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BOTANY

Sarcogyne balochistanensis sp. nov. (Acarosporales, Acarosporaceae) from Pakistan

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Abstract—In the present study, *Sarcogyne balochistanensis* sp. nov. is described and illustrated. The taxon is characterized by pale-white farinose thallus, polysporine type apothecia with quite taller hymenium, light brown sub-hymenium, large-wider asci and comparatively elongate and wider ascospores, these characters distinguish it from other species of the genus with a carbonized epihymenium. The ITS and LSU based phylogenetic analyses also support the identity of this species as new to science. A complete taxonomic treatment including description based on two collected material is provided along with molecular phylogenic analyses.

Keywords: Balochistan, lichenized fungi, taxanomy

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INTRODUCTION

The family Acarosporaceae occurs around the world, usually in xerothermic habitats on rock or in biotic soil crusts (Magnusson, 1929). The family currently includes 12 genera and 259 species (Lucking et al., 2017). The genus *Sarcogyne* Flot, is characterized by polyspory, simple and hyaline ascspores, lecideine-apothecia, and bitunicate but non-fissitunicate asci with a nonamyloid tholus (Knudsen and Standley, 2007; Magnusson, 1935). The genus is generally found growing on calcareous, non-calcareous rocks and in biotic soil crusts in the northern and southern hemisphere and can have either an endolithic or epilithic thallus. There are approximately 97 described species in the genus worldwide based on our estimation (Index Fungorum, 2020). Two species of this genus have been reported from Pakistan i.e. *S. privigna* (Ach.) A. Massal. from Kalam (Swat) and *S. regularis* Körb from Khanspur (Aptroot and Iqbal, 2012).

Balochistan is the largest province of Pakistan in terms of land area, forming the southwestern region of the country, but has not been fully explored lichenologically. Only nine, lichens have been reported from this province so far. During a survey to explore lichen flora of Balochistan, we collected specimens of *Sarcogyne*. Morpho-anatomical and Phylogenetic analyses confirmed its identity as novel species.

MATERIALS AND METHODS

Morpho-Anatomical and Chemical Characterization

Specimens were examined macro and micro morphologically with a stereomicroscope (Meiji Techno, EMZ-5TR, Japan) and a compound microscope (SWIFT M4000-D). Standard microscopy and spot tests (Hall, 1979) were used for identification. Free hand sections of apothecia and thallus mounted in water were used for anatomical study done by preparing and observing the slides of hand-cut apothecial sections (mounted in water) under microscope. Minimum twenty measurements in water were made for each diagnostic feature.

Molecular Characterization and Phylogenetic Analysis

For molecular studies, DNA isolation was done followed by amplification of target internal transcribed spacer region (ITS nrDNA) and large subunite (LSU) sequencing and phylogeny for accurate identification.

DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted directly from a portion of thallus with apothecia from each specimen using a modified 2% CTAB method (Gardes and Bruns, 1993). The ITS-nrDNA region (Internal Transcribed Spacer of the nrDNA) was amplified using the primers pair i.e. ITS1F forward primer (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS4 reverse primer (5' TCCTCCGCT-

