

## Standard Paper

# A new species of *Aspicilia* (Megasperaceae), with a new lichenicolous *Sagediopsis* (Adelococcaceae), from the Falkland Islands

Alan M. Fryday<sup>1</sup> , Timothy B. Wheeler<sup>2</sup>  and Javier Etayo<sup>3</sup> 

<sup>1</sup>Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824, USA; <sup>2</sup>Division of Biological Sciences, University of Montana, Missoula, Montana 59801, USA and <sup>3</sup>Navarro Villoslada 16, 3° dcha., 31003 Pamplona, Navarra, Spain

## Abstract

The new species *Aspicilia malvinae* is described from the Falkland Islands. It is the first species of *Megasporaceae* to be discovered on the islands and only the seventh to be reported from South America. It is distinguished from other species of *Aspicilia* by the unusual secondary metabolite chemistry (hypostictic acid) and molecular sequence data. The collections of the new species support two lichenicolous fungi: *Endococcus propinquus* s. lat., which is new to the Falkland Islands, and a new species of *Sagediopsis* with small perithecia and 3-septate ascospores c. 18–20 × 4–5 µm, which is described here as *S. epimalvinae*. A total of 60 new DNA sequences obtained from species of *Megasporaceae* (mostly *Aspicilia*) are also introduced.

**Key words:** DNA sequences, *Endococcus*, *Lecanora masafuerensis*, lichen, southern South America, southern subpolar region

(Accepted 18 March 2021)

## Introduction

Species of *Megasporaceae* Lumbsch *et al.* are surprisingly scarce in the Southern Hemisphere. Whereas 97 species are known from North America (Esslinger 2019), 104 from Russia (Urbanavichus 2010), 40 from Svalbard (Øvstedal *et al.* 2009) and 16 from the British Isles (Fletcher *et al.* 2009), only six species have been reported from Australia (McCarthy 2016), seven from New Zealand (Galloway 2007) and only three from each of South Africa (Fryday 2015) and Antarctica (Øvstedal & Lewis Smith 2001). In southern South America, six taxa have been reported from Argentina (*Aspicilia cinerea* (L.) Körb., *A. mendozae* Räsänen, *Circinaria caesiocinerea* (Nyl. ex Malbr.) A. Nordin *et al.*, *C. calcarea* (L.) A. Nordin *et al.*, *Lobothallia alphoplaca* (Wahlenb.) Hafellner and *Megaspora verrucosa* (Ach.) Arcadia & A. Nordin; Calvelo & Liberatore 2002) and, because *Lecanora masafuerensis* Zahlbr. appears not to be a species of *Aspicilia* as was suggested by Galloway & Quilhot (1998), only two (*C. calcarea* and *Megaspora verrucosa*) from Chile (Galloway & Quilhot 1998). None have previously been reported from the Falkland Islands (Fryday *et al.* 2019). Here we describe a new species that is known from three localities on the Falkland Islands, along with a lichenicolous fungus that is present on the holotype collection.

**Author for correspondence:** Alan Fryday. E-mail: fryday@msu.edu

**Cite this article:** Fryday AM, Wheeler TB and Etayo J (2021) A new species of *Aspicilia* (*Megasporaceae*), with a new lichenicolous *Sagediopsis* (*Adelococcaceae*), from the Falkland Islands. *Lichenologist* 53, 307–315. <https://doi.org/10.1017/S0024282921000244>

© The Author(s), 2021. Published by Cambridge University Press on behalf of the British Lichen Society. This is an Open Access article, distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is unaltered and is properly cited. The written permission of Cambridge University Press must be obtained for commercial reuse or in order to create a derivative work.

## Materials and Methods

### Morphological methods

Gross morphology was examined under a dissecting microscope and apothecial characteristics by light microscopy (compound microscope) on hand-cut sections mounted in water, 10% KOH (K), 50% HNO<sub>3</sub> (N) or Lugol's reagent (0.15% aqueous IKI). Thallus sections were investigated in water, K and Lugol's reagent. Ascospore measurements of the new species are given as (minimum value–)mean ± standard deviation(–maximum value). Thalline chemistry was investigated by thin-layer chromatography following the methods of Orange *et al.* (2001), and nomenclature of apothecial pigments follows Meyer & Printzen (2000).

**Additional comparative specimens examined.** *Lecanora masafuerensis*. **Chile:** Juan Fernandez Islands: Mas Afuera, Quebrada de las Vacas, near two waterfalls of stream in narrow section of canyon, 1965, H. A. Imshaug 36869, 36872; *ibid.*, Quebrada de las Casas, narrow section, 1965, H. A. Imshaug 36697, 36698, 36704 (MSC); *ibid.*, Quebrada de las Casas, 10 iii 1917, C. & I. Scottsberg s. n. (NY—isotype).

### Molecular methods

**Taxon sampling.** The majority of specimens used in this study were collected between 2007–2017, but further specimens were obtained from herbarium loans and the collecting efforts of other researchers (Table 1). Additional sequences included in the analysis were downloaded from GenBank (Table 1).

**Table 1.** Voucher information and GenBank Accession numbers of sequences used for construction of the phylogenetic tree in Fig. 3. Newly introduced sequences are in bold.

