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Standard Paper

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

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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.

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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_3 (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	*Mcm7*
Aspicilia abbasiana	*Ismayil & Abbas* 20111154 (HMAS-L)	Heilongjiang, China	Ismayil *et al.* (2015)	NR_158307	–	–	–
A. angelica	*Wheeler* 5468 (hb. Wheeler)	Montana, USA	TW213	**MW435332**	**MW447407**	**MW424813**	**MW435341**
A. aurantiaca	*Wheeler* 7091 (hb. Wheeler)	California, USA	TW220	**MW447387**	**MW447401**	**MW424807**	**MW435335**
A. boykinii	*Wheeler* 7274 (hb. Wheeler)	Montana, USA	TW277	**MW447394**	**MW447409**	**MW424815**	**MW435343**
A. cinerea	*Wheeler* 7214 (hb. Wheeler)	Montana, USA	TW210	**MW447398**	**MW447413**	**MW424819**	**MW435347**
A. cinerea	*Wheeler* 6277 (hb. Wheeler)	Finnmark, Norway	TW219	**MW447391**	**MW447405**	**MW424811**	**MW435339**
A. cuprea	*Knudsen* 16336 (hb. Wheeler)	California, USA	TW331	**MW447385**	**MW447399**	**MW424805**	**MW435333**
A. cuprea	*Owe-Larsson* 9112 (UPS)	California, USA	Nordin *et al.* (2007, 2010)	EU057902	HM060750	HM060712	–
A. cyanescens	*Owe-Larsson* 9151 (UPS)	California, USA	Nordin *et al.* (2007, 2010)	EU057904	HM060745	HM060707	–
A. dudinesis	*Nordin* 6036 (UPS)	Torne Lappmark, Sweden	Nordin *et al.* (2007, 2010)	EU057906	HM060748	HM060710	–
A. epiglypta	*Nelson* s. n. (hb. Wheeler)	Hoffellsjokull, Iceland	TW276	**MW447396**	**MW447411**	**MW424817**	**MW435345**
A. fumosa	*Wheeler* 3844 (hb. Wheeler)	Montana, USA	TW224	**MW447395**	**MW447410**	**MW424816**	**MW435344**
A. knudsenii	*Wheeler* 6798 (hb. Wheeler)	Montana, USA	TW245	**MW447386**	**MW447400**	**MW424806**	**MW435334**
A. malvinae	*Fryday* 11433 (MSC)	East Falkland, Falkland Islands	TW260	**MW447392**	**MW447406**	**MW424812**	**MW435340**
A. pacifica	*Knudsen* 9241 (hb. Wheeler)	California, USA	TW334	**MW447393**	**MW447408**	**MW424814**	**MW435342**
A. santamonicae	*Wheeler* 6648 (hb. Wheeler)	California, USA	TW230	**MW447388**	**MW447402**	**MW424808**	**MW435336**
Circinaria calcarea	*Nordin* 5888 (UPS)	Oland, Sweden	Nordin *et al.* (2007, 2010)	EU057898	HM060743	HM060705	–
Lepra albescens	*Schmitt* s. n. (ESS-20967)	Bohemia, Czech Republic	Schmitt *et al.* 2001	AF329177	AF329176	AF329175	–
Lobothallia melanaspis	*Nordin* 6622 (UPS)	Jämtland, Sweden	Nordin *et al.* (2010, 2011)	HQ259272	HM060726	HM060688	–
L. praeradiosa	*Wheeler* 3414 (hb. Wheeler)	Montana, USA	TW269	**MW447389**	**MW447403**	**MW424809**	**MW435337**
Oxneriaria permutata	*Wheeler* 4463 (hb. Wheeler)	Alaska, USA	TW296	**MW447390**	**MW447404**	**MW424810**	**MW435338**
O. supertegens	*Owe-Larsson* 9002 (UPS)	Troms, Norway	Nordin *et al.* (2007, 2010)	EU057936	HM060742	HM060704	–
O. virginea	*Wheeler* 7153 (hb. Wheeler)	Montana, USA	TW240	**MW447397**	**MW447412**	**MW424818**	**MW435346**

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).

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Table 2. Primers used in this study.