Table 1. GenBank Accession Number of sequences used in phylogentic analysis

Accession no	Specimen name	Voucher	Country	Primer
LN810867.1	*Timdalia intricate*	"Westberg P114 (S)"	Sweden	ITS+ LSU
LN810866.1	*Timdalia intricate*	"Westberg SAR92 (LD)"	Sweden	ITS+ LSU
LN810856.1	*Sarcogyne hypophaea*	"Westberg SAR198 (S)"	Sweden	ITS+ LSU
LN810857.1	*Sarcogyne hypophaea*	"Pykala 23561 (H)"	Finland	ITS+ LSU
LN810849.1	*Sarcogyne algoviae*	"Westberg 08-276 (S F122564)"	Norway	ITS+ LSU
LN810850.1	*Sarcogyne algoviae*	"Westberg 08-168 (S F122537)"	Norway	ITS+ LSU
OQ269757	*Sarcogyne balochistaniensis*	**LAH626345**	**Pakistan**	**ITS**
OQ269755	*Sarcogyne balochistanensis*	**LAH36060**	**Pakistan**	**ITS**
OQ269822	*Sarcogyne balochistanensis*	**LAH36061**	**Pakistan**	**ITS**
OQ269756	*Sarcogyne balochistanensis*	**LAH36061**	**Pakistan**	**LSU**
OQ269754	*Sarcogyne balochistanensis*	**LAH36060**	**Pakistan**	**LSU**
MG196102.1	*Sarcogyne* sp.	"Pykaelae 22279 (H)"	Finland	ITS+ LSU
MG196103.1	*Sarcogyne* sp.	"Pykaelae 28542 (H)"	Finland	ITS+ LSU
LN810851.1	*Sarcogyne arenosa*	"Knudsen 11102 and Sagar (S)"	USA: California	ITS+ LSU
LN810861.1	*Sarcogyne regularis*	"Westberg 08-034 (S F119830)"	Sweden	ITS+ LSU
LN810860.1	*Sarcogyne regularis*	"Westberg 08-102 (S F121703)"	Norway	ITS+ LSU
LN810854.1	*Sarcogyne distinguenda*	"Westberg 08-305 (S F120452)"	Sweden	ITS+ LSU
LN810855.1	*Sarcogyne distinguenda*	"Haugan H3852 (O L17425)"	Norway	ITS+ LSU
LN810864.1	*Sarcogyne* sp.	"Westberg 3106 (LD)"	Sweden	ITS+ LSU
LN810865.1	*Sarcogyne* sp.	"Westberg 08-271 (S)"	Norway	ITS+ LSU
LN810778.1	*Acarospora laqueata*	"Westberg 10-170 (S F177761)"	Switzerland	ITS+ LSU
MK372318.1	*Sarcogyne* sp.	"PRM:Wheeler 5678"	USA: Montana	ITS+ LSU
MK372316.1	*Sarcogyne* sp.	"Hb. Wheeler 1996"	USA: Montana	ITS+ LSU
MK372313.1	*Sarcogyne* sp.	"Hb. Wheeler 5674"	USA: Montana	ITS+ LSU
MK372310.1	*Sarcogyne* sp.	"UCR<USA-CA>:Knudsen 15854"	"USA: California"	ITS+ LSU
MK372319.1	*Sarcogyne* sp.	"Hb. Wheeler 5743"	USA: Montana	ITS+ LSU
MK372309.1	*Sarcogyne* sp.	"UCR<USA-CA>:McCarthy 2414"	Canada	ITS+ LSU
MK372314.1	*Sarcogyne* sp.	"PRM:Wheeler 5971"	USA: Montana	ITS+ LSU
LN810853.1	*Sarcogyne clavus*	"Berglund SAR220 (S)"	Sweden	ITS+ LSU
LN810859.1	*Sarcogyne hypophaeoides*	"Westberg 08-139 (S F123697)"	Norway	ITS+ LSU
MK948466.1	*Sarcogyne* sp.	"UCR:Knudsen 3620"	USA: California	ITS+ LSU
MG196101.1	*Sarcogyne* sp.	"Pykaelae 22273 (H)"	Finland	ITS+ LSU
MG196100.1	*Sarcogyne* sp.	"Pykaelae 27626 (H)"	Finland	ITS+ LSU
MK702076.1	*Sarcogyne* sp.	"2007000S"	China	ITS+ LSU
MK694771.1	*Sarcogyne algoviae*	"20070151"	China	ITS+ LSU
AY853393.2	*Sarcogyne regularis*	"gren 423 (UPS)"	Sweden	ITS+ LSU
DQ374145.1	*Sarcogyne privigna*	"Moberg 6599 (UPS)"	Sweden	ITS+ LSU
MK372321.1	*Sarcogyne* sp.	"PRM:Wheeler 7512"	Canada	ITS+ LSU
MK372308.1	*Sarcogyne* sp.	"NY:Knudsen 1240"	"USA: California"	ITS+ LSU
MK372315.1	*Sarcogyne* sp.	"Hb. Wheeler 6056"	USA: Montana	ITS+ LSU
MK372323.1	*Sarcogyne* sp.	"NY:Harris 60908"	"USA: Michigan	ITS+ LSU
MK372320.1	*Sarcogyne* sp.	"Hb. Wheeler 5783"	"USA: Montana"	ITS+ LSU
MK372322.1	*Sarcogyne* sp.	"NY:Harris 56545"	Canada	ITS+ LSU
MK372317.1	*Sarcogyne* sp.	"NY:Buck 56600"	Canada	ITS+ LSU
MH857702.1	*Sarcogyne similis*			ITS+ LSU
LN810858.1	*Sarcogyne hypophaeoides*	"Westberg 08-002"	Sweden	ITS+ LSU
LN810852.1	*Sarcogyne clavus*	"Obermayer 09129"	Sweden	ITS+ LSU

