Chiang Mai J. Sci. 2022; 49(3): 641-653.
https://doi.org/10.12982/CMJS.2022.051
Journal homepage : http://epg.science.cmu.ac.th/ejournal/

Research Article

A New Tropical Species of *Lycoperdon* Subgenus *Morganella* (Agaricales, Basidiomycota) From Yunnan Province, China

Lei Ye [a,b,e], Samantha C. Karunarathna*[a,b,f], Huili Li [a,b], Dhanushka N. Wanasinghe [a,b], Jaturong Kumla [c], Jianchu Xu [a,b], Mahesh C. A. Galappaththi [d] and Peter E. Mortimer*[a]

[a] Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, China

[b] CIFOR-ICRAF China Program, World Agroforestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, China

[c] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

[d] Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka

[e] The Research Institute of Alpine Economic Plant, Yunnan Academy of Agricultural Sciences, Lijiang 674100, China

[f] Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan 655011, People's Republic of China

*Author for correspondence; e-mail: peter@mail.kib.ac.cn, samanthakarunarathna@gmail.com

Received: 25 November 2021
Revised: 2 May 2022
Accepted: 2 May 2022

ABSTRACT

Two specimens of *Lycoperdon* were collected in 2012 and 2013 during surveys conducted in evergreen broadleaf forests of Xishuangbanna, Yunnan Province, China. Macro- and micro-morphological characteristics, together with analyses of combined ITS and LSU sequence data, showed that five specimens represent a single new species and is thus named as *Lycoperdon mengsongense*. The new species is distinguished from the known taxa in *Lycoperdon* by its greyish white to dark grey exoperidium covered by minute and blackish grey granules, smooth to wrinkled endoperidium, non-chambered subgleba and echinate basidiospores. Comprehensive morphological descriptions, color photographs of macro- and micro- characteristics and a phylogenetic tree to show the placement of the new species are provided. In addition, the new species is compared with closely related taxa in *Lycoperdon* subgenus *Morganella*.

Keywords: Lignicolous puffballs, Lycoperdaceae, phylogeny, taxonomy

1. INTRODUCTION

Lycoperdon is a widespread genus, found in both northern and southern Hemispheres. Dictionary of Fungi estimates that there are about 50 species in the genus, [1]. Index Fungorum lists eight subgenera viz. *Apioperdon*, *Arenicola*, *Bovistaria*, *Bovistella*, *Lycoperdon*, *Morganella*, *Utraria* and *Vascellum* under the genus *Lycoperdon* [2]. Zeller [3] proposed the genus *Morganella* Zeller with *Morganella mexicana* as the type species but the genus *Morganella* was not generally accepted until Kreisel and Dring gave it a clear-cut circumscription in 1967 [4]. Kreisel and Dring [4] included 7 species, while two more species were added by Ponce de León [5] in a monograph that, unfortunately,

was poorly illustrated. Previous studies showed that the genus has received special attention in South America [6, 7]. In the phylogenetic analyses of Alfredo et al. [7], *Morganella* was treated as a subgenus of *Lycoperdon* (in order to avoid being a paraphyletic genus) based on a cladistic taxonomy of Lycoperdaceae with ITS-LSU phylogenetic analysis. *Lycoperdon arenicola* was included in the subgenus *Morganella*, but this placement was questionable given that species with capillitium do not fit the subgenus *Morganella* [8].

To date, 21 species are listed under the subgenus *Morganella* in Index Fungorum [2] but more than half of them were synonymized or excluded. Twelve species are still classified in the genus of *Lycoperdon*, and one species in *Apioperdon*, while eight species of *Morganella* have sequence data in GenBank. Zeller [3] separated *Morganella* from *Lycoperdon* by the presence of true capillitium. The work of Kreisel and Dring [4] and Ponce de León [5] added more species to the genus *Morganella*. However, with the development of molecular phylogeny, based on ITS and LSU sequence data, Larsson and Jeppson [8] proposed that *Morganella* could be a distinct subgenus of *Lycoperdon,* and a recent study from Alfredo et al. [7] also confirmed this.

During surveys conducted in evergreen broadleaf forests of Xishuangbanna, Yunnan Province, China, two specimens of *Lycoperdon* were collected. Further morphological and molecular characterizations revealed that these two specimens belong to a distinct new species.

2. MATERIALS AND METHODS

2.1 Sampling Site

Two *Lycoperdon* collections (Table 1) were collected in an evergreen broadleaf forest during the rainy season (June to October) of 2012 and 2013 in Mengsong Village, Jinghong City, Yunnan Province, China. The average altitude of the sampling plot is 1656 meters. Photographs of the fresh basidiocarps were taken *in situ* and the number of basidiocarps, the odor of basidiocarps, forest type of the basidiocarps, substrate type of the basidiocarps growing on, and location data were recorded. Fresh basidiocarps were wrapped in aluminum foil and taken to the laboratory where macro-morphological characteristics were recorded, and then placed in a food dryer at 40 °C until they are completely dehydrated. Specimens were then sealed in labeled plastic bags. Kornerup and Wanscher [9], was followed for the color terms. All specimens were deposited in the herbarium of the Kunming Institute of Botany (HKAS), Chinese Academy of Science, China. Facesoffungi numbers and Index Fungorum numbers were obtained as detailed in Jayasiri et al. [10] and Index Fungorum [2].

2.2 Micro-morphological Study

Free hand sections of the dried specimens were mounted in 5 % KOH and Lactophenol+Cotton blue for light microscopic observations. Basidiospores and tissue system measurements were made using a calibrated ocular micrometer. All micro-morphological characteristics were observed under 10×, 20×, 40× and 100× objective lenses of a Nikon compound microscope (Nikon Model Eclipse Ni-U). Basidiospore size, colour, shape, and the hyphae of the paracapilitillium, endoperidium, stalk, gleba and basidiospores were recorded and photographed. The side views of at least 30 basidiospores were used to calculate the size range. The range of paracapilitillium cell size was calculated from at least 30 hyphae. Colour photo plates were edited in Adobe Photoshop CS3. For scanning electron microscope (SEM), basidiospores were mounted on specimen holders using double-sided tape, double-coated with gold/platinum and viewed through a ZEISS sigma 300 scanning electron microscope.