Primer name	Primer sequence (5′–3′)	Reference
ITS1F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns (1993)
ITS4	TCCTCCGCTTATTGATATGC	White *et al.* (1990)
LrlecF	CCTCAGTAACGGCGAG	Schneider *et al.* (2015)
LR7	TACTACCACCAAGATCT	R. Vilgalys (unpublished)
mtSSU1	AGCAGTGAGGAATATTGGTC	Zoller *et al.* (1999)
mtSSU3R	ATGTGGCACGTCTATAGCCC	Zoller *et al.* (1999)
MCM7for	CGTCACTACAAAACAATTCACC	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
Mcm7	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

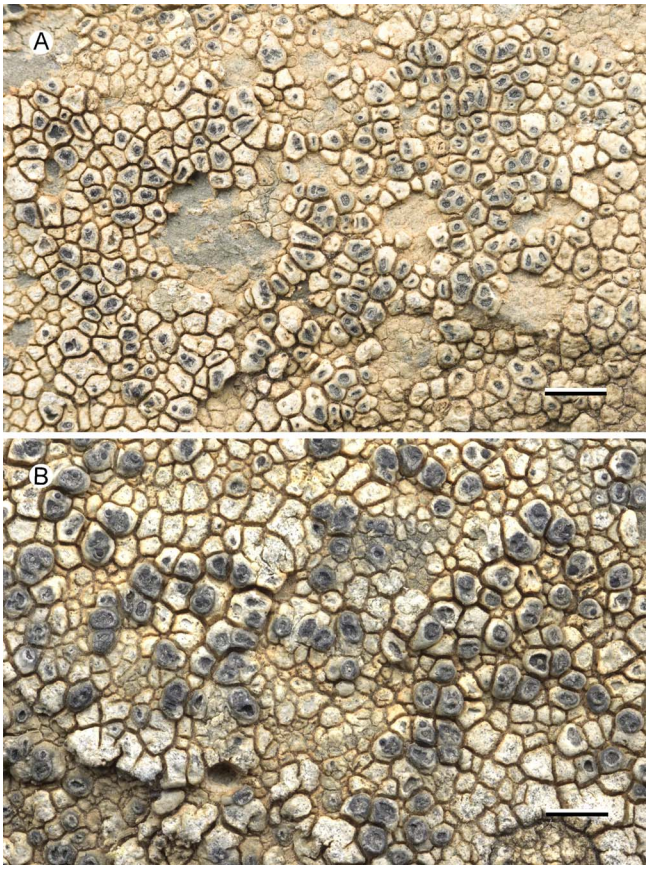

Fig. 1. *Aspicilia malvinae* (*Fryday* 11433, holotype). A, thallus with immersed apothecia. B, thallus with ±emergent apothecia. Scales = 2 mm. In colour online.

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 values ≥ 70% within one locus and the same group of taxa was supported ≥ 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 malvinae 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

