Integrative taxonomy reveals a new species, *Nephroma orvoi*, in the *N. parile* species complex (lichenized Ascomycota)

EINAR TIMDAL, MARTIN WESTBERG, REIDAR HAUGAN, TOM H. HOFTON, HÅKON HOLIEN, JAMES D. M. SPEED, TOR TØNSBERG and MIKA BENDIKSBY

Timdal, E., Westberg, M., Haugan, R., Hofton, T. H., Holien, H., Speed, J. D. M., Tønsberg, T. & Bendiksby, M. 2020. Integrative taxonomy reveals a new species, *Nephroma orvoi*, in the *N. parile* species complex (lichenized Ascomycota). *Graphis Scripta* **32** (4): 70–85. Oslo. ISSN 2002-4495.

Our knowledge of the diversity and distribution of many groups of lichens remains poor due to unclear species boundaries and challenging species identification. We have studied the medium sized to large foliose lichen *Nephroma parile*, which is known to be heterogeneous in chemistry and genetics. Our aim has been to assess the potential presence of evolutionary significant units within the *Nephroma parile* species complex that may be worthy of recognition at species level. Using phylogenetic analysis of the fungal DNA-barcode marker (ITS) in combination with studies of morphology and chemistry, we discover two distinct lineages in the *N. parile* species complex. For the strongly supported clade that corresponds to chemotype II, we describe the new species *Nephroma orvoi*. The new species is known from Norway, Sweden, Finland, Switzerland, Canada (Alberta and British Columbia), USA (Washington), and Greenland.

Einar Timdal, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway. Email: einar.timdal@nhm.uio.no (corresponding author).
Martin Westberg, Museum of Evolution, Uppsala University, Norbyvägen 16, SE-752 36 Uppsala, Sweden. Email: martin.westberg@em.uu.se.
Reidar Haugan, Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, NO-0318 Oslo, Norway. Email: reidar.haugan@lichen.no.
Tom H. Hofton, BioFokus, Gaustadalléen 21, NO-0349 Oslo, Norway. Email: tom@biofokus.no.
Håkon Holien, Nord University, Faculty of Bioscience and Aquaculture, P.O.Box 2501, NO-7729 Steinkjer, Norway and Dept. of Natural History, NTNU University Museum, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway. Email: hakon.holien@nord.no.
James D.M. Speed, Dept. of Natural History, NTNU University Museum, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway. Email: james.speed@ntnu.no.
Tor Tønsberg, University Museum of Bergen, Department of Natural History, P.O.Box 7800, NO-5020 Bergen, Norway. Email: tor.tonsberg@uib.no.
Mika Bendiksby, Dept. of Natural History, NTNU University Museum, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway. Email: mika.bendiksby@ntnu.no.

Introduction

The identification of many lichen species can be challenging due to the lack of distinguishing morphological characters, and DNA-based studies have revealed extensive numbers of unrecognized species in lichenized fungi (e.g., Molina et al. 2011, Lücking et al. 2014, Bendiksby et al. 2015). The large foliose and widespread lichen species *Nephroma parile* (Ach.) Ach. (Nephromataceae, Peltigerales) comprises three chemotypes (James & White 1987) and eight

 Published 2020-10-11 by The Nordic Lichen Society, http://nhm2.uio.no/lichens/NLS.

genotypes (Fedrowitz et al. 2012), and may represent a species complex. *Nephroma parile* s. lat. is a temperate to arctic-alpine species that is widespread and circumpolar, occurring throughout the Northern Hemisphere and in southern South America (James & White 1987, Vitikainen 2007). The genus *Nephroma* comprises abom ut 36 species (Lücking et al. 2017), of which several are restricted to pristine, old growth forests. These species are important indicator species for areas with rich biodiversity and high conservation value. As long as the species level remains the basic unit for natural resource and biodiversity management, species delimitation remains an important endeavour. This study is a further contribution into the crucial work of discovering, delimiting and describing species.

In a study of the genus *Nephroma* in Europe and Macaronesia, James & White (1987) found six major hopane triterpenoids, as identified by thin-layer chromatography (TLC), relevant to the taxonomy of the genus: **T1** (7*β*-acetoxyhopan-22-ol; peltidactylin), **T2** (15*α*-acetoxyhopan-22-ol; dolichorrhizin), **T3** (hopane-6*α*,22-diol; zeorin), **T4** (hopane-7*β*,22-diol), **T5** (hopane-15*α*,22-diol), and **T6** (hopane-6*α*,7*β*,22-triol). The six compounds occur in various, more or less species-specific permutations in most species of the genus. In the sorediate species *N. parile*, three chemotypes ('races') were recognized: Race 1 and 2 containing T2, T3, and T5, and race 3 containing T1, T3, T4, and T6. The distinction between race 1 and 2 was based on additional minor compounds. Race 1 is the widely distributed chemotype in the Northern Hemisphere, race 2 occurs in southern South America, and race 3 was known to James & White (1987) from a few collections from Switzerland, Greenland, and Canada (Alberta and British Columbia). The lectotype (H-ACH 1468B) was found to belong to race 1. James & White (1987) discussed possible correlations between chemotype and morphotype (faveolation on the upper surface, corticate soredia, subtomentose lower surface), but concluded that only one species containing three chemotypes should be recognized 'at present'.

A morphologically rather similar species, *N. isidiosum* (Nyl.) Gyeln., differs morphologically mainly in forming terete or coralloid isidia. It contains terpenoids T4 (major and constant), T1 (sometimes absent or trace only), methylgyrophorate (constant), gyrophoric acid (sometimes absent or trace only), and pigments (James & White 1987). The species is not closely related to *N. parile*, however, as it belongs in the *N. helveticum* Ach. complex (Piercey-Normore et al. 2006, Wang et al. 2013).

In the Nordic Lichen Flora, Vol. 3, Vitikainen (2007), reported two chemotypes of *N. parile* in the area and referred to them as chemotypes I and II. The former corresponds to race 1 sensu James & White (1987) and is the common chemotype. The latter corresponds to race 3 and was said to occur in Northern Norway and Finland (no locality or specimen cited). Vitikainen (2007) stated that the taxonomic status of chemotype II should be further studied as it is characterized by soredia tending to be dark, corticate, and concentrated to ridges of the reticulate upper surface, and by the lower surface being covered by short blackish tomentum.

Fedrowitz et al. (2012) studied symbiont selectivity in *Nephroma* on a global scale. Among the 60 ITS sequences of *N. parile* presented, they recognized eight genotypes, named NP1–NP8 (Fedrowitz et al. 2012, Appendix S1). No phylogeny of the genotypes, information on the genetic distances between them, or data on secondary chemistry were given.

At a workshop in Varanger, North Norway, in 2014, partly for collecting lichen species for a reference database of DNA barcode sequences (the OLICH project; Marthinsen et al. 2019), one of us (TT) collected a *Nephroma parile* s. lat. which turned out to differ genetically from other sequences of the species produced by OLICH. Subsequent examination showed the specimen belonged in chemotype II and genotype NP7. This correlation prompted us to search for other specimens of chemotype II in fungarium material, mainly in BG, O, TRH, and UPS, and to further sequence *N. parile* under the OLICH project. Independently, one of us (MW), had sequenced

Nephroma specimens in order to investigate morphologically deviating specimens of *N. parile* in northern Sweden. Also independently, one of us (THH) collected candidates for rare *Nephroma* species in Norway, especially *N. helveticum* and *N. isidiosum* (ca. 20 collections, mostly during fieldwork for action plan for *Heterodermia speciosa* [Hofton 2020]), which resulted in material relevant for this study. We merged our results and report on the findings here, which includes the description of a new species, *Nephroma orvoi*.

