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Fuscidea lightfootii and *F. pusilla* (*Fuscideaceae*, Umbilicariomycetidae, Ascomycota), two similar but genetically distinct species.

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Abstract: The two corticolous species *Fuscidea lightfootii* (Sm.) Coppins & P. James and *F. pusilla* Tønsberg are morphologically and chemically similar and it has been suggested that they are conspecific. We investigated the interspecific relationship between *F. lightfootii* and *F. pusilla* using ITS, LSU and mtSSU rDNA. The combined multigene phylogeny shows that these species are genetically distinct. They are similar in ascocarp anatomy but in thallus morphology and substratum preferences there may be slight differences between them. Moreover, *F. pusilla* displays a broader ecological range than *F. lightfootii*. Even though some morphotypes appeared distinct and may be assigned to one of the two species with some degree of certainty, the use of DNA sequencing is recommended for their identification. Epitypes are designated for both species.

Key words: cryptic species, lichenized ascomycetes, medially constricted ascospores, sorediate lichens, sterile lichens

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Introduction

Wirth & Vězda (1972) introduced the crustose genus *Fuscidea* V. Wirth & Vězda for species with a brown or grey areolate thallus, conspicuous brown prothallus and lecideine, brown to black-brown apothecia with asci of the *Fuscidea*-type containing eight, simple or 1-septate, mostly ellipsoid, sometimes medially constricted ascospores. The genus comprises *c.* 40 saxicolous and corticolous species, occurring on acidic substrata worldwide, mostly in areas with cool and maritime climates. Two corticolous, sorediate species, *Fuscidea lightfootii* (Sm.) Coppins & P. James and *F. pusilla* Tønsberg, are similar in thallus morphology and chemistry (see e.g. Gilbert *et al.* 2009). The commonly accepted distinguishing features are the presence (*F. lightfootii*) or absence (*F. pusilla*) of apothecia (Kantvilas 2001; Gilbert *et al.* 2009) and their geographical distribution (Tønsberg & Johnsen 2008).

Fuscidea lightfootii, described from the north of Ireland by Smith & Sowerby (1805), is usually fertile and has a thallus morphology that varies in colour, shape and size of the areoles, and the degree of soredia production. Its currently accepted distribution range includes Western Europe (Kalb & Hafellner 1992; Tønsberg & Johnsen 2008), Yunnan, China (www.tropicallichens.net), Brazil (Aptroot 2002) and Tasmania (Kantvilas 2001, 2004). It is not known from North America (Tønsberg 2002; Fryday 2008).

Fuscidea pusilla, described from Norway by Tønsberg (1992), is characterized as a small (less than 1 cm), sterile, sorediate crust occurring in colonies forming a mosaic of more or less confluent thalli. It occurs in Europe, in areas with continental as well as oceanic climates, and North America (Tønsberg 1993; Fryday 2008; Lendemer 2011).

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Fuscidea lightfootii and *F. pusilla* have been regarded as impossible to distinguish when sterile. They may qualify as cryptic species that are morphologically identical but genetically distinct from one another (see e.g. Crespo & Pérez-Ortega 2009). Tønsberg & Johnsen (2008) suggested that *F. lightfootii* and *F. pusilla* may be conspecific and Gilbert *et al*. (2009) proposed *F. pusilla* as a morph of *F. lightfootii* that forms small, sterile rosettes. Tønsberg & Johnsen (2008), Gilbert *et al*. (2009) and Lendemer (2011) recommended a taxonomic treatment of these species using molecular methods. Genes from two genomes of ribosomal DNA (i.e. mitochondrial and nuclear) may be sufficient for species delimitation (see e.g. Spribille *et al*. 2011; Bendiksby & Timdal 2013; Resl *et al*. 2016).

Bylin *et al*. (2007) investigated the taxonomic position of the family *Fuscideaceae* by studying seven different species of *Fuscidea*, including one specimen of *F. lightfootii* and two of *F. pusilla*. Their results showed that both species were located in the *Fuscidea*-group with high support in a maximum parsimony analysis and, within this clade, they were located in two separate subgroups.

Several papers deal with the phylogenetic relationships between sterile, sorediate and fertile taxa, so-called species pairs, to test if they are conspecific. For example, Spribille *et al*. (2011) investigated the taxonomy of the often sterile *Mycoblastus alpinus* (Fr.) Kernst. and the mostly fertile *M. affinis* (Schaer.) T. Schauer. Resl *et al*. (2016) studied the *Rinodina degeliana* Coppins (sorediate)/ *R. subpariata* (Nyl.) Zahlbr. (esorediate, fertile) species complex. In these two studies, the species of interest were shown to be conspecific. In contrast, Bendiksby *et al*. (2015) showed that sterile, sorediate specimens of the *Calvitimela aglea* complex were two distinct lineages, impossible to distinguish morphologically but differentiated in chemistry and ecology.

The hypothesis for this study was that *F. lightfootii* and *F. pusilla* are conspecific (Tønsberg & Johnsen 2008). The objective was to clarify the interspecific relationship between *F. lightfootii* and *F. pusilla* using ITS, LSU and mtSSU rDNA.

Materials and Methods

Taxon sampling

The material for this study came from herbarium collections in BG, HO and MSC, and from recently collected material from Norway, the USA (Alaska), Czech Republic, Great Britain, Ireland and Poland. Specimens collected by the authors were deposited in BG. The specimens are listed in Table 1. All specimens were subjected to thin-layer chromatography (TLC) according to the method described by Culberson & Kristinsson (1970), Culberson (1972) and Menlove (1974). All three solvents (A, B′ and C) were used; glass plates and solvent C were used for the detection of fatty acids.

DNA extraction, PCR amplification and sequencing

DNA was extracted from apothecia (fertile specimens) or soredia (sterile specimens) of *Fuscidea lightfootii* and *F. pusilla* using the DNeasy Plant Mini Kit (Qiagen). Primers for amplification were as follows: 1) ITS, ITS1f (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990), 2) LSU, nuLSU-155-5′ (Döring *et al*. 2000) and nuLSU-1125-3′ (Vilgalys & Hester 1990), and 3) mtSSU, mtSSU1 and mtSSU3R (Zoller *et al*. 1999). The PCR mixture consisted of 1× GeneAmp® PCR Buffer II (Applied Biosystems), 2·5 µM $MgCl_2$ (Applied Biosystems), 20 µM dNTPs (Promega), 0·6 µM of each primer, 0·036U AmpliTaq® DNA Polymerase (Applied Biosystems), 5·0 µl of genomic DNA extract and distilled water to a total volume of 25 µl.

