



Two new species of *Termitomyces* (Agaricales, Lyophyllaceae) from China and Thailand

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Abstract

Termitomyces floccosus and *T. upsilocystidiatus* nov., are introduced from China and Thailand based on morphological characteristics and molecular phylogenetic analyses. *Termitomyces floccosus* is characterized by a squamulose pileus and stipe, and utriform cheilocystidia. *Termitomyces upsilocystidiatus* is characterized by Y-shaped cheilocystidia. Phylogenetic analyses of combined nrSSU and nrLSU sequence data confirmed that the two taxa are distinct *Termitomyces* species. Comprehensive descriptions, colour photographs and a phylogenetic tree showing the position of the two new species are provided.

Keywords: 2 new species, phylogeny, Southeast Asia, taxonomy

Introduction

The genus *Termitomyces* R. Heim (1942a: 72) (Lyophyllaceae, Agaricales) comprises more than 30 species (Aanen *et al.* 2002, Frøslev *et al.* 2003, He *et al.* 2019). *Termitomyces* species develop a symbiotic relationship with termites of the subfamily Macrotermitinae and all species grow on the fungal comb in termite nests (Heim 1942b, Ye *et al.* 2019). In the field, *Termitomyces* are characterized by their more or less long (up to 60 cm long, Pegler & Vanhaecke 1994) pseudorrhiza connected to the comb (very few species lack this characteristic), the special structure of the perforatorium, usually free lamellae, and pinkish spore print. Microscopically, the cheilocystidia and pleurocystidia of *Termitomyces* species are typically clavate or obovoid to pyriform, sometimes utriform.

Termitomyces species, including *T. le-testui* (Pat.) R. Heim (1942b: 109), *T. schimperi* (Pat.) R. Heim (1942b: 114), *T. eurrrhizus* (Berk.) R. Heim (1942b: 140), *T. microcarpus* (Berk. & Broome) R. Heim (1942b: 128) and *T. heimii* Natarajan (1979: 853) are valued edible mushrooms that are commonly found in the markets of tropical Africa and Asia (Parent & Thoen 1977, Mondal *et al.* 2004, Chandra *et al.* 2007, Ye *et al.* 2019).

Earlier, most taxonomists studied *Termitomyces* species based only on morphology; studies involving both phylogeny and morphology were produced from 2002 onwards (Rouland-Lefèvre *et al.* 2002, Aanen *et al.* 2002, Taprab *et al.* 2002, Frøslev *et al.* 2003, Sawhasan *et al.* 2011, Oyetyayo 2012, Saré *et al.* 2014, Mossebo *et al.* 2017,

Ye L *et al.* 2019). One of the outcomes of these studies was the confirmation that *Sinotermitomyces* is synonymous with *Termitomyces* (Frøslev *et al.* 2003). From a study of the coevolutionary relationship between *Termitomyces* and termites based on phylogenetic analyses, at least five migrations of *Termitomyces* between Asia and Africa were inferred (Aanen *et al.* 2002).

Fungal diversity in Thailand and, more broadly, in the Greater Mekong Subregion is high, with many new taxa of various ranks published in recent years (see e.g., Hyde *et al.* 2018, Thongbai *et al.* 2018). However, in spite of the value of most *Termitomyces* species in human consumption, materials from Thailand and the Greater Mekong Subregion have been poorly studied and may be expected to reveal new species. This paper is a result of ongoing studies on *Termitomyces* from Thailand and southwestern China. Based on evidence from morphological observations and phylogeny, two new species are introduced, with macro- and micromorphological descriptions, colour photographs and molecular work.

Material and methods

Morphological study

Specimens were collected from Yunnan Province, China, and Nakhon Si Thammarat Province, Thailand. Upon collection in the field, they were wrapped in aluminium foil or kept separately in a plastic collection box. The specimens were described macroscopically in the laboratory; colour was determined following Kornerup & Wanscher (1978). Specimens were thereafter thoroughly dried at 60°C in a food drier, stored in sealed plastic bags, and deposited in Mae Fah Luang University herbarium (MFLU) and Yunnan Academy of Agricultural Sciences herbarium (YNNKY). Dried tissue was excised with a razor blade under a dissecting microscope, transferred onto slides, and mounted in 5% KOH solution. Microscopic anatomical and cytological characteristics including basidia, basidiospores and cystidia were observed and photographed using a Nikon eclipse 80i microscope at magnifications up to ×1000. The notation [x/y/z] specifies that measurements were made on x basidiospores from y basidiomata and z collections. 40–50 basidiospores, 20 basidia and 10 cystidia were measured. Basidiospore dimensions are given as (a)b–m–c(d) where a and d refer to the lower and upper extremes of all measurements respectively, b–c the range of 95% of the measured values, and m the average value Q, which is the length/width ratio of basidiospores, is given as $Q_m \pm$ standard deviation, where Q_m is the average Q of all basidiospores. The description was submitted to the Faces of Fungi database (Jayasiri *et al.* 2015).

