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A new species in the genus *Circinaria* (Lichenized Ascomycetes: Megasporaceae) from Pakistan

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Abstract

A crustose species *Circinaria semicontorta* is described here as new to science. It is characterized by a whitish areolate to subverrucose thallus with a thinning, cracked margin, not forming true lobes, areoles with raised whitish pseudocyphellae containing black ostioles of conidiomata, and long pycnoconidia, 12–25 µm. The phylogenetic analysis based on ITS sequences confirms placing the new species within *Circinaria*.

Key words: Dassu, Khyber Pakhtunkhwa, Kohistan, Swat, phylogeny, taxonomy

Introduction

Circinaria is a genus in Megasporaceae (Pertusariales) resurrected by Nordin *et al*. (2007, 2010). It is the second largest genus in the family with currently 44 accepted species worldwide (http://www.indexfungorum.org/). The expected number of species in *Circinaria* is considerably higher, partly because some taxa, if examined with molecular tools, will likely still need to be transferred from *Aspicilia* (Nordin *et al.* 2010, Roux *et al.* 2016, Ren & Zhang 2018, Paukov *et al.* 2019) and based on preliminary reviews of morphological, anatomical, and molecular data new species remain to be discovered (Owe-Larsson *et al.* 2011, Ismayil *et al.* 2019, McCarthy & Elix 2020).

Circinaria is characterized by large (20–39 µm long), broadly ellipsoid to globose, usually uniseriate spores, 1–4 per ascus. Few species have 6 to 8 spores per ascus arranged in two rows (Magnusson 1939, Foucard 2001). Aspicilin is the secondary lichen metabolite known in some species of *Circinaria* only. Similarly, albeit characteristic not for all taxa, pseudocyphellae are known within Megasporaceae solely in *Circinaria* (Nordin *et al.* 2010). The genus has a high morphological diversity including crustose, subsquamulose, subfoliose, fruticose, and vagrant thalli (Sohrabi *et al.* 2013). This diversity of life forms was attributed to ontogenetic stages (Elenkin 1901), ecological modifications (Kunkel 1980) or different species (Mereschkowsky 1911). Molecular studies proved close relationships between vagrant and erratic taxa showing an increasing complexity of morphological structure in different branches of phylogenetic trees (Owe-Larsson *et al.* 2011).

Only two species of the genus, viz. *Circinaria contorta* (Hoffm.) A. Nordin, Savić & Tibell (2010: 1341) and *C. caesiocinerea* (Nyl. ex Malbr.) A. Nordin, Savić & Tibell (2010: 1341); have previously been reported from Pakistan (Aptroot & Iqbal 2012). A third species, *Circinaria thorstenii* R. Zulfiqar & Khalid (2023: 4) was described only recently (Zulfiqar & Khalid 2023). During our investigation of lichen diversity of Pakistan, a new crustose *Circinaria* was found. It is here described as *Circinaria semicontorta* R. Zulfiqar, H.S. Asghar, K. Habib & Khalid *sp. nov.* and we show its position in the phylogenetic tree of the genus.

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Materials and methods

Specimen Collection, Morphological and Chemical Characterization

Specimens were collected during surveys of different sites across Pakistan, including sites in the Kohistan District, Azad Jammu and Kashmir and Swat, they are deposited in LAH, at the Institute of Botany of the University of the Punjab, Lahore. Duplicates were sent to the herbarium of the Iranian Research Organization for Science and Technology (ICH).

Thalli were examined micro- and macroscopically with a Meiji Techno EMZ-5TR stereomicroscope and a Swift M4000-D compound microscope. Anatomical observations were carried out by hand-cut sections of the thallus and apothecia, mounted in water. Ascospore measurements were taken at 100 × magnification. Secondary chemistry was analyzed using spot tests and thin-layer chromatography using Solvent System C following Orange *et al.* (2001).

DNA extraction, PCR amplification and sequencing

DNA was extracted directly from portions of thalli with apothecia using a modified 2% CTAB method (Gardes & Bruns 1993). Extracted DNA was used for PCR amplification of the ITS nrDNA marker. The ITS region was amplified using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). The amplified DNA fragments (PCR product) were visualized in 1% agarose gel using an ethidium bromide through a gel documentation system (Sambrook & Russel 2001). The amplified fragments were then sequenced by TSINGKE Biotechnology Co., Ltd. (China).

Phylogenetic Analysis

Forward and reverse sequences of ITS regions were obtained and a final sequence of each specimen was assembled using BioEdit v. 7.2.5 (Hall 1999) and matched with other online DNA sequences available through BLAST at NCBI server (https://www.ncbi.nlm.nih.gov/guide/) (Altschul *et al.* 1990).

