

A Survey of *Termitomyces* (Lyophyllaceae, Agaricales), Including a New Species, from a Subtropical Forest in Xishuangbanna, China

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ABSTRACT

A survey of mushrooms was conducted in Xishuangbanna, Yunnan Province, China, in the rainy season (May to October) of 2012, 2013, and 2014, during which 16 specimens of *Termitomyces* were collected. Preliminary macro- and micro-characteristics, together with ITS sequence data, showed that four of the specimens belonged to a new species (*Termitomyces fragilis*), while the other 12 belonged to *T. aurantiacus*, *T. eurhizus*, *T. globules*, *T. microcarpus*, and *T. bulborhizus*. In this paper, *T. fragilis* is introduced as a species new to science based on morphological characterization and phylogenetic analyses. Macro- and micro- morphological descriptions, color photographs and line drawings of the new species, and a phylogenetic tree to show the placement of the new species are provided. *T. fragilis* is then compared with other closely related taxa in the genus *Termitomyces*.

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1. Introduction

In 1942, R. Heim described specimens of a mushroom genus that exhibited a close symbiotic relationship with termites (Isoptera) [1]. In recognition of this termite-fungal mutualistic association, the genus was named as *Termitomyces* [2–6]. *Termitomyces* are highly sought-after mushrooms, due to their high culinary value, providing many rural households with additional income as well as nutrition [7–15].

Various species of *Termitomyces* are widely distributed in Africa and Southeast Asia [16–19], and several taxa have also been recorded in Central America [20]. Molecular phylogenetic analysis reveals that *Termitomyces* forms a monophyletic clade in Agaricales [21–23], although genetically they exhibit a degree of differentiation between Asia and Africa [6]. The southern part of the Yangzi River basin in China is rich in wild fungal resources such as *Termitomyces* [13,24–26]. In the southwestern province of Yunnan, *Termitomyces* mushrooms are locally famed for their taste and association with termites. Locally they are known as “Jizong”, meaning chicken-mushroom (due to the texture of the mushroom flesh), or “Yizong”, meaning ant-planted mushroom [25,27–29].

Xishuangbanna is a tropical county located in south Yunnan Province, China, with abundant

tropical vegetation resources providing suitable habitats for a wide range of insects, and more specifically, termites [30]. Xishuangbanna is also home to a high diversity of *Termitomyces* species, which are collected for consumption and for sale in markets, providing additional income to rural households [31–33].

As a result of previous studies, more than 400 samples of *Termitomyces* have been deposited in different fungal herbaria in China [13], but sequence data, especially nuclear ribosomal internal transcribed spacer (ITS) gene, is largely unavailable in GenBank, and there are few publications relating to this genus. Many of the past research studies investigating *Termitomyces* have aimed to study their relationship with termites, ignoring the taxonomy and phylogeny of this genus [22,34–39]. Recent DNA-based studies of *Termitomyces* are limited; for example, in Asia, Sawhasan et al. published DNA data for 13 species of *Termitomyces* from Thailand, and Adhikari and Durrieu published DNA data for five species of *Termitomyces* from Nepal, and Mossebo et al. published new taxa result inferred from combined nuclear ribosomal large subunit (nLSU) and mitochondrial small subunit ribosomal DNA (mtSSU-rDNA) sequences of *Termitomyces* [40–42]. There are, however, no further DNA-based publications of *Termitomyces* in Asia.

Based on the need for further research on the *Termitomyces* genus, we set out to study the composition of *Termitomyces* species in a subtropical rainforest in Xishuangbanna, Yunnan Province, China. In this study, we provide molecular based descriptions of the new species; morphological descriptions of all the collected specimens; and a record of the related habitats.

2. Materials and methods

2.1. Specimen collection and macro morphological descriptions

All 16 specimens were collected during the rainy season (May to October), from 2012 to 2014, in Mengsong, Xishuangbanna, Yunnan Province, China. The vegetation cover at the collection sites was dominated by *Camellia sinensis* var. *assamica*, *Castanopsis mekongensis*, *Schima wallichii*, and *Quercus acutissima*. Specimens were photographed *in situ*, then gathered and wrapped in aluminum foil, or kept separately in a collecting box in order to avoid mixing or crushing of specimens, and finally returned to the laboratory for further analysis and characterization. Odor and color changes upon bruising were recorded at the time of collection. Description of macro-characteristics, chemical testing, and further photographing of fresh samples were carried out soon after returning to the field station each day. This was done in accordance with the methodology described by Largent [43,44]. The Kornerup and Wanscher method was used to describe color terms, where a specific code is assigned to an observed color (chromotaxy) [45]. Specimens were dried below 40 °C in a food drier, sealed in plastic bags, and deposited in the Kunming Institute of Botany herbarium (HKAS). Facesoffungi numbers and Index Fungorum numbers were obtained as detailed in Jayasiri et al. and Index Fungorum [46,47].

