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Homoplasy rather than intergeneric hybridisation explains mammillose leaf cells in *Schistidium mammillosum* sp. nov. (Grimmiaceae)

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Abstract

We present a peculiar new species in the Grimmiaceae, *Schistidium mammillosum,* which is distinct in having mammillose leaf cells and pluristratose lamina. These features are rare in mosses but shared by a few *Grimmia* species including sympatric *G. alpestris*. Sporophyte characteristics of the new taxon, in contrast, indicated a species of *Schistidium* and suggested a possible intergeneric hybrid origin. Nuclear ITS and plastid *rps4–trnF* sequence data consistently resolved the new taxon within species of *Schistidium,* thus contradicting the hybridogenic hypothesis. We suggest that the shared feature of mammillose cells evolved independently in the two genera in adaptation to similar environmental conditions, possibly prolonged snow cover. Supported by morphological similarity, our phylogenetic analyses indicate a sister relationship of *S. mammillosum* and *S. marginale* in the Confertum clade. *Schistidium mammillosum* colonises siliceous rocks in the alpine and nival zone and is to date known from the French Alps and a single locality in Switzerland.

Key words: Alps, Bryophytes, Convergence, *Grimmia*, Mosses, Taxonomy

Introduction

Bryophytes are the second most diverse group of land plants after flowering plants but the evolutionary mechanisms that drive speciation are less explored. However, hybridisation and introgression seem to be important drivers (Sawangproh & Cronberg 2021). Similar to flowering plants and other groups of organisms, the formation of non-sterile hybrids in bryophytes is counteracted by phylogenetic distances (Anderson & Snider 1982). Accordingly, hybridisation (i.e., the formation of hybrid sporophytes) has mostly been observed between congeneric species and the putative parental species of extant taxa that emerged from hybridisation events are usually congeneric (e.g., Barbulescu *et al.* 2017; Beike *et al.* 2014; Buchbender *et al.* 2014; Sawangproh *et al.* 2020). However, a few examples of intergeneric hybridisation have been observed in different moss families in the wild. Natcheva & Cronberg (2004) and Wettstein (1924) successfully induced intergeneric hybridisation in vitro. The meiotic spores of intergeneric hybrid sporophytes were often not examined and where they were, they were most often found to be either aborted or non-viable. In a few cases, however at least a small portion of the spores were viable (Natcheva & Cronberg 2004; Wettstein 1924), stressing the potential of intergeneric hybridisation for speciation processes. Furthermore, extant species that show morphological traits of different genera have been suggested to originate from intergeneric hybridisation (Medina *et al.* 2018; Ochi 1972) and Medina *et al.* (2019) provided genetic data that support this scenario in the Funariaceae Schwägrichen (1830: 43) for *Entosthodon hungaricus* (Boros 1925: 73) Loeske (1929: 115).

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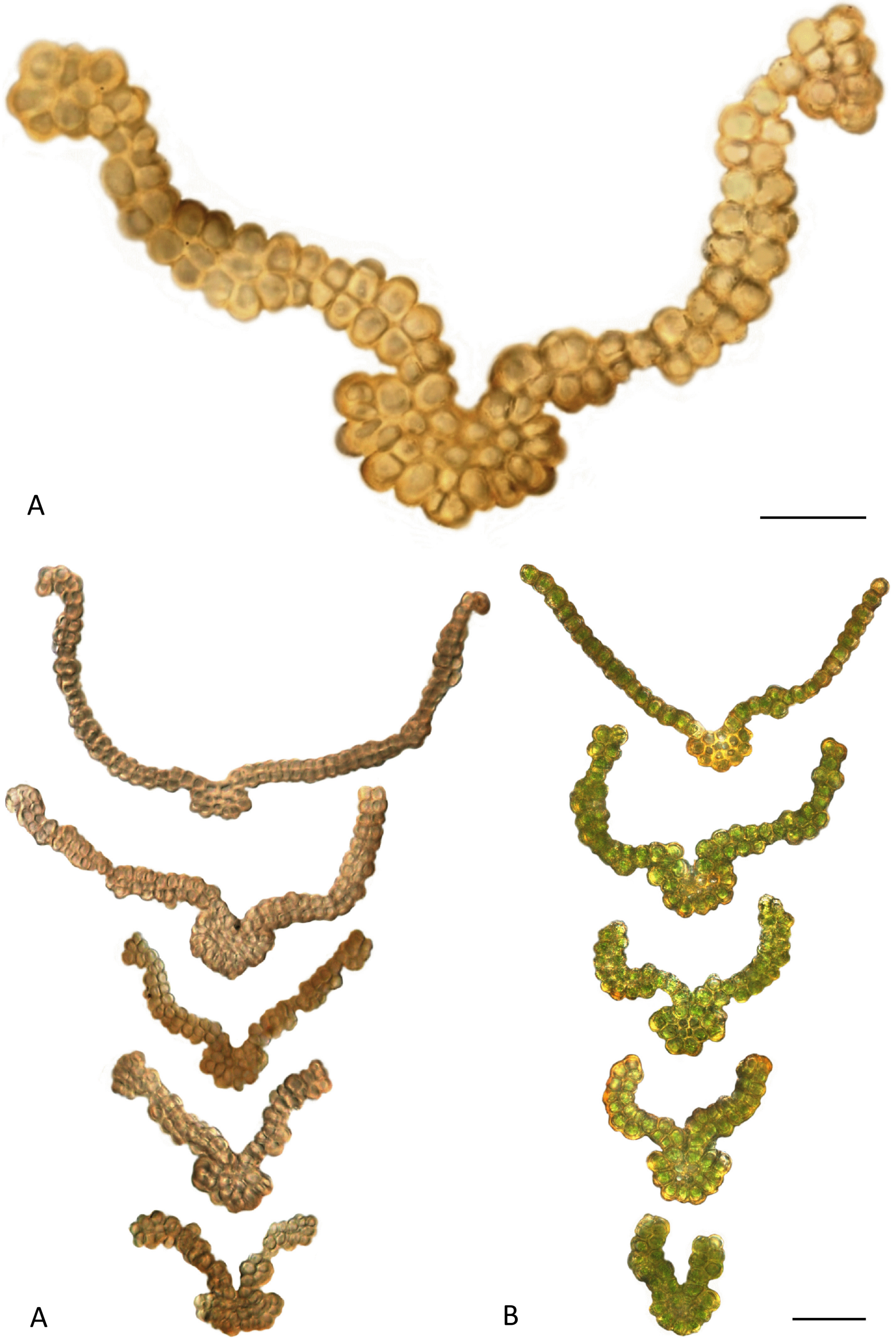

FIGURE 1. Leaf cross sections of *Schistidium mammillosum* (A; *F. Bonte 19L1656*) and *Grimmia alpestris* (B; *T. Kiebacher 2947*) Scale bars: 50 µm.

Here we explore an example of a species that has characteristics consistent with two genera in the Grimmiaceae Arnott (1825: 19). This family comprises mostly colonist species (Dierssen 2001) that are suggested to hybridise relatively frequently compared to species with long-lived life strategies (Natcheva & Cronberg 2004). The potential for hybridisation in the Grimmaceae is evidenced by examples of interspecific hybrid sporophytes in *Grimmia* Hedwig (1801: 75) and *Racomitrium* Bridel (1819: 78) (Cardot 1890; Culmann 1926; Loeske 1930; Wettstein 1924), and by molecular data suggesting reticulate evolution between different sections of *Grimmia* (Hernández-Maqueda *et al.* 2008a).

In the course of the taxonomic work on the genus *Schistidium* Bruch & Schimp. in Bruch *et al.* (1845: 93), we came across peculiar plants collected in the French and Swiss Alps belonging to an undescribed species that showed morphological traits of both of the two largest genera of the Grimmiaceae, *Grimmia* and *Schistidium*. While superficially resembling *Schistidium confertum* (Funck 1820: 18) Bruch & Schimp. in Bruch *et al.* (1845: 99), the leaf cross section revealed impressively mammillose leaf cells, a feature entirely unknown to us in *Schistidium* but expressed similarly in *Grimmia* (Figure 1). This morphological discordance, coupled with the well-known potential for hybridization in the Grimmiaceae, and the co-occurrence of *Grimmia* species exhibiting this characteristic, prompted us to investigate whether a hybridization event could underlie this phenomenon. Gametophytic characteristics of the new species were particularly similar to *G. alpestris* (Weber & Mohr 1807: 110) Schleicher (1807: 29) and this species grew intermixed with the new taxon at almost all of its localities. In addition to the mammillose leaf cells, a feature shared also with *G. caespiticia* (Bridel 1819: 77) Juratzka (1882: 172) and a few other *Grimmia* species, the new taxon also has a pluristratose leaf lamina (Figure 1), characteristic of many *Grimmia* species but rather uncommon in *Schistidium*. Conversely, the sporophytes are deeply immersed in the perichaetial leaves and the seta is straight, features known only in a few *Grimmia* species but characteristic in *Schistidium*. Also, the columella falls with the operculum, another character defining most species of *Schistidium*.