Thermal cycling parameters for the PCR reaction were as follows. For ITS, initial denaturation at 94 °C for 5 min, followed by 40 cycles starting with denaturation at 94 °C for 30 s, annealing with a 63–58 °C touchdown procedure decreasing 1 °C per cycle, ending at 57 °C for 30 s, 72 °C for 1 min 45 s, and a final elongation at 72 °C for 10 min. For LSU, initial denaturation at 94 °C for 5 min, followed by 40 cycles starting with denaturation at 94 °C for 30 s, annealing at 58–55 °C for 30 s, and polymerization at 72 °C for 1 min 45 s decreasing 1 °C per cycle for the first 6 cycles, and a final elongation at 72 °C for 10 min. For mtSSU, initial denaturation at 94 °C for 5 min, followed by 40 cycles starting with denaturation at 94 °C for 30 s, touchdown of the annealing temperature, decreasing from 62–56 °C for the first 6 cycles ending at 56 °C for 30 s, polymerization at 72 °C for 1 min 45 s, and a final elongation at 72 °C for 10 min.

PCR products were visualized on a 1% RedGel-stained agarose gel under UV light and purified using Exo-Sap-IT (GE Heathcare). Amplification primers were used for direct sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and run on an ABI Prism 3700 XL DNA Analyzer (Applied Biosystems) at the DNA Sequencing Laboratory, University of Bergen, Norway. Sequences were assembled using SeqMan II, version 4.05 (DNASTAR). GenBank Accession numbers are given in Table 1.

TABLE 1. *List of voucher specimens used in the phylogenetic analysis of* Fuscidea lightfootii *and* F. pusilla *with their GenBank Accession numbers (see Fig. 1).*

				GenBank Accession number		
Species	Locality	Substratum	Collection/Herbarium number	ITS	nuLSU	mtSSU
Fuscidea appalachensis	USA: Maine, Piscataquis Co.	granitic rock	MSC0050551	—	MG699076	MG669083
F. austera	Scotland: South Aberdeenshire	siliceous rock	MSC0050558	KY874026	KY874045	KY874033
F. cyathoides	Norway: Hordaland, Sotra	siliceous rock	BG-L-96931	KY874018	KY874038	KY874030
F. gothoburgensis	Norway: Hordaland, Fana	siliceous rock	BG-L-100245	KY874024	KY874042	KY874036
F. intercincta	Norway: Hordaland, Fjell	siliceous rock	BG-L-96939	MG669003	MG699077	MG669084
F. kochiana	Norway: Hordaland, Bergen	siliceous rock	BG-L-96940	KY874023	KY874041	KY874031
F. lightfootii	England: Cleethorpes Country Park	wooden fence	MRDS118545	MG669004	—	—
F. lightfootii	England: Doveridge, Derbyshire	*Salix* sp.	MRDS118544	MG669005	—	—
F. lightfootii	Ireland		*H. Hertel* 39511	—	—	EF659764
F. lightfootii	Norway: Rogaland, Finnøy	*Betula* sp.	BG-L-92376	MG669006	—	—
F. lightfootii	Norway: Rogaland, Finnøy	*Alnus glutinosa*	BG-L-92374	MG669007	—	—
F. lightfootii	Norway: Rogaland, Rennesøy	*Salix caprea*	BG-L-87100	MG669008	MG699078	MG669085
F. lightfootii	Norway: Rogaland, Rennesøy	*Salix aurita*	BG-L-96924	MG669009	—	—
F. lightfootii	Norway: Rogaland, Rennesøy	*Salix aurita*	BG-L-96926	MG669010	—	—
F. lightfootii	Norway: Rogaland, Sokndal	*Salix caprea*	BG-L-99466	MG669011	—	—
F. lightfootii	Norway: Rogaland, Stavanger	*Salix aurita*	BG-L-98608	MG669012	—	—
F. lightfootii	Norway: Rogaland, Stavanger	*Prunus* sp.	BG-L-99465	MG669013	—	—
F. lightfootii	Norway: Rogaland, Time	*Salix aurita*	BG-L-98609	MG669014	—	—
F. lightfootii	Norway: Rogaland, Vindafjord	*Salix caprea*	BG-L-100387	—	—	MG699086
F. lightfootii	Norway: Rogaland, Vindafjord	*Alnus glutinosa*	BG-L-100388	—	—	MG699087
F. lightfootii	Norway: Rogaland, Vindafjord	*Alnus glutinosa*	BG-L-100389	MG669015	MG669079	MG669088
F. lightfootii	Scotland: V.C. 82, East Lothian	*Salix* sp.	MSC0050473	MG669016	—	—
F. pusilla	Czech Rep.: S Bohemia, Šumava Mts.	*Picea* sp.	PRA 16645	MG669017	—	—
F. pusilla	Czech Rep.: W Bohemia, Chocenice-Měcholupy	*Fraxinus excelsior*	BG-L-100308	MG669018	—	—
F. pusilla	England: N of Hennock, Devon	*Salix* sp.	MRDS118546	MG669019	—	—
F. pusilla	Ireland: Co. Kildare, nr Athy	on twigs	MRDS102062	MG669020	—	—
F. pusilla	Ireland: Co. Waterford, Mt Congreve	*Larix* sp.	MRDS109586	MG669021	—	—
F. pusilla	Norway: Buskerud, Nes	*Alnus incana*	BG-L-98628	MG669022	—	—
F. pusilla	Norway: Buskerud, Nes	*Alnus incana*	BG-L-98625	MG669023	—	—
F. pusilla	Norway: Hedmark, Åmot	*Betula* sp.	BG-L-96935	MG669024	—	—
F. pusilla	Norway: Hedmark, Åmot	*Betula* sp.	BG-L-96936	MG669025	—	—
F. pusilla	Norway: Hedmark, Åmot	*Betula* sp.	BG-L-96937	MG669026	—	—
F. pusilla	Norway: Hedmark, Åmot	*Betula* sp.	BG-L-96938	KY874025	KY874040	KY874032
F. pusilla	Norway: Hordaland, Bergen	*Betula* sp.	BG-L-96927	MG669027	—	—
F. pusilla	Norway: Hordaland, Bergen	*Betula* sp.	BG-L-96928	MG669028	—	—
F. pusilla	Norway: Hordaland, Bergen	*Betula* sp.	BG-L-96929	MG669029	—	—
F. pusilla	Norway: Hordaland, Bergen	*Betula* sp.	BG-L-96930	MG669030	—	—
F. pusilla	Norway: Nordland, Alstahaug Sandnessjøen	*Betula pubescens*	BG-L-98886	MG669031	—	—
F. pusilla	Norway: Nordland, Brønnøy	*Betula pubescens*	BG-L-98663	MG669032	—	—

TABLE 1 (*continued*).

