
BOTANY

Acarospora sultanii sp. nov. (Acarosporaceae, Lichen Forming Ascomycota) from Darel Valley, Gilgit Baltistan, Pakistan

Muhammad Shahid Iqbal^a, * and Abdul Nasir Khalid^a

^a Fungal Biology and Systematics Research Laboratory, Institute of Botany,
University of the Punjab, Qaid-e-Azam Campus, Lahore, 54590 Pakistan

*e-mail: mshahidiqbal012@gmail.com

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Abstract—A new species in the genus *Acarospora* (Acarosporaceae, lichenized ascomycetes), from Darel Valley, Gilgit Baltistan, Pakistan, is described and illustrated here under the name *Acarospora sultanii*. It is characterized by brown to blackish white thallus, thicker epihymenium 40–60 µm, taller hymenium 120–160 µm, and larger ascospores 15–25 × 5–9 µm. Phylogenetic analysis based on ITS-nrDNA sequencing placed our species relative to *A. nodulosa*. Descriptions and images of the new species are provided, as well as a key for Pakistani species of *Acarospora*.

Keywords: phylogenetic analyses, taxonomy, lichen diversity, internal transcribed spacer

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INTRODUCTION

The lichen genus *Acarospora* A. Massal. (Acarosporaceae, Acarosporales, Lecanoromycetes) is characterized by polyspored asci with simple hyaline ascospores, ascomata that are immersed, pseudolecianorine or lecideine, and crustose thalli that are areolate to squamulose growing on rock, soil, and occasionally wood and anthropogenic substrates (Magnusson, 1929; Knudsen, 2007, 2021a). Many species of *Acarospora* are pioneers, and they are found all over the world, typically in xerothermic or arid habitats. The genus *Acarospora* is a nonmonophyletic group, according to phylogenetic analyses, and a multigene global survey will be necessary to elucidate its diversity and evolutionary relationships (Westberg et al., 2015; Knudsen et al., 2020, 2021a, 2021b, in press). The genus contains approximately 1080 species in Index Fungorum (Index..., 2022). From Pakistan, three species of *Acarospora* have been reported so far, *Acarospora anomala* H. Magan, *A. cervina* (A. Massal), *A. impressula* Th. Fr. (Aptroot & Iqbal, 2012). The purpose of this paper is to explore the taxonomy and distribution of the new species *Acarospora sultanii* from Darel Valley, Gilgit Baltistan, Pakistan.

MATERIALS AND METHODS

Morphological and Chemical Studies

For the purpose of observing morphological characters, a stereomicroscope was used (Meiji Techno, EMZ-5TR, Japan). For anatomical studies, free hand sections of the apothecia were cut, put on glass slides

with water, and examined and 5% KOH as mounting media (K), 10% water, and inspected under a compound microscope (MX4300H, Meiji Techno Co., Ltd., Japan). The secondary chemistry was examined using thin layer chromatography (with solvent C) and spot tests with potassium hydroxide KOH (10%) and calcium hypochlorite C-test reagents, in accordance with the methodology suggested by Orange et al. (2001).

Molecular Characterization

Dried materials were used to extract genomic DNA using the 2% CTAB method (Gardes & Bruns, 1993). The internal transcribed spacer (ITS) of the nrDNA was amplified using the ITS1F/ITS4 primer combination in accordance with the amplification technique (Khan et al., 2018). On a 1.2% agarose gel, the amplified DNA fragments could be seen, and the PCR results were sequenced (Sambrook & Russell, 2001). The Basic Local Alignment Search Tool (BLAST) network service of the National Center for Biotechnology Information (NCBI) was used to compare nucleotide sequences for phylogenetic analysis (Altschul et al., 1990). The MAFFT v7 tool was used to perform multiple sequence alignment with all parameters set to default values (Katoh & Standley, 2013). The start and end of alignments at a conserved site are condensed. Gaps were treated as missing data. Phylogenetic analysis was performed in MEGA 6 by creating Maximum Likelihood (ML) trees at 1000 Bootstraps based on the Kimura 2 model (Tamura et al., 2013). As

