

FIRST PHYLOGENETIC AND SCANNING ELECTRON MICROSCOPIC (SEM) ANALYSIS OF *Lycoperdon perlatum* (LYCOPERDACEAE, AGARICALES) FROM PAKISTAN

A. Iqbal¹, H. Ali^{*2}, S. Sarwar^{*2}, I. Krisai-Greilhuber³, N. Yousaf⁴, M. Fiaz⁵ M. Hanif⁴ and A. Majid⁵

¹Department of Botany, Lahore College for Women University, Lahore, Pakistan

²Institute of Botany, University of the Punjab, Lahore, Pakistan

³Department of Botany and Biodiversity Research, University of Vienna, Austria

⁴Department of Botany, Government College University, Lahore, Pakistan

⁵Department of Botany, Hazara University, Mansehra, Pakistan

*Corresponding authors' E-mail: hiraali3110@gmail.com; Samina_boletus@yahoo.com

ABSTRACT

Fungi of the family Lycoperdaceae are an important component of forests ecosystem of both ecological and economic value. Among the best known is the puffball fungus, *Lycoperdon perlatum*, which exhibits a wide range of phenotypic variation and has a widespread distribution. In the current study, specimens of *L. perlatum* were collected from the soil around the rhizospheric zone of pine trees in District Mansehra, Khyber Pakhtunkhwa, Pakistan. These samples were examined using both traditional morphological approaches and modern molecular genetic techniques, combining both traditional and advanced methods for a more comprehensive analysis. Basidiomata were initially off-white to yellow, after maturity olivaceous-brown and pear-shaped. There are whitish warts on the exoperidium which can be easily rubbed off, leaving a characteristic areolate pattern of small granules on the underlying endoperidium. The identification of species was confirmed using DNA sequencing alongside detailed microscopic examination of the basidiomata, scanning electron microscopy (SEM) of spores, and phylogenetic analysis. This combined approach provided a thorough description of *L. perlatum* and confirmed its taxonomic placement. These findings add to the growing documentation of macrofungal diversity in Pakistan and emphasize the important ecological roles of puffball fungi in supporting forest health and nutrient cycling.

Keywords: Agaricales, Basidiomycota, ITS, Phylogeny, Soil Fungi, Taxonomy

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0>)

<https://doi.org/10.36899/JAPS.2026.4.0097>

Published first online May 12, 2026

INTRODUCTION

Moist temperate forests of Pakistan have been recognized as most important biodiversity hotspots, having a variety of plant, fungal, and animal species that contribute a very significant role in the ecological balance of the region. Although these areas are very important, they remained among the least studied biomes in the world in terms of diversity of the macromycetes (Mirjam, 2010). Lycoperdaceae is a large family of puffballs with six genera: *Apioperdon*, *Bovista*, *Bovistella*, *Bryoperdon*, *Calvatia* and *Lycoperdon* (Aman *et al.*, 2022). Persoon defined *Lycoperdon* as a genus in 1796a with *Lycoperdon perlatum* as type species. Members of this genus are distinguished by basidiomata ranging from subglobose to pyriform, mostly with a prominent well-developed sterile base and spore dispersal through an apical pore. This genus has been thoroughly studied by various mycologists (Ahmad, 1952; Demoulin, 1971; Hollos, 1904; Kreisel, 1962; Perdeck, 1950) and is composed of 342 legitimate taxa in current use all over the world (Jeppson *et al.* 2012; Kirk *et al.* 2008; <https://www.mycobank.org/>). Phylogenetics have challenged this concept of the genus and sometimes species from *Bovistella*, *Handkea*, *Morganella*, and *Vascellum* (Bates 2004; Bates *et al.* 2009; Gube 2009; Jeppson *et al.* 2012; Krüger *et al.* 2001; Larsson and Jeppson 2008) are included in a large genus *Lycoperdon* *sensu lato*. Currently most species of *Lycoperdon* subgenera *Apioperdon*, *Bovistella*, *Lycoperdon*, *Morganella*, *Utraria* and *Vascellum* are mostly considered as separate genera.

In many cases, cryptic species occur that cannot be reliably distinguished by macro- or micromorphology alone (Cao *et al.*, 2012; Larsson and Jeppson 2008; Sotome *et al.*, 2013). To address this issue, molecular identification has become essential for resolving taxonomic relationships (Hibbett *et al.*, 1997; Larsson and Jeppson, 2008; Moncalvo *et al.*, 2002; Morgado *et al.*, 2013). Molecular data have proven valuable in understanding phylogenetic relationships, taxonomic diversity and morphological complexity in gasteroid fungi (Cui *et al.*, 2015; Dentinger *et al.*, 2010; Wu *et al.*, 2014).

Climatic conditions in Pakistan favor the natural development and growth of gasteroid fungi. However, only 14 *Lycoperdon* species (*L. atropureum*, *L. curtisii*, *L. echinella*, *L. excipuliforme*, *L. glabrescens*, *L. lahorensis*, *L. molle*, *L. perlatum*, *L. pratense*, *L. pseudocurtisii*, *L. rimulatum*, *L. setiferum*, *L. subterranea* and *L. umbrinum*) have been reported from localities such as Bahrain (Swat), Changla gali, Khanspur (Abbottabad), Mansehra, Patriata (Murree), Shogran, and Sharan (Kaghan Valley) (Davis *et al.*, 2012; Aman *et al.*, 2022). Most of these identifications were based solely on morphology, with limited molecular characterization and little phylogenetic discussion. Many species are difficult to identify morphologically, especially in early developmental stages. Therefore, this study employs an integrated approach combining morphological and molecular genetic analyses to achieve precise species identification. It is the first study of *L. perlatum* by molecular means from Pakistan, validates previous morphological reports and contributes to filling a gap in regional fungal biodiversity data.

