A new species of the genus *Anamylopsora* (*Baeomycetaceae; Ascomycota*) from Deosai National Park, Gilgit-Baltistan, Pakistan

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Abstract

A novel lichen species occurring on rocks was collected from three different localities within Deosai National Park, Gilgit-Baltistan, Pakistan. Phylogenetic analyses of the nrDNA ITS and nuLSU regions revealed that it clustered within the genus *Anamylopsora*. Further chemical and morpho-anatomical analyses confirmed its uniqueness, and it is described here as a new species under the name *A. pakistanica*. The distinguishing characters are: an irregularly squamulose appressed thallus on rocks without rhizines; an epinecral layer up to 25 μm thick; ascospores that are hyaline, simple, thick-walled with a smooth surface; septate paraphyses with a pigmented apical cell in a gel-like matrix; globose to subglobose pycnidia with hyaline and bacilliform pycnidiospores. In particular, the species is distinguished from other members of the genus by morpho-anatomical features including the coloration of the thalli, the presence of a thick lower cortex (up to 100 μm), and the presence of simple, thick-walled ascospores. Specimens were found at altitudes up to 4587 m, the highest elevation yet reported for *Anamylopsora*. A key and comparison to all existing species of the genus *Anamylopsora* is also given.

Keywords: *Anamylopsoraceae*; arctic-alpine; Asia; lichens; systematics

(Accepted 11 May 2023)

Introduction

The genus *Anamylopsora* Timdal was established in 1991 based on the type specimen of *A. pulcherrima* (Vain.) Timdal (Timdal 1991). Initially, it was described as *Lecidea pulcherrima* Vain. in 1888 (Vainio 1888) but then Elenkin transferred it to the genus *Psora* due to the saxicolous and squamulose characters of the thallus, renaming it *Psora pulcherrima* (Vain.) Elenkin (Elenkin 1904). Since the species also deviated from the genus *Psora* in a number of characteristics (e.g. having a non-amylloid tholus and hymenial gelatine, lacking anthraquinones in the hymenium, and having a different type of upper cortex and pycnidium), Timdal therefore established the monotypic new genus *Anamylopsora* in the family *Lecideaceae* (Timdal 1991). *Lecidea* (1984) had previously synonymized *Lecidea hedini* Magnusson with *L. pulcherrima* and when proposing the genus *Anamylopsora* he also synonymized *L. undulata* H. Magn. with *A. pulcherrima* (Timdal 1991). In 1995, Lumbsch then established a new family *Anamylopsoraceae*, which differed from the *Lecideaceae* and *Psoraceae* due to the presence of gynnecarpous ascoma development and stipitate apothecia (Lumbsch et al. 1995). Later, the family *Anamylopsoraceae* was synonymized with *Baeomycetaceae* based on multi-gene phylogenetic analyses and currently *Anamylopsora* is included in the family *Baeomycetaceae* (Baeomycetales) (Resl et al. 2015).

To date, three species of *Anamylopsora* are recognized: *A. altaica* Ahat et al. from China, *A. pruinosa* D. L. Liu & X. L. Wei from China, and *A. pulcherrima* (Vain.) Timdal from Russia and North America. All of these species have been reported from high-altitudinal regions at elevations up to 3900 m (Timdal 1991; Zuo et al. 2018; Ahat et al. 2019; Esslinger 2021). The Gilgit-Baltistan region of Pakistan, formerly known as the Northern Areas, is a highly mountainous region which includes parts of four great mountain ranges, namely the Himalaya, Hindukush, Karakoram and Pamir ranges. Besides mountains, this region is also famous for the spectacular Deosai Plateau, an almost isolated tract of land located north-west of Skardu and the neighbouring Kargil sector of Indian-administered Kashmir (Mock & O’Neill 2002). Deosai National Park is the second highest plateau in the world, covering an area of 2240 km² with an altitudinal range between 3500–5200 m a.s.l. and located between the Himalaya and Karakorum ranges in Pakistan (Usman et al. 2021). Previously, only a small number of lichens have been documented from the Deosai Plains including *Acarospora anatolica* H. Magn., *Psora himalayana* (C. Bab.) Timdal, *Psora vallesiaca* (Schaer.) Timdal and *Pyrenodesmia micromontana* (Frolow et al.) Hafellner & Türk (Knudsens & Kocourková 2015; Frolow et al. 2016; Hafellner & Türk 2016; Timdal et al. 2016). A further species, *Placidium deosaiense* Usman & Khalid, was also recently described from this locality (Usman et al. 2021). Here we describe another new species from Deosai National Park, based on phylogenetic analyses and the presence of unique morpho-anatomical and chemical characteristics. This study is a continuation of efforts to unveil the
lichen flora of high-altitude areas of Pakistan to provide information about biodiversity and support conservation efforts.

