM002	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160256	1953
M03	TERM003	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160257	1938
M05	TERM004	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160258	721
M012	TERM005	HOPEA	Wang NamKhiao, Nakhon Ratchasrima	MN160259	1229
M013	TERM006	HOPEA	Wang NamKhiao, Nakhon Ratchasrima	MN160260	1967
M015	TERM007	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160261	186
M016	TERM008	HOPEA	Wang NamKhiao, Nakhon Ratchasrima	MN160262	669
M017	TERM009	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160263	617
M018	TERM010	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160264	624
M019	TERM011	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160265	668
M023	TERM012	EUCA	Wang NamKhiao, Nakhon Ratchasrima	MN160266	624
M024	TERM013	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160267	1966
M026	TERM014	EUCA	Wang NamKhiao, Nakhon Ratchasrima	MN160268	171
EU611	TERM015	EUCA	Wang NamKhiao, Nakhon Ratchasrima	MN160269	1897
EU612	TERM016	EUCA	Wang NamKhiao, Nakhon Ratchasrima	MN160270	1897
EU613	TERM017	EUCA	Wang NamKhiao, Nakhon Ratchasrima	MN160271	1897
Mix611	TERM018	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160272	1856
Mix612	TERM019	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160273	1212
Mix613	TERM020	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160274	1898
Mix614	TERM021	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160275	1214
Mix615	TERM022	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160276	1898
SKR00	TERM023	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160277	159
SKR01 DEF	TERM024	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160278	543
SRK02 DEF2	TERM025	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160279	1930
SKR03 Acacia	TERM026	ACACIA	Wang NamKhiao, Nakhon Ratchasrima	MN160280	1859
SKR04 DEF	TERM027	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160281	186
SKR05 Acacia	TERM028	ACACIA	Wang NamKhiao, Nakhon Ratchasrima	MN160282	984
SKR07 DEF2	TERM029	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160283	1894
SKR08 DEF2	TERM030	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160284	1904
SKR09 Acacia	TERM031	ACACIA	Wang NamKhiao, Nakhon Ratchasrima	MN160285	186
SKR071	TERM032	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160286	183
SKR086 DEF9	TERM033	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160287	1089
SKR612	TERM034	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160288	1858
SKR613	TERM035	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160289	1832
SKR614	TERM036	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160290	1879
TA1	TERM037	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160291	301
TA2	TERM038	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160292	280
TA3	TERM039	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160293	705
TAP	TERM040	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160294	424
TAT	TERM041	DDF	Wang NamKhiao, Nakhon Ratchasrima	MN160295	421
Ter_Mix	TERM042	plantation	Wang NamKhiao, Nakhon Ratchasrima	MN160296	1881
Ter1M	TERM043	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160297	1938
Ter2M	TERM044	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160298	691
Ter3M	TERM045	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160299	967
Ter4M	TERM046	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160300	1952
Ter5	TERM047	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160301	775
WNK611	TERM048	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160302	1900
WNK612	TERM049	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160303	1900
WNK613	TERM050	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160304	1901
WNK614	TERM051	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160305	1577
WNK615	TERM052	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160306	1645
WNK617	TERM053	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160307	1656
WNK618	TERM054	DEF	Wang NamKhiao, Nakhon Ratchasrima	MN160308	1656
M08	TERM055	TEAK	Thong Pha Phum, Kanchanaburi	MN160309	1213
Ter1_Kan	TERM056	TEAK	Thong Pha Phum, Kanchanaburi	MN160310	581
Ter6	TERM057	TRF	Kantang, Tang	MN160311	1222
Ter7	TERM058	TRF	Kantang, Tang	MN160312	1898
Ter8	TERM059	TRF	Kantang, Tang	MN160313	2001
Ter9	TERM060	TRF	Kantang, Tang	MN160314	2015
Ter10	TERM061	TRF	Kantang, Tang	MN160315	2003
M021	TERM062	DDF	Mueang, Sukhothai	MN160316	618

Note: DDF: dry dipterocarp forest; DEF: dry evergreen forest; TRF: tropical rain forest; EUCA: Eucalyptus plantation; TEAK: Teak plantation; ACACIA: *Acacia* spp. plantation; HOPEA: *Hopea odorata* plantation

