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Research article

Hymenopellis areolata (Physalacriaceae: Agaricales), a new species from Margalla Hills National Park, Islamabad, Pakistan

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Abstract. During exploratory surveys of the fungal diversity in Margalla Hills National Park, Islamabad, we collected a new species of the genus *Hymenopellis* R.H.Petersen. This is the second report of any species of this genus from Pakistan. *Hymenopellis areolata* F.Razzaq & Khalid sp. nov. is characterized by an areolate pileus, small basidiospores, and transitional pileipellis (hymeniderm and epithelium) with small pileocystidia. Molecular phylogenetic analyses of the nucleotide sequences of nrITS and nrLSU regions, and morphological data support the description of this new species. A comparison with other closely related species confirmed that the newly described species is distinct from others.

Keywords. Basidiomycota, Islamabad, phylogeny, Physalacriaceae, taxonomy.

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Introduction

The family Physalacriaceae was initially defined by Corner (1970) and later revised by Berthier (1985). The monophyly in this family was confirmed through molecular analyses (Moncalvo *et al.* 2002; Matheny *et al.* 2006). Later, a revision on the systematics of the family Physalacriaceae was published, incorporating morphological and molecular phylogenetic data of the *Oudemansiella/Xerula* complex (Petersen & Hughes 2010). Physalacriaceae belongs to the order Agaricales with typified genus *Physalacria* (Peck 1882). Currently, around 21 genera are recognized worldwide within this family (Park *et al.* 2017). The distinguishing feature of this family is the presence of various types of basidiocarps, including secotioid, agariciod, corticoid, cantharelloid and clavarioid (Henkel *et al.* 2010; Moreae *et al.* 2015). This family is differentiated by a monomictic hyphal system having generative hyphae with clamp connections, smooth and thin-walled various-shaped basidiospores, and clavate basidia with 2–4 basidiospores (Cannon & Kirk 2007). Members in the family are typically saprobic, growing on decaying wood and leaves, although a few species are parasitic (Cannon & Kirk 2007).

The *Oudemansielloid/Xeruloid* (OX) group comprises saprotrophic mushrooms that are widespread in forests throughout the world. The taxonomy of this group is complex, and its generic classification has undergone numerous revisions in recent decades. Previously, Wang *et al*. (2008) and Yang *et al*. (2009) recognized only three genera in this complex; *Xerula* Maire, *Oudemansiella* Speg. (including *Hymenopellis* R.H.Petersen, *Dactylosporina* (Clémençon) Dörfelt, *Oudemansiella* s. str., *Mucidula* Pat., *Ponticulomyces* R.H.Petersen, and *Protoxerula* R.H.Petersen) and an unnamed clade including *Paraxerula americana* (Dörfelt) R.H.Petersen but this study was without molecular analysis. The systematics of OX taxa has since been extensively revised, resulting in the establishment of eight genera according to Petersen & Hughes (2010). These genera include *Hymenopellis*, *Dactylosporina*, *Oudemansiella*, *Mucidula*, *Paraxerula* R.H.Petersen, *Ponticulomyces*, *Protoxerula* and *Xerula* (Petersen & Hughes 2010). The revision by Petersen & Hughes (2010) was based on a comprehensive morphological study including the reexamination of a large number of specimens and molecular phylogenetic analysis using rDNA data. This revision is widely accepted (He *et al.* 2019; Wijayawardene *et al.* 2020) and serves as the basis for our taxonomic approach.

The genus *Hymenopellis* is characterized by a plane to slightly convex pileus, usually brown to olive brown, with a viscid-glutinous and slimy surface, large and smooth basidiospores, interaction with buried wood, the presence of a rooting stipe or pseudorrhizae swollen at the ground line and slender to broadly cylindrical caulocystida (Petersen & Hughes 2010). Basidiocarps of this genus can be found either growing solitary or gregarious on dead or buried hardwood and sometimes on exposed, well-decayed wood. They can appear as growing from the ground due to their long pseudorhiza resembling a deep-tap root, which attaches to the decayed wood underground (Petersen & Hughes 2010). Currently, there are 58 accepted taxa in *Hymenopellis* listed in *Index Fungorum* (accessed 7 Feb. 2022). The members of this genus are widely distributed in Eastern and North America, as well as other continents (Niego *et al.* 2021).

Several species of *Hymenopellis* were initially documented in Asia, including *H. amygdaliformis* (Zhu L. Yang & M. Zang) R.H.Petersen and *H. velata* (Zhu L. Yang & M. Zang) R.H.Petersen in China, *H. aureocystidiata* (R.H.Petersen & Nagas.) R.H.Petersen, *H. japonica* (Dörfelt) R.H.Petersen, *H. orientalis* (R.H.Petersen & Nagas.) R.H.Petersen, and *H. vinocontusa* (R.H.Petersen & Nagas.) R.H.Petersen in Japan, and *H. endochorda* (Berk. & Broome) R.H.Petersen in Sri Lanka (Petersen & Hughes 2010). *Hymenopellis chiangmaiae* (R.H.Petersen & Nagas.) R.H.Petersen was first reported in Thailand but later synonymized under *H. raphanipes* (Berk.) R.H.Petersen by Petersen & Hughes (2010). Additionally, various species have been discovered in Australia including *H. eradicata* (Kalchbr.) R.H.Petersen, *H. gigaspora* (Cooke & Massee) R.H.Petersen, *H. mundroola* (Grgur.) R.H.Petersen, *H. superbiens* (Berk.) R.H.Petersen, *H. trichofera* (R.H.Petersen) R.H.Petersen, and *H. variabilis* (R.H.Petersen) R.H.Petersen. Other species such as *H. radicata* (Relhan) R.H.Petersen have a global presence, occurring in Europe, North America, North Africa, and parts of Western Asia and Minor Asia (Ronikier 2003; Petersen & Hughes 2010). *Hymenopellis raphanipes* was first described in India (Berkeley 1850) and has also been recorded in Australia, China, India, Japan, and Thailand (Yang & Zang 1993; Pegler & Young 1986; Petersen & Nagasawa 2006; Petersen & Hughes 2010). The only *Hymenopellis* species previously reported from Pakistan is *H. ahmadii* (Dörfelt) R.H.Petersen (Petersen & Nagasawa 2006).

