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Species delimitation, bioclimatic range, and conservation status of the threatened lichen *Fuscopannaria confusa*

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Abstract: *Fuscopannaria confusa* is a rare lichen restricted to very humid localities in boreal forests. Two *Fuscopannaria* species, *F. ahlneri* and *F. mediterranea*, and *Parmeliella parvula* are morphologically problematic to distinguish from *F. confusa*. Our aim with the present study was to evaluate the taxonomic status of *F. confusa* and thereby clarify its conservation status in Norway. By phylogenetic analysis of multi-locus DNA sequences, we show that *F. confusa* is genetically well distinguished from *F. ahlneri, F. mediterranea*, and *P. parvula*. *Fuscopannaria confusa* should therefore be treated as a separate species. A species distribution modelling analysis indicates that *F. confusa* has a slightly continental but potentially wide geographic distribution in Norway. However, suitable localities are continuously being destroyed by clear-cut logging and hydroelectric power development. Because of the decline in suitable habitats, *F. confusa* should be regarded as highly threatened in Norway and listed as EN (endangered) at the national level.

Key words: comparative DNA sequence analysis, distribution modelling, taxonomy, *Parmeliella*

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Introduction

In conservation biology, it is of basic importance to recognize the appropriate conservation units, which are usually species or various populations with divergent properties. It is problematic to devise good conservation strategies if the basic conservation units remain unknown. In lichens, there are many species with unclear boundaries (e.g. Kroken & Taylor 2001; Crespo & Lumbsch 2010). Characters such as morphology and secondary chemistry are often insufficient to differentiate closely related lineages of lichens (Lumbsch & Leavitt 2011). Molecular data have revolutionized our understanding of the evolution of lichenized fungi (see review by Printzen 2010 and references therein). Phylogenetic analysis of DNA sequence data, preferably from several unlinked loci, is a useful approach to unravel species boundaries (Taylor *et al.* 2000; Kroken & Taylor 2001; Lumbsch & Leavitt 2011).

The lichen *Fuscopannaria confusa* P. M. Jørg is difficult to distinguish morphologically from two other *Fuscopannaria* species, *F. ahlneri* P. M. Jørg and *F. mediterranea* (Tav.) P. M. Jørg., as well as from *Parmeliella parvula* P. M. Jørg. These species are characterized by greyish to brownish, small-squamulose thalli attached to a bluish black hypothallus. Moreover, they are sorediate and often without apothecia (see Fig. 1A). The genus *Fuscopannaria* comprises *c.* 46 species and *Parmeliella c.* 150 species (http://www.indexfungorum.org/). Both genera belong in the family *Pannariaceae*, order *Peltigerales*. However, molecular investigations have shown that *Fuscopannaria* and

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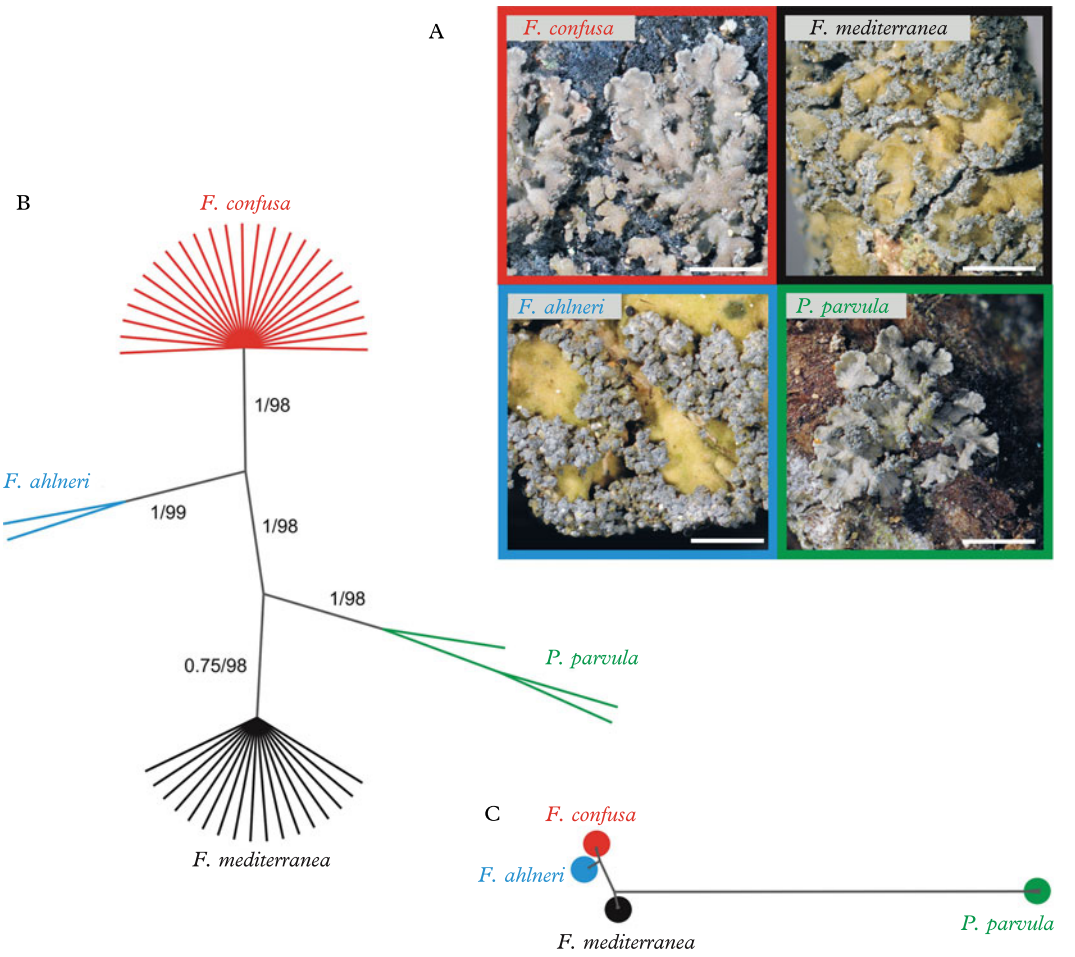