Material and Methods

Sampling site

Surveys were conducted in Deosai National Park and its adjacent areas during May and September 2019 as part of the Ph.D. research work of the corresponding author. For a more detailed description of the sampling site, see Usman et al. (2021). Vouchered specimens are deposited in the Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Morpho-anatomical and chemical studies

Methods for the examination of external morphology, macroscopic and microscopic characters and their measurements, and colour reactions of the thallus using potassium (K), sodium hypochlorite (C), sodium hypochlorite following potassium (KC) and Lugol’s solution (I) follow Usman et al. (2021). For detection of lichen secondary metabolites, thin-layer chromatography (TLC) with solvents A and G were used, as described by Orange et al. (2010). Measurements are given as (min⁻) ± SD (−max), where ‘min’ and ‘max’ are the extreme values observed,  the arithmetic mean and SD the standard deviation.

Molecular and phylogenetic studies

Nuclear DNA was extracted using a GF1 Plant DNA extraction kit according to the manufacturer’s instructions (Vivantis, Selangor Darul Ehsan, Malaysia). Primers used during amplifications were ITS1F and ITS4 for the ITS region, with LR0R and LR5 for the nuLSU region (White et al. 1990; Gardes & Bruns 1993). Polymerase chain reaction (PCR) conditions adapted from those of Gardes & Bruns (1993) were followed according to Usman & Khalid (2020). The PCR amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and then sent for sequencing at TsingKe, China, using the aforementioned primers.

Forward and reverse sequences of ITS and nuLSU regions were assembled using BioEdit v. 7.2.5 (Hall 1999) and compared with sequences on GenBank (https://www.ncbi.nlm.nih.gov/). A comprehensive representation of currently available sequences used for the phylogenetic analyses are presented in Table 1, together with GenBank Accession numbers, country distribution and reference. The sequences used in the ITS and LSU dataset were retrieved from GenBank based on inclusion of all published

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Table 1. Sequences of lichen taxa used in the ITS phylogenetic analyses of the genus Anamylopsora, with voucher information, GenBank Accession numbers and associated references. Newly generated sequences are shown in bold. * = outgroup.
sequences from the genus *Anamylopsora* (Zuo et al. 2018; Ahat et al. 2019) together with unpublished *Anamylopsora* sequence data showing 79% or greater nucleotide identity. Sequences of *Baeomyces rufus* (Huds.) Rebent. (AF448457 from China) were used as outgroup given that this is phylogenetically the closest genus to *Anamylopsora* and has been used as outgroup previously in *Anamylopsora* publications (Zuo et al. 2018; Ahat et al. 2019).

The final alignments were carried out in Clustal W implemented in BioEdit (Hall 1999). The maximum likelihood phylogram was inferred in RAxML-HPC2 using XSEDE (v. 8.2.10) with 1000 bootstrap replicates. The GTR + GAMMA nucleotide substitution model was used on the CIPRES Web Portal, following verification using jModelTest v. 2.1.6 and the Akaike information criterion (Akaile 1974; Darriba et al. 2012). Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2012). Newly generated sequences were deposited in GenBank and the sequence alignment files for the phylogenetic trees are available in the Supplementary Material (available online).

**Results**

During field sampling within Deosai National Park, an apparently novel lichen was identified on stones. Three thalli were collected from different locations for morphological and phylogenetic analyses (see below for precise locations). Sectioning revealed further details of the anatomy, as described below.

