TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2018) 42: 123-133

doi:10.3906/bot-1705-21

Xanthagaricus pakistanicus sp. nov. (Agaricaceae): first report of the genus from Pakistan

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Received: 10.05.2017 • **Accepted/Published Online:** 25.09.2017 • **Final Version:** 11.01.2018

Abstract: *Xanthagaricus pakistanicus* is described as a new species from lowland northern Pakistan, based on morphological and molecular data. It is characterized by a yellowish pileus, covered with dark brown squamules, a stipe with yellowish fibrils, globose basidiospores, and pileal squamules made up of pseudoparenchymatous epithelium with encrusted walls. Molecular phylogenetic trees were inferred based on nucleotide sequences of the internal transcribed spacer (ITS1-5.8S-ITS2) region and 28S nuclear ribosomal DNA. In phylogenetic analyses the genus *Hymenagaricus* sensu lato (s. l.) was inferred as a nonmonophyletic group and recovered in two monophyletic clades, consisting of species of the genera *Xanthagaricus* and *Hymenagaricus* sensu stricto (s. str.). On account of the pileal squamule structure (pseudoparenchymatous epithelium) and yellowish basidiospores, and the phylogenetic position in the *Xanthagaricus* clade the new species *X. pakistanicus* belongs to the genus *Xanthagaricus*. Morphoanatomical comparison with the known species of *Xanthagaricus* is provided. We also made six new combinations in *Xanthagaricus*: *X. calicutensis*, *X. epipastus*, *X. ochraceoluteus*, *X. rufomarginatus*, *X. subaeruginosus*, and *X. taiwanensis*. With this study the number of known species in the genus *Xanthagaricus* increases to 19.

Key words: Agaricales, Malakand, new taxon, phylogeny, taxonomy

1. Introduction

The name *Xanthagaricus* originally was put forward by Heinemann as a subgenus of *Hyemagaricus* Heinem s. l. (Heinemann and Little Flower, 1984). It was subsequently elevated to the status of an independent genus by Little Flower et al. (1997), a poorly cited work. Taxonomic and phylogenetic relationships of species of *Xanthagaricus* (Heinem) Little Flower, Hosag. & T.K.Abraham, and *Hymenagaricus* s. str. have not been resolved because molecular data for the described species were limited. Morphological characters are primarily used to distinguish species of *Xanthagaricus* from those of *Hymengaricus* s. str. The genus *Hymenagaricus* is typified by *H. hymenopileus* (Heinem.) Heinem. (Heinemann, 1981) and *Xanthagaricus* by *X. flavidorufus* (Berk. & Broome) Little Flower, Hosag. & T.K.Abraham (Little Flower et al., 1997). In *Hymenagaricus* s. str. species, the pileus disc is often covered with a single large squame, made up of hymeniform hyphae, and the basidiospores are brownish. While in *Xanthagaricus*, the squamules on the pileus are disrupted as the basidioma matures and the pileus disc is usually covered with more than one large squamule, consisting of hymeniform or pseudoparanchymatous cells, and the basidiospores are yellowish (Heinemann and Little Flower, 1984; Little Flower et al., 1997). These characters have led to the inclusion of 12 species in the genus *Xanthagaricus* (Little Flower et al., 1997; Reid and Eicker, 1998), mostly distributed in equatorial paleotropics (Ge et al., 2008).

During the exploration of basidiomycetous fungi of Pakistan in 2014, a species was encountered that differs macro- and microscopically from other species of *Xanthagaricus*. A detailed description and illustration of the new species and comparison with allied taxa are provided. This is the first report of *Xanthagaricus* in Pakistan. In this study we also made six new combinations.

2. Materials and methods

2.1. Sampling and morphoanatomical characterization

Specimens were collected from Malakand district of Khyber Pakhtunkhwa, Pakistan, in September 2014. Basidiomata were photographed, tagged and field notes

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were made. Munsell (1975) was used for determination of color. Specimens examined in this study are deposited in the Herbaria of Hazara University Mansehra, Pakistan (HUP), University of Swat, Pakistan (SWAT), and University of the Punjab, Lahore, Pakistan (LAH).

For anatomical studies slides were prepared in 5% aqueous KOH (w/v). Microscopic features such as size and shape of basidiospores, basidia, cheilocystidia, and squamule structure were studied under a light microscope (MX4300H, Meiji Techo Co., Ltd., Japan) with at least 20 structures measured in each instance. In the case of basidiospores, 44 spores were measured through 1000× magnification with a calibrated optical micrometer and measurements were rounded to the nearest 0.5 µm.

2.2. DNA extraction, PCR amplification, and DNA sequencing

We extracted genomic DNA using the DNeasy Plant Mini Kit (Qiagen, Redwood City, CA, USA). We amplified nuc rDNA internal transcribed spacer (ITS1-5.8S-ITS2) and 28S loci, using the primer combination ITS1F/ITS4; LR0R/LR5 (White et al., 1990; Gardes and Bruns, 1993). For PCR amplification, we followed Hussain et al. (2017). PCR products were purified using the QIAquick PCR Purification kit (Qiagen). Sequencing was performed with the same PCR primers using the Big Dye Sequencing Kit v.3.1 on an ABI-3730-XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences produced for this study have been deposited in GenBank (Table 1).

Table 1. Voucher numbers, geographic origins, and GenBank Accession numbers for the specimens included, in boldface are sequences produced in this study.