Species	Locality	Substratum	Collection/Herbarium number	GenBank Accession number		
				ITS	nuLSU	mtSSU
F. pusilla	Norway: Nordland, Brønnøy	*Betula pubescens*	BG-L-98665	MG669033	—	—
F. pusilla	Norway: Nordland, Brønnøy	*Betula pubescens*	BG-L-98667	MG669034	—	—
F. pusilla	Norway: Nordland, Brønnøy	*Betula pubescens*	BG-L-98666	MG669035	—	—
F. pusilla	Norway: Nordland, Vefsn	*Alnus incana*	BG-L-98927	MG669036	—	—
F. pusilla	Norway: Nordland, Vefsn	*Alnus incana*	BG-L-98928	MG669037	—	—
F. pusilla	Norway: Nordland, Vega	*Betula pubescens*	BG-L-98868	MG699038	—	—
F. pusilla	Norway: Nord-Trøndelag, Snåsa	*Alnus incana*	BG-L-98648	MG699039	—	—
F. pusilla	Norway: Oppland, Øyer	*Alnus incana*	BG-L-98635	MG699040	—	—
F. pusilla	Norway: Oslo, Bekkelagshøgda	*Prunus* sp.	BG-L-100190	MG699041	—	—
F. pusilla	Norway: Oslo, Bekkelagshøgda	*Malus* sp.	BG-L-100189	MG699042	—	—
F. pusilla	Norway: Rogaland, Flekkefjord	*Betula* sp.	BG-L-99464	MG699043	—	—
F. pusilla	Norway: Rogaland, Sauda	*Betula pubescens*	BG-L-98953	MG699044	—	—
F. pusilla	Norway: Sogn og Fjordane, Årdal	*Alnus incana*	BG-L-98689	MG699045	—	—
F. pusilla	Norway: Sogn og Fjordane, Eid	*Betula pubescens*	BG-L-98949	MG699046	—	—
F. pusilla	Norway: Sogn og Fjordane, Eid	*Alnus incana*	BG-L-99128	MG699047	—	—
F. pusilla	Norway: Sogn og Fjordane, Eid	*Alnus incana*	BG-L-100304	MG699048	—	—
F. pusilla	Norway: Sogn og Fjordane, Førde	*Betula* sp.	BG-L-98962	MG699049	—	—
F. pusilla	Norway: Sogn og Fjordane, Førde	*Alnus incana*	BG-L-98963	MG699050	—	—
F. pusilla	Norway: Sogn og Fjordane, Førde	*Betula pubescens*	BG-L-98964	MG699051	—	—
F. pusilla	Norway: Sogn og Fjordane, Førde	*Betula pubescens*	BG-L-98965	MG699052	—	—
F. pusilla	Norway: Sogn og Fjordane, Vågsøy	*Betula pubescens*	BG-L-99119	MG699053	—	—
F. pusilla	Norway: Sør-Trøndelag, Åfjord	*Picea abies*	BG-L-100198	MG699054	—	—
F. pusilla	Norway: Sør-Trøndelag, Midtre Gauldal	*Alnus incana*	BG-L-98644	MG699055	—	—
F. pusilla	Norway: Sør-Trøndelag, Midtre Gauldal	*Alnus incana*	BG-L-98646	MG699056	—	—
F. pusilla	Norway: Sør-Trøndelag, Rennebu	*Sorbus aucuparia*	BG-L-98640	MG699057	—	—
F. pusilla	Norway: Telemark, Notodden	*Betula* sp.	BG-L-98012	MG699058	—	—
F. pusilla	Norway: Telemark, Notodden	*Salix caprea*	BG-L-100191 (A)	MG699059	—	—
F. pusilla	Norway: Telemark, Notodden	*Salix caprea*	BG-L-100191 (B)	MG699060	—	—
F. pusilla	Norway: Telemark, Notodden	*Salix caprea*	BG-L-100191 (C)	MG699061	—	—
F. pusilla	Poland: Kotlina Sandomierska, Lasy Janowkie	*Pinus* sp.	MRDS109347	MG699062	—	—
F. pusilla	Sweden: Vestmanland		*G. Thor* 18058	—	—	EF659767
F. pusilla	Sweden: Uppland		*G. Thor* 18063a	—	—	EF659765
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Alnus incana*	BG-L-100192	MG699063	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Alnus incana*	BG-L-100193	MG699064	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Alnus incana*	BG-L-100194	MG699065	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Alnus incana*	BG-L-100195	MG699066	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Betula* sp.	BG-L-100196	MG699067	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Salix* sp.	BG-L-100197	MG699068	—	—

TABLE 1 (*continued*).

Species	Locality	Substratum	Collection/Herbarium number	GenBank Accession number		
				ITS	nuLSU	mtSSU
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Salix* sp.	BG-L-100200	MG699069	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Co.	*Salix* sp.	BG-L-100302	MG699070	—	—
F. pusilla	USA: Alaska, Lake & Peninsula Borough	*Salix* sp.	BG-L-100199	MG699071	—	—
F. pusilla	USA: Alaska, Lake Clarc National Park	*Abus viridis*	BG-L-100188	MG699072	—	—
F. verruciformis	Japan: Honshu, Prov. Shinano (Pref. Nagano)	*Betula ermaanii*	BG-L-91741	MG699073	MG669080	MG699089
Maronea constans	Tasmania: Tiddlewantie Gorge	*Boronia anemonifolia*	HO: 557799	MG699074	MG669081	MG669090
Ropalospora lugubris	USA: Katahdin	granitic rock	MSC0050548	MG699075	MG669082	MG669091

Phylogenetic analyses

MUSCLE (Edgar 2004*a*, *b*) implemented in Geneious version 8.1.8 (Biomatters Ltd.) was used to align sequences, with the 65% similarity option (Gap penalty = 14·5, Gaps extension penalty = 5), followed by manual adjustment. Ambiguous positions were manually removed from the alignment prior to the analyses. *Ropalospora lugubris* (Sommerf.) Poelt was used as an outgroup.

