A new species of *Sagiolechia* (Sagiolechiaceae) from Norway, with lirelliform ascomata and 1-septate ascospores

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The new species *Sagiolechia monoseptata* is described from the northern boreal zone of Norway where it was found on a shaded siliceous rock under a boulder in a north-facing, small, steep scree. It is characterized by the pale greyish-green thallus, black, lirelliform ascomata, and 1-septate ascospores of (11–) 12–16 × (4–) 5–6 (–7) µm, all features unique within the genus. Despite the unusual combination of morphological characters, phylogenetic analyses using nuLSU, mtSSU and *RPB*2 sequences place the new lichen in the genus *Sagiolechia* with full support.

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Introduction

The lichen flora of Fennoscandia is among the best known in the world thanks to a concentration of taxonomic expertise for a long time in these countries. Nevertheless, between 2004 and 2019, c. 20 species on average were added every year to the checklist of the lichen forming and lichenicolous fungi of Fennoscandia to reach the impressive total of 3155 species (Ekman et al. 2019). Recent fieldwork by the second author (TT) in Norway revealed an unknown lichen species growing on a shaded rock under boulders in a N-facing, steep scree facing a mire. Morphological examination and multi-loci phylogenetic studies leaved no doubts that the species belongs in the genus *Sagiolechia*.

Sagiolechia was introduced by Massalongo (1854) for the single species originally described as *Sagedia protuberans* Ach. The genus is mainly distributed in the Northern Hemisphere where it is widespread. It includes currently five accepted species: *Sagiolechia atlantica* Henssen, *S. parasitica* Alstrup & E.S. Hansen, *S. phaeospora* Fryday & T. Sprib., *S. protuberans* (Ach.) A. Massal. and *S. rhexoblephara* (Nyl.) Zahlbr. (Vězda 1967, Henssen 1995, Alstrup & Hansen 2001, Spribille et al. 2020). All species are saxicolous, including one lichenicolous on a saxicolous lichen and one growing mainly on saxicolous mosses. The genus is poorly defined by dark brown to black and epruinose apothecia, a carbonized prominent often crenate excipulum, mainly simple or partly branched-anastomosing paraphyses in a dense matrix, an amyloid hymenium, cylindrical-clavate 8-spored asci and septate, usually hyaline ascospores often with a perispore, and the absence of secondary metabolites (Vězda 1967, Henssen 1995, Alstrup & Hansen 2001, Gilbert et al. 2009, Spribille et al. 2020). It includes species usually lichenized with a trentepohlioid photobiont but one

species has a single-celled green alga considered as non-trentepohlioid and another is a lichenicolous fungus. The family Sagiolechiaceae was introduced to accomodate the genera *Rhexophiale* and *Sagiolechia* (Baloch et al. 2010). The genus *Rhexophiale* was reintroduced for *Sagiolechia rhexoblephara* by Henssen (1995) based on differences in the ontogeny of its ascomata compared to *S. protuberans*. However, phylogenetic results placed *Rhexophiale* within a clade with *Sagiolechia phaeospora* and *S. protuberans*, challenging the distinction of the two genera (Spribille et al. 2020).

This paper aims to describe as new to science a species of *Sagiolechia* from Norway and to determine its phylogenetic position using nuLSU, mtSSU and *RPB*2 sequences.

Material and Methods

The three years old herbarium material is deposited in BG with a duplicate in NY. The external morphology was studied and measured using an Olympus SZX12 stereomicroscope. Macroscopic photographs were made with a Keyence VHX-5000 Digital Microscope and a VH-Z20R/W/T lens. Hand-cut sections and squash preparations of thallus were mounted in water, a 5% aqueous potassium hydroxide solution (K), or in Lugol's iodine solution (1% I_2) without (I) or with K pre-treatment (KI) and studied using an Olympus BX51 compound microscope. Measurements were made in water. Microscopic photographs were prepared using an Olympus BX51 compound microscope, fitted with an Olympus SC50 digital camera. Colour reactions of the thallus were studied using K, common household bleach (C), crystals of para-phenylenediamine dissolved in ethanol (PD) and long wave UV (366 nm). Lichen secondary metabolites were investigated using thin-layer chromatography (TLC) according to the methods of Culberson & Kristinsson (1970), Culberson (1972), and Menlove (1974), with glass plates in all three solvents allowing for the detection and identification of fatty acids.