Material and Methods

The specimens were studied morphologically using dissecting microscopes. Thin-layer chromatography (TLC) was performed on 337 collections of *N. parile* housed, or to be housed, in the following fungaria: BG (86), LD (6), O (187), S (1), TRH (34), TROM (1), UPS (19), and WTU (3). Nordic specimens morphologically fitting the description of chemotype II by Vitikainen (2007) were given priority. TLC was performed in accordance with the methods of Culberson (1972) and Menlove (1974), using solvent system C. Most were analysed on aluminium plates, and glass plates were used occasionally.

All steps from DNA extractions and PCR amplification through sequencing and editing of the 20 OLICH sequences produced for this study (MT943600–MT943619) were performed at the Canadian Centre for DNA Barcoding (CCDB; http://www.ccdb.ca), using the primer pair ITS5/ITS4 (White et al. 1990). Eight sequences (MT940894–MT940901) were produced in our own labs; the seven Swedish sequences following the methods of Wedin et al. (2009), using the primer pair ITS1f/LR3, and one specimen (MT940900) following the procedure of Bendiksby et al. (2015) and the primers ITS5/ITS4. All ITS sequences of *Nephroma parile* in GenBank (78) were downloaded on 2020-05-15. Three additional sequences of *N. areolatum* P. James & F.J. White, *N. hensseniae* P. James & F.J. White, and *N. sulcatum* P. James & F.J. White were downloaded to function as outgroup, selected based on the Macaronesian *Nephroma* phylogeny of Sérusiaux et al. (2011).

A total of 109 sequences were preliminary aligned in BioEdit (Hall 1999) using its bundled software ClustalW (Thompson et al. 1994). The alignment was manually adjusted and ends were trimmed. In order to ease the analysis and presentation, any duplicate (i.e., identical) GenBank sequences from the same country was removed from the dataset unless it represented a specimen with known secondary chemistry. We analyzed the data phylogenetically using Bayesian, likelihood, and parsimony methods as described in Bendiksby et al. (2015).

We used ecological niche modelling to assess the climate niche of *Nephroma orvoi* in Fennoscandia. As occurrence data we used all examined specimens except the one from North America. As independent climate data we used bioclimate variables downloaded from WorldClim (Fick & Hijmans 2017). Three climate variables were selected to represent main axes of Fennoscandian climate variations. These were mean temperature of the warmest quarter, annual precipitation, and precipitation seasonality (Speed & Austrheim 2017). To account for spatial bias in the distribution of occurrence records we used 1000 randomly selected GBIF records of the Lecanoromycetes from Norway and Sweden with a coordinate precision of < 1000 m (GBIF.org 2020). We used an ensemble modelling approach using general linear model, general additive model and random forest methods. These were selected as a range of approaches that avoid overcomplexity (Guisan et al. 2017). Five-fold cross validation was carried out to evaluate the models, and ensemble modelling was weighted on the basis of each model's AUC (area under curve) statistic. The mean AUC was 0.80 indicating good performance. The model predictions were extrapolated over the whole of Fennoscandia.

Table 1. Specimens used in the phylogenetic reconstruction in this study, with ID as in Fig. 2, current species identification, genotype (GT) according to Fedrowitz et al. (2012), chemotype (CT), collection information, and GenBank ID. GenBank IDs in bold mark sequences produced for this study.

ID	Species	GT	CT	Country, province	Collection	GenBank ID
1	*N. orvoi*	NP7		Finland, Enontekiön Lappi	Mt Saana; Rikkinen JR08S1R	JN857282
2	*N. orvoi*		II	Norway, Sør-Trøndelag	Oppdal, Dovrefjell, Stølådalen; Sørensen 4615 (O L-48419)	**MT940900**
3	*N. orvoi*		II	Norway, Finnmark	Båtsfjord, Skogdalen; Tønsberg 43619 (O L-195875)	KY266927
4	*N. orvoi*		II	Norway, Finnmark	Sør-Varanger, Kiltjønnan; Hofton 17290 (O L-225689)	**MT943616**
5	*N. orvoi*		II	Norway, Nordland	Grane, Litlelva; Hofton 16027 (O L-225687)	**MT943603**
6	*N. orvoi*		II	Norway, Nord-Trøndelag	Lierne, Dalaberget; Hofton 14140 (O L-196490)	**MT943601**
7	*N. orvoi*		II	Norway, Oppland	Vågå, Veogjelet; Hofton 17159 (O L-225688)	**MT943617**
8	*N. orvoi*		II	Norway, Sør-Trøndelag	Oppdal, Vårstigen; Hofton 17408 (O L-225690)	**MT943608**
9	*N. orvoi*		II	Norway, Sør-Trøndelag	Oppdal, Drivstusætra; Hofton 17411 (O L-225691)	**MT943612**
10	*N. orvoi*		II	Sweden, Lule lappmark	Jokkmokk, Padjelanta, Allak; Berglund (hb Berglund)	**MT940896**
11	*N. orvoi*		II	Sweden, Lule lappmark	Jokkmokk, Padjelanta, Staloluokta, Westberg s.n. (LD)	**MT940897**
12	*N. orvoi*		II	Sweden, Lycksele lappmark	Tärna, Mt Gierevaartoe; Westberg 2770 (LD)	**MT940898**
13	*N. orvoi*			Sweden, Pite lappmark	Arjeplog, Skärrimvägge; Odelvik 06388 (S F60937)	**MT940894**
14	*N. orvoi*		II	Sweden, Torne lappmark	Karesuando, Pältsan; Westberg P124 (S F283561)	**MT940901**
15	*N. parile*	NP6		Canada, British Columbia	3051 UBC	HQ455101
16	*N. parile*	NP5		Canada, British Columbia	09-02 UBC	HQ455102
17	*N. parile*			Canada, British Columbia	Spahats Creek	KC437594
18	*N. parile*			Canada, British Columbia	17 km NE of Smithers; Rui & Timdal 18115 (O L-223759)	**MT943605**
19	*N. parile*	NP6		Canada	Canada E; Ahti 60210	AY124148
20	*N. parile*	NP1		Canada, Quebec	Coffey & Freebury KU1003	JN857254
21	*N. parile*	NP5		Canada	Canada SW; Vitikainen 13242	AY124145
22	*N. parile*			China, Inner Mongolia	20124452B	JX867674

