



Multi-locus phylogeny supports the placement of *Endocarpon pulvinatum* within *Staurothele* s. str. (lichenised ascomycetes, Eurotiomycetes, Verrucariaceae)

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Abstract

Within the lichen family Verrucariaceae, the genera *Endocarpon*, *Willeya* and *Staurothele* are characterised by muriform ascospores and the presence of algal cells in the hymenium. *Endocarpon* thalli are squamulose to subfruticose, whereas *Willeya* and *Staurothele* include only crustose species. *Endocarpon pulvinatum*, an arctic-alpine species newly reported for Iceland, is one of the few *Endocarpon* with a subfruticose thallus formed by long and narrow erected squamules. Molecular phylogenetic analyses of four loci (ITS, nrLSU, mtSSU, and *mcm7*) newly obtained from *E. pulvinatum* specimens from Iceland, Finland and North America does not confirm its current classification within the mostly squamulose genus *Endocarpon*, but instead supports its placement within the crustose genus *Staurothele*. The new combination *Staurothele pulvinata* is therefore proposed here. It includes also *E. tortuosum*, which was confirmed as a synonym of *E. pulvinatum* based on a single sequence.

Keywords: subfruticose *Staurothele*, *Endocarpon tortuosum*, Verrucariales, *mcm7*

Introduction

Verrucariaceae is a family of mainly lichenized ascomycete fungi comprising more than 50 genera (Kirk *et al.* 2008). Recent molecular studies have helped refine generic boundaries within the family and resulted in the description (e.g., *Hydropunctaria* C. Keller, Gueidan & Thüs, *Parabagliettoa* Gueidan & Cl. Roux, and *Wahlenbergiella* Gueidan & Thüs [Gueidan *et al.* 2009]) or resurrection (e.g., *Sporodictyon* A. Massal. [Savić *et al.* 2008, Savić & Tibell 2009] and *Willeya* Müll. Arg. [Gueidan *et al.* 2014]) of several genera. The genus *Endocarpon* Hedw. (a member of Verrucariaceae) contains squamulose or subfruticose species that have muriform ascospores and algal cells in their hymenial cavity (hymenial algae). This genus was recently shown to be sister to *Willeya*, also characterised by muriform ascospores and hymenial algae, but differing by its crustose thallus morphology (Gueidan *et al.* 2014, Gueidan & Lendemer 2015). Species of *Willeya* were previously placed in *Staurothele* Norman (Verrucariaceae), a genus also characterised by having hymenial algae, muriform spores and crustose thalli. Although deep relationships within Verrucariaceae remain unresolved or poorly supported, it is clear that *Staurothele* and the *Endocarpon*-*Willeya* clade do not share a most recent common ancestor (Gueidan *et al.* 2007, 2009, 2014). Morphologically, these two lineages differ in their ascospore pigmentation: ascospores of the *Endocarpon*-*Willeya* group are light brown, at most, at maturity, whereas ascospores of *Staurothele* s. str. (a monophyletic group of *Staurothele*, including the type species and excluding some endolithic species that belong to a different lineage; see Gueidan *et al.* 2009) are dark brown at maturity.

The lichen genus *Endocarpon* has never been the focus of any comprehensive molecular phylogenetic revision. As a result, the morphology-based classification of many species within this genus remains uncorroborated by phylogenetic analyses (Harada 1993, McCarthy 2001, Breuss 2002). *Endocarpon pulvinatum* Th. Fr. is characterised by long and narrow erected and overlapping brown squamules, that form subfruticose thalli (Fig 1). The species was described from material collected in northern Norway (Fries 1860) and has an arctic-alpine distribution (Clauzade & Roux 1985, Breuss 2002). The southernmost known locality is in Baja California, Mexico where it was found at 2800 meters

above sea level (Breuss 2002). The species is circumpolar, i.e., it has been reported from Fennoscandia (Santesson *et al.* 2004), Greenland (Alstrup 1981), North America (Goward & Thor 1992) and Russia (Kopaczewska *et al.* 1977). It was discovered in Iceland in 1996 by the first author while searching for *Dermatocarpon* species around a lake fed by a glacier river. *Endocarpon pulvinatum* is widely distributed in Iceland although it is by no means common. Most of the Icelandic locations are in the vicinity of glaciers or glacier rivers (Fig 1). For example, it has been found growing on nunataks in the Vatnajökull glacier. *Endocarpon tortuosum* Herre, a morphologically similar species described from Nevada, U.S.A. by Herre (1911), is considered to be a synonym of *E. pulvinatum* based on morphological data (Breuss 2002). Both species present cushion-like thalli formed of erected squamules, but the lobes of these squamules tend to be more dissected in *E. pulvinatum*. A preliminary study based on Icelandic specimens of *Endocarpon pulvinatum* suggested that this species belonged to *Staurothele* (Heiðmarsson *et al.* 2012). The preliminary study, however, included only Icelandic specimens. Based on the revision of the material of *E. pulvinatum* kept at OSU, additional specimens from North America were selected and sequenced. Newly obtained molecular data for Icelandic and North American specimens were subjected to phylogenetic analyses in order to confirm the phylogenetic placement of *E. pulvinatum* within the family Verrucariaceae and to revisit the synonymy between *E. pulvinatum* and *E. tortuosum*.