Margalla Hills National Park (MHNP) is a mountain range located to the north of the capital city of Pakistan, Islamabad (72°55 E and 33°43 N). It is located in the foothills of the lower Himalayas with an approximate altitude of 450 to 1580 m a.s.l. (Jabeen *et al.* 2009) covering an area of around 17 386 hectares. The Park's topography is rugged and primarily consists of limestone geology (Nasir & Akhter 1987). The soil of the MHNP is wind-deposited, colluvial, and fine-textured varying from yellowish

brown to dark brown in color (Hijazi 1984) and the climate is semi-arid to sub-tropical with an annual rainfall of about 1200 mm. During winter, the temperature drops below zero while in summer the temperature rises to 42 °C (Hussain 1986; Khalid *et al.* 2015). The main ecological regions are subtropical scrub deciduous forests and subtropical coniferous evergreen pine forests with *Pinus roxbughii* Sarg., *Dodonaea viscosa* (L.) Jacq., *Olea europaea* L. subsp. *cuspidata* (Wall. & G. Don) Cif., *Morus alba* L., *Senegalia modesta* (Wall.) P.J.H.Hurter and *Dalbergia sissoo* Roxb. as the dominant vegetation.

In this study, we propose a new species of *Hymenopellis* discovered from Margalla Hills National Park, based on morpho-anatomical and molecular phylogenetic analyses using nrITS and nrLSU markers. This research contributes to our ongoing efforts in exploring the macrofungal diversity of this region.

Material and methods

Collections and morpho-anatomical characterization

Samples were collected during visits to the Margalla Hills National Park (MHNP) in Pakistan during 2019–2020. Fresh basidiomata of macrofungi were collected and photographed in the field using a Nikon D70 camera fixed with a Nikkor macro lens (18–77 mm). For preliminary identification, important macro-morphological characters, such as length, width, shape, surface features, and colors were noted. Samples were air-dried or with the help of a fan heater. Dried specimens have been submitted to the Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

For a morphological characterization, color notations were allocated according to Munsell Color Chart (1975), while for morpho-anatomical descriptions, we followed Vellinga (2001). For microscopic observations, a trinocular Compound Microscope (CXRII, Lambomed, Labo America Inc., Fremont, CA, USA) was used. Slides from different parts of the basidiocarps were prepared and mounted in 5% potassium hydroxide. Congo red was used for contrast purposes. For basidiospores, at least 60 measurements were noted from each collection while for basidia, cheilocystidia, pleurocystidia, and stipe and pileus elements, 20 elements were measured. Dimensions were symbolized as (a–)b–c(–d); here 'a' and 'd' are denoted as extreme values in parentheses and 'b–c' encloses 90% of the measured values. The succeeding abbreviations were used; l presented as length, w was width, avl as average length, awl as the average width of structures under observation, basidiospores length/width ratio was designated as Q (Bas 1969; Yu *et al.* 2020) and their average as Qav.

DNA extraction, PCR, and sequencing

For DNA extraction, a 2% modified CTAB protocol was followed (Bruns 1995). The primer pairs of ITS1F/ITS4 and LROR/LR5 were used to amplify the ITS (White *et al.* 1990; Gardes & Bruns 1993) and LSU regions (Vilgalys & Hester 1990). The thermal profile of the PCR was initial denaturation for 1 min at 94°C, then denaturation for 35 cycles for one min at 95°C, annealing for one min at 54°C followed by an extension step of three min at 72°C with a final extension for 8 min at 72°C. Successfully amplified products of each specimen were sent for sequencing to TsingKe, China. Successful sequences created in this study were submitted to GenBank.

Phylogenetic analyses

Consensus sequences for both ITS and nrLSU were created by aligning the forward and reverse primer reads in BioEdit software ver. 7.2.5 (Hall 1999). The consensus sequences were searched for homology using BLAST (Basic Local Alignment Search Tool) at NCBI (https://www.ncbi.nlm.nih.gov/) and closely related sequences were downloaded from GenBank. From nrITS-based phylogeny, *Strobilurus albipilatus* (Peck) V.L. Wells & Kempton was selected as an outgroup, while in the combined (ITS and LSU) phylogeny, *Xerula pudens* (Pers.) Singer and *Paraxerula americana* (Dörfelt) R.H.Petersen (Hao

Table 1 (continued on next page). Taxa used in phylogenetic analyses. Newly generated sequences are shown in **bold.**