A concatenated data set of ITS1, 5.8S, ITS2, LSU and mtSSU was used to study the interspecific relationships between *F. lightfootii* and *F. pusilla*. Because of differences in substitution rates among ITS1, 5.8S and ITS2, it was decided to treat these as separate partitions with individual substitution rates. The best-fit substitution models for individual fragments were identified by a likelihood ratio test (Huelsenbeck & Crandall 1997) incorporated in the software jModelTest version 2.1.7 (Posada 2008). The best-fit models with the lowest AIC scores were chosen for analyses (Table 2). Individual trees were inspected for conflicts on nodes with values >70%, using the results from the maximum likelihood analysis. The analyses were performed under the same settings as described below. One significant conflict between *Maronea* A. Massal. and the clade containing *F. pusilla* and *F. verruciformis* Mas. Inoue was detected in the LSU tree. We did not exclude any taxa and thus combined all data matrices in one final concatenated alignment.

The phylogenetic analysis of the concatenated data set was performed with Bayesian Inference using Markov chain Monte Carlo (MCMC) as implemented in MrBayes version 3.2.1 (Ronquist & Huelsenbeck 2003). Two parallel runs of MCMC, each with four chains, starting from a random tree and using the default temperature of 0·2, were performed for six million generations. Gaps were treated as a fifth character state. Trees were sampled every 10th generation, including branch lengths. To test whether the MCMC chains had converged, the average standard deviation of split frequencies (ASDSF) of two parallel runs was monitored. The generations before the ASDSF had reached 0·01 were deleted as burn-in. A 50% majority-rule consensus tree was constructed from 540 000 trees and visualized in Geneious. Branches were considered significantly supported when posterior probabilities were ≥ 0·95.

TABLE 2. *Best-fit models calculated for individual and concatenated data sets. The number of parsimony-informative and conservative sites are given.*

nrDNA gene	Number of characters (informative/constant sites)	Best-fit model
ITS1	180 (56/97)	SYM + G
5.8S	156 (12/139)	SYM + I
ITS2	198 (69/93)	HKY + G
LSU	1043 (146/799)	GTR + I + G
mtSSU	704 (74/566)	GTR + I + G

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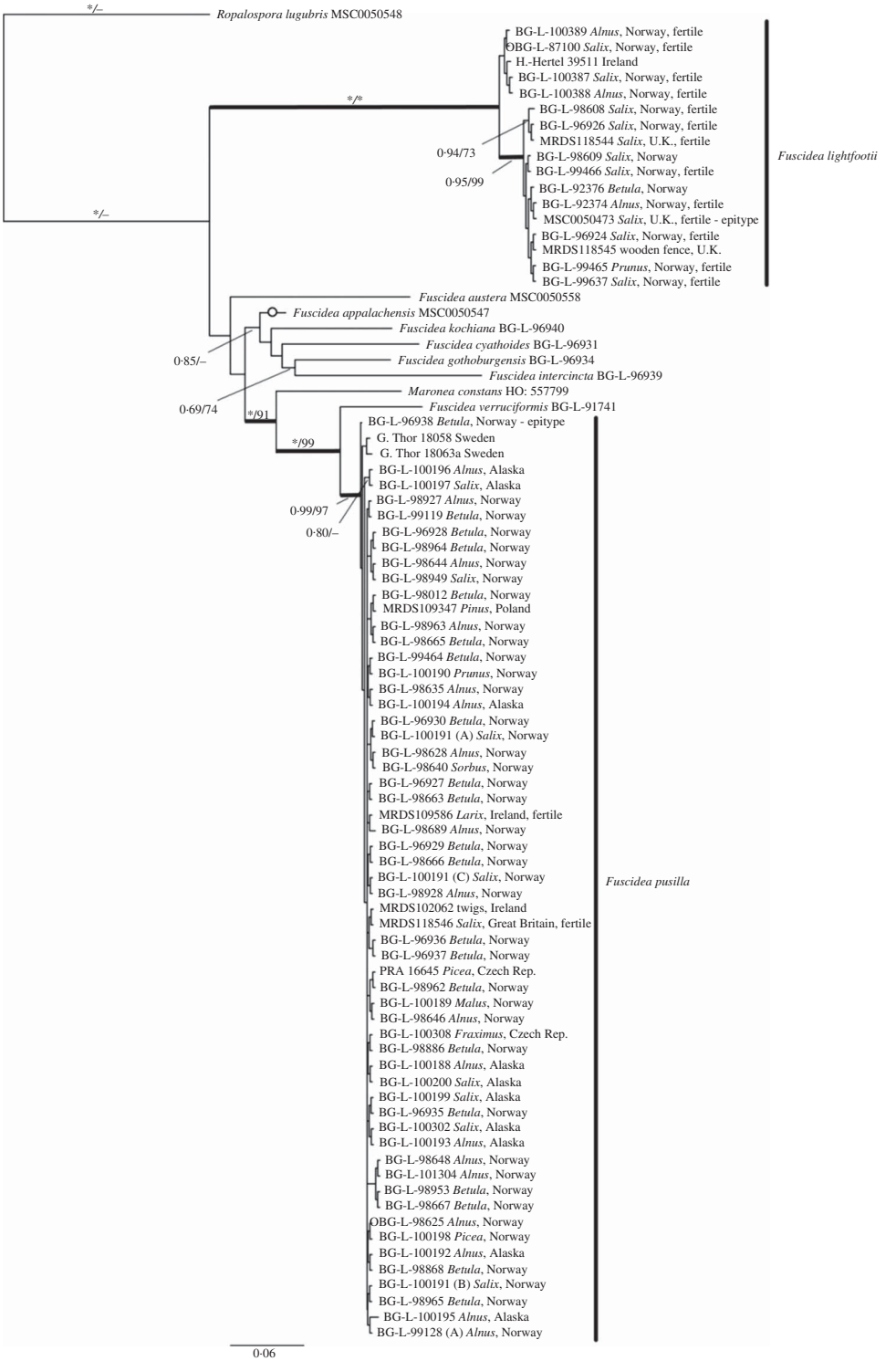

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The concatenated data set was used for the ML tree reconstruction and the branch support calculation in the program RAxML version 7.2.8 alpha (Stamatakis 2014) implemented in Geneious. Bootstrapping was carried out on 1000 replicates under the GTR + I + G model. Only clades with bootstrap values >70% were considered to be significant. The PTP (Poisson Tree Processes) model (Zhang *et al.* 2013) was run for species delimitation in the ML tree based on the concatenated data set. The default options were applied and 200 000 MCMC generations were used, with the outgroup removed. The species tree was plotted using the PhyloMap visualisation (Zhang *et al.* 2011).