FIG. 1. A, photographs of *Fuscopannaria confusa*, Hofton 08452 (O), *F. mediterranea*, Hofton 08445 (O), *F. ahlneri*, Holien (TRH L-10368), and, *Parmeliella parvula*, Holien (TRH L-9978); it is not possible to distinguish *Fuscopannaria confusa* from *P. parvula* by thallus morphology; both *F. confusa* and *P. parvula* have greyer squamules than *F. mediterranea* and *F. ahlneri*. Scales = 0·5 mm. B, unrooted molecular tree showing that *Fuscopannaria confusa* is genetically distinct from *F. ahlneri*, *F. mediterranea*, and *Parmeliella parvula*. The unrooted tree (transformed to equal branch lengths) is a 50 % majority rule consensus topology from a Bayesian phylogenetic analysis of a concatenated dataset with 54 accessions of four taxa and three unlinked genetic regions. The single most parsimonious tree (identical in topology) was 190 steps long and had a rescaled consistency index of 0·96 and a homoplasy index of 0·03. Branch labels indicate posterior probabilities from Bayesian analysis/jackknife support values from parsimony resampling. C, same as B, but with branch lengths proportional to molecular change. In colour online

Parmeliella are not phylogenetic sisters, each being more closely related to other genera in the family (Ekman & Jørgensen 2002; Miądlikowska *et al.* 2006; Wedin *et al.* 2009).

Most documented findings of the morphotaxon *F. confusa* are from Fennoscandia, but it is known also from European Russia (Hermansson & Kudryatseva 1995), two localities in the Alps (Vonarburg & Zimmermann 2006), and some scattered localities in North America (Jørgensen 2000). *Fuscopannaria confusa* seems to be associated with brook ravines, and especially the spray zone of waterfalls. Prior to this study, *F. confusa* was known from nine Norwegian localities (documented in university herbaria), mainly

in central Norway. We have collected material, tentatively assigned to *F. confusa*, from several additional localities, mainly in south-east and central Norway (Table 1; Fig. 2).

The specialized ecology and few known occurrences make it likely that *F. confusa* is a threatened species in Norway. During preparation of the former Norwegian red list (Timdal *et al.* 2006), however, the authors found it difficult to identify collections of this complex and hence to evaluate the conservation status of *F. confusa*. For that reason it was given the status NE (not evaluated). Our principal aim with the present study is to test, by use of molecular data, whether *F. confusa* is a distinct species or rather falls within the variation of *F. ahlneri*, *F. mediterranea* or even *P. parvula*. We also calculate the potential regional distribution of *F. confusa* in Norway through species distribution modelling. Finally, in light of the new data, we propose a new conservation status of *F. confusa* for the Norwegian red list, which has already been adopted based on preliminary results of this study (Timdal *et al.* 2010).

