Hymenopellis areolata (Physalaciaceae: 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, Physalaciaceae, taxonomy.

Introduction

The family Physalaciaceae 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 Physalaciaceae was published, incorporating morphological and molecular phylogenetic data of the Oudemansiella/Xerula complex (Petersen & Hughes 2010). Physalaciaceae belongs to the order Agaricales with typified genus Physalacia (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, agaricioid, 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 Specg. (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 17386 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 roxburghii* 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* (Dörffelt) R.H.Petersen (Hao
Table 1 (continued on next page). Taxa used in phylogenetic analyses. Newly generated sequences are shown in **bold**.

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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, capitulate,
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 pleuro-
cystidia (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 furredaceus 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 
capitate 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.

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>H. areolata</em></th>
<th><em>H. japonica</em></th>
<th><em>H. raphanipes</em></th>
<th><em>H. rubrobrunnescens</em></th>
<th><em>H. furfuracea</em></th>
<th><em>H. ahmadii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pileus</td>
<td>Plano-concave to applanate, covered with flat scales, dark brown to dull brown</td>
<td>Shallowly convex, radially black veins radiating from the disc and extending to the margin, very dark brown</td>
<td>Shallowly convex to shallowly concave, intricately wrinkled, dark brown to brown-black over a disc or when mature</td>
<td>Shallowly convex, outward nago to rugulose, snuff brown, blackish brown to tawny olive</td>
<td>Shallowly umbonate to umbonate or rarely umbilicate, slightly rugulose at edges of umbo to radially rivulose brown to dark brown</td>
<td>Convex, umbo dark brown, outward neutral gray-brown</td>
</tr>
<tr>
<td>Perforatorium</td>
<td>Subumbilicate</td>
<td>Shallowly umbo</td>
<td>Shallowly umbo</td>
<td>Shallowly umbonate</td>
<td>Umbonate to rarely umbilicate</td>
<td>Shallowly umbonate</td>
</tr>
<tr>
<td>Pseudorhizae</td>
<td>Short</td>
<td>Long</td>
<td>Tapering rapidly, carrot to turnip-shaped</td>
<td>Slightly inflated, sometimes long as stipe, dauciform</td>
<td>Hardly inflated, tapering downward gradually</td>
<td>Tapering to sharply tapering downward, channelled</td>
</tr>
<tr>
<td>Spores</td>
<td>11.5–17.5 × 10.0–16.0 µm, broadly ellipsoid to subglobose</td>
<td>12–20.5 × 10.5–18 µm, subglobose to broadly ovate</td>
<td>(13–)14–20 × 10–18 µm, ellipsoid, very broadly ellipsoid to subglobose, occasionally globose</td>
<td>10–16–18.5 × 7–12 µm, elongate ovoid to sublimoniform</td>
<td>12–17–20 × 9–14 µm, broadly ellipsoid</td>
<td>(12.5–)14–22 × 12–18–20 µm, globose to subglobose</td>
</tr>
<tr>
<td>Cheilocystidia</td>
<td>30.0–122.0 × 7.59–33.0 µm</td>
<td>42–150–235 × 7–37 µm</td>
<td>26–200 × 10–39 µm</td>
<td>32–160 × 8–36 µm</td>
<td>52–110 × 11–22 µm</td>
<td></td>
</tr>
<tr>
<td>Pleurocystidia</td>
<td>25.0–61.0 × 7.0–12.0 µm</td>
<td>83–171 × 14–55 µm</td>
<td>80–124–170 × (18–)22–35 µm</td>
<td>(80–)90–175 × 27–47 µm</td>
<td>109–115 × 27–32 µm</td>
<td></td>
</tr>
<tr>
<td>Pileipellis</td>
<td>A transition between hymeniderm and epithelium, mostly sphaero-pedunculate</td>
<td>An isohymenoderm, subglutinous, pedicellate, broadly clavate, obpyriform to sub-sphaero-pedunculate pileocystidia and extended pileal hairs</td>
<td>Constructed of pedicellate, narrowly to broadly clavate, sub-sphaero-pedunculate pileocystidia and extended pileal hairs</td>
<td>Composed of pedicellate, commonly broadly clavate to sphaero-pedunculate pileocystidia and extended pileal hairs</td>
<td>Constructed of one to two variables; strongly pedicellate, broadly clavate to sphaero-pedunculate pileocystidia and pileal hairs (may be present or not)</td>
<td>A hymeniderm constructed of a single element; pedicellate, elongate-clavate never sphaero-pedunculate pileocystidia</td>
</tr>
<tr>
<td>Habit and Habitat</td>
<td>Solitary on subtropical forests of Margalla Hills, Islamabad, Pakistan</td>
<td>Subtropical and temperate Asia; collection in coniferous woods, another under a deciduous tree</td>
<td>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</td>
<td>Eastern North America from the southern Appalachian Mountains to southeastern Canada; mixed hardwood-&lt;em&gt;Tsuga&lt;/em&gt; forest</td>
<td>Eastern North America, solitary to scattered, on soil or litter in mixed deciduous forests (eastern United States, eastern and maritime Canada)</td>
<td>Pakistan; in the ground with a long, rooting base</td>
</tr>
</tbody>
</table>
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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