ASPICILIA FUMOSA (MEGASPORACEAE), A NEW RECORD FOR PAKISTAN AND THE FIRST IN EURASIA

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ABSTRACT

A new record, *Aspicilia fumosa*, is being reported for the first time from Pakistan in an examination of *Aspicilia* species in Darel Valley. A description of the species is provided together with details on its distribution, habitat and phylogeny.

RESUMEN

Se cita por primera vez un nuevo registro de *Aspicilia fumosa*, en Pakistán en un examen de las especies de *Aspicilia* en el valle de Darel. Se describe la especie, así como su distribución, hábitat y filogenia.

Key Words: Darel, Gilgit Baltistan, lichen flora, phylogenetic analyses, taxonomy

INTRODUCTION

The lichen-forming ascomycete genus *Aspicilia* A. Massal. belongs to the lichenized family Megasporaceae (Pertusariales). In its traditional circumscription it has a worldwide distribution and covers 200–250 species (Sohrabi et al. 2013). However, recently, based on molecular studies of a limited number of species, the genus is being split and in the currently accepted taxonomy of the lichenized family Megasporaceae (Pertusariales) eight genera are recognized: *Aspicilia* A. Massal., *Circinaria* Link (Sohrabi et al. 2013), *Lobothallia* (Clauzade & Cl. Roux) Hafellner (Paukov et al. 2019), *Megaspora* (Clauzade & Cl. Roux) Hafellner & V. Wirth, *Sagedia* Ach (Nordin et al. 2010), *Teuvoa* Sohrabi & S. Leavitt (Sohrabi et al. 2013), *Aspiciliella* M. Choisy (Zakeri et al. 2017) and *Oxneriaria* S.Y. Kondr. & Lőkös (Haji Moniri et al. 2017).

Previously from Pakistan four species of this genus have been reported viz. *Aspicilia desertorum* (Kremp.) Mereschk., *A. nigromaculata* Fayyaz, Afshan, Niazi & Khalid, *A. persica* (Müll. Arg.) Sohrabi & *A. subconfluens* (Müll. Arg.) Hue (Aptroot & Iqbal 2012; Din et al. 2023; Fayyaz et al. 2022). Only two, *A. nigromaculata* and *A. persica* (Din et al. 2023; Fayyaz et al. 2022), were subjected to molecular studies and proven to belong to *Aspicilia* in modern sense. During a study of the lichen flora of Darel Valley, Gilgit Baltistan, Pakistan, another species of *Aspicilia* in modern sense was discovered. It is described below using morphological and molecular methods.

MATERIAL AND METHODS

Morphological and chemical studies.—The specimens were collected in 2022 during a lichen survey of various sites in Darel valley, Gilgit Baltistan, Pakistan. A stereomicroscope (Meiji Techno, EMZ-5TR, Japan) was used to examine morphological characteristics. For further identification, standard microscopy and spot tests (Hale 1979) were utilized. A compound microscope (MX4300H, Meiji Techno, Japan) was used to examine sections. The apothecia were placed in tap water and examined at various magnifications for anatomical characterization and measurements. For each diagnostic characteristic, at least twenty measurements were taken.

Molecular characterization.—For molecular analysis, DNA was extracted from air dried and cleaned thalli using a GF1 Plant DNA extraction kit, following the instructions of the manufacturer (Vivantis, Selangor Darul Ehsan, Malaysia). For qualitative examination of total extracted DNA, 1% agarose gel electrophoresis was employed (Voytas 2000). A thermal cycler (Bio-RAD T100) was used to amplify certain rDNA

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regions like ITS and LSU. Primers used for amplifications were ITS1F 50 -CCT GGT CAT TTA GAG GAA GT A A-3 0 and ITS4 50 -TCC GCT CTA TTG ATA TGC-30 for the ITS region (Gardes & Bruns 1993; White et al. 1990). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA and USA) and then submitted to TsingKe, China for sequencing utilizing the aforesaid ITS1F and ITS4 amplicons for forward and reverse sequencing. To reconstruct forward and reverse sequences, the BioEdit sequence alignment editor was utilized (Hall 1999). The nucleotide sequence comparison was carried out using the National Centre for Biotechnology Information's (NCBI) (https://www.ncbi.nlm.nih.gov/) Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/) (Altschul et al. 1990). MAFFT v.7 (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used for the multiple sequence alignment, with all parameters set to default levels (Katoh & Standley 2013). The phylogenetic tree was created utilizing the MEGA 6.0 programme (Tamura et al. 2013) and the ML approach based on the Kimura 2-parameter model. The "rapid bootstrapping" option with 1,000 repetitions was used to assess nodal support. Newly generated sequences were deposited in GenBank (https://submit.ncbi.nlm.nih.gov/subs/genbank/).