Species	Geographic origin	Voucher number	GenBank	
			ITS	28S
Agaricus aff. *campestris*	USA	6242	HM488744	
A. bingensis	Benin	ADK1992	KJ540954	
	Togo	C3155	KJ540950	
A. bisporatus	Thailand	Contu1	AF432882	
	Togo	C3181	KJ540949	
A. deserticola	USA	ASAT99-233-15	HM488748	HM488766
	USA	Smith	HM488747	
A. diminutivus	USA	ecv2360	AF482831	AF482877
A. heterocystis		Goossens5066	JF514522	
A. inapertus	USA	ecv2339	AF482834	AF482878
A. megacystidiatus	Thailand	MFLU-2012-0996	KF305947	
	Thailand	MFLU-2012-1004	KF305946	
A. pseudopratensis	Israel	HAI 0213	AJ884626	
	USA	ecv3768	HM488746	
A. subsaharianus	Tanzania	Z1	KM360157	
	Belgium	ADK4732	JF440300	
	Belgium	ADK4733	JF440301	
A. trisulphuratus	China	LAPAF7	KM657924	KR006605
	Thailand	ecv3868	HM488749	
A. xanthodermus	USA	ML 1	DQ182529	
A. sp.	Thailand	ecv3870	HM488743	HM488767
A. sp.	USA	ecv3244	ecv3244	
A. sp.	USA	6253	HM488745	
A. sp.	Thailand	ecv3614	HM488742	
Chlorophyllum rachodes	Germany	M.Enderle	AF482849	AY176345
Clarkeinda trachodes	Thailand	ecv3550	HM488751	
	Thailand	ecv3838	HM488750	HM488771
Coniolepiota spongodes	Thailand	Png012	HM488756	HM488774

Table 1. (Continued).

	Thailand	ecv3816	HM488754	
	Thailand	ecv3898	HM488755	
C. aff. *spongodes*	Thailand	ecv3613	HM488757	
Eriocybe chionea	Thailand	ecv3560	HM488752	HM488773
	Thailand	ecv3616	HM488753	HM488772
Heinemannomyces splendidissima	Thailand	ecv3586	HM488760	
Hymenagaricus ardosiicolor	Togo	LAPAF9	JF727840	
	Tanzania	Z4	KM360160	
H. cf. *kivuensis*	Burundi	BR6089	KM982454	
H. mlimaniensis	Tanzania	ZNM A	XXXXXX	
H. sp.	Thailand	ZRL3103	KM982450	KM982452
H. sp.	Thailand	LD2012186	KM982451	KM982453
Micropsalliota arginophaea	China	SFSU zrl 2089	HM436616	HM436578
	China	SFSU zrl 2091	HM436613	HM436580
M. pseudoarginea	China	SFSU zrl 3069	HM436643	
M. pusillissima	China	SFSU zrl 3047	HM436645	HM436594
M. subalba	China	SFSU zrl 2080	HM436646	HM436596
M. subarginea	China	SFSU zrl 2052	HM436612	HM436573
Xanthagaricus epipastus	China	SFSU zrl 3045	HM436649	HM436609
X. pakistanicus	**Pakistan**	**LAH SH 207 Holotype**	**KY621555**	**KY621554**
	Pakistan	**HUP SH 315**	**KY621556**	
	Pakistan	**SWAT SH 389**	**KY621557**	
X. taiwanensis	Taiwan	AFTOL-ID 1383	DQ490633	DQ457680
	Taiwan	Chen3636	DQ006271	DQ006270

Consensus sequences were generated from both, forward and reverse primer reads in BioEdit v 7.2.5, and then homology search was performed at NCBI using BLAST (https://blast.ncbi.nlm.nih.gov). According to Vellinga et al. (2011), *Hymenagaricus* s. l. belongs to the '*Agaricus* clade of Agaricaceae', and so DNA datasets were constructed from sequences of that clade. We sampled the monotypic genus *Heinemannomyces splendidissima* Watling in the analysis because this genus belongs to the *Agaricus* clade. DNA sequences were multiple aligned and assembled in CLUSTAL X 2.1 (Larkin et al., 2007). *Chlorophyllum rachodes* (Vittad.) Vellinga was selected as outgroup.

2.3. Phylogenetic analyses

Phylogenetic inference was conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods. MP analyses were performed in PAUP4.0b10 (Swofford, 2004) with a heuristic search of 1000 replicates with random stepwise addition using tree-bisection-reconnection (TBR) branch swapping and starting from trees obtained by the stepwise addition of sequences. ML analyses were run in RAxML-HPC v.8 on XSEDE via CIPRES Science Gateway (Miller et al., 2010). Rapid bootstrap analysis/search for best-scoring ML tree (-f a) was configured. For the bootstrapping phase, the GTRCAT model was selected. One thousand rapid bootstrap replicates were run. For Bayesian inference, we used BEAST 1.6.2 (Drummond and Rambaut, 2007) with a Markov chain Monte Carlo (MCMC) coalescent approach. For tree prior, a Yule-type speciation model (Gernhard, 2008) was used in all simulations, and the starting tree was randomly generated. Four independent runs were undertaken. Chain length was 10 million generations, with a sampling frequency of 1000. Tracer 1.6 (Rambaut et al., 2014) was used to check the effective sample size (ESS), and burn in values were adjusted to achieve an overall ESS of ≥200. A maximum clade credibility tree with 20% burn in was generated using TreeAnnotator 1.6.2 (Drummond and Rambaut, 2007).

Nodes were considered strongly supported when maximum parsimony bootstrap (MPB) and maximum likelihood bootstrap (MLB) percentages were ≥70% (Simmons and Norton, 2014), and Bayesian posterior probability (BPP) was ≥0.95.

3. Results

The ITS dataset is 454 characters. The optimal ML tree was found with a log likelihood score of –6824.234562. In the ITS phylogenetic tree, taxa of Agaricaceae were recovered in the '*Agaricus* clade of Agaricaceae' along with the *Micropsaliota* clade reflect the recent phylogeny of the family as proposed by Vellinga et al. (2011) (Figure 1). The *Agaricus* clade of Agaricaceae, gray highlighted, further consisted of subclades: *Agaricus* L., *Hymenagaricus* s. str., *Xanthagaricus*, *Clarkeinda* Kuntze, *Coniolepiota* Vellinga, and *Eriocybe* Vellinga. The monotypic genus *Heinemannomyces splendidissima* nested in *Hymenagaricus* s. str. clade. Statistical support for the *Agaricus* clade of Agaricaceae was strong (MLB 100%; MPB 96% and BPP 1), and excellent for the *Micropsalliota* clade (MLB 100%; MPB 100% and BPP 1). The representative genera *Xanthagaricus* and *Hymenagaricus* s. str. were recovered in two sister clades with strong statistical support (MLB 95%; MPB 98%; BPP 0.97 for *Xanthagaricus* and MLB 95%; MPB 95%; BPP 1 for *Hymenagaricus* s. str.). Statistical support for the specimens that represent the new species *X. pakistanicus* was maximal (MLB 100%; MPB 100% and BPP 1).