Molecular techniques: A well-preserved and three years old herbarium specimen lacking any visible symptoms of fungal infection was used for DNA isolation. Hand-made sections of the hymenium or of the thallus were used for direct PCR as described in Ertz et al. (2015). The material was placed directly in microtubes with 20 µl H_2O. Amplification reactions were prepared for a 50 µl final volume and the lichen material as detailed in Ertz et al. (2018). A targeted fragment of c. 0.8 kb of the mtSSU rDNA was amplified using primers mrSSU1 and mrSSU3R (Zoller et al. 1999), a fragment of about 0.9 kb of the *RPB*2 protein-coding gene was amplified using primers *fRPB*2-7cF and *fRPB*2-11aR (Liu et al. 1999) and a fragment of about 1 kb at the 5'end of the nucLSU rDNA was amplified using primers LIC15R (Miadlikowska et al. 2002) and LR6 (Vilgalys & Hester 1990). Both strands were sequenced by Macrogen® using amplification primers, and with the additional primer LR3 (Vilgalys & Hester 1990) for nuLSU. Sequence fragments were assembled with Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to 'Megablast' searches to verify their closest relatives.

Taxon selection and phylogenetic analyses: One dataset of nuLSU, mtSSU and *RPB*2 sequences was assembled for placing the newly sequenced taxon in a phylogeny of the order Ostropales s. lat. (now split into Graphidales, Gyalectales, Ostropales s. str., Thelenellales and Odontotrematales; Kraichak et al. 2018, Lücking 2019). The closest relatives of the new sequences based on BLAST searches were retrieved from GenBank, in particular all available taxa belonging to the genus *Sagiolechia* that appeared to be the genus with the highest percent identity. Additional taxa were selected mainly from Baloch et al. (2010) in order to include an exhaustive list of taxa belonging to

Table 1. Species names, voucher specimens and GenBank accession numbers for the specimens included in the phylogenetic inference depicted in Fig. 1. Sequences newly generated in this study are in bold.