Species (current name)	Locality	Voucher/ strain no.	GenBank accession no.	
			nrITS	nrLSU
Oudemansiella australis	Australia	RV95/297	AF321472	–
Oudemansiella australis	Australia	RV95/416	AF321473	–
Oudemansiella australis	Australia	RV95/852	AF321475	–
Oudemansiella australis	China	PDD71151	AY960999	AY960991
Oudemansiella canarii	Puerto Rico	RVPR100	AF321479	–
Oudemansiella canarii	Costa Rica	RV96/35	AF321477	–
Oudemansiella canarii	USA: Mississippi	TENN62802	GQ892793	–
Oudemansiella canarii	China	HKAS45444	AY804301	AY804288
Oudemansiella canarii	China	HKAS5021	AY804298	AY804287
Hymenopellis chiangmaiae	China: Guizhou Provence	TENN 57273	GU980125	–
Hymenopellis chiangmaiae	China: Guizhou Provence	TENN 57273	GU980126	–
Hymenopellis raphanipes	China	HKAS93083	KX688237	–
Hymenopellis raphanipes	China	HKAS71518	KX688242	–
Hymenopellis raphanipes	China	HKAS75607	KX688234	–
Hymenopellis raphanipes	China	HKAS69220	KX688241	–
Hymenopellis raphanipes	China	HKAS 42503	GU980130	–
Hymenopellis raphanipes	China	HKAS95781	KX688232	KX688259
Hymenopellis raphanipes	China	HKAS95783	KX688238	KX688265
Hymenopellis raphanipes	China	HKAS95782	KX688236	KX688263
Hymenopellis raphanipes	China	HKAS38682	KX688243	KX688270
Hymenopellis raphanipes	China	HKAS39593	KX688244	KX688271
Hymenopellis raphanipes	China	HKAS80141	KX688235	KX688262
Hymenopellis japonica	China	HKAS83175	KX688226	KX688253
Hymenopellis japonica	China	HKAS61674	KX688225	KX688252
***Hymenopellis areolata* sp. nov. holotype**	**Pakistan: Margalla Hills**	**MH691/LAH37573**	**OQ438118**	**OQ438162**
***Hymenopellis areolata* sp. nov.**	**Pakistan: Margalla Hills**	**MH69/LAH37574**	**OQ438119**	**–**
Hymenopellis rubrobrunnescens	USA: North Carolina	TENN52479	GQ913371	–
Hymenopellis rubrobrunnescens	USA: North Carolina	TENN52654	GQ913372	HM005112
Hymenopellis limonispora	USA: Tennessee	TENN61379	GQ913403	HM005134
Hymenopellis limonispora	USA: Tennessee	TENN59438	GQ913406	HM005133
Hymenopellis megalospora	USA: New York	TENN51257	GQ913411	–
Hymenopellis incognita	USA: Texas	TENN58768	GQ913424	HM005105
Hymenopellis incognita	USA: Missouri	TENN60228	GQ913419	–
Hymenopellis sinapicolor	USA: Arkansas	TENN56566	GQ913351	–
Hymenopellis sinapicolor	USA: Arkansas	TENN56566	GQ913352	–
Hymenopellis rugosoceps	USA: Tennessee	TENN60604	GQ913394	HM005117
Hymenopellis rugosoceps	USA: Tennessee	TENN57307	GQ913395	HM005116
Hymenopellis radicata	Sweden: Västergötland	TENN 57277	GQ913379	HM005122
Hymenopellis radicata	Sweden: Västergötland	TENN62837	GQ913375	HM005125
Hymenopellis radicata	Austria: Vienna	TENN59329	GQ913380	–
Hymenopellis orientalis	Japan	TMI_2IX2002	GQ913397	–
Hymenopellis orientalis	Japan	TMI_2IX2002	GQ913398	–
Hymenopellis orientalis	Japan	TMI_2IX2002	GQ913396	–
Hymenopellis orientalis	China	HKAS67938	KX688227	KX688254
Hymenopellis orientalis	China	HKAS70323	KX688228	KX688255
Hymenopellis furfuracea	USA: North Carolina	TENN60702	GQ913366	–

Table 1 (continued).

Species (current name)	Locality	Voucher/ strain no.	GenBank accession no.	
			nrITS	nrLSU
Hymenopellis furfuracea	USA: North Carolina	TENN50230	GQ913365	–
Hymenopellis furfuracea	USA: Tennessee	TENN61678	GQ913364	–
Hymenopellis furfuracea	USA: Tennessee	TENN 59876	GQ913367	HM005126
Hymenopellis furfuracea	USA: Tennessee	TENN61671	GQ913362	HM005101
Hymenopellis furfuracea	China	HKAS59927	KX688224	KX688251
Hymenopellis furfuracea	China	HKAS 93109	KX688223	KX688250
Hymenopellis vinocontusa	Japan	TMI7669	GQ913370	–
Hymenopellis superbiens	Australia	MEL2291946	GQ913361	–
Hymenopellis superbiens	Australia	MEL2291946	GQ913360	–
Hymenopellis trichofera	Australia	MEL2293664	GQ913354	–
Hymenopellis gigaspora	Australia: NSW, Jervis Bay	TENN50056	GQ913358	–
Hymenopellis gigaspora	Australia: NSW, Jervis Bay	TENN50050	GQ913359	–
Hymenopellis gigaspora	Australia	REH8676	GQ913357	HM005121
Hymenopellis colensoi	New Zealand	ZT12902	HM005139	HM005119
Hymenopellis colensoi	New Zealand	ZT12902	HM005140	HM005119
Paraxerula americana	USA: New Mexico	CLO4746	HM005142	HM005094
Paraxerula americana	USA: New Mexico	CLO4744	HM005141	–
Paraxerula hongoi	Japan	C 60612	HM005144	–
Xerula pudens	Spain	C 63308	HM005155	–
Xerula pudens	Austria: vic. Vienna	TENN59208	HM005154	HM005097
Strobilurus albipilatus	USA: Washington	TENN52255	GQ892806	–
Strobilurus albipilatus	USA: Washington	TENN52275	GQ892805	–

et al. 2016; Wu *et al.* 2020). The final nrITS dataset consisted of 51 sequences and the combined (nrITS and LSU) dataset contained 32 sequences including outgroup taxa (Table 1). Multiple sequences were aligned using MUSCLE ver. 3.8 (Edgar 2004) and webPRANK (Löytynoja & Goldman 2010) and manually edited where required.