The combined ITS-28S dataset comprises 37 sequences, with 1560 characters. The results of phylogenetic analyses of the combined dataset are summarized in Figure 2. The *Agaricus* clade of Agaricaceae, light-blue highlighted, was recovered with strong statistical support (MLB 93%; MPB 94% and BPP 0.98). *Hymenagaricus* s. l. is represented as nonmonophyletic, and splits in the *Xanthagaricus* and *Hymenagaricus* clades. The representative species *X. pakistanicus* was recovered in the *Xanthagaricus* clade with excellent statistical support (MLP 100%; MPB 100% and BPP 1).

3.1. Taxonomy

Xanthagaricus pakistanicus Hussain, Afshan and Ahmad sp. nov. **Figures 3–4**

MycoBank: MB 820099

Type: PAKISTAN. Khyber Pakhtunkhwa: Qaldara Dargai, 34°50′13″N, 71°89′46″E, alt. 510 m, on soil as saprotrophs, 02 September 2014, Shah Hussain (LAH SH 207, **holotype**).

Etymology: The specific epithet refers to the holotype locality of this species.

Diagnosis: The distinguishing features of *Xanthagaricus pakistanicus* are brownish yellow, large, globose to broadly ellipsoid basidiospores 7–7.5 × 6.5–7 µm; pileal squamules made up of a pseudoparenchymatous epithelium, consisting of globose to circular or ellipsoid, brilliant brown elements, with encrusted walls.

Pileus 10–20 mm diam, initially campanulate, becoming hemispherical to conico-convex, subumbonate; surface dry, light orange yellow (7.5YR 9/8) to moderate orange yellow (7.5YR 7/8), at first covered by smooth, deep yellow (2.5Y 6/10) to vivid yellow (5Y 7/14) pellicle, as the pileus expands disrupting the pellicle except at the subumbonate disc, where it is retained as one or more large, dark yellowish brown squamules, with small, scattered, dark brown squamules toward the margin; margin deflexed with yellowish remnants of membranous annulus. **Lamellae** free, brownish pink (7.5YR 7/2) to grayish yellowish pink (2.5YR 7/2), broadly ventricose, with 1–3 series of lamellulae. **Stipe** 10–15 × 1–2 mm, central, equal, attenuate toward the base, hollow; surface covered with yellowish squamules or fibrils, without a prominent annulus. **Flesh** brown, **Odor** pungent.

Basidiospores (6.5)7–7.5(8) × (6)6.5–7(7.5) µm, [avX = 7.2 × 6.7 µm, Q = 1.0–1.2, avQ = 1.1], globose to broadly ellipsoid, brownish yellow, without germ-pore, slightly thick-walled. **Basidia** 11.5–17.5 × 5–8 µm, 2- or 4-spored, cylindrical to clavate. Cheilocystidia 12–16 × 6.3–7.7 µm, clavate to cylindrical, hyaline, thin-walled. Pleurocystidia absent. **Squamules** of pileipellis are pseudoparenchymatous epithelium, with globose to circular or ellipsoid elements; terminal cells subglobose to rounded, 20–47 × 18–44 µm, brilliant brown, with encrusted walls. **Stipe covering** consisted of clavate to subclavate hyphae, dark brown, thin-walled, with rounded ends, 13–19 × 3–5 µm. **Clamp** connections absent.

Habitat and distribution: In small groups, saprotrophic on nutrient rich loamy soil, in subtropical pine forests, fruiting during rainy season of Aug–Sep. It is so far only known from lowlands of northern Pakistan.

Additional specimens examined: PAKISTAN. Khyber Pakhtunkhwa: Qaldara, Malakand, scattered in groups, on soil, 20 September 2014 (SH-315), Shah Hussain (HUP SH 315, **paratype**); Kharkai, Malakand, 22 September 2014 (SH-389), Shah Hussain (SWAT SH 389, **paratype**).

3.2. New combinations

3.2.1. ***Xanthagaricus calicutensis*** (Heinem. & Little Flower) Hussain comb. nov.

MycoBank: MB 822311

Basionym: *Hymenagaricus calicutensis* Heinem. & Little Flower, Bull. Jard. Bot. Natn. Belg. 54(1-2): 163 (1984).

This species was originally described from Kerala, India, by Heinemann and Little Flower (1984). In the original description this species was placed in *Hymenagaricus* subgen. *Xanthagaricus* on the presence of sub-hymeniform pileal elements and yellowish basidiospores. Subgen. *Xanthagaricus* has been elevated to the status of independent genus and so we proposed a new combination for *Hymenagaricus calicutensis* under *Xanthagaricus calicutensis*.