RESULTS

Phylogenetic analysis.—For phylogenetic analyses of the collected *Aspicilia* specimens, the target region comprising ITS1, 5.8S and ITS2 of nrDNA was amplified and sequenced by using ITS1F and ITS4 primers. Our collection, DR–208, clustered with all sequences identified as *Aspicilia fumosa* Owe-Larss. & A. Nordin (Fig. 1). Altogether 32 ITS rDNA sequences were analyzed, including 31 sequences obtained from the NCBI GenBank (https://www.ncbi.nlm.nih.gov/). There were 531 characters in the alignment file of which 335 were conserved, 181 variables, 133 parsimony informative and 48 were singleton variants. *Aspicilia fumosa* is morpho-anatomically and molecularly characterized in this study and verified by ITS data as a new record for Pakistan.

TAXONOMIC TREATMENT

Aspicilia fumosa Owe-Larss. & A. Nordin, Lichen Flora of the Greater Sonoran Desert Region (Tempe) 3:87. 2007. (**Fig. 2**).

Thallus type.—Crustose.

Description.—Thallus areolate, 0.2–0.5 mm thick, 5 to 10 cm across, epruinose. **Areoles:** 0.5 to 1.2 mm across, angular to irregular or aggregated, mostly convex, contiguous, separated by distinct cracks, uniformly colored and without differentiated marginal zone, with whitish grey to blackish grey, dull to shiny upper surface. **Lower surface:** grayish brown to blackish white, completely attached to the substrate. **Prothallus:** thin, continuous, found on thallus edges, blackish to blackish brown, 0.2 to 0.5 mm wide. **Upper cortex:** 28–38 µm thick, smooth, upper layer brown to dark brown, hyaline adjacent to algal layer, paraplectenchymatous, textura subglobularis to globularis, cells 9–14 µm in diam. **Algal layer:** continuous, 60 to 80 µm thick, lacking below the apothecia. **Photobiont cells:** subglobose to globose, 15–25 µm. **Medulla:** 70–110 µm thick, white.

Apothecia: aspicilioid, frequent, 0.5–1 mm in diam., angular to rounded. **Disc:** black, mostly plane, slightly convex to concave, pruinose to epruinose. Thalline margin: concolorous to thallus. **Exciple:** 50–70 µm wide. **Epihymenium:** light brown to olivaceous brown, 18–28 µm high. **Hymenium:** hyaline, 110–200 µm high. **Paraphyses:** 80–150 × 3.5–4.5 µm. **Hypothecium:** hyaline, 63–73 µm high. **Asci:** clavate, 62–72 × 15–25 µm, 8-spored. **Ascospores:** hyaline, simple, 24–28 × 13 –19 µm, ellipsoid to subellipsoid. Pycnidia and conidia not observed.

Spot tests.—K–, C–, KC–.

TLC.—No substance detected.

Habitat.—Found on calcareous rock, collected in a cold, semi-arid climate area at an altitude of 2,000 m a.s.l. The climate has typically cold desert characteristics, with severe winters (usually with moderate to heavy snowfall) and dry summers. Average annual precipitation in the valley is 100–300 mm, mostly occurring during winter and early spring in the form of snow.

