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COMMUNICATION

MORPHOLOGICAL AND MOLECULAR PHYLOGENETIC STUDIES ON *BATTARREA PHALLOIDES* (AGARICALES): A NEW REPORT TO INDIAN MYCOBIOTA

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COMMUNICATION

Morphological and molecular phylogenetic studies on *Battarrea phalloides* (Agaricales): a new report to Indian mycobiota

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Abstract: The Scaly-stalked Puffball *Battarrea phalloides* (Dicks.) Pers. is recorded for the first time in India. The fungus is reported from many countries across the continents and typically uncommon and rare in all regions. It is Red Listed in most of the European countries and is under assessment in IUCN Global Fungal Red List Initiative. The Indian sample of *B. phalloides* is reported from Kadur Taluk of Chikkamagaluru District, Karnataka with morpho-molecular data.

Keywords: Elaters, Morpho-molecular, nrITS, Red List, Scaly-stalked Puffball.

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Competing interests: The authors declare no competing interests.

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Author contribution: RK carried out the research work, wrote the article. MK guided in every step and corrected mistakes in the article.

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INTRODUCTION

Battarrea phalloides (Sandy Stilt Ball, Sandy Stilt Puffball, Scaly-stalked Puffball), previously known gasteromycete in Battarreaceae (Corda 1842), and now a distinctive saprobic basidiomycetous agaric fungus, easily recognizable with a scaly lacerated stem growing up to 40cm in height, forming a reddish-brown spore case inside a thin greyish skin. It is rare, uncommon and occurs in small scattered populations or sometimes even appears as single basidiomata.

Battarrea phalloides is red-listed in several European countries and is one of the non-lichenized fungi afforded legal protection by being included in schedule 8 of the Wildlife and Countryside Act, 1981 in the United Kingdom (Jeffries & McLain 2004). The species is currently under assessment for addition to the IUCN: The Global Fungal Red List Initiative (http://iucn.ekoo.se/iucn/species_view/159853).

Sixteen species have been described in the genus *Battarrea* Pers. since 1801 (Index Fungorum, http://www.indexfungorum.org/) and most of them are conspecific to *Battarrea phalloides*. Early taxonomic discussions about the worthiness of morphological characters for separating *B. phalloides* and *B. stevenii* were evaluated using modern phylogenetic approach by Martin & Johannesson (2000), Martin et al. (2013) and Jefferies & McLain (2014), the shreds of evidence suggest both taxa are conspecific. In addition, Martin & Johannesson (2000) considered spore ornamentation as a non-molecular character for lineage recognition and depicted three main lineages phylogenetically, they have differences in their spore ornamentation as—(a) spores with anastomosing truncate ridges, (b) finely verrucose, and (c) finely reticulate.

The present study describes *B. phalloides* as a new report to Indian mycobiota based on morphological characters and multigene phylogenetic analysis.

Materials and Methods

The Scaly-stalked Puffball like basidiomata of *Battarrea phalloides* were collected from Aladahalli Village (13.546N & 75.875E) of Kadur Taluk (Figure 1), Western Ghats region of Karnataka during July 2019.

Sampling and morphological characterization

The sporomas of different stages were collected and phenotypic characters were recorded using a field key (Atri et al. 2017). Microscopic characters were recorded using a light microscope (Olympus CH20i) and the sporocarps were shade-dried and stored in the Department of Botany, Kuvempu University for further