Cyto-nuclear discordance is commonly used as an indicator of hybridisation, because organellar genomes are usually inherited maternally (Greiner *et al.* 2015). We thus targeted nuclear and plastid loci to assess if the morphological inconsistency is consistent with a hybridisation event and to explore the phylogeny of the new species.

Material and methods

Data sets

To investigate the relationship of nuclear and plastid markers of the new taxon to the genera *Grimmia* and *Schistidium*, we built on the phylogenetic treatment of the Grimmiaceae of Hernández-Maqueda *et al.* (2008a; b) because Hernández-Maqueda *et al.* (2008b) provided a sampling of taxa and specimens across the Grimmiaceae for the plastid *rps4-trnF* region similar to the one for the ITS region of Hernández-Maqueda *et al.* (2008a). We complemented these datasets with the following accessions to form our datasets of the *rps4-trnF* region (dataset 1) and the ITS region (dataset 2). We included six accessions of the new taxon, three (two for ITS) accessions of *G. alpestris* including two from a mixed stand with the new taxon (I0088, I0191; Table 1) and three of *G. caespiticia*. Because species of *Schistidium* were underrepresented in the original datasets and because we noticed morphological similarities of the new taxon with the *Schistidium* Confertum clade (Ignatova *et al.* 2010), we also included three species from this clade, one accession of *S. confertum*, three of *S. echinatum* Ignatova & H.H.Blom in Ignatova *et al*. (2010: 211) (two for *rps4-trnF*) and one of *S. succulentum* Ignatova & H.H.Blom in Ignatova *et al*. (2010: 216).

To examine the phylogenetic relationships of the new taxon at the infrageneric level we compiled a third dataset (dataset 3) comprising the ITS region only, excluding other genera and including additional species of *Schistidium*. Analysing this third dataset independently from the dataset covering the entire family had the advantage of much lower variability and hence a more robust alignment. The ITS region is most commonly used in *Schistidium* providing adequate resolution at the species level. In contrast, the utility of plastid markers is hindered by low interspecific variation (Milyutina *et al.* 2010). Nonetheless, we aimed to test the utility of the *rpl16* and *matK* loci (not considered by Milyutina *et al.* 2010), which have proved to be informative at the species level in a number of moss families (e.g., Hedenäs 2014, 2019, 2020; Hofbauer *et al.* 2016) but we were unsuccessful in amplifying them (gel electrophoresis of PCR products showed either no or multiple bands) using the protocols provided by Hedenäs (2020) and Hofbauer *et al.* (2016). Because a blast search with ITS sequences of the new taxon revealed similarities to the *Schistidium* Confertum clade, we used accessions of this clade available on GenBank including accessions of our previous and ongoing work (Kiebacher 2020, Kiebacher & Blom in press). Furthermore, we included the newly-generated *Schistidium* accessions (Table 1), and one accession each of *S. crassipilum* Blom (1996: 224), *S. frigidum* Blom (1996: 181) and *S. tenuinerve* Ignatova & H.H.Blom in Ignatova *et al*. (2010: 220) retrieved from GenBank, as the outgroup.

TABLE 1. Specimen sampling of *Grimmia* and *Schistidium* for phylogenetic analyses. GenBank accession numbers: *rps4-trnL*, *trnL-trnF*, ITS.

Taxon	Isolate ID	Voucher information	GenBank
G. alpestris	I0088	France, Hautes-Alpes, Saint-Véran, 44.66239°N 6.94368°E, 13 July 2021*, T. Kiebacher 2947* (admixture to *S. mammillosum;* SMNS STU BR-0062335)	OR801307, OR785140, -
G. alpestris	I0191	France, Savoie, Bourg-Saint-Maurice, 16 August 2022, *F. Bonte 2022-549* (admixture to *S. mammillosum*; see taxonomy section)	OR801308, -, OR666117
G. alpestris	I0195	Switzerland, Bern, Meiringen, 46.67972°N 8.13752°E, 08 August 2012, *T. Kiebacher 1363* (SMNS STU BR-0062328)	OR801309, -, OR666118
G. caespiticia	I0196	Italy, South Tyrol, Ratschings, 46.88114°N 11.26447°E, 09 October 2021, *T. Kiebacher 3069* (SMNS STU BR-0062338)	OR801311, -, OR666120
G. caespiticia	I0089	Spain, Ávila, Sierra de Gredos, 40.26794°N 5.18854°W, 14 July 2019, *T. Kiebacher 2420* (SMNS STU BR-0062330)	OR801310, OR785141, OR666119
G. caespiticia	I0197	Switzerland, Bern, Innertkirchen, 46.73281°N 8.44605°E, 05 September 2020, *T. Kiebacher 3192* (SMNS STU BR-0062339)	OR801312, -, OR666121
S. confertum	I0081	France, Hautes-Alpes, Abriès-Ristolas, Col Lacroix, 27 July 2021, *A. Hodgson 09/098* (priv. herb. A. Hodgson)	-, -, OR666122
S. echinatum	I0094	France, Hautes-Alpes, Saint-Véran, 44.66378°N 6.93702°E, 14 July 2021*, T. Kiebacher 2979* (SMNS STU BR-0062337)	OR801313, -, OR666124
S. echinatum	I0143	Switzerland, Grison, Arosa, 46.82916°N 9.80536°E, 02 September 2022, *T. Kiebacher 3455* (SMNS STU BR-0062340)	OR801314, -, OR666125
S. echinatum	I0001	Switzerland, Wallis, Saas-Fee, 46.08360°N 7.89586°E, 05 September 2017, *T. Kiebacher 1711* (SMNS STU BR-0062329)	-, -, OR666123
S. mammillosum	I0083	France, Hautes-Alpes, Saint-Véran, *T. Kiebacher 2943* (see taxonomy section)	OR801315, OR785142, OR666126
S. mammillosum	I0084	France, Hautes-Alpes, Saint-Véran, *T. Kiebacher 2945* (see taxonomy section)	OR801316, OR785143, OR666127
S. mammillosum	I0085	France, Hautes-Alpes, Saint-Véran, *T. Kiebacher 2949* (see taxonomy section)	OR801317, OR785144, OR666128
S. mammillosum	I0193	France, Isère, La Combe de Lancey, *F. Bonte 2022-299* (see taxonomy section)	OR801320, -, OR666131
S. mammillosum	I0192	France, Savoie, La Léchère, *F. Bonte 19E956* (see taxonomy section)	OR801319, -, OR666130
S. mammillosum	I0095	Switzerland, Vaud, Bex, *A. Vanderpoorten, F. Collart, T. Kasprzyk, M. Vincent & L. Bourrat s.n.* (see taxonomy section)	OR801318, -, OR666129
S. succulentum	I0129	France, Hautes-Alpes, Saint-Véran, 44.66355°N 6.93762°E, 14 July 2021, *T. Kiebacher 2975* (SMNS STU BR-0062341)	OR801321, -, OR666132

Laboratory protocols

We extracted genomic DNA from dried plant tissue with the NucleoSpin Plant II Mini kit (Machery-Nagel, Germany) and amplified the nuclear ITS region (partial sequence of 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and partial sequence of 26S rRNA gene) and the plastid *rps4–trnF* region (partial sequence of *rps4* gene, *rps4–trnT* spacer, *trnT*, *trnT–trnL* spacer, *trnL* including intron, *trnL–trnF* spacer and partial sequence of *trnF*) using external and internal primers (Table 2). Reagents and volumes for the PCR reaction were the same as is described in Kiebacher *et al.* (2021), except that we used a 2 µg/ml solution of bovine serum albumin instead of water. We used a temperature profile of 5 minutes initial denaturation at 95°C, cycling as specified in Table 2, and a final elongation step of 10 minutes at 72°C. Amplicons were commercially sequenced by LGC Genomics (Germany) using amplification primers.

TABLE 2. Primers and PCR cycling used for amplifying of the plastid *rps4–trnF* and the nuclear ITS region.

