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Rediscovery of *Pholiota microspora* After 175 Years from Darjeeling Hills, India, Based on Morphological Data and Phylogenetic Analyses

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ABSTRACT

In the present study, a unique agaricoid *Pholiota microspora* (Berk.) Sacc., which was originally reported from Darjeeling, India by Berkley as *Agaricus microsporus* in the year 1850 based on Hooker's collection deposited in Kew is rediscovered during our field survey from Darjeeling Hills. Detailed macro- and microscopic examination, coloured photographs, and support by phylogenetic analyses and comparison with previously reported morphologically similar species are provided.

Keywords: Pholiota, Phylogenetic analyses, Rediscovery, Taxonomy

INTRODUCTION

Darjeeling District is the northernmost district of West Bengal, India, located in the foothills of the Himalayas. The Hills of Darjeeling belong to the Eastern Himalayan range and are situated between 21.95–29.45°N latitude and 82.70–100.31°E longitude, covering an area of 524,190km^2 (Dutta *et al.,* 2020). Generally, the warm and temperate climate of this region favoured rich vegetation. The Darjeeling Himalayas show their own climatic peculiarities caused by their geographical locations and a wide range of altitudinal variations that create a better habitat for the luxuriant growth of macrofungi.

The genus *Pholiota* (Fr.) P. Kumm belongs to the family Strophariaceae within the order Agaricales (Basidiomycota). It is a well-defined fungus distributed worldwide as a wood-rotting genus representing ca. 157 species (Smith and Hesler, 1968; Lee *et al.,* 2020; Tamang *et al.,* 2024). The species of *Pholiota* are characterized by yellow or brown pileus with dry, viscid or slimy caps; smooth basidiospores, thin or thick-walled with large, distinct, small, or rather indistinct germ pore; rusty-brown to yellow-brown spore deposits; pleurocystidia present or absent; cheilocystidia always present as lepto- or chrysocystidia and a pileipellis composed of a layer of cutis or ixocutis of narrow, cylindrical and inflated hyphae (Noordeloos, 2011; Lee *et al.,* 2020; Cosinglio,2024). Till now only 25 species of *Pholiota* have so far been recorded from India (Farook *et al*, 2013; Chuzho and Dkhar, 2020; Tamang *et al.,* 2024) amongst which six species of *Pholiota,* namely, *P. aurivella (*Batsch. ex Fr.) Kummer, *P. microspora* (Berk.) Sacc. and *P. squarrosa* (Oeder) P. Kumm., *P. multicingulata* Horak*, P. fasciculata* J. Tamang & K. Acharya, and *P. himalayensis* J. Tamang & K. Acharya have been reported from Darjeeling hills, West Bengal (Manjula, 1983 and Tamang *et al.,* 2024). While studying the macrofungal flora in the hilly region of West Bengal, we came across several unique macrofungi, including the specimen of *Pholiota*. Detailed macro- and microscopic examination supported by ITS-based phylogenetic analyses revealed one of our collected specimens to be *Pholiota microspora* (Berk.) Sacc., belonging to the Strophariaceae family. An extensive literature survey revealed that this specimen was reported in the year 1850 by Berkley from Darjeeling Hills, West Bengal, India (Manjula, 1983; Berkeley,1850). Hence, the present finding reports the rediscovery of *P. microspora* after 175 years in Darjeeling Hills, West Bengal.

MATERIALS AND METHODS

Morphological observations

Specimens studied were collected from the Darjeeling hills of West Bengal during field trips

conducted in 2019-2023. The macroscopic description, along with the field photographs of the fresh samples, was taken and noted in their habitat. For drying and preservation of specimens, the protocol of Pradhan *et al*. (2015) was followed. In our description for colour notation, the Methuen Handbook of Colour was used (Kornerup and Wanscher 1978). To observe the microscopic characteristics, a thin hand-made section from dried material was prepared and transferred onto a slide and rehydrated in 5% KOH solution, followed by staining in Congo red. At least thirty basidiospores were measured, and their mean value (underlined) was provided. For basidiospores, the abbreviation avL is used for the average length and avW for the average width of the basidiospores; Q represents the length/width ratio of individual basidiospores, and Q_m represents the average Q of all the basidiospores. After proper identification, the sample was deposited in the Calcutta University Herbarium (CUH) with the accession number CUHAM766 for further reference.