2.2. Micro morphological description

Micro-morphological features were documented by examining dried specimens following the methods of Largent [48]. For micro-morphological examination, sections were cut with a razor blade from dried specimens and mounted on slides in 5% KOH and Congo red, and then observed, measured and illustrated using a compound microscope (Zeiss Axioskop 40). In the description of the basidiospores, “*n*” indicates the number of basidiospores (20 basidiospores per collection) which were measured; [a/b/c] in the taxonomic description refer to the following: a = number of basidiospores measured, b = number of fruiting bodies and c = number

of collections; Lm = mean basidiospore length over a population of basidiospore; Wm = mean basidiospore width over a population of basidiospore; Q = “Length/width ratio” (l/w) of a basidiospore in side view; Qm = average Q of all basidiospores measured.

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted from three dried specimens of the new species using a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P. R. China). The ITS gene region of the new species was amplified. Amplification was performed in 25 µl volumes containing 1.0 µl template DNA, 9.5 µl double distilled water, 1.0 µl of each primer (ITS1/ITS4) [49] and 12.5 µl of 2× power Taq PCR Master Mix (A pre-mix and ready to use solution, including 0.1 Units/µl Taq DNA Polymerase, 500 µM dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCl (pH8.3), 100 mM KCl, 3 mM MgCl₂, stabilizer and enhancer. The reaction was carried out with 35 cycles under the following conditions: denaturation (95 °C, 30 s), annealing (52 °C, 30 s), extension (72 °C, 1 min), and final extension (72 °C, 10 min). The primers used for sequencing the whole ITS region were the same as those used in White et al. [49]. Amplified products were confirmed with 1% agarose gel electrophoresis stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (Beijing Bai Mai Hui Kang Biological Engineering Technology Co., Beijing, P.R. China). The newly generated sequence data of the new species were deposited in GenBank.

2.4. Sequence alignment and phylogenetic analyses

The taxon information and GenBank accession numbers used in the molecular analyses are listed in Table 1. All the available reliable nrITS sequences were retrieved from the GenBank. Sequences for each species were aligned using Clustal X [50]. Alignments were manually adjusted to allow for maximum sequence similarity. Gaps were treated as missing data. Unweighted maximum parsimony (MP) analysis was performed using PAUP 4.0b10 [51]. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max trees were unlimited, branches of zero length were collapsed, and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa [52].

Bayesian phylogenetic analyses were performed with MrBayes 3.2.2 [53–55]. Four Markov chains were run for 1,000,000 generations and sampled every 100th generation, resulting in 1000 trees. Those trees sampled prior to searches reaching a split deviation frequency value reaching 0.01 were discarded as the burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (PP) of the individual clades. Trees were viewed in Treeview and exported to Photoshop [56].

3. Results

3.1. Taxonomy of *Termitomyces fragilis*

Among the 16 specimens that were collected from the study plots (see Table 2), four specimens are

Table 1. Taxa information and GenBank accession numbers of *Termitomyces* specimens used in the molecular phylogenetic analysis. New sequences are in black bold.