RESULTS AND DISCUSSION

Molecular analysis

MP tree showed the *Termitomyces* samples in this study were clustered together with sequences from GenBank (Figure 1). Six clades were clustered according to species. Nine *Termitomyces* samples identified as *T. fuliginosus* were grouped with *T. fuliginosus* sequences from other countries such as Viet Nam and China, *T. eurrhizus*, and *Termitomyces* sp. Group8 from Taprab et al. (2002). Fifteen *Termitomyces* samples identified as *T. cylindricus* were clustered with *Termitomyces* sp. Group2 and *T. cylindricus* from Thailand and Indonesia in the database. Similarly, 12 *Termitomyces* samples identified as *T. microcarpus* were grouped with *Termitomyces* sp. Group3 and *T. microcarpus* from Thailand and India. Likewise, TERM055 and TERM062 were also grouped with their identified species, *T. heimii* and *T. clypeatus*, respectively. On the other hand, *Termitomyces* samples that morphological characteristics confidently identified as *T. striatus*, showed no match with the only one ITS sequence of *T. striatus* in the database, and grouped with *Termitomyces* sp. Group7 from Taprab et al. (2002). Since there is only one ITS sequence but several 28S sequences of *T. striatus*, we performed another phylogenetic tree of 28S from some *Termitomyces* species (Figure 2). The result from this tree showed that the samples identified as *T. striatus* in this study were related to other 28S of *T. striatus* from GenBank but with some degree of intraspecific distance.

Morphological description

Termitomyces cylindricus S.C. He 1985 (Figure 3.A-F)

Pileus 5-7 cm diam., conic to convex then convexo-applanate to plane when mature, with an obtusely rounded perforatorium; surface white to cream, and yellow-brown at the center and paler toward margin, smooth and glabrous, margin straight, often radially splitting when mature. *Lamellae* free, white to cream and becoming pinkish when mature, 0.4 cm wide, crowded with lamellulae. *Stipe* 5-8.7 cm long, 0.7-1 cm wide, central, cylindrical to subcylindrical, surface white, smooth or longitudinally striate, solid fibrous. *Annulus* absent. *Pseudorhiza* 3-7 cm long, 0.3-0.5 cm thick, cylindrical to subcylindrical, surface white, smooth or longitudinally striate, solid. *Spore deposit* white. *Basidiospores* 5.5-10 x 4-7 µm, ovoid to ellipsoid; smooth and thin-walled, inamyloid. *Basidia* 7.5-22.5 x 5-8 µm, clavate, bearing four sterigmata, inamyloid. *Hymenophoral trama* regular, 55-70 µm wide, inamyloid. *Cheilocystidia* crowded, 20-46.3 x 13-30 µm, clavate to pyriform, smooth and thin-walled, inamyloid. *Pleurocystidia* 20-47.5 x 10-28 µm, similar to cheilocystidia *Pileipellis* a repent epicutis of narrow, radial hyphae, 1.5-2.5 µm diam.

Specimens examined:— Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat environmental research station, in dry evergreen forest (TERM024, TERM025, TERM027, TERM029, TERM030, TERM032, TERM033, TERM044, TERM045, TERM046), Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat environmental research station, in Acacia sp. plantation (TERM026, TERM028, TERM031), Saraburi: Sao Hai, under *Dipterocarpus alatus* trees (TERM004), Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat silviculture research station, dry dipterocarp forest (TERM039).

Termitomyces clypeatus R. Heim 1951 (Figure 3.G-J)

Pileus 6-10 cm diam., at first pointed conical then broadly convex to plane, with a dark prominent spiniform perforatorium; surface greyish brown to ochraceous brown, dark at the center and paler towards the margin when mature, fibrillose and silky, margin at first incurved then straight, often splitting. *Lamellae* free, white to cream, 0.8-1.0 cm wide, crowded with lamellulae. *Stipe* 6.5-12 cm long, 1-2 cm wide, central, cylindrical to subcylindrical, occasionally forming slightly thickening at stipe base, surface white with longitudinal fibrillose, solid. *Annulus* absent. *Pseudorhiza* 3-10 cm long, 0.3-1.0 cm thick, tapering towards the base, surface white to cream, smooth, solid. *Spore deposit* not found. *Basidiospores* 5-8 x 3-6 µm, ovoid to ellipsoid; smooth and thin-walled, inamyloid. *Basidia* 12-20 x 5-8 µm, clavate to subcylindric, bearing four sterigmata, inamyloid. *Hymenophoral trama* regular, up to 100 µm wide, inamyloid. *Cheilocystidia* crowded, 17-18 x 9-10 µm, clavate to pyriform, smooth and thin-walled, inamyloid. *Pleurocystidia* crowded, 23-42 x 9-30 µm, similar to cheilocystidia *Pileipellis* a repent epicutis of narrow, radial hyphae, 2.0-4.0 µm diam.

Specimen examined:— Sukhothai: Mueang, Ban Kluai (TERM062).