Materials and Methods

We analyzed two collections of *F. ahlneri*, 29 collections of *F. confusa*, 17 collections of *F. mediterranea*, and five collections of *P. parvula*; most were sampled through fieldwork covering all major parts of Norway and a few older herbarium specimens (Table 1). The species names given in Table 1 represent our post-analysis conclusions. Several specimens could not be identified in the field (collected as sp. or cf.; indicated in Table 1). These were re-examined after the more morphologically distinct specimens had been sequenced and tested with TLC. Most of the specimens sampled for this study were collected during species inventories in various habitats throughout Norway (less so in the far north).

DNA was extracted from these specimens using a 2% CTAB (cetyltrimethylammonium bromide) miniprep protocol (Murray & Thompson 1980). DNA fragments from three different regions were amplified: the nuclear ribosomal internal transcribed spacer (nrITS), an anonymous nuclear region [presumably Chalcone Synthase (*CHS*)], and a part of the mitochondrial ribosomal small subunit (mtSSU) region.

The nrITS and mtSSU regions were amplified and sequenced using the primer pairs ITS5/ITS4 and MS1/MS2, respectively (White *et al.* 1990), and the partial *CHS* region using the primers CHS1F and CHS1R (James & Vilgalys 2001). The polymerase chain reaction (PCR) was performed in 25-µl reactions (5 µl of 1/100 dilution of DNA extract added) using PuReTaq *Ready-To-Go™ PCR Beads* (GE Healthcare Limited, Buckinghamshire, UK) and the following cycle conditions: 4 min at 94°C, 34 cycles of 25 s at 94°C, 30 s at 55°C, 90 s at 72°C, and a final elongation step at 72°C for 10 min. PCR products were purified using 2 µl of a 1-in-10 dilution of ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA) to 8 µl PCR product and otherwise following the provider's instructions. Cycle sequencing was performed by the CEES ABI-laboratory (http://www.bio.uio.no/ABI-lab/) using the ABI BigDye Terminator sequencing buffer and v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA). Sequences were processed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All sequence chromatograms were controlled manually and sequence alignments established in BioEdit (Hall 1999). All new sequences are deposited in GenBank, and the accession numbers are listed in Table 1.

We performed both parsimony and Bayesian phylogenetic analyses on each genetic region separately and in combination, and always with insertions/deletions coded as missing. Also expanded datasets that included taxa with similar sequences obtained through BLAST searching our self-generated sequences against public DNA sequence databases, were analyzed. TNT (Goloboff *et al.* 2008) was used for parsimony analyses and MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) for Bayesian analyses. We performed parsimony heuristic searches with one thousand random addition sequences and TBR branch swapping. For parsimony jackknifing, we used 10 000 replicates, 36% removal probability, and absolute frequencies as output. The jModelTest (Posada 2008) was used to estimate the optimal models of nucleotide substitution for the various genetic regions. We ran two independent Bayesian runs with five chains (4 heated) for 10 million generations and summarized after discarding about 25% as burn-in (approximately the point where the standard deviation of split frequencies fell below 0·015). We used 50% majority rule consensus trees to calculate posterior probabilities.

We examined 12 specimens of *F. confusa*, two specimens of *F. ahlneri*, four specimens of *F. mediterranea*, and two specimens of *P. parvula* (Table 1) for secondary metabolites using standard thin-layer chromatographic techniques (TLC), in accordance with the methods of Culberson (1972), modified by Menlove (1974) and Culberson & Johnson (1982).

In light of the DNA-sequence and TLC results, the morphology of the specimens were re-examined.

We performed ecological distribution modelling for *F. confusa* in Norway using Maxent version 3.1.0 (Phillips *et al.* 2006; Phillips & Dudík 2008) with default settings. Maxent is a maximum-likelihood modelling method based on the maximum entropy principle (Jaynes 1957). Maxent was chosen because it has proven to be one of the most reliable distribution modelling methods currently available (Elith *et al.* 2006; Phillips *et al.* 2006; Guisan *et al.* 2007; Elith & Graham 2009). Input for the distribution model was location co-ordinates of the collected and analyzed material listed in Table 1, and the two first

TABLE 1. *Specimens used in this study with voucher information (taxon name, voucher number, herbarium, locality with coordinates, year of collection, and name of collector) and GenBank accession numbers. All sequences listed were generated for the present study*

