Acta Botanica Brasilica, 2022, 36: e2021abb0225
doi: 10.1590/0102-33062021abb0225

Original article

A new pruinose lichen species in genus *Lobothallia* (Megasporaceae, lichen forming Ascomycota) from Pakistan

Asma Ashraf[1*], Kamran Habib[1] and Abdul Nasir Khalid[1]

Received: July 13, 2021
Accepted: March 23, 2022

ABSTRACT

A new species in the genus *Lobothallia* (Megasporaceae, lichenized ascomycetes), from Margalla Hills, Pakistan, is described and illustrated here under the name *Lobothallia densipruinosa*. It is characterized by contiguous, clustered, densely pruinose dark olive apothecia, conidia 6-10 µm long, and large areoles and lobes. Phylogenetic analysis based on ITS-nrDNA sequencing placed our species relative to *L. pruinosa*. Descriptions and images of the new species are provided, as well as a key for the 21 species of *Lobothallia* known to the world.

Keywords: Lichenized ascomycetes, Megasporaceae, molecular systematics

Introduction

Lobothallia is a lichenized genus in the family Megasporaceae. Initially, it was introduced as a subgenus of *Aspicilia* (Clauzade & Roux 1984). Later on, Hafellner (1991) established it as a new genus within Aspiciliaceae (now Megasporaceae) to accommodate taxa with lobate thalli *i.e.*, *L. alphoplaca*, *L. melanaspis, L. praeradiosa* and *L. radiosa* (Schmitt *et al.* 2006; Nordin *et al.* 2010). However, the advance of molecular study has broadened the concept of the genus to also include non-lobate crustose species (Nordin *et al.* 2010; Kou *et al.* 2013). The delimitations of the genus given in Nordin *et al.* (2010) include taxa with immersed to appressed or constricted sessile apothecia, asci with a non-amyloid tholus (*Aspicilia*-type), unbranched paraphyses, simple, hyaline spores and bacilliform conidia.

During the last decade, nine species have been added in the genus (Indexfungorum 2021). The genus is represented by twenty species worldwide. From Pakistan, three species have been reported so far *i.e.*, *L. alphoplaca*, *L. praeradiosa* and *L. radiosa* from KP and AJ&K (Ahmad 1965; Aptroot & Iqbal 2012).

During the investigation of the material collected from Margalla Hills, Pakistan, the authors found samples of genus *Lobothallia* with distinct morpho-anatomy that did not fit with any of the previously described species of this genus, designated here as *Lobothallia densipruinosa* sp. nov. We present a brief diagnosis, description, images, and phylogeny based on ITS sequence data as well as the key to all known species of the genus.

1 Fungal Biology and Systematics Research Lab, Institute of Botany, University of the Punjab, 54590, Lahore, Pakistan

* Corresponding author: asmakamran9@gmail.com

Material and methods

Morphological and Chemical Studies

The core material for this study was collected by the authors after obtaining permission from the Forest Department of the study site and deposited in LAH Herbarium, Institute of Botany, University of the Punjab, Lahore, viz; voucher numbers LAH 36790, LAH 36791, LAH 36949, LAH 36950 and LAH 36951. Morphological characters were observed under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan). For anatomical analyses, free hand sections of the apothecia were cut, mounted on glass slides using water and 5 % KOH as mounting media (K), 10 % water and examined under compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan). The secondary chemistry was analyzed using spot tests with potassium hydroxide KOH (10 %) and calcium hypochlorite C-test reagents and thin layer chromatography (with solvents C) according to the method proposed by Orange *et al.* (2001).

Molecular Characterization

Genomic DNA was isolated from dried specimens using the 2 % CTAB protocol (Gardes & Bruns 1993). The ITS region (Internal Transcribed Spacer of the nrDNA) was amplified using the ITS1F/ITS4 primer pair following the amplification protocol of Khan *et al.* (2018). The amplified DNA fragments were observed in 1.2 % agarose gel (Sambrook & Russell 2001) and PCR products were sequenced.