Table 1. Voucher specimens and NCBI GenBank accession numbers of the sequences used in the phylogenetic analysis

Species names	ITS GenBank accession numbers	Voucher name	Origin
<i>Acarospora nodulosa</i>	MK123362	20120619-04-02	China
<i>Acarospora nodulosa</i>	MK503440	18-59300	China
<i>Acarospora nodulosa</i>	MK502188	18-59677	China
<i>Acarospora nodulosa</i>	MK501747	20151180	China
<i>Acarospora nodulosa</i>	MK503442	18-58662	China
<i>Acarospora nodulosa</i>	MN511030	—	Spain
<i>Acarospora nodulosa</i>	MN511038	—	Spain
<i>Acarospora nodulosa</i>	MN511040	—	Spain
<i>Acarospora nodulosa</i>	MN511024	—	Spain
<i>Acarospora nodulosa</i>	LN810789	Westberg 10-215 (S F177732)	Spain
<i>Acarospora nodulosa</i>	MN511065	—	Spain
<i>Acarospora nodulosa</i>	MN511041	—	Spain
<i>Acarospora nodulosa</i>	MN511029	—	Spain
<i>Acarospora nodulosa</i>	MN511025	—	Spain
<i>Acarospora placodiiformis</i>	MN511168	—	Spain
<i>Acarospora placodiiformis</i>	MN511164	—	Spain
<i>Acarospora placodiiformis</i>	MN511165	—	Spain
<i>Acarospora placodiiformis</i>	MN511166	—	Spain
<i>Acarospora placodiiformis</i>	MN511167	—	Spain
<i>Acarospora placodiiformis</i>	LN810795	Westberg 10-211 (S F177733)	Spain
<i>Acarospora sultanii</i>	OP950856	LAH37629	Pakistan
<i>Acarospora schleicheri</i>	LN810801	Sweat & Yansky KGS1196 (UPS L-162697)	USA
<i>Acarospora schleicheri</i>	MH555388	Leavitt 16-548 BRY-C	USA
<i>Acarospora schleicheri</i>	HQ650721	—	—
<i>Acarospora schleicheri</i>	DQ525529	—	—
<i>Acarospora schleicheri</i>	MZ922059	Leavitt 19092	USA
<i>Acarospora schleicheri</i>	ON794213	Knudsen 15599	USA
<i>Acarospora schleicheri</i>	MK503461	20120924	China
<i>Acarospora schleicheri</i>	MF134872	20120924	China
<i>Glypholecia scabra</i>	FJ919810	—	—
<i>Glypholecia scabra</i>	FJ919809	—	—
<i>Pycnora sorophora</i>	MN483141	Sebernegg s.n. & Mayrhofer, 05 May 2011 (GZU)	Austria

an outgroup, *Pycnora sorophora* (Vain.) (MN483141) was selected. Table 1 lists the samples' details as well as the GenBank Accession numbers.

RESULTS

Phylogenetic Analysis

The new ITS nrDNA sequences are nested within the phylogenetic branch of the genus *Acarospora*, representing the species unknown yet, described here as *Acarospora sultanii* sp. nov. Altogether 32 ITS rDNA sequences were analyzed including sequences from GenBank. There were 457 characters in the alignment

file of which 351 were conserved, 104 variables, 24 parsimony informative and 80 were singleton variants. The closest sequences of *Acarospora nodulosa* showing 84% similarity in the tree which are separate from our described species.

Taxonomy

Acarospora sultanii M.S. Iqbal & Khalid sp. nov.
MYCOBANK # MB846808.

Etymology. The epithet “*sultanii*” honors the renowned Pakistani pioneer mycologist Sultan Ahmad (1910–1983).