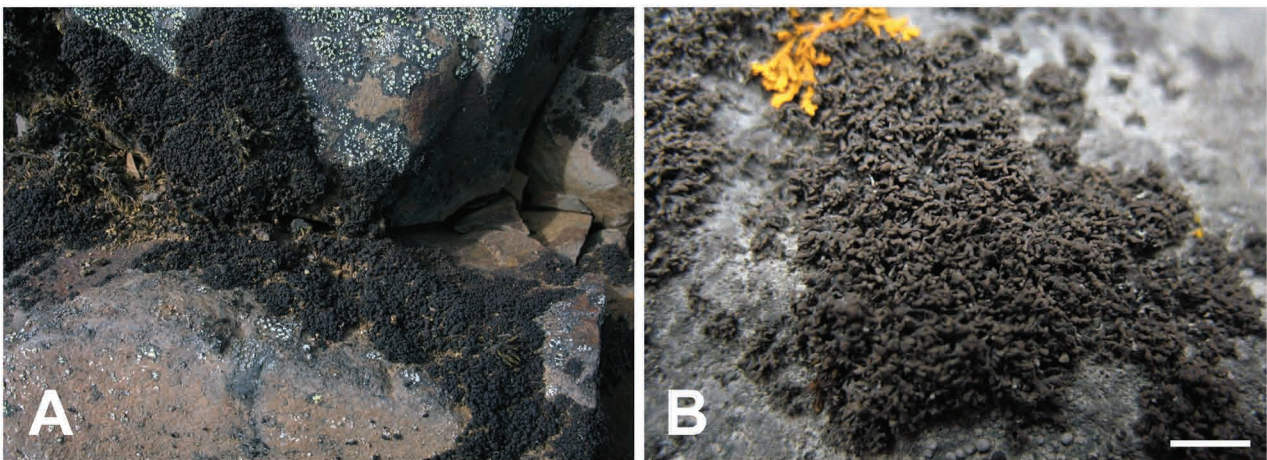


FIGURE 1. General habit of *Endocarpon pulvinatum*. A. Large thalli found on the nunatak Esjubjörg in southeastern Iceland (LA31862). B. Close-up on specimen SH300 collected near a glacier river in Northern Iceland. Bar = 5 mm. Photos Starri Heiðmarsson.

Materials and methods

DNA extractions, amplifications and sequencing

DNA was extracted from 14 specimens of *Endocarpon pulvinatum* and two specimens identified as *E. tortuosum* based on the morphology of the squamules. Specimens were originally collected from Finland, Iceland and the U.S.A. and are available at AMNH, H and OSU (Table 1). Genomic DNA was obtained using a protocol modified from Zolan & Pukkila (1986), as described in Gueidan *et al.* (2007). DNA extracts were checked with gel electrophoresis and, for each sample, the band intensity was used to choose the appropriate genomic DNA dilution for amplification. Four markers were amplified: the internal transcribed spacer (ITS) region, the large subunit of the nuclear ribosomal RNA gene (nrLSU), the small subunit of the mitochondrial ribosomal RNA gene (mtSSU), and the minichromosome maintenance component 7 (*mcm7*). For the three ribosomal markers (ITS, nrLSU, mtSSU), 1 µl of a 1/10 or 1/100 dilution of genomic DNA was added to the following PCR mix: 2.5 µl PCR buffer 10 NH₄ (Bioline, London, UK), 1.5 µl of MgCl₂ (50 mM), 0.5 µl dNTP (100 mM), 1 µl primers (10 µM), 0.5 µl DNA polymerase Bioline BioTaq (5 U/µl⁻¹), and water to a total volume of 25 µl. For *mcm7*, 2 µl of each primer (10 µM) were used instead of 1 µl. Primers and PCR conditions are described in Table 2. PCR product clean-up and sequencing were partly carried out by the sequencing facility of the Natural History Museum in London using PCR Clean-up Filter Plates (Millipore, Billerica, MA), BigDye chemistry and an ABI 3730xl sequencing machine (Applied Biosystems, Carlsbad, CA, USA). Other PCR products were cleaned and sequenced at Macrogen (Seoul, Korea) and at the Duke University Genome Sequencing and Analysis Core Facility of the Institute for Genome Sciences and Policies (Durham, North Carolina, USA). The internal primers ITS2 and ITS3 (White *et al.* 1990) were used to sequence ITS amplicons, and LR3, LR5,

LR6, LR3R, LR5R and LR6R (Vilgalys & Hester 1990) were used to sequence nrLSU amplicons. PCR products with multiple bands were cloned using a TOPO-TA cloning kit according to the manufacturer's instructions (Invitrogen, Carlsbad, California, USA).