Region Amplified	Primer Name & Sequence (5´-3´)	Reference	PCR cycling	
rps4-trnL5´Exon	F: rps4-166F-Schist GTAGTTGTCGTTCTGTTATTCCATAATGAA	This study (modification of Hernández-Maqueda *et al.* 2008b)	40×	30 sec 95°C 40 sec 54°C 1.5 min 72°C
	R: trnL5ex-Schist-R CGTCTACCAATTCCGCCATACC	This study (modification of Hernández-Maqueda *et al.* 2008b)		
rps4-trnT	F: rps4-166F-Schist	This study	40×	30 sec 95°C 40 sec 54°C 1 min 72°C
	R: A-Rbryo-Schist TCAGAGCACCGCAYTTGTAATG	This study (modification of Hernández-Maqueda *et al.* 2008b)		
*trnT-trnL5´*Exon	F: A-Fbryo-Schist CATTACAARTGCGGTGCTCTGA	This study (modification of (Hernández-Maqueda *et al.* 2008b)	40×	30 sec 95°C 40 sec 57°C 1 min 72°C
	R: trnL5ex-Schist-R	This study		
trnL-trnF	F: TabC	Taberlet *et al.* 1991	35×	60 sec 94°C 50 sec 48°C 90 sec 72°C
	R: TabF	Taberlet *et al.* 1991		
ITS1–5.8S rRNA–ITS2	F: ITS-m-18S	Spagnuolo *et al.* 1999	45×	30 sec 95°C 40 sec 57°C 2.5 min 72°C
	R: 25S-ITS54-R: TCCTCCGCTTAGTGATATGCTTAAACTCAGC	This study (modification of Stech and Frahm 1999)		
ITS1	F: m18S:	Spagnuolo *et al.* 1999	40×	30 sec 95°C 40 sec 57°C 1.5 min 72°C
	R: ITS2JK	Kučera *et al.* 2019		
ITS2	F: ITS3	White *et al.* 1990	40×	30 sec 95°C 40 sec 55°C 1.5 min 72°C
	R: 25S-ITS54-R	This study		

Alignment and phylogenetic analyses

We used GeneStudio v2.2.0.0. (available from www.genestudio.com) to edit raw sequences and aligned edited sequences of the *rps4–trnF* region with the AlignSeqs function of the DECIPHER package version 2.22.0 (Wright 2016) in R version 4.3.1 (R Core Team 2023) and ITS sequences using the Q-INS-i strategy in the online interface of MAFFT v7.511 (Katoh & Standley 2013). We used the default settings of the software and manually edited the generated alignments considering inversions in the *rps4-trnLF* alignment as specified by (Hernández-Maqueda *et al.* 2008b). ITS sequences exhibited high variability between genera and the variable parts were difficult to align. In editing the alignment, we therefore generally adopted a conservative strategy and positionally separated regions where homology was unclear. We scored indels of the alignments using simple coding (Simmons & Ochoterena 2000) and performed phylogenetic analyses using Bayesian inference (BI) in MrBayes v3.2.6 (Ronquist *et al.* 2012) and maximum likelihood (ML) in RAxML v8.2.4 (Stamatakis 2014), on the combined plastid (*rps4–trnF* region) and ITS data separately. Because of the discordance between the topologies inferred from plastid and ITS data (datasets 1 and 2) regarding the placement of *Schistidium* and subgenera and individual species of *Grimmia* (discussed by Hernández-Maqueda *et al.* 2008a) we did not proceed to merge these two datasets for a combined analysis. For BI we specified a GTR+G+I model, a sample frequency of 100, stop rule set to yes with critical value for the topological convergence diagnostic (stopval) set to 0.01 and default settings for all other parameters. We verified convergence of the two runs using Tracer v1.6.0 (Rambaut *et al.* 2013) to check that most ESS values exceeded 200 and that none was <100. To archive these criteria for the analysis of dataset 3 we set the stop rule to no and run the analysis for 1 million generations. We calculated a fifty percent majority rule consensus tree after discarding the first 25% of trees as burn-in in MrBayes. For ML analyses we also specified the GTR+G+I model and assessed support for the nodes of the best scoring tree out of 50 independent ML runs using the thorough bootstrapping algorithm with the extended majority rule bootstopping criterion. We summarised the support of nodes from the two approaches using TreeGraph 2 (Stöver and Müller 2010) displaying the BI topology.

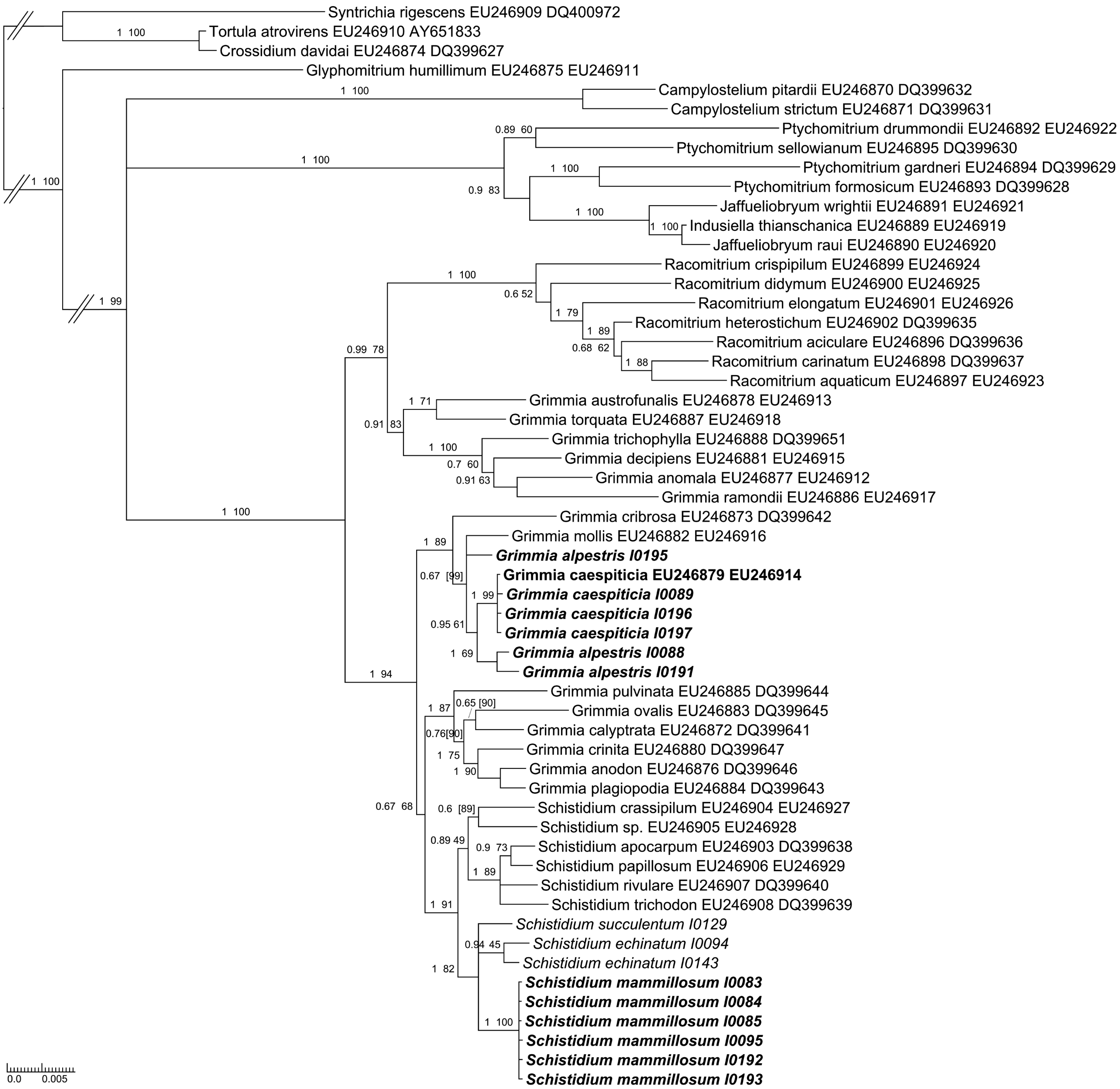

FIGURE 2. Bayesian inference (50% majority rule consensus tree) on the plastid *rps4-trnF* region (dataset 1). Numbers above branches are posterior probabilities followed by bootstrap support values ≥ 50 obtained from maximum likelihood analysis of the same dataset. *S*pecies with mammillose leaf cells are highlighted in bold. Italics denote newly-generated sequences (followed by isolate IDs; Table 1), and codes following other accessions are GenBank accession numbers.

Results

The characteristics of the three alignments are summarized in Table 3. Overall, the number of variable nucleotide sites was high, comprising about 29% of the length of the *rps4-trnF* (dataset 1) and 57% of the ITS (dataset 2) alignment of accessions from across the Grimmiaceae, and 19% of the ITS alignment focusing on the *Schistidium* Confertum clade (dataset 3).