**Phylogenetic analyses**

DNA sequences from three different thalli (LAH37090, LAH37091, LAH37092) were successfully obtained after PCR amplification for the ITS (c. 625 bp) and nuLSU (c. 908 bp) regions. Distinct, well-supported clades were recovered for both the ITS (Fig. 1) and nuLSU regions (Supplementary Material Fig. S1, available online). There was no conflict in the unique position of our taxon in both trees, which was distinct from all previously submitted sequences in GenBank. Note that LSU sequences were not available for any of the previously published species of the genus *Anamylopsora*. Therefore, only unpublished sequences (which may be described in the future) obtained directly from GenBank on the basis of sequence similarity close to our taxon were included in the LSU phylogenetic tree (Supplementary Material Fig. S1). Clade names were provisionally assigned as described below.

The final ITS phylogram (Fig. 1) consisted of 25 sequences; 24 of these formed an ingroup clade B distinct from *Baeomyces rufus,*

![Figure 1](https://doi.org/10.1017/S002428292300018X) Published online by Cambridge University Press
which formed the outgroup clade A. Within clade B, clade C consisted of seven sequences belonging to *A. pruinosa* and four sequences (MN545147, MN545148, MN545149 and MN545150) named here as *Anamylopsora* sp. which are available from GenBank but so far unpublished in a formal publication. Clade E comprised two sequences of *A. pulcherrima*. Our novel taxon, named *A. pakistanaica* here, formed a separate clade G (containing all three thalli) alongside clade H. Clade H contained three sequences of *Anamylopsora altaica* and four unpublished sequences of *Anamylopsora*. It is noted that all previously reported sequences of species described from the genus *Anamylopsora* are different from our novel taxon, including *A. altaica, A. pruinosa* and *A. pulcherrima*, with differences of 44, 31 and 22 base pairs, respectively.

A separate phylogenetic tree was constructed based on LSU sequence data from available sequences in GenBank (Supplementary Material Table S1, available online). The LSU phylogram (Supplementary Material Fig. S1) consisted of nine sequences, eight of which formed an ingroup clade distinct from *Baeomyces Rufus* forming the outgroup clade. The analyses showed the separate position of our taxon in both phylogenetic trees, a position further supported by morpho-anatomical and chemical evidence as described below.

**Taxonomy**

*Anamylopsora pakistanaica* Usman & Khalid sp. nov.

MycoBank No.: MB 843629

Differing from *A. altaica* by having larger squamules, up to 3 mm diam. (vs normally ≤ 1 mm diam. for the latter), a light brown to dark brown upper surface (vs white to whitish grey), the presence of an epinecral layer up to 25 μm thick (vs absent), thick-walled ascospores with smooth surfaces (vs thin-walled with warty surfaces) and immersed, non-marginal pycnidia (vs marginal).

Type: Pakistan, Gilgit Baltistan, Deosai National Park, saxicolous, on calciferous rock, *c*. 4216 m a.s.l., 35°11.12′N, 75°12.99′E, 13 May 2019, M. Usman DEO-01 (LAH37090—holotype). GenBank Accession nos.: ON175977 (ITS) and ON175979 (nuLSU).

(Figs 2 & 3)

*Thallus* with irregular squamules, (290–)347 ± 54(–400) μm thick at margins, appressed on rocks with soil present between thalli; squamules 0.7–3 mm diam., slightly overlapping. *Soredia* and *isidia* absent, pruinose upper surface bright brown to dark brown; margin whitish, entire to subentire, usually upturned; lower surface white to dirty white near margins, without rhizines, lacking well-developed lower cortex. The thallus is heteromeric, epinecral layer hyaline, up to 25 μm thick; upper cortex paraplectenchymatous (25–)67 ± (17–)100 μm thick, brown to light brown; algal layer (211–)1289 ± 119(–427) μm thick, continuous, unicellular, globose to subglobose (3–)4.3 ± 0.5(–6) μm diam., medulla (246–)346 ± 21(–395) μm thick; lower cortex hyaline, up to 100 μm thick near margins, while 5 mm thick towards the centre near the rock surface.