Altogether, 11 new nrITS sequences were generated for this study. Voucher information is provided in Table 1. *Megaspora cretacea* Gasparyan, Zakeri & Aptroot (2016: 248) was chosen as an outgroup for rooting the phylogenetic tree. The data matrix was aligned in MAFFT, version 7 (https://mafft.cbrc.jp/alignment/server/), using progressive G-INS-1 method (Katoh *et al.* 2019) and manually corrected in Bioedit v. 7.2.5 (Hall 1999). The optimal substitution model was inferred using the Modeltest algorithm (Darriba *et al.* 2020), implemented in raxmlGUI 2.0 (Kozlov *et al.* 2019, Edler *et al.* 2021). General time reversible (GTR+G+I) was selected as the optimal model. Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget & Simon 1999) was performed using Beast 2.6.6 (Bouckaert *et al.* 2019). The chain length was defined using ESS values in Tracer 1.7.2 (Rambaut *et al.* 2018). Two independent runs of BEAST were made with a chain length of 7,000,000 and every 1,000th generation was recorded. Tree files from two independent runs were combined in LogCombiner 2.6.6, a part of the BEAST 2 package (http://beast2.cs.auckland.ac.nz/). A maximum clade credibility tree with mean node heights was inferred with a 25% burn-in fraction and posterior probability of 0.5. Tree files were visualized with FigTree v.1.4.2 (Rambaut 2012). The final Maximum Likelihood phylogram and 1,000 rapid bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis 2014), implemented in raxmlGUI 2.0 (Edler *et al.* 2021), with the GTRGAMMA+I model of substitution. The tree topology is taken from Maximum Likelihood inference. Bootstrap support values and BMCMC posterior probability were noted on the best-scoring tree.

TABLE 1. Species used in this study. Vouchers, their geographical origin, and GenBank accession numbers are listed. GenBank accession numbers of the newly obtained sequences are in bold.

Species	ITS GenBank Accession number	Voucher number (herbarium)	Country	Reference
Circinaria affinis	HQ171237	Kulakov 1408 (hb. John, 9911)	Russia	Sohrabi *et al.* 2011
C. affinis	HQ389194	Abbas 20081364 (H)	China	Sohrabi *et al.* 2013
C. affinis	HQ389196	Kulakov 1408B (M)	Russia	Sohrabi *et al.* 2013
C. alpicola	JQ797524	Ringel & Jaschhof 5183 (H)	Kyrgyzstan	Sohrabi *et al.* 2013

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TABLE 1. (Continued)