DNA isolation, PCR and sequencing

Genomic DNA isolation from the studied material and PCRs was performed at the Yunnan Academy of Agricultural Sciences, China. Genomic DNA was isolated from dry specimens using Ezup Column Fungi Genomic DNA extraction Kit following the manufacturer's protocol. Primers pairs (Forward / Reverse) for PCR were respectively LR0R (5'-GTACCCGCTGAAGC-3') / LR5 (5'-ATCCTGAGGGAACTTC-3') (Vilgays & Hester 1990) for the nrLSU region and SSUFW105 (5'-TCGCGTTAGCA TCGTTACT AGT-3') / SSUREV475 (5'-GCCAGAGACGCGAACGTTAGTCG-3') (Aanen *et al.* 2002) for the mrSSU region. PCRs were carried out using a C1000 Thermal Cycler (Bio-Rad) with cycling programmes as follows. For nrLSU: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 1.30 min, and a final extension at 72 °C for 10 min. For mrSSU: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 40 °C for 30 s, extension at 72 °C for 54 s, with a final extension at 72 °C for 10 min. The PCR amplicons were sent to Sangon Biotech (Shanghai) for Sanger sequencing in both directions using the PCR primers.

Sequence alignment and phylogenetic analyses

Sequences of a total of 37 specimens representing 25 species (Table 1), including *Lyophyllum decastes* (Fr.) Singer (1949: 165), *L. ambustum* (Fr.) Singer (1943: 105) and *L. semitale* (Fr.) Kühner (1938: 211) as outgroup (Frøslev *et al.* 2003, Saré *et al.* 2014, Mossebo *et al.* 2017), were used in the phylogenetic analyses. The sequences of the two new *Termitomyces* species generated in this study were submitted to the NCBI database. We first used the Basic Local Alignment Search Tool (BLAST) against the GenBank database to check that the newly generated sequences were not the result of PCR amplification of contaminant DNA, and to find taxa with closely related sequences, which were

then retrieved for inclusion in the phylogenetic analyses data set. Sequences were aligned using MAFFT version 7 (Katoh *et al.* 2013) first and then adjusted by hand in BioEdit v.7.0.4 (Hall 2007). Maximum Likelihood (ML) analysis was performed using RAxML-HPC2 v. 8.2.12 (Stamatakis 2014) as implemented on the Cipres portal (Miller *et al.* 2010), with the GTR+G+I model and 1,000 rapid bootstrap (BS) replicates for both genes. A reciprocal 70 % bootstrap support approach was used to check for conflicts between the tree topologies from individual genes. As there was no significant incongruence in topology between the ML trees, the nrLSU and mrSSU sequences were combined using SequenceMatrix (Vaidya 2011) for two-gene, partitioned phylogenetic analyses. For Bayesian Inference (BI), the best substitution model for each partition was determined with the program MrModeltest 2.2 (Nylander 2004) to be GTR+G+I for both nrLSU and mrSSU. Bayesian analysis was performed using MrBayes ver. 3.2.7a (Ronquist 2011) as implemented on the Cipres portal (Miller *et al.* 2010). Two runs of six chains each and sampled every 200 generations were stopped after 485,000 generations, when the average standard deviation of split frequencies went below 0.01. Parameters and tree samples were summarized with a burn-in fraction of 0.25, which was checked against the log likelihood by sampled generation plot. A clade was considered to be strongly supported if showing a bootstrap support value (BS) $\geq 70\%$ and/or a posterior probability (PP) ≥ 0.90 .

TABLE 1. Taxa, voucher specimens and gene information about the sequences used in the phylogenetic analyses; new taxa are in bold; “T” following a species name indicates that the specimen is the holotype of that species