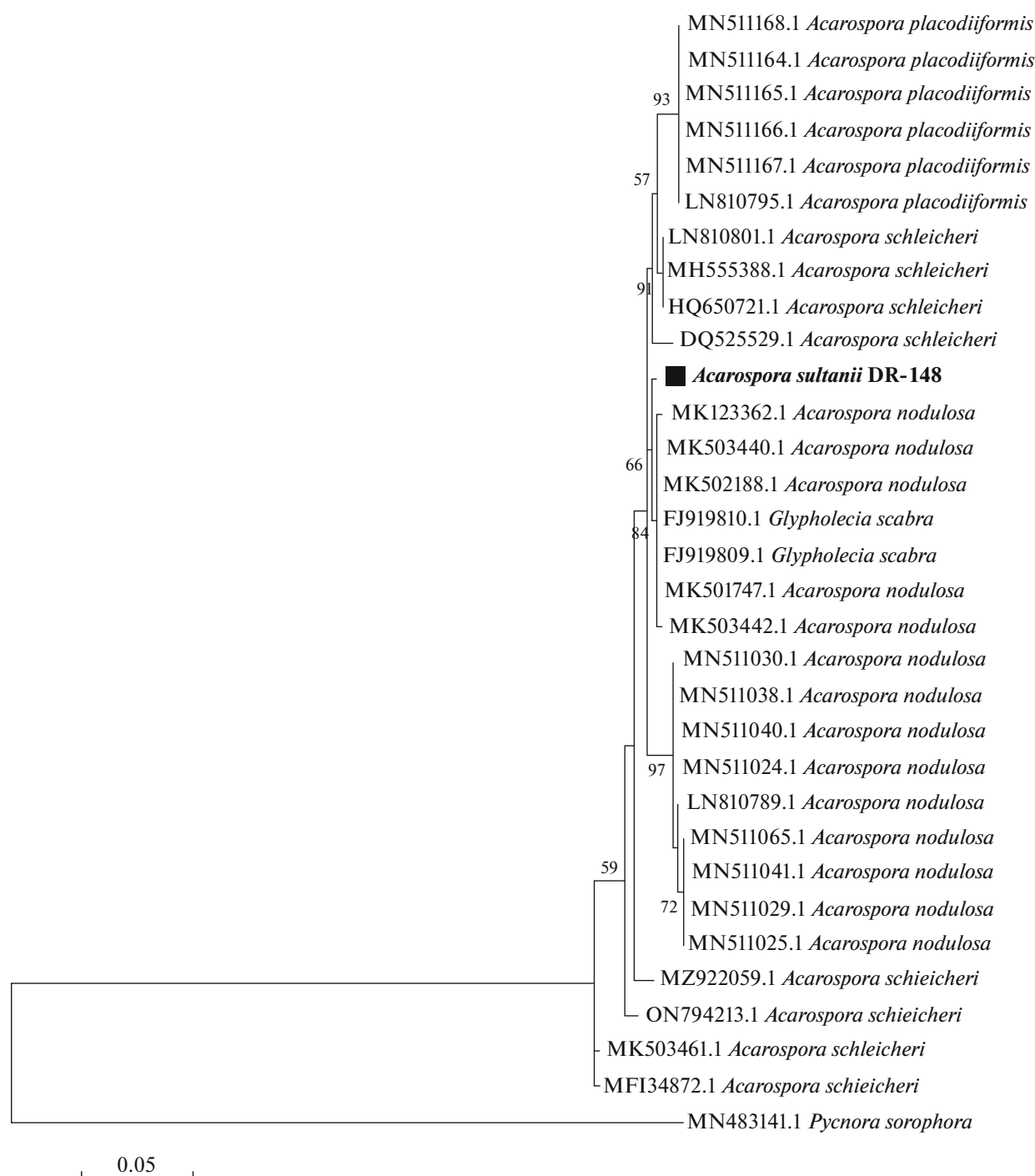


Fig. 1. Most likely phylogenetic relationship of ■ *Acarospora sultanii* sp. nov. with their associated taxa inferred from the nrITS region on rooting with *Pycnora sorophora* (MN483141) as an outgroup.