Species	Voucher	LSU	mtSSU	RPB2
Orceolina kerguelensis	Kerguelen, Poulsen 456 (C)	AF274116	AF381561	DQ366256
Absconditella sphagnorum	Czech Republic, Palice 17 Feb 02 (hb. Palice) for LSU and mtSSU; Palice 11146 (S) for RPB2	AY300824	AY300872	HM244777
Acarosporina microspora	CBS 338.39; AFTOL-ID 78	AY584643	AY584612	AY584682
Carestiella socia	Norway, Wedin 7194 (UPS)	AY661687	AY661677	HM244782
Claviradulomyces dabeicola	IMI 393994	GQ337897	GQ337898	-
Coccomycetella richardsonii	Sweden, Baloch SW068 (S)	HM244761	HM244737	HM244785
Coenogonium leprieurii	Kauff pa04021998-522 (hb. Kauff)	AF465442	AY584698	AY641032
Coenogonium luteum	Ryan 31430 (ASU)	AF279387	AY584699	AY641038
Coenogonium pineti	Italy, Thor 19164 (UPS)	AY300834	AY300884	HM244786
Cryptodiscus cladoniicola	Denmark, Faroe Islands, Kocourková *et al.* (H)	KY661653	KY661675	-
Cryptodiscus pallidus	Sweden, Östergötland, Baloch SW174 (S)	FJ904680	FJ904702	HM244789
Diploschistes cinereocaesius	AFTOL-ID 328	DQ883799	DQ912306	DQ883755
Fissurina insidiosa	AFTOL-ID 1662	DQ973045	DQ972995	DQ973083
Geisleria sychnogonoides	GESY7510	KF220304	KF220306	-
Glomerobolus gelineus	OSC 100192; AFTOL-ID 1349	DQ247803	DQ247783	-
Graphis librata	El Salvador, Lücking 28001	HQ639636	HQ639621	JF828945
Gyalecta fagicola	Sweden, Delin L-163179 (UPS)	-	HM244753	HM244807
Gyalecta flotowii	Sweden, Svensson 679 (UPS)	HM244764	HM244740	HM244794
Gyalecta friesii	AFTOL-ID 4926; UBC:Björk 05-973	KJ766566	KJ766400	-
Gyalecta geoica	Sweden, Svensson 664 (UPS)	HM244765	HM244741	HM244795
Gyalecta herculina	Czech Republic, Palice s.n. (F)	FJ941886	-	HM244779
Gyalecta hypoleuca	Austria, Hafellner 63694 (UPS)	AF465453	HM244742	AY641060
Gyalecta jenensis	Lutzoni 98.08.17-6 (DUKE)	AF279391	AY584705	AY641043
Gyalecta russula	Sweden, Hermansson 14140 (UPS)	HM244759	HM244735	HM244780
Gyalecta schisticola	Gueidan & Miadlikowska (DUKE)	-	KJ766401	KJ766974
Gyalecta truncigena	Sweden, Nordin 5851 (UPS)	HM244766	HM244743	HM244796
Gyalecta ulmi	Scheidegger 30.05.1998 (DUKE)	AF465463	AY584706	AY641044

Gyalidea praetermissa	Sweden, Svensson 949 (UPS)	HM244768	HM244745	HM244798
Ingvariella bispora	Spain, Llimona s.n. (BCNLich 17183)	HQ659185	HQ659174	-
Karstenia rhopaloides	Denmark, Laessøe 12881 (S)	FJ904685	FJ904707	HM244791
Myeloconis erumpens	New Caledonia, Lumbsch 8233 (F)	KJ449338	KJ449328	-
Odontotrema phacidiellum	Sweden, Gilenstam 2625 (UPS)	HM244769	HM244748	HM244802
Odontotrema phacidioides	Morocco, Kolařík s.n., ex hb. Palice 11440 (S)	HM244770	HM244749	HM244803
Ostropa barbara	Sweden, Wedin & Baloch SW071 (S); EB85	HM244773	HM244752	HM244806
Petractis luetkemuelleri	Nimis & Tretiach 2000 (TSB for LSU); Geletti & Tretiach 1995 (TSB for RPB2)	AF465454	-	AY641061
Phlyctis agelaea	Nordin 3028 (UPS)	AY853381	AY853332	-
Phlyctis argena	AFTOL-ID 1375	DQ986771	DQ986880	DQ992458
Porina aenea	Sweden, Arup & Baloch SW154 (S)	-	HM244754	HM244808
Porina byssophila	Sweden, Nordin 5990 (UPS)	-	HM244755	HM244809
Porina lectissima	Sweden, Arup & Baloch SW152 (S)	HM244774	HM244756	HM244811
Protothelenella sphinctrinoidella	Antarctica, Livingston Island, Lumbsch 19031d (F)	AY607735	AY607747	-
Ramonia sp.	Palice 3178 (hb. Palice); as "Palice 2336"	AY300871	AY300921	-
Sagiolechia monoseptata	Norway, Tønsberg 47117 (BG)	**MT822680**	**MT822679**	**MT822157**
Sagiolechia phaeospora	USA, Alaska, Spribille 38406 (MSC)	MN460229	MN508297	-
Sagiolechia protuberans	Sweden, Nordin 5893 (UPS)	HM244775	HM244757	HM244812
Sagiolechia rhexoblephara	Sweden, 2002, Palice s.n. (hb. Palice)	AY853391	AY853341	-
Schizoxylon albescens	Sweden, Gilenstam 2696a (UPS), Wedin 7919 (UPS)	DQ401144	DQ401142	HM244813
Sphaeropezia capreae	Gilenstam 2560 (UPS); GG2560	AY661684	AY661674	-
Sphaeropezia lyckselensis	Sweden, Gilenstam 2651 (UPS)	JX266158	JX266156	-
Stictis radiata	Jamie Platt 222 (OSC, DUKE)	AF356663	AY584727	AY641079
Thelenella antarctica	Antarctica, Livingston Island, Lumbsch 19006a (F)	AY607739	AY607749	-
Thelotrema lepadinum	India, Lumbsch 19744i	JX421652	JX421365	JX420850
Xyloschistes platytropa	AFTOL-ID 4891	KJ766680	KJ766517	-

