

# A New Species of Lichen Genus *Acarospora* (Acarosporaceae, Lichen Forming Ascomycota) from Darel Valley, Gilgit Baltistan, Pakistan

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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 pakistanica*. It is characterized by thicker epihymenium 30–60 µm, taller hymenium 100–150 µm, and larger ascospores 5–7 × 1–3 µm. A detailed description of this taxon is provided.

**Keywords:** evolutionary relationships, taxonomy, novel species, geographic distribution

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## INTRODUCTION

The lichen genus *Acarospora* A. Massal. (Acarosporaceae, Acarosporales, Lecanoromycetes) is distinguished by simple hyaline ascospores, polyspored asci, submerged ascomata, pseudolecanorine or lecideine, and crustose thalli that are areolate to squamulose (Magnusson, 1929; Knudsen, 2007, 2021a). *Acarospora* species are widespread pioneers that are often found in xerothermic or dry settings. According to phylogenetic analyses, the genus *Acarospora* is a non-monophyletic group, thus a multigene worldwide survey will be required to clarify 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, four species of *Acarospora* have been reported so far, *Acarospora anomala* H. Magan, *A. cervina* (A. Massal), *A. impressula* Th.Fr. and *A. sultanii* M.S. Iqbal & Khalid (Aptroot and Iqbal, 2012; Iqbal et al., 2022, in press). This study aims to investigate the taxonomy and geographic distribution of the novel species of genus *Acarospora* from Darel Valley, Gilgit Baltistan, Pakistan.

## MATERIALS AND METHODS

### *Morphological and Chemical Studies*

A stereomicroscope was utilized to observe morphological characteristics (Meiji Techno, Japan, EMZ-5TR). Free hand sections of the apothecia were cut, placed on glass slides with water, and viewed using a compound microscope while also being mounted with 5% KOH as mounting media (K) and 10% water (MX4300H, Meiji Techno Co., Ltd., Japan). Using

thin layer chromatography (with solvent C) and spot tests with potassium hydroxide KOH (10%) and calcium hypochlorite C-test reagents, the secondary chemistry was investigated. Orange et al. (2001).

### *Molecular Characterization*

The 2% CTAB approach was used to extract genomic DNA from dried materials (Gardes & Bruns, 1993). According to the amplification method, the internal transcribed spacer (ITS) of the nrDNA was amplified using the ITS1F/ITS4 primer pair (Khan et al., 2018). The amplified DNA fragments were visible on a 1.2% agarose gel, and the PCR outcomes were sequenced (Sambrook & Russell, 2001). Nucleotide sequences were compared for phylogenetic analysis using the Basic Local Alignment Search Tool (BLAST) network service of the National Center for Biotechnology Information (NCBI) (Altschul et al., 1990). Multiple sequence alignment was carried out using the MAFFT v7 programme, with all parameters left at their default settings (Katoh & Standley, 2013). At a conserved site, alignment starts and ends are compressed. The absence of data was viewed as a gap. By building Maximum Likelihood (ML) trees at 1000 Bootstraps based on the Kimura 2 model, phylogenetic analysis was carried out in MEGA 6 software (Tamura et al., 2013). As an outgroup, *Pycnora soro-phora* (Vain.) (MN483141) was selected. Table 1 lists the samples' details as well as the GenBank Accession numbers.