Species	Collector number (hb)	Locality	DNA Voucher/ Publication	nuITS	nuLSU	mtSSU	<i>Mcm7</i>
<i>Aspicilia abbasiana</i>	Ismayil & Abbas 20111154 (HMAS-L)	Heilongjiang, China	Ismayil <i>et al.</i> (2015)	NR_158307	–	–	–
<i>A. angelica</i>	Wheeler 5468 (hb. Wheeler)	Montana, USA	TW213	<b>MW4435332</b>	<b>MW447407</b>	<b>MW424813</b>	<b>MW435341</b>
<i>A. aurantiaca</i>	Wheeler 7091 (hb. Wheeler)	California, USA	TW220	<b>MW447387</b>	<b>MW447401</b>	<b>MW424807</b>	<b>MW435335</b>
<i>A. boykinii</i>	Wheeler 7274 (hb. Wheeler)	Montana, USA	TW277	<b>MW447394</b>	<b>MW447409</b>	<b>MW424815</b>	<b>MW435343</b>
<i>A. cinerea</i>	Wheeler 7214 (hb. Wheeler)	Montana, USA	TW210	<b>MW447398</b>	<b>MW447413</b>	<b>MW424819</b>	<b>MW435347</b>
<i>A. cinerea</i>	Wheeler 6277 (hb. Wheeler)	Finnmark, Norway	TW219	<b>MW447391</b>	<b>MW447405</b>	<b>MW424811</b>	<b>MW435339</b>
<i>A. cuprea</i>	Knudsen 16336 (hb. Wheeler)	California, USA	TW331	<b>MW447385</b>	<b>MW447399</b>	<b>MW424805</b>	<b>MW435333</b>
<i>A. cuprea</i>	Owe-Larsson 9112 (UPS)	California, USA	Nordin <i>et al.</i> (2007, 2010)	EU057902	HM060750	HM060712	–
<i>A. cyanescens</i>	Owe-Larsson 9151 (UPS)	California, USA	Nordin <i>et al.</i> (2007, 2010)	EU057904	HM060745	HM060707	–
<i>A. dudinesis</i>	Nordin 6036 (UPS)	Torne Lappmark, Sweden	Nordin <i>et al.</i> (2007, 2010)	EU057906	HM060748	HM060710	–
<i>A. epiglypta</i>	Nelson s. n. (hb. Wheeler)	Hoffellsjokull, Iceland	TW276	<b>MW447396</b>	<b>MW447411</b>	<b>MW424817</b>	<b>MW435345</b>
<i>A. fumosa</i>	Wheeler 3844 (hb. Wheeler)	Montana, USA	TW224	<b>MW447395</b>	<b>MW447410</b>	<b>MW424816</b>	<b>MW435344</b>
<i>A. knudsenii</i>	Wheeler 6798 (hb. Wheeler)	Montana, USA	TW245	<b>MW447386</b>	<b>MW447400</b>	<b>MW424806</b>	<b>MW435334</b>
<i>A. malvinae</i>	Fryday 11433 (MSC)	East Falkland, Falkland Islands	TW260	<b>MW447392</b>	<b>MW447406</b>	<b>MW424812</b>	<b>MW435340</b>
<i>A. pacifica</i>	Knudsen 9241 (hb. Wheeler)	California, USA	TW334	<b>MW447393</b>	<b>MW447408</b>	<b>MW424814</b>	<b>MW435342</b>
<i>A. santamonicae</i>	Wheeler 6648 (hb. Wheeler)	California, USA	TW230	<b>MW447388</b>	<b>MW447402</b>	<b>MW424808</b>	<b>MW435336</b>
<i>Circinaria calcarea</i>	Nordin 5888 (UPS)	Oland, Sweden	Nordin <i>et al.</i> (2007, 2010)	EU057898	HM060743	HM060705	–
<i>Lepra albescens</i>	Schmitt s. n. (ESS-20967)	Bohemia, Czech Republic	Schmitt <i>et al.</i> 2001	AF329177	AF329176	AF329175	–
<i>Lobothallia melanaspis</i>	Nordin 6622 (UPS)	Jämtland, Sweden	Nordin <i>et al.</i> (2010, 2011)	HQ259272	HM060726	HM060688	–
<i>L. praeradiosa</i>	Wheeler 3414 (hb. Wheeler)	Montana, USA	TW269	<b>MW447389</b>	<b>MW447403</b>	<b>MW424809</b>	<b>MW435337</b>
<i>Oxneriaria permutata</i>	Wheeler 4463 (hb. Wheeler)	Alaska, USA	TW296	<b>MW447390</b>	<b>MW447404</b>	<b>MW424810</b>	<b>MW435338</b>
<i>O. supertegens</i>	Owe-Larsson 9002 (UPS)	Troms, Norway	Nordin <i>et al.</i> (2007, 2010)	EU057936	HM060742	HM060704	–
<i>O. virginea</i>	Wheeler 7153 (hb. Wheeler)	Montana, USA	TW240	<b>MW447397</b>	<b>MW447412</b>	<b>MW424818</b>	<b>MW435346</b>

**DNA isolation and sequencing.** Total DNA was extracted from samples of 10–15 healthy apothecia and surrounding tissue. Two 3 mm steel beads were added to the sample tubes and frozen at –80 °C for 1 h. Samples were then mounted on the TissueLyser II (Qiagen, Germany) and ground in 30 s intervals for 1–2 min at 30/hz. DNA was extracted using the Qiagen DNeasy Plant Mini

Kit (Qiagen, Germany) according to the manufacturer's instructions except for the following modifications: in the first step, samples were incubated in lysis buffer for 1 h and vortexed every 10 mins; in the final step, the samples were eluted in 50 µl AE buffer twice. DNA quantity was tested on an Implen Nanodrop (Implen, München, Germany).

**Table 2.** Primers used in this study.

Primer name	Primer sequence (5'–3')	Reference
ITS1F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns (1993)
ITS4	TCCTCCGCTTATTGATATGC	White <i>et al.</i> (1990)
LrlecF	CCTCAGTAACGGCGAG	Schneider <i>et al.</i> (2015)
LR7	TACTACCACCAAGATCT	R. Vilgalys (unpublished)
mtSSU1	AGCAGTGAGGAATATTGGTC	Zoller <i>et al.</i> (1999)
mtSSU3R	ATGTGGCACGTCTATAGCCC	Zoller <i>et al.</i> (1999)
MCM7for	CGCTACTACAAAACAATTCCACC	This study
MCM7rev	CGCCCATCTCTTTTGTGAC	This study

**Table 3.** PCR protocols used in this study for given loci.

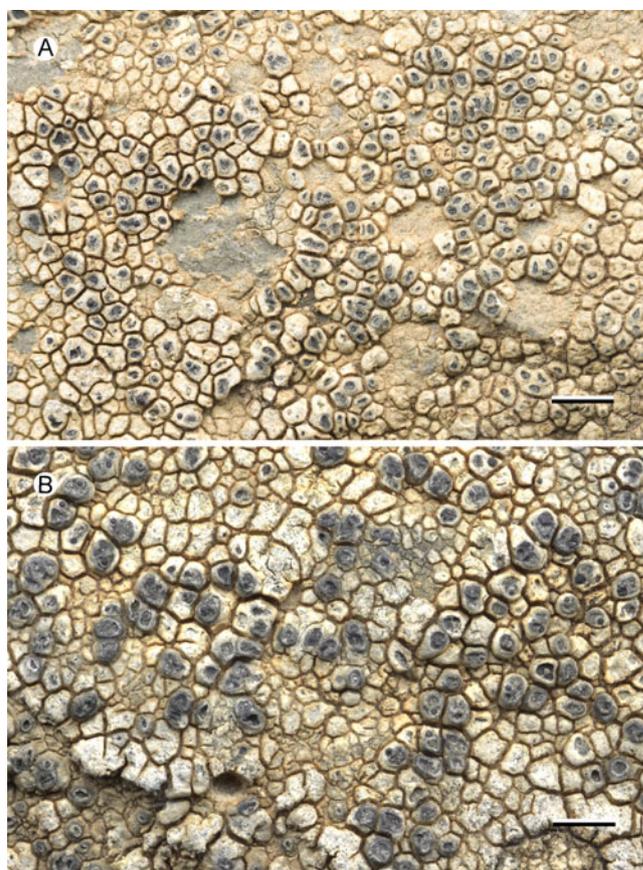
Locus	Initial denaturation	35 cycles of:	Final extension
nuITS	2 min at 94 °C	94 °C for 1 min, 54 °C for 1 min, 72 °C for 45 s	72 °C for 7 min
nuLSU	4 min at 95 °C	94 °C for 1 min, 54 °C for 1 min, 72 °C for 45 s	72 °C for 5 min
mtSSU	4 min at 95 °C	94 °C for 1 min, 54 °C for 1 min, 72 °C for 45 s	72 °C for 5 min
<i>Mcm7</i>	4 min at 95 °C	95 °C for 30 s, 50 °C for 40 s, 72 °C for 1 min	72 °C for 5 min

Standard PCR amplifications were conducted in 25 µl reaction volumes using Ready-To-Go PCR Beads (GE Healthcare, UK) following the manufacturer's recommendations. All primers used in this study are listed in Table 2.