23	*N. parile*	NP1		Finland	Oksane VI 2 2	AY124149
24	*N. parile*	NP1		Finland	KF142	HM448779
25	*N. parile*	NP1		Finland	KF172	HM448780
26	*N. parile*	NP2		Finland	Rikkinen JR08J4D	JN857275
27	*N. parile*	NP3		Finland	Rikkinen JR08S1P	JN857276
28	*N. parile*	NP1		Great Britain, Scotland	Fedrowitz KUS3	JN857263
29	*N. parile*	NP1		Greenland	Hansen 22 June 1998	AY124147
30	*N. parile*	NP1		Japan	Thor GT23856	JN857253
31	*N. parile*	NP4		Japan	Frisch 10/Jp3	JN857277
32	*N. parile*			Japan	YO8948	KJ150376
33	*N. parile*			Japan	KFJp13B	KJ150377
34	*N. parile*	NP6		South Korea	Hur 030384	DQ066708
35	*N. parile*	NP1		Norway	Kaasalainen U371	JN857261
36	*N. parile*	NP6		Norway	Kaasalainen U372	JN857280
37	*N. parile*		I	Norway, Buskerud	Rollag, Bjønnhølfjellet; Hofton 17215 (O L-225692)	**MT943600**
38	*N. parile*		I	Norway, Nordland	Leirfjord, Svartdalsvatnet; Løfall L11267 (O L-147913)	**MT943618**
39	*N. parile*		I	Norway, Nordland	Træna, Sanna; Løfall L10783 (O L-130855)	**MT943604**
40	*N. parile*		I	Norway, Nordland	Vefsn, Vikdalen; Klepsland JK06-L202 (O L-168625)	**MT943611**
41	*N. parile*		I	Norway, Nordland	Sømna, Vik, Sømna kirke; Løfall L10841 (O L-130913)	**MT943614**
42	*N. parile*		I	Norway, Nord-Trøndelag	Meråker, Langneset; Bratli 7758 (O L-168087)	**MT943615**
43	*N. parile*		I	Norway, Nord-Trøndelag	Steinkjer, Hatlinghus; Bratli & Holien 7773 (O L-168105)	**MT943602**
44	*N. parile*		I	Norway, Rogaland	Sauda, øvre Molla; Oddane (O L-182090)	**MT943607**
45	*N. parile*		I	Norway, Sogn og Fjordane	Luster, Fortunsdalen; Bøthun (O L-184059)	**MT943619**
46	*N. parile*		I	Norway, Sør-Trøndelag	Ørland, Storfosna; Haugan WG3-0157 (O L-196031)	**MT943609**
47	*N. parile*		I	Norway, Telemark	Seljord, Åmotsdal; Rui & Timdal 13725 (O L-200852)	**MT943610**
48	*N. parile*		I	Norway, Troms	Bardu, N for Setermoen; Arnesen (TROM L-1340014)	**MT943606**
49	*N. parile*	NP1		Sweden	Frisch AF1/npa	JN857270
50	*N. parile*	NP6		Sweden	Fedrowitz KU499	JN857281
51	*N. parile*		I	Sweden, Södermanland	Eknäset; Bohman (O L-200864)	**MT943613**
52	*N. parile*		I	Sweden, Torne lappmark	Westberg 3161 (LD)	**MT940899**

53	*N. parile*		Sweden, Åsele lappmark	Odelvik 4225 (S L62511)	**MT940895**
54	*N. parile*	NP5	USA, Montana	615 LG 216	HQ455099
55	*N. parile*		USA, Oregon	AFTOL 131	HQ650698
56	*N. areolatum*		Madeira	Sérusiaux s.n. (LG DNA 34)	HQ455057
57	*N. hensseniae*		Azores, Pico	Sérusiaux s.n. (LG DNA 278)	HQ455075
58	*N. sulcatum*		Canary Islands, La Palma	1985-01-20, Rajalin	AY124146

Results

The results of the morphological studies corroborate the observations by James & White (1987) and Vitikainen (2007) that the two chemotypes show minor variation in faveolation on the upper surface, development of cortex in the soredia, and in development of tomentum on the lower surface. The observations are summarized under *Notes*, in the Taxonomy section.

Among the 337 specimens examined by TLC, we found 57 of chemotype II. The remaining were of chemotype I (except for a few specimens of other chemotypes, which, by closer inspection, were found to belong to other *Nephroma* species). The two chemotypes were easily identified in standard solvent system C (Fig. 1), and we found no need for solvent system G, which was preferred by James & White (1987) for a wider set of *Nephroma* taxa.

In total, we generated 28 new DNA sequences for the present study. Among the 78 downloaded GenBank sequences, a total of 50 were duplicates and excluded. We also excluded GenBank sequence JN857283, due to likely contamination (i.e., the singleton representing genotype NP8 in Fedrowitz et al. 2012). Hence, the final DNA sequence alignment was reduced to 58 sequences (Table 1), which included three of outgroup and 55 *N. parile* accessions. These 55 accessions included seven of the eight genotypes in Fedrowitz et al. (2012; i.e., 21 DNA sequences), six GenBank sequences with unknown genotype, and our own 28 sequenced DNA samples (GenBank accession numbers MT940894–MT940901, MT943600–MT943619). The alignment was 548 basepairs long and the ingroup contained 17 parsimony informative characters. The General Time Reversible model of nucleotide substitution with gamma distribution and invariable sites (GTR+G+I) was the estimated best fit model. Resultant phylogenetic hypotheses from the Bayesian, likelihood, and parsimony analyses were congruent and consisted of two strongly supported sister clades (Fig. 2: the Bayesian MCC with branch support from all analyses), which are here named *N. parile* (41 sequences; PP=1, BS=100, JK=99) and *N. orvoi* (14 sequences, PP=0.84, BS=100, JK=94). There is also genetic variation within each of the two main clades, but this variation receives less support, is short branched, and with the available data, paraphyletic. All specimens of chemotype I (blue) occur in the *N. parile* clade, and all of chemotype II (orange) in the *N. orvoi* clade (Fig. 2).

The specimens of chemotype II were collected exclusively in boreal to arctic-alpine habitats (Fig. 4), whereas those of chemotype I also occurred in nemoral and hemiboreal (boreonemoral) habitats in Norway and Sweden.

The climate niche of *N. orvoi* was mainly determined by temperature of the warmest quarter (variable importance mean ± standard error; 0.81 ± 0.01). Annual precipitation and precipitation