MATERIALS AND METHODS

During mycological field expeditions across the Khyber Pakhtunkhwa (KPK) region of Pakistan, a *Lycoperdon* specimen from moist temperate forests of Pakistan, mixed with coniferous trees was collected in August 2022. Field observations were recorded for fresh basidiomata and digital photos were taken in situ. Colours were assigned according to Munsell (1975). The basidiomata were air dried using a fan heater and then stored in paper bags for further lab analysis.

Macroscopic and microscopic analysis: Basidiomata were studied both macro- and microscopically following the methods described by Stanek (1958) and Sunhede (1989, 2012). Fresh basidiomata were studied for shape and colour, surface ornamentation of the exo- and endoperidium, gleba colour, presence or absence of subgleba, sterile base texture, and rhizomorph morphology. Plectological examination was carried out using a CXRII, Labomed, Labo America Inc. Fremont CA 94536 USA microscope. Small fragments of basidiomata were mounted in lactic acid, Melzer's reagent, Trypan-blue or KOH, and analyzed for basidiospore dimensions (length, width, shape, and cytoplasm contents), capillitium hyphae, peridium, exoperidial elements, exoperidial hyphae, endoperidial hyphae including their colour reactions. Spore measurements are based on 30 spores. Reported values indicate the minimum - maximum range of spore lengths and width. Q denotes the length/width ratio calculated for each spore, while Q_m denotes the average of these ratios (length/width ratio of an individual spore). Spore morphology was further analyzed using scanning electron microscopy (SEM) to confirm surface ornamentation and fine structural details.

Molecular phylogenetic studies: Genomic DNA was extracted from the basidiomata with the Thermo Scientific GeneJET Plant Genomic DNA Purification Kit. The integrity and quality of DNA was checked using agarose gel electrophoresis and visualized with a Bio-Rad Gel Doc 2000 system. Nano-Drop spectrophotometer yielded a concentration of 502.9 ng/ μ l and a 260/280 ratio of 2.01 and 260/230 ratio of 1.97 (Fig. 1). Internal transcribed spacer (ITS) region was amplified by using the universal primers ITS1F, ITS4 and ITS4B whereas the large subunit (LSU) region was amplified using the primers LR0R and LR5 (White *et al.*, 1990; Khan and Javaid, 2021). PCR products were purified enzymatically using ExoSAP IT, where exonuclease I removes residual primers and shrimp alkaline phosphate degrades excess dNTPs, followed by heat inactivation of the enzymes (Voglmayr and Jaklitsch, 2008; Werle *et al.*, 1994). Sequencing was performed with the assistance of experts at the Department of Botany and Biodiversity Research, University of Vienna, using ABI PRISM Big Dye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Warrington, UK), and cycle sequencing reactions were analyzed on an ABI 3730xl Genetic Analyzer (Applied Biosystems). Consensus sequence was assembled in BioEdit sequence alignment editor version 7.2.5 (Hall, 1999). Sequence identity was confirmed by BLAST (<http://www.ncbi.nlm.nih.gov/>) search against the NCBI nucleotide database. For phylogenetic reconstruction, sequences of closely related *Lycoperdon* species were retrieved from GenBank. Multiple sequence alignment was conducted using the MUSCLE tool at EMBL-EBI (<http://www.ebi.ac.uk/>), followed by manual refinement in BioEdit (Hall, 1999) limited to correcting clear misalignments and trimming ambiguous terminal regions, ensuring accurate positional homology. Phylogenetic analysis was performed in MEGA11 (Tamura *et al.*, 2021) using Maximum Likelihood method under the Jukes-Cantor model (Jukes and Cantor, 1969). Bootstrap support was calculated from 1000 replicates, with values below 50% omitted from the final tree.

RESULTS

Lycoperdon perlatum Pers. (Figures 2-5)

Fig. 1. Measurement of Nucleic Acid Absorbance using Nano-Drop Spectrophotometer.



Fig. 2. A-D. *Lycoperdon perlatum*. A-D Basidiomata. Bars: = 2cm for A-D.