Amplifications were carried out in an Eppendorf Mastercycler Pro thermal cycler (Eppendorf North America, New York, USA) and performed using the protocols in Table 3. PCR products were cleaned using the Qiagen PCR Purification Kit (Qiagen, Germany) or Agencourt AMPure XP beads (Beckman Coulter, Inc., Brea, CA, USA), following the manufacturers' instructions, and were visualized on 1% agarose gel stained with ethidium bromide. Sequencing reactions were performed by Eurofins Genomics (Louisville, KY, USA).

**Sequence alignment.** Sequences were quality checked and sequence ends were manually trimmed in AliView (Larsson 2014; <http://www.ormbunkar.se/aliview/>). Each sequence was checked against the NCBI nucleotide database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to verify that the desired organism was sequenced. Alignments were visually checked in AliView and minor misalignments were manually adjusted.

**Phylogenetic analyses.** Maximum likelihood (ML) trees for each locus (not shown) were constructed in raxmlGUI 2.0 (Stamatakis 2014; Edler *et al.* 2020), and the bootstrap support values for each clade were compared. Using a 70% bootstrap value threshold, clades were compared and conflict was assumed to be significant when a monophyletic group was supported with bootstrap

**Fig. 1.** *Aspicilia malviniae* (Fryday 11433, holotype). A, thallus with immersed apothecia. B, thallus with ±emergent apothecia. Scales = 2 mm. In colour online.

values  $\geq 70\%$  within one locus and the same group of taxa was supported  $\geq 70\%$  as non-monophyletic within another locus (Mason-Gamer & Kellogg 1996). Because no strongly supported conflicts were observed between the four loci, downstream relationships and analyses were performed on the concatenated dataset. Analyses were run using raxmlGUI 2.0 to reconstruct a maximum likelihood concatenated 4-locus tree. We used *Lepra albescens* as the root and ran 1000 thorough ML bootstraps with the model set to GTRGAMMAI.

## The Species

### *Aspicilia malviniae* Fryday & T. B. Wheeler sp. nov.

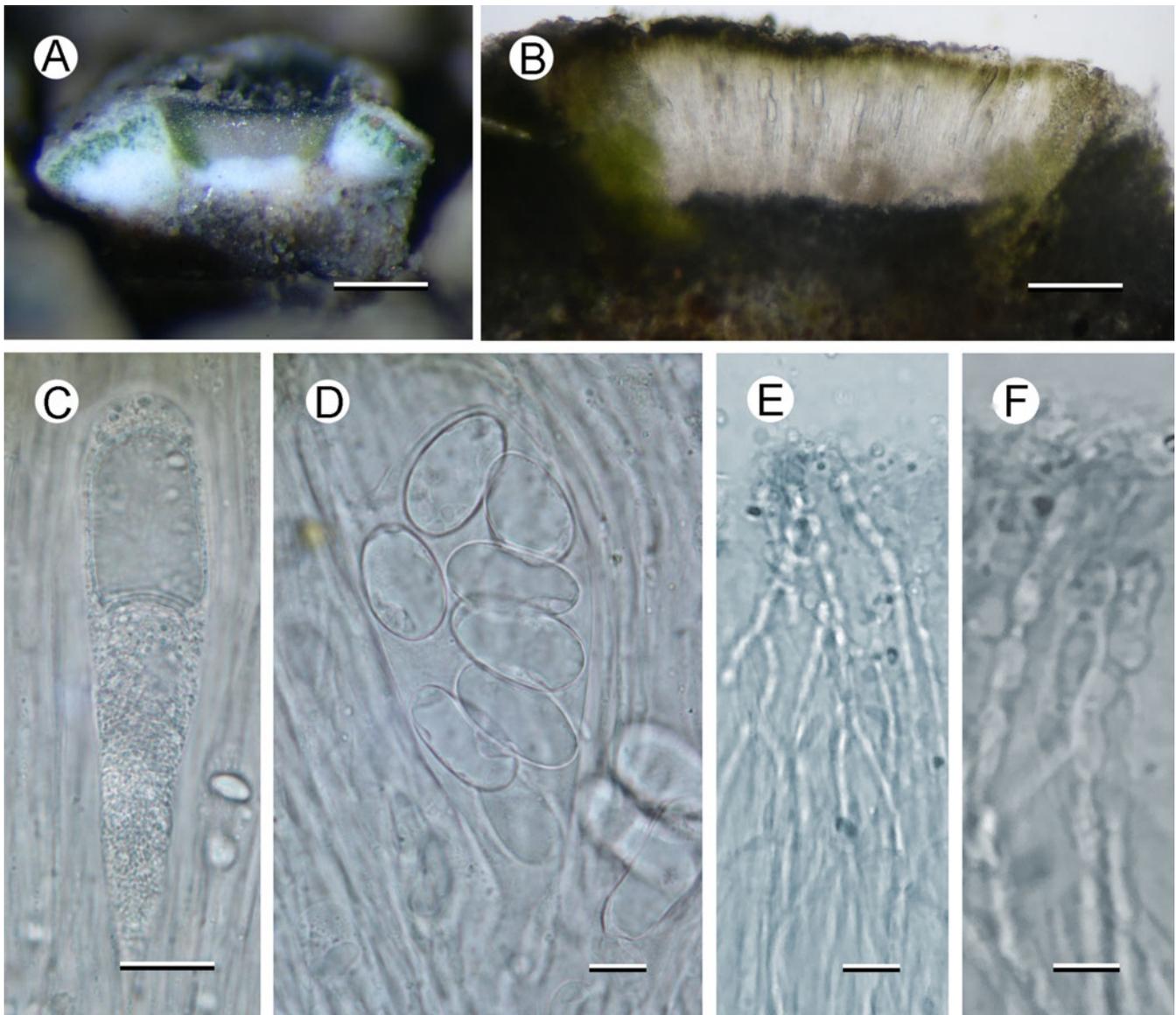
Mycobank No.: MB 839030

Distinguished from other species of *Aspicilia* by the thalline chemistry (hypostictic acid) and sequence data.

Type: Falkland Islands, East Falkland, Lafonia, 3.5 km west of Walker Creek, east side of stream north of road, 51.97705°S, 58.82285°W, 21 m, low dolerite outcrop in *Empetrum* heath above stream, 12 November 2015, A. M. Fryday 11433 & A. Orange (MSC0057604—holotype).

(Figs 1 & 2)

*Thallus* effuse, several centimetres across, cream to grey, areolate; *areoles* angular, convex, 0.2–0.5 mm across, usually contiguous



**Fig. 2.** *Aspicilia malviniae* (A–C, E & F, Fryday 11433, holotype; D, Fryday 11008). A, section through thallus and apothecium. B, section of apothecium. C, immature ascus. D, mature ascus with ascospores. E & F, paraphyses showing constricted septa in upper part (F). Scales: A = 2 mm; B = 100  $\mu$ m; C & E = 20  $\mu$ m; D & F = 10  $\mu$ m. In colour online.

but sometimes singular or in small groups on a black hypothallus, areoles separated by deep cracks. *Prothallus* black, fimbriate at the thallus edge, 0.5 mm wide. *Upper cortex* c. 50  $\mu$ m thick with a thin epinecral layer 10–25  $\mu$ m thick, upper 25  $\mu$ m of cortex grey in visible light due to medium-sized crystals that only partially dissolve in K, crystals often most frequent between the cortex and the epinecral layer; cortical cells not observed in pale-coloured areoles but grey areas had cortical cells 5–6  $\mu$ m diam. with a thin, pale grey-brown cap; *lateral cortex* pale brown due to numerous minute crystals that do not dissolve in K, slightly POL+, cells 5–8  $\mu$ m diam. *Photobiont* layer c. 25  $\mu$ m thick, cells chlorococcoid, 6–16  $\mu$ m diam.