*Apothecia* marginal, lecideine, up to 0.5 mm diam. when single, up to 1.7 mm diam. in cluster form, often globose; disc dark brown to black, shiny, epruinose, sometimes cracked; *ephy membranes* brown, (8–)12 ± 3(–15) μm thick; *hymenia* (102–)121 ± 37(–170) μm high. *Subhymenia* hyaline, up to 155 μm thick; *hymenium* black, up to 458 μm high. *Paraphyses* (47–)10.1 ± 1.3 (–63) × (1.9–)1.6 ± 0.7(–2.9) μm, sepalate, with pigmented apical cells in a gel-like matrix. *Asci* narrowly clavate to subcilindrical, tholus amyloid, 8-spored, (67–)75.5 ± 6(–86) × (8.5–)11.8 ± 2.8(–13.5) μm. *Ascospores* hyaline, simple, ellipsoid, thick-walled, (7.9–)10.1 ± 1.3(–11.9) × (5.3–)6.6 ± 0.7(–7.9) μm, I/w ratio (1.3–)1.53 ± 0.17(–1.8) μm, simple with smooth surface.

*Pycnidia* immersed in the medulla and in the upper cortex forming light brown outgrowths on the squamules, globose to subglobose, (300–)324 ± 35(–352) μm diam. *Conidia* hyaline and bacilliform (3.5–)4 ± 0.5(–4.9) × (0.7–)0.97 ± 0.15(–1.16) μm, I/w ratio (3.3–)4.25 ± 0.75(–5.6) μm.

**Chemistry.** Thallus upper surface K+ red, KC+ black, C–; upper cortex K+ red, KC+ black, C–; medulla K+ yellowish brown, KC+ brown, C–; algal layer K+ black, KC+ dark black, C–; apothecial disc I+ blue, K–, KC–, UV–. Secondary metabolites detected were atranorin, norstictic acid, salazinic acid whilst stictic acid was absent.

**Etymology.** The specific epithet *pakistanaica* (Latin) refers to Pakistan, the country of the type locality.

**Distribution.** The species has so far been found only infrequently on stones between 4008–4587 m a.s.l. in well-drained locations in Deosai National Park, Gilgit Baltistan, Pakistan.

**Additional specimens examined.** Pakistan: Gilgit Baltistan: Deosai National Park, saxicolous, on calciferous rock, *c*. 4587 m a.s.l., 35°04′36″N, 75°13′16.31″E, 2019, M. Usman DEO-57 (LAH37091—paratype; GenBank Accession nos.: ON175977 (ITS) and ON175980 (nuLSU)); *ibid.*, saxicolous, on calciferous rock, 4008 m a.s.l., 35°54′49.84″N, 75°32′37.03″E, 2019, M. Usman & K. Habib GPS-2 (LAH37092—paratype; GenBank Accession no. (ITS): MW418153).

**Discussion.** High altitudinal regions such as Deosai National Park offer specialized habitats for the evolution and growth of lichen species (Khan & Jan 2018; Usman et al. 2021). The lichen flora has previously been partially investigated using classical morphology for identification, with a variety of lichens described including catapyreneoid genera found commonly as part of biological soil crusts (Aptroot & Iqbal 2012). By contrast, we now describe a new saxicolous species. Superficially, the new species resembles *Anamylopsora altaica*, due to the thallus shape and presence of black apothecia (Ahat et al. 2019). It also shares some common characteristics with the two other remaining species of the genus, *A. pulcherrima* and *A. pruinosa*, including dark brown to black marginal apothecia, a pruinose thallus, globose to subglobose and unicellular algal cells, clavate to subcilindric asci and hyaline bacilliform pycnidiospores (Timdal 1991; Zuo et al. 2018).

However, our novel taxon *Anamylopsora pakistanaica* is clearly different from these taxa since it forms a separate clade based on ITS and nuLSU DNA sequence divergence, and phylogenetically is a sister group to *A. altaica* with strong bootstrap support. This proposal is supported by morpho-anatomical characters which distinguish the species, including coloration of the thalli, the presence of a thick lower cortex up to 100 μm, up to 5 mm thick towards the centre near the rock surface and the presence of simple thick-walled ascospores as discussed below.
Figure 2. Anamylopsora pakistanaica sp. nov. holotype (LAH37090). A, dry form on rock (arrows). B-D, cross-section of thallus viewed under stereomicroscope. E, thallus with apothecium and pycnidia (arrows). Scales: A = 10 mm; B, D & E = 500 μm; C = 1 mm. In colour online.
*Anamylopsora pakistanica* has a light to dark brown-coloured thallus upper surface and the apothecia have a thin epihymenium up to 15 μm thick, whereas *A. altaica* has a white to whitish grey thallus upper surface and an epihymenium up to 30 μm thick. Further morpho-anatomical details of *A. pakistanica* include a continuous thick medulla, 325–367 μm in depth, which contrasts...
that in A. altaica (only 190–280 μm deep), A. pruinosa (112–250 μm deep), and A. pulcherrima which has a discontinuous medulla. Anamylopsora pakistanaica also has a thicker algal layer, 229–360 μm in depth, compared to that in A. altaica (135–195 μm), A. pruinosa (50–150 μm) and A. pulcherrima (120–220 μm). In addition, A. pakistanaica has a thick hymenium, 90–151 μm, in contrast to that present in A. altaica (95–115 μm), A. pruinosa (75–100 μm) and A. pulcherrima (60–100 μm) (Timdal 1991; Zuo et al. 2018; Ahat et al. 2019).