Termitomyces fuliginosus R. Heim 1942 Figure 3.K-P)

Pileus 5-8 cm diam., conic to convex then convexo-applanate to plane when mature, with a spiniform at first and bluntly pointed perforatorium when mature; surface golden brown to brownish orange, radially fibrillose and shiny, margin at first incurved then straight, often splitting. *Lamellae* free, white to cream, 0.5 cm wide, crowded with lamellulae. *Stipe* 8-10 cm long, 1.0-1.3 cm wide, central, cylindrical or slightly bulbous at stipe base, surface cream to pale brown covering with pale brown fibrillose, solid fibrous. *Annulus* membranous ephemeral and often absent after the initial stage, attached to the upper of stipe. *Pseudorhiza* 10-20 cm long, 0.3-0.5 cm thick, cylindrical to slender, surface white, smooth or longitudinally striate, solid. *Spore deposit* white. *Basidiospores* 4.5-7 x 3-4.5 µm, ellipsoid; smooth and thin-walled, inamyloid. *Basidia* 16-20 x 6-8 µm, clavate, bearing four sterigmata, inamyloid. *Hymenophoral trama* regular, 50-72 µm wide, inamyloid. *Cheilocystidia* crowded, 18-24 x 10-11 µm, clavate, smooth and thin-walled, inamyloid. *Pleurocystidia* scattered to rare, 20-30 x 8.5-11 µm, similar to cheilocystidia. *Pileipellis* a repent epicutis of narrow, radial hyphae, 1.0-2.5 µm diam.

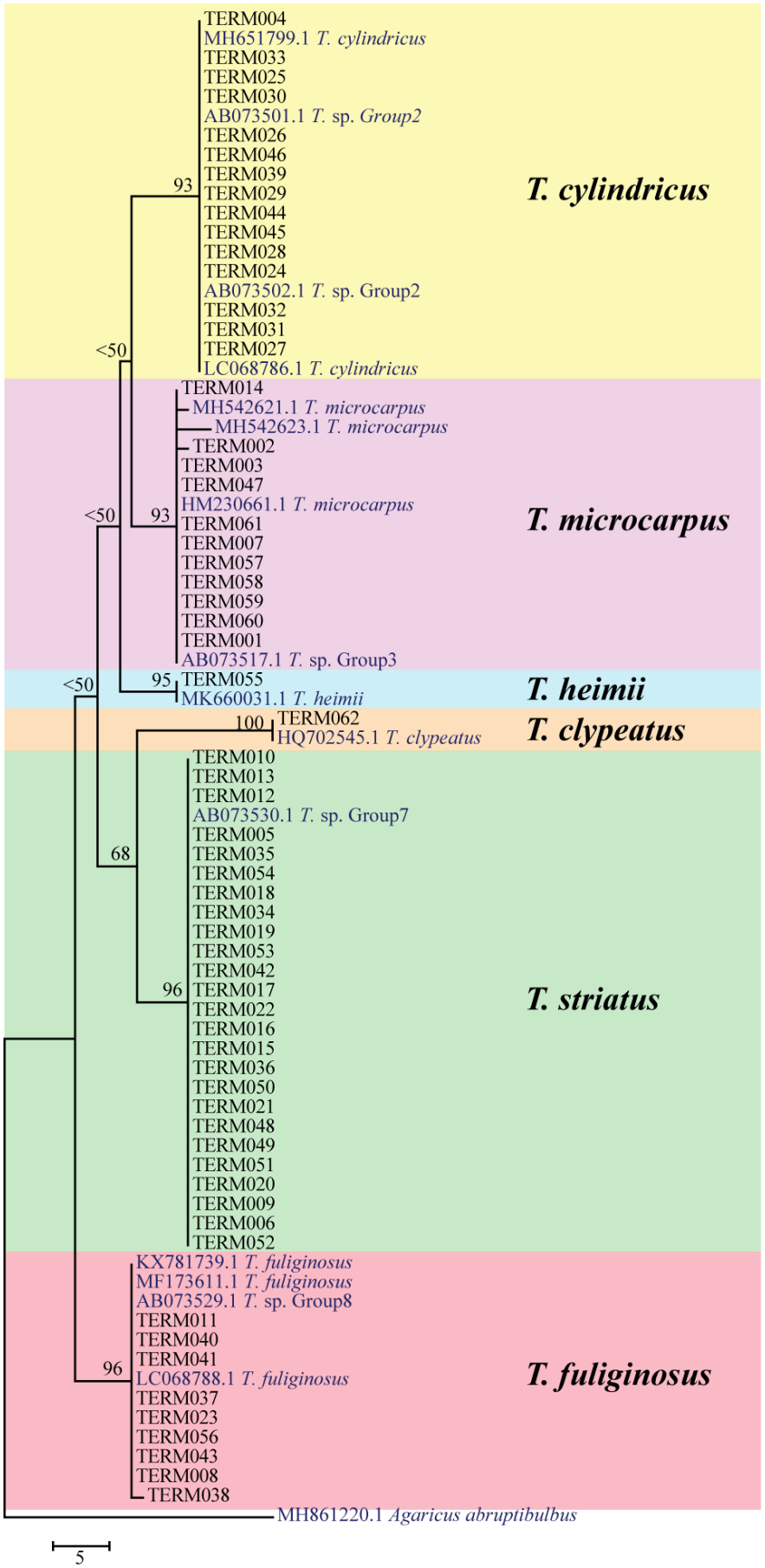

Figure 1. Phylogenetic tree based on ITS1-5.8S-ITS2 of *Termitomyces* sequences in this study and from GenBank (blue letter) using the Maximum parsimony method. *Agaricus abruptibulbus* is used as an outgroup. Numbers at the node are bootstrap scores of 1000 replicates.