**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 americana</i>	MH555373	Leavitt 16-589 BRY-C	USA
<i>Acarospora americana</i>	MH555374	53542 BRY-C	USA
<i>Acarospora americana</i>	MH555372	Leavitt 18-019 BRY-C	USA
<i>Acarospora anomala</i>	LN810758	Westberg 10-106 (S)	Sweden
<i>Acarospora anomala</i>	LN810759	Westberg 10-108 (S)	Sweden
<i>Acarospora bullata</i>	GU184112	Huneck 14 Apr 1991 (B 60 0118983)	Germany
<i>Acarospora bullata</i>	GU184110	Huneck-Poelt 21-5-1993 (GZU)	Germany
<i>Acarospora bullata</i>	DQ374126	Lumbsch & Huneck (BM)	Germany
<i>Acarospora badiofusca</i>	GU184121	Reeb VR 2-IX-00/23 (DUKE)	Spain
<i>Acarospora badiofusca</i>	LN810762	Nordin 5552 (UPS L-124833)	Sweden
<i>Acarospora badiofusca</i>	GU184120	Reeb VR 9-VII-98/11 (DUKE)	France
<i>Acarospora badiofusca</i>	LN810763	Nordin & Owe-Larsson 36 (UPS)	Sweden
<i>Acarospora bohlinii</i>	MK495370	16-51001	China
<i>Acarospora bohlinii</i>	MK494182	16-51041	China
<i>Acarospora bohlinii</i>	MK500837	16-53261	China
<i>Acarospora bohlinii</i>	MK494916	16-53603	China
<i>Acarospora bohlinii</i>	MK372063	20151034	China
<i>Acarospora bohlinii</i>	MK496510	18-58493	China
<i>Acarospora bohlinii</i>	MK500835	18-58199	China
<i>Acarospora bohlinii</i>	MK496051	18-58591	China
<i>Acarospora cervina</i>	KY439884	—	Iran
<i>Acarospora cervina</i>	KY439880	—	Iran
<i>Acarospora fuscata</i>	DQ374128	Purvis (BM)	United Kingdom
<i>Acarospora fuscata</i>	DQ374127	Crewe 58 (UPS)	Sweden
<i>Acarospora fulva</i>	MF134868	2014052A	China
<i>Acarospora fulva</i>	MK500857	2014052A	China
<i>Acarospora fulva</i>	MK500844	20130235	China
<i>Acarospora gwynnii</i>	MF138061	Sancho (MAF-lich 21272)	Antarctica
<i>Acarospora gwynnii</i>	MF138063	Sancho (MAF-Lich 21274)	Antarctica
<i>Acarospora gwynnii</i>	MF138062	Sancho (MAF-Lich 21270)	Antarctica
<i>Acarospora glaucocarpa</i>	MK811746	O-L-201295	Norway
<i>Acarospora impressula</i>	LN810776	Westberg 08-107 (S F121708)	Norway
<i>Acarospora irregularis</i>	MK996290	ACA73	—
<i>Acarospora insolata</i>	LN810777	Westberg 06-022 (LD)	Sweden
<i>Acarospora laqueata</i>	DQ842014	—	—
<i>Acarospora laqueata</i>	LN810778	Westberg 10-170 (S F177761)	Switzerland
<i>Acarospora malouina</i>	MF138059	MSC:Fryday 11338	Falkland Islands
<i>Acarospora malouina</i>	MF138056	MSC:Fryday 11351	Falkland Islands
<i>Acarospora malouina</i>	MF138058	MSC:Fryday 11346	Falkland Islands
<i>Acarospora nodulosa</i>	MK502188	18-59677	China
<i>Acarospora nodulosa</i>	MK503440	18-59300	China
<b><i>Acarospora pakistanica</i></b>	<b>OQ108365</b>	<b>LAH37628</b>	<b>Pakistan</b>
<i>Acarospora pulvinata</i>	MK503441	18-58401	China
<i>Acarospora rosulata</i>	MK996282	ACA40	—

Table 1. (Contd.)

Species names	ITS GenBank Accession Numbers	Voucher name	Origin
<i>Acarospora rugulosa</i>	LN810799	Westberg 10-099 (S F177975)	Sweden
<i>Acarospora rugulosa</i>	LN810798	Westberg 08-119 (S F123671)	Norway
<i>Acarospora rugulosa</i>	DQ374134	Foucard 3322 (UPS)	Sweden
<i>Acarospora rugulosa</i>	DQ374147	Wedin 6548 (UPS)	Sweden
<i>Acarospora rugulosa</i>	EU870642	UPS:Wedin 6527	Sweden
<i>Acarospora schleicheri</i>	MF134872	20120924	China
<i>Acarospora socialis</i>	MH555370	56088B BRY-C	USA
<i>Acarospora socialis</i>	MH555369	56088A BRY-C	USA
<i>Acarospora stapfiana</i>	MF134871	20130040	China
<i>Acarospora stapfiana</i>	MF134870	20110389	China
<i>Acarospora sinopica</i>	DQ374139	Agren 388 (UPS)	Sweden
<i>Acarospora sinopica</i>	DQ374138	Tibell 22676 (UPS)	Sweden
<i>Acarospora strigata</i>	MH555402	Leavitt 6791 BRY-C	USA
<i>Acarospora strigata</i>	MH555390	Leavitt 17-114 BRY-C	USA
<i>Acarospora tianshanica</i>	MK503500	20179371	China
<i>Acarospora tianshanica</i>	MG838742	20130069	China
<i>Acarospora umbilicata</i>	LN810808	Tibell 23532 (UPS L-136981)	Sweden
<i>Acarospora versicolor</i>	LN890277	Westberg 08-205 (S F268463)	Norway
<i>Acarospora versicolor</i>	LN890276	Westberg 08-092 (S F268460)	Norway
<i>Acarospora wahlenbergii</i>	MK812641	O-L-184363	Norway
<i>Acarospora wahlenbergii</i>	MK811926	O-L-184419	Norway
<i>Pycnora sorophora</i>	MN483141	Sebernegg s.n. & Mayrhofer, 05 May 2011 (GZU)	Austria