TABLE 1. Taxon sampling for *Endocarpon pulvinatum* and *E. tortuosum*. Specimens were obtained from OSU, AMNH and H. Collectors were Bruce McCune (BMC), Starri Heidmarsson (SH), Juha Pykälä (specimen SH347) and Roger Rosentreter (RR). Coll. = Collection.

Species	Coll. number (Herbarium)	Locality	Coll. year
<i>Endocarpon pulvinatum</i>	BMC 15912 (OSU)	USA, Montana, Glacier county, East Flattop Mountain, Glacier National Park	1986
<i>Endocarpon pulvinatum</i>	BMC 18884 (OSU)	USA, Oregon, Harney county, near Stinkingwater Pass	1991
<i>Endocarpon pulvinatum</i>	BMC 20686 (OSU)	USA, Montana, Beaverhead county, Pioneer Mountains, in narrow gorge of Canyon Creek	1993
<i>Endocarpon pulvinatum</i>	BMC 26544 (OSU)	USA, Oregon, Lake county, Hart Mountain National Antelope Refuge	2002
<i>Endocarpon pulvinatum</i>	BMC 27176-A (OSU)	USA, Wyoming, Fremont county, cliffs above Warm Spring Creek	2003
<i>Endocarpon pulvinatum</i>	BMC 27176-B (OSU)	USA, Wyoming, Fremont county, cliffs above Warm Spring Creek	2003
<i>Endocarpon pulvinatum</i>	BMC 27947 (OSU)	USA, Oregon, Wallowa county, near camp at Ice Lake, Wallowa Mountains	2005
<i>Endocarpon pulvinatum</i>	BMC 29161 (OSU)	USA, Montana, Lake county, cliffs above Upper Goat Creek	2007
<i>Endocarpon pulvinatum</i>	BMC 32483 (OSU)	USA, Oregon, Lake county, Hart Mountain National Antelope Refuge	2002
<i>Endocarpon pulvinatum</i>	RR 3686 (OSU)	USA, Idaho, Gooding county, Coyotte Creek road	1985
<i>Endocarpon pulvinatum</i>	RR 14724 (OSU)	USA, Idaho, Owyhee county, 2 km West of North Fork of the Owyhee River crossing	2001
<i>Endocarpon pulvinatum</i>	SH 300 (AMNH)	Iceland, INo, Skagafjörður fjord, by Héraðsvötn glacier river, Villinganes (LA31498)	2009
<i>Endocarpon pulvinatum</i>	SH 314 (AMNH)	Iceland, INo, Skagafjörður fjord, by Héraðsvötn glacier river, Teigakot (LA31511)	2009
<i>Endocarpon pulvinatum</i>	SH 347 (H)	Finland, EnL, Enontekiö, Kilpisjärvi, Saana nature reserve, Pykälä 43945	2011
<i>Endocarpon tortuosum</i>	BMC 12394 (OSU)	USA, Montana, Flathead county, Glacier National Park, near summit of Mount Oberlin	1982

TABLE 2. Primers and PCR conditions used to amplify ITS, nrLSU, mtSSU, and *mcm7*.

Regions	Primers	Publications	PCR conditions
ITS	ITS1F	Gardes & Bruns (1993)	5 min 94°C, 35X (1 min 94°C, 1 min 53°C, 1 min 72°C), 7 min 72°C
	ITS4	White <i>et al.</i> (1990)	
<i>mcm7</i>	<i>mcm7</i> -709for	Schmitt <i>et al.</i> (2009)	10 min 94°C, 38X (45 sec 94°C, 50 sec 56°C, 1 min 72°C), 5 min 72°C
	<i>mcm7</i> -1348rev	Schmitt <i>et al.</i> (2009)	
mtSSU	mtSSU1	Zoller <i>et al.</i> (1999)	3 min 94°C, 35X (1 min 94°C, 1 min 52°C, 90 sec 72°C), 7 min 72°C
	mtSSU3R	Zoller <i>et al.</i> (1999)	
nrLSU	LR0R	Rehner & Samuels (1994)	1 min 95°C, 35X (45 sec 95°C, 40 sec 52°C, 150 sec 72°C), 10 min 72°C
	LR7	Vilgalys & Hester (1990)	