Species name	GenBank number	Source
<i>Lyophyllum connatum</i>	HE819396	Unpublished
<i>L. infumatum</i>	JF908334	Osmundson et al. [65]
<i>Termitomyces albiceps</i>	HM230658	Unpublished
<i>T. aurantiacus</i>	JN585945	You et al. [66]
<i>T. bulborhizus</i>	HM230663	Unpublished
<i>T. cartilagineus</i>	AF079070	Unpublished
<i>T. clypeatus</i>	HQ702547	Unpublished
<i>T. cylindricus</i>	LC068786	Unpublished
<i>T. eurrhizus</i>	KJ620056	Unpublished
<i>T. eurrhizus</i>	KU179194	Zhang et al. [67]
<i>T. fragilis</i>	KY214475	this study
<i>T. fragilis</i>	KY214476	this study
<i>T. fragilis</i>	KY214477	this study
<i>T. fuliginosus</i>	LC068788	Unpublished
<i>T. heimii</i>	KP943503	Unpublished
<i>T. medius</i>	KJ768983	Unpublished
<i>T. microcarpus</i>	HM230661	Unpublished
<i>T. microcarpus</i>	KP780436	Unpublished
<i>T. radicans</i>	LC068787	Unpublished
<i>T. sp.</i>	AB051882	Katoh et al. [34]
<i>T. sp.</i>	AB051879	Katoh et al. [34]
<i>T. sp.</i>	AF321375	Rouland-Lefevre et al. [23]
<i>T. sp.</i>	AB073519	Unpublished
<i>T. sp.</i>	AB051880	Katoh et al. [34]
<i>T. striatus</i>	AF321367	Rouland-Lefevre et al. [23]
<i>T. umkowaan</i>	KJ703245	Unpublished

Table 2. List of *Termitomyces* species recorded in subtropical forests in Xishuangbanna, Yunnan, China. Information regarding the species, habitat, and local names is provided.

Species of <i>Termitomyces</i>	specimen code	Notes of <i>Termitomyces</i> fruiting habitat, local name and associated plant information
<i>T. aurantiacus</i>	HKAS88903	Fruiting on soil substrates, local name is “red Jizong”. Found in mixed tea plantation dominated by <i>Camellia sinensis</i> var. <i>assamica</i> and <i>Castanopsis calathiformis</i> trees.
<i>T. bulborhizus</i>	HKAS88900	Fruiting on soil substrates, with rich tree leaf-litter covering, local name is “yellow skin Jizong” or “yellow Jizong”. Found in secondary forest dominated by <i>Schimawallichii</i> (Candolle) Korthals, <i>Dendrocalamus membranaceus</i> Munro and <i>Pueraria</i> trees.
	HKAS88902	
	HKAS88908	
	HKAS88910	
<i>T. eurrhizus</i>	HKAS88914	Fruiting on soil substrates, with rich tree leaf-litter covering, local name is “big Jizong” or “chicken mushroom”. Found in mixed tea plantation dominated by <i>Camellia sinensis</i> var. <i>assamica</i> (J. W. Masters) Kitamura and <i>Castanopsis calathiformis</i> (Skan) Rehder and E. H. Wilson trees.
	HKAS88911	
	HKAS88915	
<i>T. globulus</i>	HKAS88904	Fruiting on soil substrates, with rich tree leaf-litter covering, local name is “big Jizong” or “round-root Jizong”. Found in mixed tea plantation dominated by <i>Camellia sinensis</i> var. <i>assamica</i> and <i>Castanopsis calathiformis</i> trees.
	HKAS88905	
<i>T. microcarpus</i>	HKAS88901	Fruiting on soil substrates, with mosses covering, local name is “Small Jizong” or “Jizong flower”. Found in secondary forest dominated by <i>Schimawallichii</i> , <i>Dendrocalamus membranaceus</i> and <i>Pueraria</i> trees.
	HKAS88907	

proposed to be a new species to science; of these, all four specimens were shown to be the same species, as described below.

Termitomyces fragilis L. Ye, Karun, J.C. Xu, K. D. Hyde and Mortimer *sp. nov.* As shown in Figure 1, Figure 2 and Figure 3.

Index Fungorum no: IF552544, FoF number: 02690

Etymology: the etymology refers to the fragile stipe of the new species.

Holotype: China. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N, 100.49433°E, alt. 1656 m, August 14 2012, Lei Ye (HKAS88912).

Diagnosis: The new species *T. fragilis* is characterized by small basidiocarps, with a hollow and fragile stipe. It grows in shallow soils that are rich in organic matter, and is associated with termites. Micro-morphologically, it is characterized by larger basidia (20–35 × 7.0–10.0 μm) and cystidia (Cheilocystidia 40–80 × 12–31 μm; Pleurocystidia 47–75 (–80) × 14–32 μm), relative to other members of the genus *Termitomyces*.