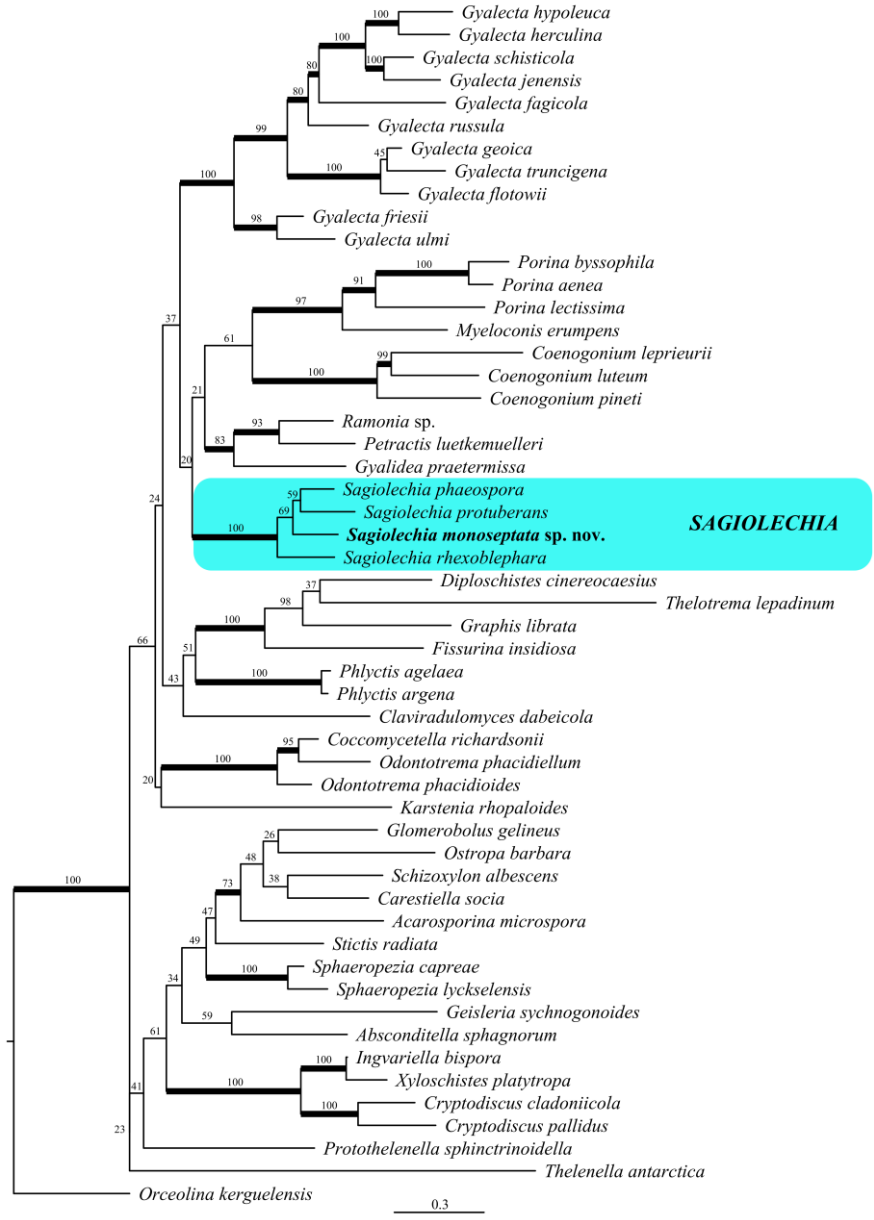

Figure 1. Phylogeny of the Ostropales s. lat. (with *Orceolina kerguelensis* as outgroup) based on a data set of nuLSU, mtSSU and *RPB*2 sequences that resulted from a RAxML analysis. Maximum Likelihood bootstrap values are shown above internal branches. Internal branches considered strongly supported (bootstrap values ≥ 70) are represented by thicker lines. A colour box highlights the lineage corresponding to the genus *Sagiolechia* and the holotype of *S. monoseptata*, newly sequenced, is in bold.

different families of the Ostropales s. lat. and for which at least two of the three loci were available to limit missing data. The sequences of taxa listed in Table 1 were aligned using MAFFT v7.402 (Katoh et al. 2002) on the CIPRES Web Portal (Miller et al. 2010) and manually improved using Mesquite 3.04 (Maddison & Maddison 2015). Terminal ends of sequences, ambiguously aligned regions, and introns were delimited manually and excluded from the datasets.

Analyses for topological incongruence among loci were carried out for the single locus datasets using a Maximum Likelihood (ML) approach with the program RAxML v.8.2.12 (Stamatakis 2014) on the CIPRES Web Portal (Miller et al. 2010) with 1000 ML bootstrap iterations (ML-BS). The GTRGAMMA model was used and node support was assessed running 1000 bootstrap replicates. A conflict was assumed significant, when two different relationships (one being monophyletic and the other being non-monophyletic) for the same set of taxa were both supported with bootstrap values ≥ 70 (Mason-Gamer & Kellogg 1996; Reeb et al. 2004). Based on this