Results

The final aligned concatenated data set comprised 11 taxa with 2283 characters, of which 1694 were constant and 359 parsimony-informative. There were 89 sequences newly generated. The Bayesian 50% majority-rule consensus (BI) tree, average branch lengths and posterior probabilities of branches for all specimens are given in Fig. 1. The average − ln likelihood of the tree was 8585·33 and the final ASDFS was 0·0031 at termination.

The bootstrap supports of the ML analysis were added to the BI consensus tree (Fig. 1). The incongruences between the BI and ML trees are indicated by an open circle in Fig. 1. The individual and final alignments, together with the resulting BI and ML trees of the concatenated data set, were deposited in treebase.org (ID: 21993). The resulting BI and ML trees showed that the species of interest were grouped in two distinctly supported clades.

Within the *Fuscidea lightfootii*-clade (PP = 1·0/ML = 100%), some genetic differentiation was shown but not corresponding to any geographical or ecological traits. Sequences of sterile and fertile *F. lightfootii* were almost identical since their pairwise identity was 99·9%. The sequences of sterile and fertile *F. pusilla* were clustered in one robust clade supported by PP = 0·99 and ML = 97% and their pairwise identity was also 99·9%.

The BI and ML supports from the PTP model of the concatenated data set showed that the two species are distinct (data not shown). In the plot reconstructed by PhyloMap, the first axis explained 83·35% of variance and the second axis explained 8·43% (see Fig. 2). *Fuscidea lightfootii* and *F. pusilla*, the species in question, were well separated from each other and occurred on different branches.

Taxonomy

Anatomical and morphological measurements are given as (smallest value–)mean(–largest value) (*n* = the number of measurements). We refrained from cutting sections from more than two apothecia of *Fuscidea pusilla* since they are very rare.

Fuscidea lightfootii (Sm.) Coppins & P. James

Lichenologist **10:** 201 (1978).—*Lichen lightfootii* Sm., in Sowerby, *English Botany* **21:** tab. 1451 (1805); type: N. Ireland [in the protologue: "north of Ireland"], *R. Scott* (BM—lectotype selected by Coppins & James (1978)); UK, Scotland: East Lothian, V.C. 82: Lammermuir Hills, Gifford, Hopes Reservoir, willow carr beside stream, 55°51′N, 2°43′W, alt. 260 m, on mature *Salix*, 30.10.2010, *A. M. Fryday* 9387 and *B. J. Coppins* (MSC0050473—epitype, designated here).

Thallus crustose, green and brown, sometimes only green (herbarium material whitish, greyish green, occasionally tinged with brown), up to 0·9 mm thick, forming rosettes to a few cm diam. on *Alnus* sp. and *Salix* sp., but on *Betula* sp. a mosaic of small, thin thalli, areolate, becoming contiguous and confluent with other thalli forming larger patches, sorediate. *Areoles* convex, sometimes strongly convex, at first esorediate, green to pale brown

FIG. 1. Phylogenetic relationships of *Fuscidea lightfootii* and *F. pusilla* displayed as a 50% majority-rule consensus tree of a B/MCMC analysis based on ITS, LSU and mtSSU sequences (–ln = 8585·33). Posterior probabilities (PP)/bootstrap support (BS) values are displayed above the branches. PP = 1·0 and BS = 100% indicated by an asterisk. Fertile specimens are indicated. Thick branches indicate well-supported clades. Open circles on a branch denote an incongruent topology with the ML tree. Sequences from *Fuscidea* specimens downloaded from GenBank lack information regarding type of phorotype and reproductive stage.

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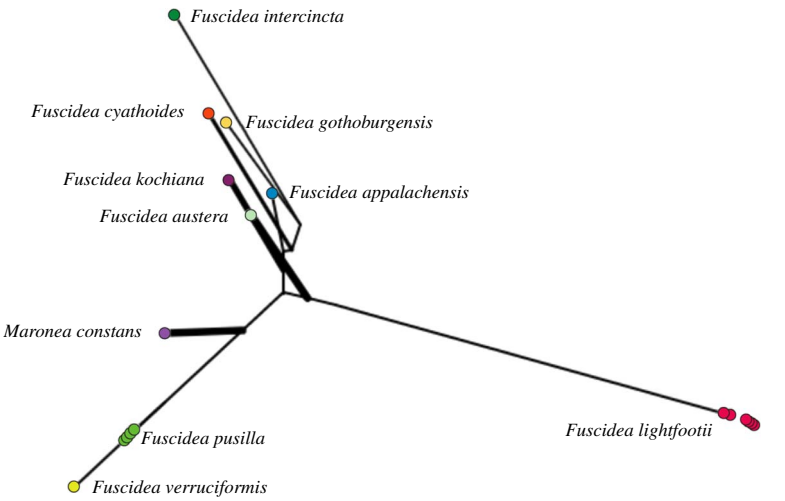

FIG. 2. PhyloMap visualization of the PTP (Poisson Tree Processes) model run for species delimitation of the ML tree based on the concatenated data set for *Fuscidea*. 200 000 MCMC generations were used, with the outgroup removed. In colour online.

and up to 0·13 mm diam., later usually sorediate and up to 0·15 mm diam., often becoming confluent. *Soralia* bursting from the apices of the areoles, green, often becoming confluent. *Soredia* green with a brown tinge, farinose, (12–)26(–31) µm diam.; *consoredia* (43–)44(–55) µm diam. *Medulla* up to 0·25 mm, I−, with crystals (in polarized light). *Prothallus* brownish or whitish, visible between the areoles and along the thallus margin. *Photobiont Apatococcus* F. Brand (Zahradníková *et al*. 2017), individual cells ≤ 24 µm diam.; walls ≤1·2 µm thick.

Apothecia ≤0·9 mm, rounded, often crenate; margin brown, thin, 0·05 mm, hyphae in section with narrow cells; *disc* black, mostly flat, occasionally convex or concave. *Epithecium* brown; *hymenium* brownish, 48–96 µm deep; *hypothecium* hyaline, ≤ 30 µm deep. *Paraphyses* (2·0–)2·6(–5·0) µm wide; tips brown, enlarged, to (3–)4(–5) µm. *Asci* clavate, of the *Fuscidea*-type, (24·0–)44·5(–60·0) × (6–)9(–13) µm. *Ascospores* simple, or occasionally 1-septate, colourless, elliptical and with median constrictions, (6–)9(–12) × (2·5–)4·0(–5·0) µm ($n = 50$).