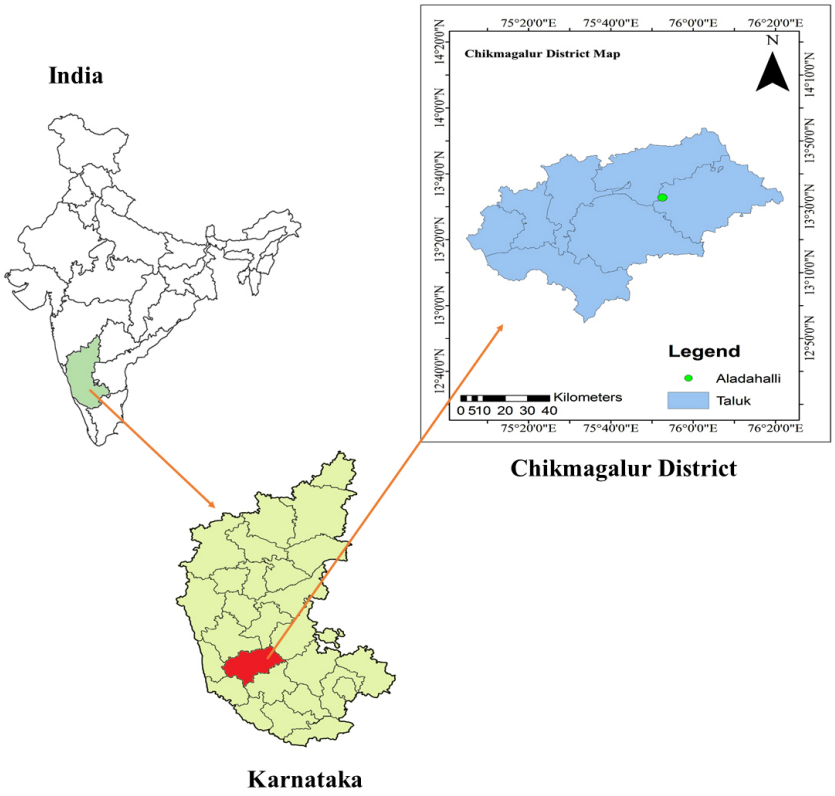

Figure 1. Geographic location of *Battarrea phalloides*

Table 1. List of primers utilized to amplify nrITS and nrLSU gene sequences.

	Primer	Sequence	Amplifying gene	T_m [°C]
1	ITS 1	TCCGTAGGTGAACCTTGCGG	nrITS	60.99
2	ITS 4	TCCTCCGCTTATTGATATGC		55.25
3	LROR	ACCCGCTGAACTTAAGC	nrLSU	52.77
4	LR5	TCCTGAGGGAAACTTCG		52.77

studies (Image 1). To identify the surface ornamentation of spores, scanning electron microscopy was carried out in ZEISS EVO CSEM.

DNA Extraction, PCR and Phylogenetic analysis

The total genomic DNA was extracted from the freshly collected sporocarp using the CTAB method (Doyle & Doyle 1987) with modifications. 100mg of inner stipe tissue was directly homogenized with 500µl of 2X CTAB extraction buffer pre-warmed to 65°C in a 1.5ml microcentrifuge tube with the help of micro-pestle, followed by vortexing and incubated in a water bath at 65°C for 1h. The sample was cooled briefly before centrifugation at 13,000rpm for 30min. To the centrifugate 3µl of RNase A (20mg/ml) was added and incubated for 10min at 37°C, followed by the addition of an equal amount of PCI (25:24:1) with slow invert mixing. The mixture was centrifuged at 10,000rpm for 10min at room temperature and the supernatant was extracted. To precipitate the DNA 500µl of ice-cold isopropanol was added and incubated overnight at 4°C, followed by centrifugation at 10,000rpm for 10min at 10°C to pellet the DNA and washed twice with 70% ethanol, drained and dissolved in 50µl of 1X TE buffer.

PCR reactions were carried out in 0.2ml PCR tubes with 50µl reaction mixture containing, 25µl double distilled water, 8µl 10X PCR buffer A (Himedia). 2.5µl of each primer, 0.5µl of Taq DNA polymerase (3U/µl), 1.5µl dNTP's mixture (Himedia) and 10µl of DNA template. The primer pair ITS 1 and ITS 4 (White et al. 1990) for nrITS region and LROR and LR5 (Vilgalys & Hester 1990) for the nrLSU region were used (Table 1). The thermal profile for nrITS amplification; 4' 94°C, 32 cycles of 30" 94°C, 1' 52°C, 1' 72°C and a final extension step of 7' 72°C, for nrLSU 5' 94°C, 30 cycles of 30" 94°C, 1' 47°C, 1' 72°C and a final extension step of 7' 72°C. The PCR products were examined on 1% Agarose gel stained with Ethidium Bromide and visualized under gel image documentation system (BioRad) followed by cleanup and sequencing.

The electropherograms of both forward and reverse sequences obtained from Eurofins Genomics India Pvt. Ltd. Bengaluru were checked and trimmed using MEGA X (Kumar et al. 2018). Consensus sequences were generated using BioEdit sequence alignment editor v.7.2.5 (Hall, CA) by Clustal W (Madeira et al. 2019). BLAST search in the GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) nucleotide database to identify the related taxa by sequence similarity and both nrITS and nrLSU sequences were deposited to GenBank with accession numbers MN450310 and MN700164, respectively.