Taxon	Voucher specimen	mrSSU	nrLSU	Reference
<i>Termitomyces aurantiacus</i>	HUYI-DM 152E	KY809186	KY809234	Willis (2007)
<i>T. bulborhizus</i>	KM128338	KY809213	KY809261	Mossebo <i>et al.</i> (2017)
<i>T. brunneopileatus</i> T	DM392	KY809225	KY809273	Mossebo <i>et al.</i> (2017)
<i>T. brunneopileatus</i>	DM394	KY809197	KY809244	Mossebo <i>et al.</i> (2017)
<i>T. clypeatus</i>	KM29920	KY809208	KY809256	Mossebo <i>et al.</i> (2017)
<i>T. clypeatus</i>	KM29150	KY809207	KY809255	Mossebo <i>et al.</i> (2017)
<i>T. entolomoides</i>	tgf10	–	AY232692	Frøslev <i>et al.</i> (2003)
<i>T. entolomoides</i>	tgf103	AY232680	AY232693	Frøslev <i>et al.</i> (2003)
<i>T. eurrhizus</i>	KM142419	KY809218	KY809266	Mossebo <i>et al.</i> (2017)
<i>T. floccosus</i> T	MFLU 19–1312	MN701029	MN633305	This study
<i>T. globulus</i>	DM770	KY809204	KY809252	Frøslev <i>et al.</i> (2003)
<i>T. heimii</i>	Muid.sn	AF357091	AF042586	Moncalvo <i>et al.</i> (2000)
<i>T. heimii</i>	KM16528	KY809205	KY809253	Mossebo <i>et al.</i> (2017)
<i>T. le-testui</i>	DM150G	KY809184	KY809231	Mossebo <i>et al.</i> (2017)
<i>T. le-testui</i>	KM128346	KY809215	KY809263	Mossebo <i>et al.</i> (2017)
<i>T. mammiformis</i>	DM25E	KY809182	KY809229	Mossebo <i>et al.</i> (2017)
<i>T. mammiformis</i>	DM25G	KY809183	KY809230	Mossebo <i>et al.</i> (2017)
<i>T. mboudaeinus</i>	DM223E	KY809189	KY809237	Mossebo <i>et al.</i> (2017)
<i>T. mboudaeinus</i>	DM223	KY809226	KY809274	Mossebo <i>et al.</i> (2017)
<i>T. medius</i>	DM372G	KY809195	KY809243	Mossebo <i>et al.</i> (2017)
<i>T. medius</i>	KM16685	KY809206	KY809254	Mossebo <i>et al.</i> (2017)
<i>T. medius</i> f. <i>ochraceus</i>	DM602B	KY809198	KY809246	Mossebo <i>et al.</i> (2017)
<i>T. microcarpus</i>	PRU3900	AF357092	AF042587	Hofstetter <i>et al.</i> (2002)
<i>T. robustus</i>	KM142418	KY809217	KY809265	Mossebo <i>et al.</i> (2017)
<i>T. robustus</i>	DM436	KY809223	KY809271	Mossebo <i>et al.</i> (2017)
<i>T. sagittiformis</i>	KM109566	KY809212	KY809260	Mossebo <i>et al.</i> (2017)
<i>T. schimperi</i>	DM24E	KY809181	KY809228	Mossebo <i>et al.</i> (2017)
<i>T. singidensis</i>	tgf74	AY232687	AY232713	Frøslev <i>et al.</i> (2003)

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TABLE 1. (Continued)

Taxon	Voucher specimen	mrSSU	nrLSU	Reference
<i>T. striatus</i>	KM142436	KY809219	KY809267	Mossebo <i>et al.</i> (2017)
<i>T. striatus</i> f. <i>bibasidiatus</i> T	DM280B	KY809193	KY809241	Mossebo <i>et al.</i> (2017)
<i>T. striatus</i> f. <i>substriatus</i> T	DM370B	KY809220	KY809268	Mossebo <i>et al.</i> (2017)
<i>T. striatus</i> f. <i>substriatus</i>	DM370G	KY809194	KY809242	Mossebo <i>et al.</i> (2017)
<i>T. subumkowaan</i>	DM260F	KY809190	KY809239	Mossebo <i>et al.</i> (2017)
<i>T. subumkowaan</i> T	DM260B	KY809227	KY809275	Mossebo <i>et al.</i> (2017)
<i>T. titanicus</i>	KM142416	KY809216	KY809264	Mossebo <i>et al.</i> (2017)
<i>T. upsilocystidiatus</i> T	MFLU 19–1289	MN636642	MN636637	This study
<i>Lyophyllum decastes</i>	JM87/16	AF357136	AF042583	Hofstetter <i>et al.</i> (2002)
<i>L. ambustum</i>	CBS450.87	AF357135	AF223214	Hofstetter <i>et al.</i> (2002)
<i>L. semitale</i>	CBS369.47	AF357124	–	Hofstetter <i>et al.</i> (2002)

Results

Phylogenetic analyses

The two-gene dataset contained 72 sequences representing 25 species. The aligned dataset comprised 965 characters including gaps (nrLSU: 1–536, mrSSU: 537–942). The phylogenetic trees generated by Bayesian and ML methods exhibited similar topologies. The phylogenetic analyses support *T. upsilocystidiatus* and *T. floccosus* as two separate species that are clearly different from the other *Termitomyces* in the tree (Fig. 1). *Termitomyces floccosus* and *T. upsilocystidiatus* are, therefore, proposed as new species, represented by the holotypes MFLU19-1312 and MFLU19-1289, respectively.