Taxon	Voucher number	Herb-arium	Municipality of origin	Latitude	Longitude	Collection year	Collector	mtSSU	nrITS	CHS
Fuscopannaria ahlneri	L-10368†	O	Namsos	64.355	11.338	2005	*H. Holien*	GU570017	GU570096	GU570063
F. ahlneri	L-10358†	TRH	Roan	64.157	10.463	2005	*H. Holien*	GU570018	GU570097	—
F. confusa	L-171935†	O	Ål	60.633	8.4039	2008	*T. H. Hofton*	GU570050	GU570124	—
F. confusa	L-171936	O	Åmot	61.202	11.215	2007	*S. Reiso*	GU570019	GU570100	GU570064
F. confusa	L-171937†	O	Hattfjelldal	65.645	14.121	2008	*T. H. Hofton*	GU570048	—	GU570085
*F. confusa**	L-171938	O	Hemsedal	60.853	8.4276	2008	*S. Reiso*	GU570043	—	—
F. confusa	L-171939	O	Hjartdal	na	na	2008	*S. Reiso*	GU570039	GU570115	GU570080
*F. confusa**	L-171940	O	Klæbu	63.254	10.642	2007	*T. H. Hofton*	GU570038	GU570114	GU570079
*F. confusa**	L-171941	O	Klæbu	63.254	10.642	2007	*T. H. Hofton*	GU570052	—	GU570086
F. confusa	L-171942	O	Midtre Gauldal	62.876	10.720	2007	*S. Reiso*	GU570011	GU570090	GU570056
F. confusa	L-171943	O	Midtre Gauldal	62.876	10.720	2007	*S. Reiso*	GU570022	GU570103	GU570066
*F. confusa**	L-171944	O	Midtre Gauldal	62.883	10.694	2007	*S. Reiso*	GU570032	GU570109	GU570075
F. confusa	L-171945	O	Nord-Aurdal	60.964	9.2033	2007	*S. Hofton*	GU570013	GU570092	GU570057
*F. confusa**	L-171946	O	Nord-Aurdal	60.922	9.2984	2007	*S. Reiso*	GU570036	—	GU570078
F. confusa	L-171947	O	Nord-Aurdal	60.916	9.2128	2007	*T. H. Hofton*	GU570055	GU570133	—
F. confusa	L-11621	O	Nordre Land	61.070	10.025	1989	*J. Holtan-Hartwig*	—	—	GU570058
F. confusa	L-171948	O	Notodden	59.696	9.1629	2008	*S. Reiso*	GU570041	—	GU570082
F. confusa	L-171919	O	Øyer	61.255	10.495	2007	*S. Reiso*	GU570014	GU570093	GU570060
F. confusa	L-171920	O	Ringebu	61.429	10.136	2007	*T. H. Hofton*	GU570020	GU570101	—
F. confusa	L-171921	O	Ringebu	61.594	10.248	2007	*S. Reiso*	GU570037	GU570113	—
F. confusa	L-171922	O	Ringebu	61.450	10.048	2007	*T. H. Hofton*	GU570054	GU570127	—
*F. confusa**	L-171923	O	Sel	61.672	9.4979	2007	*S. Reiso*	GU570034	—	—
F. confusa	L-171924	O	Sel	61.669	9.4276	2007	*T. H. Hofton*	GU570053	GU570126	—
F. confusa	L-11620	O	Grong	64.223	12.044	1984	*T. Tønsberg*	—	—	GU570059
F. confusa	L-171925†	O	Stor-Elvdal	61.500	11.087	2007	*T. H. Hofton*	GU570025	GU570106	GU570069
F. confusa	L-171926	O	Stor-Elvdal	61.442	10.874	2006	*T. Hofton*	GU570026	—	GU570070
F. confusa	L-171927	O	Stor-Elvdal	61.443	10.878	2006	*T. Hofton*	GU570027	GU570107	GU570071
*F. confusa**	L-171928	O	Tinn	60.016	8.6238	2008	*S. Reiso*	GU570040	GU570116	GU570081
F. confusa	L-171929	O	Tinn	59.867	9.0204	2008	*S. Reiso*	GU570042	GU570117	—
*F. confusa**	L-171930	O	Tinn	59.855	9.0223	2008	*S. Reiso*	GU570044	GU570118	GU570083
*F. confusa**	L-171931	O	Tinn	59.875	9.0244	2008	*S. Reiso*	GU570045	—	GU570084
F. mediterranea	L-136674	O	Etne	59.766	6.135	2004	*T. H. Hofton et al.*	—	GU570129	—
F. mediterranea	L-65747†	O	Namdalseid	64.261	11.518	1999	*A. Solås*	—	GU570130	GU570087
F. mediterranea	L-118798†	O	Narvik	68.552	17.590	2002	*H. Bratli* & *Bjørklund*, P.	—	GU570128	—
F. mediterranea	L-171949	O	Nord-Aurdal	60.964	9.2033	2007	*T. H. Hofton*	GU570012	GU570091	—
F. mediterranea	L-171950	O	Øyer	61.255	10.495	2007	*S. Reiso*	GU570015	GU570094	GU570061