Species	ITS GenBank Accession number	Voucher number (herbarium)	Country	Reference
C. alpicola	JQ797552	Ringel 5137 (H)	Kyrgyzstan	Sohrabi *et al.* 2013
C. alpicola	JQ797556	Litterski 4848 (H)	Kyrgyzstan	Sohrabi *et al.* 2013
C. arida	EU057905	Owe-Larsson 8770 (UPS)	USA	Nordin *et al.* 2007
C. arida	HQ406800	Owe-Larsson 8759 (UPS)	USA	Owe-Larsson *et al.* 2011
C. arida	HQ406801	Knudsen 2046 (UPS)	USA	Owe-Larsson *et al.* 2011
C. aschabadensis	JQ797519	Borisova s.n. (LE)	Turkmenistan	Sohrabi *et al.* 2013
C. aschabadensis	GU289916	Borisova s.n. (LE)	Turkmenistan	Sohrabi *et al.* 2013
C. caesiocinerea	EU057897	Tibell 22612 (UPS)	Sweden	Nordin *et al.* 2007
C. caesiocinerea	FJ532372	Orange 17594 (NMW)	UK	Unpublished
C. calcarea	EU057898	Nordin 5888 (UPS)	Sweden	Nordin *et al.* 2007
C. calcarea	HQ406804	Nordin 5914 (UPS)	Sweden	Owe-Larsson *et al.* 2011
C. calcarea	LT671468	Roux *et al.* 25256 (UPS)	France	Roux *et al.* 2016
C. contorta	LT671470	Fröberg 09–44i (UPS)	Sweden	Roux *et al.* 2016
C. contorta	EU057900	Nordin 5895 (UPS)	Sweden	Nordin *et al.* 2007
C. contorta	HQ406805	Tibell 23702 (UPS)	Sweden	Owe-Larsson *et al.* 2011
C. cerebroides	JQ797534	Ringel 5138 (H)	Kyrgyzstan	Sohrabi *et al.* 2013
C. cerebroides	JQ797553	Ringel 5184 (H)	Kyrgyzstan	Sohrabi *et al.* 2013
C. digitata	HQ171230	Ringel & Jaschhof 5185 (H)	Kyrgyzstan	Sohrabi *et al.* 2011
C. digitata	HQ171236	Ringel & Jaschhof 5185-B (H)	Kyrgyzstan	Sohrabi *et al.* 2011
C. esculenta	HQ406803	Owe-Larsson 9824 (UPS)	Russia	Owe-Larsson *et al.* 2011
C. esculenta	JQ797510	Owe-Larsson 9796 (UPS)	Russia	Sohrabi *et al.* 2013
C. esculenta	JQ797511	Owe-Larsson 9796 (UPS)	Russia	Sohrabi *et al.* 2013
C. fruticulosa	HQ171227	Kulakov s.n. (hb. John 9913)	Russia	Sohrabi *et al.* 2011
C. fruticulosa	MK347508	Paukov 3074 (UFU)	Russia	Paukov *et al.* 2019
C. fruticulosa	**OR523873**	Ren 4084 (SDNU)	**China**	**This paper**
C. fruticulosa	**OR523874**	Ren 4052 (SDNU)	**China**	**This paper**
C. fruticulosa	**OR523872**	Ren 3251 (SDNU)	China	**This paper**
C. gyrosa	JQ797528	Sohrabi 10401A (hb. M. Sohrabi)	Iran	Sohrabi *et al.* 2013
C. gyrosa	JQ797532	John 11984A (M)	Turkey	Sohrabi *et al.* 2013
C. gyrosa	JQ797557	MAF-Lich 15363 (H)	Spain	Sohrabi *et al.* 2013
C. hoffmanniana	LT671465	Nordin 5917 (UPS)	Sweden	Roux *et al.* 2016
C. hoffmanniana	LT671466	Fröberg 09–44c (UPS)	Sweden	Roux *et al.* 2016
C. hispida	HQ171233	Sohrabi 15099 (Herb. M. Soharbi)	Iran	Sohrabi *et al.* 2011
C. hispida	HQ171235	Ochirova s.n. (LE)	Russia	Sohrabi *et al.* 2011
C. hispida	HQ389197	Sohrabi 10212b (hb. M. Sohrabi)	Iran	Sohrabi *et al.* 2013
C. hispida	**OR523875**	**Ren 3250 (SDNU)**	**China**	**This paper**
C. jussuffii	JQ797518	Esnault 2033 (GZU)	Algeria	Sohrabi *et al.* 2013
C. jussuffii	JQ797521	Vězda: Lich. Sel. Exs. No. 2381 (H)	Morocco	Sohrabi *et al.* 2013
C. lacunosa	JQ797517	Abbas 940003 (H)	China	Sohrabi *et al.* 2013
C. lacunosa	JQ797520	Peregoudov s.n. (LE)	Kazakhstan	Sohrabi *et al.* 2013
C. lacunosa	**OR523876**	**Ren 4051 (SDNU)**	**China**	**This paper**
C. rogeri	HQ171231	Rosentreter 16373 (SRP)	USA	Sohrabi *et al.* 2011
C. rogeri	HQ171232	Rosentreter 16333 (SRP)	USA	Sohrabi *et al.* 2011
C. rogeri	MZ536742	Di Meglio 305 (OSC)	USA	McCune & Di Meglio 2021

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TABLE 1. (Continued)

Species	ITS GenBank Accession number	Voucher number (herbarium)	Country	Reference
C. rostamii	JQ797527	Sohrabi 10212 (IRAN)	Iran	Sohrabi *et al.* 2013
C. rostamii	JQ797541	Sohrabi 9364 (IRAN)	Iran	Sohrabi *et al.* 2013
C. semicontorta	**OQ398460**	**Khalid & Habib KSH-17 (LAH36686)**	**Pakistan**	**This paper**
C. semicontorta	**OQ398461**	**Khalid, Asghar & Habib s.n. SL-07**	**Pakistan**	**This paper**
C. semicontorta	**OQ398462**	**Habib & Khalid KH-210 (LAH38221)**	**Pakistan**	**This paper**
C. semicontorta	**OQ398463**	**Habib & Khalid KH-201 (LAH38219)**	**Pakistan**	**This paper**
C. semicontorta **(Holotype)**	**OQ398464**	**Habib & Khalid KH-20 (LAH38220)**	**Pakistan**	**This paper**
C. semicontorta	**OR916309**	**Wahab Bot-20171-HM-10**	**Pakistan**	**This paper**
C. thorstenii	OK239678	Wahab 20170	Pakistan	Unpublished
C. thorstenii	OP650909	M.Usman CH-76 (LAH37791)	Pakistan	Zulfiqar & Khalid 2023
C. thorstenii	OP650910	Habib & Khalid KH-53 (LAH37793)	Pakistan	Zulfiqar & Khalid 2023
C. thorstenii	OP650912	Habib & Khalid KH-208 (LAH37797)	Pakistan	Zulfiqar & Khalid 2023
Megaspora cretacea	KX253975	Gasparyan 600199170 (B)	Armenia	Zakeri *et al.* 2016