The *rps4-trnF* and ITS phylogenies of the Grimmiaceae derived from datasets 1 and 2 were largely consistent with results of Hernández-Maqueda *et al.* (2008a; b) and chiefly showed the same incongruences between the two datasets. The accessions of the new taxon, however, were consistently resolved in *Schistidium*.

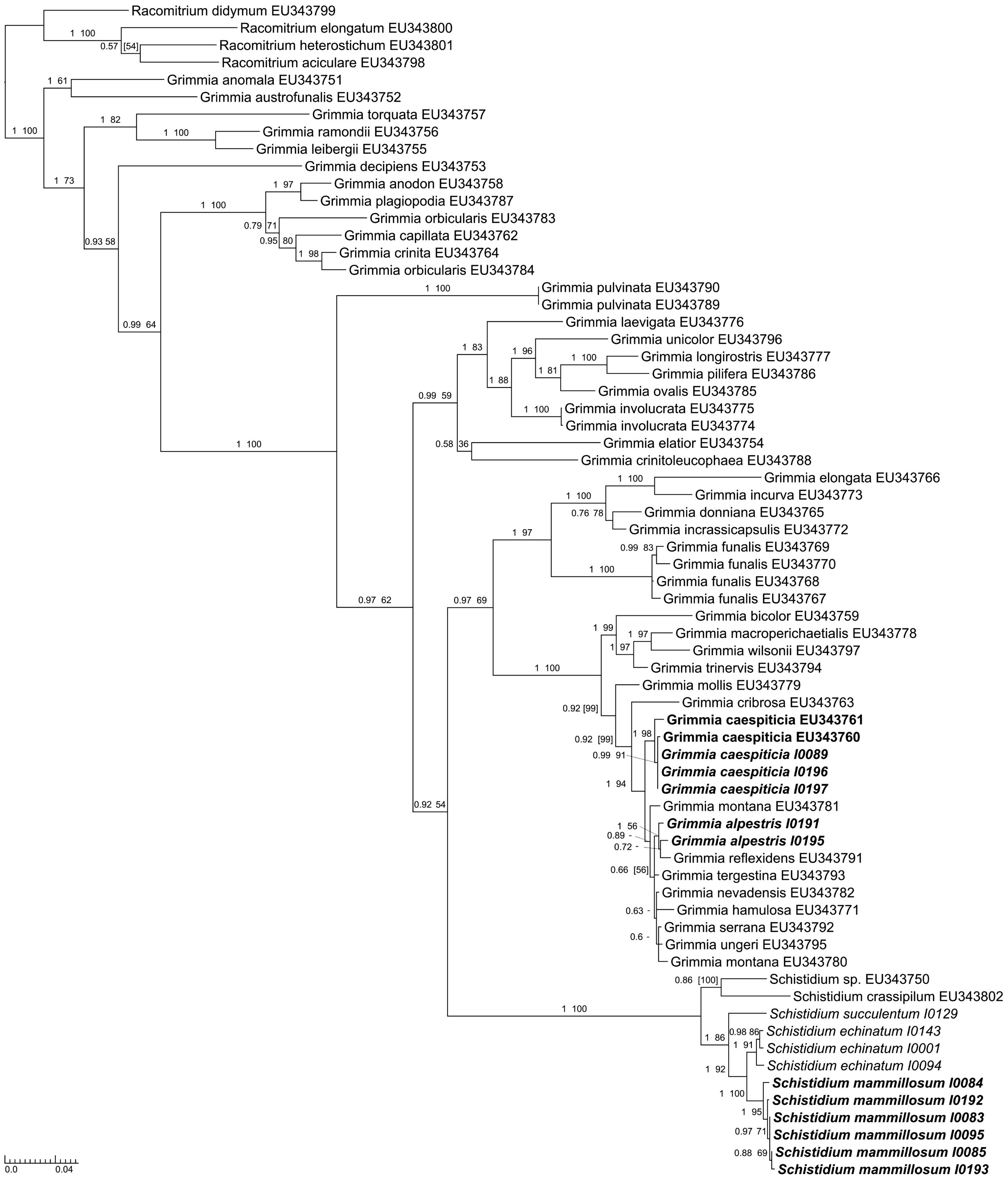

FIGURE 3. Bayesian inference (50% majority rule consensus tree) on the nuclear ITS region (dataset 2). Numbers above branches are posterior probabilities followed by bootstrap support values ≥ 50 obtained from maximum likelihood analysis of the same dataset. *S*pecies with mammillose leaf cells are highlighted in bold. Italics denote newly-generated sequences (followed by isolate IDs; Table 1), and codes following other accessions are GenBank accession numbers.

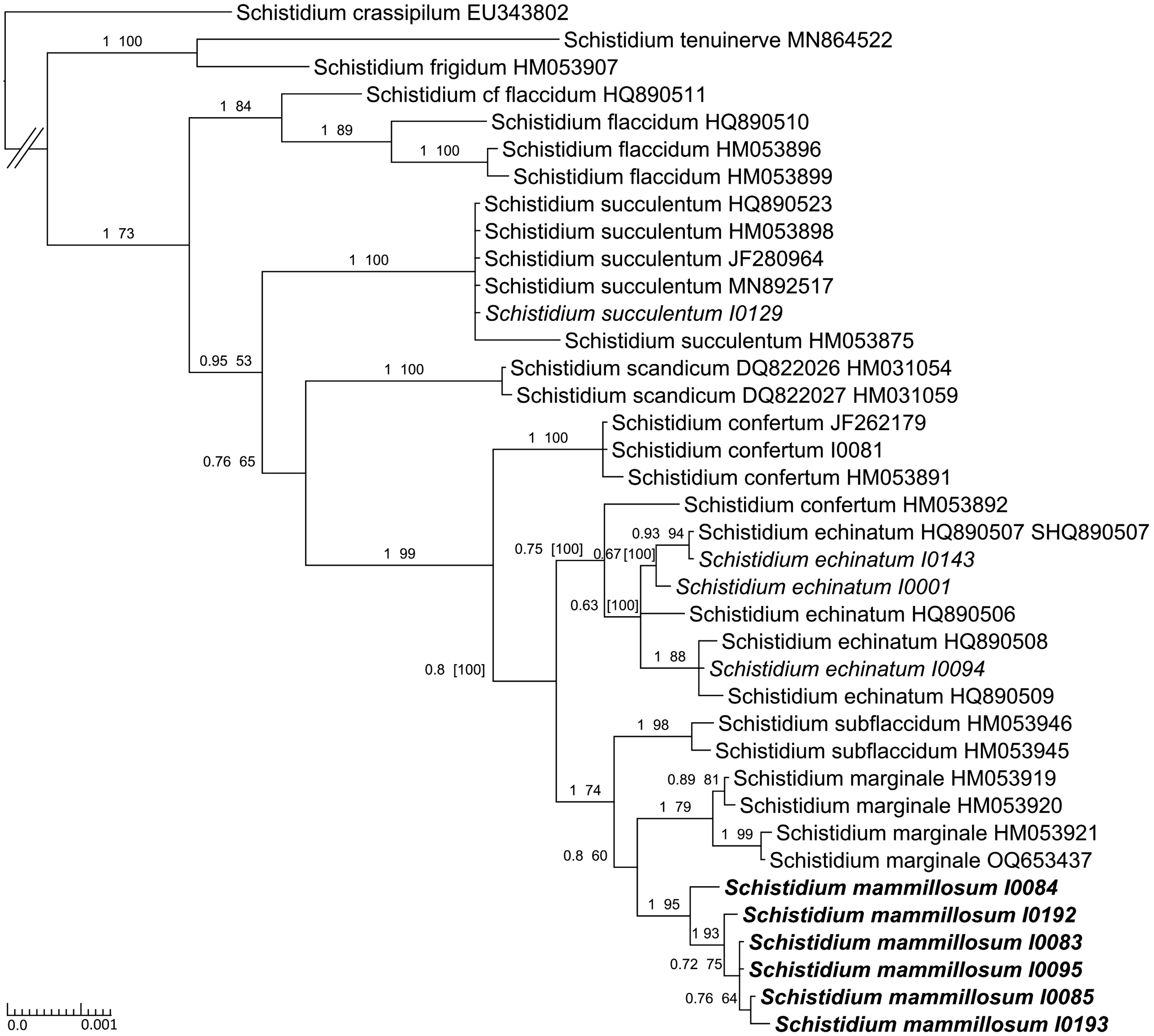

FIGURE 4. Bayesian inference (50% majority rule consensus tree) on the nuclear ITS region of species of the *Schistidium* Confertum clade (dataset 3). Numbers above branches are posterior probabilities followed by bootstrap support values ≥ 50 obtained from maximum likelihood analysis of the same dataset. *Schistidium mammillosum* is highlighted in bold. Italics denote newly-generated sequences (followed by isolate IDs; Table 1), and codes following other accessions are GenBank accession numbers.