Table 1. *Continued*

Taxon	Voucher number	Herb-arium	Municipality of origin	Latitude	Longitude	Collection year	Collector	mtSSU	nrITS	CHS
*F. mediterranea**	L-171951	O	Ringebu	61.594	10.248	2007	*S. Reiso*	GU570046	GU570121	—
*F. mediterranea**	L-171952	O	Sel	61.672	9.4979	2007	*S. Reiso*	GU570024	GU570105	GU570068
*F. mediterranea**	L-171953	O	Sel	61.676	9.5035	2007	*S. Reiso*	GU570035	GU570112	GU570077
F. mediterranea	L-9539†	TRH	Steinkjær	64.026	11.348	2004	*H. Holien*	GU570016	GU570095	GU570062
F. mediterranea	L-6285	TRH	Steinkjær	64.019	11.353	2001	*H. Holien*	—	GU570120	—
F. mediterranea	L-139789	O	Surnadal	63.096	8.9311	2005	*G. Gaarder*	—	GU570131	GU570088
F. mediterranea *	L-171954	O	Tinn	59.991	8.7784	2007	*S. Reiso*	GU570033	GU570111	GU570076
F. mediterranea	L-171955	O	Tinn	59.877	9.0219	2008	*S. Reiso*	—	GU570119	—
F. mediterranea	L-135117†	O	Tokke	59.442	7.7762	2004	*E. Timda*l	—	GU570132	GU570089
F. mediterranea	L-171956†	O	na	na	na	2008	*T. H. Hofton*.	GU570047	GU570122	—
*F. mediterranea**	L-171957†	O	na	na	na	2008	*T. H. Hofton*	GU570049	GU570123	—
F. mediterranea	L-171958†	O	na	na	na	2008	*T. H. Hofton*	GU570051	GU570125	—
Parmeliella parvula	L-10171†	TRH	Leksvik	63.702	10.608	2005	*H. Holien*	—	GU570099	—
P. parvula	L-55763†	O	Ørland	63.633	9.4333	1998	*R. Haugan*	—	—	GU570074
*P. parvula**	L-171932	O	Osen	64.262	10.580	2007	*S. Reiso*	—	GU570110	—
cf. P. parvula	L-33812	O	Overhalla	64.509	12.084	1998	*E. Rolstad* & *Gaarder*, G.	GU570031	—	—
P. parvula	L-9978	TRH	Verdal	63.72	11.61	2003	*H. Holien*	—	GU570098	—

* Specimens collected as uncertain or unknown species within the complex and named after careful morphological investigation after the DNA results were known.
† Specimens examined for secondary metabolites using standard thin-layer chromatographic technique.