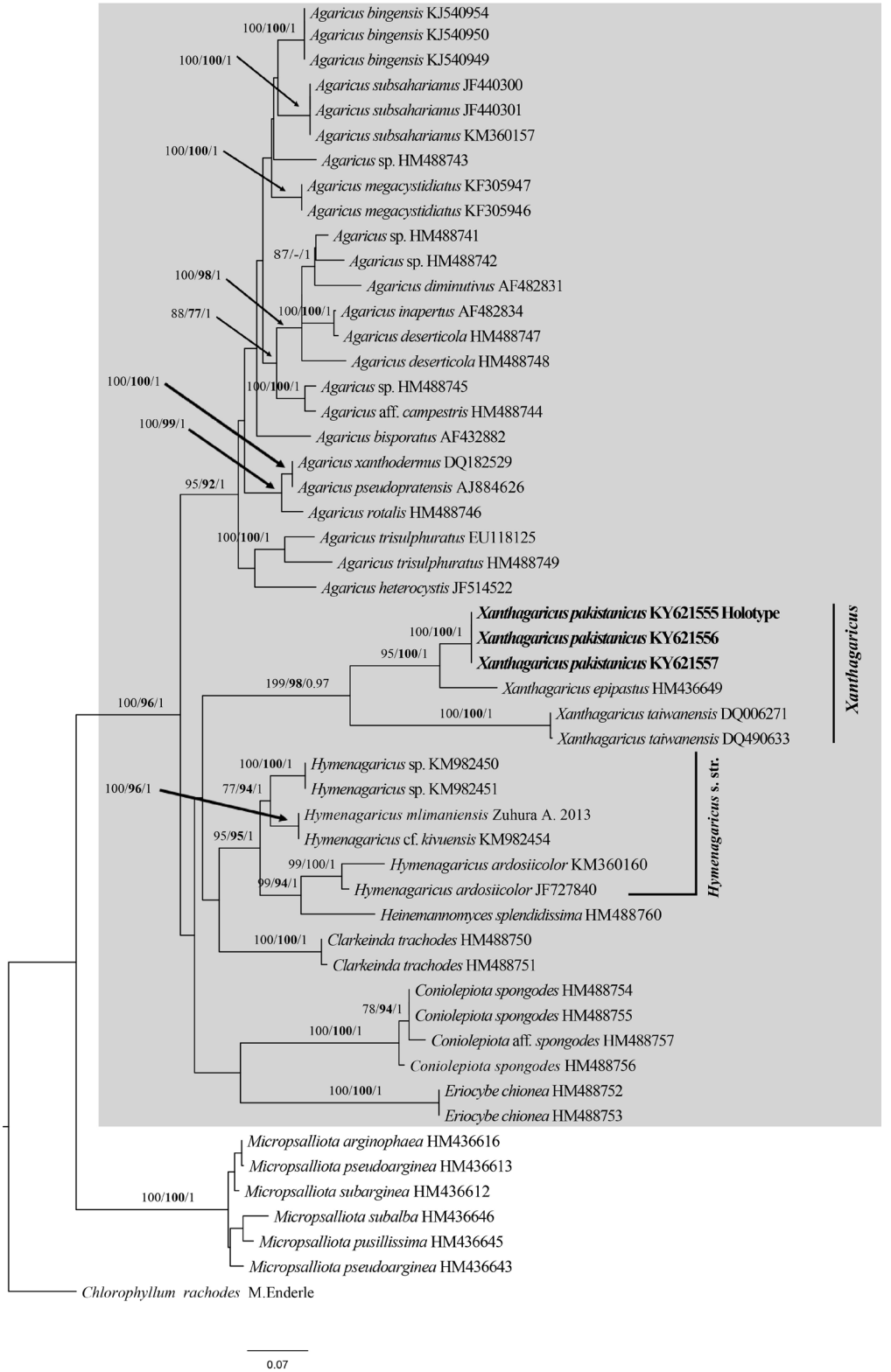

Figure 1. Phylogenetic inference of *Xanthagaricus pakistanicus* (in boldface) with other taxa of Agaricaceae inferred from 52 ITS sequences, with GenBank accessions following the species name. The *Agaricus* clade of Agaricaceae is gray highlighted. Bootstrap support values (>70% significant) above branches are the maximum likelihood bootstrap (MLB), maximum parsimony bootstrap (MPB) in boldface, and Bayesian posterior probabilities (BPP; >0.95).