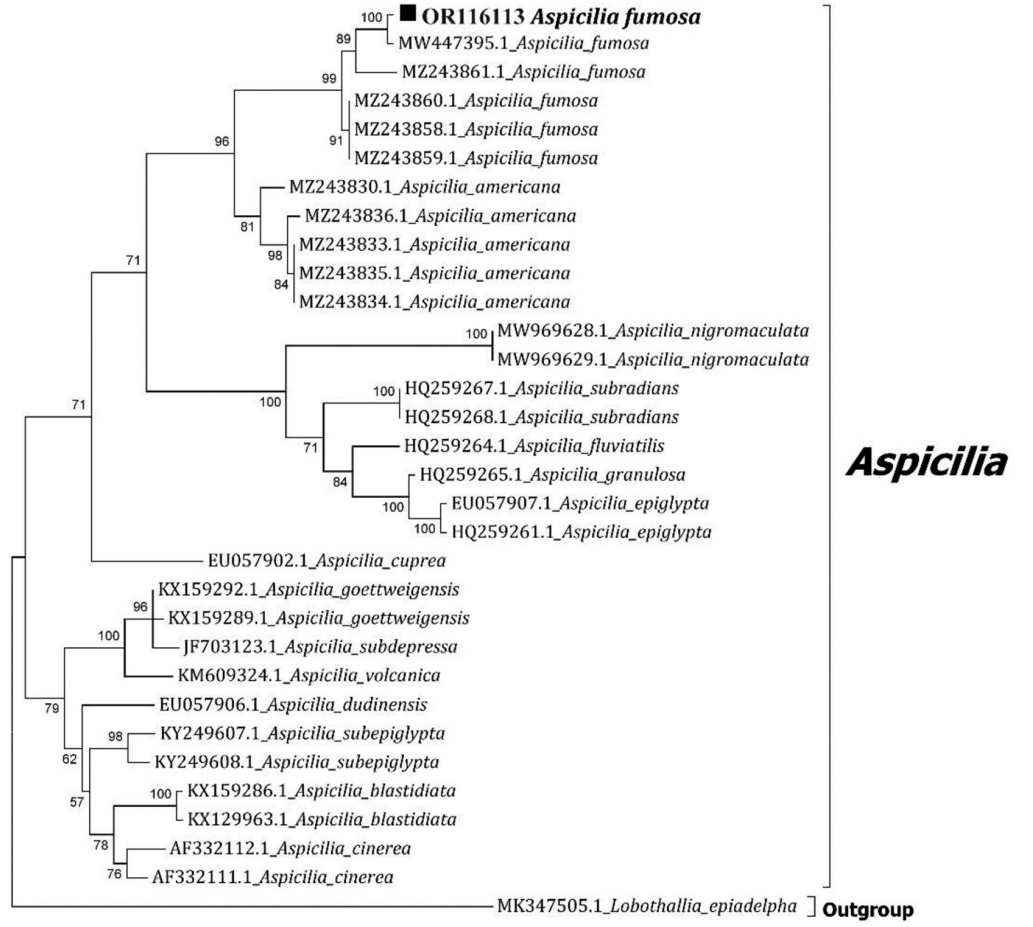

Fig. 1. Molecular phylogenetic analysis of *Aspicilia* A. Massal. members by the maximum likelihood method based on rDNA sequences, including ITS1, 5.8S and ITS2. Numbers below branch node represent ML bootstrap (> 50%) based on 1000 replicates. Sequences generated from Pakistani collections are marked with black box (■).

Voucher Specimen.—**PAKISTAN:** Gilgit Baltistan, Darel Valley 35°37 N, 73°27′E, elev. 2,000 m, on rocks, 10 Aug 2022, *Muhammad Shahid Iqbal DR–208* (ITS GenBank accession number OR116113).

Discussion.—The morpho-anatomical features of the Pakistani collection agree with the published description of *A. fumosa* from North America. *Aspicilia fumosa* is so far known only from the western USA (https://www.gbif.org/search?q=*Aspicilia*%20*fumosa*, 27 Jan. 2024), and the record from Pakistan is the first in Eurasia. The species of the genus *Aspicilia* in modern sense show only very slight morphological differences, and the most reliable way of identification is by sequencing ITS. Accordingly the new record of *Aspicilia fumosa* was verified primarily by molecular analysis. Morphological comparison with the first description (Nash et al. 2007) showed no significant differences: it mentions convex, mostly pale grey areoles; absence of lichen substances; large hymenium (140–200(–260) µm) and ascospores (16–)18–22(–25) × (9–)10–14(–16) µm). However, the upper surface seems to be more whitish grey to blackish grey upper surface (vs. partly brown-grey to olive-grey or light brown) and growing on calcareous rock (vs. siliceous rock) (Nash et al. 2007). Also the apothecia seem to be more often surrounded by a swollen thallus ring. Conidia were not found in our specimen.