Results

The resulting Maximum Likelihood and Bayesian trees (Figure 1) are concordant with the ITS tree published by Sohrabi *et al*. (2013). The basal clades include crustose species *Circinaria arida* Owe-Larss., A. Nordin & Tibell (2011: 240); *C. calcarea* (L.) A. Nordin, Savić & Tibell (2010: 1341); *C. caesiocinerea* (Nyl. ex Malbr.) A. Nordin, Savić & Tibell (2010: 1341); *C. contorta* (Hoffm.) A. Nordin, Savić & Tibell (2010: 1341); *C. hoffmanniana* (S. Ekman & Fröberg ex R. Sant.) A. Nordin (2016: 179); *C. laxilobata* G. Ismayil, A. Abbas & S.Y. Guo (2019: 26); *C. serenensis* (Cl. Roux & M. Bertrand) A. Nordin (2016: 179) (both not shown in the picture), and *C. thorstenii* R. Zulfiqar & Khalid (2023: 4); however the topology of this group is not statistically supported and needs further study. Six newly obtained ITS nrDNA sequences belong to the basal part of the tree with crustose species and formed a supported group sister to *Circinaria thorstenii* recognizing a new species, described below as *Circinaria semicontorta sp. nov.*

The Species

Circinaria semicontorta R. Zulfiqar, H.S. Asghar, K. Habib & Khalid *sp. nov*. MycoBank No.: MB849956

Circinaria semicontorta has a whitish areolate to subverrucose thallus with a thinning, cracked margin, it has areoles with raised whitish pseudocyphellae containing black ostioles of conidiomata, with 12–25 µm long pycnoconidia, and it lacks of secondary metabolites.

Type:—PAKISTAN. Khyber Pakhtunkhwa Province, Kohistan: Dassu, on calcareous rocks, 841 m a.s.l, 35°59′ N 73° 61′ E, 9 Jul 2020, K. Habib & A.N. Khalid KH-20 (Holotype LAH38220).

(Figure 2)

Thallus crustose, areolate to sub-verrucose, discrete, c. 10 cm across, 200–300 µm thick in section with a thinning radially cracked marginal part. *Areoles* contiguous, rarely discrete, irregular, or angular to rounded, weakly concave to plane and convex, pruinose, 0.5–1.2 mm wide, marginally elongated, giving a lobate view, wrinkled (resembling tree branches), up to 1.5 mm long. *Prothallus* absent. *Pseudocyphellae* present, indistinctly papilliform, whitish. *Thallus color* whitish-grey when dry, greenish grey when wet. *Upper cortex* paraplectenchymatous, 20–30 µm thick, cells rounded, 4–8 µm in diameter. *Algal layer* even, continuous, 50–60 µm thick, photobiont chlorococcoid, 6–12 µm in diameter. *Medulla* prosoplectenchymatous, 100–190 µm thick, hyphae white, 2–3 µm wide.