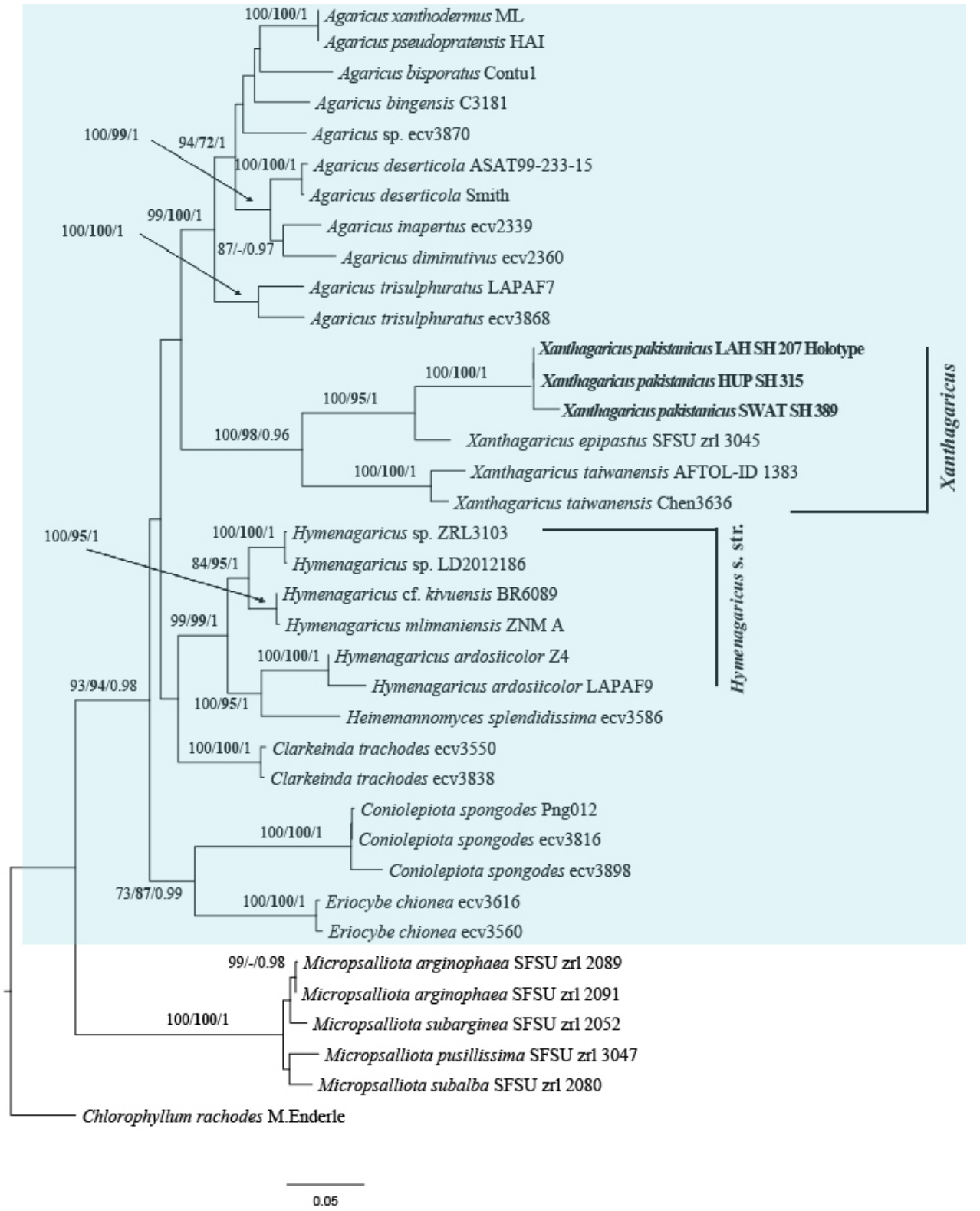

Figure 2. Maximum likelihood phylogeny of *Xanthagaricus* and allied genera based on combined ITS-28S dataset; the new species *X. pakistanicus* are represented in boldface, with voucher numbers following the species name. The *Agaricus* clade of Agaricaceae is light blue highlighted. Bootstrap support values (>70%) are presented in order MLB, MPB in boldface (based on 1000 bootstrap replicates, respectively) and Bayesian posterior probabilities (BPP) > 0.95.

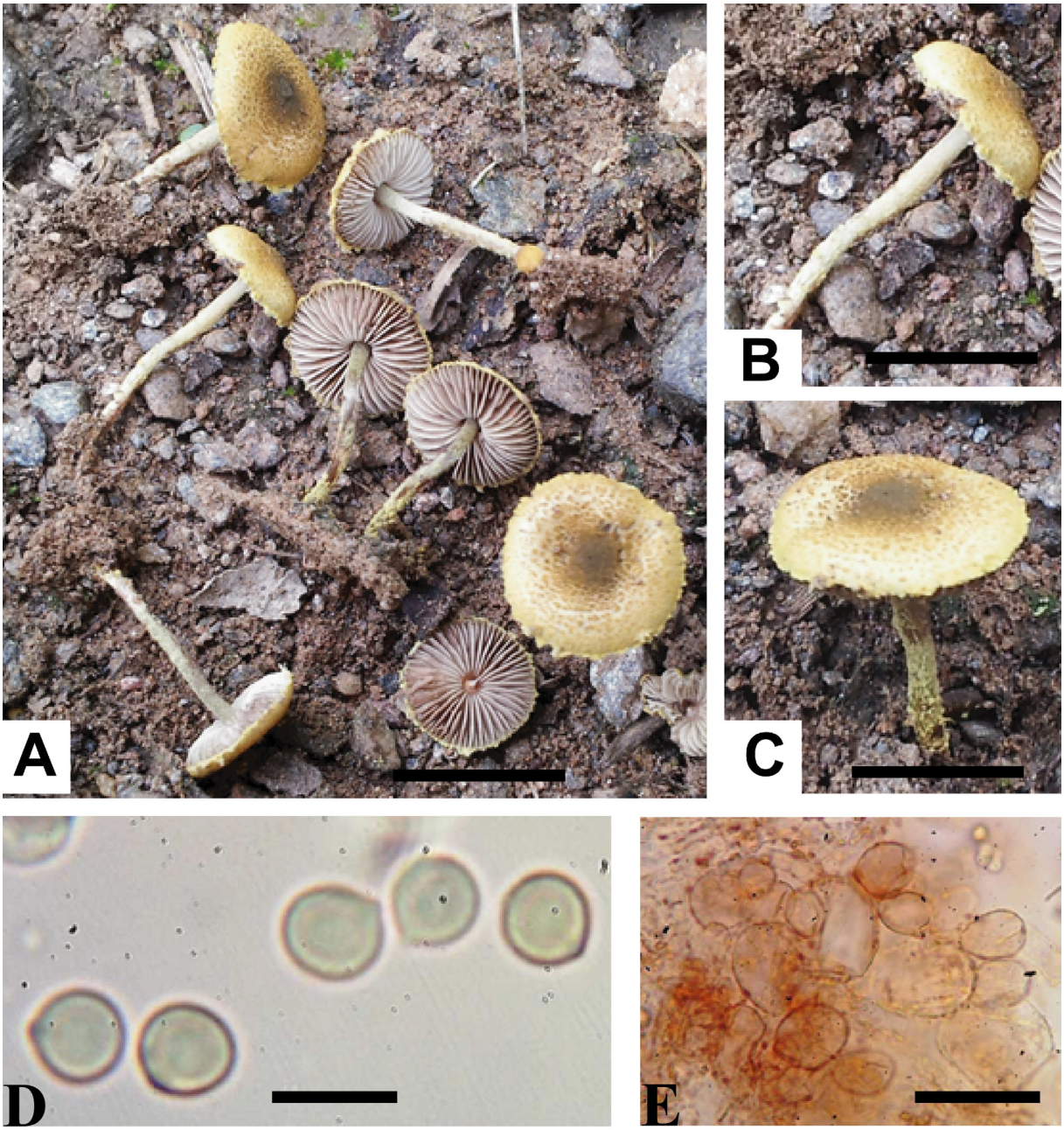

Figure 3. *Xanthagaricus pakistanicus*. A: Holotype (LAH SH 207), B: Subumbonate pileus (LAH SH 207), C: Stipe with yellowish fibrils (LAH SH 207), D: Basidiospores, E: Pileipellis. Scale bars: A–C = 20 mm, D–E = 8 µm.