*Apothecia* abundant, usually immersed (aspicilioid) but sometimes becoming sessile with a prominent proper margin, black, 0.4–0.5 mm diam. when mature, usually 1 per areole, (occasionally two, rarely three); usually  $\pm$ round, rarely oblong or linear, angular if > 1 per areole; *thalline margin* not apparent, formed by the thalline areole; *proper margin* densely pruinose when

immature, becoming epruinose, 0.1 mm wide; *disc* black, slightly concave, pruinose when immature, becoming epruinose when mature. In section, *exciple* up to 250  $\mu$ m wide at the surface, tapering to nothing where it merges with the subhymenium, pale brown but darker at the surface that is N+ green, composed of narrow (1–1.5  $\mu$ m wide) conglutinated hyphae that become wider (c. 5  $\mu$ m) and cellular with constricted septa for the final 4–5 cells towards the cortex. *Hymenium* 140–160  $\mu$ m, I+ slowly yellow and after c. 5 minutes greenish blue; *paraphyses* very fine c. 1  $\mu$ m, the upper 20–25  $\mu$ m (epihymenium) wider (5  $\mu$ m) with constricted septa, cells globose to oblong, 5  $\mu$ m wide by 5–7  $\mu$ m long; *epihymenium* olive-brown (K+ brown, N+ bright aeruginose; Caesiocinerea-green); *subhymenium* hyaline, I+ slowly (after c. 5 minutes) dark blue, composed of  $\pm$ vertically aligned hyphae with the same kind of medium-sized hyaline crystals as in the cortex, merging with the hypothecium. *Hypothecium* hyaline, I+ slowly (after c. 5 minutes) bluish mauve, composed of thick (c. 5  $\mu$ m) randomly organized hyphae. *Asci* cylindrical

when immature, *c.* 110 × 20 µm, becoming broadly clavate when mature, 80–90 × 40–45 µm; *ascospores* 8 per ascus, hyaline, broadly ellipsoid, (22–)24.55 ± 1.63(–30) × (11–)14.65 ± 1.53(–18) µm, l/w ratio (1.28–)1.69 ± 0.19(–2.18), *n* = 20.

*Pycnidia* not observed.

**Chemistry.** Thallus C–, K–, KC–, Pd–, but in section slowly K+ yellow. TLC (solvent C): hypostictic acid (red spot at *R<sub>f</sub>* 4), faint red spot at *R<sub>f</sub>* 1 (probably subhypostictic acid), UV(after charging)++ cream spot at *R<sub>f</sub>* 8.

**Notes.** Of the species of *Aspicilia* reported from the Southern Hemisphere, only two (viz. *Lecanora* (*Aspicilia*) *masafuerensis* and *A. mendozae*) are candidates for an earlier name for our species. However, both are reported to have much smaller ascospores than our new species: those of *L. masafuerensis* are given as 10–18 × 6–8 µm (Zahlbruckner 1924) and those of *A. mendozae* as 9–14 × 8–9 µm (Räsänen 1941). Examination of specimens in MSC identified as *L. masafuerensis* and an isotype in NY, revealed slightly larger ascospores than those reported by Zahlbruckner (16.7–22.4 × 8.6–9.5 µm), but the specimens also had a dilute brown epihymenium, apparently *Porpidia*-type asci and are probably referable to *Xenolecia spadicomma* (Nyl.) Hertel. In addition, our species contains hypostictic acid as its primary secondary metabolite, which is an uncommon metabolite in lichens and, to the best of our knowledge, found as a major constituent in species of *Megasporaceae* only in the Chinese species *A. abbasiana* S. Y. Kondr. *et al.* (Kondratyuk *et al.* 2016; syn. *A. volcanica* Ismayil *et al.* (Ismayil *et al.* 2015)). The ascospores of this species are a similar size ((13–)16–23(–26) × (10–)13–16 µm) to those of *A. malvinae* and it also occurs on igneous rock. However, it differs in the paraphyses being distinctly moniliform for their whole length, not just the upper cells as in our species, and in the thallus containing stictic and constictic acids in addition to hypostictic acid, which are apparently absent from the thallus of our species. In addition, *A. abbasiana* is phylogenetically closely related to *A. cinerea* (Fig. 3) and so is quite distant from our species.

The four collections of this species were all collected from rocks close to streams, the holotype and the two collections from West Falkland (*Fryday* 11008, *Orange* 23271) from near the coast, and the other collection from East Falkland (*Fryday* 11431) from a somewhat higher altitude (67 m) inland. The two collections from East Falkland were on dolerite and although the site from which both the West Falkland collections were made, the Patricia Luxton NNR, is also primarily dolerite, at least one of the collections (*Fryday* 11008) was from sandstone. Unfortunately, the other collection from East Falkland (*Orange* 23171) was not accessible for this study.

The holotype and one of the West Falkland collections (*Orange* 23271) had a nearly identical ITS sequence and the three coastal collections were also morphologically similar. The inland collection (*Fryday* 11431) was also morphologically similar although the paraphyses appeared to be slightly more moniliform (the area with constricted septa extending through the upper 5–6 cells, whereas for the other collections only the upper 3–4 cells had constricted septa) but this appears to be a variable character. However, the presence of hypostictic acid, an uncommon substance in *Aspicilia*, as a major substance in all three available collections (*Fryday* 11008, 11431 & 11433) strongly suggests that they represent a single taxon. Mature asci and ascospores were infrequent in the holotype collection and the other collection

from East Falkland (*Fryday* 11431), although these were frequent in a collection from West Falkland (*Fryday* 11008).

*Aspicilia malvinae* is in a highly supported group outside of the *A. cinerea* clade and was recovered as sister to the ‘cyanescens’ clade and the ‘americana’ clade, but with no support (Fig. 3). This ambiguity arises as the single gene trees of ITS and LSU place *A. malvinae* either within the unsupported ‘americana’ group or within the unsupported ‘cyanescens’ group, respectively. The relationships within *Aspicilia* s. str. are unresolved, not due to gene tree discordance, because none of the single gene trees are supported at any of these conflicting nodes, but due to a lack of data from within the group. More sampling is needed to resolve the relationships between the clades within *Aspicilia* s. str. However, the separation of *A. malvinae* from the *A. cinerea* clade, which contains *A. abbasiana*, the only other *Aspicilia* species containing hypostictic acid as a major substance, is highly supported. The relationships between the genera *Lobothallia*, *Circinaria*, *Oxneriaria* and *Aspicilia* are also highly supported here (Fig. 3).

