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Termitomyces srilankensis sp. nov. (*Lyophyllaceae*, *Agaricales*), a new species from Sri Lanka

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Abstract: Based on morphological and phylogenetic support, the new agaric species *Termitomyces srilankensis* is described from samples collected from the wet zone in Sri Lanka. The species is described with pictures of basidiomes and main micro-characters while molecular identification was used to verify the new species in comparison with the closest taxa. The new species is characterised by broad, convex to applanate pileus with tiny pointed perforatorium, crowded lamellulae of 3 tiers, and ellipsoid basidiospores. A molecular phylogenetic analysis based on ITS sequencing data verifies and separates *T. srilankensis* from *T. fuliginosus*, *T. globulus* and *T. heimii*.

INTRODUCTION

The genus *Termitomyces* R. Heim, type species *Termitomyces striatus* (Beeli) R. Heim, belongs to the family *Lyophyllaceae* Jülich which comprises 19 genera (Wijayawardene *et al*. 2022) with *Lyophyllum* P. Karst as the type. Taxa of this family are commonly characterized by siderophilous granule-filled basidia except the genus *Ossicaulis* Readhead & Ginns and most species of the genus *Clitocybe* (Fr.) Staude (Hofstetter *et al*. 2002; Singer 1986; Clémençon 1974, 1978, 1984; Kuhner 1938). Its member species have been recorded as saprobes or plant parasites and are mostly distributed in north-temperate and arctic areas (Bellanger 2015; Vesterholt & Ludwig 2012; Singer 1986). Some species of *Termitomyces* are well known for their edibility.

Species of *Termitomyces* form an obligate symbiotic or mutualistic association with the fungus-feeding termites (Aanen *et al.* 2002; Aanen & Eggleton 2005). The fruiting bodies of *Termitomyces* are the main source of food for fungus-growing termites of family *Macrotermitinae* which are exclusively found in Africa and Southeast Asia (Aanen *et al.* 2002; Aanen 2006). Kirk *et al*. (2008) reported approximately 30 taxa of *Termitomyces* and 102 taxa are listed in the Index Fungorum (2023).

Apart from being popular as a seasonal culinary delicacy, species of *Termitomyces* are known to contain medicinal properties such as antioxidants, immunomodulators, antitumorals and antimicrobials that are used to treat neurodegenerative disorders (Teke *et al*. 2018; Hsieh & Ju 2018).

MATERIALS AND METHODS

Sampling Site

The specimen was collected from Kegalle, Sabaragamuwa province located in the wet zone of Sri Lanka which receives a mean annual rainfall of over 2,500 mm, with a strong contribution from the south-western

monsoons. The mean annual temperature is in the range of 28–33°C while the relative humidity is in the range of 85–91%. The specimens were collected mostly from home gardens and bear lands.

Sample Collection

Basidiomata were collected in 2020 and 2021 and photographed with a XD5 digital camera while they were in the natural habitat and after being separated from the habitat, using a Canon XD5 digital camera. Collected basidiomata were cleaned as much as possible to remove soil or attached debris. Specimens were wrapped separately in aluminum foil to prevent spore contamination and damage. The specimens were taken to the laboratory for further studies.

Morphological Studies

Macro-morphological characteristics such as size, shape, and structure of the pileus and stipe were recorded. The colour terminology used for macro-morphological identification followed Kornerup & Wanscher (1978). The specimens were dried with a portable dryer at 40°C for 24–48 hours and sealed in zip-lock plastic bags containing silica gel as a desiccant to control humidity. All the herbarium specimens were deposited in the Fungarium of the University of Ruhuna (FUOR), Sri Lanka and duplicated in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (HKAS). Micro-morphological observations of dry specimens were carried out with free hand sections. Slides were prepared with distilled water. In addition, 3–5% KOH, Congo red, and 3–5% NH4(aq) were used to investigate some morphological characters where necessary. Morphological characters were observed and photographed using a compound light microscope (Nikon Model Eclipse Ci–s) attached to a Canon 550D digital camera. The measurements were taken with the Tarosoft Image Frame Work program, while images used for figures were processed with Adobe Photoshop CS3 (Version 15.0.0, Adobe®, San Jose, CA, USA) extended version 10.0 (Adobe Systems, San Jose, CA, USA).