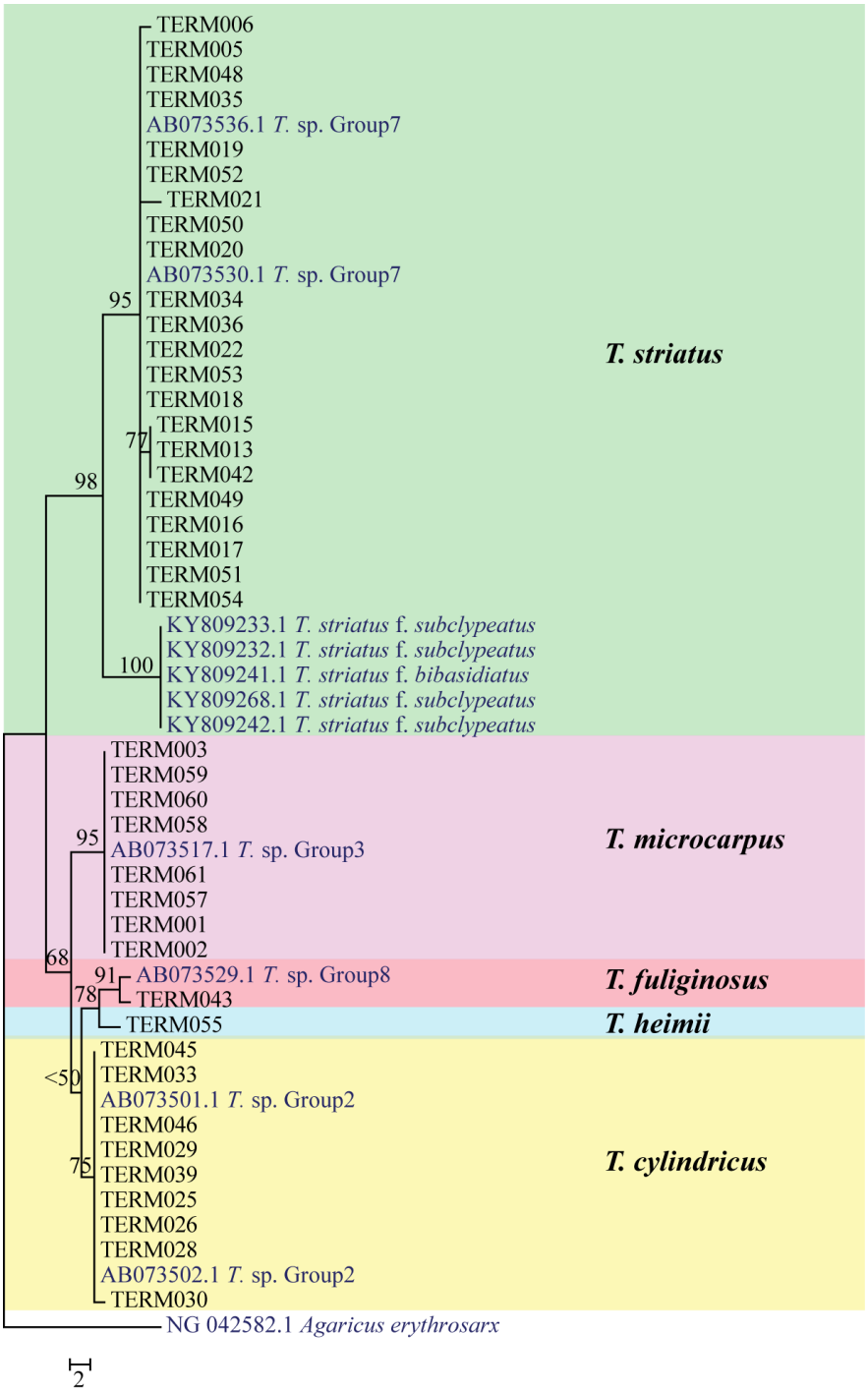

Figure 2. Phylogenetic tree based on 28S rDNA of *Termitomyces* sequences in this study and from GenBank (shown in blue letter) using the Maximum parsimony method. *Agaricus erythrosarx* is used as an outgroup. Numbers at the node are bootstrap scores of 1000 replicates

Specimens examined:— Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat environmental research station, in dry evergreen forest (TERM043), Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat silviculture research station, dry dipterocarp forest (TERM037, TERM038), Nakhon Ratchasrima: Wang Nam Khiao, Thaplan National park, in *Hopea odorata* plantation (TERM008), Nakhon Ratchasrima: Wang Nam Khiao, Wang Nam Khiao Forestry Student Training Station, in dry evergreen forest (TERM011), Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat environmental research station, in dry evergreen forest (TERM023), Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat silviculture research station, dry dipterocarp forest (TERM040, TERM041), Kanchanaburi: Thong Pha Phum, teak plantation (TERM056).