The chloroplast data (dataset 1, Figure 2) placed *Racomitrium* and *Grimmia* sect. *Dryptodon* (Brid. 1826: 191) Müll. Hal. (1849: 797) in a clade (posteriori probability 0.99, ML bootstrap support 78) sister to a clade including all other *Grimmia* species and *Schistidium*. Within this clade, all species of *Schistidium* including the new taxon were resolved in a supported clade (1, 91) sister to *Grimmia* sect. *Grimmia* (1, 87). The *Grimmia* species with mammillose leaf cells (*G. alpestris* and *G. caespiticia*) were placed with support (1, 89) in the subgenus *Orthogrimmia* Schimper (1856: 48) which was the sister clade to the *Schistidium*/*Grimmia* sect. *Grimmia* clade.

The ITS tree, using *Racomitrium* as the outgroup (dataset 2, Figure 3), resolved *Schistidium* including the new taxon nested within *Grimma* in a fully supported (1, 100) and highly divergent clade. The sister clade (supported by 0.97, 69) of *Schistidium* was the subgenus *Orthogrimmia* and within *Orthogrimmia*, the *Grimmia* species with mammillose leaf cells were included in the fully supported clade of section *Montanae* I. Hagen (1909: 16).

The *rps4–trnF* sequences of the accessions of the new taxon were identical except for one indel in the *trnL–trnF* spacer (note that this locus was available only for three of the six accessions; Table 1) and differed from all other *Schistidium* accessions in 4 synapomorphic point mutations. In ITS, the new taxon exhibited higher intraspecific variation (Figures 3–4). The differences comprised six variable singleton nucleotide sites and six indels and only for two of the accessions (I0083 and I0095; Table 1) the sequences were identical. The analysis of the *Schistidium* Confertum clade (dataset 3) resolved the new taxon as sister to *S. marginale* H.H.Blom in Blom *et al.* (2016: 211).

TABLE 3. Characteristics of alignments.

Dataset	Length	Variable nucleotide sites	Parsimony informative nucleotide sites	Indels coded
(1) *rps4-trnF* (Grimmiaceae)	1652	479	337	228
(2) ITS (Grimmiaceae)	1698	970	685	779
(3) ITS (*Schistidium* Confertum clade)	954	184	100	117

Taxonomy

Schistidium mammillosum Kiebacher, A.Hodgson & H.H.Blom *sp. nov.* (Figures 1, 5–7)

Diagnosis:— *Schistidium mammillosum* within the genus is distinctive in its (1) small size, the possession of (2) mammillose cells on the leaf lamina and upper costa, (3) one to three(four)-stratose lamina, (4) muticous apices or hair-points developed as short yellowish awns, (5) thick, two to four(five)-stratose leaf margins in the upper part of the leaves, (6) dorsally strongly-projecting costae, (7) cylindric urns, (8) short rectangular to transversely elongated exothecial cells of capsules, (9) ≥ 4 stomata, and (10) perfect peristome teeth.

Type:—FRANCE. Hautes-Alpes: Saint-Véran, ca. 450 m SE of Refuge de la Blanche, at the base of the rock wall SE of the refuge, 2563 msm, 44.6633°N, 6.94273°E, base of WSW-facing inclined rock wall, mid-long snow cover because of snow accumulation at base of the wall, siliceous greenish rock, 13 July 2021, *T. Kiebacher 2945* (Holotype: SMNS STU BR-0062333, Isotypes: JE 04010738, S B328541.

Paratypes:— FRANCE. Alpes-Maritimes: Belvédère, Vallon de la Fous, 2689 msm, 44.11053°N, 7.42071°E, S-facing rocks, granite, 25 July 2023, *T. Kiebacher 4309* (SMNS STU BR-0062342). *Loc. cit.*, 2694 msm, 44.11076°N, 7.42147°E, S-facing rocks, granite with chlorite, 25 July 2023, *T. Kiebacher 4310* (SMNS STU BR-0062343). Hautes-Alpes: Ristolas, Crête des Lauze, ca. 2400 msm, sur roches acides, schisteuses, 08 July 2021, *V. Hugonnot 2021458* (*priv. herb.* V. Hugonnot). Saint-Véran, ca. 300 m SE of Refuge de la Blanche, at the base of the rock wall SE of the refuge, 2552 msm, 44.66406°N, 6.94332°E, base of W-facing inclined rock wall, mid-long snow cover because of snow accumulation at base of the wall, siliceous greenish rock, 13 July 2021, *T. Kiebacher 2942* (SMNS STU BR-0062331, Z 000144855). *Loc. cit.*, ca. 350 m SE of Refuge de la Blanche, at the base of the rock wall SE of the refuge, 2557 msm, 44.66387°N, 6.94311°E, base of W-facing inclined rock wall, long snow cover because of snow accumulation at base of the wall, wet rock fissures, siliceous greenish rock, 13 July 2021, *T. Kiebacher 2943* (BOZ BRYO 9597, SMNS STU BR-0062332). *Loc. cit.*, ca. 400 m SE of Refuge de la Blanche, 2584 msm, 44.66539°N, 6.94448°E, base of W-facing inclined rock wall, mid-long snow cover because of snow accumulation at base of the wall, siliceous greenish rock, 15 July 2021, *T. Kiebacher 2949* (SMNS STU BR-0062336). *Loc. cit.*, ca. 500 m SE of Refuge de la Blanche, 2622 msm, 44.66239°N, 6.94368°E, base of W-facing inclined rock wall, mid-long snow cover because of snow accumulation at base of the wall, siliceous greenish rock, 13 July 2021, *T. Kiebacher 2946* (SMNS STU BR-0062334). *Loc. cit.,* E of Refuge de la Blanche, near path to Lacs Blanchet, below Lac Blanchet Inférieur, ca. 2550 msm, 44.664°N, 6.943°E, at base of schist crags on mountain slope, schist, likely calcareous, 25 July 2017, *A. Hodgson 09/027* (priv. herb. A. Hodgson). Vallouise-Pelvoux, Vallouise Pelvoux, a 160 m au Nord du lieu dit vallon Clapouse, 2559 msm, paroi fraiche a ruisselements temporaires, 09 August 2019, *F. Bonte 19L1656* (*priv. herb.* F. Bonte). Isère: La Combe de Lancey, Belledonne, sud du col de la Sitre, 2135 msm, paroi siliceuse, 16 July 2022, *F. Bonte 2022-299* (*priv. herb.* F. Bonte). Venosc, Ecrins, sous le col de la Muzelle, 2409 msm, fissure de paroi siliceuse, 19 July 2018, *F. Bonte 18M068* (*priv. herb.* F. Bonte). Savoie: La Léchère, Lauzière, 440 m au sud de la combe de la Valette, 2075 msm, paroi siliceuse à écoulements temporaires, 08 July 2019, *F. Bonte 19E956* (*priv. herb.* F. Bonte). Bourg-Saint-Maurice, Beaufortin, sous l'aiguille de Bellaval au sud, 2659 msm, paroi siliceuse exposée au sud, 16 August 2022, *F. Bonte 2022-549* (*priv. herb.* F. Bonte). SWITZERLAND. Vaud: Bex, 2265 msm, 46.21465°N, 7.08103°E, 16 September 2019, *A. Vanderpoorten, F. Collart, T. Kasprzyk, M. Vincent & L. Bourrat s.n.* (LG).