3.2.2. *Xanthagaricus epipastus* (Berk. & Broome) Hussain comb. nov.

MycoBank: MB822313

Synonyms: *Hymenagaricus epipastus* (Berk. & Broome) Heinem. & Little Flower, Bull. Jard. Bot. Natn. Belg. 54(1-2): 166 (1984).

Lepiota flavidorufa (Berk. & Broome) Petch, Ann. Roy. Bot. Gard. Peradeniya 6: 310 (1917).

Stropharia epipasta (Berk. & Broome) Sacc., Syll. Fung. 5: 1018 (1887).

Basionym: *Agaricus epipastus* Berk. & Broome, Journ. Linn. Soc., Bot. 11: 553 (1871).

The type specimen of this species was described from Sri Lanka and was initially named under *Agaricus* (Berkeley and Broom, 1871). Then it was transferred to *Stropharia* by Saccardo (1887), and Petch (1917) treated

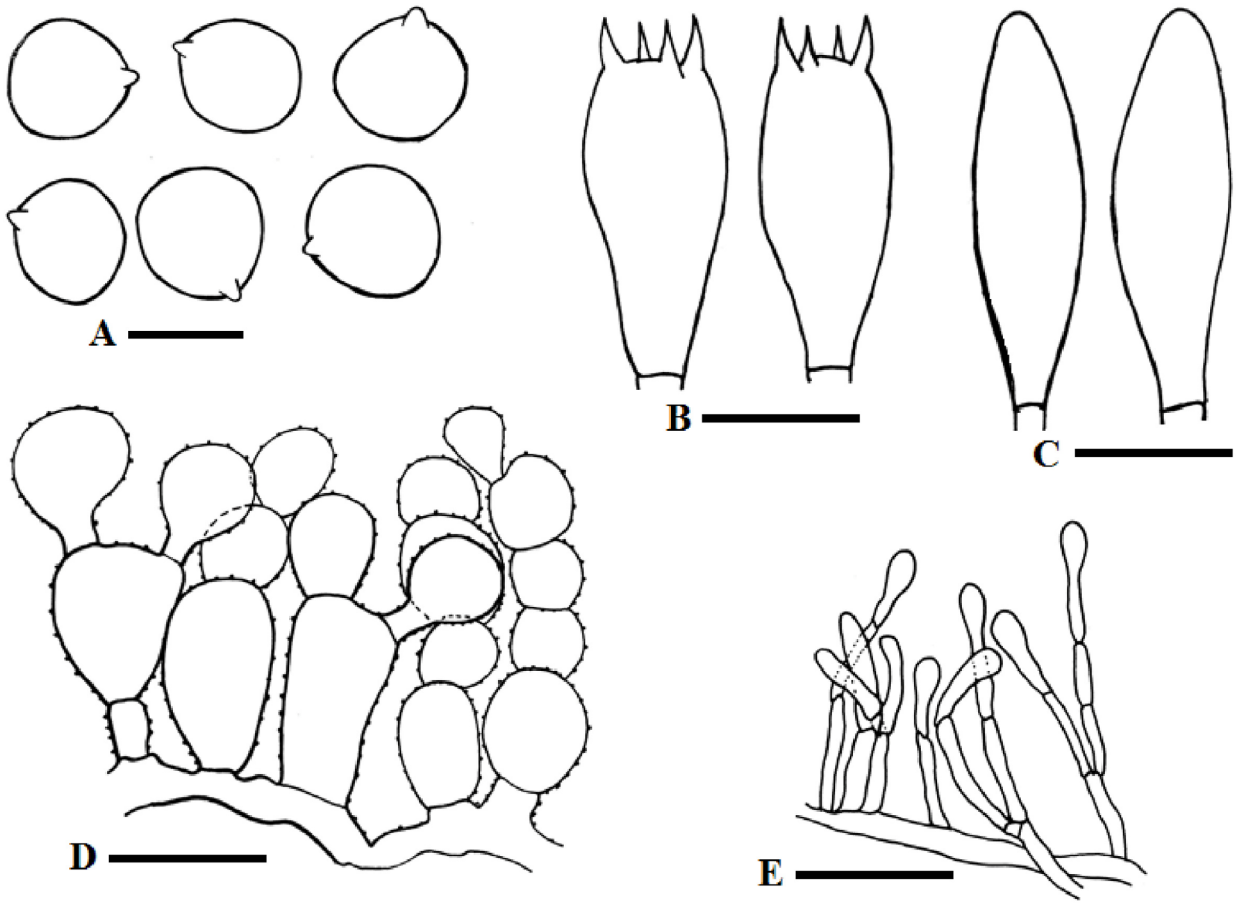

Figure 4. Anatomical features of *Xanthagaricus pakistanicus* (LAH SH 207). A: Basidiospores, B: Basidia, C: Stipe covering, D: Cheilocystidia, E: Pileal squamules. Scale bars: A = 5 µm, B–E 7 µm.

it under the genus *Lepiota*. Heinemann and Little Flower (1984) transferred this species to the genus *Hymenagaricus* subgen. *Xanthagaricus*. In our phylogenetic analyses this species clustered in the *Xanthagaricus* clade and so we proposed a new combination of *Xanthagaricus epipastus*.

3.2.3. *Xanthagaricus ochraceoluteus* (D.A.Reid & Eicker) Hussain comb. nov.

MycoBank: MB822312

Basionym: *Hymenagaricus ochraceoluteus* D.A.Reid & Eicker, S. Afr. J. Bot. 64(6): 357 (1998).

The type specimen of this species was described from South Africa. This species has the characters of *Xanthagaricus* and so we proposed the new combination under that generic name.

3.2.4. *Xanthagaricus rufomarginatus* (D.A.Reid & Eicker) Hussain comb. nov.

MycoBank: MB 822314

Basionym: *Hymenagaricus rufomarginatus* D.A.Reid & Eicker, S. Afr. J. Bot. 64(6): 357 (1998).

This species was originally described from South Africa; we proposed the new combination because this species belongs to *Xanthagaricus*.