Pileus (1.2–)2.2–4.5(–5.4) cm, cuspidate, conical becoming applanate when mature, with small and obtusely pointed perforatorium; surface brownish gray (9D2) or grayish brown (5D3) at center; mid of center is darker: brownish gray (9E2) or gray (7E1); reddish gray (9B2), brownish-gray (6C2), grayish-white (1B1), light gray (1C1) elsewhere and mixed with tiny white filamentous striate (1A1); glabrous and smooth, paling toward margin, radially striate; margin straight, splitting when mature. **Lamellae** free, (1.0)2.0–4.0(5.0) mm wide, white (1A1) when young and becoming pinkish white (7A2) when mature, crowded, with lamellulae. **Stipe** (2.7) 4.5–7.0(7.5) × (0.1)0.2–0.4(0.5) cm, central, cylindrical, equal; the base of stipe abrupt or forming a root-like terminal of (1.5–)2.7–4.5(–7.2) × (0.05–)0.15–0.25(–0.4) cm size underground level, in



Figure 1. *Termitomyces fragilis*. (A–C) Mature basidiocarps in the field (A: HKAS88912, B: HKAS88909, C: HKAS88906); (D) Young basidiocarps in the field (HKAS88913), Scale bars: Lengths of each bar are A = 5 cm, B = 2 cm, C = 2.5 cm, D = 0.5 cm.

contact with termites' fungi-garden and mixed substrates such as wood, soil and litter; surface white (1A1), smooth and glabrous, longitudinally striate, fibrous and fistulose to broadly fistulose, frangible, of longitudinally parallel hyphae, thin-walled and hyaline, 2.0–7.0(10) μm diam. **Context** narrow, fleshy, white (1A1), (0.5–)1–2.5(–4) mm wide, of inflating hyphae, thin-walled and hyaline, normal hyphae 2.5–7.5 μm diam. **Basidiospore** colorless, ovoid to ellipsoid, [100/7/4] 9.0–10.0(–10.5) \times 5.5–6.5(–7.5) μm , $Q = 1.0\text{--}2.0$, $Q_m = 1.2 \pm 0.2$; thin-walled and subhyaline. **Basidia** 20–35 \times 7.0–10.0 μm , clavate, bearing four sterigmata, thin-walled, subhyaline. **Cheilocystidia** 40–80 \times 12–31 μm , clavate to pyriform, thin-walled and hyaline. **Pleurocystidia** 47–75(–80) \times 14–32 μm , similar to cheilocystidia. **Pileipellis** comprising 2 layers, lower layer cells are much bigger than upper layer, hyphae 2–5 μm diam in upper layer, in lower layer, hyphae 10–35 μm diam, radial hyphae, ovoid, a repent epicutis of narrow, repent, thin-walled.

Habitat and Distribution: Usually scattered (few solitary) on soil with decaying litter under which

termites have built their nests, associated with *Camellia sinensis* and *Castanopsis calathiformis* trees, Yunnan Province, China.

Material examined: China. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N, 100.49433°E, alt. 1656 m, August 14 2012, *Lei Ye* (HKAS88912, *holotype*, Accession KY214475); *Ibid.*, Mengsong Village, September 20 2012, *Lei Ye* (HKAS88909, *paratype*, Accession KY214476); *Ibid.*, Mengsong Village, June 23 2013, *Lei Ye* (HKAS88906, *paratype*, Accession KY214477); *Ibid.*, Mengsong Village, August 14 2012, *Lei Ye* (HKAS88913, *paratype*); *Ibid.*

Notes: The new species *T. fragilis* morphologically closely resembles the small basidiocarps of *T. microcarpus*, *T. tylerianus*, and *T. clypeatus* (see discussion).

Taxonomy of the remaining *Termitomyces* specimens (Basidiocarps see Figure 4)

Termitomyces aurantiacus (R. Heim) R. Heim, *Termites et Champignons* (Paris): 56 (1977)

Note: Termite-symbiotic fungi, soil habitat, local name is “red Jizong”. Edible.