Alignments and phylogenetic analyses

Samples for which we obtained more than one marker were included in a multi-locus analysis (Table 3). The analysis also included reference taxa from Verrucariaceae for which new or published sequences of *mcm7* were available (James *et al.* 2006, Gueidan *et al.* 2007, 2008, 2011, Wang *et al.* 2014, Yuzon *et al.* 2015), in order to represent most genera or species groups within the family. The dataset comprised 43 specimens representing Verrucariaceae and two species of *Capronia* Sacc. (Chaetothyriales) as an outgroup. New sequences were edited using Sequencher v. 5.4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and manual alignments of all sequences were done using Mesquite v. 3.04 (Maddison & Maddison 2011). Ambiguous regions were delimited as described in Lutzoni *et al.* (2000) and, together with the introns, were excluded from the phylogenetic analyses. Amino acid sequences were used to help align nucleotide sequences from the protein-coding gene *mcm7*. To test for congruence, each locus was first subjected to a bootstrap analysis separately using maximum likelihood (ML) (RAxML VI-HPC v.8.2.9; Stamatakis *et*

al. 2005, 2008), as implemented on the CIPRES Web Portal (<http://www.phylo.org>; Miller *et al.* 2010). The markers ITS (mostly represented by the 5.8S region, as ITS1 and ITS2 could not be unambiguously aligned across the family and were, therefore, excluded from the phylogenetic analysis), nrLSU, and mtSSU were recognized as three separate partitions, and each codon position in *mcm7* was analysed as a separate partition. A GTRCAT model was applied to all markers and partitions. Support values were obtained using a fast bootstrap analysis of 1,000 pseudoreplicates. Resulting topologies were compared for a potential conflict among loci using a 70% reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996). If no conflict was detected, the four loci regions were concatenated and the combined dataset analysed using a Bayesian approach with MrBayes v. 3.2.6 (Ronquist *et al.* 2011), as implemented on the CIPRES Web Portal. Models of evolution were estimated for each locus using the corrected Akaike Information Criterion as implemented in jmodeltest v.2.1.7 (Guindon & Gascuel 2003, Darrriba *et al.* 2012). The model settings for the six partitions were: the first codon position of *mcm7* (GTR+G+I), the second codon position of *mcm7* (GTR+G), the third codon position of *mcm7* (GTR+G), ITS (SYM+G+I), mtSSU (GTR+G+I) and nrLSU (GTR+G+I). Two analyses of four chains were run for 5 million generations and trees were sampled every 500 generations. For the two runs, the convergence of parameters was checked using Tracer v. 1.6 (Rambaut *et al.* 2014). All runs converged on the same average likelihood score and topology. A burn-in sample of 5,000 trees was discarded for each run. The remaining 10,000 trees were used to estimate the posterior probabilities with the “compute consensus” command in PAUP* (Swofford 2002). The consensus tree was computed with the sumt command in MrBayes and visualised in PAUP*. Additional support values were obtained from a tree search and a RAxML fast bootstrap analysis of 1,000 pseudoreplicates using GTRCAT model applied to the same six partitions.

TABLE 3. Taxon and gene sampling for the multi-locus dataset assembled for the placement of *E. pulvinatum* within the Verrucariaceae. Two species of *Capronia* are used as an outgroup. GenBank numbers in bold represent sequences generated as part of this study. A dash represents missing data. *the *mcm7* sequence of *Capronia semiimmersa* comes from the strain MUCL 39979. **nrLSU and mtSSU sequences for *Endocarpon pusillum* Z07020 were obtained from genomic data available in GenBank (APWS01000000; Wang *et al.* 2014).