DNA Extraction, PCR Amplification, and Sequencing

The genomic DNA of dried specimen was extracted from dried samples using a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). The nuclear ribosomal internal transcribed spacer (nrITS) and large subunit (nrLSU) regions were amplified using the primer pair ITS5/ITS4 and LROR/LR5 (Vilgalys 1990; White 1990). The amplification process was carried out for total volume of 25 µL comprising 1.0 µL of template DNA, 9.5 µL of double-distilled water, 1.0 µL of each primer, and 12.5 µL of 2× Power Taq PCR Master Mix. The latter consisted of a premixed, ready to use solution that included 0.1 Units/ µLTaq DNA polymerase, 500 µm of dNTP mixture each (dATP, dCTP, dGTP, and dTTP), 20 mM of Tris–HCl pH 8.3, 100 mMKCl, 3 mM of MgCl2, stabilizer, and enhancer. During the polymerase chain reaction (PCR), each sample underwent 35 cycles according to the following settings: denaturation (95°C, 30 s), annealing (52°C, 30 s), extension (72°C, 1 min), and final extension (72°C, 10 min). Amplified products were confirmed on a 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., P.R. China). The nucleotide sequence data were deposited in GenBank.

Sequence Alignment and Phylogenetic Analyses

The obtained sequences were checked and assembled using BioEdit 7.0.9.0 (Hall 1999) and compared with those available in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) on the BLAST algorithm. Taxon information applied in the molecular work is listed in Table 1. The ITS dataset comprises 35 sequences, including 33 *Termitomyces* sequences from GenBank which include the type species of the genus, *T. striatus*. *Lyophyllum connatum* and *Lyophyllum infumatum* were chosen as the outgroup taxa for ITS phylogenetic trees. The ITS sequence data were analyzed using maximum likelihood (ML), and Bayesian analyses. The reconstruction of ML analysis was performed using raxmlGUI v.0.9b2 with the model GTRGAMMA. A Bayesian analysis was conducted with MrBayes v. 3.1.2 (GTR+I+G model) to valuate posterior probabilities (PP) by Markov chain Monte Carlo sampling (BMCMC). Sequences for each strain were aligned using Clustal X (Thompson *et al.* 1997). Ambiguously aligned regions were excluded from all analyses. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. The final tree (Fig. 2) was displayed in FigTree v1.4.0 (Rambaut 2012) and then copied to Microsoft PowerPoint 2013 and converted to jpeg files using Adobe Photoshop CS3 Extended 10.0 (Adobe Systems, San Jose, CA, USA).

TAXONOMY

Termitomyces srilankensis A.N. Ediriweera, P. Voto, S.C. Karunarathna & H. Kularathne, sp. nov. (Fig. 1)

Typus: Sri Lanka, Sabaragamuwa Province, Kegalle District, ectomycorrhizal on humus soil mixed with clay with a high moisture level, abundant with termites and termite nests, 2nd August 2021, H. Kularathne and A.N. Ediriweera, Herb. FUOR0016AGS, GenBank accession number ON685313, Index Fungorum number: IF 553494. Isotype: HKAS123147.

Etymology: The specific epithet derives from "Sri Lanka", where the species was first detected.

Diagnosis: This taxon is characterized by a 9–13 cm broad, weakly radially fibrillose pileus with tones of yellowish and grey, a stipe that is tapering downwards to an abrupt rooting base, subamygdaliform to lacrymoid spores of (10.41) 10.75–11.44 (11.67) × (4.12) 4.18–5.12 (5.45) µm, clavate to ellipsoid or ellipsoid-utriform cheilocystidia of (21.12) 22.18–23.48 (23.98) × (6.08) 6.21 - 6.92 (7.04) µm, and pyriform pleurocystidia of (36. 89) 38.56 – 39.94 (40.04) × (26.89) 27.17 – 28.19 (28.87) µm.