Phylogenies were inferred by Maximum Likelihood analyses using RAxML ver. 8.1.11 under the GTRCAT model on the online CIPRES Science Gateway Portal ver. 3.1 (Miller *et al.* 2010; Stamatakis 2014) with 1000 bootstrap replicates for quick bootstrapping. The final phylogram was displayed in FigTree ver. 1.4.3 (Rambaut 2014) and exported to editing software Adobe Illustrator CC (2022) ver. 16.0.2.

Results

The amplified fragment size of the ITS region was 655 bp. The nrITS alignment comprised 864 positions, out of which 450 were conserved, 386 were variables, 335 were parsimony informative, and 51 were singletons. The nrITS sequences of the new species show 94% sequence similarity with *H. raphanipes* (KX688237) from China and 93% similarity with *H. rubrobrunnescens* (Redhead, Ginns & Shoemaker) R.H.Petersen (GQ913372). The phylogenetic tree (Fig. 1) shows that the newly generated sequences of *H. areolata* sp. nov. falls within the same clade comprising *H. japonica*, *H. raphanipes*, *O. canarii* (Jungh.) Höhn., and *O. australis* G.Stev. & G.M.Taylor with moderate support (BS 76%). The findings indicate that *Hymenopellis* is paraphyletic, consistent with the results of Petersen & Hughes (2010).

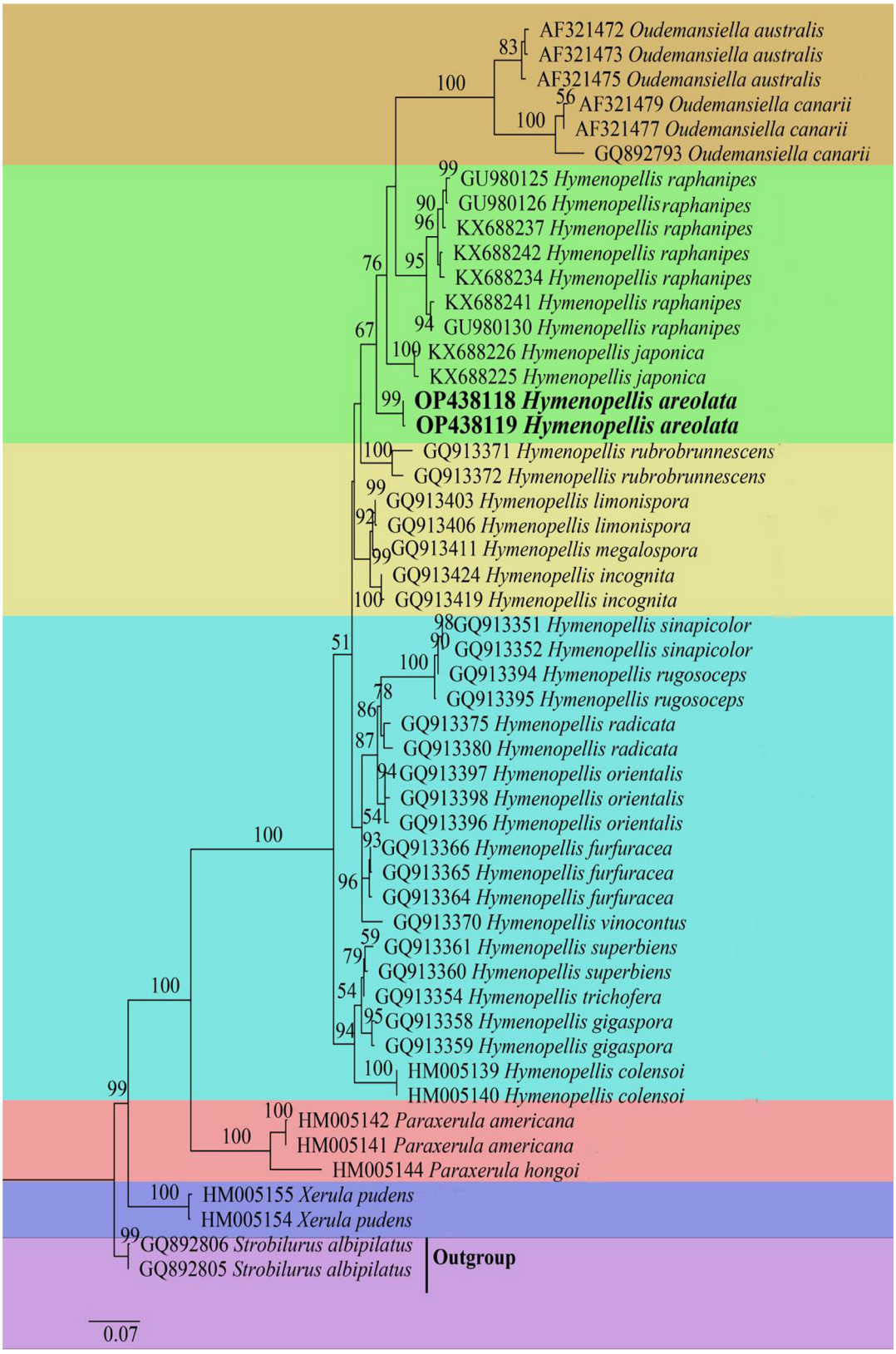

Fig. 1. Maximum Likelihood (ML) phylogram of *Hymenopellis areolata* F.Razzaq & Khalid sp. nov. (OQ438118 and OQ438119) inferred from ITS dataset. ML bootstrap proportional values were mentioned on the nodes. The newly obtained sequences are highlighted in **bold**.