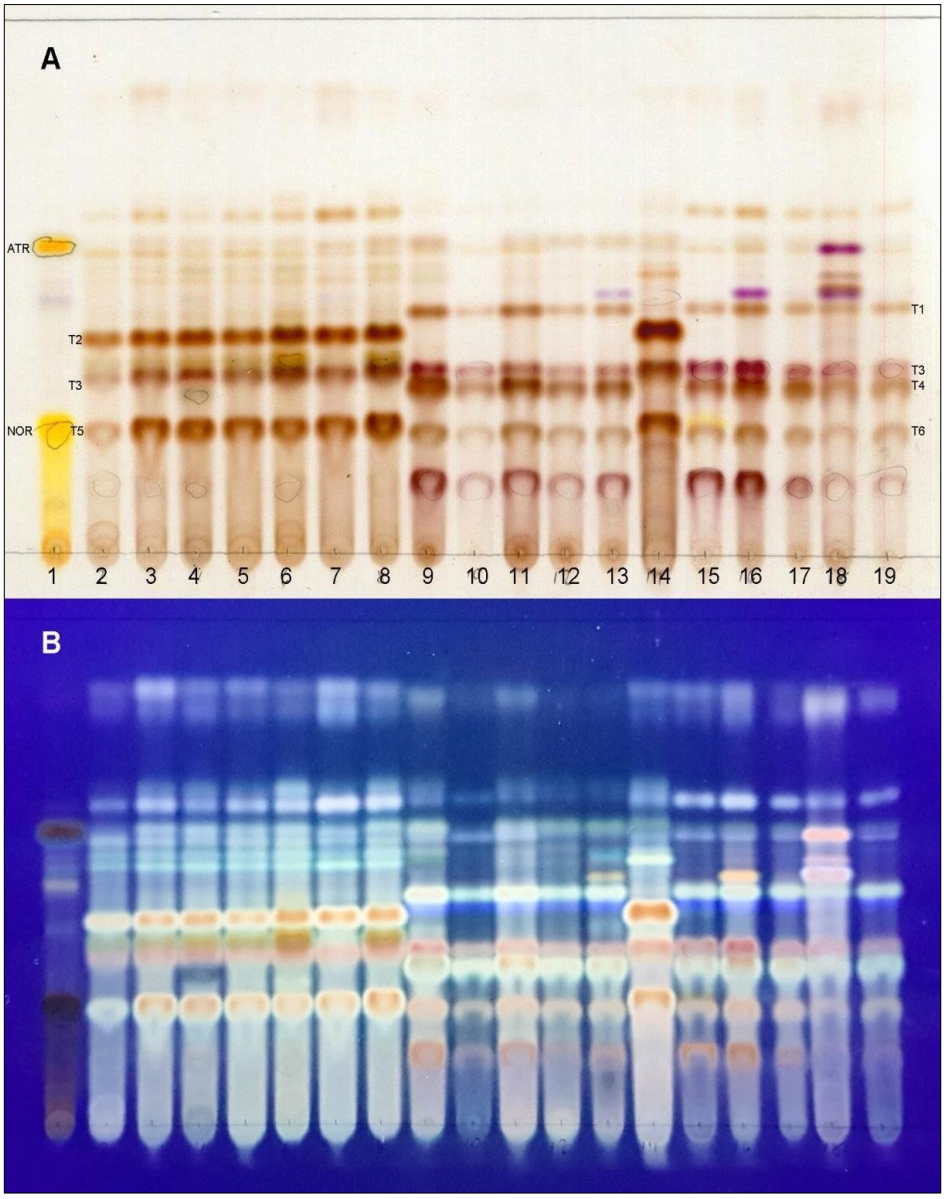

Figure 1. Results from thin-layer chromatography of 18 samples of *Nephroma parile* s. lat. Chromatogram in solvent system C shown in visible light (A) and in UV 366 nm (B), both after treatment with sulphuric acid and heat. Lane 1: references, atranorin (ATR) and norstictic acid (NOR); lanes 2–8 and 14: *Nephroma parile* s. str.; lanes 9–13 and 15–19: *N. orvoi*. The triterpenoid codes, T1–T6, are according to James & White (1987). Lane 2: O L-168105; lane 3: O L-200852; lane 4: O L-196031; lane 5: O L-168625; lane 6: O L-168087; lane 7: O L-225401; lane 8: O L-201429; lane 9: O L-48420; lane 10: O L-196490; lane 11: O L-42011; lane 12: O L-42035; lane 13: O L-42097; lane 14: O L-225692; lane 15: O L-225688; lane 16: O L-225690; lane 17: O L-225691; lane 18: O L-225687; lane 19: O L-225689.

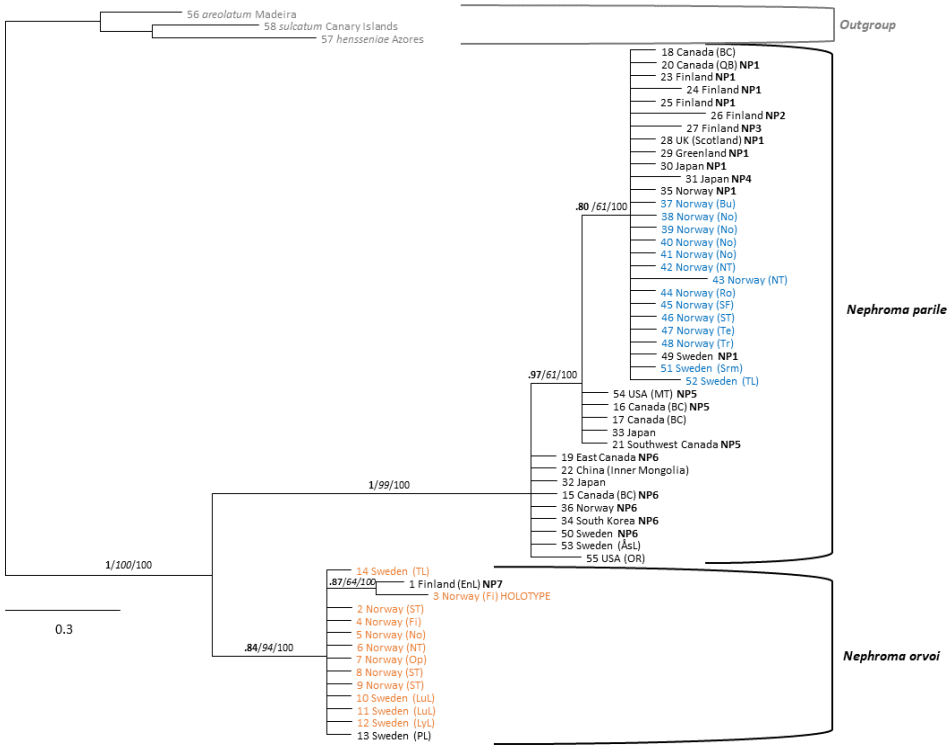

Figure 2. Phylogenetic hypothesis based on a Bayesian analyses of 55 aligned DNA sequences from the *Nephroma parile* species complex, with *N. areolatum*, *N. hensseniae*, and *N. sulcatum* used as outgroup. Support values are reported as posterior probabilities (PP) / parsimony jackknifing (JK) / bootstrap (BS) above branches. The sequence numbers refer to those in Table 1, and the NP1–NP7 denotation refers to the *N. parile* genotypes of Fedrowitz et al. (2012). Blue terminals: chemotype I; orange terminals: chemotype II; black terminals: unknown chemotype.

seasonality were less important (variable importance 0.13 ± 0.02 and 0.15 ± 0.02 respectively). Climate niche suitability decreased with temperature of the warmest quarter and increased with precipitation seasonality (Fig. 5). Model predictions across Fennoscandia show regions of high climate suitability for *N. orvoi* in the northern parts of Fennoscandia, and in the Scandes mountain chain (Fig. 6). Southern and lowland regions have low suitability.

Discussion

As part of our ongoing work of discovering, delimiting, DNA barcoding, and describing lichen species, we have performed this synergistic, integrative taxonomic study of the chemotypically and genetically heterogeneous species complex that *N. parile* s. lat. for some time has been known to represent (e.g., James & White 1987, Fedrowitz et al. 2012). Our aim has been to assess the potential

presence of multiple evolutionary significant units within this medium to large foliose lichen that may deserve recognition at species level. Our phylogenetic results (Fig. 2), based on the fungal DNA-barcode marker (ITS) in combination with studies of morphology and chemistry, reveal two distinct (long-branched and strongly supported) genetic lineages in the *N. parile* species complex. These two main clades seem to correlate with two known chemotypes (I and II, respectively). Based on an integrated evaluation of the following three aspects, we conclude that *N. parile* s. lat. consist of at least two evolutionary significant units that deserve recognition at species level: (1) phylogenetic support (*N. parile* PP=1, JK=99, and *N. orvoi* PP=0.84, JK=94), (2) the correlation between clades and chemotypes, and (3) the morphological and geographical trends distinguishing the chemotypes (James & White 1987, Vitikainen 2007, supported by our observations). The only currently regarded synonym of *N. parile* at species rank known to us is *N. subparile* Gyeln. (Wetmore 1960). In the protologue, the distinguishing feature given is a glabrous, not pubescent, lower surface (Gyelnik 1930). This does not fit the morphology of chemotype II. As no other name seems to be available, we hereby describe the strongly supported clade that corresponds to chemotype II as the new species *N. orvoi.* All 57 specimens of chemotype II were identified as *N. orvoi* and are listed under *Specimens examined*, and those of chemotype I were identified as *N. parile* (not shown, except for those that were sequenced; Table 1). The new species is so far known from Norway, Sweden, Finland, Switzerland, Canada (Alberta and British Columbia), USA (Washington), and Greenland.

For the genus *Nephroma*, as for most other ascomycete taxa, the fungal DNA-barcode marker (ITS) seems to efficiently distinguish taxa at what we like to consider as an appropriate level for species recognition, corroborating statements by Lohtander et al (2002) and Fedrowitz et al. (2012).