DNA extraction and PCR amplification

DNA was isolated from dried specimens with the XcelGen Fungal gDNA isolation Kit (Xcelris Genomics, Ahmedabad, India) following the manufacturer's instructions. The universal primers ITS1 and ITS4 were used for amplification (White *et al.,* 1990) of nuclear ribosomal internal transcribed spacer (nrDNA ITS) on the Applied Biosystems®2720 automated thermal cycler following the amplification protocol as described by Thapa *et al.,* (2023). The PCR product was checked in 2% agarose gel, and the positive reaction-giving samples were sent to Barcode Biosciences (Bangalore, India) for Sanger sequencing. The newly generated sequence in this study was submitted to GenBank (www.ncbi.nlm.nih.gov) with accession number OM818654.

Sequence alignment and phylogenetic analyses:

The phylogenetic analyses were conducted using the newly generated sequences along with the closely related sequences retrieved from GenBank by using a Blastn algorithm and using data from previously published articles (Tamang *et al.,* 2024). *Agrocybe* species were selected as outgroups for constructing a phylogenetic tree following Tamang *et al.,* 2024**.** A final aligned data matrix consisting of 38 nrITS sequences was imported to MEGA v.7.0 (Kumar *et al.,* 2016) and aligned using MUSCLE (Edgar, 2004). The maximum likelihood (ML) analyses were performed using raxmlGUI 2.0 (Edler *et al.,* 2020) with 1000 bootstrap replicates and GTRGAMMA as a substitution model. Bayesian analyses were performed using MrBayes v. 3.2.7 (Ronquist *et al.,* 2012) using Monte Carlo Markov Chains (MCMC) analyses (Geyer, 1991) following the parameters given by Vishal *et al.* (2021). Phylogenetic trees were edited in FigTree v.1.4.4 (Rambaut, 2012) and Microsoft PowerPoint 2019. Maximum likelihood bootstrap (MLBS) and Bayesian posterior probabilities (PP) values over 70% and 0.9 are presented in the resulting tree **(Figure 1).**

RESULTS

Phylogenetic analyses

The molecular phylogenetic analyses were performed using the ITS nrDNA dataset of 38 sequences. The dataset also includes our newly generated sequences and outgroup taxa. The final aligned matrix consisted of 421 distinct alignment patterns with 24.16% completely undetermined characters and gaps. The topology of the phylogenetic tree generated from Bayesian analyses showed a topology similar to that of the tree generated from the ML analyses. Hence, the ML tree with an optimization likelihood value of -4435.504314 is shown in **Figure 1.** The phylogenetic analyses conducted using the nrITS region sequence data revealed that the sequence of our present Indian collection of *P. microspora* clustered closely with the sequence of the same taxon reported from China and Japan with significant support values (100% BS and 1.00PP), indicating strong phylogenetic relationships **(Figure 1).**

Taxonomy

Pholiota microspora (Berk.) Sacc., *Syll. fung. (Abellini)* **5**: 742, 1887 **(Figure 2)**

=*Agaricus microsporus* Berk., Hooker's Journal of Botany and Kew Garden Miscellany **2**: 86 (1850)

Pileus 16–38 mm broad, convex when young to broadly convex to plano-convex with age; pileus surface smooth, slightly velvety, viscid, becoming orange grey (6B2–6B3) to greyish orange (6B3) at the centre, pale yellow (3A3) to dull yellow (3B3) at the margin, margin not striate. Lamellae adnate, 3–4 mm broad, crowded, broad to ventricose; pale-red (8A3) in colour; edges wavy to slightly eroded with age. Stipe 20–41mm long, 2–4 mm wide at apex,