Termitomyces heimii Natarajan 1979 (Figure 4.A-C)

Pileus 0.8-3.5 cm diam., parabolic to campanulate then plane to uplifted when mature, with rounded perforatorium; surface white to cream and dark at the center when mature,

velar squamulose on surface, margin at first incurved then straight, often splitting. *Lamellae* free, white to creamy, 1 mm wide, crowded with lamellulae. *Stipe* 1.3-4 cm long, 0.5-2 cm wide, central, cylindrical to subcylindrical, surface white to cream, smooth and glabrous, solid. *Annulus* membranous at first, forming the persistent double annulus on the upper of stipe, surface white to cream. *Pseudorhiza* 7-20 cm long, 0.5-1.5 cm thick, cylindrical, surface white to cream, smooth, leathery, hollow. *Spore deposit* not found. *Basidiospores* 6-9.5 x 4-6 µm, ovoid to ellipsoid; smooth and thin-walled, inamyloid. *Basidia* 18-19 x 5-8 µm, clavate to subcylindric, bearing four sterigmata, inamyloid. *Hymenophoral trama* regular, up to 100 µm wide, inamyloid. *Cheilocystidia* crowded, 21-35 x 14-20 µm, clavate to pyriform, smooth and thin-walled, inamyloid. *Pleurocystidia* rare, 20-34 x 11-18 µm, similar to cheilocystidia *Pileipellis* a repent epicutis of narrow, radial hyphae, 2.0-4.5 µm diam.

Specimen examined:— Kanchanaburi: Thong Pha Phum, teak plantation (TERM055).

Termitomyces microcarpus (Berk. & Broome) R. Heim 1942 (Figure 4.D-F)

Pileus 1.0-1.5 cm diam., conic then plane to uplifted when mature, with small obtuse perforatorium; surface white to cream and dark or yellowish-brown at the center when mature, smooth and glabrous, margin straight, often radially splitting when mature. *Lamellae* free, white to creamy, 1 mm wide, crowded with lamellulae. *Stipe* 4-6 cm long, 0.2-0.5 cm wide, central, cylindrical, surface white to cream, smooth and glabrous. *Annulus* absent. *Pseudorhiza* forming a root-like at stipe base under ground level. *Spore deposit* white. *Basidiospores* 4.5-8 x 3-5 µm, ovoid to ellipsoid; smooth and thin-walled, inamyloid. *Basidia* 15-30 x 5-10 µm, clavate to subcylindric, bearing four sterigmata, inamyloid. *Hymenophoral trama* regular, 50-60 µm wide, inamyloid. *Cheilocystidia* rare, 30-40 x 10-17.5 µm, clavate to pyriform, smooth and thin-walled, inamyloid. *Pleurocystidia* rare, 25-35 x 10-17.5 µm, similar to cheilocystidia. *Pileipellis* a repent epicutis of narrow, radial hyphae, 2.0-4.0 µm diam.

Specimens examined:— Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat environmental research station, in dry evergreen forest (TERM001, TERM002), Nakhon Ratchasrima: Wang Nam Khiao, Wang Nam Khiao Forestry Student Training Station, in dry dipterocarp forest (TERM003, TERM007, TERM014), Trang: Kachong, oil palm plantation (TERM047, TERM057, TERM058, TERM059, TERM060, TERM061).

Termitomyces striatus (Beeli) R. Heim (Figure 4.G-K)

Pileus 2-8 cm diam., conic to convex then plane when mature, with small and pointed perforatorium; surface light brown to greyish brown and dark at the center when mature, usually radially fibrillose, shiny, margin at first incurved then straight, often splitting. *Lamellae* free, white to creamy, 3-5 mm wide, crowded with lamellulae. *Stipe* 4.5-11 cm long, 0.5-1.5 cm wide, central, cylindrical to subcylindrical, occasionally swelling on its upper part and tapering at the base to form pseudorhiza, surface whitish, longitudinally striate, solid. *Annulus* absent. *Pseudorhiza* 7-20 cm long, 0.5-1.5 cm thick, tapering at the base; surface white to pale grey, smooth. *Spore deposit* pinkish. *Basidiospores* 5-8.5 x 4-6 µm, ovoid to ellipsoid; smooth and thin-walled, inamyloid. *Basidia* 15-22 x 4-6 µm, clavate to subcylindric, bearing four sterigmata, inamyloid. *Hymenophoral trama* regular, 40-100 µm wide, inamyloid. *Cheilocystidia* crowded, 20-32 x 6-17 µm, clavate to pyriform, smooth and thin-walled, inamyloid. *Pleurocystidia* crowded, 17-32 x 6-16 µm, similar to cheilocystidia, sometime subcylindrical or digitate. *Pileipellis* a repent epicutis of narrow, radial hyphae, 2.0-5.0 µm diam.