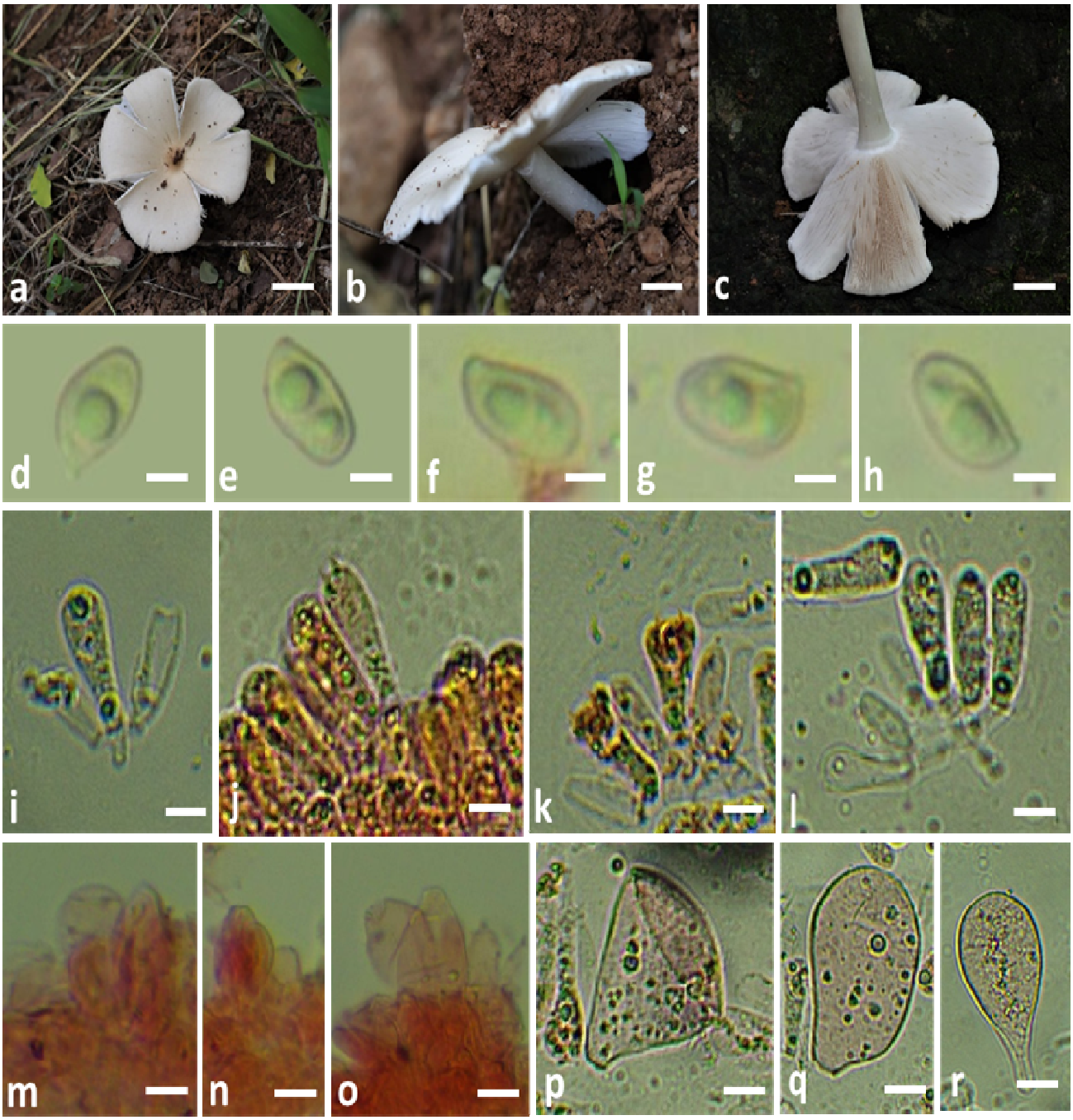

Fig. 1 (previous page). Morphology of *Termitomyces srilankae* (HKAS123147/FUOR0016AGS): (a -c) Mature basidiome; (d-h) Basidiospores; (i-l) Basidia; (m-o) Cheilocystidia; (p-r) Pleurocystidia. Scale bars: (a- c) = 3 cm, (d, h) = 3 µm, (i-r) = 5 µm. All images by A.N. Ediriweera.