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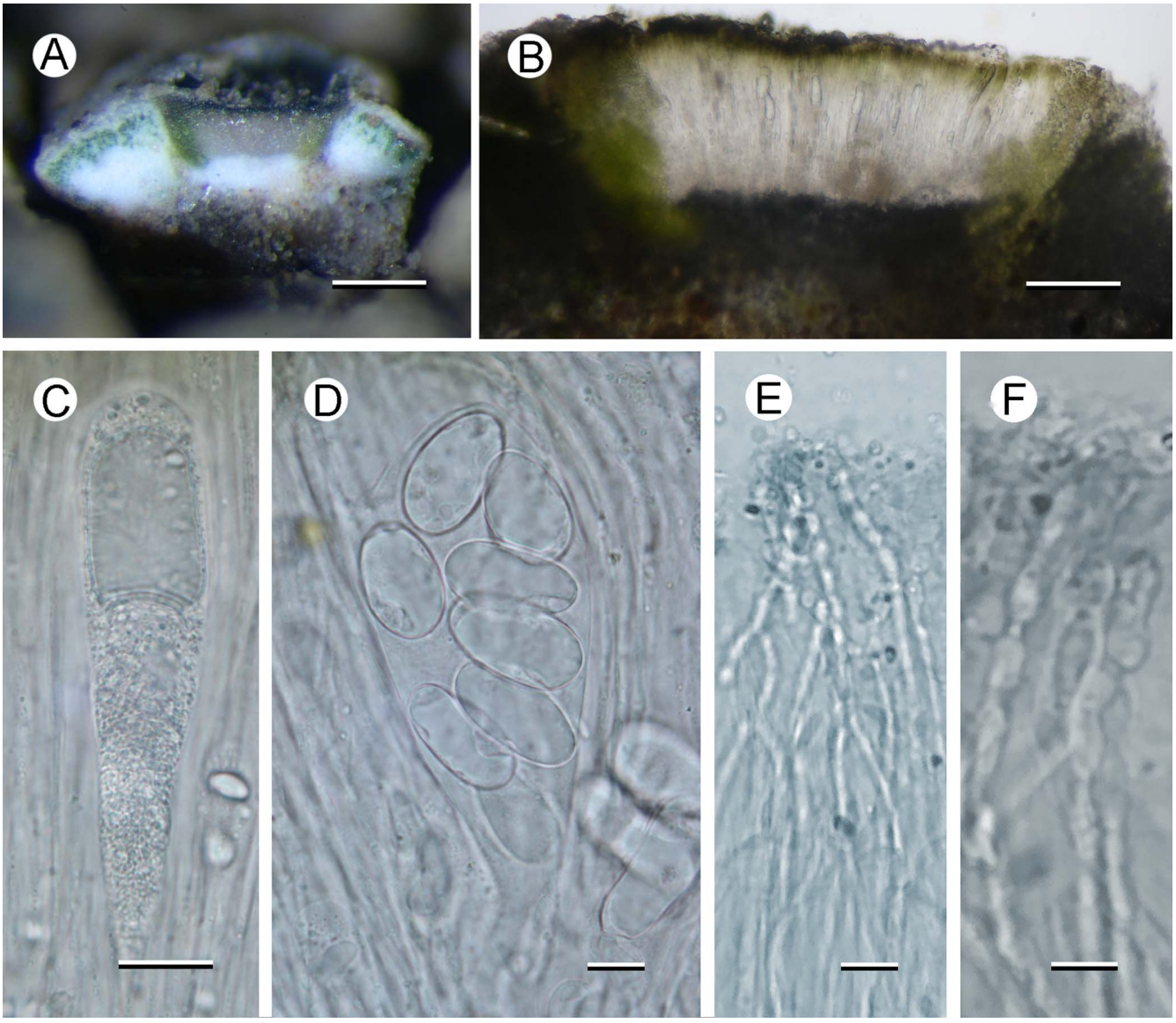

Fig. 2. *Aspicilia malvinae* (A–C, E & F, *Fryday* 11433, holotype; D, *Fryday* 11008). A, section through thallus and apothecium. B, section of apothecium. C, immature ascus. D, mature ascus with ascospores. E & F, paraphyses showing constricted septa in upper part (F). Scales: A = 2 mm; B = 100 μm; C & E = 20 μm; D & F = 10 μm. 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 μm thick with a thin epinecral layer 10–25 μm thick, upper 25 μm 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 μm 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 μm diam. *Photobiont* layer *c.* 25 μm thick, cells chlorococcoid, 6–16 μm 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 ±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 μm 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 μm wide) conglutinated hyphae that become wider (*c.* 5 μm) and cellular with constricted septa for the final 4–5 cells towards the cortex. *Hymenium* 140–160 μm, I+ slowly yellow and after *c.* 5 minutes greenish blue; *paraphyses* very fine *c.* 1 μm, the upper 20–25 μm (epihymenium) wider (5 μm) with constricted septa, cells globose to oblong, 5 μm wide by 5–7 μm long; *epihymenium* olive-brown (K+ brown, N+ bright aeruginose; Caesiocinerea-green); *subhymenium* hyaline, I+ slowly (after *c.* 5 minutes) dark blue, composed of ±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 μm) randomly organized hyphae. *Asci* cylindrical

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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_f 4), faint red spot at R_f 1 (probably subhypostictic acid), UV(after charring)++ cream spot at R_f 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 Usborne, 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