TATTGATATGC 3') (White et al., 1990), while the LSU region was amplified using the primer pair i.e. LR5 (5' TCCTGAGGGAAACTTCG 3') and LROR (5' ACCCGCTGAACTTAAGC 3') following the amplification protocol of Khan et al. (2018).

The amplified DNA fragments (PCR products) were visualized with the help of 1% agarose gel using ethidium bromide through Gel documentation system (Sambrook and Russel, 2001). The amplified product was then sequenced.

Phylogenetic Analysis

Bidirectional sequences (ITS1 and ITS4) and (LROR and LR5) were reassembled by using BioEdit software (Hall, 1999). The sequences were retrieved using Basic Local Alignment Search Tool (BLAST) analysis. Maximum percent identification and query coverage of sequences with related taxa was found out. Multiple sequences (including sequences retrieved from GenBank) were aligned using MAFFT (multiple alignment using fast fourier transform) software with default parameters. The phylogenetic tree was executed by software MEGA 7.0 (Kumar et al., 2016). The evolutionary history was retrieved with Maximum Likelihood Method based on Kimura 2-parameter. The model was selected by searching best DNA model for ML analysis in MEGA 10 (Kumar et al., 2016). One thousand rapid bootstrap replicates were run to infer the evolutionary history of the species. *Timdalia intricata* (LN810867.1 and LN810866.1) was selected for rooting purpose of tree (Czarnota and Guzow-Krzemińska, 2018) as out group.

RESULTS

Phylogenetic Analysis

Sequences generated from Pakistani collection were used as a reference to BLAST against GenBank data. Three new ITS and LSU-rDNA sequences nested within the Phylogenetic branch of the genus *Sarcogyne,* representing the species unknown yet, described here as *Sarcogyne balochistanensis* sp. nov. A total of 45 sequences have been analyzed including 42 from GenBank. The data matrix had 1112 unambiguously aligned nucleotide positions among which 689 were constant, 400 variables, 275 parsimony-informative and 123 were singletons variants.

Sarcogyne balochistanensis sp. nov. appeared to be a sister species of *Acarospora lequeata*, within the clade of *Sarcogyne*. The result largely agrees with the analyses of Westberg et al. (2015), who found *Sarcogyne* clade including both *Sarcogyne* and *Acarospora* species. The clade of *Sarcogyne* lacks support for the basel branches. The analyses represent independent position of the Pakistani collections, as formed separate clade with in the *Sarcogyne* group (Fig. 1).

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Etymology. The species epithet *balochistanensis* (Latin) refers to the locality Balochistan province of Pakistan from where samples were collected.

Diagnoses. Distinguish from other species of the genus with a carbonized epihymenium by thinly rimose-areolate thallus, slightly endosubstratic at margins, slightly pruinose *Polysporina*-type apothecia, quite taller hymenium and asci, taller and light brown subhymenium, and comparative elongate and wider ascospores (4–7.2 × 3–4 μm).

Thallus. Crustose, epilithic, rimose, thinly rimose-areolate at center, 0.1–0.5 mm thick, forming conspicuous extended patches, 4–6 cm across, coating the substrate, farinose, differentiated by very thin cracks, unstratified, intermingled with rock particles, emergent from the rock, slightly endosubstratic at margins, non-lobate. **Upper surface:** dull, pruinose, off-white to pale yellow, unchanged when wet.