Diagnosis. *Acarospora sultanii* differs from its closely related species brown to blackish white thallus, thicker epihymenium 40–60 μ m, taller hymenium 120–160 μ m, and larger ascospores 15–25 $\times$ 5–9 μ m.

Holotype. Pakistan: Gilgit Baltistan, Darel Valley 35°37' N, 73°27' E, elev. 1843 m, on rocks, October 21, 2020, Muhammad Shahid Iqbal DR-148

(LAH37629), (ITS GenBank accession number OP950856).

Description. Thallus: crustose, squamulose-areolate, obscure castaneous, indeterminate, effuse, mostly enlarged patches, dull, somewhat shiny, epruinose, 13–14 cm in length. **Squamules:** 1–1.5 mm, up to 0.4 mm thick, often opaque, contiguous, lobate,

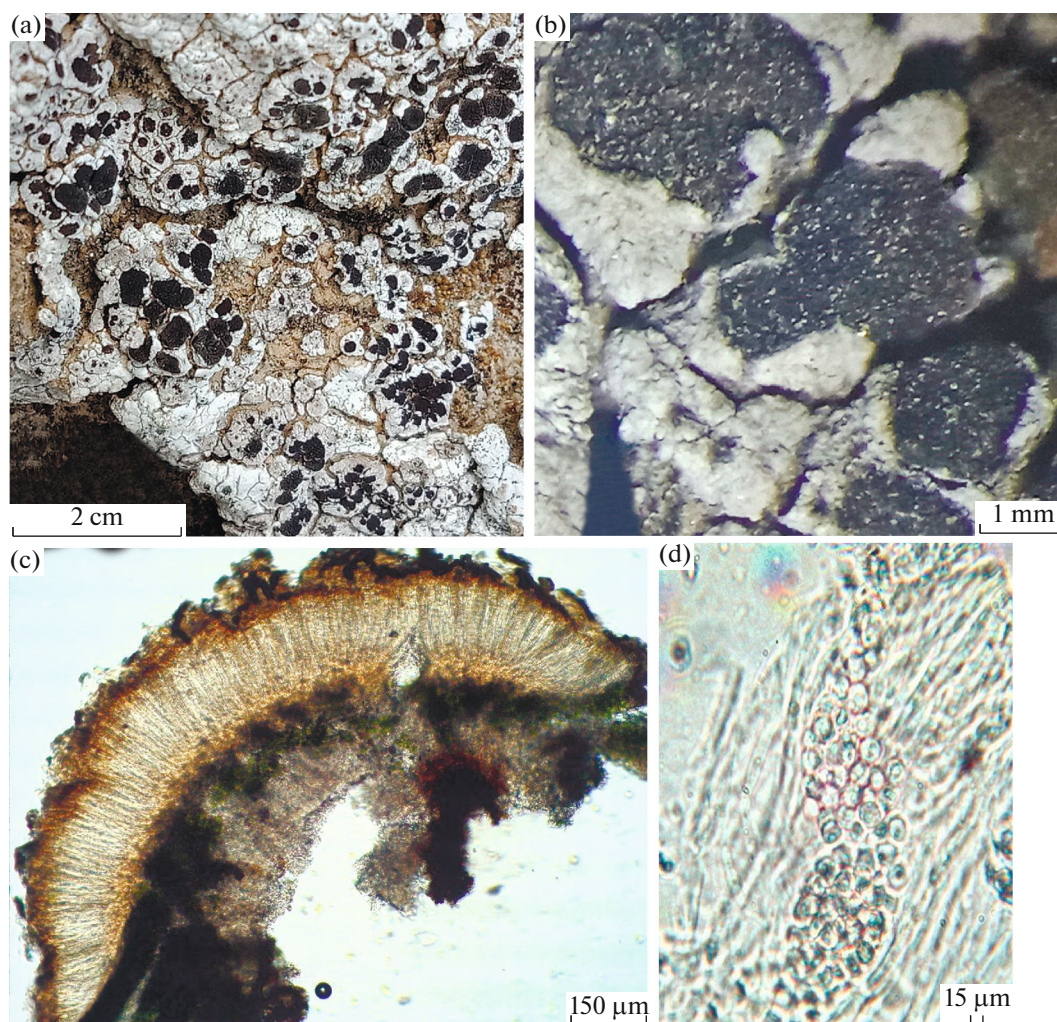


Fig. 2. (a) Habitus of *Acarospora sultanii* (Holotype, LAH37629); (b) Apothecia on Thallus; (c) Cross section of Apothecia; (d) Ascospores.