Species	Collection number	ITS	nrLSU	mtSSU	<i>mcm7</i>
<i>Bagliettoa baldensis</i>	CG1760	KM371427	-	KM371555	KM371502
<i>Dermatocarpon bachmannii</i>	SH155	KY697124	KY773245	KY773543	KY782250
<i>Dermatocarpon cf. meiohyllizum</i>	SH199	KY697125	KY773246	KY773544	KY782251
<i>Dermatocarpon minutum</i>	CG387	KY769535	EF469160	-	KY769565
<i>Dermatocarpon minutum</i>	SH175	KY697126	KY773247	KY773545	KY782252
<i>Endocarpon pallidulum</i>	SJ4028	DQ826735	DQ823097	FJ225674	-
<i>Endocarpon pulvinatum</i>	BMC 20686	KY769529	KY769543	KY769560	-
<i>Endocarpon pulvinatum</i>	BMC 26544	KY769530	KY769544	KY769561	-
<i>Endocarpon pulvinatum</i>	BMC 27947	KY769532	KY769547	KY769562	KY769566
<i>Endocarpon pulvinatum</i>	BMC 27176-A	-	KY769545	KY769563	KY769567
<i>Endocarpon pulvinatum</i>	BMC 32483	KY769534	KY769549	-	KY769568
<i>Endocarpon pulvinatum</i>	RR 3686	KY769535	KY769550	KY769564	-
<i>Endocarpon pulvinatum</i>	SH300	-	KY773276	KY773546	-
<i>Endocarpon pulvinatum</i>	SH314	KY697127	KY773277	KY773547	KY782253
<i>Endocarpon pusillum</i>	CG470	KY769556	EF643754	FJ225677	-
<i>Endocarpon pusillum</i>	Z07020	HM237334	genome**	genome**	XM_007808116
<i>Henrica melaspora</i>	AA62248	KY769557	EF413601	FJ225678	KY769569
<i>Heteroplacidium fuscum</i>	CG582	KY769558	EF643793	-	KY769570
<i>Hydropunctaria aractina</i>	SH310	KY697128	KY773248	KY773548	KY782254
<i>Hydropunctaria maura</i>	SH233	KY697129	KY773249	KY773549	KY782255
<i>Hydropunctaria rheitrophila</i>	SH304	KY697130	KY773250	KY773550	KY782256
<i>Hydropunctaria scabra</i>	SH273	KY697131	KY773251	KY773551	KY782257
<i>Parabagliettoa cyanea</i>	CG1761	KM371423	-	KM371552	KM371497
<i>Placidium arboreum</i>	CG579	KY769559	EF643765	-	KY769571
<i>Placopyrenium canellum</i>	CG808	EU010250	EF643784	FJ225694	KY769572
<i>Sporodyction schaeerianum</i>	SH298	KY697132	KY773252	KY773552	KY782258
<i>Staurothele areolata</i>	SH154	KY697133	KY773278	KY773553	KY782259
<i>Staurothele areolata</i>	SH268	KY697134	KY773279	KY773554	KY782260
<i>Staurothele fissa</i>	SH194	KY697135	KY773280	KY773555	KY782261
<i>Staurothele fissa</i>	SH248	KY697136	KY773281	KY773556	KY782262

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TABLE 3. (Continued)

Species	Collection number	ITS	nrLSU	mtSSU	<i>mcm7</i>
<i>Staurothele fissa</i>	SH260	KY697137	KY773282	KY773557	KY782263
<i>Verrucaria aethiobola</i>	SH184	KY697138	KY773253	KY773558	KY782264
<i>Verrucaria aethiobola</i>	SH197	KY697139	KY773254	KY773559	KY782265
<i>Verrucaria alpicola</i>	SH228	KY697140	KY773255	KY773560	KY782266
<i>Verrucaria ceuthocarpa</i>	SH236	KY697141	KY773256	KY773561	KY782267
<i>Verrucaria degelii</i>	SH315	KY697142	KY773257	KY773562	KY782268
<i>Verrucaria latebroso</i>	SH183	KY697143	KY773258	KY773563	KY782269
<i>Verrucaria latebroso</i>	SH210	KY697144	KY773259	KY773564	KY782270
<i>Verrucaria margacea</i>	SH163	KY697145	KY773260	KY773565	KY782271
<i>Verrucaria margacea</i>	SH202	KY697146	KY773261	KY773566	KY782272
<i>Verrucaria muralis</i>	SH160	KY697147	KY773262	KY773567	KY782273
<i>Wahlenbergiella mucosa</i>	SH271	KY697148	KY773263	KY773568	KY782274
<i>Wahlenbergiella tavaresiae</i>	CG1101	HQ822056	HQ822059	HQ822057	KY769573
Outgroup - <i>Capronia pilosella</i>	WUC28	DQ826737	DQ823099	FJ225725	JN993262
Outgroup - <i>Capronia semiimmersa</i>	WUC244	AF050259	FJ358226	FJ225726	JN993261*

Samples of *E. pulvinatum* for which we obtained only ITS or both ITS and nrLSU were analysed separately (Table 4). These datasets were restricted to the genus *Staurothele* s. str. and comprised both newly obtained and already published sequences of *Staurothele* species (Table 4), including *S. areolata* (Ach.) Lettau, *S. clopima* (Wahlenb.) Th. Fr., *S. dendritica* V. Wirth, *S. drummondii* (Tuck.) Tuck, *S. fissa* (Taylor) Zwackh and *S. frustulenta* Vain. to investigate the putative synonymy between *E. tortuosum* and *E. pulvinatum*. For ITS, ambiguous regions were delimited as described in Lutzoni et al. (2000) and, together with the introns, were excluded from the phylogenetic analyses. The marker nrLSU did not have introns and was alignable across its length. Each dataset was analysed using maximum likelihood with RAxML VI-HPC v.8.2.9, as implemented on the CIPRES Web Portal. Within ITS, three partitions corresponding to ITS1, 5.8S and ITS2 were specified. A GTRCAT model was implemented for all partitions and tree search and fast bootstrap analyses of 1,000 pseudoreplicates were completed. Unrooted trees were visualised using FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree>). Trees shown in Figs 2–4, and their respective datasets, were deposited in TreeBase (ID20693).