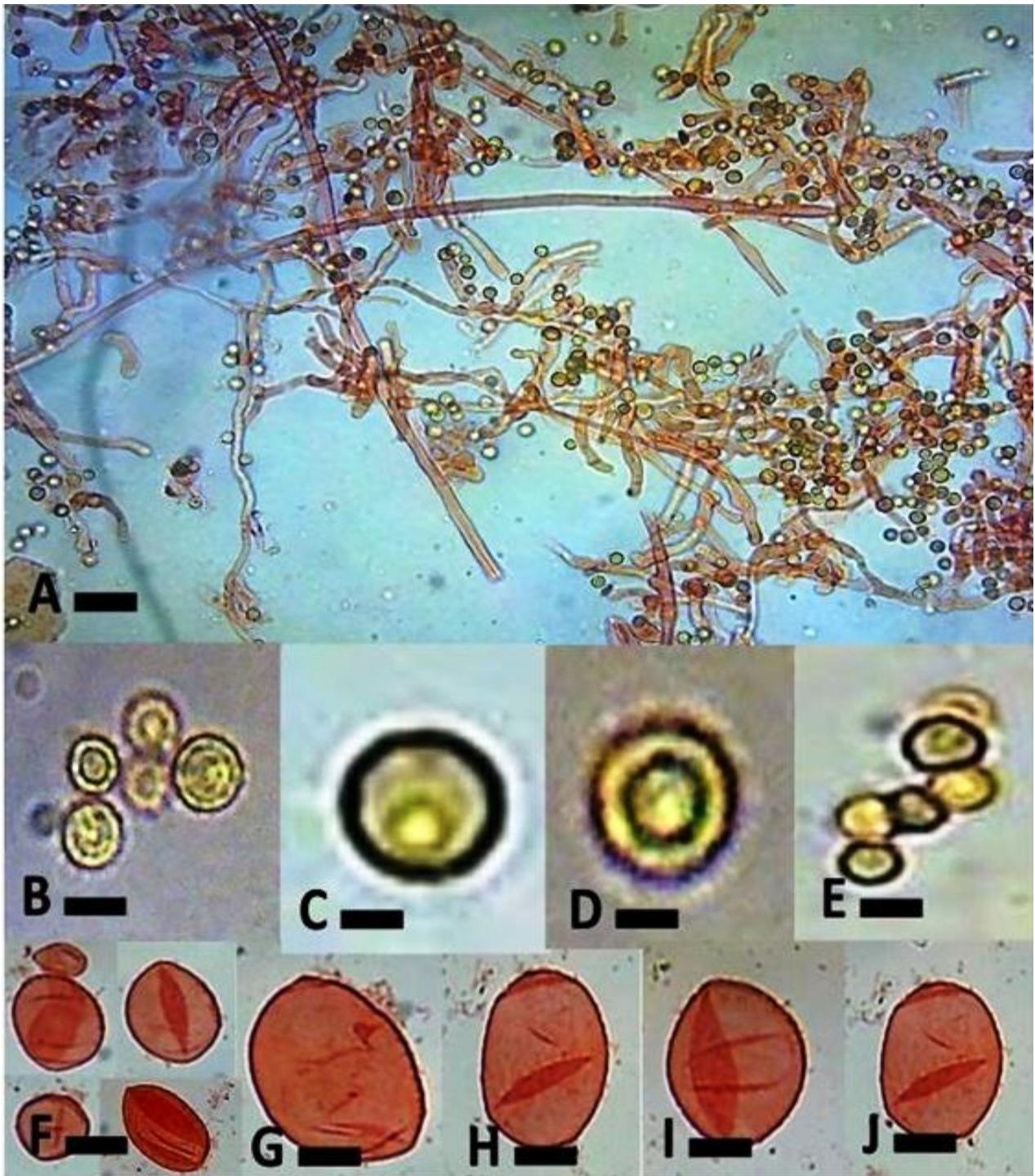


Fig. 3. A-D. *Lycoperdon perlatum*. A, C-E. Basidiospores and capillitial hyphae. B-E. Spores at 100 \times with oil emersion. F-J. Exoperidial sphaerocysts. Bar: = 10 μ m for A-E; 20 μ m for F-J.