criterion, no conflict was detected and the nuLSU, mtSSU and *RPB*2 datasets were concatenated. A ML analysis was performed on the three-locus dataset using RAxML v.8.2.12 (Stamatakis 2014) on the CIPRES Web Portal (Miller et al. 2010) with 1000 ML bootstrap iterations (ML-BS). The three-locus dataset was divided in five partitions (nuLSU, mtSSU, *RPB*2/1st, *RPB*2/2nd, *RPB*2/3rd positions) with the GTRGAMMA model. *Orceolina kerguelensis* (Tuck.) Hertel was used as the rooting taxon. ML-BS ≥ 70 were considered to be significant. Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2012). The dataset and tree were deposited in TreeBASE as submission 26692.

Results

Phylogenetic analysis: Three new sequences (1 nuLSU, 1 mtSSU, 1 *RPB*2) were obtained for this study and 134 additional sequences (48 nuLSU, 50 mtSSU, 36 *RPB*2) were retrieved from GenBank (Table 1). The resulting concatenated matrix of the Ostropales s. lat. consisted of 53 OTUs and of 2333 (898 for nuLSU, 559 for mtSSU, 876 for *RPB*2) unambiguously aligned sites respectively. The RAxML tree obtained from the combined three-locus analysis of the Ostropales s. lat. dataset is shown in Fig. 1. The main well-supported lineages are in accordance with the results obtained by Baloch et al. (2010) and Spribille et al. (2020). The new species is nested in the genus *Sagiolechia* with full support.

Taxonomy

Sagiolechia monoseptata **Ertz & Tønsberg, sp. nov.** Fig. 2

MycoBank: MB 838445.

Diagnosis: A species of *Sagiolechia* characterized by a pale greyish-green thallus, black lirelliform ascomata, and 1-septate ascospores of (11–) 12–16 × (4–) 5–6 (–7) µm.