Taxonomy

Termitomyces floccosus S.M. Tang, Raspé & S.H. Li, *sp. nov.* (Fig. 2)

Mycobank: MB 833128, Faces of Fungi: 06725

Etymology:—the epithet ‘floccosus’ refers to the floccose squamules on the pileus and stipe.

Holotype:—THAILAND, Nakhon Si Thammarat Province, Thung Song District, Tham Yai, 8°10.17′N, 99°44.31′E, 130 m a.s.l., 27 April 2019, collected by *Song-Ming Tang* (MFLU19-1312, holotype!).

Diagnosis:—*Termitomyces floccosus* differs from similar *Termitomyces* species by the white, labile and floccose squamules on the pileus, the broadly conical and poorly individualised perforatorium, and the whitish pseudorrhiza.

Description: *Pileus* 5–6 cm diam., plano-convex, with a broadly conical perforatorium with obtuse apex; surface brownish orange (6C4–6C5) at center, then pale brownish grey (6C2 to 6B2 to 5A–B2), gradually slightly paler toward margin, with white, sparse, and labile floccose squamules; context white, up to 0.6 cm thick under perforatorium. *Lamellae* free, 2–3 mm broad, close, white (1A1) to pinkish white with age; margins fimbriate, concolorous; lamellulae in 1–2 tiers. *Stipe* 2–2.3 × 0.7–0.8 cm, central, subcylindrical, solid, off-white to pale grey, exannulate but more or less densely covered with greyish squamules below a narrow smooth apical. *Pseudorrhiza* strongly tapering; surface whitish to greyish white, longitudinally striate; context solid, fibrous. *Odour* slightly fragrant. *Taste* sweet. *Spore print* pinkish.

Basidiospores [48/2/1] (5.5)5.8–5.98–7.6(8.0) × (3.0)3.2–3.52–4.4 μm, Q = 1.71 ± 0.17, ovoid to ellipsoid, thin-walled, hyaline. *Basidia* (15.0)15.6–22.1(23.0) × (4.5)4.8–6.3(6.8) μm, clavate, sometimes flexuose, with two sterigmata. *Pleurocystidia* 32.0–43.2 × 6.2–6.7 μm, mostly fusiform, sometimes utriform, thin-walled, hyaline. *Cheilocystidia* 25.8–40.4 × 8.5–12.5 μm, mostly utriform, sometimes fusiform. *Hymenophoral trama* 150–200 μm

wide, regular, of thin-walled and hyaline hyphae, 10.2–17.0 µm wide. *Hymenial* layer 200–360 µm wide. *Pileipellis* a cutis, 200–335 µm thick, of radially oriented hyphae, 11.0–12.7 µm wide. *Pseudorrhiza* context of thin-walled, hyaline hyphae, 14.0–23.0 µm wide.

Habitat and distribution: Gregarious on forest soil, above subterranean termitarium. Currently known only from the type locality in Thailand, Nakhon Si Thammarat Province.