2.3 DNA Extraction, PCR and Sequencing

Total genomic DNA was extracted from dry basidiocarps of two different collections (HKAS101876 and HKAS88251). Basidiocarps were ground into a fine powder with liquid nitrogen

Table 1. Names, strain numbers, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses. Sequences derived in this study are in black bold. Ex-type strains are mentioned with superscripted "T".

Taxon	Strain/voucher number	GenBank accession	
		ITS	LSU
Lycoperdon albostipitatum	INPA 239563 ^{T}	KU958363	KU958364
Lycoperdon albostipitatum	UFRN-Fungos 2249	KU958361	KU958362
Lycoperdon albostipitatum	UFRN-Fungos 2569	KU958357	KU958358
Lycoperdon albostipitatum	UFRN-Fungos 2572	KU958359	KU958360
Lycoperdon arenicola	UFRN-Fungos 1006 ^{T}	KU958303	KU958304
Lycoperdon arenicola	UFRN-Fungos 649	KU958297	KU958298
Lycoperdon arenicola	UFRN-Fungos 656	KU958291	KU958292
Lycoperdon arenicola	UFRN-Fungos 657	KU958299	KU958300
Lycoperdon arenicola	UFRN-Fungos 729	KU958295	KU958296
Lycoperdon arenicola	UFRN-Fungos 864	KU958293	KU958294
Lycoperdon arenicola	UFRN-Fungos 941	KU958305	KU958306
Lycoperdon arenicola	UFRN-Fungos 1367	KU958287	KU958288
Lycoperdon arenicola	UFRN-Fungos 1510	KU958289	KU958290
Lycoperdon arenicola	UFRN-Fungos 2567	KU958285	KU958286
Lycoperdon arenicola	UFRN-Fungos 2581	KU958301	KU958302
Lycoperdon fuligineum	INPA 239561	KU958351	KU958352
Lycoperdon fuligineum	UFRN-Fungos 606	KU958347	KU958348
Lycoperdon fuligineum	UFRN-Fungos 371	KU958353	KU958354
Lycoperdon fuligineum	UFRN-Fungos 1768	KU958327	KU958328
Lycoperdon fuligineum	UFRN-Fungos 1971	KU958321	KU958322
Lycoperdon fuligineum	UFRN-Fungos 1972	KU958323	KU958324
Lycoperdon fuligineum	UFRN-Fungos 2560	KU958333	KU958334
Lycoperdon fuligineum	UFRN-Fungos 2561	KU958335	KU958336
Lycoperdon fuligineum	UFRN-Fungos 2562	KU958337	KU958338
Lycoperdon fuligineum	UFRN-Fungos 2563	KU958345	KU958346
Lycoperdon fuligineum	UFRN-Fungos 2566	KU958341	KU958342
Lycoperdon fuligineum	UFRN-Fungos 2571	KU958329	KU958330
Lycoperdon fuligineum	UFRN-Fungos 2575	KU958325	KU958326
Lycoperdon fuligineum	UFRN-Fungos 2578	KU958331	KU958332
Lycoperdon fuligineum	UFRN-Fungos 2579	KU958349	KU958350
Lycoperdon fuligineum	UFRN-Fungos 2582	KU958339	KU958340
Lycoperdon fuligineum	UFRN-Fungos 2586	KU958343	KU958344

Table 1. (Continued)

Taxon	Strain/voucher number	GenBank accession	
		ITS	LSU
Lycoperdon mengsongensis	**HKAS101876**	**MH311860**	**MH311865**
Lycoperdon mengsongensis	**HKAS88251** [T]	**MH311863**	**MH311868**
Lycoperdon nudum	TENN59070	AF485065	-
Lycoperdon nudum	ICN 154541	KU958317	KU958318
Lycoperdon nudum	UFRN-Fungos 1765 [T]	KU958319	KU958320
Lycoperdon nudum	UFRN-Fungos 1766	KU958315	KU958316
Lycoperdon nudum	UFRN-Fungos 2565	KU958311	KU958312
Lycoperdon nudum	UFRN-Fungos 2568	KU958313	KU958314
Lycoperdon oblongatum	UFRN-Fungos 2570 [T]	KU958355	KU958356
Lycoperdon puiggarii	CMU-Mor3	KX064241	MW832383
Lycoperdon purpurascens	MEL:2382736	KP012918	KP012918
Lycoperdon purpurascens	CMU55-Ly1	KC414581	-
Lycoperdon sosinii	VLA 15520	KC591769	-
Lycoperdon sp.	ICN177118	KU958371	KU958372
Lycoperdon sp.	UFRN-Fungos 655	KU958307	KU958308
Lycoperdon sp.	UFRN-Fungos 2554	KU958309	KU958310
Lycoperdon subincarnatum	TNS Kasuya B286	KF551244	-
Lycoperdon subincarnatum	Culture 414	AJ237626	-
Lycoperdon subincarnatum	Isolate 48	KM373265	-
Tulostoma calcareum	GB MJ6965 [T]	NR_164015	KU519087
Tulostoma kotlabae	Mrazek1300	DQ112629	DQ112629
Tulostoma squamosum	MJ5467	DQ415732	DQ415732

and DNA was extracted using a Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China) following the manufacturer's protocol. DNA used as a template for PCR was stored at 4 °C while a portion of DNA was duplicated at -20 °C for long-term storage.