Type: Norway, Nordland: Grane, Trolldalen, 65.25035°N, 13.32185°E, 300 m elev., on shaded rock under a boulder in N-facing, small, steep scree facing an intermediate fen, 23 June 2017, Tor Tønsberg 47117 (BG L-105249, holotype; NY, isotype).

Etymology: The species epithet refers to the 1-septate ascospores.

Description: Thallus thin, pale greyish-green when dry, smooth or with some granules. Prothallus absent. Photobiont trentepohlioid, but no distinct orange bodies observed in fresh material; cells in

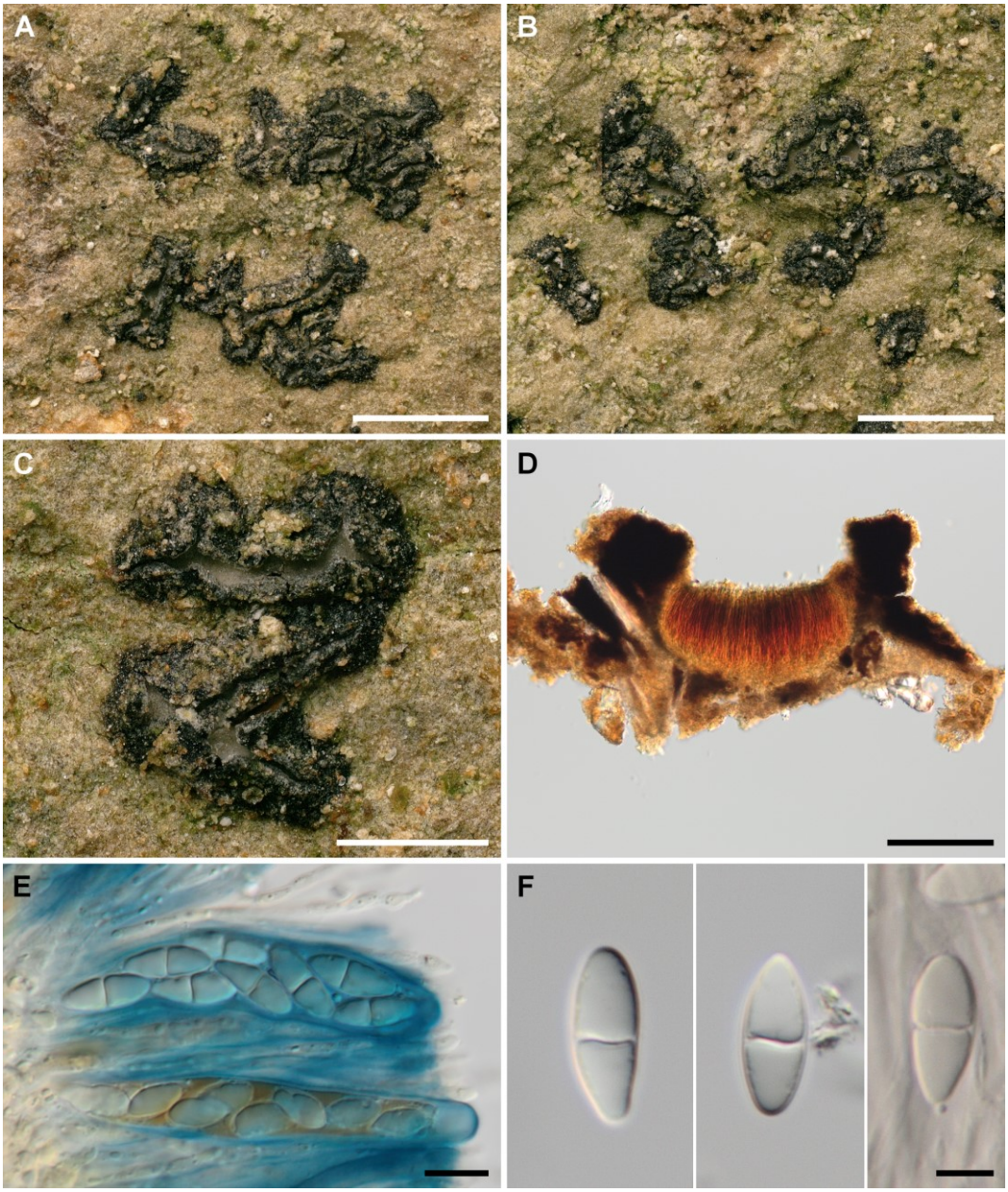

Figure 2. *Sagiolechia monoseptata* (holotype). **A–C** Thallus with ascomata. **D** Section of ascoma in lugol. **E** Asci (upper one with mature ascospores; lower one with immature ascospores) and paraphyses in KI. **F** Ascospores in water. Scale bars: A–B=1 mm, C=500 µm, D=100 µm, E=10 µm, F=5 µm. Photos D. Ertz.

short chains of c. 2–4 (–6) cells, ± rounded, ellipsoid to oblong, 11–23 × 7–19 µm. Ascomata black, epruinose, semi-sessile, without constricted base, when young ±urceolate and rounded or angular, opening with lobes sometimes appearing as black tooths, soon becoming elongate-lirelliform, frequently with 1 to 3 branches, 0.5–2 × 0.3–0.5 mm, sometimes 2–3 contiguous forming clusters up to 2 mm diam.; hymenial disc white, deeply immersed in ascomata and remaining mostly covered by the black excipulum. Excipulum black, somewhat crenate, 35–45 µm laterally, sometimes thicker in