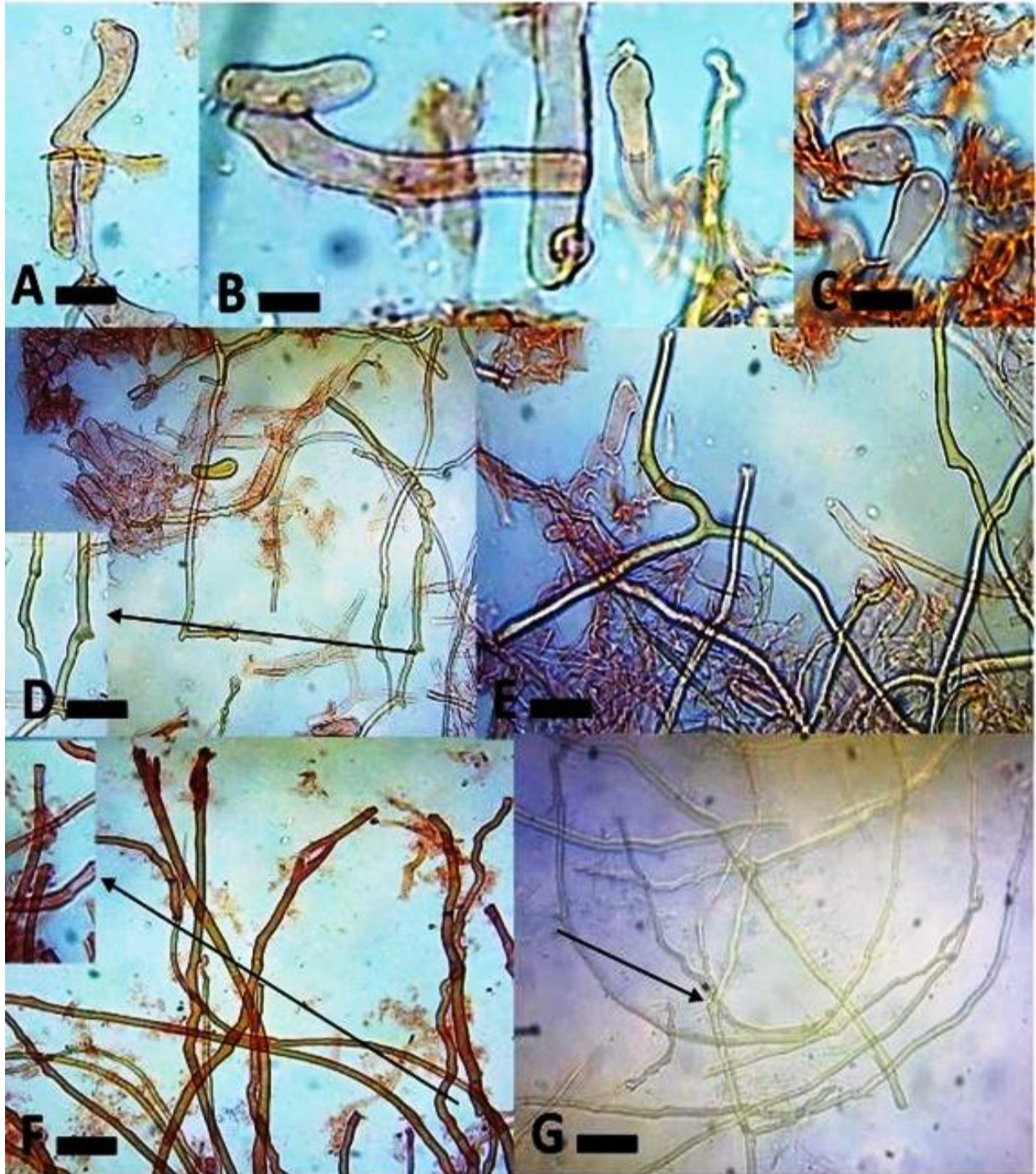


Fig. 4. A-G. *Lycoperdon perlatum*, A. Exoperidial hyphae B. Capillitial hyphae. C-G. Endoperidial hyphae. Bar: = 10 μ m for A and B, = 20 μ m for C-G.

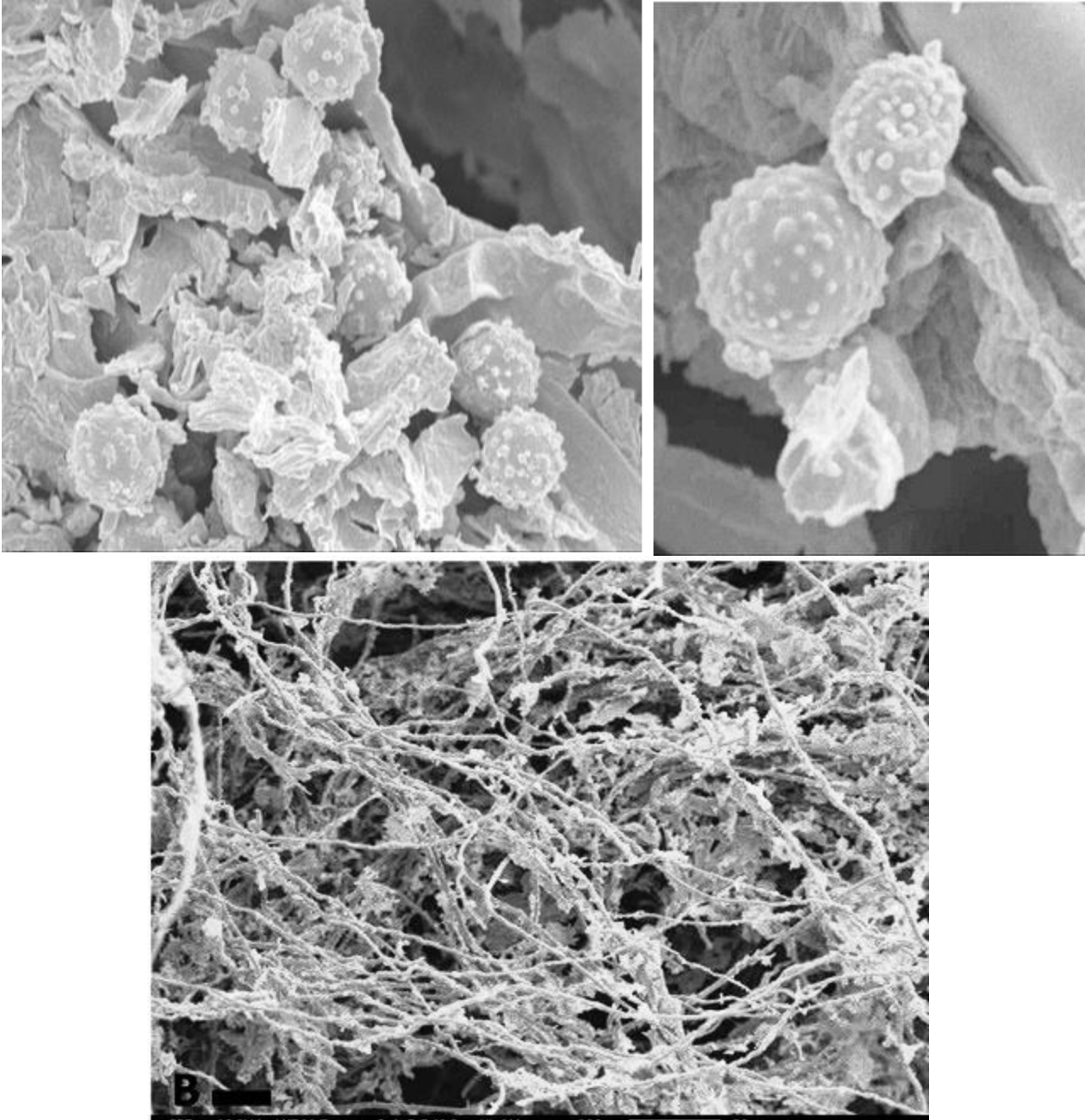


Fig. 5. *Lycopodium perlatum*. Scanning Electron Microscope images showing ornamented basidiospores and hyphae.