DNA sequence data were obtained from the partial sequences of two ribosomal genes. Primers ITS5/ITS4 [11] and LR0R/LR5 [12] were used to amplify DNA sequences of internal transcribed spacers (ITS1-5.8S-ITS2) and partial 28S large subunit rDNA (LSU) respectively. Polymerase chain reaction (PCR) was carried out on a volume of 25 µl which contained 12.5 µl of 2 × Power Taq PCR MasterMix (Bioteke Co., China), 1 µl of each primer, 1 µl genomic DNA and 9.5 µl deionized water. PCR thermal cycler programs for all gene regions were programmed with an initial denaturation at 94 °C for 3 min and a final extension at 72 °C for 10 min. ITS and LSU gene amplifications were followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 55 °C for 40 seconds and extension at 72 °C for 1 min. All the PCR products were observed on 1% agarose electrophoresis gels stained with ethidium bromide (30 minutes at 220V). Amplified PCR

fragments were sent to a commercial sequencing provider (Beijing Tsingke Biological Technology Co., Ltd.). Nucleotide sequence data acquired were deposited in GenBank (Table 1).

2.4 Molecular Phylogenetic Analyses

2.4.1 Sequencing and sequence alignment

Newly generated sequences from ITS and LSU regions were analysed with 54 sequences of closely related taxa retrieved from GenBank (Table 1). Sequences with high similarity indices were determined from a BLAST search to find the closest matches with taxa in *Lycoperdon subg. Morganella*. Multiple alignments of all consensus sequences, as well as reference sequences were automatically generated with MAFFT v. 7 [13], and manually corrected where necessary using BioEdit v. 7.0.5.2 [14].

2.4.2 Phylogenetic analyses

The single-locus datasets were examined for topological incongruence among ITS and LSU for members of the analyses. Alignments were concatenated and subjected to maximum-likelihood (ML), maximum parsimony (MP) and Bayesian (BI) phylogenetic analyses.

CIPRES Science Gateway platform [15] was used to perform RAxML and Bayesian analyses. ML analyses were made with RAxML-HPC2 on XSEDE v. 8.2.10 [16] using GTR+GAMMA swap model with 1000 bootstrap repetitions. Evolutionary models for Bayesian analysis were selected independently for each locus using MrModeltest v. 2.3 (Nylander, 2004) [17] under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10 and GTR+I+G was selected as the best fit model for all three analyses. MrBayes analyses were performed setting GTR+I+G, 2 M generations, sampling every 100 generations, ending the run automatically when the standard deviation of split frequencies dropped below 0.01 with a burn-in fraction of 0.25. MP analysis was conducted with PAUP v. 4.0b10 [18] inferring trees with the heuristic search option with TBR branch swapping and 1000 random sequence additions. The robustness of equally parsimonious trees was evaluated by 1000 bootstrap replications. Alignment gaps were treated as missing characters in the analysis, where they occurred in relatively conserved regions. Tree scores, including consistency index, retention index, rescaled consistency index and homoplasy index (CI, RI, RC and HI) were also calculated for all the trees generated under different conditions as measures of homoplasy in the data. Kishino-Hasegawa tests [19] were performed in order to determine whether trees were significantly different. ML, MP bootstrap values equal to or greater than 70 %, and the posterior probability in BI (BYPP) equal to or greater than 0.95 are given above each node of the tree. Phylograms were visualized with FigTree v1.4.0 program [20] and reorganized in Microsoft power point (2007). Finalized alignment and the phylogenetic tree were deposited in TreeBASE, submission ID: 25389 (http://www.treebase.org/).

2.4.3 Pairwise homoplasy index

Pairwise homoplasy index (PHI) test was performed by SplitsTree4 to determine the recombination level within phylogenetically closely related species by using a concatenated dataset of closely related species [21–23]. Pairwise homoplasy index results lower than 0.05 ($\Phi_w < 0.05$), indicate the presence of significant recombination in the dataset. Relationships between closely related taxa are visualized by constructing splits graphs from concatenated datasets, using the Log-Det transformation and splits decomposition options.

3. Results

3.1 Phylogenetic Analyses

The final concatenated alignment (LSU and ITS) of *Lycoperdon subg. Morganella* comprised 54 sequences with 1613 characters, including the new taxon proposed in this study and outgroup taxa. *Tulostoma calcareum* (GB MJ6965), *T. kotlabae* (Mrazek1300) and *T. squamosum* (MJ5467) were used as outgroup taxa as per the previous studies [7].

RAxML analysis of the combined dataset yielded the best scoring tree (Figure 1) with a final ML optimization likelihood value of -6628.866393. The matrix had 588 distinct alignment patterns, with 14.25 % of undetermined characters or gaps. Parameters for the GTR+I+G model of the combined loci were as follows: Estimated base frequencies; A = 0.25474, C = 0.195998, G = 0.272665, T = 0.276597; substitution rates AC = 0.898977, AG = 2.590529, AT = 1.303699, CG = 0.800718, CT = 3.41451, GT = 1.00; proportion of invariable sites I = 0.482628; gamma distribution shape parameter α = 0.529567. ML bootstrap support values were mapped on the tree as the first value (Figure 1). MP analyses generated a maximum of two equally most parsimonious trees, the first of which is shown in Figure 1 (Length = 802, CI = 0.721, RI 0.916, RC = 0.66, HI = 0.279), and MP bootstrap support values were mapped on the tree as the second value (Figure 1). From analysed characters, 1202 were constant, 127 were variable and parsimony-uninformative and 284 were parsimony-informative. Bayesian analysis ran 485000 generations before the average standard deviation for split frequencies reached below 0.01 (0.009252). The analysis generated 4851 trees (saved every 100 generations) from which 3639 were sampled after 25 % of the trees were discarded as burn-in. Alignment contained a total of 590 unique site patterns. ML phylogeny (Figure 1) showed the same terminal clades as those presented in the MP and BI phylogeny.

Two newly generated sequences of *Lycoperdon mengsongens*e (HKAS88251, HKAS101876), formed a well-supported clade (100% ML, 98 MP and 1.00 BYPP, Figure 1) with remaining species of *Lycoperdon* subg. *Morganella* (Figure 1). *Lycoperdon mengsongens*e constituted a monophyletic clade with *Lycoperdon puiggarii* (CMU-Mor3) *L. purpurascens* (CMU55-Ly1, MEL 2382736) and *L. sosinii* (VLA 15520) with 100% ML, 100 MP and 1.00 BYPP support values (subclade A, Figure 1) sister to *L. oblongatum* UFRN-Fungos 2570 and *L. subincarnatum* (Isolate 48, Culture 414, TNS Kasuya B286). Among them, *Lycoperdon mengsongens*e has a close phylogenetic affinity to *L. purpurascens* (CMU55-Ly1) with 100% ML, 100 MP and 1.00 BYPP support values (Figure 1). The two strains of *Lycoperdon purpurascens* (CMU55-Ly1, MEL 2382736) were not monophyletic within subclade A (Figure1).