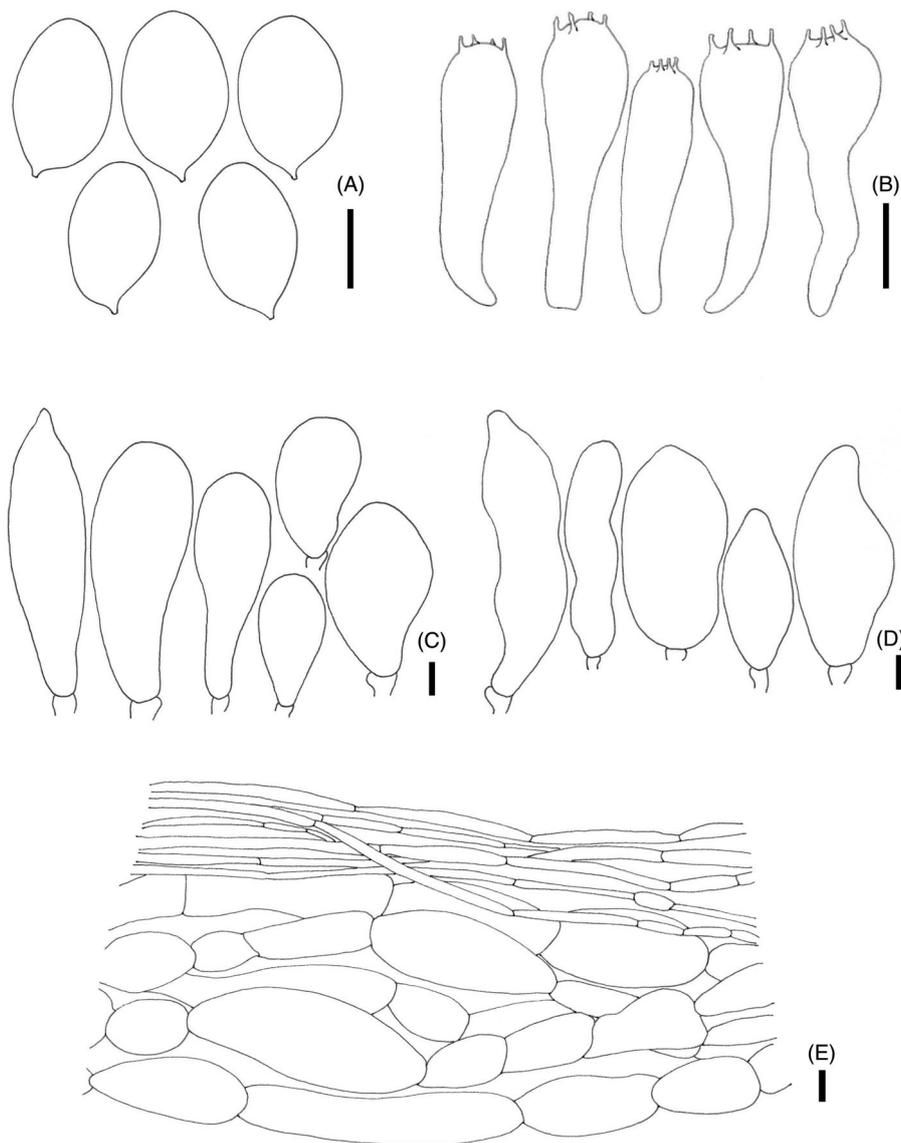


Figure 2. *Termitomyces fragilis* (A) Basidiospores (Bar = 5 μm); (B) Basidia (Bar = 10 μm); (C) Cheilocystidia (Bar = 10 μm); (D) Pleurcystidia (Bar = 10 μm); (E) Pileipellis (Bar = 10 μm).

Material examined: China. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N, 100.49433°E, alt. 1656 m, July 11 2013, *Lei Ye* (HKAS88903, *paratype*); *Ibid.*

Termitomyces bulborhizus T.Z. Wei, Y.J. Yao, Bo Wang and Pegler, *Mycol. Res.* 108(12): 1458 (2004)

Note: Termite-symbiotic fungi, soil habitat, leaf-litter substrates covering, local name is “yellow skin Jizong” or “yellow Jizong”. Edible (high culinary value).

Material examined: CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N, 100.49433°E, alt. 1656 m, October 11 2012, *Lei Ye* (HKAS88910, *paratype*); *Ibid.* August 29 2013, *Lei Ye* (HKAS88908, *paratype*); *Ibid.* August 04 2014, *Lei Ye* (HKAS88914, *paratype*); *Ibid.* Mengsong Village, August 29 2014, *Lei Ye* (HKAS88900, *paratype*); *Ibid.* September 05 2014, *Lei Ye* (HKAS88902, *paratype*); *Ibid.*

Termitomyces eurrhizus (Berk.) R. Heim [as “eurhizus”], *Arch. Mus. Hist. Nat. Paris, ser. 6* 18: 140 (1942)

Note: Termite-symbiotic fungi, soil habitat, leaf-litter substrates covering, local name is “big Jizong” or “big chicken mushroom”. Edible (high culinary value).