TABLE 4. Taxon sampling for the two single-locus datasets ITS and nrLSU for the genus *Staurothele* assembled for determining the identity of *E. tortuosum* in relation to *E. pulvinatum*. GenBank numbers in bold represent sequences generated as part of this study. A dash represents missing data.

Species	Collection number	ITS	nrLSU
<i>Endocarpon pulvinatum</i>	BMC15912	KY769527	KY769541
<i>Endocarpon pulvinatum</i>	BMC18884	KY769528	KY769542
<i>Endocarpon pulvinatum</i>	BMC20686	KY769529	KY769543
<i>Endocarpon pulvinatum</i>	BMC26544	KY769530	KY769544
<i>Endocarpon pulvinatum</i>	BMC27176-A	-	KY769545
<i>Endocarpon pulvinatum</i>	BMC27176-B	KY769531	KY769546
<i>Endocarpon pulvinatum</i>	BMC27947	KY769532	KY769547
<i>Endocarpon pulvinatum</i>	BMC29161	KY769533	KY769548
<i>Endocarpon pulvinatum</i>	BMC32483	KY769534	KY769549
<i>Endocarpon pulvinatum</i>	RR3686	KY769535	KY769550
<i>Endocarpon pulvinatum</i>	RR14724	-	KY769551
<i>Endocarpon pulvinatum</i>	SH300	-	KY773276
<i>Endocarpon pulvinatum</i>	SH314	KY697127	KY773277
<i>Endocarpon pulvinatum</i>	SH347	KY765294	-
<i>Endocarpon tortuosum</i>	BMC12394	KY769536	KY769552
<i>Staurothele areolata</i>	SH154	KY697133	KY773278
<i>Staurothele areolata</i>	SH268	KY697134	KY773279
<i>Staurothele areolata</i>	W1112	-	JN573790
<i>Staurothele clopima</i>	W1235	-	JN573792
<i>Staurothele clopima</i>	SS087	EU553513	EU598712
<i>Staurothele dendritica</i>	WB13227A	KY769537	KY769553
<i>Staurothele dendritica</i>	WB13227B	KY769538	KY769554

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TABLE 4. (Continued)

Species	Collection number	ITS	nrLSU
<i>Staurothele drummondii</i>	CG831	KY769539	EF643774
<i>Staurothele fissa</i>	SH194	KY697135	KY773280
<i>Staurothele fissa</i>	SH248	KY697136	KY773281
<i>Staurothele fissa</i>	SH260	KY697137	KY773282
<i>Staurothele fissa</i>	CG586	KY769540	EF643775
<i>Staurothele fissa</i>	W1137	-	JN573793
<i>Staurothele fissa</i>	W1367	-	JN573794
<i>Staurothele frustulenta</i>	AA53935	DQ826736	DQ823098

Results

Multi-locus dataset

Apart from the relationship among the three samples of *Staurothele fissa*, no strong conflict was detected among the four single-locus topologies. Because incongruence is not unexpected within species, the four datasets were concatenated. The combined dataset contained a total of 2,895 characters (273 from ITS, 1,333 from nrLSU, 728 from mtSSU, and 561 from *mcm7*), of which 1,136 characters formed distinct alignment patterns (106 from ITS, 377 from nrLSU, 301 from mtSSU, and 352 from *mcm7*). The proportion of gaps and completely undetermined characters was 10.89 %.

Similar to previous molecular studies on Verrucariaceae (Gueidan *et al.* 2007, 2009, Savić *et al.* 2008, Muggia *et al.* 2010, Prieto *et al.* 2012), the backbone of the phylogeny, including the relationships among genera within Verrucariaceae remains unstable (low PP and BS), but most genera or small groups of species were well supported (Fig 2). The results show with strong confidence that the subfruticose species *Endocarpon pulvinatum* is nested within the crustose genus *Staurothele* (1.00 PP and 100% BS), i.e., outside the genus *Endocarpon*.

Single-locus datasets

The datasets included 24 sequences and 543 characters for ITS and 29 sequences and 1,400 characters for nrLSU. The ITS alignment contained 133 distinct alignment patterns and 5.17% of gaps and completely undetermined characters. The nrLSU alignment represented 187 distinct alignment patterns and 7.34% of gaps and completely undetermined characters. Both gene trees show a close relationship between *E. pulvinatum*, *Staurothele areolata*, *S. frustulenta* and *S. drummondii* (Figs 3 & 4; 80% and 99% BS, respectively). A single representative of *E. tortuosum* (BMC 12394, Table 4) was nested within the *E. pulvinatum* clade in both the ITS and nrLSU phylogenies (Figs 3 & 4).