Molecular phylogenetic analysis was performed by using nrITS and nrLSU sequences separately. Datasets of 17 nrITS sequences (Table 2) and 15 nrLSU sequences (Table 3) including those retrieved from the NCBI GenBank are used to assess the alignment confidence score in the GUIDANCE web server (http://guidance.tau.ac.il) by MAFFT algorithm (Katoh et al. 2019) to construct 100 alternative guide trees. Using GUIDANCE outputs the columns showing less than 93% confidence scores are removed and aligned in BioEdit v.7.2.5. The alignment file obtained is further used to analyze the maximum likelihood in RAxML GUI v.2.0.0.0 using the GTRGAMMA+I model as suggested by jModelTest

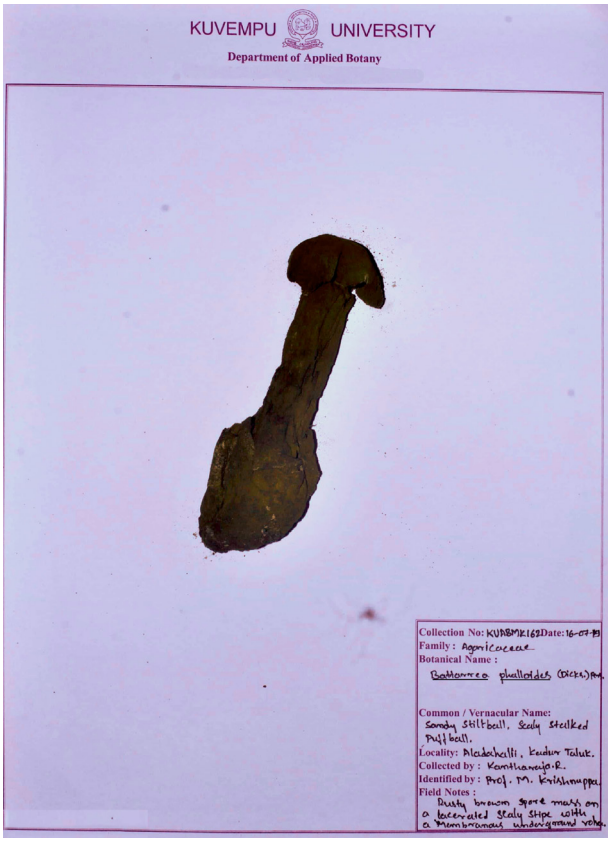

Image 1. Specimen submitted to herbarium.

v.2.1.10 (Darriba et al. 2012) with 1,000 bootstrap replications. The best trees obtained are inferred by Mr. Bayes.

RESULTS

Taxonomy

***Battarrea phalloides* (Dicks.) Pers.,**

MycoBank No.: 159853

GenBank Accession No.: MN450310 (nrITS), MN700164 (nrLSU).

Basionym: *Lycoperdon phalloides* Dicks., Fasciculus plantarum cryptogamicarum Britanniae 1: 24 (1785)

Etymology: The specific epithet *phalloides* refers to the similarity of volva with genus *Phallus.*

Basidiomata medium to large, 20–30 cm in length (Images 2 & 3). Spore case 3–5.2 cm diam. Greyish membranous skin when young, shedding to become convex rusty brown abundant spore mass at maturity (Image 4). *Stipe* 10-25cm in length, 1.8–3 cm diam., light brownish, hairy to lacerated scaly, base include underground membranous volva. Gleba pulverulent includes capillitia and elaters. Spores 5–7×4–6 µm, globose to almost elliptical (Image 5), finely reticulate (Image 6), inamyloid in Melzer's reagent. Elaters 50–80+ µm long 4–7µm wide, cylindrical to fusiform, annular to spiral thickenings (Image 6), ochraceous in KOH.

Ecology: Saprophytic, growing alone or scattered in dry sandy soil. Cited twice in July and August 2019 in Kadur Taluk (13.546N & 75.875E).

Specimens: India, Karnataka, Chikmagaluru District, Kadur Taluk, 28 July 2019 (KUABMK-162) and 15 August 2019, Kantharaja R & Krishnappa M.