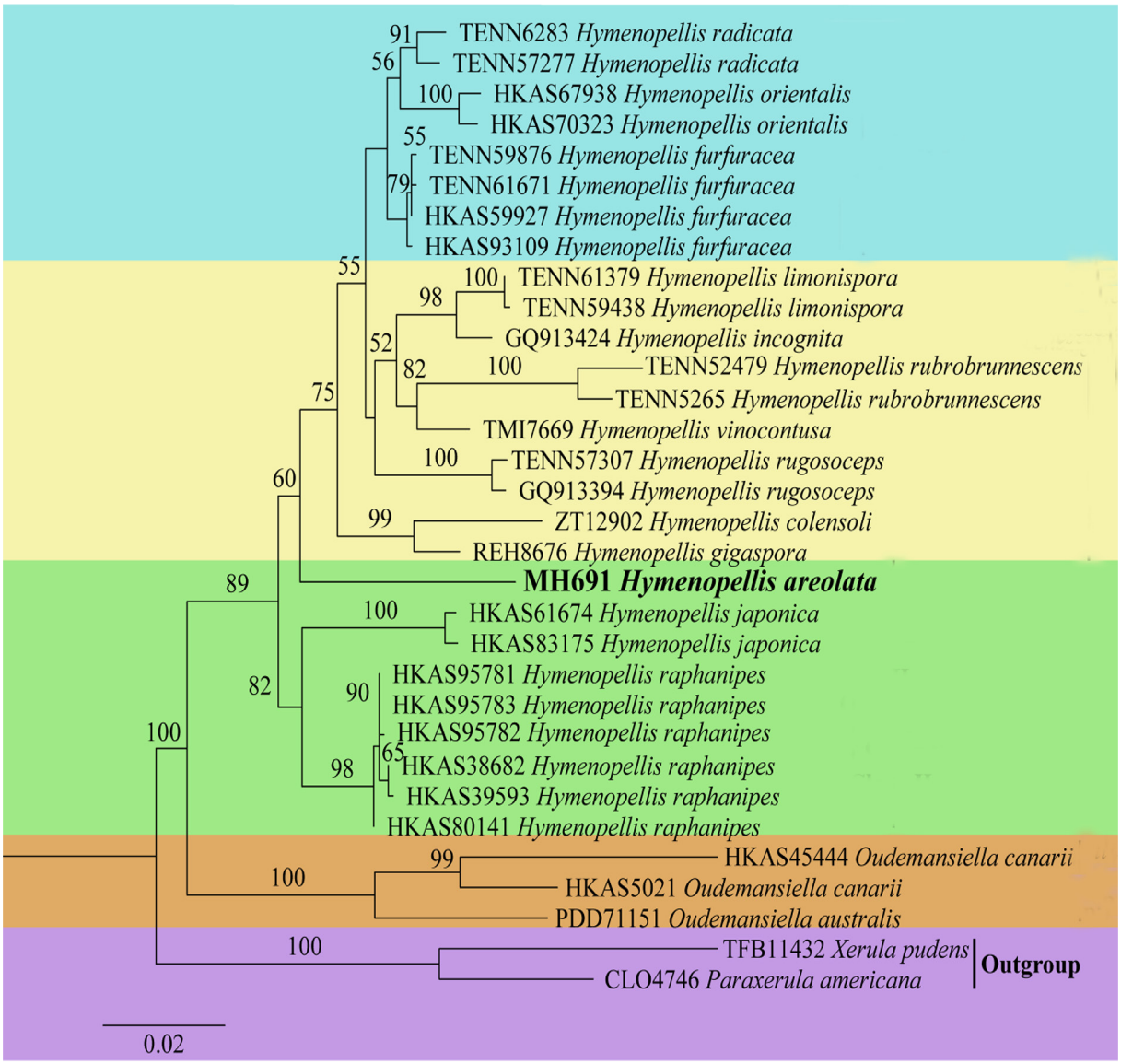

Fig. 2. Maximum Likelihood (ML) phylogram of *Hymenopellis areolata* F.Razzaq & Khalid sp. nov. (MH691) inferred from combined ITS and nrLSU dataset. ML bootstrap proportional values are presented above the nodes. The newly obtained sequences are highlighted in **bold**.

The nrLSU sequence of *Hymenopellis areolata* sp. nov. was 928 bp long. The combined (nrITS and LSU) alignment contained 2132 positions, 1474 were conserved, 351 were variables, 202 were parsimony informative, and 142 were singletons. In the combined phylogram (Fig. 2), the new species also formed a distinct lineage.