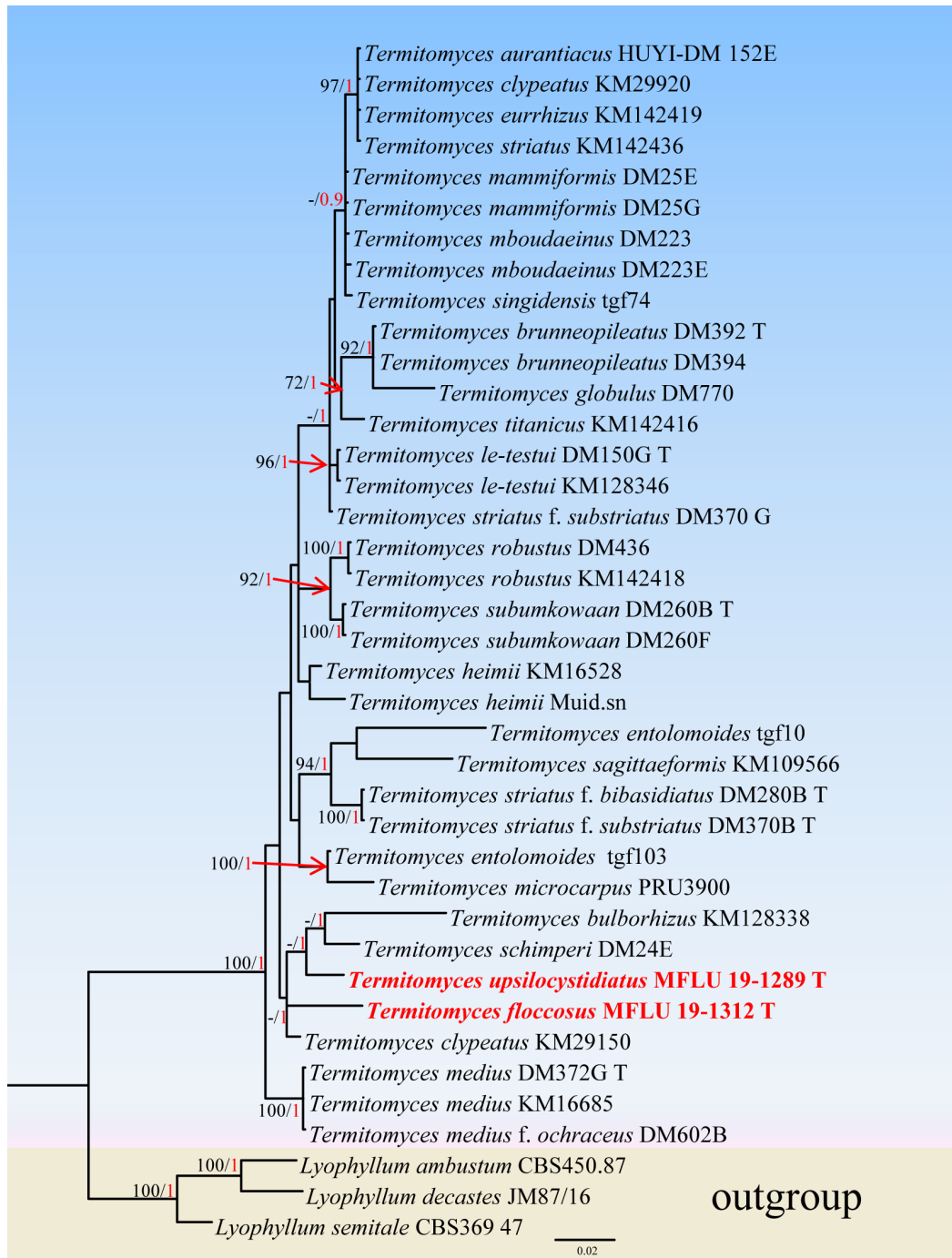


FIGURE 1. Majority rule consensus “Bootstrap consensus tree of *Termitomyces* from Bayesian analysis of combined mrSSU and nrLSU sequences, rooted with *Lyophyllum decastes*, *L. ambustum* and *L. semitale*. Bayesian Posterior Probability (PP) ≥ 0.9 and Bootstrap Support (BS) values $\geq 70\%$ are indicated at the nodes (BS/PP). The two new species are in red. “T” refers to sequences from type specimens.

Notes:—Only a handful of *Termitomyces* species with squamules on the pileus and stipe have been described to date. These are *T. lanatus* R. Heim (1977: 97), *T. magoyensis* Otieno (1964: 112), *T. mammiformis* R. Heim (1942b: 147), *T. reticulatus* Van der Westh. & Eicker (1990: 928), *T. schimperi*, and *T. singidensis* Saarim. & Härk (1994: 16) (Heim 1977, Tibuhwa *et al.* 2010). The first two species are somewhat similar to each other and differ from *T.*

floccosus by their blackish brown farinose squamules over a whitish pileipellis, while the latter is characterized by whitish squamules over a pale brownish grey pileipellis. Moreover, *T. lanatus* produces a dark brown pseudorrhiza, whereas that of *T. floccosus* is white. *Termitomyces magoyensis* also shows a prominent annulus, which is not present in *T. floccosus*. *Termitomyces mammiformis* is similar to *T. floccosus* in having labile, pale squamules on the pileus, as well as in basidiome size, but the perforatorium is markedly different in the two species. In the former, it is prominent, clearly delimited, cylindrico-conical, scrobiculate, and dark brown, whereas in the latter, it is low, broadly conical, not clearly delimited, and brownish orange. *Termitomyces singidensis* and *T. floccosus* both have pale squamules on the pileus, but the pileipellis of the former is deep brown, whereas it is much paler in *T. floccosus*. Moreover, *T. singidensis* has a sharply pointed perforatorium, whereas *T. floccosus* has a broadly conical, obtuse perforatorium. Finally, *T. reticulatus* is quite different from *T. floccosus* in having umber brown scales over a white to silvery pileipellis, and, most remarkably, a prominent annulus, which is lacking in *T. floccosus*. Phylogenetically, *T. floccosus* collections clustered with *T. schimperi*, *T. bulborhizus* T.Z. Wei, Y.J. Yao, Bo Wang & Pegler (2004: 1458) (KM128338), and *T. clypeatus* R. Heim (1951: 207) (KM29150) but without statistical support.