Image 2. *Battarrea phalloides* in habitat.

Image 3. Specimen with membranous volva.

Image 4. Gleba with rusty brown spore mass.

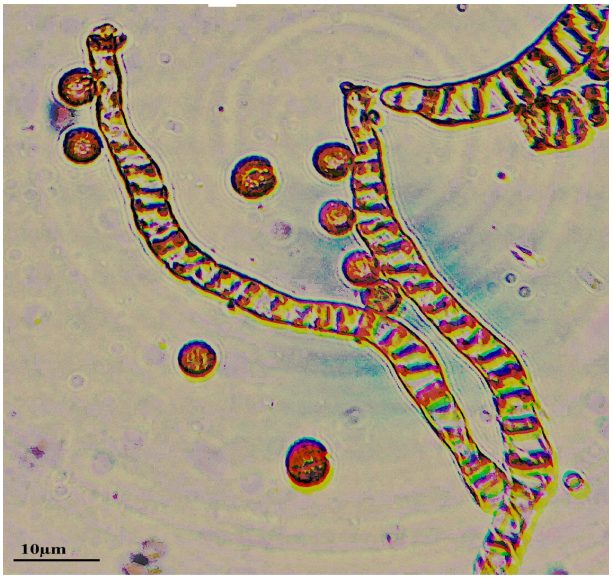

Image 5. Basidiospores and Elaters (scale 10µm). © R. Kantharaja.

Table 2. List of species, geographic origin and GenBank accession numbers of nrITS sequences used in molecular phylogeny analysis.

	Species	Geographic origin and year	GenBank accession number
1	*Battarrea phalloides*	Spain, 2013	HF913784
2	*Battarrea phalloides*	Spain, 2013	HF913785
3	*Battarrea phalloides*	USA, 2017	MF422608
4	*Battarrea phalloides*	UK, 2005	DQ184685
5	*Battarrea stevenii*	Spain, 1999	AF215655
6	*Battarrea phalloides*	UK, 2005	DQ184690
7	***Battarrea phalloides***	**India, 2019**	**MN450310**
8	*Battarrea stevenii*	UK, 2005	DQ184688
9	*Battarrea phalloides*	UK, 2005	DQ184687
10	*Tolustoma calongei*	Spain, 2016	KU518973
11	*Tolustoma kotlabe*	Sweden, 2005	DQ112629
12	*Tolustoma obesum*	Sweden, 2016	KU518987
13	*Tolustoma obesum*	Sweden, 2016	KU518988
14	*Tolustoma grandisporum*	Sweden, 2016	KU519003
15	*Tolustoma grandisporum*	Sweden, 2016	KU519006
16	*Tolustoma grandisporum*	Sweden, 2016	KU519001
17	*Lycoperdon perlatum*	China, 2007	EU622257

Table 3. List of species, geographic origin and GenBank accession numbers of nrLSU sequences used in molecular phylogeny analysis.

	Species	Geographic origin and year	GenBank accession number
1	*Chlorophyllum agaricoides*	China, 2017	MG742020
2	*Chlorophyllum agaricoides*	Spain, 2015	KR233498
3	*Chlorophyllum agaricoides*	China, 2017	MG742021
4	*Chlorophyllum agaricoides*	Spain, 2015	KR233494
5	*Chlorophyllum olivieri*	China, 2017	MG742037
6	*Chlorophyllum olivieri*	China, 2017	MG742036
7	*Disciseda bovista*	Hungary, 2018	MK277947
8	*Tolustoma fimbriatum*	Hungary, 2018	MK278635
9	*Tolustoma albicans*	Hungary, 2018	MK278628
10	*Tolustoma macrocephala*	USA, 2002	AF518663
11	*Tolustoma simulans*	Hungary, 2018	MK278639
12	*Tolustoma simulans*	Hungary, 2018	MK278634
13	***Battarrea phalloides***	**India, 2019**	**MN700164**
14	*Battarrea lacinata*	USA, 1999	AF208534
15	*Lycoperdon ericaeum*	Japan, 2014	KU507401

Phylogenetic Analysis

The specimen KUABMK-162 was subjected to molecular identification initially based on sequences of the nrITS region via. BLAST search analysis in the GenBank database and found >99% similarity with unpublished sequences (DQ184690, DQ184688, and DQ184687). The maximum likelihood analysis using RAxML and MrBayes drawn by the GTRGAMMA+I model as suggested by jModelTest v.2.1.10 confirms the closest relation of newly generated sequences with *Battarrea phalloides* with 97% bootstrap support (Figure 2). Due to unavailability of nrLSU sequences of *B. phalloides* the generated nrLSU sequences were found clustered with *Battarrea lacinata* (Figure 3).