Taxonomy

Phylum Basidiomycota R.T. Moore
Class Agaricomycetes Doweld
Order Agaricales Underw.
Family Physalacriaceae Corner
Genus *Hymenopellis* R.H.Petersen

Hymenopellis areolata F.Razzaq & Khalid sp. nov.
MycoBank: MB 847568
Figs 3–4

Diagnosis

Differs from *H. japonica* and *H. raphanipes* by its smaller basidiospores (11.5–17.5 × 10.0–16.0 µm). It differs from *H. japonica* in having an areolate pileus surface, and transitional pileipellis (hymeniderm and epithelium), and differs from *H. raphanipes* in having a subumblicate, applanate pileus, along with the color and presence of clamp-connections.

Etymology

The specific epithet '*areolata*' refers to the areolate surface of the pileus.

Type material

Holotype

PAKISTAN • Punjab Province, Margalla Hills, Islamabad, 72°55 E, 33°43 N, at 1580 m a.s.l.; Aug. 2019; *Abdul Nasir Khalid*, *MH-691* (LAH37573); GenBank (ITS: OQ438118, LSU: OQ438162).

Additional specimen examined

PAKISTAN • Punjab Province, Margalla Hills, 72°55 E, 33°43 N, at 1580 m a.s.l.; sub-tropical, found on moist and calcareous soil, during monsoon season, solitary or in small groups; Sep. 2019, *Abdul Nasir Khalid*, *MH69* (LAH37574); GenBank (OQ438119).

Description

Basidiomata medium-sized to large, solitary, and radicating. *Pileus* 8.0–10.0 cm in diam., plano-concave to applanate, subumbilicate at the center, covered with flat scales, uplifted, irregular, dark brown (7.5YR3/4) to dull brown (7.5YR5/4), hard, surface dry and dull, areolate, margins striate (Fig. 3A, C). *Lamellae* adnate with teeth, close to subdistant, ventricose, broad, cream to whitish in color, thick, margins entire. Lamellulae frequent, 5.0–7.0 between two lamellae (Fig. 3B). *Stipe* 8.0–16.0 × 0.7–1.0 cm including pseudorhizae, central, equal but slightly broader towards the base, cylindrical, light gray (2.5Y8/1) to dark grayish yellow (2.5Y4/2), whitish in upper part and with no or very small scales, strigose and rigid, short pseudorhizae present (Fig. 3D). *Annulus* and *volva* absent. *Taste* and *odor* were not observed.

Basidiospores (11.5–)12.5–17(–17.5) × (10.0–)11.0–16.0 µm, avl × avw = 14.8 × 12.3 µm, Q = 1.02–1.42 µm, Qav = 1.20 µm, broadly ellipsoidal to subglobose, apiculate, multiguttulate, smooth, thin-walled, pale yellow in 5% KOH (Fig. 4A). *Basidia* (35.5–)37.0–56.0(–59.0) × (10.5–)10.5–18 (–18.5) µm, clavate, with 2–4 sterigmata, guttulate, with basal clamp, thin-walled, pale yellow in 5% KOH (Fig. 4B). *Cheilocystidia* (30.0–)31.0–118.0(–122.0) × (7.5–)9.0–27.0(–33.0) µm, avl × avw = 68.2 × 15.8 µm, polymorphic, narrowly utriform to utriform, narrowly clavate, lageniform, capitate,

Fig. 3. Basidiocarps of *Hymenopellis areolata* F.Razzaq & Khalid sp. nov., holotype (LAH37573). Photos by Abdul Nasir Khalid.