## 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 pakistanica* sp. nov. Altogether 66 ITS rDNA sequences were analyzed including sequences from GenBank. There were 522 characters in the alignment file of which 296 were conserved, 218 variables, 185 parsimony informative and 33 were singleton variants. The closest sequences of *Acarospora bohlinii* showing 99% and *Acarospora laqueata* 87% similarity in the tree which are separate from our described species.

*Taxonomy*

*Acarospora pakistanica* M.S. Iqbal, Abbas & Khalid sp. nov.

MYCOBANK # MB 847011.

**Etymology.** The epithet “*pakistanica*” refers to the type locality Pakistan.

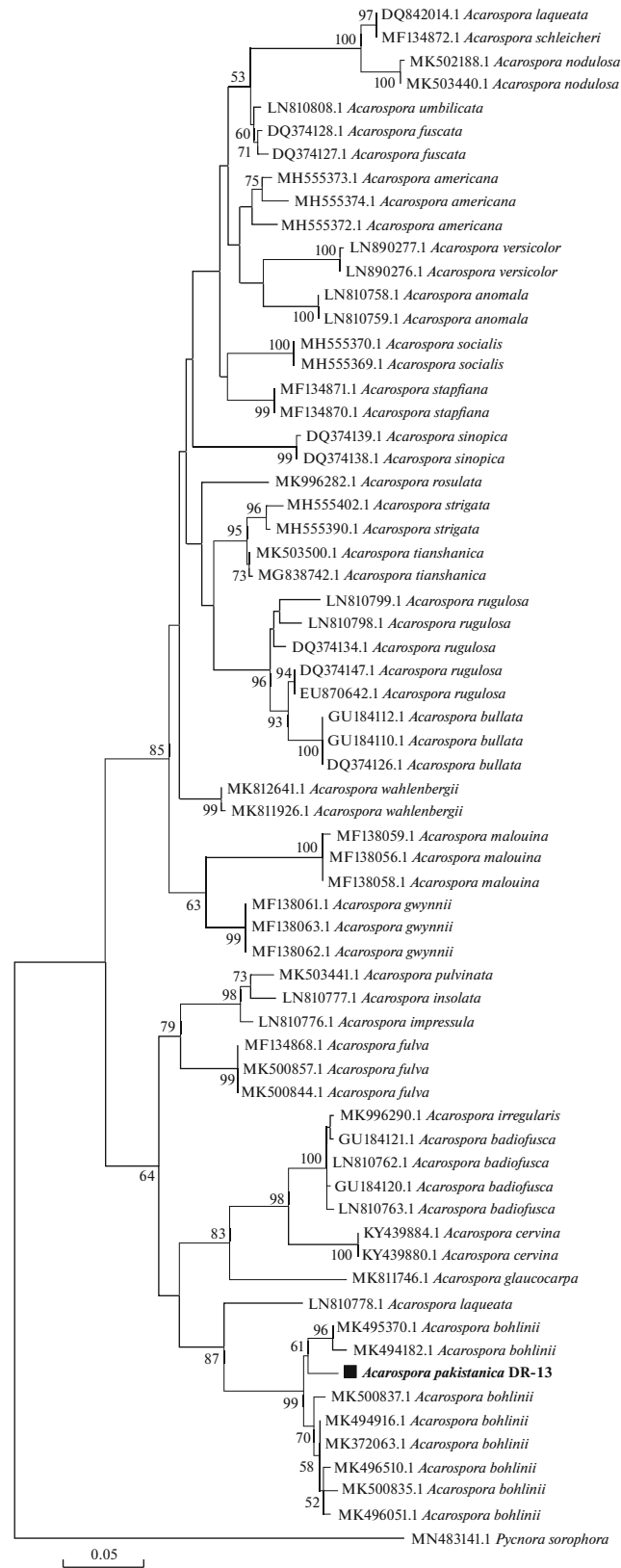
**Diagnosis.** *Acarospora pakistanica* differs from its closely related species by thicker epihymenium 30–