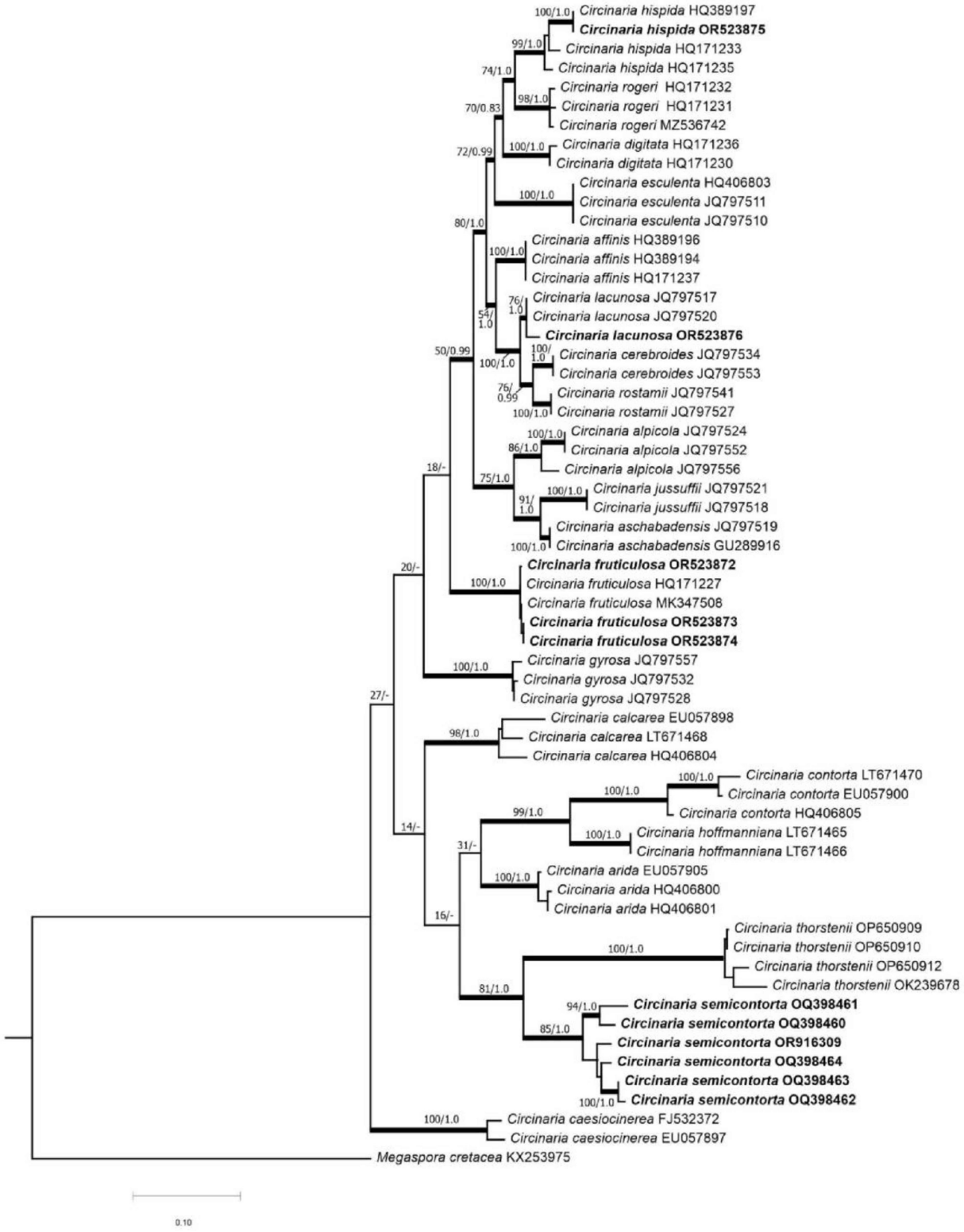

FIGURE 1. Maximum likelihood (ML) phylogeny of selected *Circinaria* ITS sequences. The reliability of each branch was tested by ML and Bayesian methods. Numbers at tree branches indicate ML bootstrap percentages (left) and Bayesian inference with the Markov chain Monte Carlo (BMCMC) posterior probabilities (right). Thicker branches indicate when the bootstrap value of ML is ≥70% or the BMCMC posterior probability is ≥0.95 or both. GenBank Accession numbers are given to serve as operational taxonomic unit (OTU) names (see Table 1). Originally produced sequences are marked in bold. *Megaspora cretacea* was used as an outgroup.