Taxonomy

Nephroma orvoi **Timdal, M. Westb., Haugan, Hofton, Holien, Speed, Tønsberg & Bendiksby, sp. nov.** Fig. 3

Mycobank: MB 836813.

Diagnosis: Differs from *N. parile* in having more faveolate, wrinkle-ridged lobes, soralia concentrated on these ridges, more persistently corticate soredia, a darker lower surface often with a thicker pseudotomentum, and in containing triterpenoids T1, T3, T4 (major), and T6.

Type: Norway, Finnmark, Båtsfjord: the top of the valley Skogdalen, 70°32.18'N, 29°41.59'E (WGS84), 237 m alt., 2014-07-02, Tor Tønsberg 43619 (=WG2-0049) (O L-195875, holotype [DNA: KY266927, TLC: terpenoids T1, T3, T4 (major), and T6]; BG L-104044, isotype).

Description: Thallus 4–10 (–13) cm diam.; lobes up to 1 (–1.5) cm wide; upper surface smooth to distinctly faveolate especially near the lobe ends, medium brown to dark brown or partly olivaceous brown, with mostly laminal, more rarely marginal, soralia which are often concentrated on the ridges; soredia granular, long remaining corticate, sometimes forming coralloid clusters; margin entire; medulla white; lower side medium brown near the lobe ends, darker brown to blackish brown in inner part, smooth, naked or pseudotomentose near the lobe ends, pseudotomentose in middle and inner part. Apothecia and conidiomata not seen. Photobiont *Nostoc*.

Chemistry: A series of triterpenoids, mainly compounds T1 (7*β*-acetoxyhopan-22-ol; peltidactylin), T3 (hopane-6*α*,22-diol; zeorin), T4 (hopane-7*β*,22-diol, the major compound), and T6 (hopane-6*α*,7*β*,22-triol), with traces of additional unknown terpenoids.

Figure 3. *Nephroma orvoi*. Holotype (O L-195875). **A**: habitus; **B**: faveolate lobe and corticate soredia; **C**: pseudotomentose lower surface. Scale: A = 5 mm, B = 2 mm, C = 2 mm.

Etymology: The species is named in honour of Dr Orvo Vitikainen on the occasion of his 80th birthday, in appreciation of his significant contribution to the taxonomy of the Peltigerales.

Habitat and distribution: The species grows on rock, shrubs, and tree trunks. Recorded phorophytes are *Alnus incana*, *Juniperus communis*, *Salix caprea*, *S.* sp. (alpine willow), and *Sorbus aucuparia.* The sites include subalpine birch forest, subalpine pine forest, boreal spruce-dominated forest, boreal rainforest (rarely), and alpine habitats up to 1400 m altitude in the Nordic countries (1900 m in USA, Washington). Most finds are from large boulders and small rock walls, clearly preferring

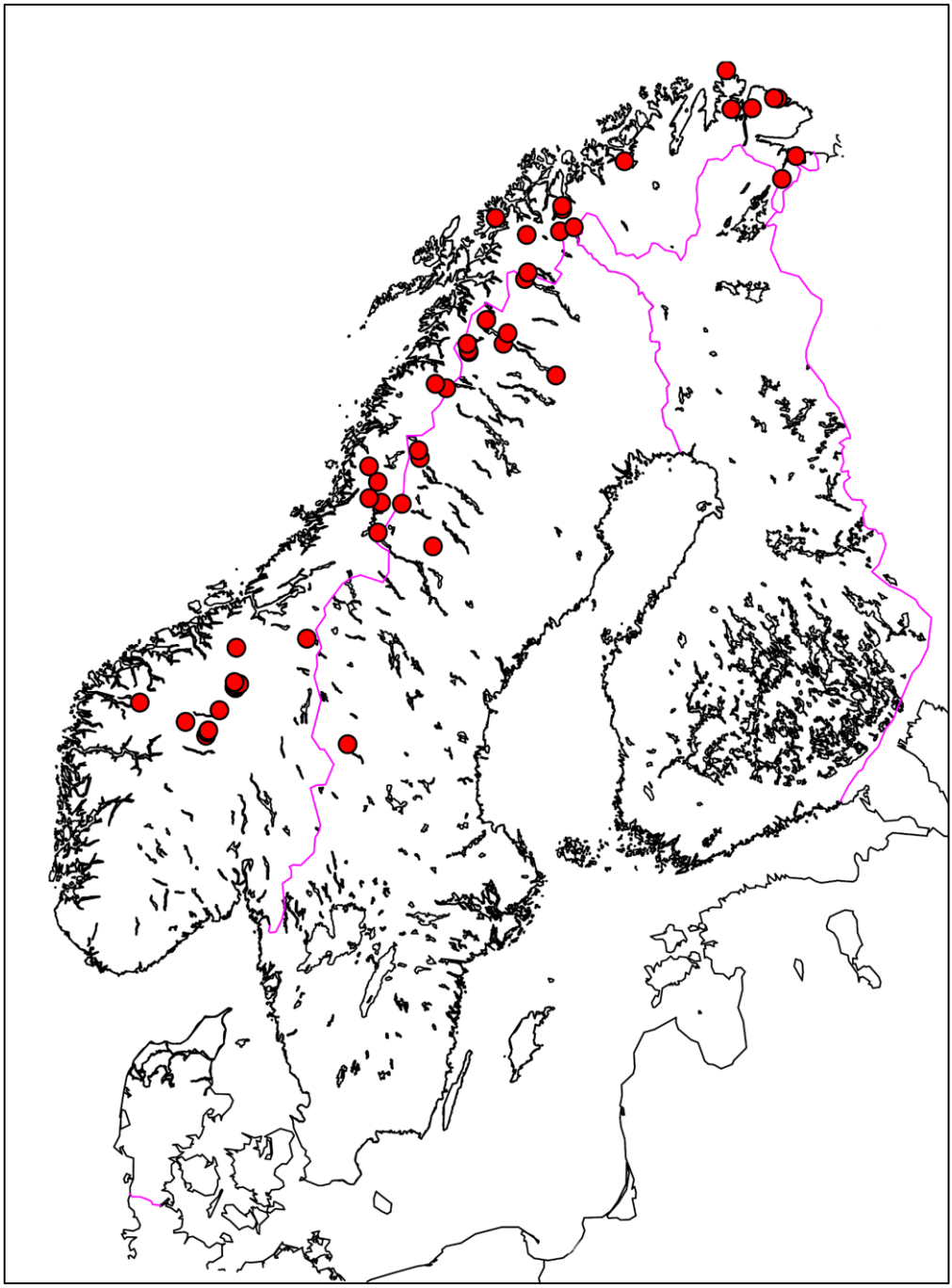

Figure 4. The distribution of *Nephroma orvoi* in the Nordic countries based on the examined specimens and GenBank ID JN857282.