60 µm, taller hymenium 100–150 µm, and larger ascospores 5–7 × 1–3 µm.

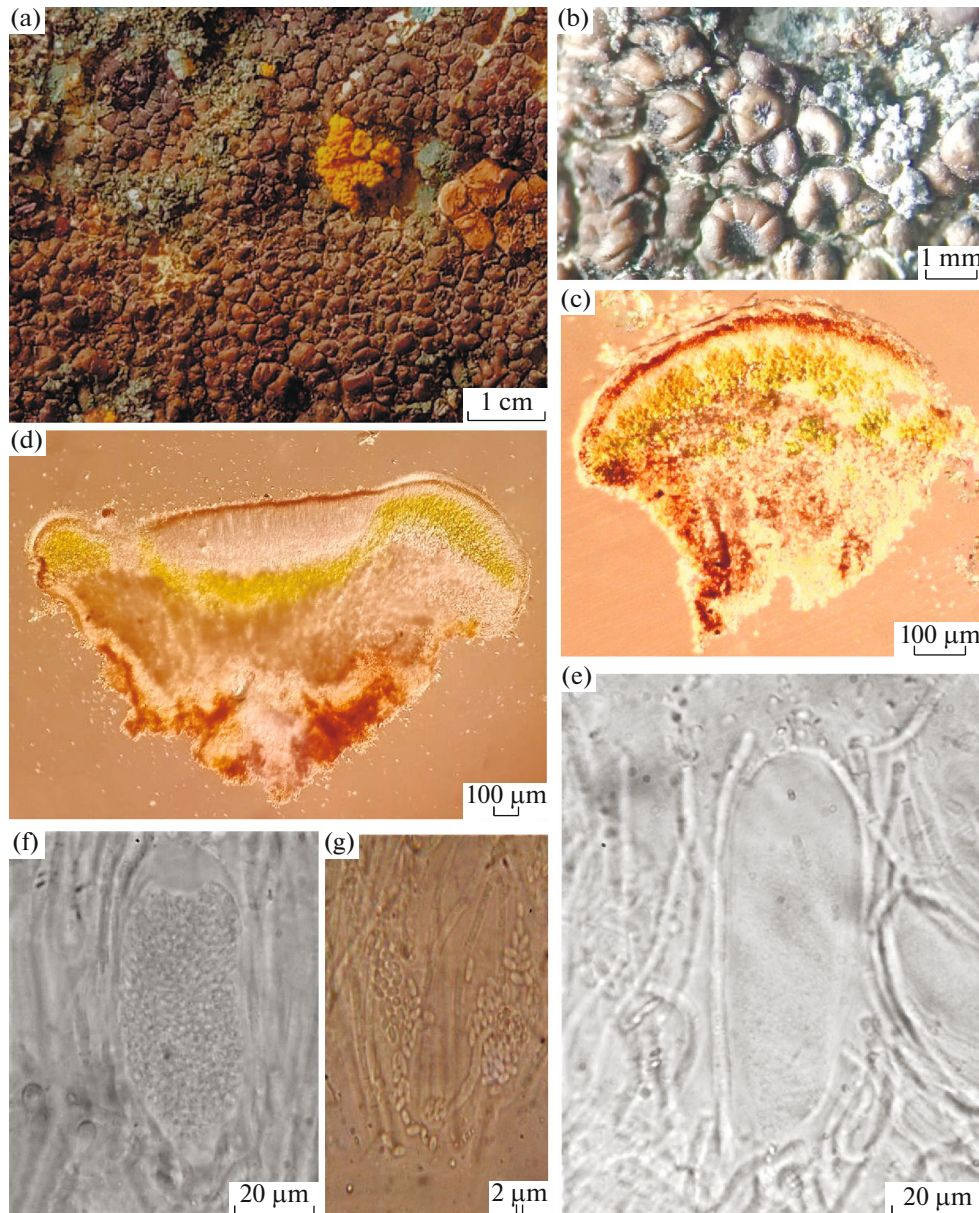
**Holotype.** Pakistan: Gilgit Baltistan, Darel Valley 35°37' N, 73°27' E, elev. 1843 m, on rocks, October 21, 2020, Muhammad Shahid Iqbal DR-13 (LAH37628), (ITS GenBank accession number OQ108365).