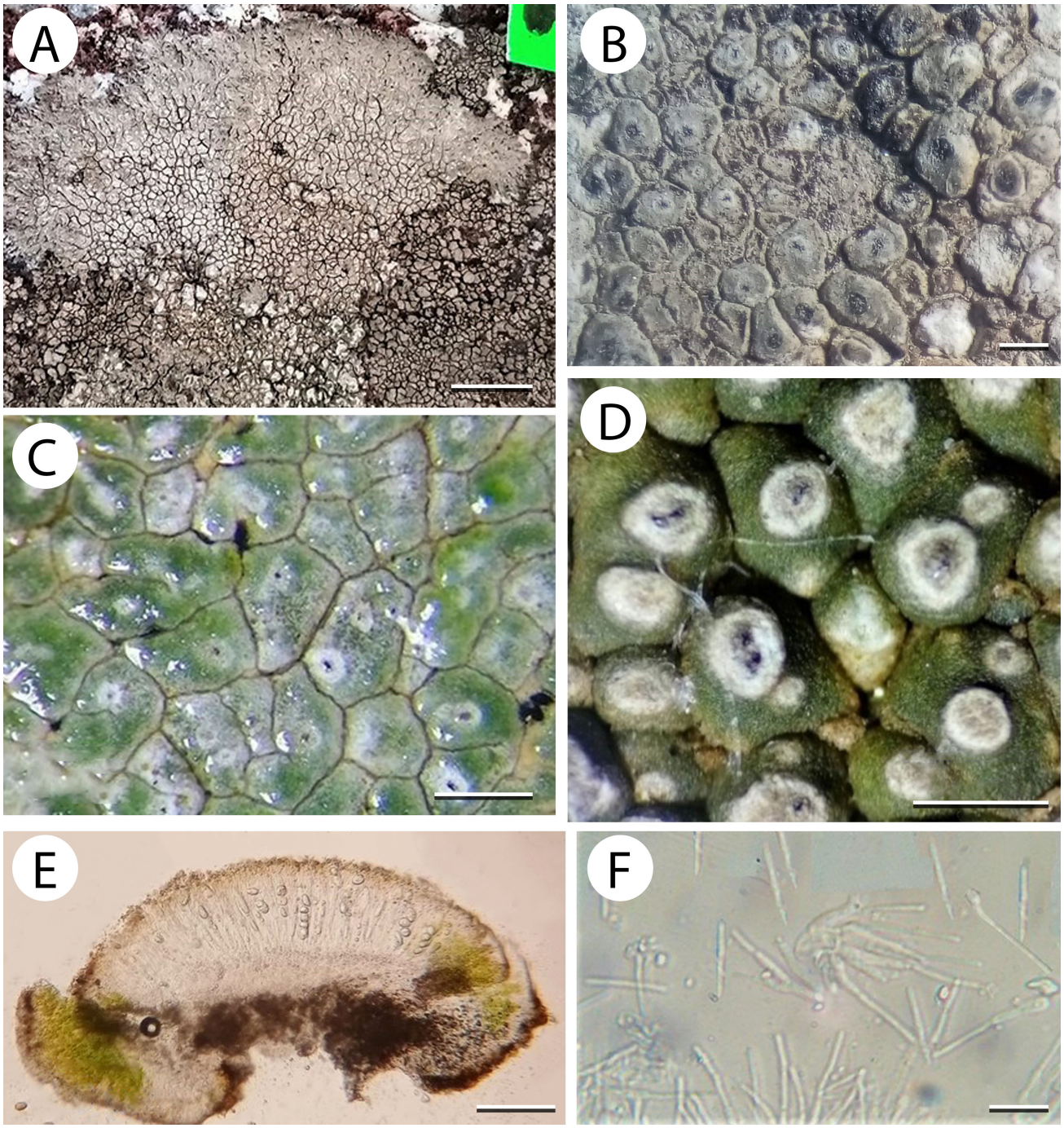

FIGURE 2. (A–F): *Circinaria semicontorta* (Holotype). A. Dry thallus B. Apothecia and areoles (dry thallus) C & D. Pycnidia in pseudocyphellae (wet). E. Section of apothecia F. Conidia. Scales: A = 2 cm, B = 0.5 mm, C = 1 mm; D = 1 mm; E = 100 µm, F = 15 µm.

Apothecia aspiciloid, common, 1–2 per areole. *Disc* black, pruinose, plane to weakly concave, rounded to rarely elongated, 0.2–0.7 mm in diameter. *Margins* rarely prominent, often forming a whitish rim. *Proper exciple* indistinct. *Thalline exciple* 90–100 µm thick. *Paraphyses* moniliform, apically branched, up to 3 µm wide at the apex. *Epihymenium* light brown, 15–25 µm tall. *Hymenium* hyaline, 90–160 µm tall. *Hypothecium* hyaline, 30–50 µm tall. *Asci* clavate, hyaline, 70–100 × 15–20 µm. *Ascospores* 4 per ascus, simple, hyaline, spherical to broadly ellipsoid, 17–25 × 15–20 µm, uniseriate.

Pycnidia immersed in pseudocyphellae, single or sometimes aggregated, punctiform, with black ostiole surrounded by a whitish margin. *Conidia* simple, hyaline, bacilliform, straight, 12–25 µm × c. 1 µm.

Chemistry: cortex and medulla, all negative. TLC: no substance detected.

Etymology: The species epithet reflects the close relationships with the morphological similar areoles of the crustose species *Circinaria contorta*.

Distribution and habitat: Circinaria semicontorta (Holotype, KH-20) is a saxicolous species growing on calcareous rocks in a dry temperate climate, at an altitude of 841 m a.s.l., in an open habitat exposed to sun and rain, with a temperature ranging from -8°C to 28°C and rainfall ranging between 700–800 mm annually. Some of the paratypes cited below (KH-210, KH-201) were found growing on silicate rocks in similar habitat as the holotype, but at an altitude of 1,811 m a.s.l. Whereas, other paratypes cited below (HM-10, KSH-17, SL-07) were found growing on silicate rocks in relatively cold climate, at an altitude of 1,598 to 2,300 m a.s.l, with an average annual rainfall varying between 1000–1650 mm.