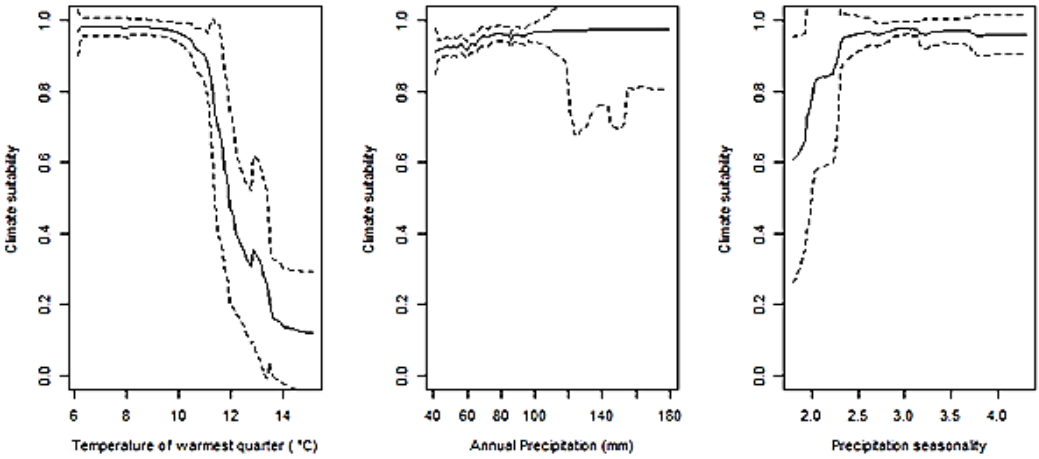

Figure 5. Response curves of *Nephroma orvoi* climate suitability along the three climatic variables. Mean and standard deviations across model methods and replicates shown as solid and dashed lines respectively.

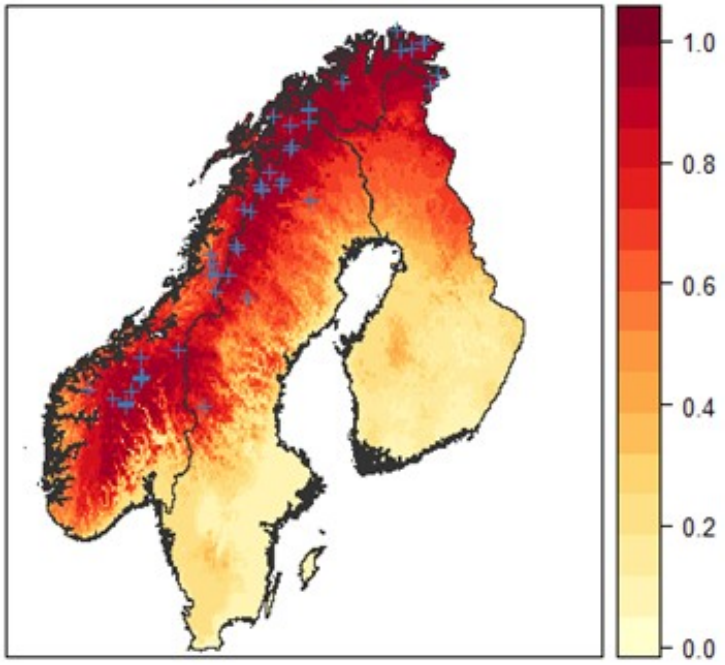

Figure 6. Climate suitability of *Nephroma orvoi* predicted across Fennoscandia. Red areas show regions with higher climatic suitability, while yellow areas have lower suitability. Mean predictions are shown across modelling methods and replicates. Species occurrence records used in the modelling are shown as blue crosses.

medium to rather strongly base-rich (but structurally hard) rocks, and it seems to belong to the Lobarion-community (along with, e.g., other *Nephroma* species, *Lobaria scrobiculata*, and *Physconia* spp.). Most finds are from well-lit 'semi-sheltered', more or less old-growth or semi-natural forest, but the species does not seem to be especially sensitive to forestry as long as sufficient forest cover remains after logging and other activities. Many finds, especially in the southern end of its range (inner south-east Norway) are from deep valleys, river canyons or other microclimatically rather sheltered sites, while in northern Scandinavia it seems to tolerate more open (alpine) situations. In the Nordic countries, it is clearly concentrated to northern boreal, continental and semi-continental forests. Its distribution is northern boreal-subalpine with a northeastern boreo-continental tendency. It seems to be absent from the lowlands (nemoral, hemiboreal, southern boreal), and also seems to avoid oceanic areas (Fig. 4).

We have examined one extra-Nordic specimen, from USA (Washington), see specimens listed below. In addition to the specimens examined by us, the species is reported from Switzerland, Greenland, and Canada (Alberta and British Columbia) (James & White 1987, as *N. parile* race 3) and from Finland (Vitikainen 2007, as *N. parile* chemotype II, and Fedrowitz et al. 2012, as *Nephroma parile* genotype NP7, GenBank ID JN857282, specimen 1 in our Fig. 2).

Notes: The species is morphologically very similar to *N. parile*, and the diagnostic morphological differences may not always lead to definite identification. *Nephroma orvoi* always has a distinct pseudotomentum, brown, corticate soredia and faveolate lobe ends (often it is also darker brown in its color). *Nephroma parile* shows variation in all these characters which are quantitative rather than qualitative; the lower side may be glabrous to pseudotomentose, the lobe ends are smooth to somewhat faveolate and the soredia are often bluish and 'soft' or brown and appearing corticated (probably in exposed situations) to various degrees, but it rarely (or never?) show all three characters at the same time. Some specimens, however, cannot be diagnosed without TLC.

Nephroma isidiosum forms more cylindrical dispersal units, isidia, but a clear distinction between those and the corticate soredia of *N. orvoi* is sometimes difficult. It also differs in forming a more distinct (longer) tomentum than the short pseudotomentum of *N. orvoi*. *Nephroma isidiosum* belongs in the *N. helveticum* Ach. complex (Piercey-Normore et al. 2006, Wang et al. 2013), however, and may be identified by the presence of the depsides methyl gyrophorate (constant) and gyrophoric acid (occasional) (James & White 1987). The terpenoids occurring in *N. isidiosum* are T4 (major) and T1 (minor to trace), like in *N. orvoi*, but T3 and T6 are absent (James & White 1987).

Additional specimens examined (paratypes): **Norway**. *Oppland*: Dovre, Jønndalsberget NØ, 61.95377°N, 9.24071°E, alt. 755 m, bjørkedominert lauvskog i Ø-vendt bratt li, lågurt-høgstaudeskog, gammelskog, på berg, 2018-09-11, T.H. Hofton 18147 (O L-227129); Lom, Skamsdal, [32V MP 606–614,431–437, alt. 700–800 m], 1924-07-21, S. Sørensen (O L-47845); Vågå, Veogjelet, 61.64892°N, 8.98977°E, alt. 919 m, furu-bjørk blandskog i grunn bekkekløft, lågurtskog, åpen naturskog, på berg, 2017-09-05, T.H. Hofton 17159 (O L-225688); Vågå, Sjoa nedenfor Griningsdalsbrua, V-side, 61.57128°N, 8.94030°E, alt. 891 m, furuskog langs elv, naturskog, på halvrikt berg, 2018-08-20, T.H. Hofton 18081 (O L-227131); Vågå, Hindseterkampen, 61.62167°N, 8.96949°E, alt. 944 m, furuskog i S-vendt li, bærlyng-lågurtskog, gammelskog, på stor steinblokk, 2018-09-22, T.H. Hofton 18234 (O L-227130; *Sogn og Fjordane*: Stryn, uphill from Oppheim, E of trail to Raksætra/Oppheimssætra, 61°53.009'N 6°47.220'E, alt. 406 m, corticolous on trunk of *Sorbus aucuparia*, 2007-09-05, T. Tønsberg, 38647 (BG L-104047); *Sør-Trøndelag*: Oppdal, Dovre, Vårstien, [32V NQ 322–334,114–167], blant mose på stein, 1928-08-25, K. Fægri (BG L-46326); Oppdal, in alp. Dovrens., Kongsvoll, [32V NQ 305–321,075–093, alt. 900–1000 m], 1934-07, E.P. Vrang (O L-48437, TRH L-20976); Oppdal, Dovrefjell nasjonalpark, ved Blesebekken i øst-området, 32V NQ 33,07, alt. 1250 m, på bakken, 1978-07-04, A.J. Sørensen 1638 (O L-48420); Oppdal, Dovrefjell nasjonalpark, stor fuglestein N for Haugtjørnin i øst-området, 32V NQ 38,14, alt. 1170 m, på humusdekket stein, 1977-07-10, A.J. Sørensen 5923 (O L-48417);