Discussion

Endocarpon pulvinatum is an arctic-alpine species with a circumpolar distribution. It is characterised by long (up to 7 mm) and narrow erected or sometimes subcylindrical squamules, forming subfruticose thalli (Breuss 2002). Although the habit of this species differs from most other *Endocarpon* (generally with non- to little-overlapping round squamules), it is similar to other *Endocarpon* species with cushion-like thalli (e.g., *E. adscendens* [Anzi] Müll. Arg.), hence its current placement within this genus. This species is shown here to belong to *Staurothele*, a genus that has, to date, comprised only crustose species. The presence of fruticose or subfruticose species within crustose lineages has already been reported from other lichen genera, such as *Lecanora*, *Lepraria*, and *Leprocaulon* (Lendemer & Hodkinson 2013, Lendemer & Tripp 2015). Despite a major difference in thallus structure, *E. pulvinatum* shows some similarities with other species of *Staurothele* (i.e., muriform ascospores and presence of hymenial algae, two traits also shared with *Endocarpon*). Moreover, at maturity, the ascospores of *E. pulvinatum* and *Staurothele* become darkly pigmented, whereas they become light brown, at most, in species of the genus *Endocarpon*. This is the first non-crustose species in the genus *Staurothele*. Within *Staurothele*, *E. pulvinatum* is part of a clade comprising other species found also exclusively at higher elevations, in the arctic, or in cooler regions of the temperate zone (*S. areolata*, *S. frustulenta*, and *S. drummondii*).