often imbricate. **Lobes:** round to indistinct, clear. **Colour:** brown to blackish white when dry, no changes when wet.

Upper cortex. 40–60 μm thick, paraplectenchymatous, globularis, upper layer dark brown, lower layer hyaline initial later off brown, cell 8–12 μm in diam. **Algal layer:** continuous, 70–140 μm thick, algal cells 6–18 μm in diam. **Medullary layer:** 40–150 thick, hyphae 1.6–3.4 μm in diam.

Apothecia: frequent, 1–4 per squamule, usually one per squamule, rounded to immersed, plane to concave, umbonate. **Disc:** black, rough, 0.3–1.5 mm across, epruinose. **Margins:** prominent, above thallus level, concolorous to disc, continuous, smooth. **Parathecium:** initially expanding around disc 20–40 μm thick outer part light blackish to somewhat glossy and inner part colorless. **Epihymenium:** off brown to dark brown, 40–60 μm tall, no change KOH. **Hymenium:** 120–160 μm tall, hyaline. **Paraphyses:** branched, 3–4 μm wide, apices unexpanded up to 4 μm wide. **Sub-**

hymenium: 20–40 μm thick, hyaline or light brown yellow. **Hypothecium:** 50–155 μm tall. **Asci:** clavate, 40–85 $\times$ 8–20 μm , multi-spored. **Ascospores:** simple, hyaline, broad to narrowly ellipsoid, 15–25 $\times$ 5–9 μm , no change in KOH. **Pycnidia:** absent. **Conidia:** absent.

Spot test: K–, C–, KC–.

Secondary metabolites: no substance detected.

Ecology. With an average annual precipitation of 100 to 300 mm, with the majority of it snow in the winter and early spring, saxicolous was discovered on the bark of coniferous trees in a dry temperate climate, fully exposed to sunlight and rain. The predominant tree species in the area are *Cedrus deodara* (Roxb. ex Lambert) G. Don, *Pinus wallichiana* A.B. Jacks, *Picea smithiana* Boiss, and *Abies pindrow* Royle. The average summer temperature is 35°C, and the average winter temperature is between and –10°C.

DISCUSSION

Our species is morphologically similar to *A. nodulosa* from China, as both species have squamulose-areolate thallus, rounded to immersed apothecia with epruinose disc of apothecia. Phylogenetically, *A. nodulosa* and *A. sultanii* are found to be strongly supported relative taxa (84% BS) (Fig. 1). The two species can be easily segregated by the character of apothecia. The apothecia of the Pakistani taxon are frequent, 1–4 per squamule, usually one per squamule, immersed, plane to concave, umbonate, 0.3–1.5 mm across, blackish rough surface when dry, no change when wet while the apothecia of the Chinese species are up to 1.3 mm across, usually one or more per areole, sometimes smaller apothecia merging into a single apothecium; sometimes apothecium elevated above squamule and lecanorine, pale red and rough surface of disc. The differences between both species also include the thicker epihymenium 40–60 µm vs. (10 µm), taller hymenium 120–160 µm vs. (50–150 µm), larger size ascospores 15–25 × 5–9 µm vs. (4–6 × (2–)3–3.6 µm), no substance detected vs (norstictic acid). Morphologically, the new species can be easily distinguished from the other species of genus with brown to blackish- white thallus i.e., *A. placodiiformis*, *A. schleicheri* and *G. scabra* by its thicker epihymenium 40–60 µm, thicker hymenium 120–160 µm, and larger ascospores 15–25 × 5–9 µm.