Material examined: CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N, 100.49433°E, alt. 1656 m, October 14 2012, *Lei Ye* (HKAS88911, *paratype*); *Ibid.* July 12 2014, *Lei Ye* (HKAS88915, *paratype*); *Ibid.*

Termitomyces globulus R. Heim and Gooss.-Font., *Bull. Jard. bot. État Brux.* 21: 216 (1951)

Note: Termite-symbiotic fungi, soil habitat, the pseudorhiza links with insect nest in deeper soil layers, local name is “big Jizong” or “Round-root Jizong”. Edible (high culinary value).

Material examined: CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N,

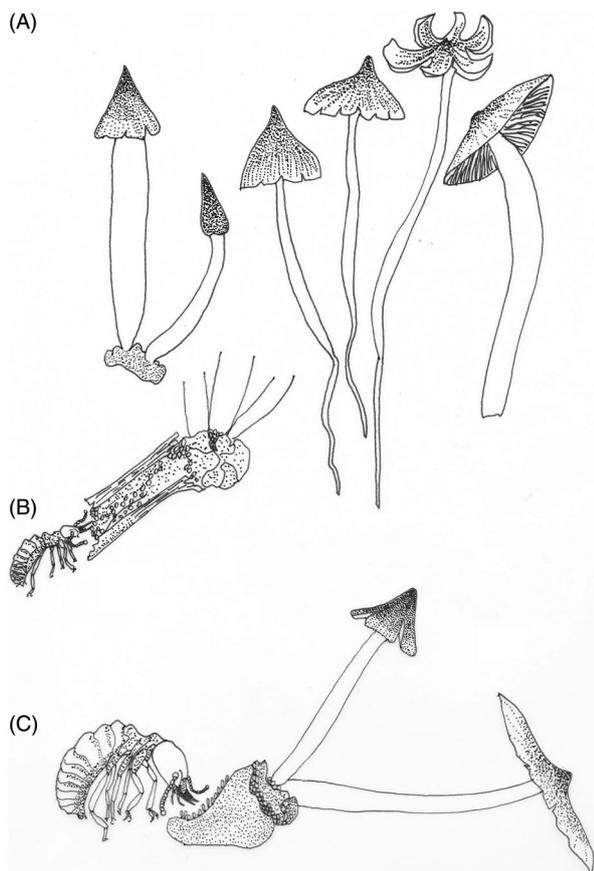


Figure 3. Illustration of the shape and habitat characteristics of *T. fragile* fruiting bodies (this picture only as a schematic view, without reference to the real proportion and morphology). (A) basidiocarps with root-like stipe base or normal base; (B) a termite (Isoptera) uses plant tissue to make a fungi-garden under the basidiocarps; (C) a termite works on a fungi-garden underground, this part of the substrate is dense and in close contact with the soil.

100.49433°E, alt. 1656 m, September 10 2013, *Lei Ye* (HKAS88904, *paratype*); *Ibid.*, Mengsong Village, September 15 2013, *Lei Ye* (HKAS88905, *paratype*); *Ibid.*

Termitomyces microcarpus (Berk. and Broome)

R. Heim, *Arch. Mus. Hist. Nat. Paris, ser. 6* 18: 128 (1942)

Note: Termite-symbiotic fungi, thin-layer soil habitat, moss substrates covering, local name is “Small Jizong” or “Jizong flower”. Edible.

Material examined: China. Yunnan Province: Xishuangbanna, Mengsong Village, 21.49894°N, 100.49433°E, alt. 1656 m, August 07 2013, *Lei Ye* (HKAS88907, *paratype*); *Ibid.*, September 01 2014, *Lei Ye* (HKAS88901, *paratype*); *Ibid.*

4. Discussion

DNA from the 16 *Termitomyces* specimens was extracted and sequenced several times using primers (ITS (ITS1, ITS5, ITS4); SSU (SSUFW105, SSUREV4757); nLSU (LROL, LR5)) and PCR protocols based on recent references. However, only the

ITS sequences from the collection of new species proved successful. The fresh samples collected in the first and second years gave good sequences, while the other older samples did not provide good sequence results, so we used only morphological descriptions to identify those specimens. The morphological characteristics clearly showed the remaining 12 specimens as belonging to five distinct, previously described species.