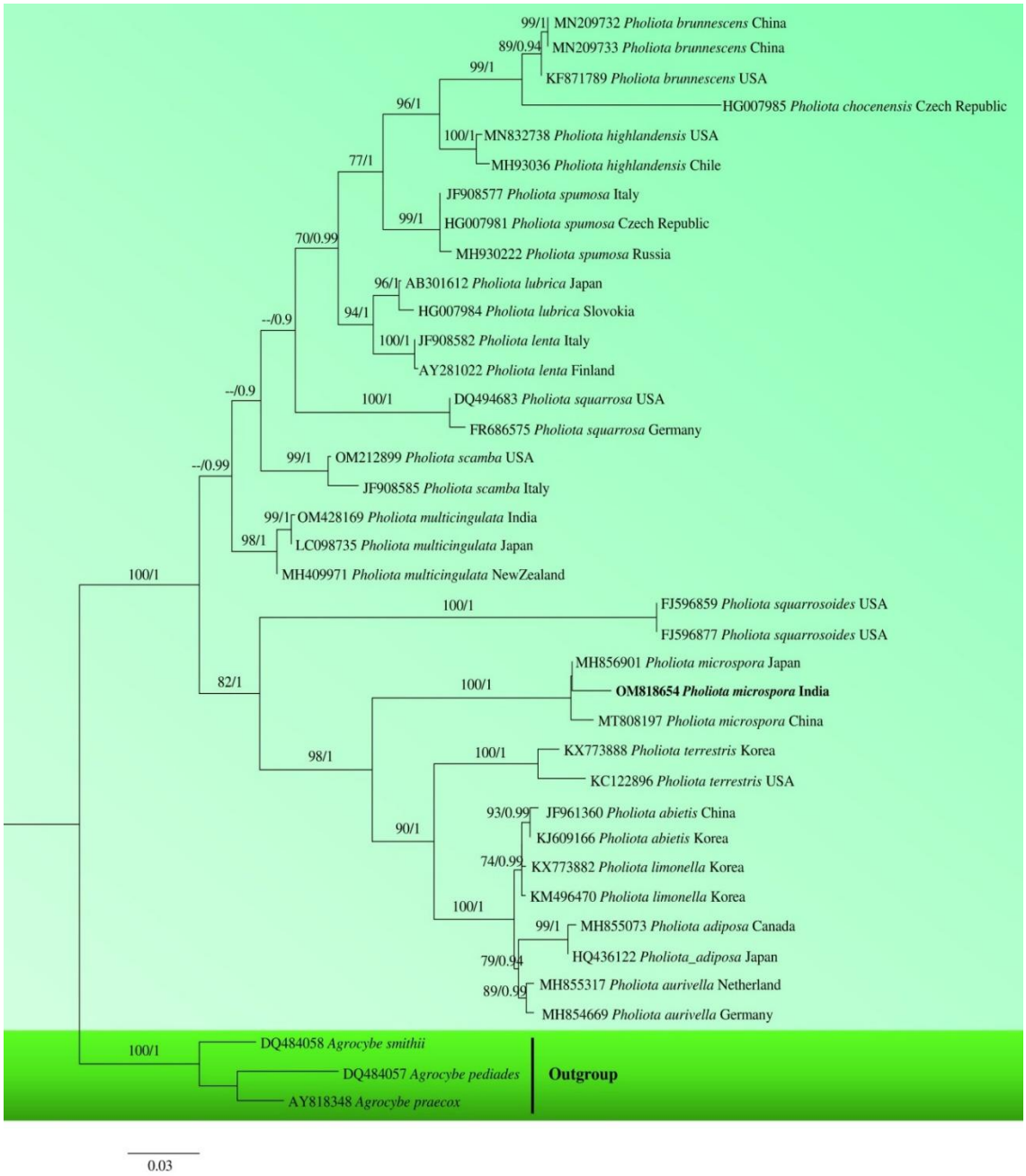

Figure 1: Maximum likelihood tree of *Pholiota microspora* inferred from nrITS data. MLBS values ≥ 70% and PP values ≥ 0.9 are shown above the nodes. The collection from Darjeeling, India is shown in bold. The scale bar represents the expected changes per site

slightly bulbous at the base; fleshy; covered with light orange (6A4) coloured appressed squamules streaked towards the base; solid to slightly stuffed with age. Annulus thin, membranaceous and flared.