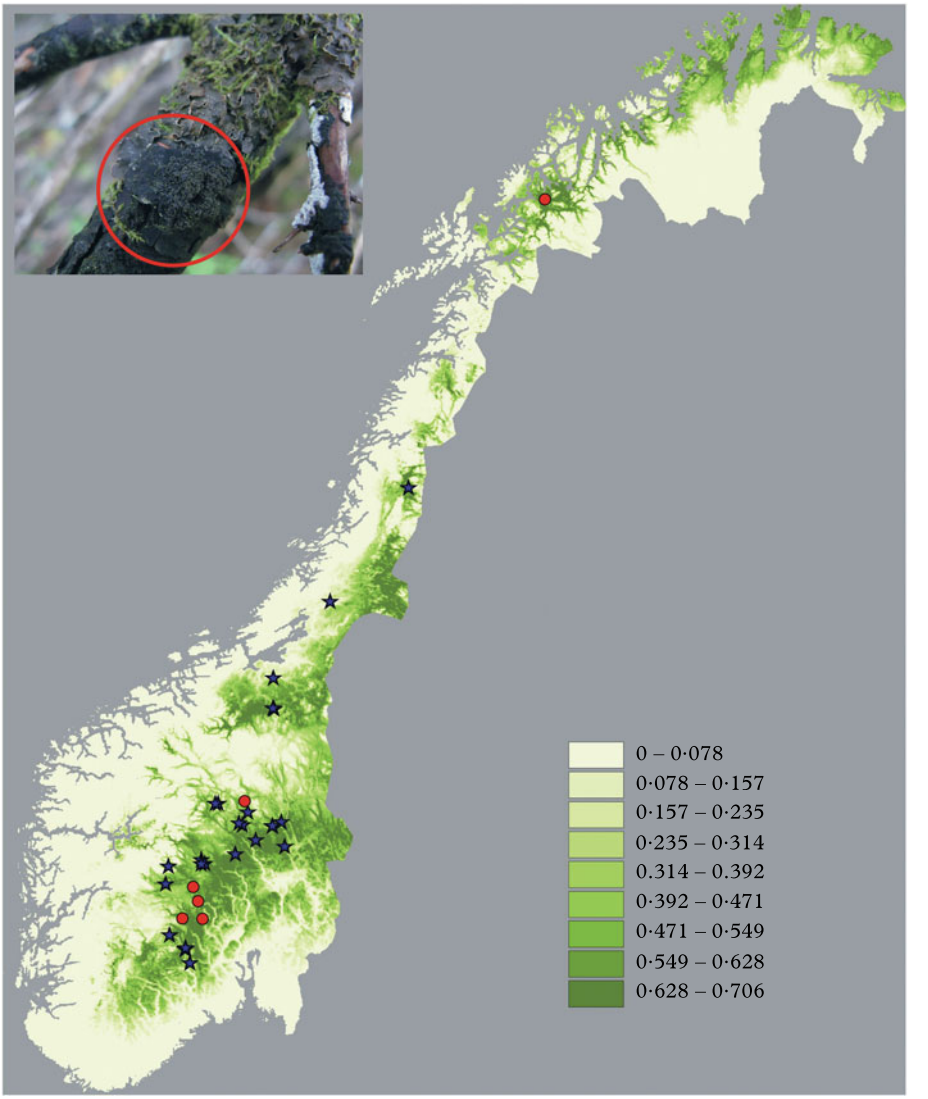

FIG. 2. Map showing habitat suitability for *Fuscopannaria confusa* according to a maximum entropy model. Shading indicates the predicted probabilities of species occurrence on a scale of 0 to 1, with darker shades showing higher predicted areas. Asterisks (*) indicate sampling sites for *F. confusa* collections analyzed; dots (•) indicate new reports of *F. confusa* after the modelling was performed. Inset: *F. confusa*, *Hofton* 0810x (O). In colour online.

principal components extracted from a set of 54 environmental variables (1 × 1 km resolution) analyzed by Bakkestuen *et al.* (2008, 2009). The first principal component represents a regional variation (gradient) from coast to inland and from oceanic/humid to continental areas. The second principal component represents a regional variation from north to south and from high to low altitudes. These two axes corresponded to the two bioclimatic gradients used in Bakkestuen *et al.*'s (2008) classification of Norway into biogeographical regions: vegetation sections (from highly oceanic to slightly continental) and vegetation zones (from nemoral to alpine).

Results

We obtained high quality DNA sequences of at least one genetic region of each accession listed in Table 1; in total, 113 new sequences

(42 nrITS, 31 *CHS*, and 40 mtSSU). We had generally lower success rate for older specimens of all species. That only single and/or different genetic regions could be amplified from some specimens (see Table 1: e.g. *Parmeliella parvula*) might indicate poor quality of the template DNA (M. Bendiksby, unpublished). Best-fit models of nucleotide substitution using the Bayesian information criterion were: K80+G for nrITS; HKY for mtSSU; JK for *CHS*. Parsimony and Bayesian analyses produced identical results. The gene trees obtained from both of these analyses were congruent, were strongly supported, and data matrices contained low or no homoplasy. With low or no homoplasy, the tree-lengths (L) depict the level of molecular variation; nrITS being the most variable (L = 178), mtSSU the second (L = 46), and *CHS* being the least variable marker of the three (L = 16). The corresponding numbers of parsimony informative characters are: 134 in nrITS, 22 in mtSSU, and eight in the *CHS* matrix. The same characteristics apply for the gene trees of the expanded datasets (those that include sequence data of additional taxa from public databases), which are presented with tree statistics and additional descriptions as Supplementary Figure 1 (SF1) in the Appendix. Among all datasets, only the expanded mtSSU dataset generated more than a single most parsimonious tree (SF1C).

In the concatenated matrix, we included all accessions for which we had sequenced the nrITS, or both *CHS* and mtSSU (see Table 1). The Bayesian 50% majority rule consensus tree (identical to the single most parsimonious tree) is presented with tree statistics in Fig. 1B. All accessions of each of the four morphospecies (*F. ahlneri*, *F. confusa*, *F. mediterranea*, and *P. parvula*) group with high support, respectively.