For phylogenetic analysis, nucleotide sequence comparison was performed using the Basic Local Alignment Search Tool (BLAST) network service of the National Centre for Biotechnology Information (NCBI) (Altschul *et al.* 1990). Closest matching sequences and those used in Paukov *et*

Table 1. Specimens used in the phylogenetic analyses. New sequences are in bold.

Species	Origin	Voucher	GenBank No
Lobothallia alphoplaca	China	Tong 20117616 (SDNU)	JX499233
L. alphoplaca	China	Wang 20117646 (SDNU)	JX476025
L. alphoplaca	Norway	Klepsland O-L-200411	MK812484
L. alphoplaca	Ukraine	SK A20 [Nadeina *et al.* (ex KW-L 68283)]	KT456207
L. brachyloba	Russia	Frolov 357 (UFU)	MK347506
L. crassimarginata	China	Tong 20122583 (SDNU)	KC007439
L. crassimarginata	China	SDNU 20122565	NR154116
L. crassimarginata	China	SDNU 20122565	JX476026
L. densipruinosa	**Pakistan**	LAH 36790	MZ871507
L. densipruinosa	**Pakistan**	LAH 36791	MZ871511
L. densipruinosa	**Pakistan**	LAH 36949	MZ871512
L. densipruinosa	**Pakistan**	LAH 36950	MZ871514
L. densipruinosa	**Pakistan**	LAH 36951	MZ871515
L. epiadelpha	Russia	UFU L-3189	MK347505
L. helanensis	China	Tong20122791(SDNU)	JX476031
L. helanensis	China	Tong 20122517(SDNU)	JX476030
L. melanaspis	Norway	Owe-Larsson 8943a (UPS)	JF825524
L. melanaspis	Sweden	Nordin 6622 (UPS)	HQ259272
L. praeradiosa	Russia	L-1264 (UFU)	MK347501
L. praeradiosa	China	XJU 20070730	KT180162
L. praeradiosa	China	SDNU 20126314	JX499232
L. praeradiosa	China	XJU 201007071	KT180160
L. praeradiosa	China	SDNU 20126355	JX499230
L. pruinose	China	SDNU:20123278	NR154117
L. pruinose	China	SDNU 20123630	JX476027
L. pruinose	China	SDNU 20123278	JX476028
L. radiosa	Sweden	Nordin 5889 (UPS)	JF703124
L. radiosa	Greece	ALV19489j558	MN989283
L. radiosa	Greece	ALV19481j550	MN172452
L. recedens	Portugal	ALV8368	MN586980
L. recedens	Sweden	Nordin 6035 (UPS)	HQ406807
L. semisterilis	China	18-59345(KUN-L)	MK778042
L. semisterilis	China	18-59322(KUN-L)	MK778039
L. semisterilis	China	18-59262 (KUN-L)	MK778040
L. subdiffracta	Russia	Frolov 178-1 (UFU)	MK347503
Megaspora verrucosa	Svalbard	ZT2013052	KP314333

al. (2019) were downloaded from GenBank for subsequent phylogenetic analysis (Tab. 1). Multiple sequence alignment was performed using program MAFFT v7 with all parameters set to default values (Katoh & Standley 2013). The beginning and end of alignments were trimmed at a conserved site. Gaps were treated as missing data. Phylogenetic analysis was performed in MEGA X (Kumar *et al.* 2018) by constructing Maximum Likelihood (ML) trees at 1000 Bootstraps based on Kimura 2 model. *Megaspora verrucosa* (Ach.) Arcadia & A. Nordin (KP314333) was chosen as an outgroup. Information on the samples together with the GenBank Accession numbers are given in Table 1.

Results

Phylogenetic Analysis

The new ITS nrDNA sequences are nested within the phylogenetic branch of the genus *Lobothallia*, representing the species unknown yet, described here as *Lobothallia densipruinosa* sp. nov. Altogether 36 ITS rDNA sequences were analyzed, including 31 obtained from the GenBank. There were 527 characters in the alignment file of which 314 were conserved, 199 variables, 143 parsimony informative and 54 were singleton variants.

The topology of the tree is almost similar to that in Paukov *et al.* (2019). In the ITS phylogram *Lobothallia densipruinosa* sp. nov. formed a sister clade with *L. pruinosa* with strong support (99 % BS). The analysis represents the independent position of the Pakistani taxon.