Application of the PHI test to the concatenated two-locus sequences (ITS and LSU) revealed the recombination level within phylogenetically related species (subclade A, Figure 1). No significant recombination events were observed (Φ_w=0.6354) between *Lycoperdon mengsongense* and phylogenetically closely related species viz. *Lycoperdon puiggarii* (CMU-Mor3) *L. purpurascens* (CMU55-Ly1, MEL 2382736) and *L. sosinii* (VLA 15520) (Figure 2).

3.2 Taxonomy

Lycoperdon mengsongense L. Ye, P.E. Mortimer, & Karunarathna *sp. nov.* Figure 3

Index Fungorum number: IF554754; *Facesoffungi number*: FoF: 04606

Etymology:—The species epithet "mengsongense" refers to the location where the type specimen was collected.

Holotype:—CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, E 100° 28' 15", N 21° 30' 49", 20 September 2012, Lei Ye (HKAS 101876)

Diagnosis:— *Lycoperdon mengsongense* is characterized by small (1–2 cm in diameter) basidiocarps; light grey to grey exoperidium, the upper half is obviously darker than the lower half of basidiocarps; endoperidium is obviously dark brown on the fully mature basidiocarps (spores have been spread); small basidiospores (2.8–3.7 μm in diameter), the surface is clearly visible with dense and short spinous protrusions, and spines are dense and short.

Description:—Basidiocarps globose or depressed globose, 10–23 mm in diameter, 11–22 mm in height, whitish, branched, cord-like rhizomorphs attached at the base, peridium double. **Exoperidium** persistent, greyish white (1B1) to

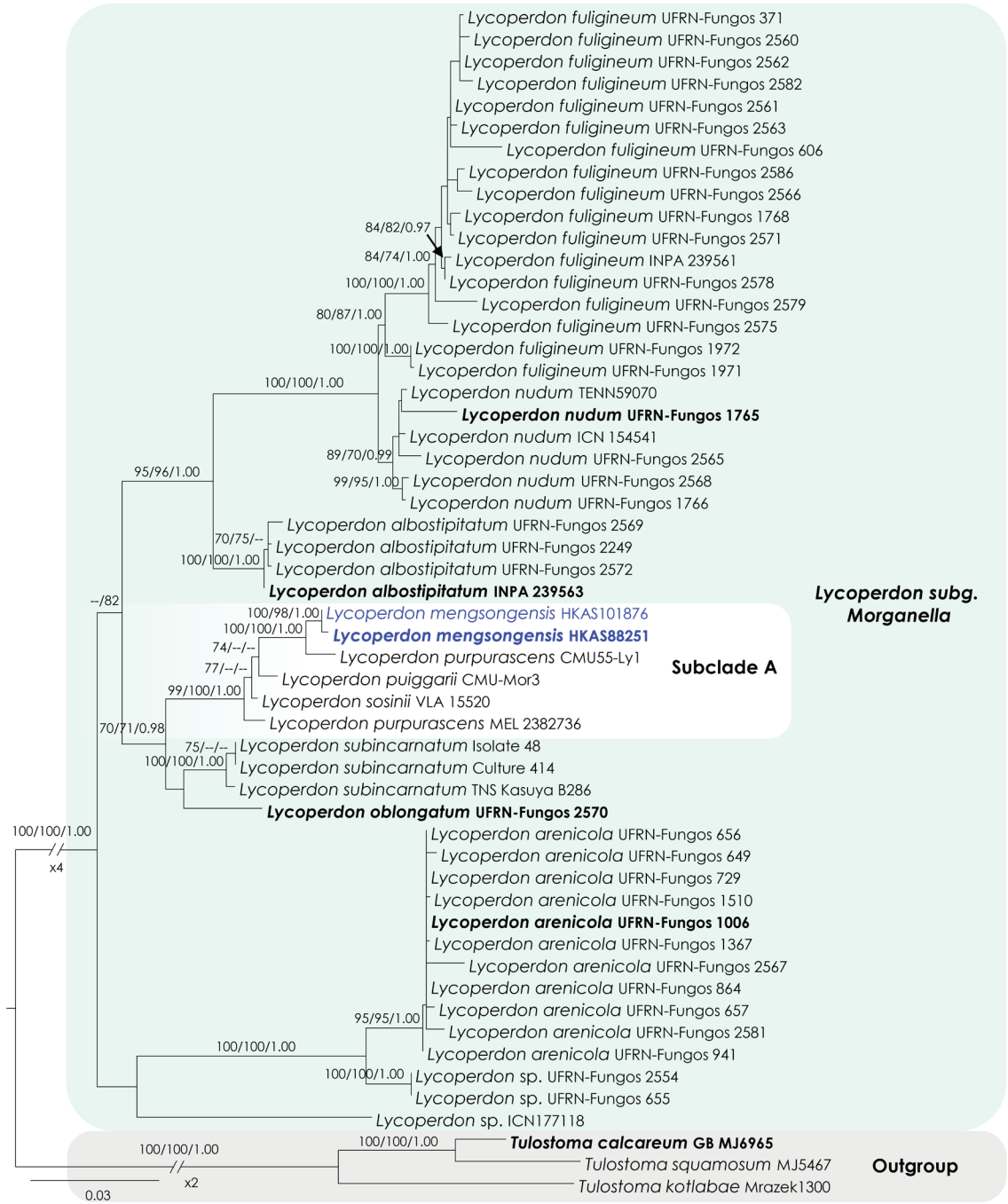

Figure 1. RAxML tree based on an integrated dataset of partial LSU + ITS DNA sequence analysis in *Lycoperdon* subg. *Morganella*. Bootstrap support values for ML, MP equal to or greater than 70%, and BYPP equal to or greater than 0.95 are shown as ML/MP/BI above the nodes. Blue represents new isolates. Species names given in bold black indicate ex-type and ex-paratype strains. The scale bar represents the expected number of nucleotide substitutions per site.