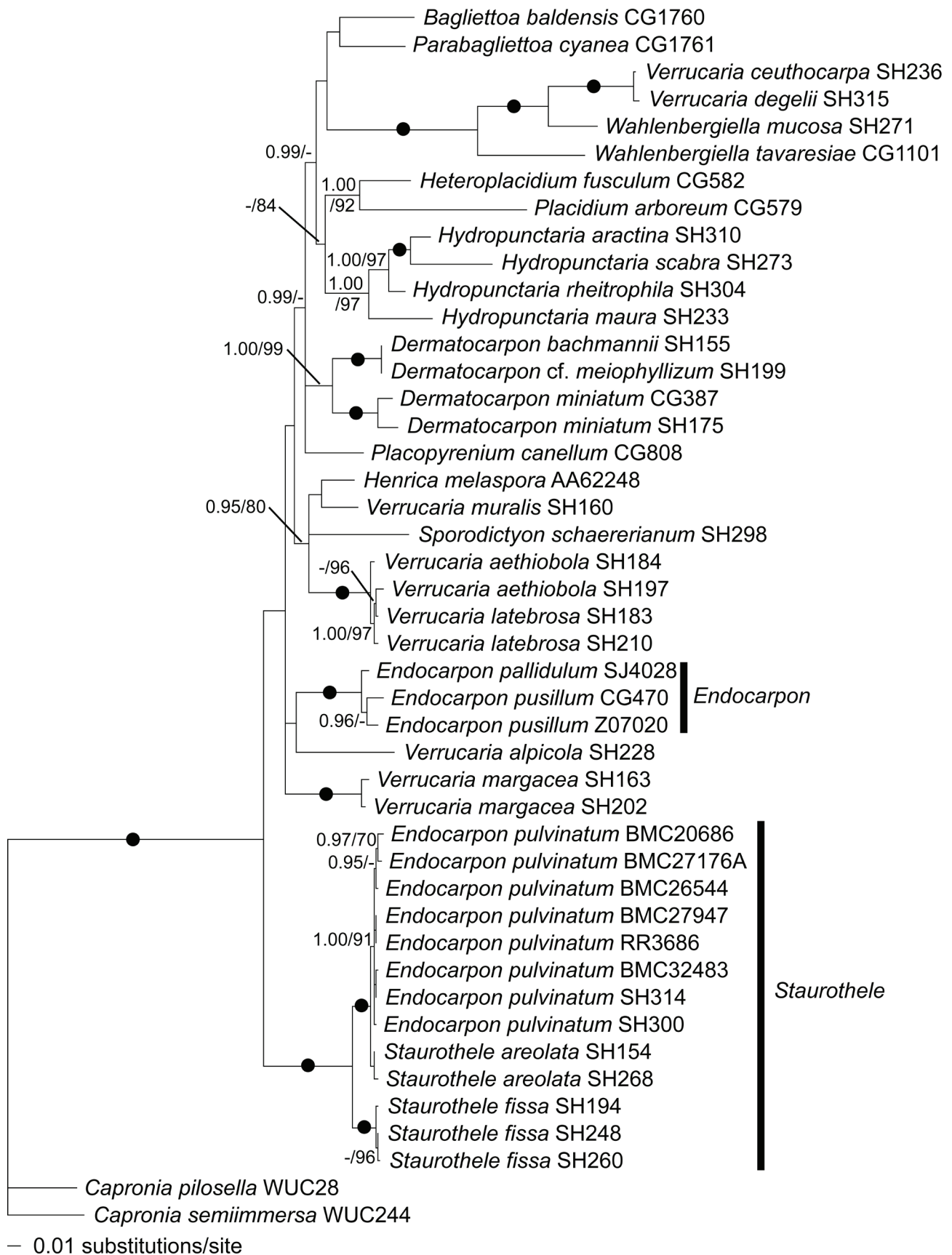


FIGURE 2. Phylogenetic placement of *E. pulvinatum* within the family Verrucariaceae resulting from a Bayesian analysis of a multi-locus dataset (ITS, nrLSU, mtSSU and *mcm7*). Support values are reported above or below the branches (posterior probability [PP]/bootstrap support [BS]). Internodes with strong support (1.00 PP and 100% BS) are indicated by a black dot. Only values of PP \geq 0.95 and of BS \geq 70% are shown. Two species of *Capronia* were used as an outgroup.

In 1911, Herre described a new species, *Endocarpon tortuosum*, based on material collected near Reno in Nevada, USA. He described the thallus of *E. tortuosum* as “small or irregular foliose squamules, tortuous and nodulose, often forming a rough, thick, and very irregular indeterminate crust; sometimes the squamules are densely compacted to form a thick, almost uniform torulose crust, while the squamules are stipitate, erect, and 7 mm or more in height. When the squamules are scattered, their lobes are much dissected, and more or less complicate, recalling *E. pulvinatum*” (Herre 1911). When the descriptions published by Herre (1911) and Breuss (2002) are compared, the thallus structure of *E. tortuosum* and *E. pulvinatum*, as well as the dimensions of their ascospores and their hymenial algae are very similar. The only difference between *E. pulvinatum* and *E. tortuosum* is the squamule lobes, which are more dissected in the former species than the latter. For these reasons the two taxa were considered conspecific by Breuss (2002). Although *E. tortuosum* is represented in our dataset by a single specimen (Montana, USA), we confirmed its identity (and synonymy) as *E. pulvinatum*. A new combination is proposed below to accommodate *E. pulvinatum* within the genus *Staurothele*.

Taxonomy

Staurothele pulvinata (Th. Fr.) Heiðmarsson comb. nov.

Mycobank MB820490

- ≡ *Endocarpon pulvinatum* Th. Fr., Nova Acta Reg. Soc. Sci. Upsal., Ser. 3, 3: 257 (1861) [1860]—Type: NORWAY. Finnmark: Nesseby, Mortensnæs, 1857, *Th.M. Fries s.n.* (UPS L-135411, holotype!).
- ≡ *Dermatocarpon pulvinatum* (Th. Fr.) Körb., Parerga lichenol. (Breslau) 4: 308 (1863).
- ≡ *Polyblastia pulvinata* (Th. Fr.) Jatta, Syll. Lich. Ital.: 652 (1900).
- = *Cornicularia mirabilis* Lynge, Skr. Svalbard Ishavet 81: 107 (1940)—Type: GREENLAND. Moskusoksefjorden: Hoelsbu, 1932, *S. Aandstad s.n.* (O, holotype, not seen). Synonymy proposed by Alstrup (1981).
- = *Pyrenothamnia spraguei* Tuck., Bull. Torrey bot. Club 10: 22 (1883)—Type: USA. Washington: eastern slope of the Cascade Mountains, alt 3500–6000 ft, 1882–1883, *T.S. Brandegees s.n.* (FH, holotype and isotypes, not seen; NY, isotype, not seen; US, isotype, not seen). Synonymy proposed by Santesson (1949).
- = *Endocarpon tortuosum* Herre, Bot. Gaz. 51(4): 288 (1911)—Type: USA. Nevada: Washoe, Reno, 1909, *A.W.C.T. Herre 24* (BRY, F, FH, MICH, NEB, US, syntypes, not seen). Synonymy proposed by Breuss (2002).

Acknowledgments

The visit to Oregon State University herbarium (OSU) and part of the molecular work on North American specimens of *Endocarpon* were funded by an internal grant from the Natural History Museum in London to CG. JM and FL acknowledge Assembling the Tree of Life project (AFToL 2, DEB-0732984) funded by the National Science Foundation. The authors would like to thank Bruce McCune for hosting CG’s visit to OSU, as well as James Lendemer and Richard C Harris for hosting CG’s visit to NY (funded by a National Science Foundation grant to James Lendemer, DEB 1145511) and for lending some material of *Staurothele dendritica*. Juha Pykälä is gratefully acknowledged for lending specimens from Finland and Mohammad Sohrabi for a specimen of *Dermatocarpon* from Iran. SH’s visit to Duke University (Lutzoni lab) and the molecular work carried out there on Icelandic specimens of *Endocarpon* and other Icelandic Verrucariaceae were funded by the Icelandic Centre for Research. Support for fieldwork in Iceland was received from the Icelandic Research Council and the Energy Research Fund of the National Power Company of Iceland.

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