Taxonomy

Lobothallia densipruinosa A. Ashraf, K. Habib & Khalid sp. nov.

MycoBank No: 840981

Etymology: The epithet refers to the discs of apothecia densely covered by a white pruina.

Diagnosis: Differs from *L. pruinosa* in having large areoles and lobes, contiguous clustered apothecia, dark olive to blackish disc covered with a dense white pruina, and larger conidia.

Material examined: PAKISTAN. Islamabad, Margalla hills, on rocks, 33.7439° N, 73.0228° E, April 04, 2020, A. Ashraf, K. Habib, MH-100. Holotype-LAH 36790, MH-111. Isotype-LAH 36800.

Description: Thallus: crustose, placodioid, areolate, up to 1.0 mm thick, 2 to 3 cm across, lobate, tightly attached to substratum; Areoles: 0.5-2 mm across, contiguous to rarely discrete, angular, irregular, plane to uneven, rim whitish; Lobes: radiating, thinning towards margins, contiguous, plane to uneven, confluent, 1-3 mm long, 1-2 mm wide; Upper surface: whitish to light greenish gray, pruinose, dull; Cortex: 20-40 µm thick, brown above, hyaline at the inner part, paraplectenchymatous, epinecral layer 10-18 µm thick; Algal layer: uneven, discontinuous, 60-150 µm thick, photobiont chlorococoid, cells globose to subglobose, 10-20 µm in diam. Apothecia: frequent, grouped, contiguous, elevated; Disc: plane, densely covered by white pruina, olivaceous-black, when dry, olivaceous when wet, initially rounded, becoming irregular or angular with age, up to 1.4 mm in diam.; Margin: continuous, prominent, up to 0.2 mm thick, concolorous with thallus; Epihymenium: brown to dark brown, 10-22 µm thick; Hymenium: hyaline, 90-125 µm tall, sometimes containing algal groups; Hypothecium: hyaline, 30-65 µm, with algal groups or continuous algal layer present below the hypothecium; Paraphyses: anastomosing 2-4 µm wide, moniliform, apical cell 4-6 µm wide; Asci: clavate, 8-spored, 60-85 × 20-30 µm; Ascospores: globose to broadly ellipsoid, simple, 10-15 × 7-11 µm; Conidia: bacilliform, 6-10 × 0.8-1.4 µm (Fig. 2).

Spot tests: Cortex: K+ yellow turns red, C-, KC-; Medulla: K+ yellow, C-, KC-. TLC: Norstictic acid

Ecology: Found in humid sub-tropical scrub forest, fully exposed to sunlight and rain, having mean max. 34.3 °C and min. 3.4 °C temperature with average rainfall 1200 mm per year.

Additional Material Examined (Paratype): PAKISTAN. Murree, on rocks, 33.9070° N, 73.3943° E, September 07, 2020, K. Habib, A. Ashraf, MH-168, MH-106-3, MH-44 Paratype-LAH 36791.

Taxonomic Remarks

Our species is morphologically similar to *L. pruinosa* from China, as both species have distinct lobate, closely adnate, pruinose thallus and a pruinose disc of apothecia. Phylogenetically, *L. pruinosa* and *L. densipruinosa* are found to be strongly supported relative taxa (99 % BS) (Fig. 1). The two species can be easily segregated by the character of apothecia. The apothecia of the Pakistani taxon are appressed and grouped into the contiguous clusters, having dark olivaceous disc when wet, blackish when dry, and covered with a white dense pruina, while the apothecia of the Chinese species are solitary, very slightly projected, with usually pruinose dark brown disc. The differences between both species also include the size of lobes (1-3 mm long, 1-2 mm wide), areoles (0.5-2 mm wide) and conidia (6-10 × 0.8-1.4 µm) which are larger in *L. densipruinosa*, compared to lobes 1-2 mm long, 0.7-1 mm wide, areoles 0.5-1 mm wide and conidia 5-7 × 1–1.3 µm in *L. pruinosa*.