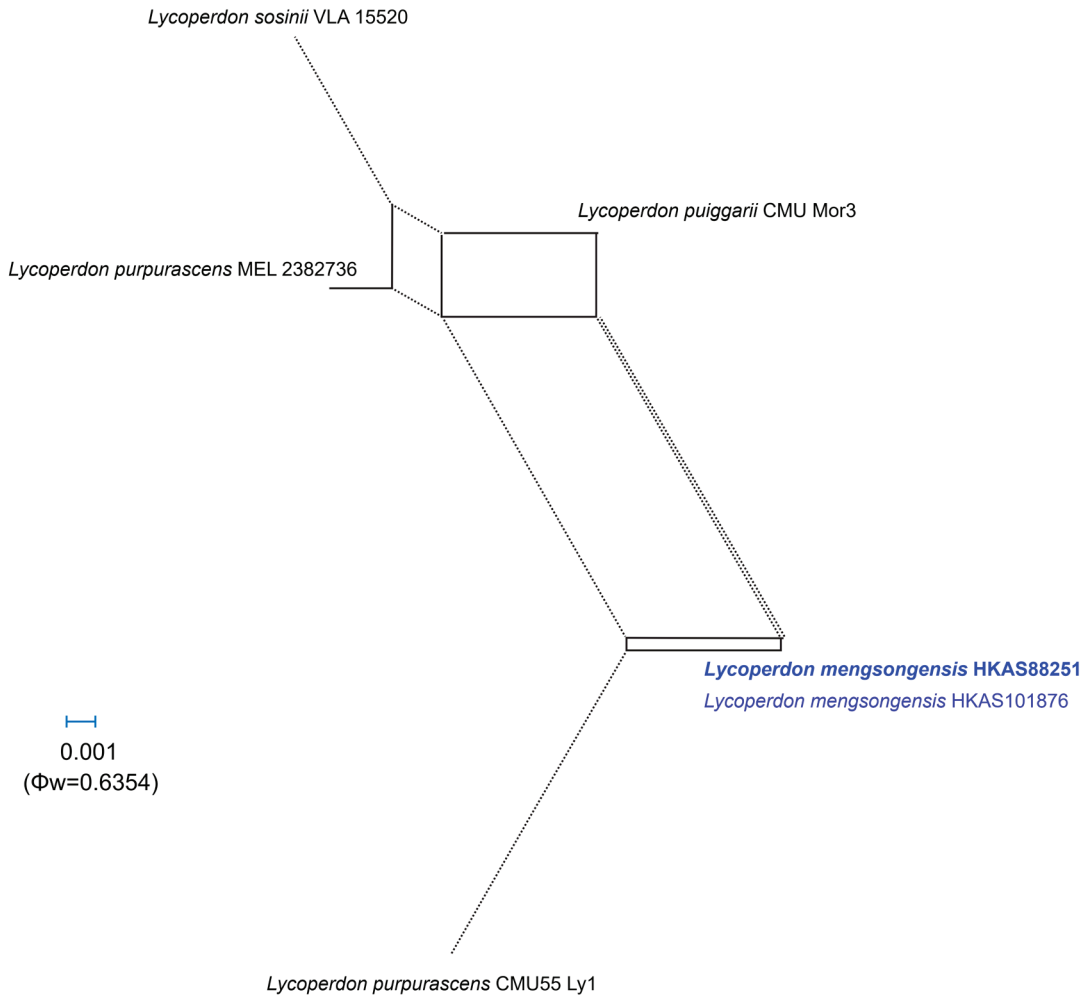

Figure 2. Split graphs showing the results of the pairwise homoplasy index (PHI) tests of *Lycoperdon mengsongensis* and closely related taxa using LogDet transformation and splits decomposition. Blue represents new isolates in this study. Species names given in bold black indicate ex-type and ex-paratype strains. PHI test results (Φ_w) ≤ 0.05 indicate significant recombination within the dataset.

dark grey (1F1), lighter below, covered by minute and blackish-grey (1G1) granules that are mainly distributed at the top area causing the color of top area to be darker than other parts, minute conical tubercles, composed of chains of globose cells, 32–67.5 μm in thickness of basidiocarp surface to endoperidium surface, tissues of exoperidium after being treated with KOH and stained with Congo red, the composed by chains of globose cells can be seen clearly, 3.5–7 × 3–7 μm in diameter of one cell, globose cell chains is dense and covers the surface, exoperidial sphaerocysts subglobose, 5–7.5 × 4–7 μm in diameter of top cell. **Endoperidium** white (1A1) to dark brown (6F8), papery, thin, smooth to wrinkled with age, forming an opening at the top for spore release,