Plants small, up to 1 cm high, green, uppermost parts sometimes yellowish-green, lower parts often blackish-green or brownish, dull or, rarely, with a fine oily sheen, forming dense, sometimes extensive, tufts. **Rhizoids** sparse at base of shoots, reddish-brown when dry, orange-brown when wet, smooth. **Stems** 100–180 µm in diameter, irregularly branched; epidermis of (1)2–3(4) layers of brownish cells with thickened walls grading into thin-walled cortical cells, outermost layer with bulging cells, central strand distinct. **Leaves** dense, arranged in often indistinct spiral rows, appressed when dry, erect to patent when moist, straight or slightly twisted around the axis in the upper part, (narrowly) triangular to triangular-lanceolate, acuminate or more rarely acute, 0.6–1.5 × 0.24–0.5 mm; apex pointed, blunt or narrowly rounded, muticous or sometimes with a short yellowish or, rarely, whitish, dentate or almost smooth awn up to 80 µm long and formed by elongated cells. **Costa** 30–65 µm wide, strong throughout but often narrower at base, strongly projecting dorsally, above often in a deep furrow in transverse section, usually percurrent, sometimes ending below apex, or excurrent in the awn, sometimes on dorsal side at apex ending in elongate and prorate cells, dorsal epidermis above base of isodiametric and usually strongly mammillose cells with single cells often more strongly protruding, rarely smooth; in transverse section at base on dorsal side broadly rounded, above elliptical to trapezoid, or irregularly angular, sometimes above with a groove on dorsal side, 3–4-stratose in lower and central part, 2–3(4)-stratose in upper part. **Margins** plane or narrowly recurved on one or both sides, crenulate to crenulate-denticulate at apex, in upper part 2–4(5)-stratose. **Lamina** at base unistratose, above irregularly 1–3(4)-stratose, pluristratose parts as irregular bands and patches of cell layers that distally sometimes end in strongly protruding cells on dorsal face; cells mostly short, esinuose and thick-walled throughout, walls of rectangular cells are partly sinuose or slightly so except at base, moderately to strongly mammillose, rarely smooth, in transverse section strongly mammillose cells often have the outer walls more strongly thickened than the intercellular walls; cells at base 10–14 µm wide, at margins oblate or isodiametric, more rarely short rectangular, often with strongly incrassate transverse walls, marginal row sometimes hyaline, towards costa usually more elongate, not forming a hyaline group; cells in lower part above the base quadrate, short rectangular or oblate, in central and upper part isodiametric and oblate, sometimes intermixed with a few short rectangular cells, often variable in size, 6–12(13) µm wide. **Perichaetial leaves** mostly straight, sometimes slightly bent inwards above the capsule, exceeding the capsule by (0.1)0.2–0.9 mm; lanceolate, ovate or elliptical, sometimes abruptly constricted in the upper part, acute to acuminate, 1.4–1.9 × 0.45–0.9 mm, occasionally irregularly undulate, at base sometimes transversely undulate, cells usually hyaline in lower part, sometimes up to mid-leaf; costa in upper part strongly projecting dorsally and sometimes S-shaped, usually distinctly narrower at base, ending below apex, percurrent, rarely excurrent; apex pointed, blunt or narrowly rounded, muticous or ending in a short, usually unistratose, yellowish awn up to 200 µm long, formed by elongate cells and denticulate to dentate at margins, rarely smooth; margins plane to narrowly recurved in upper half, rarely more broadly recurved. **Vaginula** with a few to > 15 uniseriate hairs up to 0.6 mm long; ochrea usually ending below the urn, sometimes reaching it. **Calyptra** often falling with the lid, cucullate, with one long slit, rarely mitriform and with 2–3 slits at base, base often reaching the margin of the lid or slightly exceeding it.

Gonioautoicous. **Antheridia** in axillary buds; perigonial leaves smaller than stem leaves, leaves below perigonia with a widened base. **Sporophytes** commonly present, usually abundant, from partly exposed in lateral view to completely immersed in perichaetial leaves. **Seta** very short, 50–180 µm long, ca. 80–120 µm thick. **Urn** yellowish-brown to light orange-brown, brown when old, glossy, often finely striolate, cylindric or shortly cylindric, 500–900(1100) × 340–680 µm, length-width ratio 1.2–1.6(1.7). **Exothecial cells** a mixture of quadrate, short rectangular and transversely elongate cells, 14–40(50) µm long and 16–45(55) µm wide, usually thin-walled, or with slightly irregularly thickened walls, without or with indistinct, sometimes distinct, corner thickenings, especially towards the mouth. **Stomata** 4–8(10) per urn. **Peristome** perfect, orange-red to brownish-red, brown when old, inner face dull, and often with a faint white dusting; teeth somewhat fragile, rather easily broken off, erect to spreading, soon recurved, straight or twisted up to 90° around the axis, triangular, sometimes with irregular outline, inserted by 20–40(50) µm and exceeding the capsule mouth by 200–270 µm, often strongly perforated above with rounded to oval perforations and splits, or irregularly-resolved, more rarely entire or subentire with small oval perforations in upper part, sometimes with semi-perforations, primary peristome layer (PPL) at margins partly overlapping the inner peristome layer (IPL), at base smooth or almost smooth, increasingly papillose upwards with small and rather distant, mostly point-shaped, papillae, above partly intermixed with elongate papillae; IPL at base with rather small and distant point-shaped or a mixture of point-shaped and elongate papillae in variably distinct oblique or sometimes vertical rows, above more densely papillose with point-shaped and elongate papillae forming an irregular pattern, or densely and finely papillose throughout. **Columella** falling with the operculum. **Operculum** convex, rostrate, uniformly orange-brown, rim similar in colour or slightly darker coloured. **Rostrum** conical, straight or slightly oblique, 160–300 µm long. **Spores** 8–12 (14) µm, somewhat irregular in shape, almost smooth, mostly with one oil globule per spore when young.

FIGURE 5. *Schistidium mammillosum*: A tufts growing on greenstone; B part of a tuft; C–D shoots with sporophytes; E vegetative shoots; F shoots in moist state. Scales: A 5 mm; B–F 200 µm. A from *A. Hodgson 09/027*; B–F *T. Kiebacher 2945* (type).

FIGURE 6. *Schistidium mammillosum*: A leaves; B basal leaf cells; C cells in central and upper part of leaf; D–E leaf apices; F stem cross section; G leaf apices of perichaetial leaves; H leaf cross sections; I–J Perichaetial and subperichaetial leaves (from left to right). Scales: A, I–J 200 µm; B–C 25 µm; D–H 50 µm. A–C, F, H from *T. Kiebacher 2945* (type); D, *F. Bonte 19L1656*; E, G, I *A. Vanderpoorten, F. Collart, T. Kasprzyk, M. Vincent & L. Bourrat s.n.*; J *T. Kiebacher 2946.*

Etymology:—The specific epithet *mammillosum* refers to the mammillose leaf cells which were to date unknown in the genus (Figures 1, 6).

Variation:—*Schistidium mammillosum* varies considerably and it seems that this variation is due to the microenvironment including seasonal variation. Plants growing within distances of a few meters can show the extremes of the variation and we suspect that differences in snow cover and humidity play an essential role for this. Most notably, the mammillosity of cells which usually differentiates *S. mammillosum* from all other species of the genus is occasionally weakly developed or even absent. However, when examining shoots from different parts of a cushion at least some leaves with more or less distinctly mammillose cells are present, e.g., on juvenile shoots in the interior of the cushion.

There is also considerable variation in perichaetial leaf shape and this coincides with the exposure of the capsule. They can be broadly (ovate-) triangular, with broad apices that only slightly overtop the capsule, and, consequently the capsules are partly exposed in lateral view. Alternatively, representing the other extreme of the variation, they are broadly lanceolate or elliptical with narrow upper parts and overtop the capsules so that they completely hide the urns.