Morphologically, the new species can be easily distinguished from the other species of genus with whitish or whitish grey thallus *i.e*: *L. subdiffracta, L. controversa*, *L. cernohorskyana*, *L. chadefaudiana, L. lacteola,* and *L. cheresina*, by its well-developed marginal lobes, elevated clustered apothecia covered by a white dense pruina and having dark olivaceous disc.

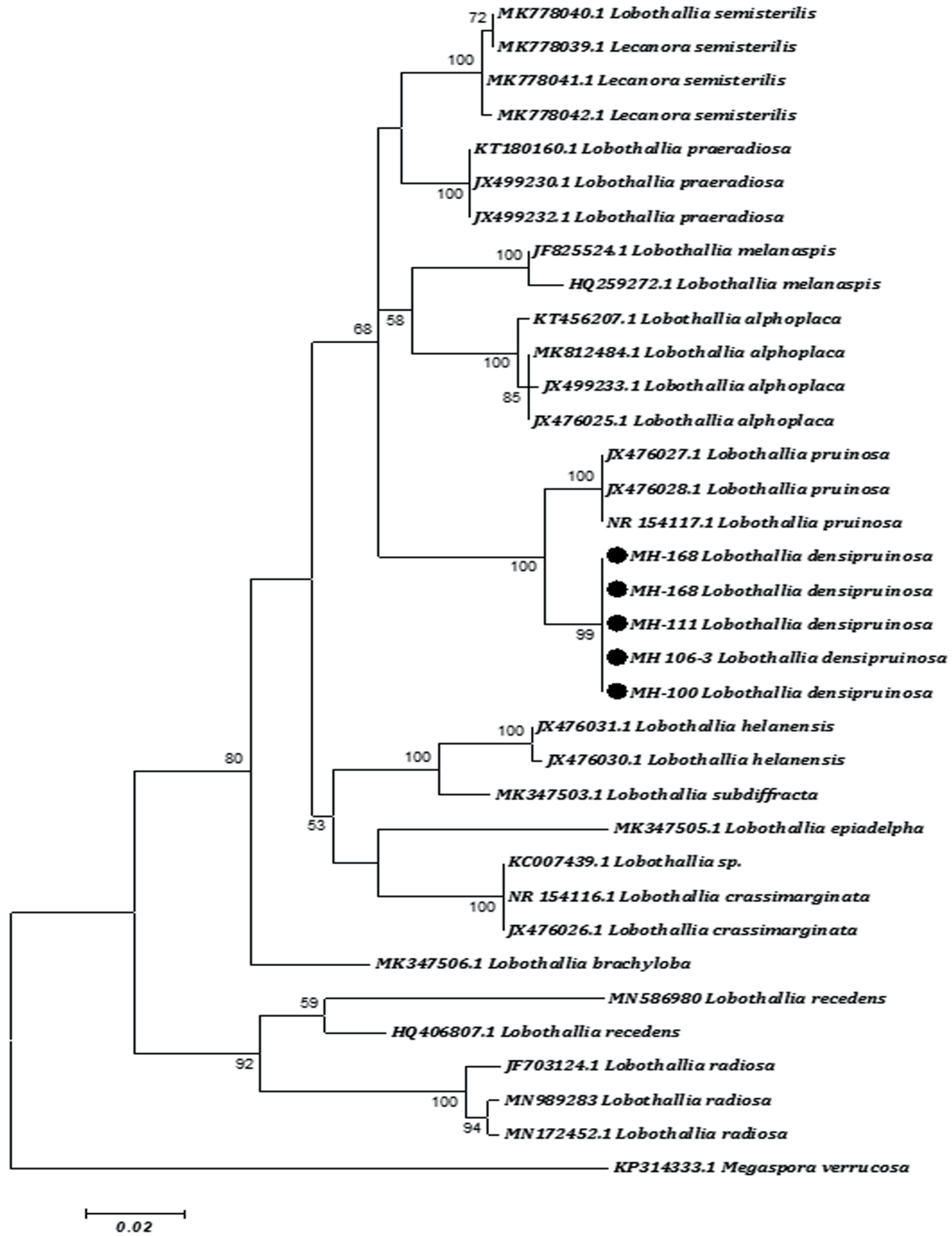

Figure 1. The Maximum Likelihood tree of newly described *Lobothallia densipruinosa* and related taxa (based on ITS sequences). Numbers on the branch node represent ML bootstrap (≥ 50) based on 1000 replicates. Sequences generated from the Pakistan collection are marked with black circle.