Furthermore, A. pakistanaica has squamules between 0.7–3 mm diam., compared to A. altaica which forms squamules ≤1(–2.5) mm diam. and A. pruinosa which has squamules of 2–3 mm diam. Anamylopsora pakistanaica forms ascii up to 82 μm in length while A. pulcherrima has larger ascii up to 125 μm in length. Anamylopsora pakistanaica also has ellipsoid ascospores with a thick-walled and smooth surface whereas A. altaica and A. pruinosa have thin-walled ascospores with a warty surface (Timdal 1991; Zuo et al. 2018; Ahat et al. 2019). Other differences of A. pakistanaica are the thick epicnidal layer up to 25 μm, whereas in A. pulcherrima the layer is only 5–10 μm thick and is absent in A. altaica. Anamylopsora pakistanaica has a thinner upper cortex, 50–84 μm thick, in contrast to A. pruinosa and A. pulcherrima where the upper cortex is 125–150 μm and 35–180 μm deep, respectively (Timdal 1991; Zuo et al. 2018; Ahat et al. 2019).

It is also noted that A. pakistanaica is saxicolous in nature and rhizines are absent, whereas A. pruinosa is terriolous (Zuo et al. 2018), providing a key differentiating character separating these species. A further difference is that A. pulcherrima produces alectorialic acid, A. pruinosa produces alectoriaic and barbatolic acids and A. altaica produces psoromic acid, whereas A. pakistanaica produces anatrinor, norstictic acid and salazinic acid (Timdal 1991; Zuo et al. 2018; Ahat et al. 2019). A final significant difference among Anamylopsora species lies in their altitudinal locations. The new species A. pakistanaica was found on rocks at a high altitude between 4008 and 4587 m a.s.l., compared to A. altaica found at 960–1087 m, A. pruinosa at 1577 m and A. pulcherrima from 550 to 3900 m (Timdal 1991; Zuo et al. 2018; Ahat et al. 2019). Based upon this combination of characters, the new species A. pakistanaica is clearly distinct.

A key to species of Anamylopsora

1 On soil, ascospores subglobose, rhizines abundant .................................................. A. pruinosa
   On rock, ascospores ellipsoid, rhizines absent .................................................. 2

2(1) Epinecral layer present, ascospore surface smooth ................................................. 3
   Epinecral layer absent, ascospore surface warty .............................................. A. altaica

3(2) Upper surface ochraceous brown, algal layer discontinuous, epinecral layer up to 15 μm thick, upper cortex up to 180 μm thick .......................................................... A. pulcherrima
   Upper surface light brown to dark brown, algal layer continuous, epinecral layer up to 25 μm thick, upper cortex up to 84 μm thick .................................................. A. pakistanaica

References


Acknowledgements. The authors are very grateful to Prof. Dr Pradeep Kumar Divakar (Departamento de Farmacología, Universidad Complutense de Madrid, Spain), Dr José Pizarro (Curator, Herbario MAF, Universidad Complutense of Madrid, Spain), and Prof. Peter Crichtend and Dr Chris Wade (School of Life Sciences, University of Nottingham, UK) for sending herbarium specimens of lichens for thin-layer chromatography and phylogenetic suggestions, respectively.

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Supplementary Material. The Supplementary Material for this article can be found at https://doi.org/10.1017/S002428292300018X.

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Prieto M, Baloch E, Tehler A and Wedin M (2013) Mazaedium evolution in the Ascomycota (Fungi) and the classification of mazaediate groups of formerly unclear relationship. Cladistics 29, 296–308.