**Additional collections examined. Falkland Islands:** *West Falkland:* Chartres, Patricia Luxton NNR, 51.72560°S, 59.98474°W, 15 m, sloping rock with *Bucklandiella* sp., 2015, *Fryday* 11008 (MSC); *ibid.*, 51.72824°S, 59.98484°W, seasonally irrigated bedrock sloping at 40°, level with ground, unshaded, aspect 60°, with *Pertusaria alterimosa*, *Pertusaria cerebrinula/spegazzinii*, *Parmelia saxatilis*, *Massalongia patagonica*, *Bucklandiella* sp., 2015, *A. Orange* 23271 (NMW). (The amount of moss suggests that this is a moist microhabitat by Falkland Islands standards). *East Falkland:* Mt Osborne, Camilla Creek, 51.716467°S, 58.898183°W, 67 m, bare, stony area in grass/*Empetrum* heath, 2015, *A. M. Fryday* 11431 (MSC).

### Lichenicolous fungi

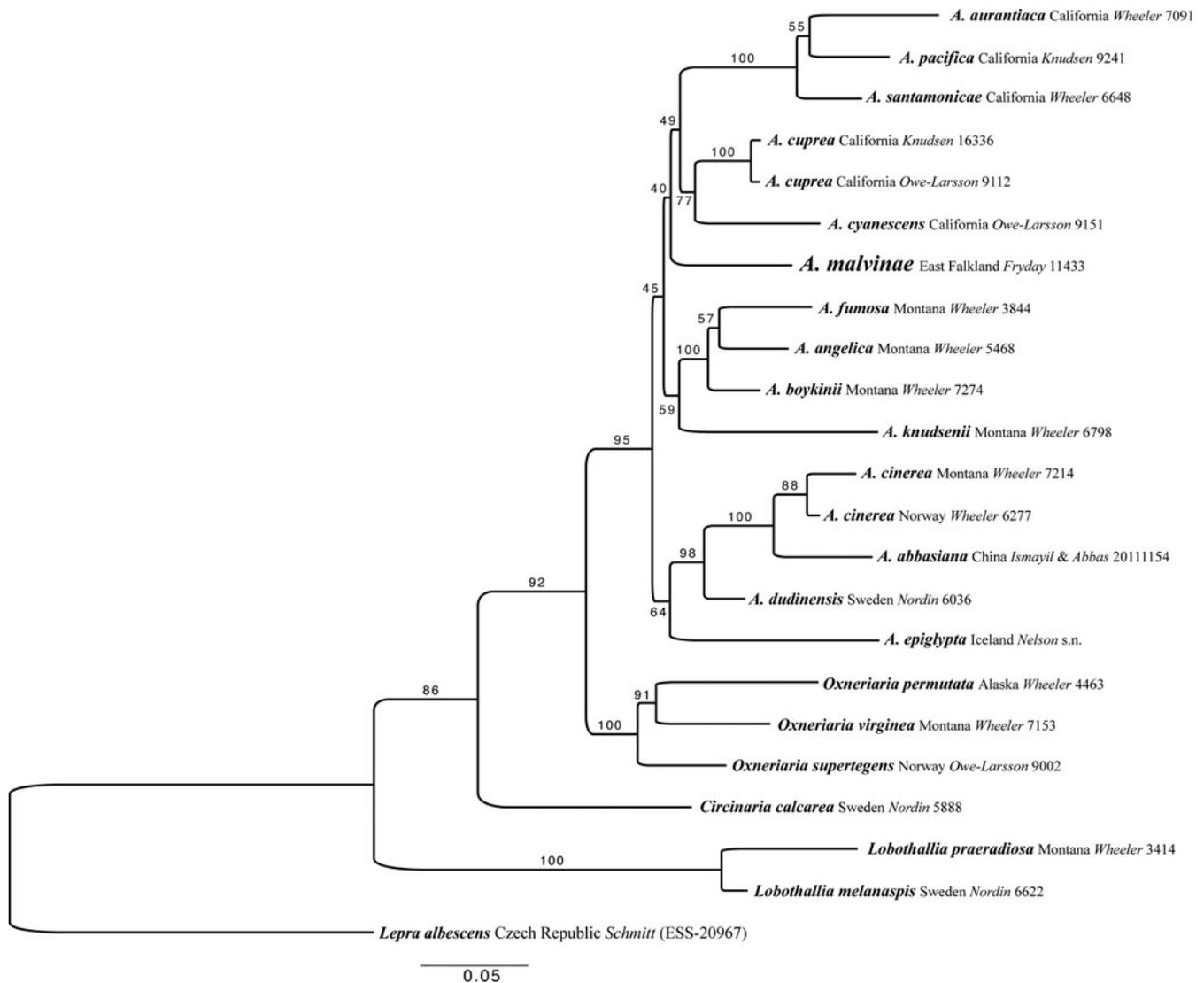
The collections of *Aspicilia malvinae* support two lichenicolous fungi: an *Endococcus* that is best accommodated in *Endococcus propinquus* s. lat. (Körb.) Trevis., reported here for the first time from the Falkland Islands, and a species of *Sagediopsis* that is frequent on the holotype and is described here as new to science.

### *Endococcus propinquus* s. lat. (Körb.) Trevis.

*Conspect. Verruc.*, 17 (1860).

*Ascomata* 0.15–0.20 mm diam. with a depressed ostiole; *ascospores* brown, 1-septate, broadly ellipsoid (8–)9.45 ± 0.76(–10) × (5.5–)6.6 ± 0.77(–8) µm; l/w ratio (1.125–)1.44 ± 0.14(–1.67); *n* = 10 (*Fryday* 11431—MSC).

Two species of *Endococcus* were reported by Diederich *et al.* (2018) as occurring only on *Aspicilia*: *E. calcaricola* (Mudd) Nyl. (as *Microthelia calcaricola* Mudd) and *E. verrucosus* Hafellner. The application of the name *Endococcus calcaricola* is uncertain; it has been included as a synonym of *E. rugulosus* Nyl. (Index Fungorum Partnership 2021), which has ascospores 13–17 × 5–8 µm (Ihlen & Wedin 2008). Hafellner (1994) gives the ascospore dimensions of *E. verrucosus* as (13–)14–17(–18) × 7–9 µm, whereas Zhurbenko & Notov (2015) report them as (7.5–)10–15 (–21) × (5–)6–8(–10) µm. Therefore, the ascospores of both these species are notably larger than those of the species reported here. Two other species were mentioned by Diederich *et al.* (2018) as occurring on several lichen genera including *Aspicilia*, and by Ihlen & Wedin (2008) as occurring on



**Fig. 3.** Maximum likelihood (ML) tree of the concatenated (ITS, LSU, mtSSU and *Mcm7*) dataset for members of *Aspicilia* and related species. Analyses were performed using raxmlGUI 2.0 (Stamatakis 2014; Edler et al. 2020). Maximum likelihood bootstrap values are shown above each branch. The newly introduced species is in larger font and voucher information for all specimens is provided (see Table 1 for further details). Abbreviation: A. = *Aspicilia*.