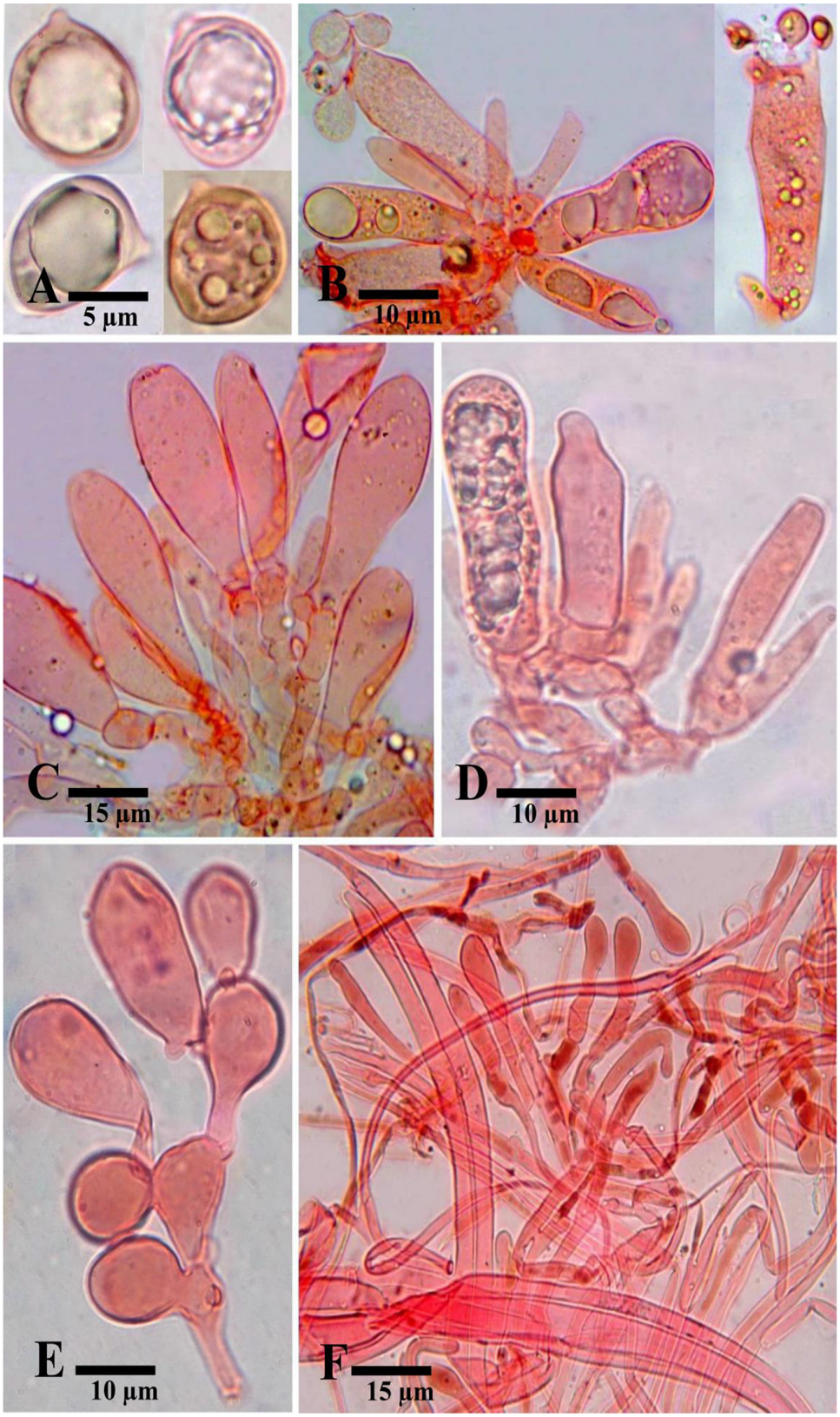

Fig. 4. Microscopic features of *Hymenopellis areolata* F.Razzaq & Khalid sp. nov., holotype (LAH37573). **A**. Basidiospores. **B**. Basidia. **C**. Cheilocystidia. **D**. Pleurocystidia. **E**. Pileus elements. **F**. Stipitipellis with caulocystidia. Photos by Fauzia Razzaq.

pale yellow in 5% KOH, thin-walled (Fig. 4C). *Pleurocystidia* (25.0–)27.0–39.0(–41.0) × (7.0–) 8.0– 10.5(–12.0) µm, avl × avw = 31.8 × 8.7 µm, utriform to narrowly utriform, conical, capitate, sometimes cylindrical, with basal clamp, pale yellow in 5% KOH and thin-walled (Fig. 4D). *Pileipellis* a transition between hymeniderm and epithelium, mostly sphaeropedunculate to subglobose, few clavate pileocystidia, 26.5–49 × 17–31.0 µm, hyphae 16.0–26.0 µm in diam., avw = 20.3 µm, septate, smooth, thin-walled, brown pigmented in 5% KOH, some hyaline, hyphal structures hyaline (Fig. 4E). *Stipitipellis* made up of septate hyphae, cylindrical, 5.0–9.0 µm in diam., avw = 6.78 µm, parallel in arrangement, clamp-connections present, pale yellow in 5% KOH. Caulocystidia (44.0–)47.0–71.0 (–73.0) × (8.5–)9.0–11.0 µm, avl × avw = 57.6 × 9.8 µm, narrowly clavate, thick-walled, with brown vacuolar pigment; clamp connections present (Fig. 4F).

Habitat

Saprobic, solitary on moist and calcareous soil.

Distribution

The new species is known only from Margalla Hill National Park in Islamabad, Pakistan.

Discussion

Hymenopellis areolata sp. nov. is distinguished by its subumbilicate, applanate, areolate pileus, small basidiospores (11.5–17.5 × 10.0–16.0 µm), small cheilo- (30.0–122.0 × 7.0–33.0 µm) and pleurocystidia (25.0–41.0 × 7.0–12.0 µm), a transitional pileipellis (hymeniderm and epithelium) and small pileocystidia (26.5–)27.0–49.0 × 17.0–31.0 µm).

In ITS based phylogenetic analysis *H. areolata* sp. nov. is related to *H. japonica*, which is found in subtropical and temperate regions of Asia. However, it can be distinguished by its smaller pileus (1.5–6.0 cm in diam.) which is shallowly convex to umbonate, with slightly raised or radially wrinkled, black reticulate veins radiating from the disc towards the margins with a slender stipe (5.5–8.5 × 0.3–0.4 cm) which is delicately furfuraceous to squamulose. Anatomically, it has larger basidiospores (12.0–20.5 × 10.5–18 µm), larger pleurocystidia (120.0–155.0 × 27.0–51.0 µm), and an ixohymenoderm pileipellis with large pileocystidia (30.0–89.0 × 15.0–45.0 µm) (Petersen & Hughes 2010).

Hymenopellis raphanipes, described from the subtropical region of China, seems to be another phylogenetically related species to *H. areolata* sp. nov. in ITS phylogram, but differs by its hairy umbo pileus, viscid and wrinkled surface, blackish brown color, reticulate black veins that radiate from the disc to the margins, and stipe surface with darker brown to olive-brown small appressed patches. Moreover, it also has larger basidiospores (13.0–)14.0–20.0 × 10.0–18.0 µm, larger cheilo- and pleurocystidia (42.0–150.0(–235.0) µm and 83.0–171.0 × 14.0–55.0 µm respectively), and larger pileocystidia (27.0–97.0 × 8.0–26.0 µm) (Petersen & Hughes 2010; Hao *et al.* 2016).