Key to species of ***Lobothallia***

Updated key to species of genus *Lobothallia* after Paukov *et al.* (2019).

1 Marginal lobes absent or indistinct; thalli may have incised, plicate margins or tiny, infrequent and irregular lobules. In radial specimens the 'lobes' are formed by cracking of the peripheral zone by splits moving from the central parts of thalli 2
Marginal lobes constant and well developed. In closely adnate specimens the splits originate mostly from the outside of the thalli 10

2 (1) On calcareous rocks. Thallus off-white, partly with yellowish or greyish tinge 3
On siliceous rocks. Thallus light grey, dark grey, olive grey to brownish 9
On soil in dry habitats. Thallus white to grey, instead of olive grey to brownish Norstictic acid. China ..... ***Lobothallia semisterilis***

3 (2) Thallus ±continuous with granules on the surface or consisting of areoles with multiple cracks and having a granular appearance. Norstictic acid or no lichen substances 4
Thallus rimose-areolate to distinctly areolate at least in the central part. Areoles not granular, with smooth or farinose upper surface. Terpenes, norstictic, stictic acid or no lichen substances 5

4 (3) Lacks lichen substances, K–. Thallus thick, up to 1·5 mm, continuous to rimose, with rough yellowish granules on the surface. Apothecia immersed, separated from the thallus by thin cracks ..... ***Lobothallia chadefaudiana***
Contains norstictic acid, K+ red. Thallus up to 0·5mmthick, rimose to areolate; areoles have a granular appearance. Apothecia appressed, becoming constricted at the bases with time ..... ***Lobothallia cernohorskyana***

5 (3) Thallus contains terpenes and ±norstictic or stictic acid, 1-4 mm thick. Apothecia without visible thalline margin ..... ***Lobothallia controversa***
Thallus contains no terpenes 6

6 (5) Thallus off-white, thick, 0·5–2·0 mm, superficial, with ± farinose, continuous or finely cracked, plicate margins, lacking long and straight radial cracks. Thalline margin finally prominent. Contains norstictic and ±connorstictic acids ..... ***Lobothallia lacteola***
Thallus whitish, commonly with grey tinge, thin to thick, sometimes semi-immersed, not farinose, with definite cracks up to the margins. Orbicular specimens usually have long, straight, open, radially arranged cracks. Lacking prominent thalline margin. Secondary metabolites absent or thallus contains stictic/norstictic acid 7

7 (6) Contains no detectable secondary metabolites ..... ***Lobothallia cheresina*** chemotype ***cheresina***
Norstictic or stictic acid present 8

8 (7) Stictic acid as a main secondary metabolite ..... ***Lobothallia cheresina*** chemotype ***justii***
Norstictic acid as a main secondary metabolite ..... ***Lobothallia cheresina*** chemotype ***microspore***

9 (2) Thallus areolate, light to dark grey to brownish, areoles often completely obscured by apothecia. Thalline margin of apothecia smooth, projecting, later receding. Disc brownish, 0.3–1 mm in diam. Chemistry without lichen substances. Europe, Caucasus ..... ***Lobothallia recedens***
Thallus areolate, slightly brownish grey or cacao grey, 2–5 apothecia per areole. Distinct thalline margin when uplifted, not receding. Disc blackish brown, 0.3–0.5 mm in diam. Chemistry Norstictic acid. Korea ..... ***Lobothallia gangwondoana***
Thallus squamulose, light to dark grey to olive grey, pruinose. Squamules with deep cracks, outer squamules usually slightly enlarged. Thalline margin incised. Disc blackish. China (Gansu, Inner Mongolia); Russia (Altai), Mongolia ..... ***Lobothallia subdiffracta***

10 (1) Thallus with stictic acid as a main lichen substance 11
Thallus with norstictic acid as a main lichen substance or secondary metabolites absent 13