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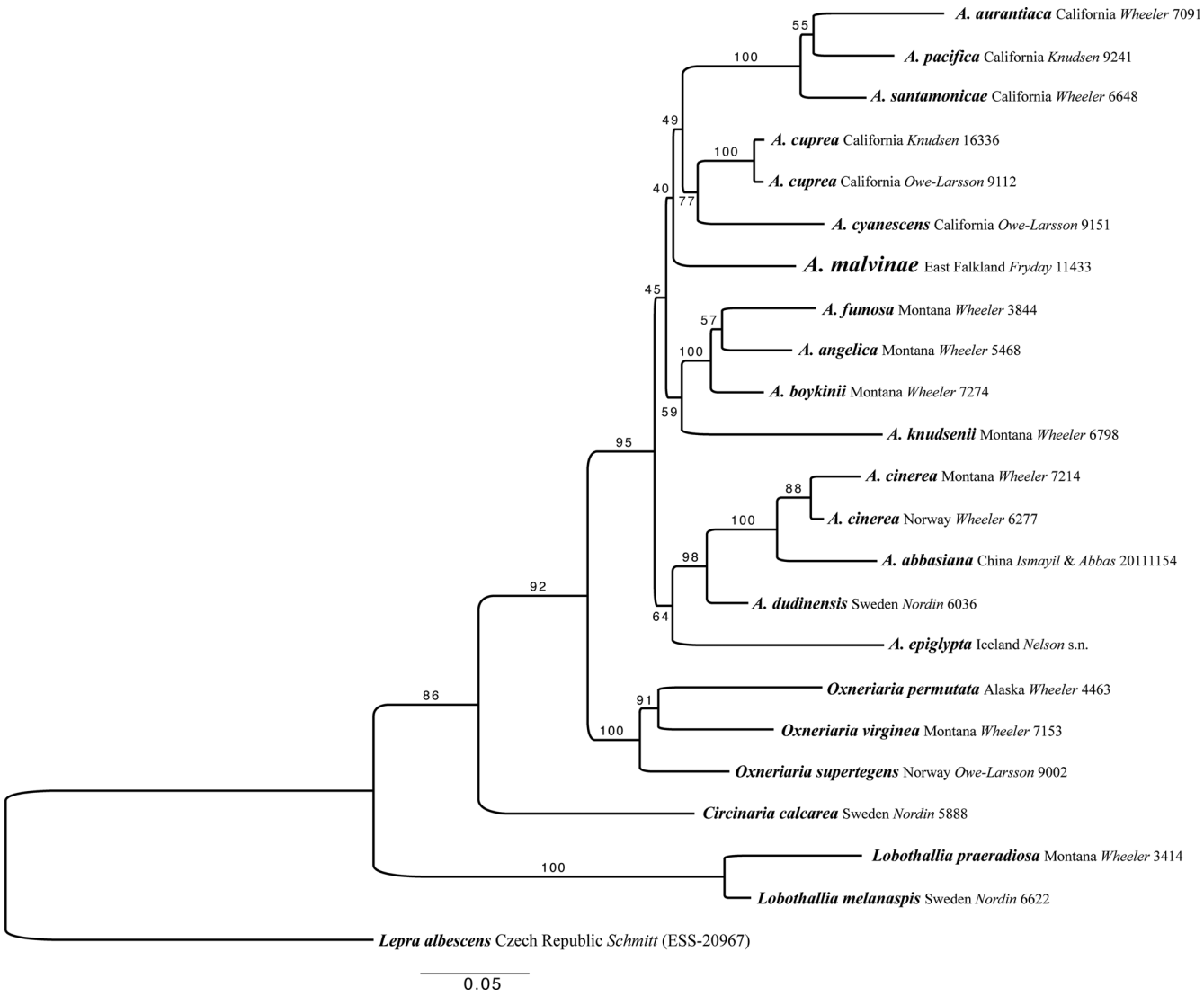

Fig. 3. Maximum likelihood (ML) tree of the concatenated (ITS, LSU, mtSSU and *Mcm7*) dataset for members of *Aspicilia* and related species. Analyses were performed using raxmlGUI 2.0 (Stamatakis 2014; Edler *et al.* 2020). Maximum likelihood bootstrap values are shown above each branch. The newly introduced 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 Usborne, Camilla Creek, 51.716467°S, 58.898183°W, 67 m, bare, stony area in grass/*Empetrum* heath, 2015, *A. M. Fryday* 11431 (MSC).

Sagediopsis epimalvinae Etayo, T. B. Wheeler & Fryday sp. nov.

MycoBank No.: MB 839031

Lichenicolous fungus growing on *Aspicilia malvinae*. 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 malvinae* 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 malvinae*. *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