Description

Basidiomata large.

Pileus 9 – 13 cm across, when young convex and cuspidate, at maturity broadly convex to applanate with a tiny pointed perforatorium, margin at maturity straight to only slightly incurved, moderately indented and radially split, surface yellowish grey (4B2) to sand colour (4B3) with a light yellow (4A4) center, almost smooth with radial white fibrils.

Lamellae free, 2 – 4 mm wide, white (1A1) when young and pale yellow (1A3) at maturity, very crowded with intermingled lamellulae of 3 tiers of 1.2, 3.2 and 4 cm, edge smooth, equal.

Stipe 9.0 – 11.0 × 1.0 – 1.2 cm, central, cylindrical but in the lower portion tapering down to a thin abruptly rooting base 2.5 – 3.5 cm long, white (1A1) to pale yellow (1A3), apex fibrillose and squamulose, elsewhere smooth and glabrous, longitudinally striate, stuffed.

Context fleshy, moderately thick, 1.5 – 2.5 mm.

Basidiospores (n=40) (10.41) 10.75 – 11.44 (11.67) × (4.12) 4.18 – 5.12 (5.45) µm, on average 11.08 × 4.63 µm, Q = 1.6 – 1.9, in front view elliptic, in side view subamygdaliform to lacrymoid, thin-walled, smooth, hyaline with a yellowish to golden colour content.

Basidia (19.73) 21.33 – 22.02 – (23.8) × (6.1) 6.14 – 7.38 (7.78) µm, on average 23.11 × 6.77 µm, 4-spored, sterigmata 2 – 3.5 µm long, clavate to sub cylindraceous, thin walled, smooth, hyaline to sub hyaline.

Cheilocystidia (21.12) 22.18 – 23.48 (23.98) × (6.08) 6.21 – 6.92 (7.04) µm, on average 22.94 × 6.38 µm, clavate to ellipsoid or ellipsoid-utriform, thin-walled, hyaline to sub-hyaline.

Pleurocystidia (36. 89) 38.56 – 39.94 (40.04) × (26.89) 27.17 – 28.19 (28.87) µm, on average 39.12 × 27.77 µm, pyriform, thin-walled, hyaline.

Ecology, Habit and Habitat: Saprotrophic, solitary, in humus soil mixed with clay with high moisture level, abundant with termites and termite nests or mounds above and below the surface soil layer.

NOTES

Termitomyces species grow on clayey soils associated with termites. Our new taxon also was collected from clay-mixed humus soil where huge networks of clefts formed by termites were present under the uppermost layer of soil during heavy rains. A differentiated shape both of the spore side view and of cheilocystidia represents some of the most peculiar features in the descriptive dataset of our new taxon which otherwise shares several morphological characters with other congeneric species. It is closely related to some unspecified *Termitomyces* species on a molecular basis with 99.55 % to 99.86 % identity in the BLAST search. Besides, our collection formed a distinct clade with the three following taxa of *Termitomyces*, originally described from tropical Africa, in the phylogenetic analysis with high bootstrap support (100 % MLBS) (Figure 4.17, Clade A): *T. fuliginosus* R. Heim (MRNo215), *T. globulus* R. Heim & Gooss. (BUMR03) and *T. eurrhizus* (Berk.) R. Heim. (WHX-2015). These three vouchers are phylogenetically closely related to our new strain (FUOR0016AGS). In the base pair (bp) comparison of ITS sequences data revealed that there are1.68%, 5.35% and 8.71% bp differences between our strain and the strains of *T. fuliginosus*, *T. globulus* and *T. eurrhizus* respectively.

Termitomyces fuliginosus, differs from *T. srilankensis* by having a cap-shaped or bell-shaped pileus at both young and mature stages with an incurved margin at maturity, and smaller and ellipsoid basidiospores 7.3 × 4.5 µm on average. The voucher MRNo215, identified as *T. fuliginosus* and reported from Thailand, which appears in our phylogram with a sufficiently high identity percentage (98.32%), is unpublished and therefore not comparable.