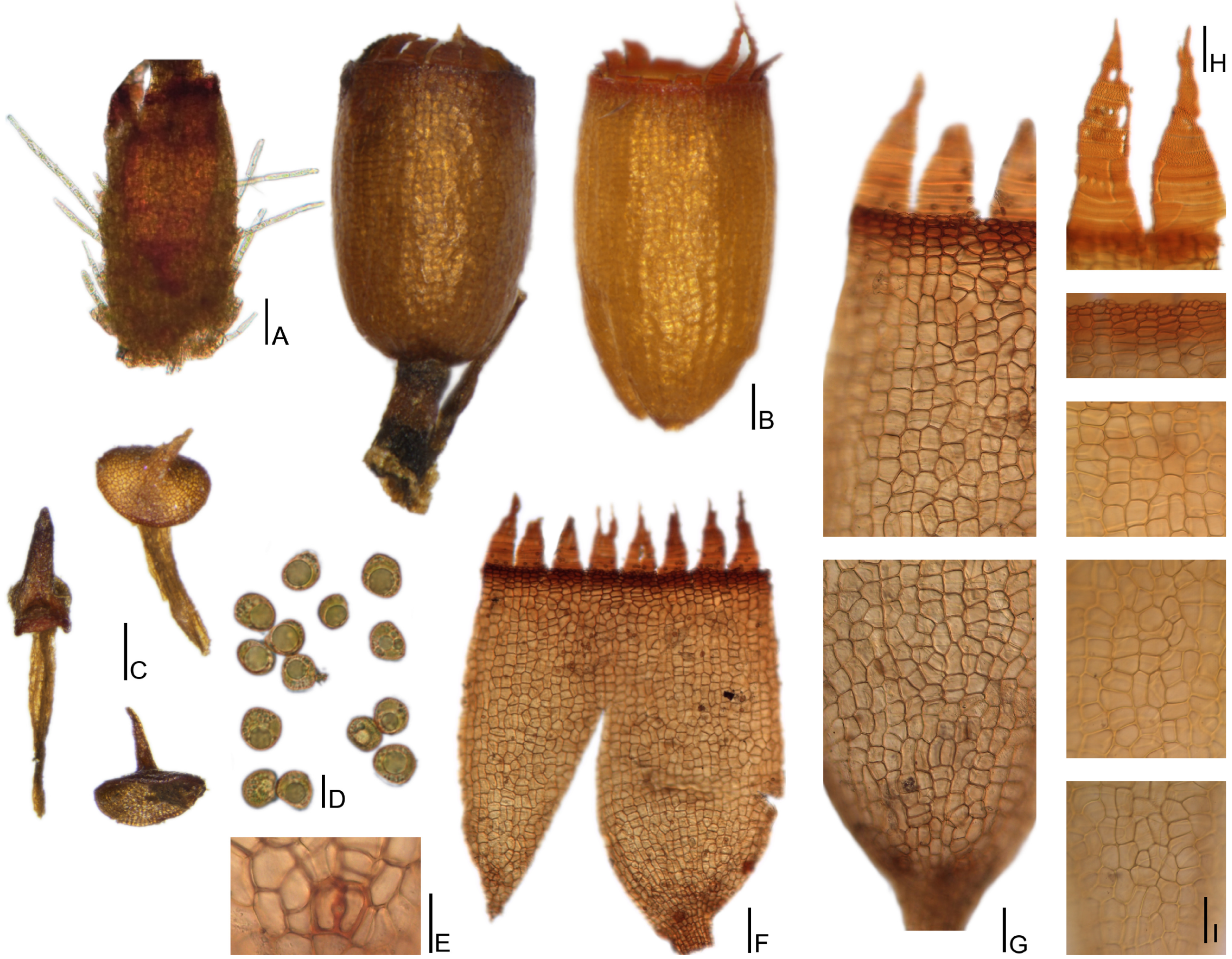

FIGURE 7. *Schistidium mammillosum*: A vaginula; B capsules; C opercula, columellae, calyptra; D spores; E stoma; F half an urn; G pattern of exothecial cells in upper and lower half of urn; H peristome teeth; I exothecial cells at mouth, in upper, central and lower part of urn. Scales: A–B, F 100 µm; C 200 µm; D 500 µm; E 25 µm G–I 50 µm. A from *A. Vanderpoorten, F. Collart, T. Kasprzyk, M. Vincent & L. Bourrat s.n.*; B–D, H *T. Kiebacher 2945* (type); E–G *T. Kiebacher 2946*; I *V. Hugonnot 2021458.*

The costa is percurrent to excurrent in vegetative leaves, but usually ends below the apex in perichaetial leaves, both in leaves that have an awn and those that do not have one. It is rarely percurrent or excurrent in the awn.

In most specimens the spores are usually no larger than 10.5 µm in diameter. Occasionally, capsules contain a variable proportion (usually < 50%) of spores as large as 12 µm or slightly more and (rarely) a few spores can be up to 14 µm in diameter. The minimum spore size is less variable and ranges from 8 to 9 µm.

Differentiation:—The mammillose leaf cells are usually sufficient to distinguish *S. mammillosum* from other species of the genus, because this characteristic is not known from any other species. Further diagnostic characteristics that are shared only with few members of the genus are the small size, the hair-points, which, when present, are formed as short yellowish awns, the extensively bistratose lamina with three-stratose spots and the often rather numerous vaginula hairs.

Schistidium mammillosum may be confused with other species of the Confertum clade. The proposed sister species, *S. marginale*, shares with *S. mammillosum* the thick leaf margins and the strong costa which is strongly projecting and sometimes dorsally grooved in the upper part of the leaf and 3–4-stratose in the lower half in both species. Superficially, however, *S. mammillosum* is more similar to *S. confertum* and *S. echinatum*, because of the small size, often light green colour and relatively short sporophytes. Morphs of these latter species with scarcely developed hair-points can easily be confused with *S. mammillosum* in the field and S. *echinatum* sometimes co-occurs.

Schistidium marginale can be distinguished from *S. mammillosum* by its larger size (stems usually > 1 cm and leaves > 1.5 mm long vs. usually < 1 cm and < 1.5 mm, respectively) and usually well-developed hyaline hair-points. The latter characteristic is in most cases also sufficient to distinguish *S. confertum* and the similar *S. echinatum*. Furthermore, these two species often have a distinct oily sheen which is usually absent in *S. mammillosum*. The stratosity of the lamina provides an additional characteristic to distinguish *S. mammillosum* from *S. marginale*, *S. confertum*, *S. echinatum* and most other species of the clade. The bistratose parts of the lamina in these species are mostly confined to the upper half of the leaf whereas in *S. mammillosum* they regularly occur also in the lower half. Furthermore, three-stratose spots in the upper part of the lamina are not rare in *S. mammillosum*, but unknown in other species of the Confertum clade except for *S. succulentum*. This latter species is similar in size to *S. mammillosum* and in having mostly muticous leaves (or, at most, short and awn-like hair-points) but differs in having strongly reduced peristome teeth that hardly exceed the capsule mouth (vs. well-developed, about 230 µm long). Two other species that share the small size and mostly muticous leaves with *S. mammillosum* are *S. atrichum* (Müll.Hal. & Kindb. in Macoun 1892: 65) Weber (1976: 106) and *S. subconfertum* (Brotherus 1929: 45) Deguchi (1979: 240). They differ in the largely unistratose lamina (vs. irregularly bi- to three-(four-)stratose). *Schistidium atrichum* additionally differs in having smaller leaves (up to 0.8 mm vs. up to 1.5 mm long) and capsules which lack stomata, while *S. subconfertum* differs in having reduced peristome teeth (vs. well-developed).

In the absence of sporophytes, *S. mammillosum* may be confused with *Grimmia alpestris*, *G. caespiticia, G. nutans* Bruch in Müller (1829: 390), or *G. reflexidens* Müller (1849: 795) which like *S. mammillosum* have, or may have, mammillose and esinuose or slightly sinuose laminal cells. These species differ from *S. mammillosum* in usually having hyaline hair-points (vs. leaves at most with yellowish awns) and leaf margins that are either flat or slightly incurved in the central and upper part of the leaf (vs. at least narrowly recurved in some leaves). *Grimmia alpestris* and *G. caespiticia* differ further in their bluish-green colour (vs. green). The characteristic of differentiated cells of the costa in transverse section in *Grimmia* versus uniform cells in *Schistidium* (e.g., Maier 2009) should be used with caution to distinguish *S. mammillosum* from *G. alpestris*. The differentiation is not always clear in *G. alpestris,* and in *S. mammillosum* the central cells can similarly form a group of smaller and more thin-walled cells. Furthermore, at the leaf base the dorsal cells are often more strongly thickened and clearly differ from thin-walled ventral cells in *S. mammillosum*.

Ecology:—*Schistidium mammillosum* occurs in the alpine and lower nival zone with known occurrences ranging from about 2000 to 2700 msm. The species mostly grows on sunny sites in S- to W-facing situations, frequently at the base of rock walls where snow accumulates and remains until summer. Some sites are characterized by temporary wetness due to runoff or water dripping from fissures. The species colonises inclined to vertical faces of siliceous rock, including greenstone, schists, and granite. The substrate conditions range from subneutral to rather acidic conditions for the genus. Species commonly found growing intermixed with tufts of *S. mammillosum* are *Grimmia alpestris*, *Hymenoloma crispulum* and *Pseudoleskea incurvata*. Less constant accompanying species observed comprise *Bartramia ithyphylla*, *Brachytheciastrum collinum*, *Dichodontium pellucidum*, *Distichium capillaceum*, *Ditrichum flexicaule*, *Pseudoleskea patens*, *Lescuraea saxicola*, *Schistidium echinatum*, *S. dupretii*, *S. rivulare*, *Syntrichia norvegica*, *Tortella tortuosa* s.l. and *T. pseudofragilis*.

Distribution:—To date, *Schistidium mammillosum* is only known from the western Alps. It is known from several sites in the French Alps and a single locality in the western part of Switzerland (Figure 8).