Specimens examined:— Nakhon Ratchasrima: Wang Nam Khiao, ThaplanNational park, in *Hopea odorata* plantation (TERM005, TERM006), Nakhon Ratchasrima: Wang Nam Khiao, Wang Nam Khiao Forestry Student Training Station, in dry evergreen forest (TERM009, TERM010, TERM048, TERM051, TERM052, TERM054), Nakhon Ratchasrima: Wang Nam Khiao, Wang Nam Khiao Forestry Student Training Station, in Bamboo plantation (TERM049, TERM050, TERM053), Nakhon Ratchasrima: Wang Nam Khiao, Wang Nam Khiao Forestry Student Training Station, in mix forest tree species plantation (TERM013, TERM018, TERM019, TERM020, TERM021, TERM022, TERM042), Nakhon Ratchasrima: Wang Nam Khiao, Wang Nam Khiao Forestry Student Training Station, in Eucalyptus plantation (TERM012, TERM015, TERM016, TERM017), Nakhon Ratchasrima: Wang Nam Khiao, Sakaerat environmental research station, in dry evergreen forest (TERM034, TERM035, TERM036).

Key to Termitomyces

1a Mature pileus generally smaller than 2.0 cm diameter……...……..*T. microcarpus*
1b Mature pileus generally greater than 2.0 cm diameter……...…….2
2a Conspicuous a persistent annulus or membranous ephemeral annulus ……...……...3
2b Annulus absent…….……...…….4
3a Pileus white to pale brown with rounded perforatorium, pseudorhiza leathery and hollow ……..*T. heimii*
3b Pileus golden brown to brownish orange with bluntly pointed perforatorium, pseudorhiza freshy fibrous and solid …..……..……...…*T. fuliginosus*
4a Pileus white to cream with obtusely rounded perforatorium, not spiniform..……...…….....*T. cylindricus*
4b Pileus ochraceous brown to greyish brown with spiniform perforatorium ….....……5
5a Perforatorium dark color, small pointed perforatorium, covering with radially striate ……...……*T. striatus*
5b Perforatorium dark color, prominent acutely perforatorium, smooth ……...…*T. clypeatus*