Oppdal, Dovrefjell nasjonalpark, ved Stølåa i vest-området, 32V NQ 30,16, alt. 1070 m, på humusdekket stein, 1977-07-15, A.J. Sørensen 4535 (O L-48411); Oppdal, Dovrefjell nasjonalpark, S-vendt skråning i Stølådalen i vest-området, 32V NQ 30,16, alt. 1400 m, på stein, 1977-07-21, A.J. Sørensen, 4615 (O L-48419); Oppdal, Vårstigen S, 62.33963°N, 9.62258°E, alt. 901 m, bjørkeskog, svak lågurtskog, halvgammel åpen skog, på rik steinblokk, 2017-10-20, T.H. Hofton 17408 (O L-225690); Oppdal, Vårstigen, Drivstusætra S, 62.34831°N, 9.63343°E, alt. 895 m, bjørkeskog, høgstaudeskog, relativt gammel skog, på stor, relativt rik steinblokk, 2017-10-20, T.H. Hofton 17411 (O L-225691); Rennebu, Jøldalshytta, [c. 62.863°N, 9.509°E], alt. 750-800 m, 1931-07, O.A. Høeg (TRH L-20958 & TRH L-20961); Tydal, Gammelvollsjøen, Røttesåsen, Ø for tjønn 630, 32V PR 365,003, alt. c. 630 m, blåbær-småbregnegranskog, på selje [*Salix caprea*], 1996-08-23, T. Prestø (TRH L-30934); *Nord-Trøndelag*: Lierne, Dalaberget, 64.68580°N, 13.75250°E, alt. 456 m, sørberg, bratt og rik lågurt-blandskog under fjellvegg, på grov, gammel selje [*Salix caprea*], 2014-09-05, T.H. Hofton 14140 (O L-196490); Røyrvik, Børgefjell National Park, Tønnefjellet, 33W VN 43,21, alt. 859–900 m, on *Salix* sp. (alpine willow), 1974-07-10, T. Tønsberg 177 (TRH L-27724); *Nordland*: Grane, Fiplingdalen, along and W of Vestfiplingdalsvegen, between Strandli and Fiplingkroken, 65.40029°N 13.63377°E, alt. 393 m, corticolous on *Sorbus aucuparia*, 2014-08-19, T. Tønsberg 44527 (BG L-104046); Grane, Majavatn, the W-facing slope of Litlfjellet, 65°09.71'N, 13°22.64'E, corticolous on *Salix caprea* in old-growth *Picea abies* forest, 2016-08-10, T. Tønsberg 46694 (BG L-101538); Grane, Litlelva, 65.61121°N, 13.29248°E, alt. 75 m, boreal gran-regnskog langs småelv, sumpskog, på gammel gråor [*Alnus incana*], 2016-08-11, T.H. Hofton 16027 (O L-225687); Saltdal, Solvågtind ovan Solvågli, sälg [*Salix caprea*] i reg. subalp., 1937-08-05, G. Degelius (O L-42011); *Troms*: Målselv, Bjørnstad i Kirkedalen, [alt. 60–200 m], 1911-05-30, B. Lynge (O L-228202); Storfjord, Lulle, [34W DB 75–77,87–89], 1911-06-23, B. Lynge (BG L-44352, O L-42035); Storfjord, Skibotndalen, SW-facing slope E of the fieldstation, 34W DB 75–76,93, alt. 140–220 m, on boulder in birch forest, 2003-08-05, H. Holien 9537 (TRH L-9787); Tranøy, Senja, SSE of Vesterfjell, along and E of road 860, W of lake Storvatnet, W facing slope of hill 220, 33W XS 0634,7865, corticolous on trunk of mature *Salix caprea* in *Betula pubescens* forest, 2010-07-14, T. Tønsberg 40252 (BG L-89257); *Finnmark*: Alta, Sakkobadne i Kåfjord, [34W EC 780–801,577–610, alt. 100–350 m], på stein, 1967-08-27, L. Ryvarden (O L-42097); Båtsfjord, ad Vesterelv sinus Syltefjord ad alpum Gaisa, J.M. Norman (O L-42119); Gamvik, Mehavn på Gorgos Njargga, [35W NU 300–317,805–834], 1906-08-22, J.J. Havaas (BG L-44338); Sør-Varanger, Fredheim i Sydvaranger, [36W UC 898–909,310–327, alt. 15–70 m], 1906-08-03, J.J. Havaas (O L-42096); Sør-Varanger, Pasvik, Kiltjørnan NE, 69.39160°N, 29.43412°E, alt. 132 m, furu-bjørk blandskog, lyngskog, urskognær, åpen skog, på stor baserik steinblokk, 2017-09-27, T.H. Hofton 17290 (O L-225689); Tana, Vesterbugt, [35W NU 295–310,190–210, alt. 1–100 m], 1920-07-15, B. Lynge & O.A. Høeg, (O L-42105); Tana, Julelven–Væderelven, [70°27'N 28°42'E], 1968-08-06, E. Dahl & H. Krog (O L-42100). **Sweden**. *Dalarna*: Särna, at the waterfall Fjätfallet, 6.5 km ESE of Särna, at the lower waterfall, north sidea, on mosses on southexposed moderately shady steep sloping rock wall, 1980-09-13, Löfgren 1081 (UPS L-146394); *Åsele lappmark*: Dorothea, c. 1700 m SO om Harrsjö, strax V om skogsbilväg, 64°32'N, 15°38'E, granskogsbryn, på bark av *Salix caprea*, 2004-06-28, Odelvik 4122 (S L61275); Vilhelmina, 15 km WNW of Klimpfjäll, 660 m S of the bridge over river Saxån, the N slope of Mt Stihke, 65.11281˚N, 14.50922˚E, alpine heath on rather calcareous soil, on a large boulder, 2017-07-26, M. Westberg ULR174 (UPS L-944930); *Lycksele lappmark*: Tärna, Dalåjve, c. 1 km NE of Klippen, boulder, 1967-08-04, R. Moberg 889 (UPS L-008164); Tärna, c. 2.8 km NNW of Västansjö, Mt Gierevaartoe, 65.77271˚N, 15.06178˚E, on E-facing, inclined rock in low alpine heath, on rock, M. Westberg 2770 (LD); *Pite lappmark*: Arjeplog, 2400 m O-OSO om riksröse Rr 232A, 1400 m OSO om Skärrim, toppen, strax V om Skärrimjåhkå, Skärrimvägge, 66.77380˚N, 15.91304˚E, fjällhed, på mossa på stenblock, 2006-08-22, Odelvik 06388 (S F60937); *Lule lappmark*: Gällivare, Suorvasjöområdet: nedanför Karnjelapakte, reg. subalp., 1922-07-12, G.E. Du Rietz (UPS L-938985); Gällivare, Suorvasjöområdet: Ruotjajauresydbranterna, 1922-07-24, G. Einar Du Rietz (UPS L-938967); Gällivare, Muddus Nationalpark, Lilla Vuosmavares SV-sluttning, *Salix caprea*, 1944-08-07, Svenonius MS128 (UPS L-549993); Jokkmokk, Näntotjåkko, sydsluttning mot Letsitjaure, på död *Juniperus*, regio alpina inf., 1936-07-24, Björkman (UPS L-623330); Jokkmokk, Padjelanta National Park, Staloluokta, 67.31762˚N, 16.69340˚E, rock, 2004-07-30, M. Westberg (LD); Jokkmokk, Padjelanta National Park, c. 2.5 km S of Staloluokta, by the river Viejejåhkå, 67.29284˚N, 16.70822˚E, among mosses on a boulder, 2004-07-30, M. Westberg 3033 (LD); Jokkmokk, Padjelanta National Park, c. 10 km N of Staloluokta, below Mt Allak, north side of Lake Virihaure. 67.40988˚N, 16.64229˚E, *Juniperus communis*,