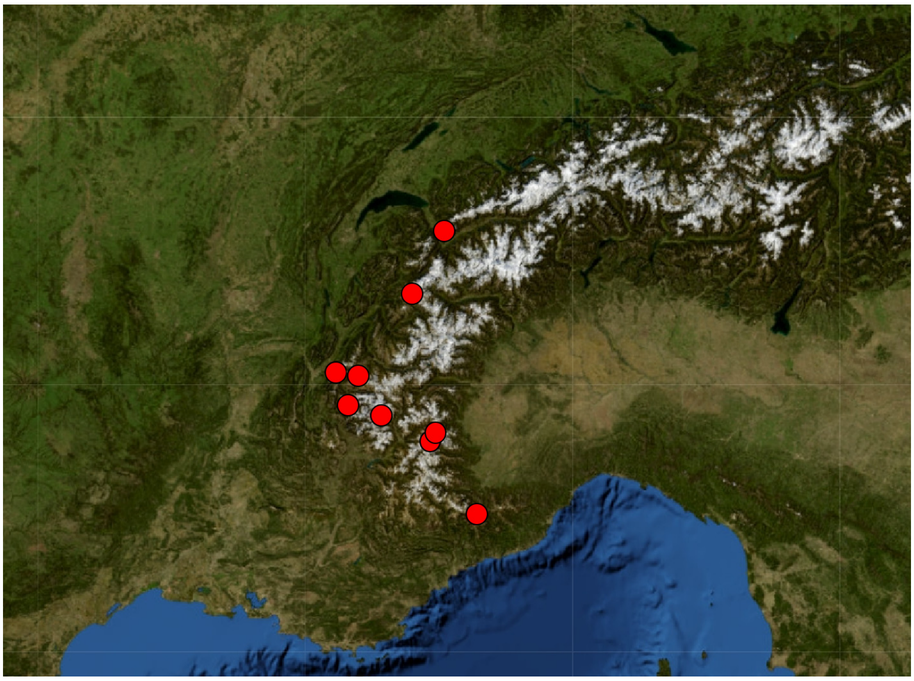

FIGURE 8. Known occurrences of *Schistidium mammillosum* in the Western Alps. Tiles courtesy of the U.S. Geological Survey (https://usgs.gov).

Discussion

The placement of the new taxon within the *Schistidium* clade in the analysis of nuclear and plastid markers does not support the hypothesis of an intergeneric hybrid origin involving a *Grimmia* species with mammillose cells and we consequently describe it as a species of *Schistidium*, *S. mammillosum*. However, intergeneric hybridization within the Grimmiaceae should not be universally dismissed. The difference in sexual conditions between *Grimmia* (mostly dioicous) and *Schistidium* (mostly monoicous) does not appear to be a fundamental barrier, as hybrid sporophytes have been observed between monoicous and dioicous *Grimmia* species (Cardot 1890; Culmann 1926; Loeske 1930; Wettstein 1924). It is possible that intergeneric hybridization played a role in the evolution of the few dioicous *Schistidium* species. We intend to explore this topic further in future studies.

Because nearly all extant taxa of *Grimmia*, along with the majority of other Grimmiaceae, lack mammillose leaf cells, the most plausible explanation for the shared morphological peculiarity of *S. mammillosum* is homoplasy, rather than assuming an ancestral state that has been lost in most species. Given the regular co-occurrence of *S. mammillosum* and *G. alpestris*, it is likely that the trait evolved independently in response to similar environmental conditions. The sites where these species grow are often characterised by prolonged snow cover and are at the same time sun-exposed and we suggest that these conditions play a role. We observed a substantial variability in the degree of mammillosity in specimens of *S. mammillosum* (see 'Variation' in the taxonomy section; Figure 6) collected within distances of few meters. At one site we tried to relate this variation to the microenvironmental conditions and observed that cells of plants growing at the very base of a rock wall tended to be more strongly mammillose than cells of plants growing further up, presumably as a result of longer snow cover. However, this is only a single observation and requires further investigation. Among other bryophyte species, the development of mammillose cells under harsh environmental conditions has for instance been observed in *Didymodon rigidulus* Hedwig (1801: 104) (Crundwell 1976; Kucera 1999).

While a number of studies address the function of leaf papillae suggesting that they are often important for surface water conductance and storage (e.g., Castaldo-Cobianchi & Giordano 1984; Jauregui-Lazo *et al.* 2023), little is known about the function of mammillose cells. Possibly, under low light conditions under the snow they act as lenses enabling photosynthesis in chloroplasts exposed to the focused light. Such a function has been suggested for protonemal cells of the luminous moss *Schistostega pennata* (Hedwig 1801: 31) Weber & Mohr (1803: 1 [verso]) (Noll 1888) and for epidermal cells of forest understory vascular plants (Bone *et al.* 1985). The pluristratose leaf lamina of *S. mammillosum* may guarantee that the focused light is captured in case the focal point is below the upper cell layer. If this hypothesis is true, it would also mean that vaulted cell walls could be restricted to the upper surface. Such a pattern occurs in *Timmia* Hedwig (1801: 176) species which typically grow at sites characterised by long snow cover or in *Timmiella* (De Notaris 1865: 100) Limpricht (1888: 590), a genus with shade tolerant species. Such a differentiation between the upper and lower surfaces may not be relevant for rock-dwelling genera such as *Grimmia* and *Schistidium*, in which the orientation of the shoots depends on the substrate's slope. An alternative explanation for the presence of mammillose cells could be that they provide protection against mechanical damage resulting from icing. They may reduce the risk of leaves sticking to the ice or serve as crystallization nuclei, promoting the formation of many small crystals on the leaf surface, as opposed to larger crystals that might inflict damage during the recurrent thawing and refreezing cycles beneath the snow layer. Köckinger & Hedenäs (2023) observed snow cover dependent facultative development of papillae on the dorsal costa of *Tortella* species, which could have such a function.

In bryophytes, many characteristics previously thought to be definitive of higher-level taxa have frequently been shown to be homoplastic, necessitating revisions to taxonomic concepts. For example, within mosses, the genus *Hypnum* Hedwig (1801: 236) was found to encompass species from different families (Kučera *et al.* 2019; Schlesak *et al.* 2018), while in liverworts, the morphological concept of *Scapania obcordata* (Berggren 1875: 96) S.W. Arnell in Arnell & Mårtensson (1959: 117) has recently been demonstrated to include taxa from different sections (Kiebacher & Urmi 2023).

The phylogenetic analysis of the Confertum clade (Ignatova *et al.* 2010) suggests a shared ancestry of *S. mammillosum* with *S. marginale*. This is supported by shared morphological characteristics and similar substrates. Along with *S. confertum*, the two species have the capacity to colonise siliceous rocks which are generally too acidic for the majority of other species in the genus. Shared features of *S. mammillosum* with the Confertum clade in general include the occurrence in mountain habitats, small size, flat hair-points in perichaetial leaves, and not or only slightly sinuose cells compared to the sister Frigidum clade.

The interspecific variability in ITS, and especially the remarkable differentiation from other species of the Confertum clade in plastid markers, suggest that *S. mammillosum* has not diverged recently. For comparison, no differentiation in ITS could be found for the morphologically well-differentiated subspecies of *S. submuticum* Zick. ex Blom (1996: 250) (Ignatova *et al.* 2010), while Milyutina *et al.* (2010) pointed out the limitations of plastid markers to differentiate taxa at the species level in *Schistidium*.

Additional taxonomic remarks

The phylogenetic analysis of the ITS region of the Confertum clade (Figure 4) provides molecular evidence for the occurrence of *S. echinatum* in France and Switzerland and may suggest its occurrence in Sweden (*S. confertum* HM053892; Hofbauer *et al.* 2016). We retrieved the latter accession from GenBank and have not studied the specimens morphologically. The accession shares synapomorphic mutations with accessions of *S. echinatum* but at the same time differs at several positions. The morphological differentiation between *S. echinatum* and *S. confertum* is not straightforward, and the variability present in specimens of this species complex requires a revision to clarify to what extent it includes further morphologically distinct and cryptic lineages.

The phylogeny of the ITS region (Figure 4) also suggests that the morphological peculiarities of specimens of *S. succulentum* from the Alps result from phenotypic plasticity rather than genetic differentiation. In earlier (Kiebacher 2020; Kiebacher & Blom 2022) and unpublished work we noticed that specimens from the Alps often lack papillae on the lamina and costa and that the lamina is sometimes largely unistratose. Contrary to this, in the Caucasus from where the species was described, leaves with a bistratose and papillose lamina and a papillose costa in the upper part are the common expression (Ignatova *et al.* 2010). The sequence of the ITS region of a specimen lacking papillae and with an almost unistratose lamina included in the analysis (I0129, Figure 4, Table 1) is identical to accessions from Caucasus and the Italian Alps.

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