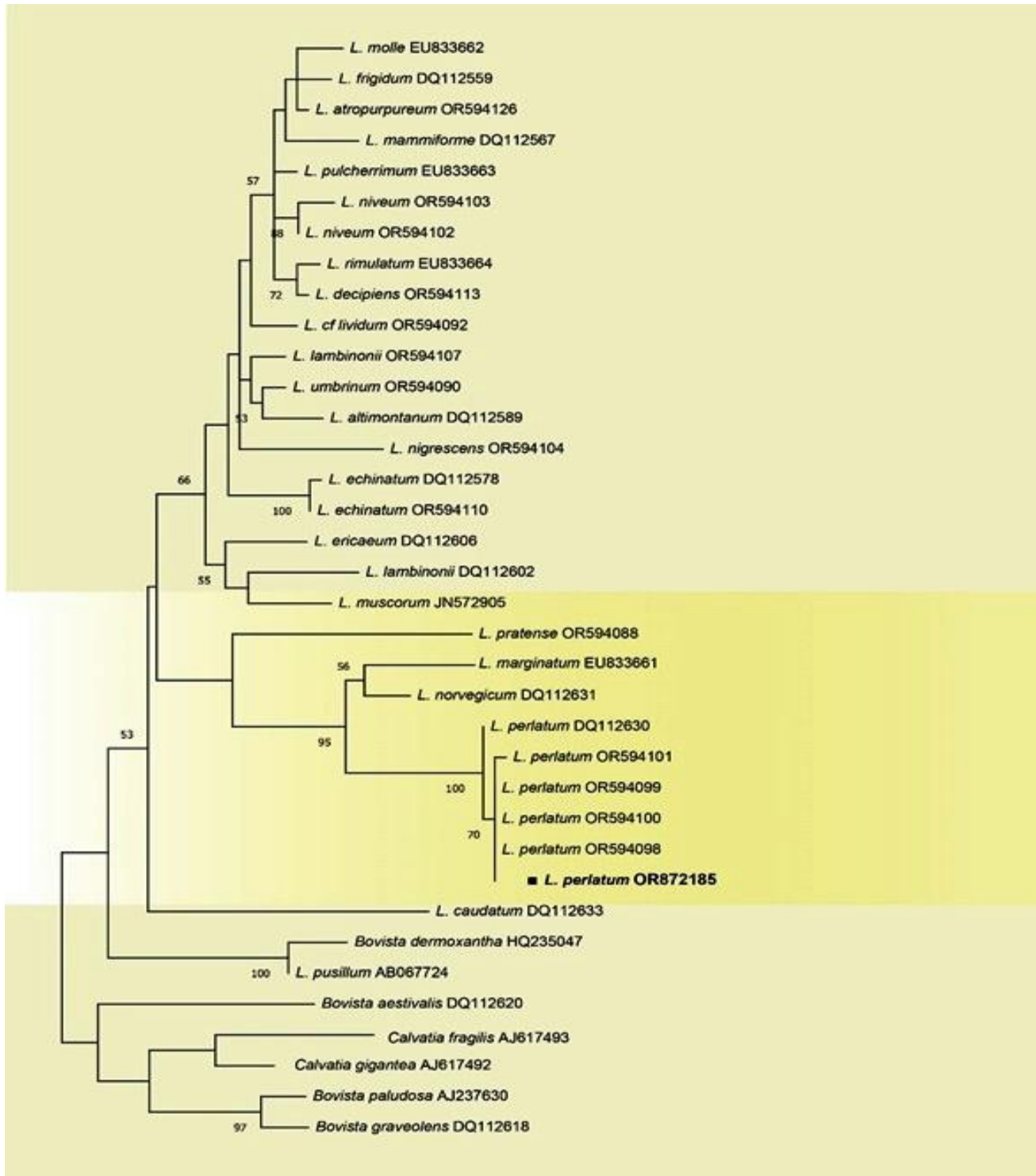


Fig. 6. Phylogenetic tree of *Lycoperdon perlatum* based on Maximum Likelihood (ML) analysis of nrITS sequences. The Pakistan sample represented by (●).

GenBank: OR872185

Macroscopic features: Basidomata pyriform, off white to light brown above, light white to yellowish at base when mature, consisting of apical spore head with gleba and lower short pseudostipe with subgleba, up to 30 mm wide and 62 mm high, gregarious or convivial, reclusive sometimes; not connected to the substratum through a mycelial cord.