2004-07-31, T. Berglund (hb Berglund); same locality and date, E-facing rocks on the shore, M. Westberg 3068 & 3080 (LD); *Torne lappmark*: Jukkasjärvi, Abisko, kulle i *Betula* skogen mellan Abisko och Paddos, rock, 1931-07-19, G. Degelius (UPS L-146530); Jukkasjärvi, north side of Lake Torneträsk, near the shore, 68.422386˚N, 18.955402˚E, mosses on schistose rock in open, subalpine *Betula* forest, 2004-08-03, M. Westberg 3182 (LD); Karesuando, Pältsan (Bealccan), S-SE-facing slope of the southern peak, c. 800 m SE of the southern peak, 69.99193˚N, 20.26533˚E, alt. c. 900 m, rock, 2011-08-04, M. Westberg P124 (S F283561). **USA.** *Washington*: Whatcom County, Skyline Divide, northwest of Mt. Baker, 48˚50'N 121˚50'W, alt. 1900 m, on alpine ridgetop, under juniper, rocky soil, 1983-08-26, B. Ryan 8962 (WTU L-3726).

Acknowledgements: We wish to thank the Norwegian Barcode of Life for funding the sequencing at the Canadian Centre for DNA Barcoding; the fungaria LD, S, WTU, and Toni Berglund for the loan of material; Fredrik Steinsbu Wasberg for running 160 TLC analyses in O; the Norwegian Biodiversity Information Centre for networking-funding to MB (the international Varanger workshop in 2014; project number 70184224); and, Jouko Rikkinen for locality information for the Finnish GenBank specimen. Sequencing of the Swedish specimens was supported by a grant to MW from the Swedish Taxonomy Initiative (Svenska Artprojektet) administered by the Swedish Species Information Centre (ArtDatabanken) and MW would also like to thank Mats Wedin (Swedish Museum of Natural History) for supporting the sequencing of specimens.

References

Bendiksby, M., Haugan, R., Spribille, T., Timdal, E. 2015. Molecular phylogenetics and taxonomy of the *Calvitimela aglaea* complex (Tephromelataceae, Lecanorales). *Mycologia* **107**: 1172–1183. https://doi.org/10.3852/14-062.

Culberson, C. F. 1972. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* **72**: 113–125. https://doi.org/10.1016/0021-9673(72)80013-X.

Fedrowitz, K., Kaasalainen, U. & Rikkinen, J. 2012. Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. *Ecology and Evolution* **2**: 2291–2303. https://doi.org/10.1002/ece3.343

Fick, S. E. & Hijmans, R. J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37:** 4302–4315. https://doi.org/10.1002/joc.5086

GBIF.org. 2020-05-18. GBIF Occurrence Download https://doi.org/10.15468/dl.bjyp9k

Guisan, A., Thuiller, W. & Zimmermann, N. E. 2017. *Habitat suitability and distribution models: with applications in R.* Cambridge University Press.

Gyelnik,V. 1930. Lichenologiai Közlemények 20–45. *Magyar Botanikai Lapok* **29**: 23–35.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Hofton, T. H. 2020. Elfenbenslav (*Heterodermia speciosa*) i Norge – status pr. 31.12.2019. *BioFokus-rapport* **2020-1**: 1–118.

James, P. W. & White, F. J. 1987. Studies on the genus *Nephroma* I. The European and Macaronesian species. *The Lichenologist* **19**: 215–268. https://doi.org/10.1017/S0024282987000239

Lohtander, K., Oksanen, I. & Rikkinen, J. 2002. A phylogenetic study of *Nephroma* (lichen-forming Ascomycota). *Mycological Research* **106**: 777–787. https://doi.org/10.1017/S0953756202006068

Lücking, R., Dal-Forno, M., Sikaroodi, M., Gillevet, P. M., Bungartz, F., Moncada, B., Yánez-Ayabaca, A., Chaves, J. L., Coca, F. L., Lawrey, J. D. 2014. A single macrolichen constitutes hundreds of unrecognized species. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 11091–11096. https://doi.org/10.1073/pnas.1403517111

Lücking, R., Hodkinson, B. P. & Leavitt, S. D. 2017. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* **119**: 361–416. http://dx.doi.org/10.1639/0007-2745-119.4.361

Marthinsen, G., Rui, S. & Timdal, E. 2019. OLICH: A reference library of DNA barcodes for Nordic lichens. *Biodiversity Data Journal* **7**: e36252. https://doi.org/10.3897/BDJ.7.e36252

Menlove, J. E. 1974. Thin-layer chromatography for the identification of lichen products. *Bulletin of the British Lichen Society* **34**: 3–5.

Molina, M. C., Del-Prado, R., Divakar, P. K., Sánchez-Mata, D. & Crespo, A. 2011. Another example of cryptic diversity in lichen-forming fungi: The new species *Parmelia mayi* (Ascomycota: Parmeliaceae). *Organisms Diversity and Evolution* **11**: 331–432. https://doi.org/10.1007/s13127-011-0060-4

Piercey-Normore, M. D., Coxson, D., Goward, T. & Goffinet, B. 2006. Phylogenetic position of a Pacific Northwest North American endemic cyanolichen, *Nephroma occultum* (Ascomycota, Peltigerales). *The Lichenologist* **38**: 441–456. https://doi.org/10.1017/S0024282906005950

Sérusiaux, E., Villarreal, A. J. C, Wheeler, T. & Goffinet, B. 2011. Recent origin, active speciation and dispersal for the lichen genus *Nephroma* (Peltigerales) in Macaronesia. *Journal of Biogeography* **38**: 1138–1151. https://doi.org/10.1111/j.1365-2699.2010.02469.x

Speed, J. D. M. & Austrheim, G. 2017. The importance of herbivore density and management as determinants of the distribution of rare plant species. *Biological Conservation* **205**: 77–84. http://dx.doi.org/10.1016/j.biocon.2016.11.030

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Vitikainen, O. 2007. Nephromataceae. *In*: Ahti, T., Jørgensen, P. M., Kristinsson, H., Moberg, R. Søchting, U. & Thor, G. (eds), *Nordic Lichen Flora, Volume 3*. Nordic Lichen Society, Uppsala, pp. 91–95.

Wang, H.-Y., Jiang, D.-F., Huang, Y.-H., Wang, P.-M. & Li, T. 2013. Study on the phylogeny of *Nephroma helveticum* and allied species. *Mycotaxon* **125**: 263–275. https://doi.org/10.5248/125.263

Wedin, M., Westberg, M., Crewe, A. T., Tehler, A. & Purvis, O. W. 2009. Species delimitation and evolution of metal bioaccumulation in the lichenized *Acarospora smaragdula* (Ascomycota, Fungi) complex. *Cladistics* **25**: 161–172. https://doi.org/10.1111/j.1096-0031.2009.00240.x

Wetmore, C. M. 1960. The lichen genus *Nephroma* in North and Middle America. *Publications of the Museum, Michigan State University, Biological Series* **1**: 369–452.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (eds), *PCR protocols: A guide to methods and applications*. Academic Press, New York, pp. 315–322.