***Termitomyces upsilocystidiatus* S.M. Tang, Raspé & K.D. Hyde, sp. nov. (Fig. 3)**

Mycobank: MB 833137, Faces of Fungi: 06724

Etymology:—the epithet ‘upsilocystidiatus’ refers to Y-shaped hymenial cystidia.

Holotype:—CHINA. Yunnan Province, Dali prefecture, Nan-Jian county. 25°02.376'N, 100°30.174'E. 1,500 m a.s.l., 3 September 2016, collected by Hong-Yan Su (MFLU19-1289, holotype!).

Diagnosis:—*Termitomyces upsilocystidiatus* sp. nov. differs from similar species by its mostly Y-shaped cheilocystidia, and bulbous stipe base.

Description:—*Pileus* 5–11.3 cm diam., depressed, with large, obtuse umbo-shaped perforatorium, frequently cleaving toward margin; surface pale brownish grey (4B2–6C2–3), gradually slightly paler toward margin, glabrous. *Lamellae* free, 0.8–1.0 cm broad, crowded, white (1A1) to pinkish white with age; margins fimbriate, irregularly eroded to cleaved at places, concolorous, lamellulae in 1–2 tiers. *Stipe* 4.5–5.5 × 1.5–1.7 cm, central, cylindrical, with bulbous base up to 3 cm, exannulate, off-white, smooth, glabrous, solid. *Pseudorrhiza* cylindrical, tapering, white, longitudinally striate, solid, fibrous. *Context* white, up to 4 mm thick under perforatorium. *Odour* slightly fragrant. *Taste* sweet. *Spore print* pinkish.

Basidiospores [49/10/2] (4.8)4.9–5.56–6.7(6.8) × (3.5)3.6–3.74–4.1(4.2) μm, Q = 1.48 ± 0.11, ovoid to ellipsoid, thin-walled, hyaline. *Basidia* (18.0)18.5–21.5(24.0) × (5.8)6.0–7.3(8.0) μm, clavate to slightly flexuose with one, two or four sterigmata. *Cheilocystidia* 36.0–59.1 × 12.3–23.0 μm, typically Y-shaped, sometimes merely gibbose or triangular, thin-walled, hyaline. *Pleurocystidia* 45.6–76.9 × 19.8–38.6 μm, fusiform. *Hymenophoral trama* 131–205 μm wide, regular, thin-walled, hyaline hyphae, 6–13 μm wide. *Hymenial* layer 300–400 μm wide. *Pileipellis* a cutis 300–440 μm thick, of radially oriented hyphae, 15.0–18.0 μm wide. *Pseudorrhiza context* of thin-walled, hyaline hyphae, 9.3–12.0 μm wide.

Habitat and distribution: Gregarious to scattered, in plantation or forest, above subterranean termitaria. Currently known only from Yunnan Province, China.

Notes:—*Termitomyces upsilocystidiatus* is morphologically distinguished from other *Termitomyces* species with greyish to greyish brown pileus by its mostly Y-shaped cheilocystidia. Macroscopically, *T. upsilocystidiatus* is most similar to *T. umkowaan* and *T. subumkowaan*, with which it shares the general aspect of the pileus (colour, shape of perforatorium, cleaving in age, and, to a lesser extent, size) and stipe (pale colour, absence of annulus and squamules, swelling at the base). However, it clearly differs from those two species by the Y-shape of the cheilocystidia, as *T. umkowaan* (Cooke & Masee) D.A. Reid (1975: 118) and *T. subumkowaan* Mossebo (2002: 118) produce mostly clavate, scattered or rare cheilocystidia (Mossebo *et al.* 2002, Van der Westhuizen & Eicker 1990). Moreover, *T. umkowaan* macroscopically differs from *T. upsilocystidiatus* by somewhat larger basidiomes (pileus up to 22 cm in diam.), while *T. subumkowaan* differs from the former two species by its blackish pseudorrhiza (Mossebo *et al.* 2002, Van der Westhuizen & Eicker 1990). Another similar species is the common *T. eurhizus*, which differs from *T. upsilocystidiatus* by the darker (umbrinous) perforatorium, the black-brown encrusted pseudorrhiza, and the obovoid to pyriform hymenial cheilocystidia (Pegler & Vanhaecke 1994). Phylogenetic analyses confirmed *T. upsilocystidiatus* is different from other sequenced *Termitomyces* species, including *T. subumkowaan* and *T. eurhizus*. *Termitomyces umkowaan*, however, was not included in the phylogenetic analyses because of the unavailability of sequences.