Basidiospores smooth, ellipsoid in shape, 4.9–6.1 × 3.4–4.1µm (avL=5.6µm, avW= 3.94µm); Q=1.3–1.5 µm, Q_m=1.41. Basidia 4-sterigmate, clavate, 15.2–18.2 × 6.4–7.6 µm. Lamellae-edge sterile. Pleurocystidia absent. Cheilocystidia present as leptocystidia, 22.23–33.6 µm, difficult to separate. Lamellae trama regular, composed of slightly inflated hyaline hyphae, 3.2–12.6 µm in diameter. Sub-hymenium layer composed of hyaline gelatinized hyphae. Caulocystidia absent. Pileipellis, an ixocutis (252 µm), consisting of cylindrical gelatinized hyphae, incrustated with brown pigments, 3.1–6.3 µm in diameter. Clamp connections present in many septa.

Figure 2: *Pholiota microspora.* a-c, Basidiomata in the field; d, Basidiospores; e-f, Basidium; g, Leptocystidia. Scale bars: a-c = 5mm; d-f = 5 µm; g = 10 µm

Habit and habitat: Caespitose on log of *Prunus cerasoides* Buch-Ham.ex D.Don.

Distribution: This taxon was previously reported from Darjeeling in West Bengal (Berkley, 1850), as *Agaricus microsporus* (Berkley, 1850; Manjula, 1983) and Japan (Neda, 2008).

Specimen examined: India, West Bengal, Darjeeling hills, Lava, 27.0863ºN & 88.6615ºE, elevation 2200 m, 21-June-2019, CUH AM766, India, Coll. J Tamang.

Additional Specimen examined: India, West Bengal, Darjeeling hills, Mulkharga, 27°10'14"N 88°42'08"E, elevation 2,304 m, 22-June-2023, Coll. J Tamang, F-60/2023 (CUH AM1025).

Edibility: Edible.

Remarks: *Pholiota microspora,* is reported here from Darjeeling Hills after Berkley's report in 1850 as *Agaricus microsporus* based on Hooker's collection deposited in Kew, India, based on morphological data and phylogenetic analyses.

DISCUSSION

Pholiota microspora is characterized by having viscid fruit bodies, smaller basidiospores, a thin membranaceous annulus, dense appressed squamulose streaks on the stipe surface, and smooth basidiospores measuring 4.9–6.1 × 3.4–4.1µm in diameter; absence of pleurocystidia and presence of cheilocystidia in the form of leptocystidia. This feature of the presence of leptocystidia was not described in the previously reported specimen of *P. microspora*. The present collection shows similarity with the previously reported holotype specimen collected by Hooker from Darjeeling, India except for having slightly larger basidiocarps (16–38mm vs 27–43mm) (Berkley, 1850; Manjula, 1983). The sample reported from Japan also shows similar micro and macro-characteristics except for having a slightly larger basidiocarp size (16–38mm vs 25–100mm) and the absence of leptocystidia (Neda, 2008).

Among morphologically related species, *Pholiota mucigera* Holec & Niemelä, described from Finland, differs in having a large size, almost white coloured

pileus, slightly larger ovoid basidiospores and absence of cystidia (Holec and Niemela, 2000). *Pholiota olivaceodisca* A.H. Sm. & Hesler is different from *P. microspora* in having a deep olive-buff pileus, the serrulate lamellae, the fibrillose to floccose-scaly stipe, and larger basidiospores (5.5–7 x 3.5–4 µm) (Neda, 2008). *Pholiota microspora var. himalayensis* differs in possessing thick-walled basidiospores and the presence of pleurocystidia (Adhikari *et al.,* 2014).

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REFERENCES

Adhikari, M.K., Watanabe, K. and Parajuli, G.P. 2014. A new variety of *Pholiota microspora* Berk. Sacc. (Agaricales) from Nepal. *Biodiversitas,* **15**:101–103; doi: 10.13057/biodiv/d150115.

Berkeley, M.J., 1850. Decades XXV to XXX. Sikkim Himalayan Fungi, collected by Dr J D Hooker. *Hooker's Journal of Botany and Kew Gardens Miscellany*, **2**: 106–112.

Chuzho, K. and Dkhar, M.S. 2020. *Pholiota polychroa* and *Porodisculus orientalis*: two new additions to wood-rotting fungi of India. *Studies in Fungi,* **5(1)**:447–451; doi：10.5943/sif/5/1/25.