Hymenopellis rubrobrunnescens, an eastern North American species, differs by a shallowly convex to umbonate pileus, outward rugose to rugulose, slightly inflated pseudorhiza, sometimes long (as long as stipe), dauciform. Microscopically, it has elongated ovoid to sublimoniform, larger fusiform-mammilate to capitulate cheilo- and lecythiform, fusiform capitate to mammilate pleurocystidia (26–200 × 10–39 µm and 80–124(–170) × (18–)22–35 µm) (Petersen & Hughes 2010).

Morphologically, *Hymenopellis furfuracea* (Peck) R.H.Petersen, occurring in mixed deciduous forests in the United States and Canada, is similar to our new species due to its brown to dark brown, rarely shallowly umbilicate pileus, white lamellae, and broadly ellipsoidal basidiospores. *Hymenopellis furfuracea* is unique due to its adnexed to sinuate-adnate with tooth lamellae, relatively large basidiospores (12–

Table 2. Comparison of important characters of *Hymenopellis areolata* F.Razzaq & Khalid sp. nov. with related species.

Characters	*H. areolata*	*H. japonica*	*H. raphanipes*	*H. rubrobrunnescens*	*H. furfuracea*	*H. ahmadii*
Pileus	Plano-concave to applanate, covered with flat scales, dark brown to dull brown	Shallowly convex, radially black veins radiating from the disc and extending to the margin, very dark brown	Shallowly convex to shallowly concave, intricately wrinkled, dark brown to brown-black over a disc or when mature	Shallowly convex, outward rugose to rugulose, snuff brown, blackish brown to tawny olive	Shallowly umbonate to umbonate or rarely umbilicate, slightly rugulose at edges of umbo to radially rivulose brown to dark brown	Convex, umbo dark brown, outward neutral gray-brown
Perforatorium	Subumbilicate	Shallowly umbonate	Shallowly umbo	Shallowly umbonate	Umbonate to rarely umbilicate	Shallowly umbonate
Pseudorhizae	Short	Long	Tapering rapidly, carrot to turnip-shaped	Slightly inflated, sometimes long as stipe, dauciform	Hardly inflated, tapering downward gradually	Tapering to sharply tapering downward, channeled
Spores	11.5–17.5 × 10.0–16.0 µm, broadly ellipsoidal to subglobose	12–20.5 × 10.5–18 µm, subglobose to broadly ovate	(13–)14–20 × 10–18 µm, ellipsoidal, very broadly ellipsoidal to subglobose, occasionally globose	10–16(–18.5) × 7–12 µm, elongate ovoid to sublimoniform	12–17(–20) × 9–14 µm, broadly ellipsoid	(12.5–)14–22 × 12–18(–20) µm, globose to subglobose
Cheilocystidia	30.0–122.0 × 7.59.0–27.0–33.0 µm	40–145 × 12–31 µm	42–150(–235) × 7–37 µm	26–200 × 10–39 µm	32–160 ×8–36 µm	52–110 ×11–22 µm
Pleurocystidia	25.0–41.0 × 7.0–12.0 µm	120–155 × 27–51 µm	83–171 × 14–55 µm	80–124(–170) ×(18–)22–35 µm	(80–)90–175 × 27–47 µm	109–115 × 27–32 µm
Pileipellis	A transition between hymeniderm and epithelium, mostly sphaero-pedunculate to subglobose, few clavate pileocystidia	An ixohymenoderm, sub-glutinous, pedicellate, broadly clavate, obpyriform to sub-sphaeropedunculate pileocystidia	Constructed of pedicellate, narrowly to broadly clavate, subsphaero-pedunculate pileocystidia, and extended pileal hairs	Composed of pedicellate, commonly broadly clavate to sphaeropedunculate pileocystidia and extended pileal hairs	Constructed of one to two variables; strongly pedicellate, broadly clavate to sphaeropedunculate pileocystidia and pilear hairs (may be present or not)	A hymeniderm constructed of a single element; pedicellate, elongate-clavate never sphaeropedunculate pileocystidia
Habit and Habitat	Solitary on subtropical forests of Margalla Hills, Islamabad, Pakistan	Subtropical and temperate Asia; collection in coniferous woods, another under a deciduous tree	Widespread from Australia to Japan, to be expected throughout Southeast Asia, including Sri Lanka; gregarious or solitary in the bamboo forest or in mixed forest	Eastern North America from the southern Appalachian Mountains to southeastern Canada; mixed hardwood-*Tsuga* forest	Eastern North America, solitary to scattered, on soil or litter in mixed deciduous forests (eastern United States, eastern and maritime Canada)	Pakistan; in the ground with a long, rooting base

17(–20) µm), large cheilocystidia (32–160 × 8–36 µm), and narrowly ten pin-shaped pleurocystidia (Petersen & Hughes 2010).

Hymenopellis ahmadii a previously reported species from Pakistan based on morpho-anatomical characterization, differs from *H. areolata* sp. nov. by a smaller pileus that is 3–3.5 cm in diam. Its pileus is convex to shallowly umbonate, and an apically tomentose to downward furfuraceous stipe surface. Additionally, it also has larger basidiospores and (12.5–)14.0–22.0 × 12.0–18.0(–20.0) µm and larger pleurocystidia (109.0–115.0 × 27.0–32.0 µm, fusiform with an extended neck and a distinct capitulum (Petersen & Hughes 2010).

Therefore, morphological and molecular phylogenetic data support the placement of *H. areolata* sp. nov. as a different species of the genus *Hymenopellis*. A table of the comparative diagnostic features of close species is given in Table 2.

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