Termitomyces fragilis is described as having small to medium basidiocarps. We made morphological comparisons between our new species and three closely related species that also have small basidiocarps: *T. microcarpus* [25], *T. tylerianus* [57], and *T. clypeatus* [58]. Basidiocarps of *T. fragilis* are morphologically very similar to *T. microcarpus*; basidiocarps of both species are small and generally, the pseudorhiza are absent. The colors of the pileus in the two species are very similar, but the color in the center part of the pileus of *T. fragilis* turns to reddish-gray upon bruising. Lamellae of *T. microcarpus* are white at first, becoming pinkish when mature, but in *T. fragilis* the lamella is white when young, becoming pinkish-white when mature. The stipe of *T. microcarpus* is solid and strong, but the stipe of *T. fragilis* is fistulose to broadly fistulose, and fragile. Basidiospores of *T. microcarpus* ($5.5\text{--}8.0 \times 3.5\text{--}5.5 \mu\text{m}$) are ovoid to ellipsoid, and smaller than in *T. fragilis*. The basidia of *T. fragilis* are bigger than those of *T. microcarpus* ($16.0\text{--}26 \times 6.0\text{--}9.0 \mu\text{m}$). The cystidia of the two species are similar, but *T. fragilis* has bigger cystidia than *T. microcarpus* (cheilocystidia $15.0\text{--}48 \times 9.0\text{--}20.0 \mu\text{m}$; pleurocystidia $23\text{--}44 \times 9.0\text{--}26 \mu\text{m}$). The diameter of pileipellis top cells of *T. fragilis* is the same as *T. microcarpus* ($2.0\text{--}5.0 \mu\text{m}$), but the second layer of cells are always wider [13]. *Termitomyces fragilis* is phylogenetically closely related to *T. microcarpus* (As shown in Figure 5), but the comparison of the ITS sequences shows there are 25 base pair differences between the two species.

Termitomyces tylerianus [57] is also morphologically closely related to *T. fragilis*. *Termitomyces tylerianus* has 1.0–2.0 cm diameter pileus, whereas in *T. fragilis* the pileus reaches a maximum 5.0 cm. The stipe of *T. tylerianus* is usually shorter than that of *T. fragilis* ($3.0\text{--}4.0 \times 0.2\text{--}0.3 \text{cm}$), and the stipe of *T. tylerianus* is solid and fibrous. Basidiospores of *T. tylerianus* ($5.0\text{--}7.0 \times 3.5\text{--}4.5 \mu\text{m}$) are smaller than those of *T. fragilis*. Basidia of *T. tylerianus* ($15.0\text{--}25.0 \times 5.5\text{--}8.0 \mu\text{m}$) are smaller than those of *T. fragilis*. The cheilocystidia or pleurocystidia are not found in *T. tylerianus* [57], but *T. fragilis* has both cheilocystidia and pleurocystidia.

Termitomyces clypeatus, another small basidiocarp species in Yunnan Province, is morphologically



Figure 4. Basidiocarps of *Termitomyces* found in Mengsong *T. aurantiacus*: A, B; *T. bulborhizus*: C, D, E, F, G; *T. eurhizus*: H, I; *T. globules*: J, K, L, M; *T. microcarpus*: N, O.

similar to *T. fragilis*. The pileus of *Termitomyces clypeatus* has a diameter of 2.5 cm, whereas in *T. fragilis* the pileus can be 2–5 cm. The stipe of *T. clypeatus* is usually longer and wider than that of *T. fragilis* (4.0–13.0 × 0.5–1.8 cm), and the stipe of *T. clypeatus* is solid. *Termitomyces clypeatus* [59] has a long (up to 22 cm), cylindrical bottom stipe, which is much more developed than that of *T. fragilis*. Basidiospores of *T. clypeatus* (6.0–9.0 × 4.0–6.0 μm) are smaller than those of *T. fragilis*. Basidia of *T. clypeatus* (15–28 × 5.5–9.0 μm) are smaller than those of *T. fragilis*. The cystidia of *T. clypeatus* are similar in shape to those of *T. fragilis*, but smaller than those of *T.*

fragilis (cheilocystidia 16–50 × 8.0–25 μm, pleurocystidia 15–74 × 9.0–25 μm).