*Aspicilia*: *E. perpusillus* Nyl., with ascospores (12–)15–25(–30) × 5–9 μm, and *E. propinquus* s. lat., with ascospores 7–11 μm long (Ihlen & Wedin 2008). Although *E. propinquus* s. str. occurs only on the thalli of *Porpidia* spp., *E. propinquus* s. lat. is widespread and has been recorded on a wide variety of crustose lichens, including *Aspicilia* (Ihlen & Wedin 2008), and this would appear to be the best accommodation for our fungus.

**Specimen examined.** Falkland Islands: East Falkland: Mt Osborne, Camilla Creek, 51.716467°S, 58.898183°W, 67 m, bare, stony area in grass/*Empetrum* heath, 2015, A. M. Fryday 11431 (MSC).

***Sagediopsis epimalviniae* Etayo, T. B. Wheeler & Fryday sp. nov.**

MycoBank No.: MB 839031

Lichenicolous fungus growing on *Aspicilia malviniae*. Similar to *Sagediopsis fissurisedens* that grows on *Aspilidea* (syn. *Aspicilia*) *myrinii*, but differing in the much smaller perithecia, 0.15–0.20

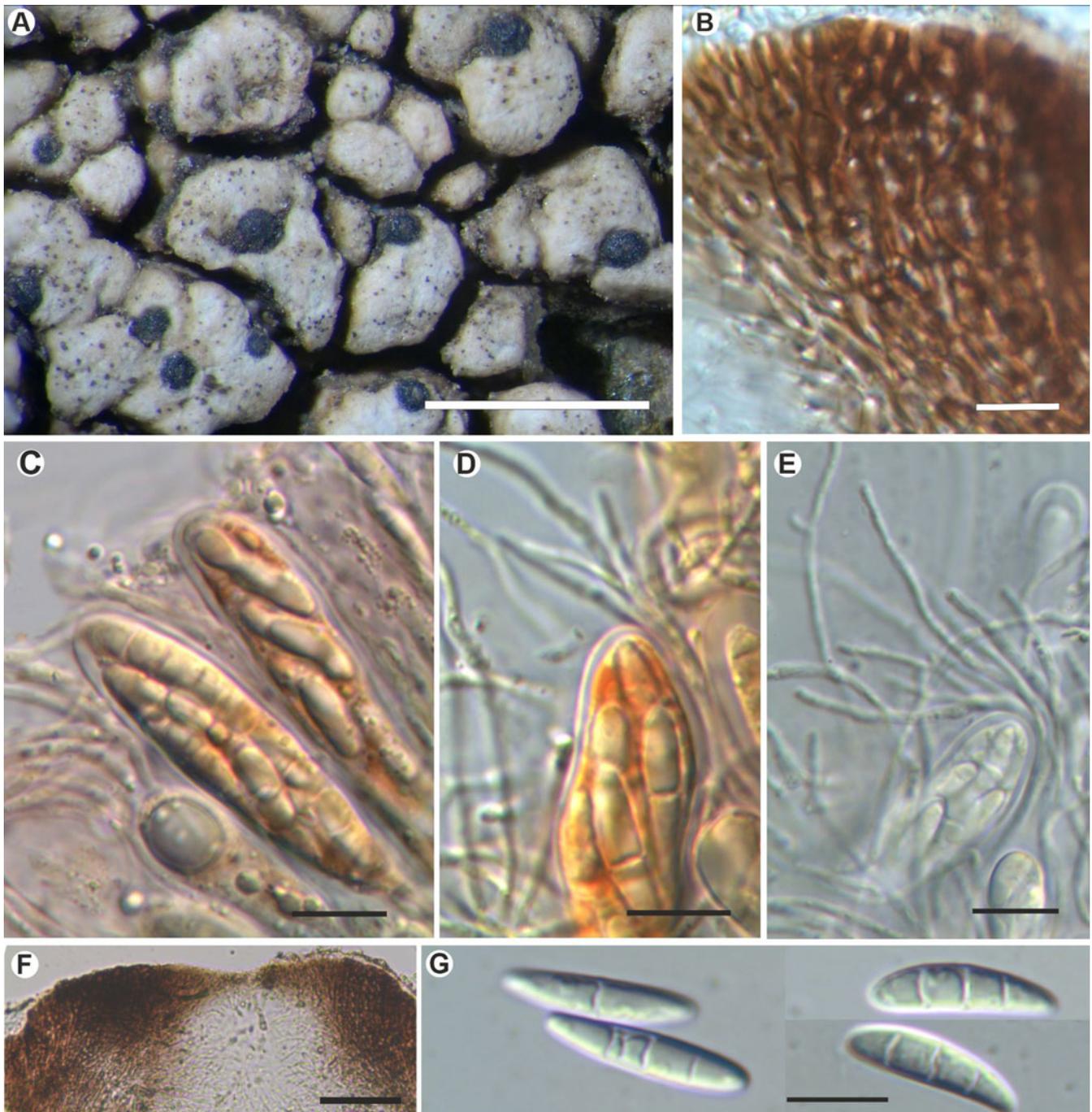
mm diam., smaller asci, 52–60 × 11–15 μm, and longer and narrower ascospores (16–)18–20(–22) × (3.5–)4–5(–6) μm.

Type: Falkland Islands, East Falkland, Lafonia, 3.5 km west of Walker Creek, east side of stream north of road, 51.97705°S, 58.82285°W, 21 m, lichenicolous on *Aspicilia malviniae* on a low dolerite outcrop in *Empetrum* heath above stream, 12 November 2015, A. M. Fryday 11433a & A. Orange (MSC0057605—holotype).

(Fig. 4)

Independent thallus not formed but apothecia production in the host is apparently suppressed, hyphae below ascomata hyaline.

Ascomata perithecioid, not clypeate but with the wall markedly thickened above, 150–200 μm diam., obovate but flattened above, not radially split, with central ostiole sometimes not visible, black, matt, immersed, finally slightly protruding from the thallus of *Aspicilia malviniae*. Exciple entire, black, greatly thickened apically giving a flat surface up to 100 μm thick, in section surrounded by a hyaline, non-cellular coat 4–5 μm thick, dark brown above to somewhat lighter brown below, K–, hyphae around ostiole



**Fig. 4.** *Sagediopsis epimalvinae* (Fryday 11433a, holotype). A, habitus of several *Sagediopsis* perithecia on areolae of *Aspicilia malvinae*. B, upper part of exciple showing elongated hyphae disposition and paraphysoids in ostiolar channel. C & D, upper part of asci and paraphyses (in I). E, ascus with simple and branched paraphyses (in water). F, upper part of a perithecium showing the thickened upper exciple and paraphysoids. G, ascospores. Scales: A = 1 mm; B–E & G = 10  $\mu$ m; F = 25  $\mu$ m. In colour online.

elongated; base and lateral parts of exciple 15–20(–35)  $\mu$ m thick, composed of several layers of more or less elongated cells with a thick cell wall and small lumina, 4–7  $\times$  1.5–2  $\mu$ m. *Paraphysoids* growing down from around ostiole, abundant, immersed in a colourless gel, simple to branched, even anastomosed, especially at the base, 10–30  $\times$  1  $\mu$ m. *Hymenial gel* I+ reddish, KI+ blue; *subhymenium* with large colourless oil droplets, 1–6  $\mu$ m diam. *Hamatecium* of persistent paraphysoids, flexuose, not thickened at the apex, septate, simple or sparingly branched, not constricted

at the septa, 1–1.5  $\mu$ m thick, with many small colourless oil droplets. *Asci* clavate to cylindrical, with a short 'foot', thin-walled laterally (1  $\mu$ m) but thicker apically, 2–3  $\mu$ m, 8-spored, 52–60  $\times$  11–15  $\mu$ m ( $n=6$ ), wall I+ reddish, turning bluish in part and KI+ pale blue, tholus I–, ascoplasm I+ orange. *Ascospores* biserial in the asci, hyaline only finally slightly brownish, ellipsoid to fusiform to slightly soleiform, with upper part wider than lower, (0–) 3-septate, not or hardly constricted at the septa, with a large oil droplet in each cell occupying most of the cell except the very

ends, thin and smooth-walled, without perispore, straight, rarely curved, (16–)18–20(–22) × (3.5–)4–5(–6) μm ( $n = 40$ ).