11 (10) Thallus light grey to dark or brownish grey, growing on non-calciphilous Aspicilia species, closely attached to the host or occasionally not parasitic. Apothecia immersed or slightly projecting, up to 0·6 mm, abundant and crowded in the central parts of thalli. Mediterranean ..... ***Lobothallia radiosa*** chemotype ***parasitica***
Thallus olive brownish to shades of brown, without hints of grey, free-living or parasitic in the early stages of development. Mature apothecia larger than 0·8 mm, sessile, constricted at the base, not crowded. South Urals (Russia), Kazakhstan, Xinjiang Autonomous Region of Chin 12

12 (11) Young thalli parasitic on Circinaria maculata, later free-living. Lobes of non-parasitic thalli loosely attached, overlapping, smooth or with unclear cracks. Apothecia with dark brown flat discs. Thalline margin permanent, thick ..... ***Lobothallia epiadelpha***
Thalli free-living, lobes firmly attached to the substratum, not overlapping, with definite deep cracks forming a reticulate pattern in exposed habitats. Apothecia with brownish black to jet black, finally convex discs. Thalline margin receding in mature apothecia ..... ***Lobothallia zogtii***

13 (10) Thalli entirely or partly loosely attached to the substratum or at least outer 1–4mm of lobes not adherent. Lobes ±overlapping. Mature apothecia sessile with constricted base 14
Thalli closely adnate to the substratum almost up to the margins, lobes not overlapping. Mature apothecia immersed to projecting (but check *Lobothallia radiosa* in couplets 19 and 21) 16

14 (13) Thallus lacks secondary metabolites. Lobes dark grey, greenish when wet, repeatedly branching. In wet habitats ..... ***Lobothallia melanaspis***
Thallus with norstictic acid. In dry habitats 15

15 (14) Thalli loosely attached to the substratum, separate lobes or even whole thalli can be detached almost intact (less evident in younger specimens). Lobes strongly convex to almost cylindrical, whitish grey, rarely with light shades of brown, side margins of lobes never arranged in parallel. Central 'areoles' bullate with strongly swollen tips and constricted bases ..... ***Lobothallia alphoplaca***
Thalli normally closely adnate to the substratum with only outer 1–4 mm of lobes not adherent (but specimens overgrowing lichens/mosses or older parts of thalli may strongly resemble the previous species as these areas are easily detachable). Lobes flat to moderately convex, grey to distinctly brownish, often with side margins arranged in parallel in the closely adnate parts. Central 'areoles' flat to moderately convex or uneven, not bullate ..... ***Lobothallia praeradiosa***

16 (13) On inundated rocks in summer-dry creeks. Thallus lead or bluish grey, without pruina. Areoles angular, apothecia dark, 1–6 on areole, 0·5–1 mm diam., immersed, immarginate or rarely with indistinct dark margins. Sardinia ..... ***Lobothallia hydrocharis***
In dry, not inundated habitats. Thalli variously coloured, grey, whitish, brownish, often pruinose. Apothecia with margins, if immarginate, less than 0·6 mm diam. Distribution various 17

17 (16) Apothecia immersed to slightly projecting, normally less than 0·6 mm 18
Mature apothecia projecting to sessile (if slightly projecting, then more than 0·6 mm) 20

18 (17) Thallus brown, white-pruinose. Lobes strongly convex, simple to dichotomous, with ±straight and parallel margins. Apothecia 1–2 on areole. Contains norstictic acid. China (Gansu) ..... ***Lobothallia hedinii***
Thallus light to dark grey, whitish, rarely brownish, pruinose or not. Lobes flat, apothecia 1–7 on areole. Contains norstictic acid or no secondary metabolites 19

19 (18) Thallus light grey, epruinose, contains norstictic acid. Apothecia immersed, circular, without margins, always smaller than the areole. Central areoles with ±smooth surface ..... ***Lobothallia brachyloba***
Thallus from chalky white to dark grey with brownish tint, pruinose when growing on limestone, without (chemotype radiosa) orwith(chemotype subcircinata) norstictic acid. Apothecia immersed to slightly projecting, finally with visible margins, crowded on central areoles and angular. Central areoles with uneven surface and crossed by depressions or cracks which finally divide them into smaller units and, in turn, have single apothecia ..... ***Lobothallia radiosa***