the upper part up to 75 µm, K–, much thinner and hyaline to brown below the hymenium, c. 5–13 µm. Hymenium hyaline, clear, (48–) 65–100 µm, I+ pale blue (mainly in the half upper part) quickly turning dark vinose red, KI+ deep blue; epihymenium not distinctly delimited; subhymenium hyaline, I+ pale orange. Paraphyses simple, 2.5–3 µm, hyaline and not distinctly enlarged at apex. Asci narrowly clavate to cylindrical, 8-spored, 65–90 × 9–12 µm, KI+ blue with a darker thin layer on the outer surface. Ascospores hyaline, 1–2 seriate in ascus, ellipsoid, 1-septate, one cell sometimes longer, (11–) 12–16 × (4–) 5–6 (–7) µm (n=16), without or with a thin, rather inconspicuous gelatinous sheath c. 0.5 µm thick. Conidiomata not observed.

Chemistry: thallus C–, K–, PD–, UV–; no lichen substances detected by TLC.

Distribution and habitat: *Sagiolechia monoseptata* is only known from the type locality in the northern boreal zone of Norway where it was found on the horizontal upper side of a shaded siliceous rock under a boulder in a N-facing, small, steep scree (Fig. 3) facing an intermediate fen. Closely associated species included *Lepraria incana* (L.) Ach. and an unidentified crustose lichen with pycnidia. Other noteworthy lichens found in the scree included *Gyalecta friesii* Flot. ex Körb. and *Psilolechia clavulifera* (Nyl.) Coppins. The scree supported small specimens of *Betula pubescens*. The fen was bordered by *Picea abies*.