Apothecia: frequent, scattered, emergent, flat to weakly concave, 0.4–1.2 mm in diam. **Disc:** rough, wrinkled, black, no change in color when wet, often mature disc with carbonized accretions, slightly pruinose, somewhat glossy, round to ellipsoid, marginate; **Margins:** thick, rough, concolorous to disc, slight glossy, quite elevated above the disc, broader than disc when ascocarp young, becoming concave, stipitate, incised, persistent. **Exciple:** black, 40–60 μm wide. **Epihymenium:** reddish brown to dark brown, 20–30 μm tall. **Hymenium:** hyaline, 130–190 μm tall, euamyloid IKI+ blue; **Subhymenium:** light brown, 70–130 μm high, Hypothecium indistinct, algal layer present beneath the hypothecium. **Paraphyses:** septate, unbranched to rarely branched, apical cell slightly swollen, 3.5–5.5 μm wid. **Asci:** clavate, hyaline, 75–110 × 20–32 μm, multi-spored; **Ascospores:** ellipsoid, 2-loculate, 4–7.2 × 3–4 μm; **Spot test:** K–, C–, KC–.

Ecology. Growing on rocks in dry temperate climate, exposed to sun and rain, maximum and minimum temperature –7 to 35°C, receive an average annual rainfall of <30 inches.

Holotype. Pakistan, Balochistan, District Killa Saifullah, Muslim Bagh, 30°50″ N, 67°44′25″ E, 1787 m. a.s.l., on rocks, August 18, 2019, Alla Ud Din, MB-16 (Holotype-LAH626345) (Isotype-LAH626346).

Additional examined (syntype). Pakistan, Balochistan, Ziarat, 30°22′51.6″ N, 67°43′37.2″ E, 2543 m a.s.l on rocks, July 2nd 2018, Alla Ud Din and A.N. Khalid, Z18-4B (LAH36060), and Z18-19C (LAH36061).

Remarks. Phylogenetically, *Acarospora lequeata* has been found the closest relative of *Sarcogyne balochistanensis* sp. nov. The morpho-anatomical characters do not support its placement with in *Aca-*