20 (17) Lobes strongly convex, short, 1–2 mm, dichotomous or simple, grey, nonpruinose. Apothecia with blackish discs and thick, permanent thalline margin (thickness equal to or more than the radius of the disc). Norstictic acid ..... ***Lobothallia crassimarginata***
Lobes flat or slightly convex, whitish, greyish to brownish or brownish grey, often pruinose. Apothecia single or crowded, with narrower margins 21

21 (20) Lobes flat, grey to brownish, short, 1–2mmlong, 0·7–1mmwide. Pruina on the margins of areoles or covering the whole surface and discs of apothecia. Apothecia solitary, dark brown, slightly projecting, discs 0·7–1·2 mm, Conidia 5–7 µm. Norstictic acid. China ..... ***Lobothallia pruinosa***
Lobes flat, whitish to light greenish gray, 1–3 mm long, 1–2 mm wide. Pruina present on the whole surface covering discs of apothecia. Apothecia grouped, dark olivaceous disc when wet, blackish when dry, elevated, disc up to 1·4 mm. Conidia 6–10 µm long. Norstictic acid. Pakistan ..... ***Lobothallia densipruinosa***

Lobes flat to moderately convex, chalky white to brownish grey, 3–5mm long, 0·5– 1·5 mm wide, with or without pruina. Apothecia initially crowded and angular, extended period immersed, later projecting to broadly sessile 0·5–1·5 mm. No lichen substances or norstictic acid. Widespread ..... ***Lobothallia radiosa***, see also couplet 19