An Updated Key to the Species of Acarospora from Pakistan

Epihymenium 40–60 µm, hymenium 120–160 µm, size of ascospores 15–25 × 5–9 µm *A. sultanii*

Epihymenium 20–30 µm, hymenium 70–110 µm, ascospores 3–5 × 1–2 µm *A. pakistanica*

Size of ascospores 3–6 × 1–2 µm *A. badiofusca*

Epihymenium 10–20 µm, hymenium 130–160 µm, size of ascospores 3–5 × 1.8–2.8 µm ... *A. kashmiriana*

Epihymenium 21–50 µm, hymenium 110–154 µm, size of ascospores 1.5–2 × 1.1–1.5 µm ... *A. kohistaniensis*

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COMPLIANCE WITH ETHICAL STANDARDS

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.

REFERENCES

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J., Basic local alignment search tool, *J. Mol. Biol.*, 1990, vol. 215, no. 3, pp. 403–410.
- Aptroot, A. and Iqbal, S.H., Annotated checklist of the Lichens of Pakistan, with reports of new records, *Herzogia*, 2012, vol. 25, no. 2, pp. 211–229.
- Gardes, M. and Bruns, T.D., ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts, *Mol. Ecol.*, 1993, vol. 2, no. 2, pp. 113–118.
<https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Katoh, K. and Standley, D.M., MAFFT multiple sequence alignment software version 7: improvements in performance and usability, *Mol. Biol. Evol.*, 2013, vol. 30, no. 4, pp. 772–780.
- Khan, M., Khalid, A.N., and Lumbsch, H.T., A new species of Lecidea (Lecanorales, Ascomycota) from Pakistan, *MycoKeys*, 2018, vol. 38, p. 25.
- Knudsen, K., Acarospora, in *Lichen Flora of the Greater Sonoran Desert Region*, Tempe, 2007, vol. 3, pp. 1–38.
- Knudsen, K., Kocourková, J., Cannon, P., Coppins, B., Fletcher, A., and Simkin, J., Acarosporales: Acarosporaceae, including the genera *Acarospora*, *Caeruleum*, *Myriospora*, *Pleopsidium*, *Sarcogyne* and *Trimmatothelopsis*, *Rev. Br. Ir. Lichens*, 2021a, vol. 12, pp. 1–25.
- Knudsen, K., Kocourková, J., Hodková, E., Malíček, J., and Wang, Y., Acarosporaceae of the Chihuahuan Desert: four Magnusson species saved from synonymy and a new yellow species, *Bryologist*, 2021b, vol. 124, pp. 533–551.
<https://doi.org/10.1639/0007-2745-124.4.533>
- Knudsen, K., Kocourková, J., Hodková, E., Malíček, J., and Wang, Y., Acarosporaceae of New Mexico: ten new species of *Acarospora* and *Sarcogyne*, *Bryologist* (in press).
- Magnusson, A.H., *A Monograph of the Genus Acarospora*, Kungliga Svenska Vetenskaps: Akademiens Handlingar, 1929, ser. 3, 7.
- Orange, A., James, P.W., and White, F.J., *Microchemical Methods for the Identification of Lichens*, British Lichen Society, 2001.
- Sambrook, J. and Russel, D.W., Rapid isolation of yeast DNA, in *Molecular Cloning: A Laboratory Manual*, Sambrook, J. and Russel, D.W., Eds., New York: Cold Spring Harbor Laboratory Press, 2001, pp. 631–632.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S., MEGA6: molecular evolutionary genetics analysis version 6.0, *Mol. Biol. Evol.*, 2013, vol. 30, no. 12, pp. 2725–2729.
- Westberg, M., Millanes, A.M., Knudsen, K., and Wedin, M., Phylogeny of the Acarosporaceae (Lecanoromycetes, Ascomycota, Fungi) and the evolution of carbonized ascomata, *Fungal Diversity*, 2015, vol. 73, pp. 145–158.
<https://doi.org/10.1007/s13225-015-0325-x>