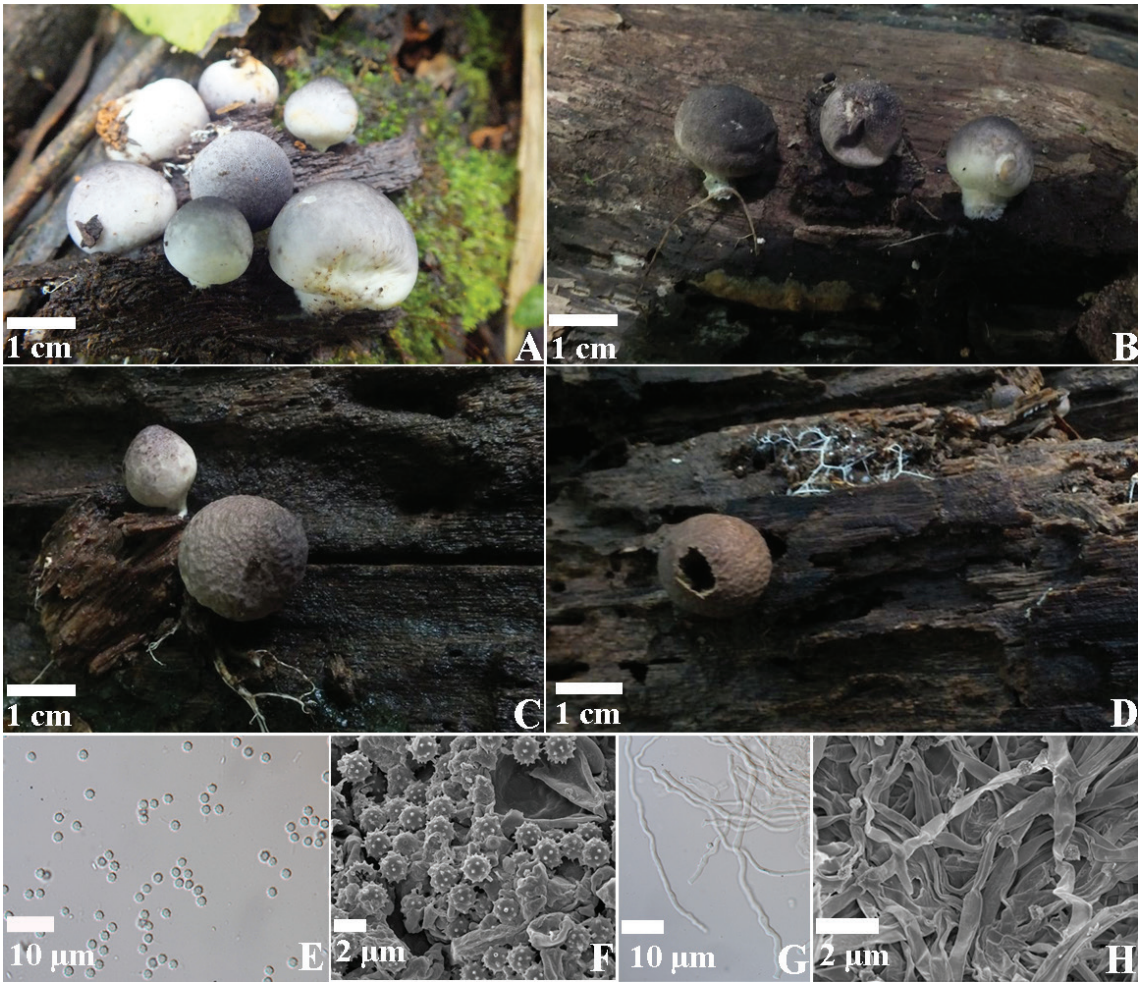

Figure 3. *Lycoperdon mengsongense*. Fresh basidiocarps of *L. mengsongense* in the field, scale bar=1 cm (A–D). Basidiospores of *L. mengsongense* with KOH taken under light microscope, scale bar=10 µm (E). Basidiospores taken under ZEISS Discovery V8 Stereoscope (scale bar=2 µm, Magnification=2001 ×) (F). *Paracapillitium* with KOH taken under a light microscope, scale bar=10 µm (G). *Paracapillitium* taken under ZEISS Discovery V8 Stereoscope, Bar=2 µm, Magnification=×1620 (H).

dehiscing by a simple pore. **Gleba** cottony and white (1A1) when young, powdery and light brown (5D7) when mature, filamentous hyphae with the size of 2.4–3.9 µm diameter. **Pseudocolumella** not observed. **Subgleba** present, not chambered, white (1A1) to light yellow (1A5), 2.5–4 mm high × 2–2.5 mm wide. **Eucapillitium** absent. **Paracapillitium** abundant, slightly branched, 2.7–3.2 µm in diameter, septate, hyaline, amorphous**,** occasionally branched, light yellow (1A5) in 5% KOH, walls <1 µm. **Basidiospores** echinate, subglobose or globose, 2.8–3.7 µm in diameter (ornamentation excluded), hyaline in 5% KOH, no changing in Melzer's reagent, with sterigma, ornamentation wrinkled and spiny margin under the SEM microscope. spine length less than 0.5 µm (Figure 3).

Habitat and distribution: —in a group, on decaying wood in evergreen broadleaf forest dominated by Elaeocarpaceae and Fagaceae, Fruiting on humus soil or large decaying logs on the ground.

Material examined:—China. Yunnan Province (100°28'30"–102°30'29" E, 21°30'16"–21°45'49" N): Jinghong City, Mengsong Village, elevation 1500 m, 20 September 2012, Lei Ye (HKAS 101876, **holotype**); *Ibid.* Lei Ye (HKAS88251, paratype), 30 July 2013. In addition to the first two collection groups that successfully obtained molecular biology data, there are three other collection groups are *Ibid.* 18 September 2012, Lei Ye (HKAS101875, paratype); *Ibid.* 6 August 2013, Lei Ye (HKAS88247, paratype); *Ibid.* 30 July 2013, Lei Ye (HKAS88254, paratype).

Notes:—The main distinguishing characteristics of *Lycoperdon mengsongense* are the dark greyish to blackish-grey exoperidium which is lighter below and covered by minute and greyish black granules that are mainly distributed on the top area; the endoperidium is smooth when young, then wrinkled with age; gleba light brown; basidiospores are small and echinate with a short pedicel (Figure 3, A–J). The phylogenetic tree showed that *L. mengsongense* has a monophyletic relationship to *L. purpurascens* (CMU55-Ly1, Figure 1). However, they have different morphological characteristics (Table 2). *Lycoperdon purpurascens* (isolate CMU55-Ly1) is a tropical collection that was found in Thailand [24], basidiocarp size of CMU55-Ly1 (12–16 × 8–12 mm) is similar to *L. mengsongense* (10–23 × 11–22 mm), mature basidiocarp size of *L. mengsongense* is similar to a type material from Bonin Island (20–30 mm diameter) [5], but the exoperidium color of the two species is quite different, dark grayish brown to blackish violet gray and deep olive-buff to olive-buff towards the base in CMU55-Ly1, the overall color is darker than *L. mengsongense*. The endoperidium of CMU55-Ly1 is mustard yellow; dark olive-buff mature gleba is also different compared to *L. mengsongense*. Morphology of basidiospores in these two species are similar, but basidiospores of CMU55-Ly1 are slightly larger (3.5–4.0 μm), and the spine length of CMU55-Ly1 is slightly longer than *L. mengsongense* (less than 0.5 μm).