Notes: Because of its whitish grey colour *Circinaria semicontorta sp. nov.* is similar to *C. thorstenii*. The new species differs by its zonate, thinning and cracked thallus periphery, its olive colour (at least when wet), the convex areoles, and the longer conidia. Convex rounded areoles of *C. semicontorta sp. nov.* may resemble *C. contorta*. The new species, however, has more closely appressed and thus flattened areoles, longer conidia and, most conspicuously, pseusocyphellae with carbonized ostioles of conidiomata.

Despite their current phylogenetic position within crustose *Circinaria, C. semicontorta sp. nov.* and *C. thorstenii* possess characters distinct from other species of this group. Like some vagrant and dwarf-fruticose species they develop pseudocyphellae which usually contain conidiomata and lack aspicilin, which is present in the most of the aforementioned crustose taxa. An important diagnostic character are also the much longer conidia, which are 11–15 µm in *Circinaria thorstenii*, but 12–25 µm in *C. semicontorta sp. nov.* The size of conidia in other crustose species in the basal groups of *Circinaria* falls within the range of 4–11 µm. These characters may indicate closer relationships of these two species with vagrant representatives of *Circinaria* and imply the necessity of using the multi-locus phylogeny to reveal their position in the tree of the genus.

Additional specimens examined (paratypes): PAKISTAN. Khyber Pakhtunkhwa, Kohistan: Razika Seo Valley, on siliceous rocks, 1,811 m a.s.l., 35°26′N 73°27′E, 9 Sep. 2020, K. Habib & A.N. Khalid KH-210 (LAH38221), KH-201 (LAH38219); Azad Jammu & Kashmir: Neelam Valley, Kel, 34°50' N 74°22' E, 2200 m a.s.l, on siliceous rocks, July 21, 2019, A. N. Khalid and K. Habib, KSH-17, (LAH36686); Swat District, Kalam valley: 35°53' N 72°49' E; 2,001 m a.s.l, on siliceous rocks, 29 August 2020, A.N. Khalid, S. Asghar & K. Habib SL-07; District Malakand, Heryankot, 34°50' N 71°90' E; on siliceous rocks, 1,598 m a. s. l., June 15, 2019, HM-10, Hira Wahab, (Bot-20171).

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215 (3): 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Aptroot, A. & Iqbal, S.H. (2012) Annotated checklist of the lichens of Pakistan, with reports of new records. *Herzogia* 25 (2): 211–229. https://doi.org/10.13158/heia.25.2.2010.211

Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F.K., Müller, N.F., Ogilvie, H.A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., Suchard, M.A., Wu, C-H., Xie, D., Zhang, C., Stadler, T. & Drummond, A.J. (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 15 (4): e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B. & Flouri, T. (2020) ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. *Molecular Biology and Evolution* 37 (1): 291–294. https://doi.org/10.1093/molbev/msz189

Edler, D., Klein, J., Antonelli, A. & Silvestro, D. (2021) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12: 373–377.