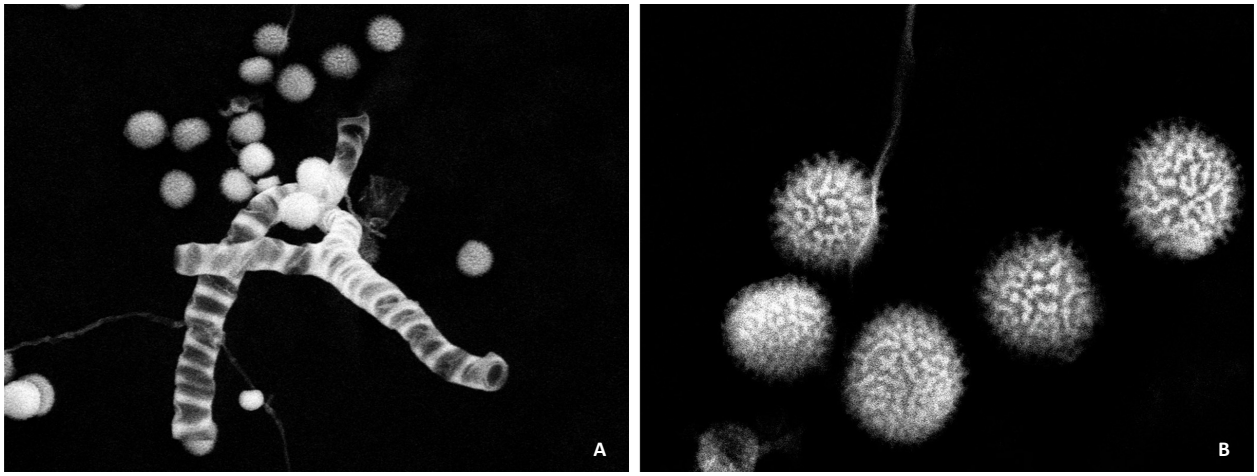

Image 6. Scanning Electron Microscopic view of A—Elaters | B—spores. © R. Kantharaja.