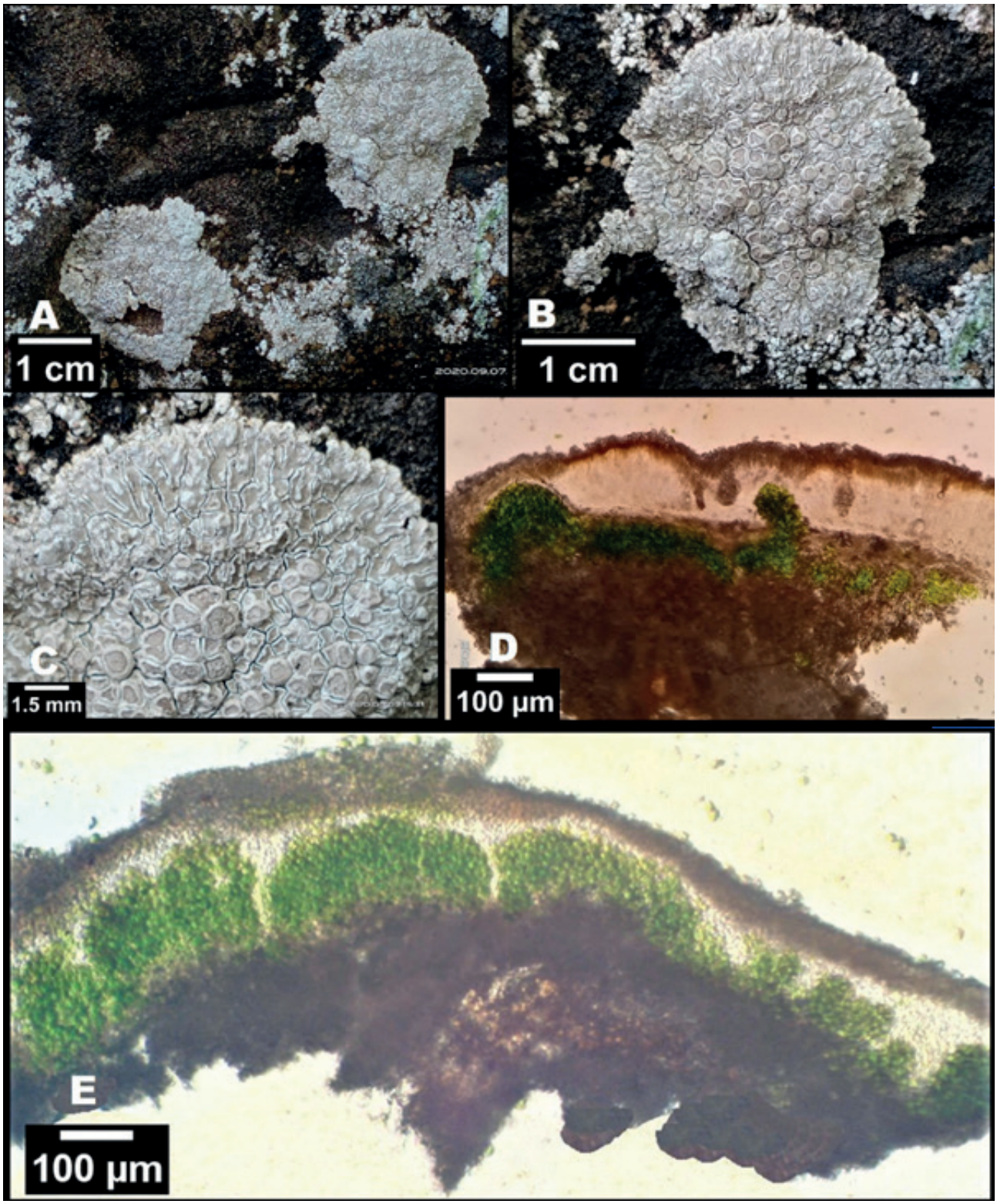

Figure 2. A-E: *Lobothallia densipruinosa* sp. nov. **A-B**: Thallus view (Holotype), **C**: closer view of lobes and apothecia, **D**: Section of apothecium, **E**: Section of thallus.

Acknowledgment

We are grateful to Prof. Dr. Alexander Paukov (Department of Biodiversity and Bioecology, Institute of Natural Sciences and Mathematics, Ural Federal University, Ekaterinburg, Russian Federation) for formal identification, valuable comments and suggestions that greatly improved the manuscript.

References

Ahmad S. 1965. A preliminary contribution to the lichen-flora of West Pakistan. Biologia 11: 21-47.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic Local Alignment Search Tool. Journal of Molecular Biology. 215 (3): 403-10.

Aptroot A, Iqbal SH. 2012. Annotated checklist of the lichens of Pakistan, with reports of new records. Herzogia 25 (2): 211-229.

Clauzade G, Roux C. 1984. Les genres Aspicilia Massal. et Bellemerea Hafellner et Roux. Bulletin de la Société Botanique de Centre-Ouest, Nouvelle Série 15: 127-141.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Hafellner J. 1991. Die Gattung Aspicilia, ihre Ableitungen nebst Bemerkungen über cryptolecanorine Ascocarporganisation bei anderen Genera der Lecanorales (Ascomycetes lichenisati). Acta Botánica Malacitana 16: 133–140.

Indexfungorum. 2021. http://www.indexfungorum.org/names/Names.asp. 01 Mar 2021.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software Version 7: improvement in performance and usability. Molecular Biology and Evolution 30: 772-780.

Khan M, Khalid AN, Lumbsch HT. 2018. A new species of *Lecidea* (Lecanorales, Ascomycota) from Pakistan. MycoKeys (38): 25-34.

Kou XR, Li SX, Ren Q. 2013. Three new species and one new record of *Lobothallia* from China. Mycotaxon 123: 241-249.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35 (6): 1547-1549.

Nordin A, Savić S, Tibell L. 2010. Phylogeny and taxonomy of *Aspicilia* and Megasporaceae. Mycologia 102: 1339-1349.

Orange A, James P, White FJ. 2001. Microchemical Methods for the Identification of Lichens. 1st ed. London, British Lichen Society.

Paukov AG, Davydov EA, Nordin A, et al. 2019. Three new species, new combinations and a key to known species of *Lobothallia* (Megasporaceae). Lichenologist 51(4): 301-22.

Sambrook J, Russell DW. 2001. Detection of DNA in agarose gels. Molecular Cloning, A Laboratory Manual, (3rd Ed.) Cold Spring Harbor Laboratory Press, New York. 5-14.

Schmitt I, Yamamoto Y, Lumbsch HT. 2006. Phylogeny of Pertusariales (Ascomycotina): resurrection of Ochrolechiaceae and new circumscription of Megasporaceae. Journal of the Hattori Botanical Laboratory 100: 753-764.