Pycnidia not observed.

Chemistry. Divaricatic acid. Spot tests: K−, C−, KC−, Pd−, UV+ bluish white (thallus).

Distribution and ecology. *Fuscidea lightfootii* is corticolous on branches and twigs, rarely trunks, of *Salix caprea* (38% of the total specimens sequenced), *S. aurita* (25%), *Alnus glutinosa* (19%), *Betula* spp. (6%) and *Prunus* (6%). It has also been found on worked timber. Revised (sequenced) material is from Great Britain and Rogaland in SW Norway; it would seem to be a species of oceanic climate. The list of species examined can be found in Appendix A (see Supplementary Material, available online).

Notes. As the likelihood of successful DNA amplification of the type specimen from 1805 is very low, a successfully sequenced specimen was designated as the epitype, following Article 9 of the International Code of Botanical Nomenclature (McNeill *et al*. 2012).

Fuscidea pusilla Tønsberg

Sommerfeltia **14:** 138 (1992); type: Norway, Hedmark: Åmot, between Åset and Bechsminne ["Åset-Bechsminne"],

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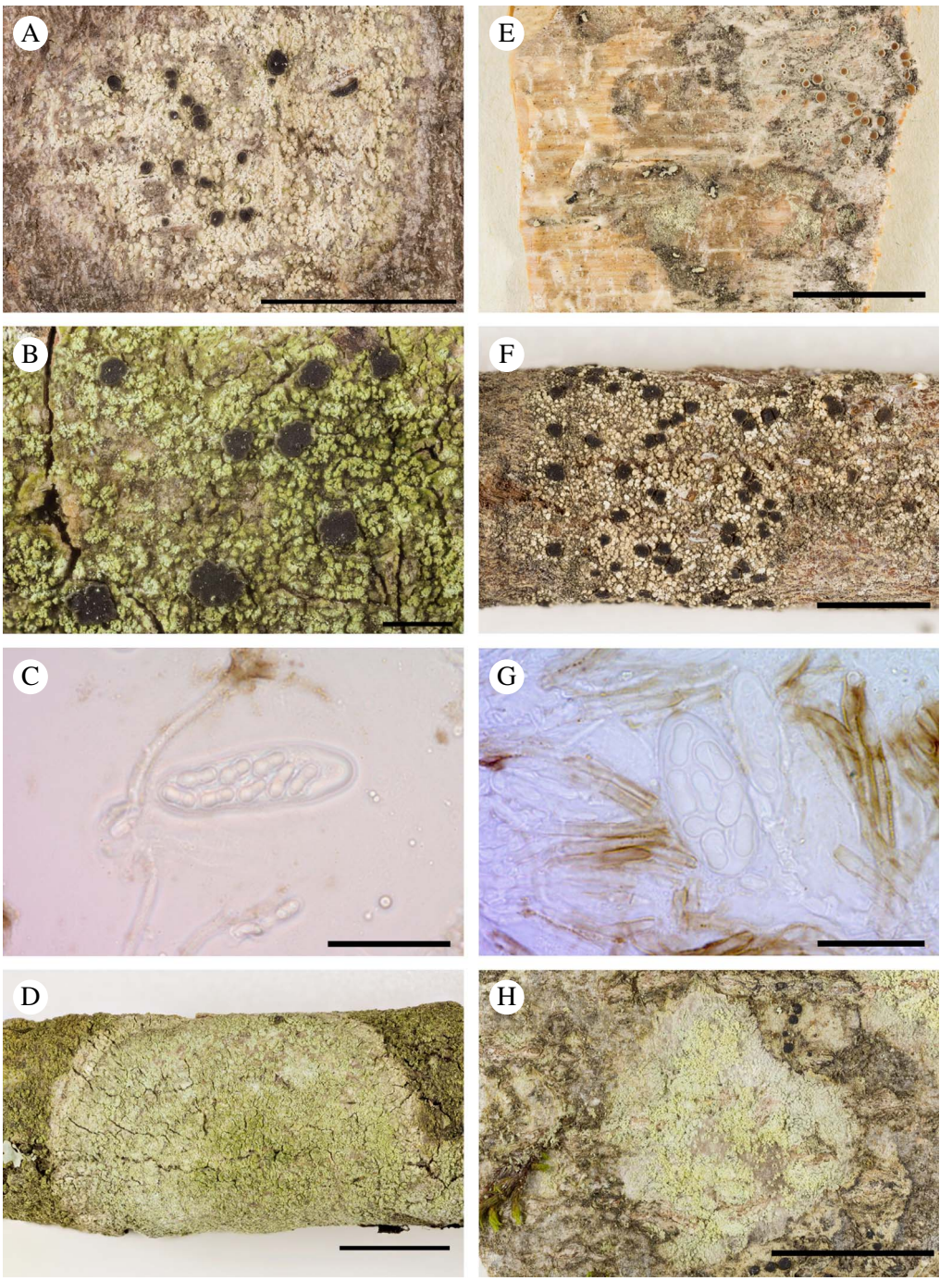

FIG. 3. A–D, *Fuscidea lightfootii*; A, fertile (*A. M. Fryday* 9387 and *B. J. Coppins* in MSC—epitype; B, fertile (*T. Tønsberg* 47026, BG-L-100387); C, asci and ascospores (same specimen as in B); D, sterile (*J. I. Johnsen*, BG-L-99466). E–H, *Fuscidea pusilla*; E, sterile (*T. Tønsberg* 40953, BG-L-96938—epitype); F, fertile (MRDS 118546 in hb. M. R. D. Seaward); G, asci and ascospores (same specimen as in F); H, sterile (*T. Tønsberg* 46018, BG-L-98963). Scales: A, D & F = 0·5 cm; B = 1 mm; C & G = 20 µm; E & H = 1 cm. Photographs: A, B, D–F, H & K by K. Abel.