*Conidiomata* not observed.

**Notes.** Our new species fits well with the genus *Sagediopsis* by having immersed perithecioid ascomata with a flattened upper zone; a thickened upper exciple formed by elongated hyphae around the ostiole; periphysoids immersed in gel growing down from around the ostiole; abundant paraphysoids intermixed with the asci; a hymenial gel reacting reddish to blue with Lugol's reagent or IKI; *Verrucaria*-type asci that are thickened apically and 8-spored; ellipsoid to fusiform, transversally septate ascospores.

No other species of *Sagediopsis* are known from *Aspicilia*, although two were reported from *Aspilidea myrinii* (Fr.) Hafellner before it was transferred from *Aspicilia* to *Aspilidea*: *S. fissurisedens* Hafellner (Hafellner 1993) and *S. aspiciliae* Nik-Hoffm. & Hafellner (Hoffmann & Hafellner 2000). *Sagediopsis fissurisedens* has several features in common with our new species: ascomata with the exciple markedly thickened above, a similar hamathecium, 8-spored asci and 3-septate ascospores. However, it differs in the much larger perithecia (0.4–0.7 mm diam.), the larger asci (60–80 × 13–17 μm) that are I–, and the slightly shorter but notably wider ascospores, 12–17 × 5–8 μm. *Sagediopsis aspiciliae* is very different, also with larger ascomata (120–360(–400) μm diam.), larger asci (60–100 × 9–13.5 μm) and ascospores that are simple to rarely 1-septate and ellipsoidal, (10–)10.5–14.1(–15) × (5–)5.2–6.9(–8) μm, but it has a hemiamyloid hymenium gel similar to *S. epimalvinae*.

*Sagediopsis* species have also been reported from other genera of *Pertusariales*. *Sagediopsis pertusariicola* Zhurb. was described growing on *Pertusaria* (Zhurbenko 2009). This species has larger, glossy perithecia, (200–)250–400(–500) μm diam., and ascospores with upper and lower parts mostly equal in width, sometimes with pointed ends. *Sagediopsis campsteriana* (Lindsay) D. Hawksw. & R. Sant. is very similar to our new species but seems to be an exclusive parasite of *Ochrolechia* sp. Hawksworth (1975) and Triebel (1993) described it with ellipsoid ascospores, (1–)3(–4)-septate, (12–)15–20(–25) × 4–6 μm. According to the most recent description by Zhurbenko (2009), its ascomata are larger, 150–250(–400) μm diam., and usually immersed to sometimes erumpent to almost superficial (a sessile, obpyriform perithecium is drawn in Hawksworth (1975)) and it has slightly larger asci, 60–73 × 10–13 μm. In all of these species growing on *Pertusariales*, a subhymenium with many colourless oil droplets has not been recorded.

Other species of *Sagediopsis* are known from species of *Lecideaceae*. *Sagediopsis aquatica* (Stein) Triebel (Rambold et al. 1990), which occurs on *Koerberiella wimmeriana* (Körb.) Stein and is known only from Europe, has ascospores (22–)27–36(–45) × (2.5–)3–3.5(–4) μm that are narrowly fusiform to acicular and acuminate at the basal end. *Sagediopsis barbara* (Th. Fr.) R. Sant. & Triebel, which is restricted to *Porpidia* spp. (Triebel 1989), has larger ascomata (to 450 μm diam.) and larger ascospores (20–)27–39.5(–46) × (3–)3.5–4.5(–5) μm. *Sagediopsis dissimilis* Triebel was described growing on *Paraporpidia leptocarpa* (Nyl.) Rambold & Hertel in Australasia (Triebel 1993) and has 0–1-septate ascospores, (7.5–)8–10.5(–12) × (4–)4.5–6(–6.5) μm.

Other species of the genus have been reported from a range of unrelated host genera. *Sagediopsis bayozturkii* Halıcı et al. was described on *Acarospora macrocyclos* (Halıcı et al. 2017). It differs from *S. epimalvinae* by its smaller perithecia (90–150 μm diam.) and smaller ascospores ((10–)11–14(–15) × 4–5 μm), as well as in

several other features. *Sagediopsis lomnitzensis* (Stein) Orange on *Ionaspis lacustris* (With.) Lutzoni and *I. odora* (Ach.) Th. Fr. (Orange 2002) has a perithecial wall that is I+ sometimes violet to blue in part, and smaller, 1-septate, halonate ascospores, (9.5–)11–18 × 5–7(–8) μm. *Sagediopsis vasilyevae* Zhurb. on *Rhizocarpon* has much larger ascospores, (37.5–)41.7–50.3(–53.0) × (2.5–)2.9–3.5(–3.8) μm (Zhurbenko & Yakovchenko 2014).

**Acknowledgements.** Fieldwork on the Falkland Islands by the first author was funded by the UK Government through DEFRA and the Darwin Initiative as part of the project *Lower Plants Inventory and Conservation in the Falkland Islands* (Reference number DPLUS017). Support for fieldwork and advice regarding landowners was provided by Falklands Conservation. We are grateful to Alan Orange (Cardiff) for sharing his unpublished sequences of *Aspicilia malvinae* with us and to Kerry Knudsen (Prague) for additional specimens included in this study. We also thank the curators of NY for the loan of their isotype of *Lecanora masafuerensis*, and the curators of BM, GB, UPS & W for details of the type specimens of this species housed in their herbaria.

**Author ORCIDs.**  Alan Fryday, 0000-0002-5310-9232; Timothy Wheeler, 0000-0003-0668-8662; Javier Etayo, 0000-0003-0392-0710.