4. Discussion

The species in sub-genus *Morganella* were reported on decaying wood or cow dung in temperate and tropical forests [5,7]. The genus is characterized by subglobose to globose, small basidiocarps, a two-layered peridium comprised of an exoperidium and endoperidium, powdery basidiospores released through an apical pore, no true capillitium and spinose basidiospores [4–6, 24, 29, 33]. Most of all known species in sub-genus *Morganella* have been found on decaying wood except for *M. stercoraria* which grows on cow dung. Like other species in *Lycoperdon* subg. *Morganella*, *L. mengsongense* was found on decaying wood in a tropical forest.

In this study, we introduce *Lycoperdon mengsongense* based on its unique macro- and micro- morphological characteristics together with the support of phylogenetic analyses results. Morphological characteristics of *L. mengsongense* clearly distinguish it from other closely related species, i.e. *L. purpurascens*, *L. sosinii*. According to Kreisel and Dring [4], it was mentioned that *Lycoperdon purpurascens* has considerable morphological variabilities across geographically different areas, but that statement was made without considering phylogenetic data.

The PHI test result (Φ_w=0.6354) of *Lycoperdon mengsongense* and its related species in *Lycoperdon* subg. *Morganella* ruled out the possibility of gene recombination interfering with the species delimitation (Figure 2). This is further evidence that *Lycoperdon mengsongense* is a new species.

Table 2. Comparison of main characteristics of *Lycoperdon purpurascens* and *L. mengsongense*.

Microstructure	*Lycoperdon purpurascens*	*Lycoperdon mengsongense*
Basidiocarps	12–16 mm diameter, 8–12 mm height, subglobose to depressed globose	10–23 mm in diameter, 11–22 mm in height, globose or depressed–globose
Exoperidium	Dark grayish brown to blackish violet gray and deep olive–buff to olive-buff to toward the base	Greyish white to dark grey, lighter below, covered by minute and blackish grey granules that mainly distributed at top area
Endoperidium	Pitted, soft, papery, mustard yellow, slightly shining, thin	White to dark brown, papery, thin, smooth to wrinkled with age
Gleba	Cottony and white when young, powdery and dark olive–buff when mature	Cottony and white when young, powdery and light brown when mature
Subgleba	Composed of compacted cells, Maize yellow	Present, not chambered, white to light yellow
Pseudocolumella	Inconspicuous	Inconspicuous
Basidiospores	Globose, (Q=1.14) 3.5–4 μm diameter without the ornamentation, minutely spiny, spines up to 0.5 μm length, with an oil drop inside, shortly pedicellate	Echinate, subglobose or globose, (Q =1.32) 2.8–3.7 μm in diameter, with sterigma, wrinkled and spiny margin and spine length less than 0.5 μm
Paracapillitium	Abundant, septate, and branched, 2.5–4.5 μm diameter, hyaline, presenting amorphous and hyaline incrustation	Abundant, slightly branched, 2.7–3.2 μm in diameter, septate, hyaline, amorphous
Habitat	On rotting wood in a tropical deciduous forest, dominated by *Castanopsis* spp.	On decaying wood in evergreen broadleaf forest

Notes: The characteristics of *Lycoperdon purpurascens* refer to Kumla et al. [24]. Some taxa are morphologically closely related to *L. mengsongense*, for example, *L. arenicola* is characterized by pyriform to turbinate basidiocarps, its exoperidium incrusted with grains of sand composing of minute spines and yellowish brown at the base to olive grown to grey at the base, abundant capillitium and was often found in sandy soil [28] ; *L. sulcatostoma* is characterized by a echinate exoperidium, which is brownish orange to light brown at the top, to pale orange towards the base, a conspicuous peristome at the top of basidiocarps and bigger basidiospores size (5–6 μm) [29]; *L. benjaminii* has greyish orange basidiocarps and a rudimentary subgleba [30]; *L. compacta* has subglobose to pyriform basidiocarps, a developed and chambered subgleba, violet to brown exoperidium when young and greyish yellow to olive yellow when mature and pseudocolumella are present [31]; *L. velutinum* has a velutineous exoperidium and bigger basidiospores (4.9–6.5 μm), umber gleba, tan subgleba, small and flattened pseudocolumella [4, 5, 7]; *L. costaricense* is characterized by pyramidal spines on a brown exoperidium, ochre yellow and reticulate-areolate endoperidium surface [6, 7, 32]; *Morganella samoensis* has a furfuraceus and brown exoperidium with minute conical tubercles, olivaceous to dark brown and obsolete subgleba [5]; *M. stercoraria* is characterized by tan to light brown exoperidium with small pluricellular spines, chambered subgleba, rare paracapillitium and grows on cow dung [5, 29]; *M. afra* has globose to pyriform basidiocarps, fuscous above and lighter below exoperidium, light brown and areolate endoperidium, chambered subgleba and light yellowish basidiospores [4, 28].

Acknowledgements

We would like to thank the National Science Foundation of China (NSFC), project codes 41761144055 and 41771063. In addition, the CGIAR Research Program 6: Forest, Trees and Agroforestry, the Kunming Institute of Botany, Chinese Academy of Science (CAS) and the Chinese Ministry of Science and Technology, under the 12th 5-year National Key Technology Support Program (NKTSP) 2013BAB07B06 integration and comprehensive demonstration of key technologies on Green Phosphate-mountain construction for providing financial support for this study. We would like to thank the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences for providing us with the use of an electron microscope. Samantha C. Karunarathna thanks CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2018PC0006) and National Science Foundation of China (NSFC) project code 31851110759. Dhanushka Wanasinghe would like to thank CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2021FYB0005) and the Postdoctoral Fund from Human Resources and Social Security Bureau of Yunnan Province. Lei Ye would like to thank Joint Project of Basic Agricultural Research in Yunnan Province (No2018FG001-032) for providing microscope and specimen handling instruction. This research was partially supported by Chiang Mai University. Fiona Worthy in the World Agroforestry Centre (ICRAF), Kunming Institute of Botany, China, is thanked for English language editing.