Dehiscence occurring through irregular rupturing of the apical part forming a pore of up to 9 mm diameter. Peridium double. Exoperidium finally olivaceous and persistent (maybe due to dry weather conditions), completely covering the endoperidium in both young and mature specimens, with small verrucae and larger pyramidal spines and presence of a distinct areolation after dehiscence of the spines, endoperidium papery and brownish ochre. Gleba white when young, becoming light greenish brown to pale with age; compact, cottony, more fluffy with age. Sterile base present, spongy and compact, light yellowish-brown (Fig. 2).

Microscopic features: Basidiospores globose, thick-walled, brown green to olivaceous, $2.9\text{--}4.8 \times 2.26\text{--}4.10 \mu\text{m}$ excluding verrucae, verrucae up to $1.2 \mu\text{m}$, yellowish in KOH (Fig. 3). Capillitium well developed, aseptate, yellowish, dichotomous branching pattern, branches straight to slightly wavy (sub-undulate) with tapering ends, diameter of hyphae up to $4 \mu\text{m}$, encrusted, pores rare (Fig. 4). Exoperidium $57.5\text{--}69.5 \mu\text{m}$, hyaline (translucent), hyphae globose to subglobose. Endoperidium hyaline, branched, aseptate, and hyphae crowded (Fig. 5).

Material examined: PAKISTAN: Khyber Pakhtunkhwa, District Mansehra, 09 Aug. 2022, gregarious, on ground, at 1225 meters above sea level, leg. S. Sarwar, SS99, (LCWUBOTHSS99).

Molecular phylogenetic characterization: BLAST analysis of the ITS region revealed >99% concordance with *L. perlatum* sequences, confirming species identity. The final ITS dataset comprised 36 sequences and 689 aligned characters, of which 532 were conserved, 152 variable, 87 parsimony informative. Phylogenetic reconstruction using Maximum Likelihood method under the Jukes-Cantor model (Jukes and Cantor, 1969) produced the best tree with a log likelihood of -2871.81 (Fig. 6). The Pakistani specimen clustered with other *L. perlatum* sequences, supported by high bootstrap values (>90%), and grouped most closely with European and Asian reference sequences. Within this clade, branch lengths were short, reflecting low intra-specific divergence and supporting the species cosmopolitan distribution. The robust support of the *L. perlatum* cluster confirms the Pakistani specimen distinct identification and stability across regions.

DISCUSSION

Biodiversity in Pakistan remains underexplored, especially for gasteroid fungi, that play a significant role in forests ecosystems. Reporting species such as *L. perlatum* provides important taxonomic foundations for strengthening regional baseline data, informs species conservation planning, and contributes to global phylogenetic datasets. *Lycoperdon perlatum*, the type species of the genus *Lycoperdon*, is widespread across the globe (Sarasini, 2005; Bates *et al.*, 2009), yet its presence in Pakistan had not been confirmed using molecular evidence. Our study provides this validation, thereby filling an important gap in knowledge of local fungal diversity. Morphologically, it has pyriform basidiomata, a verruculose and areolate exoperidium pattern, consistent with previous descriptions (Breitenbach and Kränzlin, 1986; Bates *et al.*, 2009), confirming our specimen as *L. perlatum*. This congruence between morphology and molecular evidence indicates the reliability of integrative approaches in fungal taxonomy.

Lycoperdon perlatum can be confused with similar species such as *L. nettyanum*, which forms more adherent granules (Ramsey, 1980) and is very rare known from the US only (no sequence available yet), *L. umbrinum*, that develops a purplish gleba and has a different exoperidium ornamentation, lacking areoles after spines are rubbed off (Davis *et al.*, 2012), and *L. foetidum* (now *Fuscospina nigrescens* (Pers.) R.L. Zhao & J.Xin Li, in Li, Cao, Phurbu, He, Zhu, Parra & Zhao 2024), that becomes darker brown when mature and has a different basidiospore ornamentation (Bates *et al.*, 2009). Another species, *Lycoperdon excipuliforme* (= *Utraria excipuliformis* (Scop.) Quél.), has significantly larger non-areolate basidiomata (Yousaf *et al.*, 2012).

In addition to morphology, our ITS sequences cluster within the *L. perlatum* clade with high bootstrap support, and are highly similar to sequences from Europe, Asia, and North America (Bates *et al.*, 2009; Kim *et al.*, 2016; Yousaf *et al.*, 2012). A similar phylogeographic pattern has been observed in Pakistan and Israel (Krakhmalnyi *et al.*, 2023), supporting the cosmopolitan distribution of *L. perlatum*. Although some minor haplotype variation occurs in regional surveys, the broad molecular uniformity across continents indicates that morphological uniformity is associated with genetic consistency.

Combined morphological and molecular data place our specimen in a broader biogeographical context and strengthen the view that *L. perlatum* is a widely distributed, phylogenetically stable lineage within Lycoperdaceae. No detailed molecular or ultrastructural analyses of Pakistani material have previously been documented for *L. perlatum*, although it has a long history and was first described by Persoon in *Obsevationes Mycologicae* (1796). This study therefore provides the first molecular and SEM examination of *L. perlatum* in Pakistan. Our specimen clustered within a well-supported *L. perlatum* clade, as found in previous research from Europe and North America (Larsson and Jeppson, 2008; Bates *et al.*, 2009). This congruence supports the worldwide distribution of the species and confirms the utility of ITS

markers to resolve gasteroid taxa. The areolate ornamentation of the exoperidium and verrucose surface features were confirmed by SEM and match those reported by Breitenbach and Kränzlin (1986).