https://doi.org/10.1111/2041-210X.13512
Elenkin, A. (1901) Lichen manna (*Lichen esculentus* Pall.) (Historical survey of literature). *Trudy Imp S.-Peterburgsk. Bot. Sada* 19: 53–99. [in Russian]
Foucard, T. (2001) *Svenska Skorplavar.* Interpublishing, Stockholm. 392 pp.
Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
Hall, T.A. (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
Ismayil, G., Abbas, A. & Guo, S.Y. (2019) A new saxicolous *Circinaria* species (Megasporaceae) from northeast China. *The Bryologist* 122 (1): 23–30.
https://doi.org/10.1639/0007-2745-122.1.023
Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20 (4): 1160–1166.
https://doi.org/10.1093/bib/bbx108
Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35 (21): 4453–4455.
https://doi.org/10.1093/bioinformatics/btz305
Kunkel, G. (1980) Microhabitat and structural variation in the *Aspicilia desertorum* group (lichenized Ascomycetes). *American Journal of Botany* 67: 1137–1144.
https://doi.org/10.1002/j.1537-2197.1980.tb07747.x
Larget, B. & Simon, D.L. (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16 (6): 750–759.
https://doi.org/10.1093/oxfordjournals.molbev.a026160
Magnusson, A.H. (1939) Studies in species of *Lecanora*, mainly the *Aspicilia gibbosa* group. Kungl. Svenska Vetenskaps-Akademiens Handlingar, ser. III 17 (5): 1–182.
McCarthy, P.M. & Elix, J.A. (2020) A new species of *Circinaria* (Megasporaceae) from New South Wales, Australia. *Australasian Lichenology* 86: 90–94.
McCune, B. & Di Meglio, J. (2021) Revision of the *Aspicilia reptans* group in Western North America, an important component of soil biocrusts. *Monographs in North American Lichenology* 5: 1–94.
Mereschkowsky, C. (1911) Excursion lichénologique dans les steppes Kirghises (Mont Bogdo). *Trudi Naturh. Ver. K. Univ. Kazan* 43: 1–42. [in Russian]
Nordin, A., Tibell, L. & Owe-Larsson, B. (2007) A preliminary phylogeny of *Aspicilia* in relation to morphological and secondary product variation. *In:* Frisch, A., Lange, U. & Staiger, B. *Lichenologische Nebenstunden. Contributions to Lichen Taxonomy and Ecology in Honour of Klaus Kalb. Bibliotheca Lichenologica* No. 96. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Berlin-Stuttgart, pp. 247–266.
Nordin, A., Savic, S. & Tibell, L. (2010) Phylogeny and taxonomy of *Aspicilia* and Megasporaceae. *Mycologia* 102 (6): 1339–1349.
https://doi.org/10.3852/09-266
Orange, A., James, P. & White, F.J. (2001) *Microchemical Methods for the Identification of Lichens.* First Edit. British Lichen Society, London, 101 pp.
Owe-Larsson, B., Nordin, A., Tibell, L. & Sohrabi, M. (2011) *Circinaria arida* sp. nova and the '*Aspicilia desertorum' complex*. *Bibliotheca Lichenologica* 106: 235–246.
Paukov, A.G., Davydov, E.A., Nordin, A., Roux, C., Şenkardeşler, A., Sohrabi, M., Vondrák, J., Frolov, I.V., Teptina, A.Y. & Shiryaeva, A.S. (2019) Three new species, new combinations and a key to known species of *Lobothallia* (Megasporaceae). *The Lichenologist* 51 (4): 301–322.
https://doi.org/10.1017/S0024282919000264
Rambaut, A. (2012) FigTree v1.4.2. Available from: http://tree.bio.ed. ac.uk/software/figtree (accessed 4 June 2024)
Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67 (5): 901–904.
https://doi.org/10.1093/sysbio/syy032
Ren, Q. & Zhang, L.H. (2018) Taxonomic studies on the genus *Circinaria* in northern China. *Mycosystema* 37 (7): 865–880.
https://doi.org/10.13346/j.mycosystema.180044
Roux, C., Nordin, A. & Bertrand, M. (2016) *Lobothallia controversa* Cl. Roux & A. Nordin sp. nov., correspondant au *Lecanora farinosa* sensu Nyl. non (Flörke) Nyl. *Herzogia* 29 (2): 586–595.

https://doi.org/10.13158/heia.29.2.2016.586

Roux, C. (2016) *Aspicilia serenensis* Cl. Roux and M. Bertrand sp. nov., new species of lichen (*A. calcarea* group, Megasporaceae). *Bulletin of the Linnean Society of Provence* 67: 165–182.

Sambrook, J. & Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual. CSHL Press. 2344 pp.

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Sohrabi, M., Ahti, T. & Litterski, B. (2011) *Aspicilia digitata* sp. nov., a new vagrant lichen from Kyrgyzstan. *Lichenologist* 43 (1): 39–46.
https://doi.org/10.1017/S0024282910000538

Sohrabi, M., Stenroos, S., Myllys, L., Søchting, U., Ahti, T. & Hyvönen, J. (2013) Phylogeny and taxonomy of the 'manna lichens'. *Mycological Progress* 12 (2): 231–269.
https://doi.org/10.1007/s11557-012-0830-1

White, T.J., Bruns, T. & Lee, S.B., Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: a Guide to Methods and Application.* San Diego: Academic Press, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zakeri, Z., Gasparyan, A. & Aptroot, A. (2016) A new corticolous *Megaspora* (Megasporaceae) species from Armenia. *Willdenowia* 46 (2): 245–251.
https://doi.org/10.3372/wi.46.46205

Zulfiqar, R. & Khalid, A.N. (2023) A novel saxicolous species of *Circinaria* (Megasporaceae, Ascomycota) from Pakistan. *Plant Systematics and Evolution* 309: 21.
https://doi.org/10.1007/s00606-023-01856-0