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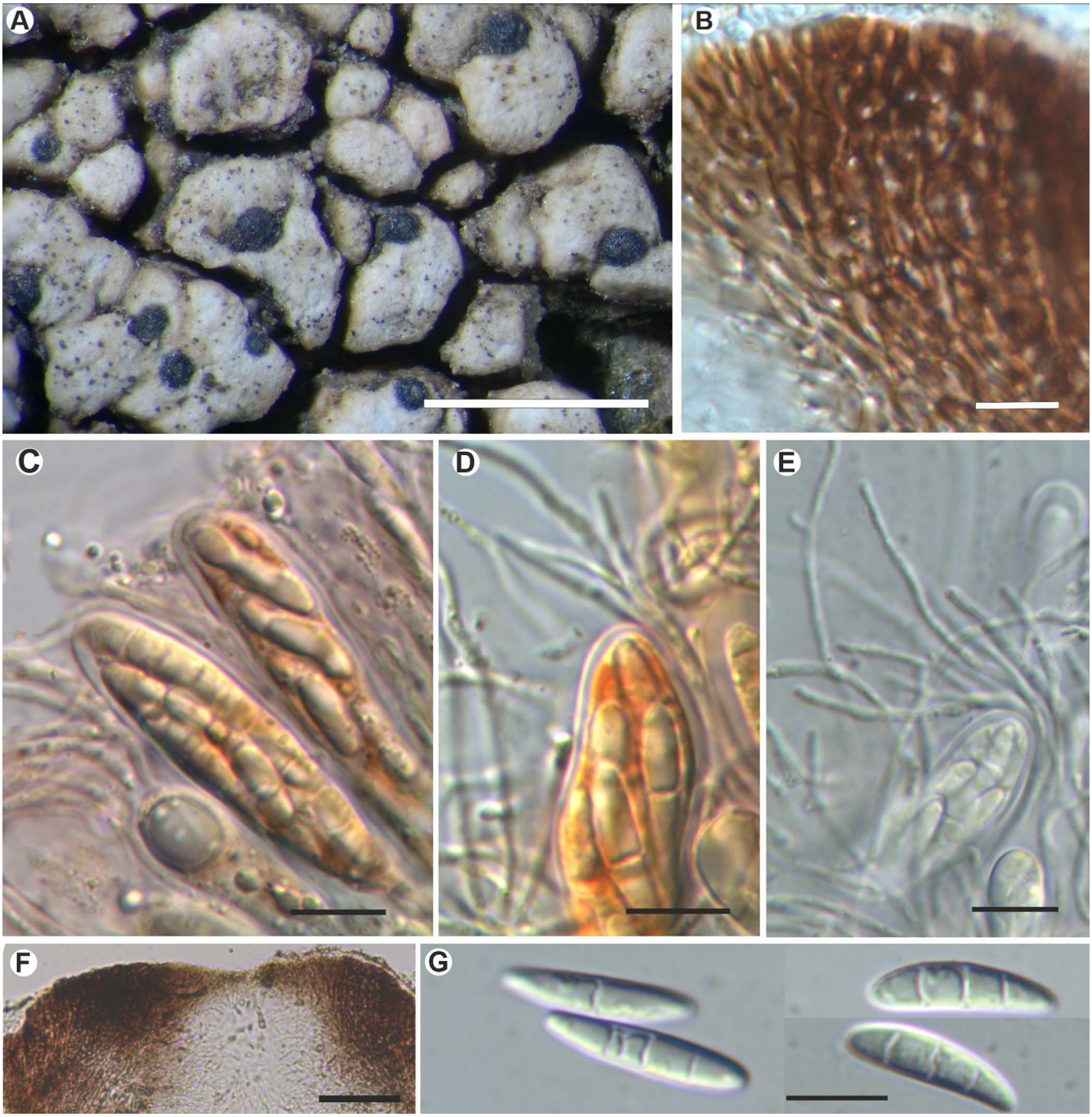

Fig. 4. *Sagediopsis epimalvinae* (*Fryday* 11433a, holotype). A, habitus of several *Sagediopsis* perithecia on areolae of *Aspicilia malvinae*. B, upper part of exciple showing elongated hyphae disposition and periphysoids in ostiolar channel. C & D, upper part of asci and paraphyses (in I). E, ascus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

elongated; base and lateral parts of exciple 15–20(–35) µm thick, composed of several layers of more or less elongated cells with a thick cell wall and small lumina, 4–7 × 1.5–2 µm. *Periphysoids* growing down from around ostiole, abundant, immersed in a colourless gel, simple to branched, even anastomosed, especially at the base, 10–30 × 1 µm. *Hymenial gel* I+ reddish, KI+ blue; *subhymenium* with large colourless oil droplets, 1–6 µm diam. *Hamathecium* of persistent paraphysoids, flexuose, not thickened at the apex, septate, simple or sparingly branched, not constricted at the septa, 1–1.5 µm thick, with many small colourless oil droplets. *Asci* clavate to cylindrical, with a short 'foot', thin-walled laterally (1 µm) but thicker apically, 2–3 µm, 8-spored, 52–60 × 11–15 µm ($n = 6$), wall I+ reddish, turning bluish in part and KI+ pale blue, tholus I–, ascoplast I+ orange. *Ascospores* biseriate 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

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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).

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