These findings support the idea that integrative approaches combining genetic and morphological data enhance species delimitation and reduce taxonomic uncertainty. Notably, the DNA confirmation of *L. perlatum* in Pakistan fills a gap in records of gasteroid fungi in underexplored habitats. Such efforts are essential for setting conservation priorities and for understanding the biological significance of these species in forest ecosystems.

Conclusion: This study demonstrates the value of integrating morphological and molecular approaches for accurate fungal identification. By documenting the characteristics of *L. perlatum* from District Mansehra, Khyber Pakhtunkhwa, we contribute to the growing knowledge of Pakistan's macrofungal diversity. These findings highlight the ecological importance of gasteroid fungi and underscore the need for continued biodiversity assessments to support conservation of forests ecosystems.

Conflicts of Interest: The authors declare no conflict of interest.

Acknowledgements: Authors would like to express sincere gratitude to Austrian Academy of Science for funding to support this research under JESH postdoc fellowship by Joint Excellence in Science and Humanities (JESH) and to University of Vienna, Department of Botany and Biodiversity Research for providing the opportunity to conduct research by using all available facilities. We are highly indebted to Mag. Dr. Michael Barfuss University of Vienna, Department of Botany and Biodiversity Research, Division of Systematic and Evolutionary Botany, Plant Evolutionary Genomics and Systematics for his help in molecular work. We are also thankful to Mag. Dr. Silvia Ulrich, University of Vienna, Department of Botany and Biodiversity Research, Division of Structural and Functional Botany for her support in SEM analysis.

REFERENCES

- Ahmad, S. (1952). Gasteromycetes of Pakistan. Punjab University Press. 150 p.
- Aman, N., A.N. Khalid and J.M. Moncalvo (2022). A compendium of macrofungi of Pakistan by ecoregions. MycoKeys 89: 171. Doi: 10.3897/mycokeys.89.81148
- Bates, S.T. (2004). Arizona members of the Geasteraceae and Lycoperdaceae (Basidiomycota Fungi). Master thesis. Arizona State University; Tempe (USA).
- Bates, S.T., R.W. Roberson and D.E. Desjardin (2009). Arizona gasteroid fungi I: Lycoperdaceae (Agaricales, Basidiomycota). Fungal Divers. 37: 153–207.
- Breitenbach, J. and F. Kränzlin (1986). Fungi of Switzerland. Vol. 2: Non-Gilled Fungi. Verlag Mykologia; Luzern. 412 p.
- Cao, Y., S.H. Wu and Y.C. Dai (2012). Species clarification of the prize medicinal *Ganoderma* mushroom “Lingzhi”. Fungal Divers. 56: 49–62. DOI 10.1007/s13225-012-0178-5
- Cui, Y.Y., B. Feng, G. Wu, J. Xu and Z.L. Yang (2015). Porcini mushrooms (*Boletus* sect. *Boletus*) from China. Fungal Divers. 81(1): 1–24. DOI 10.1007/s13225-015-0336-7
- Davis, R.M., R. Sommer and J.A. Menge (2012). Field guide to mushrooms of Western North America. University of California Press, Berkeley. 372 p.
- Demoulin, V. (1971). Les Lycoperdales de l'Europe occidentale et central. Bull. Jard. Bot. Natl. Belg. 41: 351–406.
- Dentinger, B.T.M., J.F. Ammirati, E.E. Both, D.E. Desjardin, R.E. Halling, T.W. Henkel, P.A. Moreau, E. Nagasawa, K. Soyong and A.F. Taylor (2010). Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). Mol. Phylogenet. Evol. 57: 1276–1292. <https://doi.org/10.1016/j.ympev.2010.10.004>
- Gube, M. (2009). Molecular phylogeny and systematics of the Lycoperdaceae. Mycol. Prog. 8(1): 45–58.
- Hall, T. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.
- Hibbett, D.S., E.M. Pine, E. Langer, G. Langer and M.J. Donoghue (1997). Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc. Natl. Acad. Sci. USA 94: 12002–12006. <https://doi.org/10.1073/pnas.94.22.12002>
- Hollos, L. (1904). Gasteromycetes Hungariae. Akademiai Kiado; Budapest.
- Jeppson, M., E. Larsson and M.P. Martín (2012). *Lycoperdon rupicola* and *L. subumbrinum*: Two new puffballs from Europe. Mycol. Prog. 11: 887–897. DOI 10.1007/s11557-011-0804-8
- Jukes, T.H. and C.R. Cantor (1969). Evolution of protein molecules. In H.N. Munro (Ed.), Mammalian Protein Metabolism. Vol. 3: 21–132. Academic Press; New York.