References

[1] Kirk P., Cannon P., Minter D. and Stalpers J., *Ainsworth & Bisby's Dictionary of the Fungi*, Wallingford, UK, 2008. DOI 10.1079/9780851998268.0000.

[2] Index Fungorum, 2021; Available at: http://www.indexfungorum.org/names (accessed May 2021).

[3] Zeller S.M., *Mycologia,* 1948; **40**: 639–668. DOI 10.2307/3755316.

[4] Kreisel H. and Dring D., *Feddes Repertorium*, 1967; **74**: 109–122. DOI 10.1002/fedr.19670740105.

[5] Ponce de León P., *Revision of the Genus Morganella (Lycoperdaceae),* Field Museum of Natural History, Fieldiana Botany, 1971; **34 (3)**: 27–44. DOI 10.5962/bhl.title.2567.

[6] Suárez V. and Wright J., *Mycologia,* 1996; **88**: 655–661. DOI: 10.2307/3761163.

[7] Alfredo D.S., Baseia I.G., Accioly T. and Silva B.D., *Mycol. Prog.*, 2017; **16**: 965–985. DOI 10.1007/s11557-017-1332-y.

[8] Larsson E. and Jeppson M., *Mycol. Res.,* 2008; **112**: 4–22. DOI 10.1016/j.mycres.2007.10.018

[9] Kornerup A. and Wanscher J.H., *Methuen Handbook of Colour*, 1978.

[10] Jayasiri S.C., Hyde K.D., Ariyawansa H.A., Bhat J. and Buyck B., *Fungal Divers.,* 2015; **74**: 3–18. DOI 10.1007/s13225-015-0351-8.

[11] White T.J., Bruns T., Lee S. and Taylor J., Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics; in Innis M.A., Gelfand D.H., Sninsky J.J., and White T.J., eds., *PCR Protocols: A Guide to Methods and Applications,* New York: Academic Press, 1990: 315–322. DOI 10.1016/b978-0-12-372180-8.50042-1.

[12] Vilgalys R. and Hester M., *J. Bacteriol.,* 1990; **172**: 4239–4246. DOI 10.1128/jb.172.8.4238-4246.1990.

[13] Katoh K., Rozewicki J. and Yamada K.D., *Brief. Bioinform.,* 2019; **20**: 1160–1166. DOI 10.1093/bib/bbx108.

[14] Hall T.A., BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series, 1991; 41: 95–98.

[15] Miller M.A., Pfeiffer W. and Schwartz T.,

Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the Extreme to the Campus and Beyond (XSEDE '12). Association for Computing Machinery, Chicago, USA, 16-20 July 2012; **39**: 1–8. DOI 10.1145/2335755.2335836.

[16] Stamatakis A., *Bioinformatics,* 2014; **30**: 1312–1313. DOI 10.1093/bioinformatics/btu033.

[17] Nylander J.A.A., *MrModeltest 2.0. Program distributed by the author*. Evolutionary Biology Centre, Uppsala University, 2004.

[18] Swofford D.L., *PAUP: Phylogenetic Analysis using Parsimony, version 4.0 b10*. Sinauer Associates, Sunderland, 2002. DOI 10.1007/978-1-4020-6754-9_12413.

[19] Kishino H. and Hasegawa M., *J. Mol. Evol.,* 1989; **29**: 170–179. DOI 10.1007/bf02100115.

[20] Rambaut A., FigTree version 1.4.0., 2012; Available at http://treebioedacuk/software/figtree/.

[21] Bruen T.C., Philippe H. and Bryant D., *Genetics,* 2006; **172**: 2665–2681. DOI 10.1534/genetics.105.048975.

[22] Huson D.H. and Bryant D., *Mol. Biol. Evol.,* 2006; **23**: 254–267. DOI 10.1093/molbev/msj030.

[23] Quaedvlieg W., Binder M., Groenewald J.Z., Summerell B.A. and Carnegie A.J., *Persoonia,* 2014; **33**: 1–40. DOI 10.3767/003158514x681981.

[24] Kumla J., Suwannarach N., Bussaban B. and Lumyong S., *Mycoscience,* 2014; **55**: 49–52. DOI 10.1016/j.myc.2013.05.002.

[25] Rebriev Y. and Bulakh E.M., *Mikol. Fitopatol.*, 2015; **49**: 293–296.

[26] Rebriev Y., *Mikol. Fitopatol.*, 2016; **50**: 302–312.

[27] Kasuya T., Phongpaichit S. and Dissara Y., *Nat. Hist. Bull. Siam Soc.*, 2006; **54**: 209–213.

[28] Alfredo D.d.S., Accioly T. and Baseia I.G., *Turk. J. Bot.,* 2014; **38**: 595–599. DOI 10.3906/bot-1307-68.

[29] Alves C.R. and Cortez V.G., *Nova Hedwigia,* 2013; **96**: 409–417. DOI 10.1127/0029-5035/2013/0078.

[30] Cortez V.G., Calonge F.D. and Baseia I.G., *Mycotaxon,* 2007; **102:** 425–429.

[31] Barbosa M.M.B., da Silva M.A., da Cruz R.H.S.F. and Calonge F.D., *Mycotaxon,* 2011; **116**: 381–386. DOI 10.5248/116.381.

[32] Morales M.I., Nassar M. and Sáenz J., *Rev. Biol. Trop.,* 1974; **21**: 221–227.

[33] Alfredo D., Leite A., Braga-Neto R. and Baseia I., *Mycosphere,* 2012; **3**: 66–77. DOI 10.5943/mycosphere/3/1/8.