**Description.** **Thallus:** crustose, saxicolous, areolates, 4 cm across. **Areole:** round to irregular, 0.3–0.6 mm across, swollen, plane to convex, determinate, angular. **Color:** off-brown to grey, unchanged when wet. **Upper Surface:** smooth, slightly pruinose, off-brown to greyish, dull, unchanged when wet. **Cortex:** 10–20 µm wide, pale brown to greyish, paraplectenchymatous, cells 13–16 µm in diameter, rounded, sometime shining and darker at the ends of margins. **Medulla:** 30–60 µm, hyphae thick, white, 2.3–2.7 µm in width. **Algal layer:** even, continuous, 100–150 µm, photobiont 10–14 µm in diameter. **Apothecia:** brownish, lecideine, 0.5–1 mm, frequent, superficial, concolorous with thallus. **Disc:** pale brown to grey, unchanged when wet, darker at the center of thallus, pruinose, smooth. **Margins:** thick, prominent, continuous, concolorous to disc.

**Epihymenium:** off-brown, 30–60 µm high. **Hymenium:** hyaline, 100–150 µm. **Hypothecium:** colourless,



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



**Fig. 2.** (a) Habitus of *Acarospora pakistanica* (Holotype, LAH37628), (b) Apothecia on thallus; (c) Section of Thallus; (d) Cross section of Apothecia; (e) Ascus; (f) Ascus with Ascospores; (g) Ascospores.

10–50 µm high. **Paraphyses:** apex swollen, capitate, branched, anastomosing, simple,  $50\text{--}80 \times 2\text{--}3$  µm. **Asci:** clavate, multisporous,  $60\text{--}90 \times 20\text{--}30$  µm. **Ascospores:** off-brown,  $5\text{--}7 \times 1\text{--}3$  µm.

**Substrate:** Rock.

**Spot test:** K–, C–, KC–.

**Secondary metabolites:** no substance detected.

#### DISCUSSION

Our species is morphologically similar to *A. laqueata* from Switzerland, as both species have crustose areolate thallus with angular areole, coun-

tinuous algal layer. Phylogenetically, *A. laqueata* and *A. pakistanica* are found to be strongly supported relative taxa (87% BS) (Fig. 1). The two species can be easily segregated by the character of apothecia. The apothecia of the Pakistani taxon are brownish, lecidine, 0.5–1 mm, frequent, superficial, concolorous with thallus, pale brown to grey, unchanged when wet, darker at the center of thallus, pruinose, smooth disc while the apothecia of the Switzerland species are cryptolecanorine, immersed, 1–2(–5) per areole, 0.2–0.6 mm across, round or angular, with a concave, reddish brown to brown-black disc. The differences between both species also include the taller hymenium

100–150  $\mu\text{m}$  vs. (80–120  $\mu\text{m}$ ), larger ascospores 5–7  $\times$  1–3  $\mu\text{m}$  vs. (4–6  $\times$  1.5–3  $\mu\text{m}$ ), smaller hypothecium 10–50  $\mu\text{m}$  vs. (30–100  $\mu\text{m}$ ). Morphologically, the new species can be easily distinguished from the other species of genus by its thicker epihymenium 30–60  $\mu\text{m}$ , taller hymenium 100–150  $\mu\text{m}$ , and larger ascospores 5–7  $\times$  1–3  $\mu\text{m}$ .

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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>
- Iqbal, M.S., Abbas, Z., and Khalid, N., *Acarospora sultanii* sp. nov. (Acarosporaceae, lichen forming Ascomycota) from Darel Valley, *Gilgit Baltistan, Pakistan*, 2022 (in press).
- 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, *MycKeys*, 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>