In the TLC analyses, we found two unknown fatty acids and one unknown triterpenoid in *F. confusa*, *F. ahlneri* and *F. mediterranea*, and no metabolites in *P. parvula*. The metabolites occurred in low concentration, but the results were reproducible and constant over all representatives of the same species investigated. We did not notice any diagnostic difference in the secondary chemistry between the three *Fuscopannaria* species.

The habitat suitability for *F. confusa* according to a maximum entropy model is shown in Figure 2. When taking only bioclimatic variation into consideration, the darker colours show higher predicted areas. The habitat suitability model obtained an area under the curve value of 0·885, which suggests a potentially useful model (1 indicates a perfect model, and 0·5 indicates a random prediction; Pearce & Ferrier 2000). The two 'composite' environmental variables (oceanic to continental areas, and north to south / high to low altitudes; see Bakkestuen *et al.* 2008 for further explanation) contributed almost equally to the performance of the model and show that *F. confusa* responds to both of the two main recognized regional bioclimatic gradients in Norway.

Discussion

Accurate species delimitation is crucial in biodiversity assessments and conservation biology. Multi-locus DNA sequencing provides an invaluable tool for robust species delimitation (Lumbsch & Leavitt 2011). In this study, we have tested, by multi-locus DNA sequencing, whether *Fuscopannaria confusa* is a species distinct from the three morphologically highly similar species, *F. ahlneri*, *F. mediterranea* and *Parmeliella parvula* (Fig. 1A). Our DNA sequence data from three unlinked genetic regions clearly show that *F. confusa* represents a separate species (Fig. 1B). Moreover, phylogenetic results from the extended datasets (those including additional taxa; SF1) show that none of the three morphologically similar species represent the closest relative of *F. confusa*. *Fuscopannaria mediterranea* is the genetically most divergent among the three *Fuscopannaria* species included (Fig. 1C), whereas *F. ahlneri* is the morphologically most readily distinguishable species by its more prolonged and wider lobes (up to 1·5 mm wide vs up to 1·0 mm in *F. mediterranea*, 0·8 mm in *F. confusa*, and 0·6 mm in *P. parvula*). Moreover, our morphological re-examination revealed that

F. mediterranea differs from *F. confusa* and *P. parvula* in forming somewhat larger, more olivaceous brown squamules with contrasting blue-grey soralia. In *F. confusa* and *P. parvula*, both squamules and soralia were uniformly grey. *Fuscopannaria confusa* and *P. parvula* remain the most difficult to separate morphologically. Jørgensen (1991) reported *P. parvula* to have isidioid soralia and *F. confusa* to have needle-like crystals in the soralia in herbarium specimens. The latter character develops slowly and reflects the presence of triterpenoids in the thallus. We failed to separate *F. confusa* from *P. parvula* on thallus morphology. *Fuscopannaria confusa* may have a somewhat darker thallus than *P. parvula*, but chromatography or DNA sequencing seems to be required for distinguishing these two species. Our TLC results support Jørgensen's (1991) report of two unknown fatty acids and one unknown triterpenoid in *F. confusa,* as well as no observable metabolites in *P. parvula*. Although in low concentration, the metabolites do seem to represent reliable characters for distinguishing between *F. confusa* and *P. parvula*. The hymenial character reported by Jørgensen (1991; i.e. colour reaction by iodine) could not be studied in our sterile material.

Although the three *Fuscopannaria* species, *F. ahlneri*, *F. mediterranea*, and *F. confusa*, are sometimes found growing side by side, they exhibit rather clear differences in ecology and distribution, *F. mediterranea* being the most divergent. Whereas both *F. ahlneri* and *F. confusa* have narrow ecological niches, being mostly *Picea*-associated and strongly hygrophilous, *F. mediterranea* has a much wider ecological amplitude and distribution. It is found in all parts of Norway (except the most northern parts) with its main occurrences in coastal areas. It is less hygrophilous and grows mainly on mature deciduous trees or sometimes on more or less calcareous rock walls. Only a few findings of *F. mediterranea* are recorded from *Picea* twigs in the spray zone of waterfalls. *Fuscopannaria ahlneri* has the most restricted distribution, and is almost totally confined to lowland, old-growth *Picea* forests of central Norway (Timdal 2011). The species belongs to an exclusive biogeographical element of boreal rainforests (Holien & Tønsberg 1996). It grows mostly on twigs of spruce, but also on *Sorbus aucuparia* and on cliffs, in extremely humid situations, especially at the bottom of ravines. *Parmeliella parvula* also grows mainly on twigs of spruce in old-growth *Picea* forests. It occurs in Norway along the west coast from Rogaland to Nordland (Timdal 2011). In contrast to *F. ahlneri*, *F. mediterranea* and *P. parvula*, the distribution of *F. confusa* in Norway is more continental.