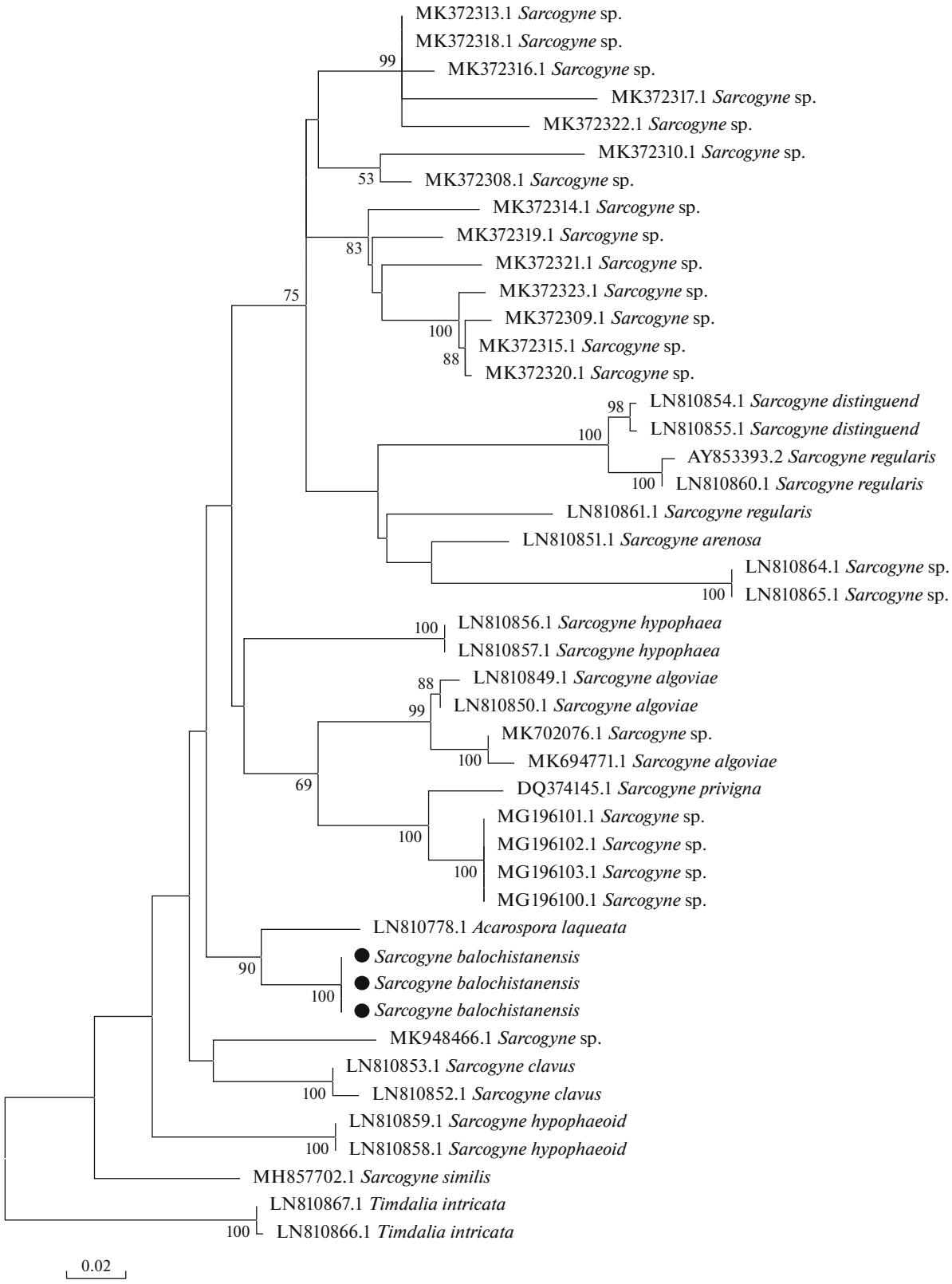

Fig. 1. Combine ITS-LSU based Molecular Phylogenetic analyses of *Sarcogyne* spp. nom. by the Maximum Likelihood method. Number below branch node represent ML bootstrap (only ≥50) based on 1000 replicates. Sequence generated from Pakistani collections are marked with ●.