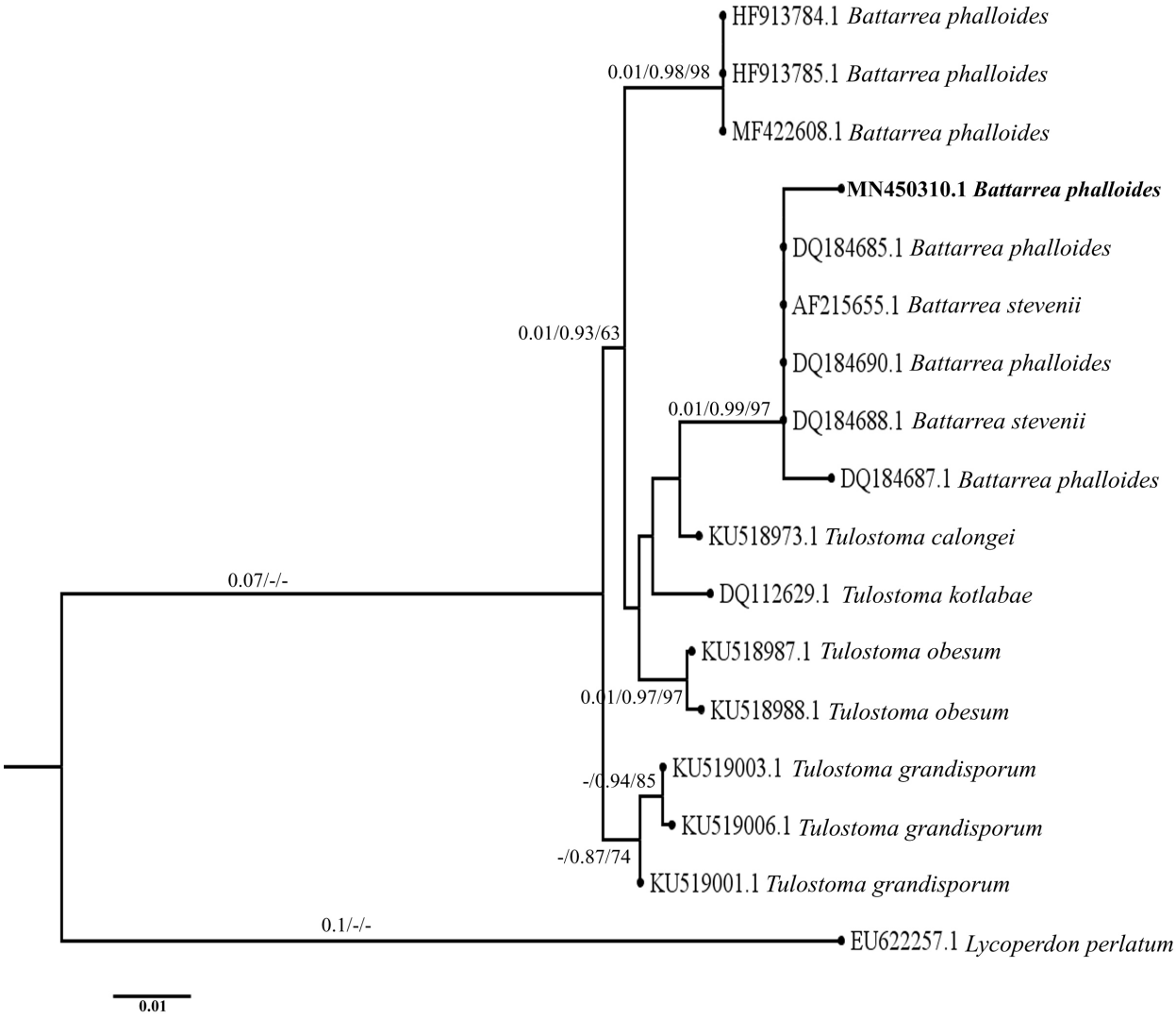

Figure 2. RAxML tree of *Battarrea phalloides* generated by maximum likelihood analysis of nrITS sequences using GTRGAMMA+I model with *Lycoperdon perlatum* as an outgroup showing bootstrap support (BS>50%) and Bayesian posterior probability values (PP>0.7). (BL/PP/BS).