Termitomyces globulus differs from our new species by having a larger pileus of 8 – 21 cm with a reddish-brown central area and a finely striate margin, smaller and ellipsoid basidiospores of 6 – 9 × 3.5 – 6 µm, larger cheilocystidia of 20 – 60 × 16 – 25 µm, and pleurocystidia of 35 – 71 × 17 – 28 µm (Heim 1951; Pegler & Vanhaecke 1994).

In addition, *Termitomyces eurrhizus* differs by having a pileus with a brown to reddish-brown surface with a straight margin, smaller basidiospores of 6 – 9 × 4 – 6 µm, larger cheilocystidia of 13 – 55 × 8 – 33 µm and pleurocystidia of 18 – 69 × 10 – 35 µm (Wei *et al*. 2009; Heim 1942; Pegler & Vanhaecke 1994).

Termitomyces heimii Natarajan, represented in the sister clade to Clade A in our phylogram, was described from the adjacent South India, it differs from *T. srilankensis* by a smaller pileus up to 10 cm broad even at maturity, convex to plano-convex and with an incurved margin, a thick annulus, much larger basidiospores (19.5 – 21 × 5.5 – 7 µm,), 2- or 4-spored basidia, rare pleurocystidia and absence of cheilocystidia (Natarajan 1979).

Pegler & Vanhaecke (1994) reports two species from Sri Lanka: *T. eurhizus* and *T. microcarpus* (Berk. & Broome) R. Heim.

Combined morphological characters and phylogenetic analyses support the description of our collection as a new species, *T. srilankensis*, in *Termitomyces*.

Taxa names	Collection ID	ITS
Lyophyllum connatum (Schumach.) Singer	SR-32	HE819396
L. infumatum (Bres.) Kühner	MCVE 10152	JF908334
Termitomyces acriumbonatus Usman & Khalid	LAH36363_MU106	MT179688
T. clypeatus R. Heim	MU19-50	FJ147329
T. clypeatus	MU25-49	HQ702547
T. cylindricus S.C. He	MRNo170	LC068786
T. cylindricus	INDO18	MH651799
T. entolomoides R. Heim	BUMR06	MK743955
T. eurrhizus (Berk.) R. Heim	WHX-2015	KU179194
T. fragilis L. Ye, Karun, J.C. Xu, K.D. Hyde & Mortimer	HKAS:88906 paratype	KY214477
T. fragilis	HKAS:88909 paratype	KY214476
T. fragilis	HKAS:88912 type	KY214475
T. fuliginosus R. Heim	MRNo215	LC068788
T. globulus R. Heim &Gooss.-Font.	BUMR03	MK743956
T. heimii Natarajan	TERM055	MN160309
T. heimii	UOC MAT MT01	KP943503
*T. intermedius*Har. Takah. &Taneyama	GDGM46325	MF488973
T. intermedius	GDGM46311	MF488972
T. medius R. Heim & Grassé	BUMR07	MK743976
T. Medius	CUH:AM080	KJ768983
T. microcarpus (Berk. & Broome) R. Heim	UOC KAUNP MK04	KP780436
T. microcarpus	MU195-46	HM230661
T. radicatus Natarajan	MRNo173	LC068787
T. sheikhupurensis Izhar, Khalid & H. Bashir	LAH36413 paratype	MT192218
T. sheikhupurensis	LAH 35710 type	NR_172179
T. sp.	HKAS 117638	MZ869839
T. sp.	HKAS 117639	MZ869840
T. sp.	HKAS 117640	MZ869843
T. sp.	HKAS 117641	MZ869844
T. sp.	OS2	AF321375
T. srilankensis sp.nov.	FUOR0016AGS	ON685313
T. striatus (Beeli) R. Heim	TERM048	MN160302
T. striatus	TERM049	MN160303
T. striatus	TERM051	MN160305
T. umkowaan (Cooke &Massee) D.A. Reid	HUH-SH5	KJ703245

Table 1. Fungal taxa, Voucher numbers and GenBank accession numbers of the sequences used in the phylogenetic analyses of ITS