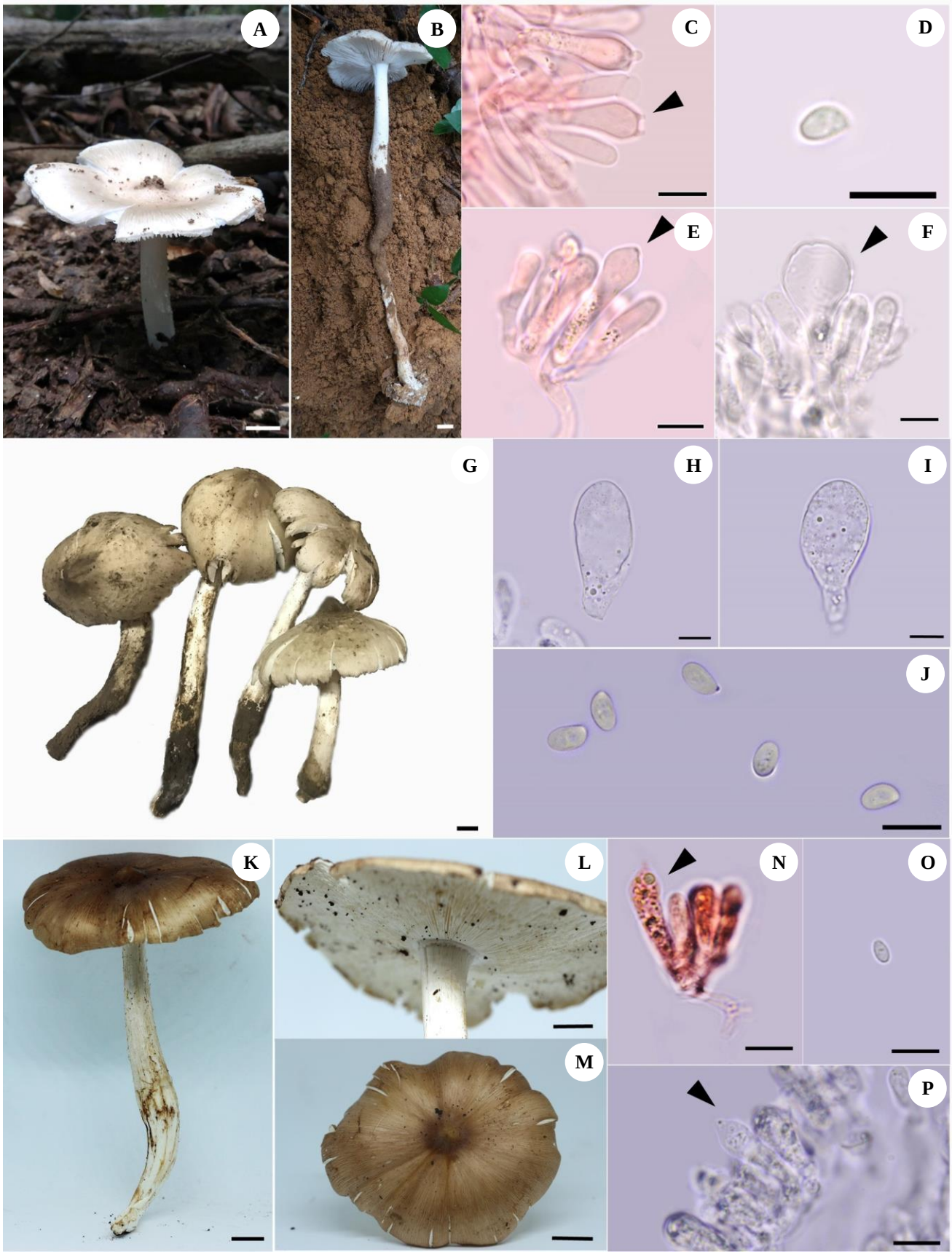

Figure 3. Fresh basidiocarps and microscopic features of *Termitomyces cylindricus* (A-F); arrowhead pointing at basidium (C); basidiospore (D); arrowhead pointing at cystidia (E, F), *Termitomyces clypeatus* (G-J); cystidium (H, I); basidiospores (J), *Termitomyces fuliginosus* (K-P); arrowhead pointing at cystidium (N, P); basidiospore (O). Scale bar: fresh basidiocarps = 1 cm; microscopic features = 10 µm