Consiglio, G. 2023. *Pholiota*, *Pyrrhulomyces* and *Flammula*. RMR, Boll. Amer 119, Anno XXXIX **2**: 3–67. doi: 10.57624/AMER.2023.10

Dutta, A.K., Paloi, S. and Acharya, K. 2020. New record of *Tulostoma squamosum* (Agaricales: Basidiomycota) from India based on morphological features and phylogenetic analyses. *Journal of Threatened Taxa*, **12(3)**: 15375–15381.

Edgar, R. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research,* **32(5)**:1792–1797.

Edler, D., Klein, J., Antonelli, A. and Silvestro, D. 2020. raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution*, **12(2)**: 373–377; doi: 10.1111/2041-210X.13512.

Farook, A.V., Khan, S.S. and Manimohan, P. 2013. Checklist of agarics (gilled mushrooms) of Kerala state. *Mycosphere,* **4(1)**:97–131.

Geyer, C.J. 1991. Markov chain Monte Carlo maximum likelihood. In: Keramidas EM (eds) Computing Science and Statistics: Proceedings of the 23rd Symposium on the Interface. Fairfax Station: Interface Foundation, 156–163.

He, M.Q., Zhao, R.L., Hyde, K.D., *et al*. 2019. Notes, outline and divergence times of Basidiomycota. *Fungal Diversity,* **99(1)**:105–367.

Holec, J. and Niemelä, T. 2000. *Pholiota mucigera* (Agaricales), a new species from a boreal old-growth forest. *Annales Botanici Fennici*, **37(2):** 79–83.

Kornerup, A. and Wanscher, J.H. 1978. Methuen Handbook of Colour. 3rd Edition. Eyre Methuen Ltd. Reprint, UK.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular biology and evolution,* **33(7)**:1870–1874

Lee, J.W., Park, M.S., Park, J.H., Cho, Y., et al. 2020. Taxonomic Study of the Genus *Pholiota* (Strophariaceae, Basidiomycota) in Korea. *Mycobiology,* **48(6):** 476–483; doi: 10.1080/12298093.2020.1831427.

Manjula, B.L. 1983. A revised list of the agaricoid and boletoid basidiomycetes from India and Nepal. *Proceedings: Plant Sciences,* **92**:81–213

Neda, H. 2008. Correct name for “nameko”. *Mycoscience,* **49**:88–91

Noordeloos, M.E. 2011. Strophariaceae s.l. Edizioni Candusso. Alassio.

Pradhan, P., Dutta, A.K. and Acharya, K. 2015. A low-cost long-term preservation of macromycetes for fungarium. Protocol Exchange; doi: 10.1038/protex.2015.026.

Rambaut, A. 2012. FigTree v 1. 4. Molecular evolution, phylogenetics and epidemiology. Edinburgh, UK: Available from: http://tree.bio.ed.ac.uk/software/ (Accessed 29 January 2025)

Ronquist, F., Teslenko, M., Mark, P., Ayres, D.L., Darling, A. *et al.,* 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systems Biology,* **61(3)**:539–42.

Smith, A.H. and Hesler, L. 1968. The North American species of *Pholiota*. New York: Hafner Publishing Company.

Tamang, J., Thapa, A., Acharya, K. 2024. Two new species of *Pholiota* (Strophariaceae) from India. *Biologia*, **79**:2639–2648; doi: 10.1007/s11756-024-01715-4.

Thapa, A., Tamang, J., Acharya, K. 2023. A new species of *Tricholomopsis* (Agaricales) from Darjeeling Hills, India. *Italian Journal of Mycology* **52(1)**:144–153; doi: 10.6092/issn.2531-7342/18381.

Vishal, V., Munda, S.S., Singh, G., Lal, S. 2021. Wild edible gasteroid fungus *Astraeus* (Diplocystidiaceae) from Jharkhand, India. *Indian Journal of Applied & Pure Biology*, **36(2):**569–579.

White, T.J., Bruns, T., Lee, S. and Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, **18(1)**:315–322; doi :10.1016/b978-0-12-372180-8.50042-1.