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along State Road 3, UTM grid ref.: 32W PN 2674 (1917 II) [*c.* 61·0805°N, 11·3359°E], alt. 240 m, on *Betula pubescens/pendula* (roadside tree), 6 August 1983, *T. Tønsberg* 8041 (BG-L-22659—holotype [vidi]; E, UPS—isotypes); Norway, Hedmark: Åmot, along and just W of State Road 3, between Åset and Bechsminne, 61°05·08′N, 11°21·07′E, alt. 240–250 m, on trunk of young *Betula* on east-facing, steep, unstable slope near gravel pit and 20 m from busy road, 4 June 2011, *T. Tønsberg* 40953 (BG-L-96938—epitype, designated here).

Thallus crustose, ≤0·32 mm thick, greyish green to green (in herbarium greyish green to green), usually forming small rosettes up to 10 mm diam. on *Betula* spp., sparingly sorediate in patches; up to 2 cm on *Alnus incana*. *Areoles* discrete, convex, up to 0·3 mm diam., easy to squash, developing beneath and penetrating through the uppermost layer of bark, becoming dissolved into soredia, especially at the thallus centre. *Soralia* green to pale yellowish with brown tinge, bursting from the apices of the areoles, irregular, becoming confluent. *Soredia* mostly farinose (10–)12(–14) µm diam.; *consoredia* (36·0–)40·5(–45·0) µm diam. *Medulla* ≤0·2 mm) or indistinct or absent, I−; crystals present. *Prothallus* distinct, pale to dark brown, visible between the areoles, sometimes ramifying the thallus. *Photobiont Apatococcus fuscideae* A. Beck & Zahradn., having globose to broadly ellipsoid cells dividing by binary fission (Zahradníková *et al*. 2017); individual cells (12–)19(–36) µm diam.; walls ≤ 2 µm thick.

Apothecia sessile, constricted at base, roundish, up to 0·9 mm diam., dark grey-brown to black; margin paler or concolorous with disc, flexuose; rim of hyphae with elongated cells, thin, 0·04 mm. *Disc* black, mostly flat, occasionally convex or concave. *Epithecium* brown; *hymenium* brownish, ≤100 µm; *hypothecium* hyaline, ≤15 µm. *Paraphyses* (1·5–)2·0(–3·5) µm wide; tips enlarged, brown, (3–)4(–6) µm. *Asci* clavate, of the *Fuscidea*-type, (30–)35(–40) × (8·0–)8·5(–11·0) µm. *Ascospores* simple, colourless, elliptical, medially constricted (6–)8(–10) × (2·5–)3·0(–4·5) µm ($n = 18$).

Pycnidia not observed.

Chemistry. Divaricatic acid. Spot tests: K−, C−, KC−, Pd−, UV+ blue-white (soralia).

Distribution and ecology. Based on the sequenced material only, *F. pusilla* is a corticolous species occurring in continental as well as oceanic climates at altitudes ranging from about sea-level to 800 m. Its presently known distribution includes Central Europe, Great Britain, Ireland, Norway and the USA (Alaska). It has been collected mainly on *Betula* spp. (38% of the specimens sequenced), *Alnus incana* (31%), *Salix caprea* (13%) and *Picea abies* (3·6%), and occasionally (less than 2%) on other phorophytes such as *Alnus viridis*, *Fraxinus excelsior*, *Larix* sp., *Malus domestica*, *Prunus* sp. and *Sorbus aucuparia* (see Table 1). Most of the specimens from *Betula* were collected on young trees with flaking bark. The list of species examined is provided in Appendix A (see Supplementary Material, available online).

Discussion

The resulting BI and ML trees demonstrate that *Fuscidea lightfootii* and *F. pusilla* are grouped in two clearly supported clades and that they are phylogenetically distinct. The hypothesis that they are conspecific, mentioned by Tønsberg & Johnsen (2008) and suggested by Gilbert *et al*. (2009), is therefore rejected. Our result agrees with Bylin *et al*. (2007), where *F. lightfootii* and *F. pusilla* appeared in different groups.

Based on the material studied here, *F. lightfootii* and *F. pusilla* differ in the size of their thalli, the species reaching a few cm in diameter and up to 10 mm in diameter, respectively. This difference is probably due to differences in phorophyte bark structure and uneven specimen sampling. For *F. lightfootii*, most collections are from *Salix* (63% of the total specimens sequenced) and *Alnus* (19%), while *F. pusilla* has most frequently been collected on *Betula* (38%) and *Alnus* (33%). The small size of the *F. pusilla* thalli is apparently due to the bark of young *Betula* trees being an unstable substratum where the uppermost, colourless layer tends to peel away. In one collection of *F. lightfootii* (*J. I. Johnsen*, BG-L-92376) from the trunk of *Betula* (see Fig. 4),

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the thalli form a mosaic of small, thin rosettes similar to those typical for *F. pusilla* when growing on this phorophyte. When growing on *Alnus* and *Salix*, *F. pusilla* thalli are thicker and may exceed 2 cm in diameter, for example *T. Tønsberg* 44828 (BG-L-98635) and *T. Tønsberg*

FIG. 4. *Fuscidea lightfootii*, sterile (*J. I. Johnsen*, BG-L-92376) resembling *F. pusilla* (Rogaland, Norway). Scale = 1 cm. Photograph by K. Abel.

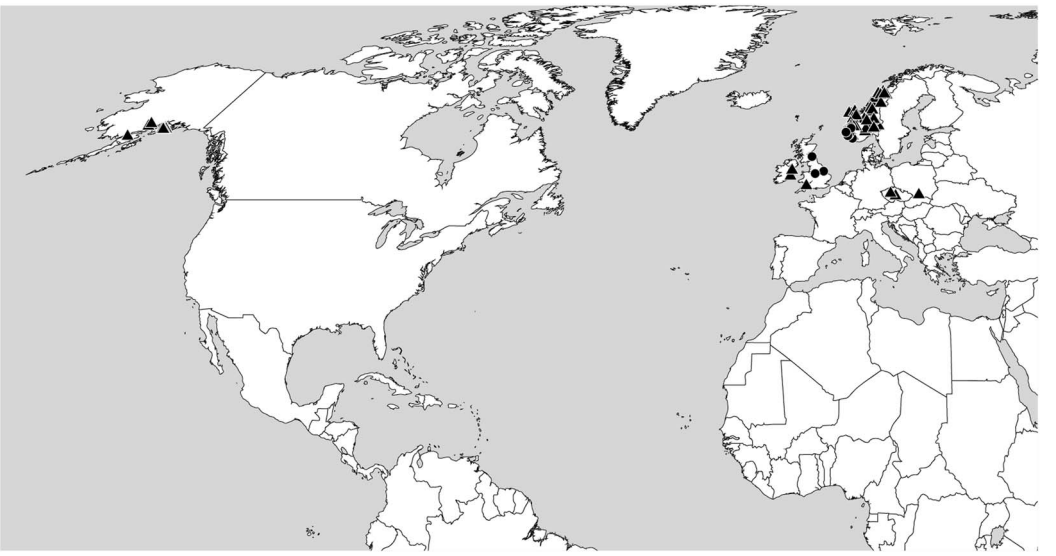

FIG. 5. Distribution of *Fuscidea lightfootii* (circles) and *F. pusilla* (triangles) based on the specimens cited and sequenced.