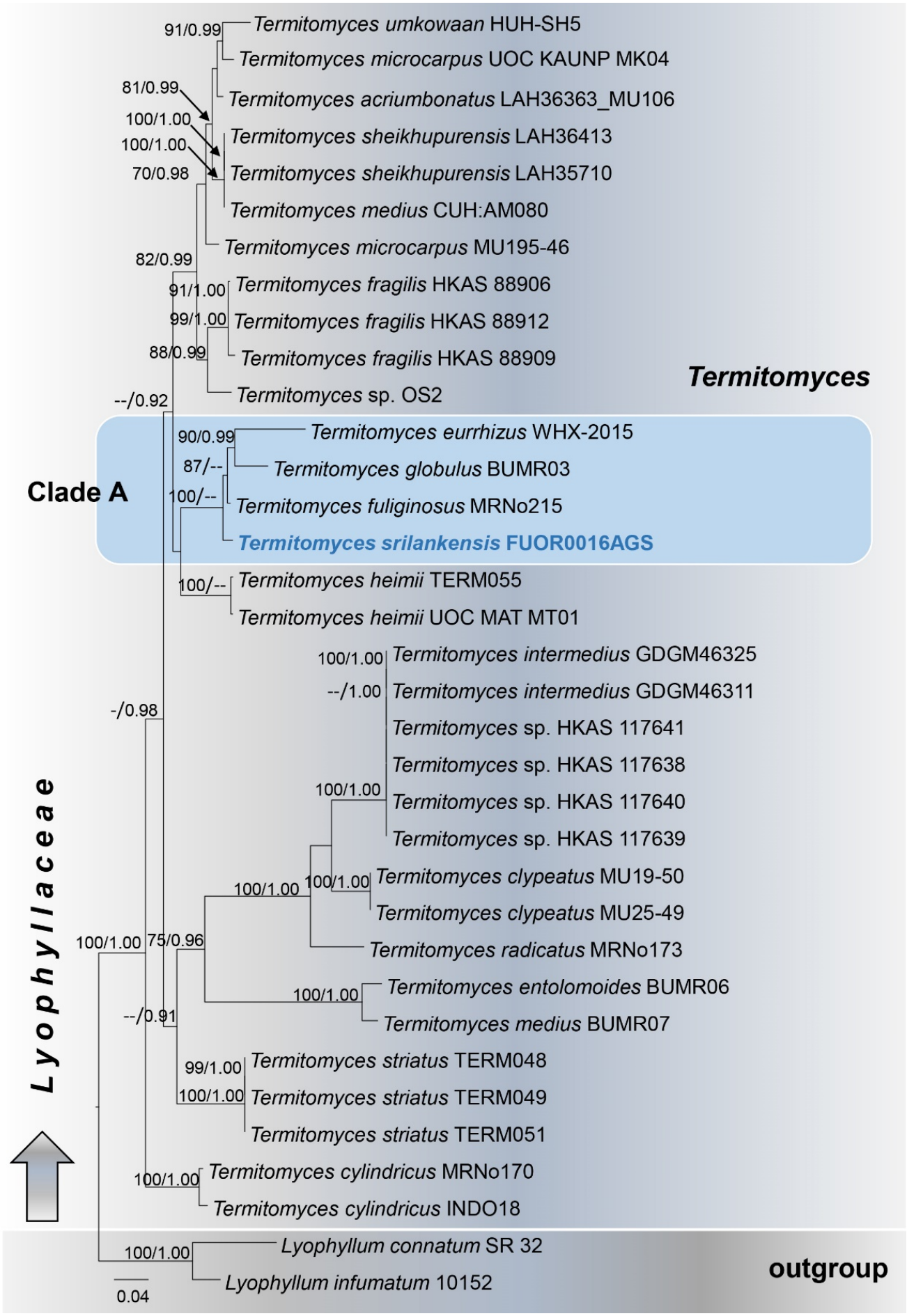

Fig. 2 (below). Phylogram generated from maximum likelihood analysis based on RAxML analysis based on the ITS sequence data including 35 strains. Bootstarp support values for maximum likelihood (ML, left), higher than 70%, and Bayesian posterior probabilities (BYPP, right) greater than 0.90 are provided. The tree is rooted with *Lyophyllum connatum* and *Lyophyllum infumatum*. The new record is printed in blue.

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