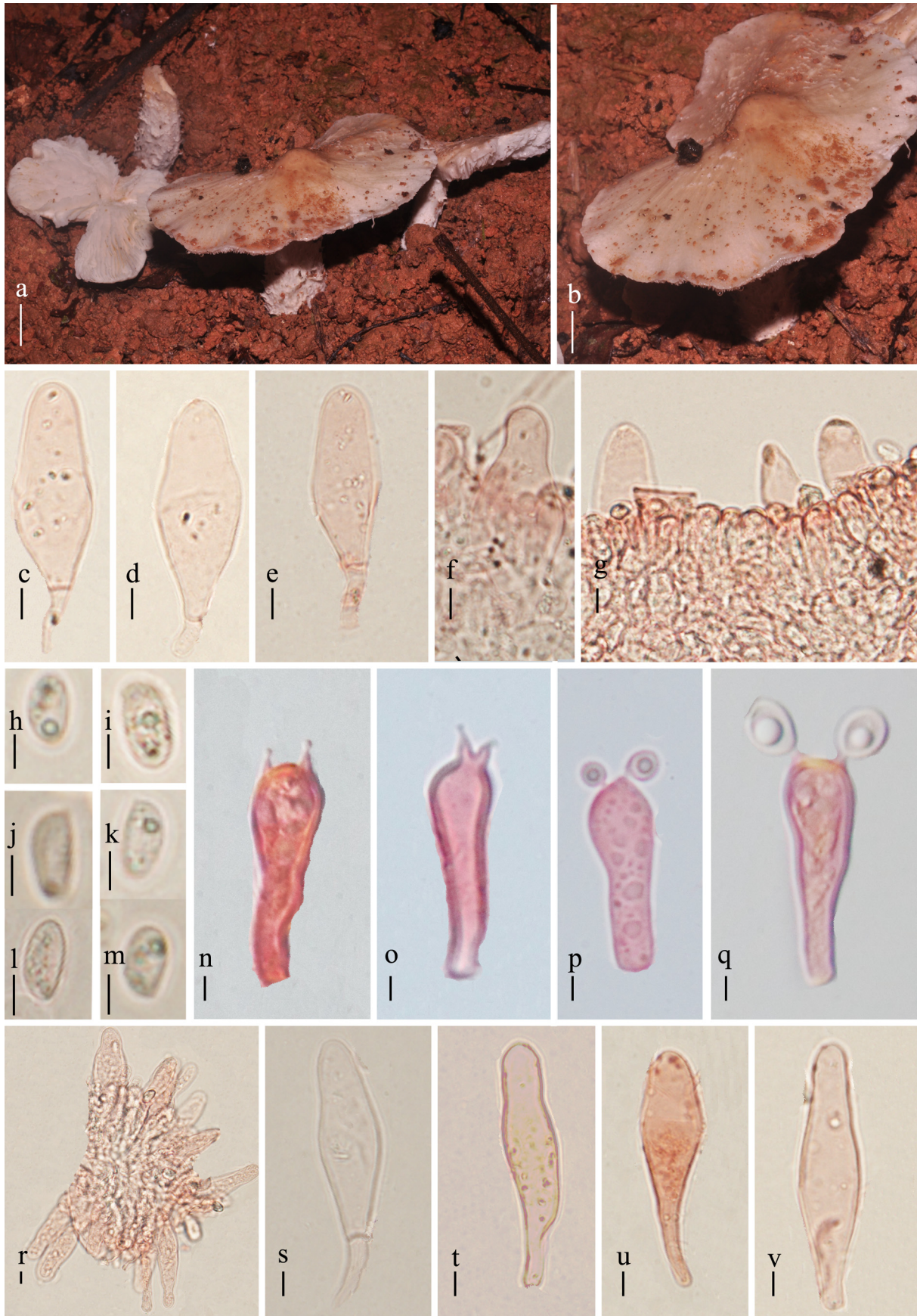


FIGURE 2. *Termitomyces floccosus* a–b. basidiomes; c–g. pleurocystidia; h–m. basidiospores; m–q. basidia; r–v. cheilocystidia. Scale bars: a–b = 1 cm; c–v = 5 μ m. Photos by: Song-ming Tang.



FIGURE 3. *Termitomyces upsilocystidiatus*. a–b. basidiomes; c–h. cheilocystidia; i–k pleurocystidia; l–p. basidia; q–u. basidiospores. Scale bars: a = 2 cm; c–k = 5 μ m, l–p = 2 μ m, q–u = 1 μ m. Photos by: Song-ming Tang.

Discussion

The genus *Termitomyces* comprises ca. 34 species (He *et al.* 2019), most of which are well-known for nutritional and medicinal values (Zhao *et al.* 2019, Maity *et al.* 2020). Fifteen *Termitomyces* species show a greyish to grey-brown pileus. Thirteen of them are known species and two are the new species introduced in this study. Major characteristics of these *Termitomyces* species are listed in Table 2.