3.2.5. *Xanthagaricus subaeruginosus* (Berk. & Broome) Hussain comb. nov.

MycoBank: MB822316

Synonyms: *Hymenagaricus subaeruginosus* (Berk. & Broome) Heinem. & Little Flower, Bull. Jard. Bot. Natn. Belg. 54(1-2): 160 (1984).

Psalliota subaeruginosa (Berk. & Broome) Petch, Ann. Roy. Bot. Gard. Peradeniya 4: 56 (1907).

Stropharia subaeruginosa (Berk. & Broome) Sacc., Syll. Fung. 5: 1013 (1887).

Basionym: *Agaricus subaeruginosus* Berk. & Broome, Journ. Linn. Soc., Bot. 11: 554 (1871).

This taxon was originally described from Sri Lanka and was placed in the genus *Agaricus* (Berkeley and Broome 1871). Later on it was transferred to *Stropharia* by Saccardo (1887). Petch (1907) transferred this species

to *Psalliota* on the basis of some greenish tints in the early stage of basidioma development and the presence of free lamellae rather than adnexed. Heinemann and Little Flower (1984) placed this species in *Hymenagaricus* subgen. *Xanthagaricus*, based on the examination of type specimens and also on the description given by Petch. We proposed a new combination for this species.

3.2.6. *Xanthagaricus taiwanensis* (Zhu L.Yang, Z.W.Ge & C.M.Chen) Hussain comb. nov.

MycoBank: MB822315

Basionym: *Hymenagaricus taiwanensis* Zhu L.Yang, Z.W.Ge & C.M.Chen, Cryptogamie Mycol 29(3): 261 (2008).

This species was originally described from Taiwan and was placed in *Hymenagaricus* subgen. *Xanthagaricus* on account of pseudoparenchymatous pileus squamules and brownish-yellow basidiospores. In our phylogenetic analyses this species was recovered in the *Xanthagaricus* clade with excellent statistical support and so we proposed the new combination.

4. Discussion

Xanthagaricus pakistanicus is characterized by dark brown pileal squamules made up of pseudoparenchymatous epithelium, with globose to circular or ellipsoid elements, having encrusted walls; and globose to broadly ellipsoid, brownish yellow basidiospores 7–7.5 × 6.5–7 µm. *Xanthagaricus epipastus* and *X. taiwanensis* are most closely related to *X. pakistanicus* among the species sampled for our phylogenetic analyses. *X. epipastus* and *X. taiwanensis* were originally described from Sri Lanka and Taiwan, respectively (Heinemann and Little Flower, 1984; Ge et al., 2008). The new species *X. pakistanicus* with globose to subglobose basidiospores can be differentiated from both *X. epipastus* and *X. taiwanensis*, which have ellipsoid spores. Based on umbonate pileus, another similar species is *X. subaeruginosus*. Type specimens of *X. subaeruginosus* were described from Sri Lanka. The pileus of *X. subaeruginosus* has some dull greenish tints in the early stage of basidioma development and the basidiospores are ovoid with a truncate base (Heinemann and Little Flower, 1984; Pegler, 1986). Similarly, *X. globisporus* (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham shared yellowish and broadly ellipsoid basidiospores with *X. pakistanicus*. However, *X. globisporus* differs from the new species by yellowish fibrillose pileal squamules, white flesh that turns red, and a dark brown stipe. Two species in the genus *Xanthagaricus* described from South Africa are *X. ochraceoluteus* differing from the new species by its bright ochre yellow pileus with yellowish background and smaller basidiospores 4.0–5.2 × 3.0–3.2 µm, and *X. rufomarginatus* with dark brown squamules on the pileus made up of a pseudoparenchymatous epithelium of angular cells and with globose to subglobose, pale yellow basidiospores (Reid and Eicker, 1998). Comparison of morphological characters of *X. pakistanicus* with regard to these and other species of *Xanthagaricus* is set out further in Table 2.

The genus *Hymenagaricus* s. l. consisted of two subgenera. i.e. subgen. *Hymenagaricus* and *Xanthagaricus*. Little Flower et al. (1997) raised the subgen. *Xanthagaricus* to the status of independent genus; unfortunately that work has been poorly cited in the taxonomy of this group of mushrooms and most of the taxonomists (Reid and Eicker, 1995, 1998, 1999; Ge et al., 2008; Tibuhwa and Mwanga, 2014) follow Heinemann and Little Flower's (1984) classification. In our phylogenetic analyses both the subgenera are monophyletic and were recovered with strong statistical support. However, *Hymenagaricus* s. l. is polyphyletic and the subgenera should be treated as different genera.

Acknowledgments

We are greatly thankful to Zuhura Mwanga (University of Dar es Salaam, Tanzania) for providing the sequence data of *Hymenagaricus mlimaneinsis*. The authors thank Dr Else C Vellinga (Department of Plant and Microbial Biology, University of California Berkeley, California, USA) for her critical review and comments during the review process. Financial support for this study was provided by the Higher Education Commission of Pakistan under the International Research Support Initiative Program (IRSIP).

Table 2. Characteristics distinguishing *Xanthagaricus pakistanicus* from the remaining species of the genus *Xanthagaricus*.