- Khan, I.H. and A. Javaid (2021). Molecular characterization of *Penicillium expansum* associated with blue mold disease of apple in Pakistan. Pak. J. Bot. 53(6): 2299-2303. DOI: [http://dx.doi.org/10.30848/PJB2021-6\(34\)](http://dx.doi.org/10.30848/PJB2021-6(34))
- Kim, C.S., J.W. Jo, Y.N. Kwag, G.H. Sung, J.G. Han, S.Y. Kim and S.O. Oh (2016). Two new *Lycoperdon* species collected from Korea: *L. albiperidium* and *L. subperlatum* spp. nov. Phytotaxa 260(2): 101-115. DOI: <https://doi.org/10.11646/phytotaxa.260.2.1>
- Kirk, P.M., P.F. Cannon, D.W. Minter and J.A. Stalpers (2008). Ainsworth & Bisby's dictionary of the fungi. 10th Ed. CABI; Wallingford. 771 p.
- Krakhmalnyi, M., O.S. Isikhuemhen, S.P. Jeppson, S.P. Wasser and E. Nevo (2023). Species Diversity of Lycoperdaceae (Agaricales) in Israel, with insights into the Phylogenetic Structure of the Family. J. Fungi (Basel) 9(10):1038. <https://doi.org/10.3390/jof9101038>
- Kreisel, H. (1962). Die Lycoperdaceae der Deutschen Demokratischen Republik. Feddes Repert. 66: 69-118.
- Krüger, D., M. Binder, M. Fischer and H. Kreisel (2001). The Lycoperdales: a molecular approach to the systematics of some gasteroid mushrooms. Mycologia 93: 947-957. <https://doi.org/10.1080/00275514.2001.12063228>
- Larsson, E. and M. Jeppson (2008). Phylogenetic relationships among species and genera of Lycoperdaceae based on ITS and LSU sequence data from north European taxa. Mycol. Res. 112: 4-22. <https://doi.org/10.1016/j.mycres.2007.10.018>
- Li, J.X., B. Cao, D. Phurbu, M.Q. He, X.Y. Zhu, L.A. Parra, and R.L. Zhao (2024). The revision of the taxonomic system of Lycoperdaceae. Mycosphere 15(1): 4919-5016. Doi 10.5943/mycosphere/15/1/22
- Mirjam, M. (2010). Mountains of the world – ecosystem services in a time of global and climate change: seizing opportunities–meeting challenges. ICIMOD. Available at: <http://www.icimod.org/publications/index.php/search/publication/708>.
- Moncalvo, J.M., R. Vilgalys, S.A. Redhead, J.E. Johnson, T.Y. James, M.C. Aime, V. Hofstetter, S. Verduin, E. Larsson, T.J. Baroni, R.G. Thorn, S. Jacobsson, H. Clemencon and O.K. Miller (2002). One hundred and seventeen clades of euagarics. Mol. Phylogenet. Evol. 23: 357-400. [https://doi.org/10.1016/S1055-7903\(02\)00027-1](https://doi.org/10.1016/S1055-7903(02)00027-1)
- Morgado, L.N., M.E. Noordeloos, Y. Lamoureux and J. Geml (2013). Multi-gene phylogenetic analyses reveal species limits, phylogeographic patterns, and evolutionary histories of key morphological traits in *Entoloma* (Agaricales, Basidiomycota). Persoonia 31: 159-178. <https://doi.org/10.3767/003158513X673521>
- Munsell, A.H. (1975). Munsell Soil Color Charts. Munsell Color; Baltimore.
- Perdeck, A.C. (1950). The genus *Lycoperdon* in the Netherlands. Meded. Bot. Tuinen Plantensyst. Utrecht 98: 1-84.
- Persoon, C.H. (1796). Observationes Mycologicae. Vol. 1. C.C. Schuppel; Leipzig.
- Ramsey, R.W. (1980). *Lycoperdon nettyana*, a new puffball from western Washington State. Mycotaxon 11(1): 185-188.
- Sarasini, M. (2005). Gasteromiceti epigei. Associazione Micologica Bresadola; Trento. 406 p.
- Sotome, K., Y. Akagi, S.S. Lee, N.K. Ishikawa and T. Hattori (2013). Taxonomic study of *Favolus* and *Neofavolus* gen. nov. segregated from Polyporus (Basidiomycota, Polyporales). Fungal Divers. 58: 245-266. DOI 10.1007/s13225-012-0213-6
- Stanek, V.J. (1958). Studies on the genus *Lycoperdon* in Czechoslovakia. Česká Mykol. 12: 84-100.
- Sunhede, S. (1989). Lycoperdaceae of North Europe. Fungiflora; Oslo. 387 p.
- Sunhede, S. (2012). Lycoperdaceae of Northern Europe: Supplement. Sven. Mykol. Tidskr. 33: 1-40. <https://doi.org/10.1080/14772000.2013.857367>
- Tamura, K., G. Stecher and S. Kumar (2021). MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38(7): 3022-3027. <https://doi.org/10.1093/molbev/msab120>
- Voglmayr, H. and W.M. Jaklitsch (2008). *Prosthecius* species with *Stegonsporium* anamorphs on Acer. Mycol. Res. 112(8): 885-905. <https://doi.org/10.1016/j.mycres.2008.01.020>
- White, T.J., T. Bruns, S. Lee and J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: A guide to methods and applications. Academic Press; San Diego. 315-322.
- Werle, E. (1994). Die Gattung *Lycoperdon* in Bayern. Hoppea, Denkschr. Regensburg. Bot. Ges. 55: 5-96.
- Wu, G., B. Feng, J. Xu, X.T. Zhu, Y.C. Li, N.K. Zeng, M.I. Hosen and Z.L. Yang (2014). Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Divers. 69:93-115. DOI 10.1007/s13225-014-0283-8
- Yousaf, N., A.N. Khalid and A.R. Niazi (2012). New records of noteworthy gasteroid fungi from Pakistan. Mycotaxon 119: 261-267. DOI: <https://doi.org/10.5248/119.261>