The original descriptions of a number of *Termitomyces* species, such as *T. albuminosus* (Berk.) R. Heim (1941:72), *T. eurrhizus*, *T. lanatus*, or *T. microcarpus* are not clear or complete enough. Moreover, according to Saarimäki *et al.* (1994), the original material of *T. lanatus* has been lost. Re-examination and more detailed descriptions of these species, accompanied by reliable phylogenetic analyses and/or, if the type is missing or in poor condition, careful epitypification is needed to more clearly and unequivocally characterize these species, and to allow for more reliable comparisons with putatively new species.

TABLE 2. Selected key morphological characteristics of *Termitomyces* species with grey to grey-brown pileus; the two new species are in boldface

Taxa	Pileus colour	Pileus diameter	Perforatorium	Stipe	Cheilocystidia	Pleurocystidia	References
<i>T. chypeatus</i> (Beeli) R. Heim	Dark grey-brown at first, then light grey	2.5–10.0 cm	Narrowly conical, acute	Cylindrical to subfusiform	Subglobose to pyriform	Subglobose to pyriform	Heim (1951)
<i>T. entolomoides</i> R. Heim	Dark bluish-grey	4.0–4.5 cm	Conical obtuse	Subbulbous, somewhat fibrous-sealy	Broadly ovoid to clavate to obpyriform	Broadly ovoid to clavate to obpyriform	Heim (1977), Pegler (1994)
<i>T. epipolius</i> (Singer) L.D. Gómez	Grey	2.5–4.2 cm	-	Pruinose	Utriform	Absent	Singer (1989)
<i>T. eurhizius</i> (Berk.) R. Heim	Umbrinous at center, elsewhere greyish-brown	6–15(24) cm	Obtusely rounded	Cylindrical to subbulbous	Obovoid to pyriform	Obovoid to pyriform	Heim (1942b)
<i>T. floccosus</i>	Brownish grey	5.0–6.0 cm	Broadly conical, obtuse	Cylindrical, squamulose	Mostly utriform	Mostly fusiform	This study
<i>T. intermedius</i> Har. Takah. & Taneyama	Grey-brown to brownish	6.0–10.0cm	Conical obtuse	Cylindrical, smooth	Absent	Clavate	Huang <i>et al.</i> (2017)
<i>T. lanatus</i> R. Heim	Grey	10.0 cm	Obtuse	Cylindrical	-	-	Heim (1977)
<i>T. mammiformis</i> R. Heim	Greyish-white	4.0–7.0 cm	Sharp and scrobiculate	Squamose	-	-	Heim (1977)
<i>T. medius</i> R. Heim & Grassé	Yellowish to brownish-grey	3.0 cm	Sharp	Cylindrical	-	-	Heim (1977)
<i>T. radicans</i> (Beeli) Natarajan	Orange-white to orange-grey	3.0–5.0 cm	Sharp	Cylindrical, smooth	Absent	Absent	Natarajan (1977)
<i>T. sagittiformis</i> (Kalchbr. & Cooke) D.A. Reid	Greyish-sepia, paler toward margin	2.0–6.0 cm	-	Subbulbous, fibrillose-striate	-	-	Kalchbrenner (1881)
<i>T. subumkwaan</i> Mossebo	Pale greyish-brown to ochraceous	Up to 12 cm	Conical obtuse to acute	Fusiform to sub-bulbous base, smooth	Clavate to pyriform	Clavate to pyriform, extremely rare	Mossebo (2002)
<i>T. titanicus</i> Pegler & Pearce	Ash-grey with dark brown patches	40–100cm	Low broad	Cylindrical, squamose	Clavate	Clavate	Pegler & Pearce (1980)
<i>T. umkwaan</i> (Cooke & Massee) D.A. Reid	Cream pale-grey to brown-buff	7.5–10.0 cm	Pointed to broadly conical	Fusiform to subbulbous base, smooth	Broadly clavate to pyriform	Broadly clavate to pyriform, few	Cooke (1889), Van der Westuizen & Eicker (1990)
<i>T. upsilocystidiatus</i>	Pale brownish grey	10.0–11.3 cm	Obtuse umbo	Sub-bulbous, smooth	Mostly Y-shaped	Oblong	This study

Based on phylogenetic analyses including seven described species of *Termitomyces*, Aanen *et al.* (2002) inferred at least five migrations between Africa and Asia. However, since the diversity of *Termitomyces* in Asia is still poorly known (Wei *et al.* 2006, Ye *et al.* 2019), the biogeographical history of the genus should be re-evaluated when the species diversity of the genus in Asia, and possibly also in Africa, has been better characterized.

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