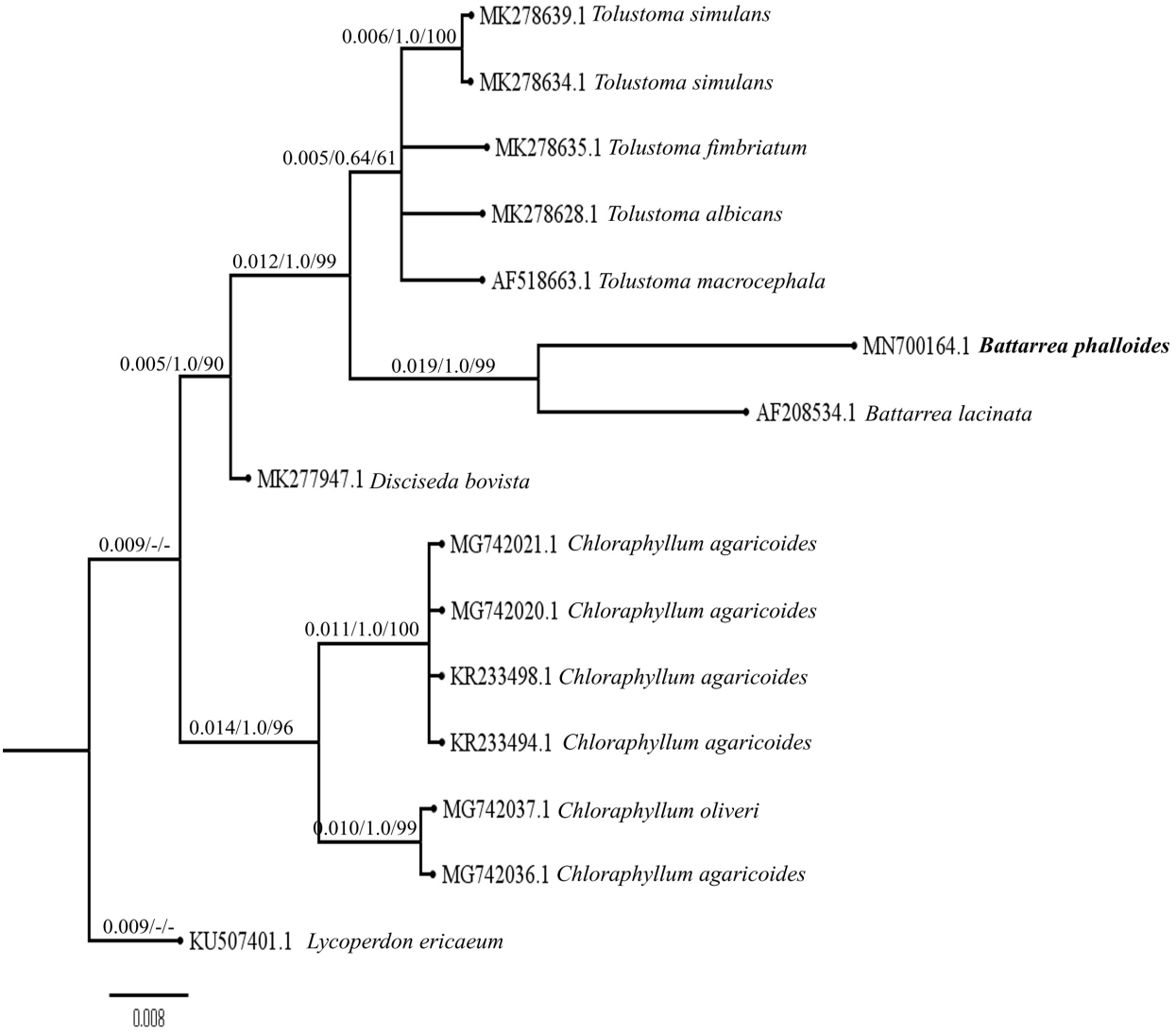

Figure 3. RAxML tree of *Battarrea phalloides* generated by maximum likelihood analysis of nrLSU sequences using GTRGAMMA+I model with *Lycoperdon ericaeum* as an outgroup showing bootstrap support (BS>50%) and Bayesian posterior probability values (PP>0.6). (BL/PP/BS).

DISCUSSION

Previous reports of *Battarrea phalloides* showed the species is found in arid and semi-arid habitats like desserts and dry Savanna (Martin & Johannessen 2000; Howladar et al. 2013; Ivancevic et al. 2016). The present study claims that *B. phalloides* is found in Chikmagalur District, Western Ghats region of Karnataka, India. The climatic conditions in Kadur Taluk support the habitat preference of the species, where the average annual rainfall (620mm) is almost similar to the dry areas. Howladar et al. (2013), stated *Battarrea phalloides* is rare everywhere but distributed worldwide, cited the reports from across continents and this report adds another vicinity of occurrence.

Martin & Johannessen in 2000 identified three main lineages in a phylogenetic study of *B. phalloides* and *B. stevenii* herbarium collections from various parts of the world by considering spore ornamentation as a non-molecular character. Contrary to this, Garrido-Benevent in 2014 tried to represent cryptic speciation and predicted the presence of three to four putative species within the *Battarrea phalloides-stevenii* complex. but, he also noted the requirement of further data to build a consistent taxonomy. The current taxonomic data according to Mycobank and Index Fungorum, however, suggests *B. stevenii* as a synonym of *B. phalloides*. In our study, the SEM image of spore confirms the presence of reticulate ornamentation which is highly similar to the previous reports.

The nrITS sequences of specimen KUABMK-162 (MN450310) is found clustered with specimens from Israel, Cyprus and UK (DQ184685, DQ184687, and DQ184690) with a well-supported bootstrap value of 97% and maximum Bayesian posterior probability value of 0.99. Based on morpho-molecular characters the present study confirms the identity of the specimen as *Battarrea phalloides* and is a new record to Indian mycobiota.

REFERENCES

Atri, N.S., M. Kaur & S. Sharma (2017). Characterization of Lamellate Mushroom - An Appraisal, 471–500pp. In: Satyanarayana, T., S. Deshmukh & B. Johri (eds.) *Developments in Fungal Biology and Applied Mycology*. Springer, Singapore.