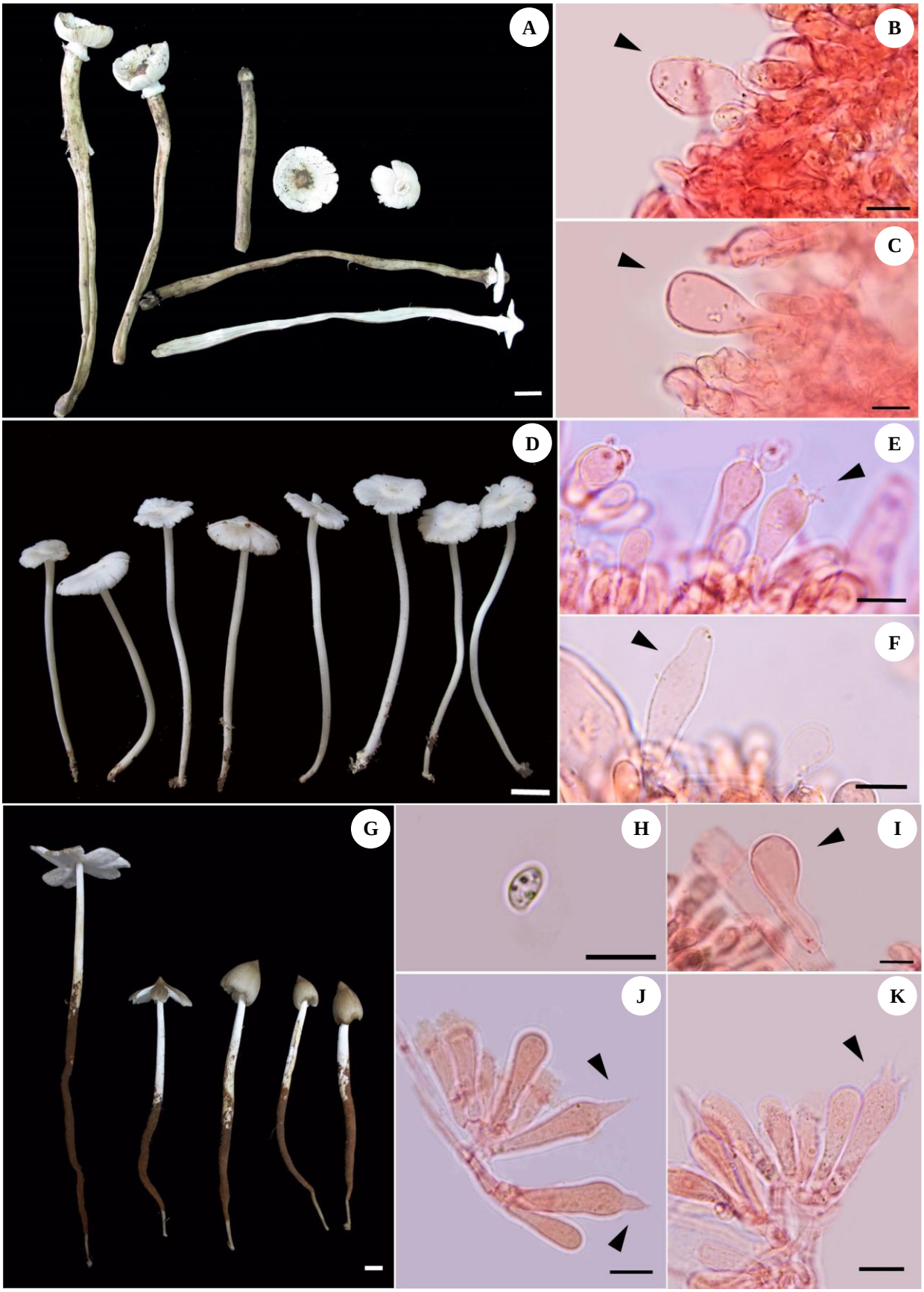

Figure 4. Fresh basidiocarps and microscopic features of *Termitomyces heimii* (A-C); arrow pointing at cystidia (B, C), *Termitomyces microcarpus* (D-F); arrowhead pointing at cystidium (E); arrowhead pointing at basidium (F), *Termitomyces striatus* (G-K); basidiospore (H); arrowhead pointing at cystidia (I, J); arrowhead pointing basidium (K). Scale bar: fresh basidiocarps = 1 cm; microscopic features = 10 µm.

Discussion

Since 1978, the surveys of Thai *Termitomyces* have been done extensively. To date, 18 species of *Termitomyces* were reported, but most of which used only morphology including macroscopic features such as size and color of the pileus, and the presence of pseudorhiza (Bels and Pataragetvit 1982; The Royal Society 1996; Pitchayangkul 1998; Sangwanit et al. 2013). Nowadays, molecular techniques have been proved to be a handy tool in fungal classification because it provides more accurate species delimitation (Taylor et al. 2000; Siddiquee et al. 2012) and thus is required for analysis of very similar species (Tibuhwa et al. 2010). Since the absence of the description key and the differences of some morphologies in Thailand from previous studies, we provided here the first diagnostic key of six Thai *Termitomyces* species (*T. clypeatus*, *T. cylindricus*, *T. fuliginosus*, *T. heimii*, *T. microcarpus* and *T. striatus*) from 62 samples. We used both morphological characteristics and molecular characteristics, because using only molecular techniques with unidentified species or without the accuracy of species identification by morphological identification can be problematic. Additionally, we investigated the evolutionary relationship of them based on phylogenetic analyses of ITS1-5.8s-ITS2 sequences (Figure 1).

Based on the description of our specimens, we found that the morphological characteristics of four *Termitomyces* species, *T. fuliginosus*, *T. heimii*, *T. microcarpus* and *T. striatus*, were corresponded with the previous description of Pegler and Vanhaecke (1994); Wei et al., (2009); Karun et al., (2013). Moreover, three of these four species (*T. fuliginosus*, *T. heimii*, *T. microcarpus*) identified by DNA barcoding showed high % identity (98-100%) from BLAST results, matching with the same described species in the database. The absence of species matched with *T. striatus* may due to the deficiency of ITS sequences of Asian *T. striatus* in the database, because only one sequence from Congo was found and this sequence is rather distantly related to our *T. striatus* samples, suggesting that our species may be classified into different subspecies. The result from the 28S tree (Figure 2) also supported that our *T. striatus* samples may belong to different subspecies with *T. striatus* from Congo since the samples were in the monophyletic clade with some genetic distance.

In addition, all samples were best hit with *Termitomyces* sequences collected from Thailand, which was studied by Taprab et al. (2002) despite no morphological characteristics of the submitted sequences to support. We also found that morphological characteristics of the other *Termitomyces* species in this study differed from previous studies, especially *T. clyeatus*. Our specimens of *T. clypeatus* do not have the acutely spiniform and dark color of perforatorium as mentioned by several reports (Pegler and Vanhaecke 1994; Wei et al. 2009; Karun et al. 2013). Likewise, the color of *T. cylindricus* in this study also differed from previous descriptions (Pegler and Vanhaecke 1994; Wei et al. 2009; Karun et al. 2013). Tibuhwa et al. (2010) reported that color is much used in identification keys to narrow the broad range of comparisons for different groups of species in the genus. Many variations of color are found within this genus from white-cream to grey, orange to brown, and rarely bluish to black between species. Thus, using pileus color might lead to misidentification of this genus. Phylogenetic tree of this study showed that most of our sequences were clustered according to species consistent with morphological identification. However, the sequence information of this genus from Thailand in the database was still little. Thus, here, we provided diagnostic keys for morphological identification together with the sequences of this study in the database for assisting more accurate identification and facilitating molecular identification by DNA barcoding of this genus in the future.

In conclusion, sixty-two samples of *Termitomyces*, which were collected from four provinces in Thailand, were identified based on morphological and molecular identification. These samples were classified into six species including *T. microcarpus*, *T. heimii*, *T. fuliginosus*, *T. aurantiacus*, *T. striatus* and *T. clypeatus*. Due to many variations in morphological features of this genus were found, the molecular identification is recommended to use together with morphological identification as the key provided here for more accurate identification of this genus.

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