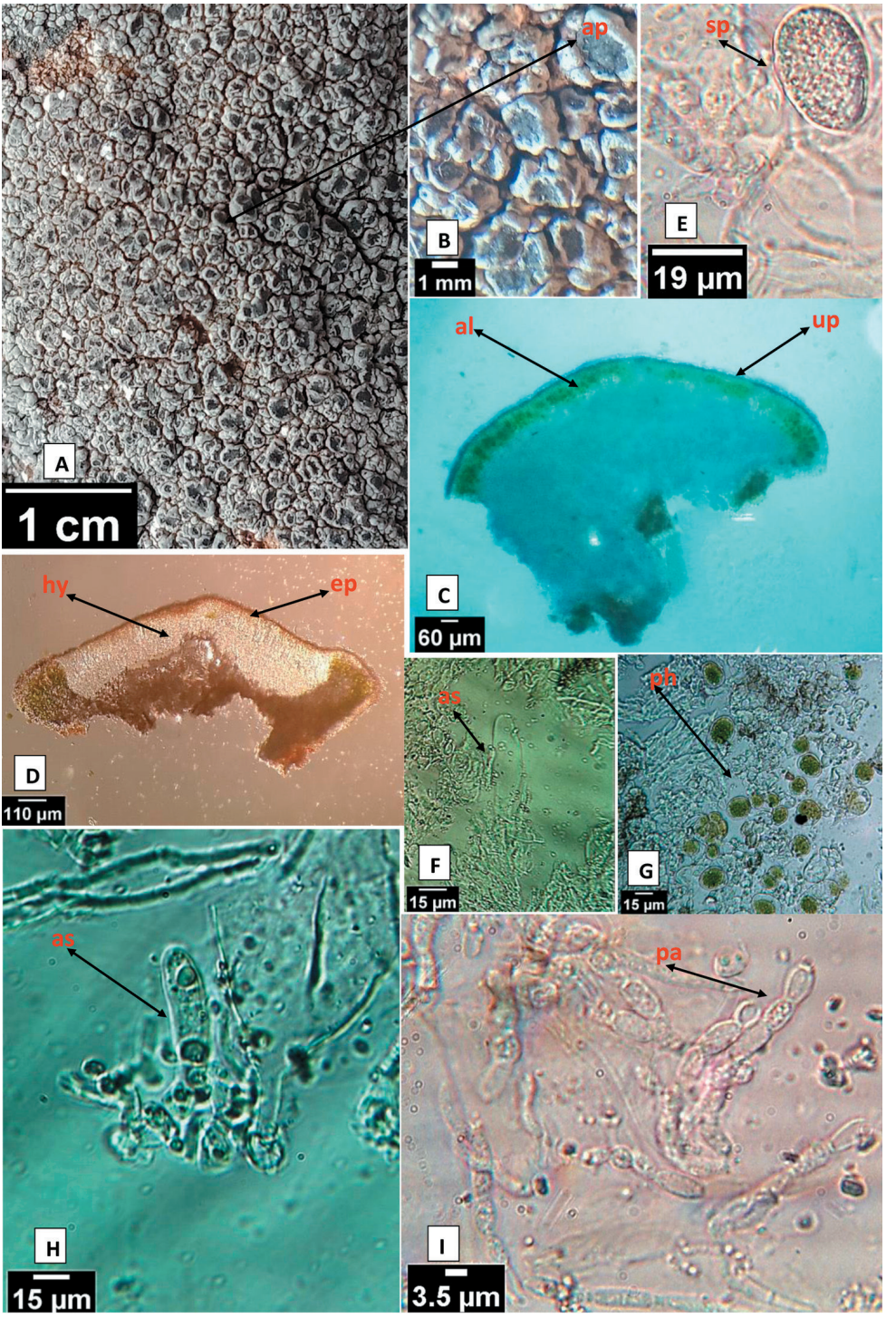

Fig. 2. *Aspicilia fumosa,* A–I: **A.** Thallus; **B.** Apothecia; **C.** Section of thallus; **D.** Cross section of apothecium; **E.** Ascospore; **F.** Asci; **G.** Photobiont cells; **H.** Asci with ascospores; **I.** Paraphyses.

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DECLARATIONS

Conflict of interest.—There are no conflicts of interest for the authors of this study.

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