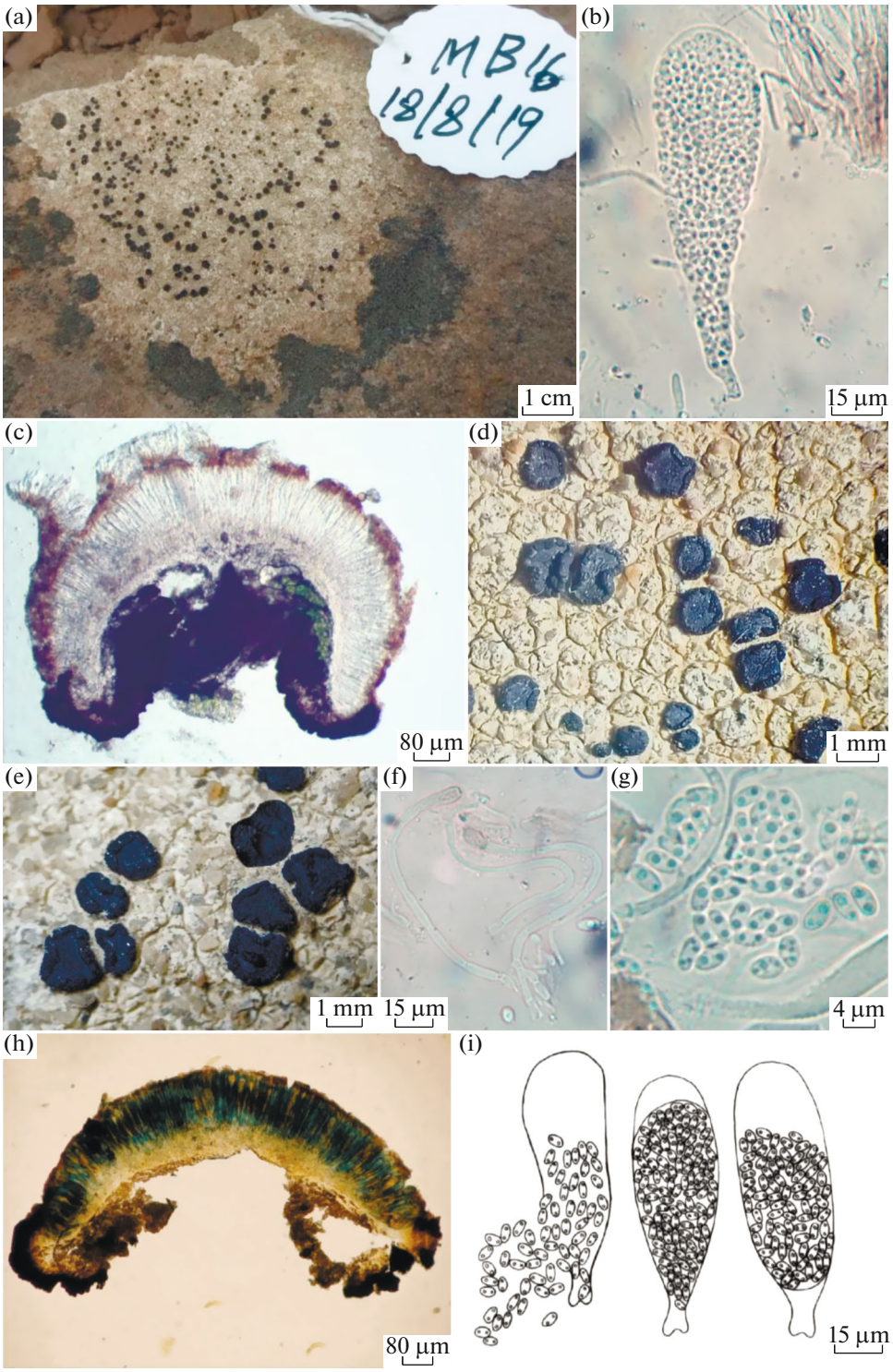

Fig. 2. (a–i) *Sarcogyne balochistanensis*; (a) Holotype; (b) Ascus; (c) Section of Apothecia; (d, e) showing thinly rimose-areolate thallus and apothecia; (f) paraphyses; (g) Ascospores; (h) Logule reaction; (i) Line drawing.

rospora, but instead support its placement with in *Sarcogyne*.

The genus *Polysporina* was characterized primarily by apothecia with carbonized epihymenial accretions and lecideine apothecia (Vězda, 1978; Kantvilas, 1998; Knudsen, 2007; Knudsen and Kocourková, 2008). The undescribed taxon with carbonized epihymenial accretions from Pakistan was included in the recent phylogeny of the family (Westberg et al., 2015a), and was recovered in *Sarcogyne*.

The comparative morpho-anatomical study showed its resemblance to *S. integra* B. de Lesd. ex H. Magn. but differs in having thinly rimose-areolate thallus at center only (vs. thick rimose areolate thallus) wrinkled black apothecia with no change when wet and mature disc with carbonized accretions (vs. disc smooth, black and reddish in colour when wet), taller hymenium (130–190 μm vs. 65–80 μm), non-amyloid and taller (70–130 μm) subhymenium (vs. 30–40 μm tall and amyloid) long-wider asci (75–110 × 20–32 μm vs. 50–70 × 10–15 μm) and ascospores (4–7.2 × 3–4 μm vs. 3–4(–6) × 2–3 μm).

Sarcogyne magnussonii B. de Lesd. has also white epilithic thallus, but it differs from *Sarcogyne balochistanensis* sp nov. in thickness of thallus (0.1–0.5 mm vs. 3 mm thick), especially in having black apothecia with epihymenial melanin accretions (*Polysporina*-type apothecia) (vs. smooth and reddish black apothecia), taller hymenium (130–190 μm vs. 80–120 μm) taller subhymenium (70–130 μm vs. 30–50 μm thick), larg and wider asci (75–110 × 20–32 vs. 60–70 × 18–20 μm).

In having mature disc rugulose with carbonized accretions, *Sarcogyne balochistanensis* is similar to *Sarcogyne albothallina* K. Knudsen, T.B. Wheeler, Kocourk. and M. Westb but differs in having farinose thallus, (vs. not farinose), IKI+ amyloid and taller (130–190 μm) hymenium (vs. hemiamyloid, 80–100 μm tall), large and wider asci, (75–110 × 20–32 μm vs. 60–70 × 10–20 μm) IKI–, taller (70–130 μm) subhymenium (vs. IKI+ 40–50 μm).

In comparison to the members of *nivea* group the Pakistani species is characterized by epillthic thalli, 0.4–1.2 mm in diameter apothecia, hymenium 130–190 μm, light brown hypothecium and ascospores 4–7 × 3–4 μm. Distinguish from the other closely related species, *Sarcogyne clavus* (DC.) Kremp., and *Sarcogyne regularis* Körb. by epilithic thalli, pure black apothecia, quite tall hymenium, and light brown hypothecium, tall and wider asci.

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COMPLIANCE WITH ETHICAL STANDARD

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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