## References

- Calvelo S and Liberatore S (2002) Catálogo de los líquenes de la Argentina [Checklist of Argentinean Lichens]. *Kurtziana* 29(2), 7–170.
- Diederich P, Lawrey JD and Ertz D (2018) The 2018 classification and checklist of lichenicolous fungi, with 2000 non-lichenized, obligately lichenicolous taxa. *Bryologist* 121, 340–425.
- Elder D, Klein J, Antonelli A and Silvestro D (2021) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12, 373–377.
- Esslinger TL (2019) A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada, version 23. *Opuscula Philolichenum* 18, 102–378.
- Fletcher A, Purvis OW and Coppins BJ (2009) *Aspicilia* A. Massal. (1852). In Smith CW, Aptroot A, Coppins BJ, Fletcher A, Gilbert OL, James PW and Wolseley PA (eds), *The Lichens of Great Britain and Ireland*. London: British Lichen Society, pp. 181–188.
- Fryday AM (2015) A new checklist of lichenized, lichenicolous and allied fungi reported from South Africa. *Bothalia* 45, 59–122.
- Fryday AM, Orange A, Ahti T, Øvstedal DO and Crabtree DE (2019) An annotated checklist of lichen-forming and lichenicolous fungi reported from the Falkland Islands (Islas Malvinas). *Glalia* 8, 1–100.
- Galloway DJ (2007) *Flora of New Zealand Lichens. Revised Second Edition Including Lichen-Forming and Lichenicolous Fungi. Volumes 1 and 2*. Lincoln, New Zealand: Manaaki Whenua Press.
- Galloway DJ and Quilhot W (1998) Checklist of Chilean lichen-forming and lichenicolous fungi [Lista patron de los líquenes y hongos liquenícolas de Chile]. *Gayana Botanica* 55, 111–185.
- Gardes M and Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2, 113–118.
- Hafellner J (1993) Über Funde von lichenicolen Pilzen und Flechten im südlichen Norwegen. *Herzogia* 9, 749–768.
- Hafellner J (1994) Beiträge zu einem Prodromus der lichenicolen Pilze Österreichs und angrenzender Gebiete. I. Einige neue oder seltene Arten. *Herzogia* 10, 1–28.
- Halıcı MG, Güllü M and Parnikoza I (2017) *Sagediopsis bayozturkii* sp. nov. on the lichen *Acarospora macrocyclos* from Antarctica with a key to the known species of the genus (Ascomycota, Adelococcaceae). *Polar Record* 53, 271–275.
- Hawksworth DL (1975) Notes on British lichenicolous Fungi, I. *Kew Bulletin* 30, 183–203.
- Hoffmann N and Hafellner J (2000) Eine Revision der lichenicolen Arten der Sammelmattungen *Guignardia* und *Physalospora*. *Bibliotheca Lichenologica* 77, 1–190.

- Ihlen PG and Wedin M (2008) An annotated key to the lichenicolous Ascomycota (including mitosporic morphs) of Sweden. *Nova Hedwigia* **86**, 275–365.
- Index Fungorum Partnership (2021) *Index Fungorum* [WWW resource] URL <http://www.indexfungorum.org>. [Accessed 4 January 2021].
- Ismayil G, Abbas A and Guo S-Y (2015) *Aspicilia volcanica*, a new saxicolous lichen from Northeast China. *Mycotaxon* **130**, 543–548.
- Kondratyuk SY, Lököš L, Park J-S, Jang S-H and Jeong M-H (2016) New *Aspicilia* species from South Korea proved by molecular phylogeny with a key to the Eastern Asian aspicilioid lichens. *Studia Botanica Hungarica* **47**, 227–249.
- Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics* **30**, 3276–3278.
- Mason-Gamer R and Kellogg EA (1996) Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae* (*Gramineae*). *Systematic Biology* **45**, 524–545.
- McCarthy P (2016) *Checklist of the Lichens of Australia and its Island Territories*. [WWW resource] URL <https://id.biodiversity.org.au/reference/lichen/30005164> [Accessed 10 November 2020].
- Meyer B and Printzen C (2000) Proposal for a standardized nomenclature and characterization of insoluble lichen pigments. *Lichenologist* **32**, 571–583.
- Nordin A, Tibell LB and Owe-Larsson BR (2007) A preliminary phylogeny of *Aspicilia* in relation to morphological and secondary product variation. *Bibliotheca Lichenologica* **96**, 247–266.
- Nordin A, Savic S and Tibell L (2010) Phylogeny and taxonomy of *Aspicilia* and *Megasporaceae*. *Mycologia* **102**, 1339–1349.
- Nordin A, Owe-Larsson B and Tibell L (2011) Two new *Aspicilia* species from Fennoscandia and Russia. *Lichenologist* **43**, 27–37.
- Orange A (2002) Lichenicolous fungi on *Ionaspis lacustris*. *Mycotaxon* **81**, 265–279.
- Orange A, James PW and White FJ (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.
- Øvstedal DO and Lewis Smith RI (2001) *Lichens of Antarctica and South Georgia: A Guide to Their Identification and Ecology*. Cambridge: Cambridge University Press.
- Øvstedal DO, Tonsberg T and Elvebakk A (2009) The lichen flora of Svalbard. *Sommerfeltia* **33**, 1–393.
- Rambold G, Hertel H and Triebel D (1990) *Koerberiella wimmeriana* (*Lecanorales, Porpidiaceae*) and its lichenicolous fungi. *Lichenologist* **22**, 225–240.
- Räsänen V (1941) La flora liquenologica de Mendoza (Argentina). *Anales de la Sociedad Científica Argentina, Buenos Aires* **131**, 97–110.
- Schmitt I, Messuti MI, Feige GB and Lumbsch HT (2001) Molecular data support rejection of the generic concept in the *Cocotremataceae* (Ascomycota). *Lichenologist* **33**, 315–321.
- Schneider K, Resl P, Westberg M and Spribille T (2015) A new, highly effective primer pair to exclude algae when amplifying nuclear large ribosomal subunit (LSU) DNA from lichens. *Lichenologist* **47**, 269–275.
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.
- Triebel D (1989) Lecideicole Ascomyceten. Eine Revision der obligat lichenicolen Ascomyceten auf lecideoiden Flechten. *Bibliotheca Lichenologica* **35**, 1–278.
- Triebel D (1993) Notes on the genus *Sagediopsis* (*Verrucariales, Adelococcaceae*). *Sendtnera* **1**, 273–280.
- Urbanavichus G (2010) *A Checklist of the Lichen Flora of Russia*. St Petersburg: Nauka.
- White TJ, Bruns TD, Lee SB and Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis MA, Gelfand DH, Sninsky JJ and White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. New York: Academic Press, pp. 315–322.
- Zahlbruckner A (1924) Die Flechten der Juan-Fernandez-Inseln. In Skottsberg C (ed.), *The Natural History of Juan Fernandez and Easter Island, Vol. II*. Uppsala: Almqvist & Wiksells, pp. 315–408.
- Zhurbenko M (2009) *Sagediopsis pertusariicola* (*Verrucariales*), a new lichenicolous ascomycete from the Arctic. *Nova Hedwigia* **88**, 549–555.
- Zhurbenko M and Notov A (2015) The lichenicolous lichen *Placocarpus americanus* and some noteworthy lichenicolous fungi from Russia. *Folia Cryptogamica Estonica* **52**, 95–99.
- Zhurbenko MP and Yakovchenko LS (2014) A new species, *Sagediopsis vasilyevae*, and other lichenicolous fungi from Zabaikal'skii Territory of Russia, southern Siberia. *Folia Cryptogamica Estonica* **51**, 121–130.
- Zoller S, Scheidegger C and Sperisen C (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *Lichenologist* **31**, 511–516.