Darriba, D., G.L. Taboada, R. Doallo & D. Posada (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8): 772.

Dentinger, B.T.M., S. Margaritescu & M. Monclavo (2010). DNA Barcoding: Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. *Molecular Biology Resources* 10: 628–633.

Doyle, J.J. & J.L. Doyle (1987). A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* 19(1): 11–15.

Ellis, J.B. & M.B. Ellis (1990). Fungi without gills (Hymenomycetes and Gasteromycetes): an identification handbook. London: Chapman & Hall.

Gardes, M. & T.D. Bruns (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

Garrido-Benavent, I. (2014). The *Battarrea phalloides-stevanii* complex: multiple sources of evidence as a strategy to unveil cryptic species within poorly characterized taxa. *Butlletí Societat Micològica Valenciana* 19: 17–35.

Guindon, S. & O. Gascuel (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Howladar, S., Y.A.G. Mahmoud & A. Meriseel (2013). *Battarrea phalloides* – new for Saudi Arabia. *Österreichische Zeitschrift für Pilzkunde* 22: 1–6.

Ivancevic, B. (2017). *Battarrea phalloides*. In: IUCN 2019. The Global Fungal Red List Initiative. Accessed on 23 August 2019. http://iucn.ekoo.se/iucn/species_view/159853/

Ivancevic, B., A. Mesic, Z. Tkalcec, I. Kusan & I. Horjan (2016). Studies on Croatian Basidiomycota 3: the first record of *Battarrea phalloides* (Agaricales) with a worldwide taxonomic review of *Battarrea* species. *Nova Hedwigia* 102: 197–209.

Jacobson, K.M., P.J. Jacobson & O.K. Miller (1999). The autecology of *Battarrea stevenii* in ephemeral rivers of southwestern Africa. *Mycological Research* 103: 9–17.

Jeffries, P. & L. McLain (2004). Synonymy between *Battarrea phalloides* and *B. stevanii*. English Nature Research Reports. Number 625.

Katoh, R. & Yamada (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20:1160-1166.

Kirk, P.M., P.F. Cannon, D.W. Minter & J.A. Stalpers (2008). *Ainsworth & Bisby's Dictionary of the Fungi, 10th Ed.* CAB International, Wallingford.

Madeira, F., Y. Park, J. Lee, N. Buso, T. Gur, N. Madhusoosanan, P. Basutkar, A.R.N. Tivey, S.C. Potter, R.D. Finn & R. Lopez (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Research* 2(47): 636–641.

Martin, K.J. & P.T. Rygiewicz (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiology* 5(28): 1–11.

Martin, M.P. & H. Johannesson (2000). *Battarrea phalloides* and *B. stevanii*, insight into a long-standing taxonomic puzzle. *Mycotaxon* 76: 67–75.

Martín, M.P., K. Rusevska, M. Dueñas & M. Karadelev (2013). *Battarrea phalloides* in Macedonia: genetic variability, distribution and ecology. *Acta Mycologica* 48: 113–122.

Senthilarasu, G. & V. Kumaresan (2016). Diversity of agaric mycota of Western Ghats of Karnataka, India. *Current Research in Environmental and Applied Mycology* 6(2): 75–101.

Shepherd, L.D. & J.A. Cooper (2017). First record of the fungus *Battarrea phalloides* (Agaricaceae) in New Zealand. *New Zealand Journal of Botany* 1–6.

Swapna, S., S. Abrar & M. Krishnappa (2008). Diversity of Macrofungi in Semi-Evergreen and Moist Deciduous Forest of Shimoga District-Karnataka, India. *The Journal of Mycology and Plant Pathology* 38(1): 21–26.

Thompson, J.D., D.G. Higgins & T.J. Gibson (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22(22): 4673–4680.

Uzunov, B. (2014). New Localities of *Battarrea phalloides* (Basidiomycota) in Bulgaria. Annual of Sofia University "St. Kliment Ohridski". Faculty of Biology. Book 2 – Botany 99: 71–75.

Vilgalys, R. & M. Hester (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology* 172: 4238–4246.

White, T.J., T. Bruns, S. Lee & J. Taylor (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics, pp. 315–322. In: Innis, M., D. Gelfand, J. Sninsky & T. White. *PCR Protocols*. Academic Press, Inc., NewYork.

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