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44774 (BG-L-100191). In the material from those phorophytes, there is no difference in size between thalli of *F. lightfootii* and *F. pusilla*.

The genetic variation within *F. lightfootii* and *F. pusilla* is very low. Only a small number of haplotypes are recognized within each of the two species and no geographical trends are found (data not shown).

Two specimens, originally identified as *F. lightfootii* based on the presence of

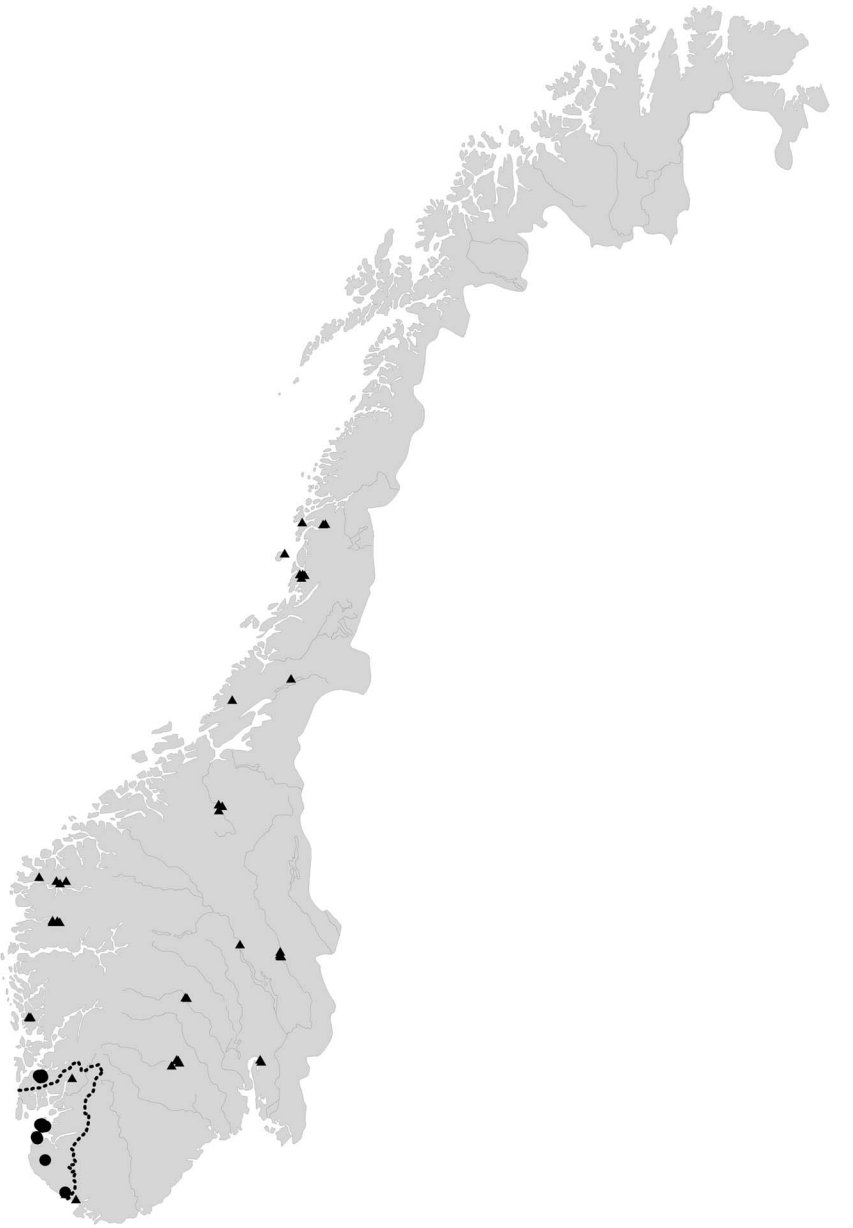

FIG. 6. Distribution of *Fuscidea lightfootii* (circles) and *F. pusilla* (triangles) in Norway based on the specimens cited and sequenced, showing their overlapping zone in or near Rogaland County (dotted line).

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apothecia, have been proved to represent fertile specimens of *F. pusilla*. These are from *Larix* in Ireland (MRDS 109586) and from *Salix* in Great Britain (MRDS 118546). Fertile specimens of *F. pusilla* have not previously been reported (e.g. Tønsberg 1992; Gilbert *et al.* 2009). Its apothecia appear to be morphologically and anatomically rather similar to those of *F. lightfootii*. In the present study, the asci of *F. pusilla* appear to be smaller (mean length = 35 µm) than those of *F. lightfootii* (mean length = 44·5 µm). As we refrained from making sections from more than two apothecia of *F. pusilla*, further data are needed to test this difference statistically.

The photobionts in *Fuscidea* have been identified as two distinct species of *Apatococcus* F. Brand (Zahradníková *et al.* 2017). *Apatococcus fuscideae*, characterized by a reticulate chloroplast, is the photobiont in most species of *Fuscidea*, including *F. pusilla*. *Fuscidea lightfootii*, on the other hand, is associated with a different species of *Apatococcus*, still undescribed. We do not know if it is possible to distinguish between these two photobionts using non-molecular methods such as cultivation or by examination in squash preparations of lichen thalli. According to Friedl & Büdel (2008), the chloroplast morphology and the life cycle of green algae in lichen thalli may differ from conspecific, free-living specimens.

We consider *F. lightfootii* and *F. pusilla* to represent cryptic species as it is apparently not possible to identify a specimen to one or the other species based on morphological methods alone. Of the two species, *F. pusilla* appears to have the broadest ecological range, occurring in both continental and oceanic areas. In the British Isles and SW Norway, *F. lightfootii* and *F. pusilla* are sympatric (see Figs 5 & 6). We conclude that *F. lightfootii* and *F. pusilla* are two distinct species, and that DNA sequencing is necessary for their identification.

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Supplementary Material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S0024282918000270

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