Taxa	Pileus shape and diam.	Stipe size	Basidiospores morphology	Squamules structure	References
Xanthagaricus brunneolus Little Flower, Hosag. & T.K.Abraham					Little Flower et al., 1997.
X. calicutensis (Heinem. & Little Flower) Hussain comb. nov.	40 mm diam, plane to convex, fibrillose squamules	45 × 4 mm	4.9–5.8 × 3.7–4.3 µm, Q = 1.29, ovoid, brownish yellow	Sub-hymeniform, 10–20 µm diam,	Heinemann and Little Flower, 1984.
X. chrysosporus (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	30 mm diam, plane to convex, with blistering squamules	30 × 2 mm	6.0–7.5 × 4.0–4.8 µm, Q = 1.52, ellipsoid, golden yellow	Sub-hymeniform, spherocystes 11–14 µm diam.	Heinemann and Little Flower, 1984.
X. epipastus ((Berk. & Broome) Hussain comb. nov.	20 mm diam, convex, with dark brown squamules	40 × 2 mm	3.7–4.7 × 2.8–3.4 µm, Q = 1.36, ellipsoid, yellow	Sub-hymeniform, globular or pear-shaped elements, 10–27 µm diam.	Heinemann and Little Flower, 1984.
X. erinaceus (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	15–20 mm, conical-convex, with shaggy squamules	30 × 1 mm	4.3–5.1 × 2.7–3.1 µm, Q = 1.60, ellipsoid, yellow	Hymeniform, clavate to pear shaped elements, 10–24 × 6–8 µm.	Heinemann and Little Flower, 1984.
X. flavidorufus (Berk. & Broome) Little Flower, Hosag. & T.K.Abraham	20–27 mm, convex, with umbonate disc, covered with blistery squamules	70 × 1 mm	4.4–5.4 × 3.0–3.5 µm, Q = 1.51, ellipsoid, light yellow	Vesicular elements, collapsed, 10 µm diam.	Heinemann and Little Flower, 1984; Little Flower et al., 1997.
X. globisporus (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	10 mm diam, conico-convex, yellowish fibrillose squamules	20 × 1 mm	4.6–5.1 × 4.1–4.8 µm, Q = 1.17, subglobose, yellowish	Hymeniform or pseudoparenchymatous with globular elements, 10–25 × 8–25 µm.	Heinemann and Little Flower, 1984.
X. gracilis (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	5–10 mm diam, convex to subumbonate, fibrillose squamulus	30–35 × 1 mm	5.0–6.3 × 3.5–4.2 µm, Q = 1.50, ellipsoid, deep yellow	Subhymeniform, with globular elements, 8–22 × 8–14 µm diam.	Heinemann and Little Flower, 1984.
X. luteolosporus (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	30–40 mm diam, plane to convex, with cottony squamules	50 × 4 mm	5.0–6.0 × 3.4–4.2 µm, Q = 1.43, ellipsoid with truncate base, brownish yellow	Hymeniform, subglobular to clavate elements, 7–30 µm diam.	Heinemann and Little Flower, 1984.
X. myriostictus (Berk. & Broome) Little Flower, Hosag. & T.K.Abraham	4–6 mm diam, plano-convex, with furfuraceous squamules	16 × 1 mm	3.7–4.4 × 2.6–3.0 µm, Q = 1.46, ellipsoid, pale yellow	Hymeniform to pseudoparenchymatous, globose elements, 9–20 µm diam, with encrusted walls.	Heinemann and Little Flower, 1984.
X. nanus Little Flower, Hosag. & T.K.Abraham					Little Flower et al., 1997.
X. ochraceoluteus (D.A.Reid & Eicker) Hussain, comb. nov.	9–12 mm, convex to companulate, bright ochre yellow squamules	30 × 1 mm	4.0–5.2 × 3.0–3.2 µm, ovoid to ellipsoid, yellowish	Pseudoparenchymatic tissue of angular, yellow brown cells, 5–15 µm diam.	Reid and Eicker, 1998.
X. pakistanicus sp. nov.	10–20 mm diam, hemispherical to conico-convex, with dark yellowish brown squamules	10–15 × 1–2 mm	7.0–7.5 × 6.5–7 µm, Q = 1.1, globose broadly ellipsoid, brownish yellow	Pseudoparenchymatous epithelium, with globose to circular or ellipsoid elements, terminal cells subglobose to rounded, 20–47 ×18–44 µm, with encrusted walls	Observed during this study
X. rubescens (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	10–16 mm diam, conico-convex, with fibrillose squamules	20 × 1 mm	4.1–4.9 × 2.9–3.4 µm, Q = 1.44, ellipsoid, yellowish	Pseudoparenchymatous, with globose to ellipsoid elements, 15–35 × 8–12 µm diam.	Heinemann and Little Flower, 1984.
X. rufomarginatus (D.A.Reid & Eicker) Hussain, comb. nov.	20 mm diam, companulate, with dark brown squamules	20 × 3 mm	4.0–4.2 × 2.5–3.0 µm, globose to subglobose, hyaline to pale yellow	pseudoparenchymatous epithelium with angular cells.	Reid and Eicker, 1998.
X. subaeruginosus (Berk. & Broome) Hussain, comb. nov.	45 mm diam, obtusely conical, with greenish brown squamules	40(90) × 2 mm	4.2–5.0 × 2.9–3.5 µm, Q = 1.43, ovoid with truncate base, yellow brown	Pseudoparenchymatous to hymeniform, with vesicular elements, 22–40 × 14–35 µm.	Heinemann and Little Flower, 1984.
X. subepipastus (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	10–15 mm diam, plano-convex, with greenish squamules,	15–20 × 1 mm	4.8–5.3 × 3.3–3.9 µm, Q = 1.35, ovoid to ellipsoid, pale yellow	Subhymeniform to pseudoparenchymatous, with globular to vesicular elements, 11–20 µm diam.	Heinemann and Little Flower, 1984.
X. taiwanensis (Zhu L.Yang, Z.W.Ge & C.M.Chen) Hussain, comb. nov.	15–35 mm diam, conico-compunulate to hemispherical, with dark brown squamules	40–70 × 6–9 mm	5.0–5.5 × 3.0–4.0 µm, Q = 1.46, ellipsoid, brownish yellow	Pseudoparenchymatous, with subglobose to oblong ellipsoid elements, 10–20 × 8–12 µm.	Ge et al., 2008.
X. viridulus (Heinem. & Little Flower) Little Flower, Hosag. & T.K.Abraham	5–15 mm diam, conico-compunulate, dark green squamules	10–25 × 1 mm	3.8–5.0 × 2.9–3.6 µm, Q = 1.35, globose to ovoid with truncate base, light yellowish brown	Pseudoparenchymatous to hymeniform, with vesicular elements, 22–40 × 14–35 µm.	Heinemann and Little Flower, 1984.

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