Discussion

Our phylogenetic results leave no doubt that the new lichen belongs in the genus *Sagiolechia* as currently circumscribed. The morphology fits rather well with that genus, including the trentepohlioid photobiont, the black somewhat crenate excipulum, mainly simple or partly branched-anastomosing paraphyses in a dense matrix, a KI+ blue hymenium, cylindrical-clavate and 8-spored asci, septate ascospores, and the absence of secondary metabolites. However, *S. monoseptata* differs from all known *Sagiolechia* species by its lirelliform ascomata, and 1-septate ascospores. *Sagiolechia protuberans*, widespread on calcareous rocks, and *S. rhexoblephara*, an arctic-alpine species growing on saxicolous bryophytes, both differ from *S. monoseptata* by rounded ascomata, a brown epihymenium and 3-septate ascospores (Zahlbruckner 1913, Vězda 1967, Henssen 1995, Gilbert et al. 2009). *Sagiolechia atlantica* described from Madeira and *S. phaeospora* described from Alaska, have whitish thalli, muriform, larger ascospores and an I+ blue hymenium (Henssen 1995, Spribille et al. 2020). In addition, *S. phaeospora* is the only species of the genus to have brown ascospores and a non trentepohlioid alga as photobiont. *Sagiolechia parasitica* is a lichenicolous fungus described from Greenland forming apothecia with a black disc and a crenulate margin, and 3-septate ascospores (Alstrup & Hansen 2001). *Sagiolechia fusiformis* (Müll. Arg.) Zahlbr. is a poorly known species described from rocks in Japan (Zahlbruckner 1905); it might belong to the genus *Catinaria* according to Vězda (1967). It differs from the new species by rounded, 0.4–0.7 mm apothecia with a widely exposed hymenial disc and 3-septate ascospores.

Figure 3. Type locality of *Sagiolechia monoseptata* in Norway (Nordland, Grane, Trolldalen) consisting of boulders in a N-facing, small, steep scree facing a fen. Photo T. Tønsberg, 28 July 2020.

The lirelliform black ascomata and 1-septate hyaline ascospores with rounded apices are reminiscent of members of the heterogeneous genus *Melaspilea*. Among them, *M. interjecta* (Leight.) A.L. Sm. inhabits siliceous rocks, but that species differs from *S. monoseptata* in having a K± olivaceous epihymenium and excipulum, an I+ yellowish hymenium, a reddish brown-black hypothecium, richly branched and anastomosed paraphysoids, shorter (47–53 µm) asci, and larger (17–23 × 7–9 µm) ascospores (Smith 1911, Sanderson et al. 2009). *Melaspilea granitophila* (Th. Fr.) Coppins also grows on siliceous rocks, but is very different from *S. monoseptata* in having much smaller (0.1–0.4 mm diam.) and rounded to somewhat elongate apothecia, an excipulum extended below into a stipe-like structure, paraphysoids, and much smaller asci (25–45 × 14–20 µm) (Coppins 1989, Sanderson et al. 2009); phylogenetic analyses place the species in the Arthoniaceae (Frisch et al. 2014). *Melaspilea tyroliensis* Szatala is known only from the type collection in Italy (Mt. Margola), on syenite. It has a thallus with white maculiform soralia, sessile lirelliform ascomata up to 1 mm long and 0.1–0.2 mm wide with an entire excipulum, I+ red hymenium, asci of 50–60 × 21–24 µm with a thick upper wall and 1-septate ascospores becoming brownish and clearly constricted in the middle (Szatala 1954, Nimis et al. 2018).

The genus *Sagiolechia* appears morphologically rather heterogeneous as it includes species with lirelliform to rounded ascomata, with hyaline or brown ascospores, with transversely septate to muriform ascospores, and with a lichenized or a lichenicolous life habit. However, with the four species currently sequenced and forming a distinct lineage, there is no evidence for a refined generic concept. *Sagiolechia rhexoblephara* is the basal taxon in the *Sagiolechia* clade (Fig. 1), which supports Henssen's (1995) hypothesis that this species differs from the generic type (*S. protuberans*) by the structure and development of the apothecia and might thus deserve a different genus, *Rhexophiale*. However, this topology is rather weakly supported and it was not recovered in the phylogeny of Spribille et al. (2020) where *S. protuberans* was the basal taxon with *S. phaeospora*

and *S. rhexoblephara* forming the ingroup. The new species described in the present study and two other probably undescribed species reported by Spribille et al. (2020) suggest that more species of *Sagiolechia* are to be discovered in the cold areas of the Northern Hemisphere.

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