Phylogenetic analyses also show that *T. fragilis* is closely related to *T. microcarpus* (as shown in Figure 5). According to the phylogenetic tree, the species most closely related to *T. fragilis* in the clade is an unidentified *Termitomyces* species from Japan. However, there are no descriptions, photo plates, or drawings available for the unidentified *Termitomyces* from Japan. This reflects the knowledge gaps which exist in *Termitomyces* research due to the limited availability of specimens, literature, and GenBank information.

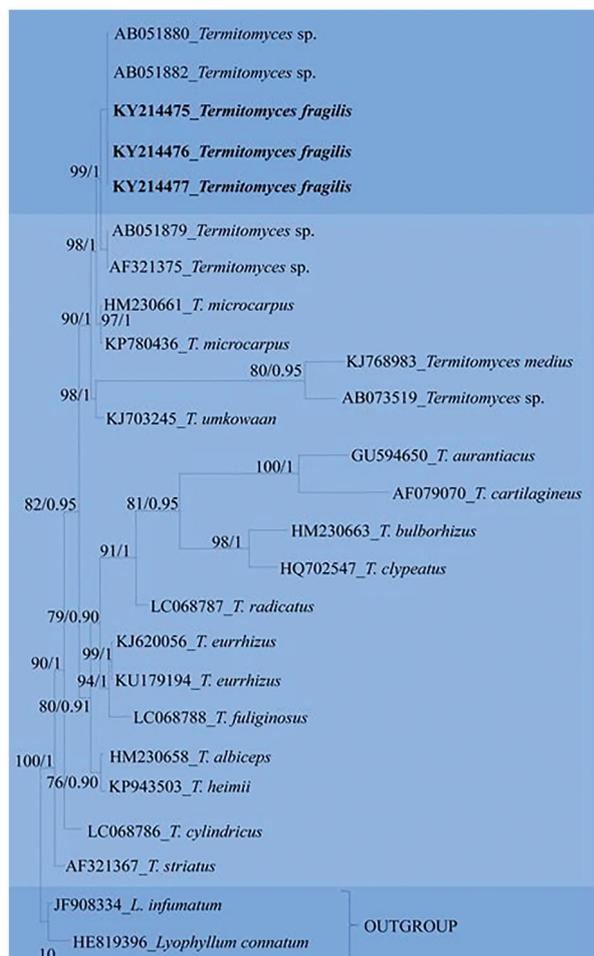


Figure 5. Phylogenetic tree showing the phylogenetic position of *Termitomyces fragilis* in comparison with all the available ITS-1-5.8S-ITS2 rDNA sequence data of *Termitomyces* in GenBank. Bootstrap support values for maximum parsimony equal or higher than 50% are defined as right side above the nodes and Bayesian posterior probabilities equal or higher than 0.90 are given left side, above the nodes. Data were analyzed with random addition sequence and unweighted parsimony, and gaps were treated as missing data. The tree is rooted with *Lyophyllum infumatum* (JF908334) and *L. connatum* (HE819396).

Additional termitomyces specimens collected

Termitomyces aurantiacus [26], *T. eurhizus*, *T. globulus* [59], *T. microcarpus* [25], *T. bulborhizus* [60] (as shown in Table 2), and *T. fragilis* were all found in Mengsong. Apart from *T. fragilis* and *T. microcarpus*, all of the aforementioned species (Figure 4) have large, solid basidiocarps, which are highly valued as edible mushrooms.

There are more than 20 *Termitomyces* species recorded in China, of which most of them have been found in Yunnan [61–63]. According to Zhang the *Termitomyces* species of Yunnan account for more than 80% of the records in China, and is close to half of the world's records, while the *Termitomyces* fruiting habitat in Yunnan is less than 10% of the world's total habitat, giving context to the degree of abundance and diversity of

Termitomyces in Yunnan [63]. This high level of abundance and diversity is likely due to the high diversity of termite species in Yunnan Province, and more specifically, Xishuangbanna County, which has the greatest diversity of termites in China [64].

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Disclosure statement

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