There are now *c.* 43 records of *F. confusa* from Norway, of which 29 are included in our present molecular and modelling investigation. Most of these 43 records are from areas of natural *Picea*-forest in continental and eastern parts of south central Norway. We wanted to calculate the potential regional distribution of *F. confusa* in Norway through species distribution modelling. The Maxent habitat model for *F. confusa* (Fig. 2) suggests, when taking only bioclimatic variation into consideration, that the species may be found also in areas further north; that is, in areas outside the range of *Picea*. Indeed, recent observations of *F. confusa*, found after the modelling was performed (T. H. Hofton, S. Reiso & P. M. Jørgensen, pers. comm.), support the reliability of the habitat model, also in northern parts of Norway (Fig. 2). In addition, *F. confusa* seems to have its main distribution in the middle boreal zone, with quite a few localities in the northern boreal zone, while being rarer in the southern boreal zone. Although collections performed for this study have doubled the total number of findings recorded, we want to emphasize that *F. confusa* is rare, also in its preferred habitats. Our hope is that this study will guide our search, and conservation efforts, for this species. We anticipate new findings in the predicted habitats.

Finally, we wanted to re-evaluate, in light of the new data, the conservation status of *F. confusa* for the Norwegian red list. Hygrophilous lichen species connected to waterfalls in forest regions seem to be very sensitive to periodically low water flow, especially during warm periods of the summer. The drainage areas of rivers supporting populations of these

species tend to be rather large, and with a large percentage of mires and swamps, which act as water reservoirs preventing very low water flow during dry periods. Many rivers in Norway lack such properties and have periodically very low water flow. Thus, potentially suitable habitats for species like *F. confusa* are restricted. As the species is confined to very small forest patches in today's landscape, it is also sensitive to small-scale stochastic natural and human-induced events that destroy the trees and cause rapid changes in sunlight and wind exposure. In addition, a large number of suitable localities have been destroyed due to clear-cut logging and especially hydroelectric power development. Habitat loss is a common and very serious threat to lichens (Wolseley 1995; Scheidegger & Werth 2009). Due to changes in the landscape, it is likely that *F. confusa* has experienced a strong population decline since the 1890s, when the first large waterfalls were developed for hydroelectric power (http://www.riksantikvaren.no/?module=Articles;action=Article.publicShow;ID=3059).

Although the overall delimitation of *F. confusa* must take into consideration additional species and geographic regions, our results clearly show that *F. confusa* must be considered and managed as a distinct and threatened species in Norway; that is, endangered (EN; high risk of extinction in the wild) based on C1 (less than 2500 individuals and 20 % reduction/5 years) and D1 (50–250 reproducing individuals) criteria (Kålås *et al.* 2010). Continued mapping of *F. confusa* in boreal regions should be undertaken to assess its global status. *Fuscopannaria confusa* might be overlooked, but despite a potentially wide geographical distribution, it is likely to be threatened throughout its distribution area because it occupies a habitat in decline.

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Appendix

SUPPLEMENTARY FIGURE. A1. A, *CHS* (single most parsimonious tree); B, nrITS (single most parsimonious tree); C, mtSSU (one of the three most parsimonious trees). Generic names for *Fuscopannaria* (*F.*) and *Parmeliella* (*P.*) are abbreviated. Results from parsimony and Bayesian analyses were similar. Branch labels indicate posterior probabilities from Bayesian analysis/jackknife values from parsimony resampling. Reported are also best-fit models of nucleotide substitution, lengths of trees, and homoplasy and rescaled consistency indices (HI and RC, respectively). In the nrITS and *CHS* phylogenies, *F. confusa* comes out as a separate and well-supported group, while in the mtSSU phylogeny, *F. confusa* groups with *F. ahlneri, F. praetermissa* and *F. leucosticta.* The mtSSU marker is a slower evolving region, and is not expected to distinguish between closely related species. Overall, the results clearly show that *F. confusa* represents a species genetically distinct from *F. ahlneri, F. mediterranea* and *Parmeliella parvula.* In colour online.