

# *Lepraria lobificans* (syn. *L. santosii*) and *L. sylvicola* new to northern Europe from Norway

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The two lichen species *Lepraria lobificans* (syn. *L. santosii*) and *L. sylvicola* are reported new to northern Europa, based on collections from Western Norway and Northern Norway, respectively. The specimens were identified by morphology, thin-layer chromatography, and the DNA barcode marker. The common species currently named *L. lobificans* in Northern Europe should be named *L. finkii*.

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## Introduction

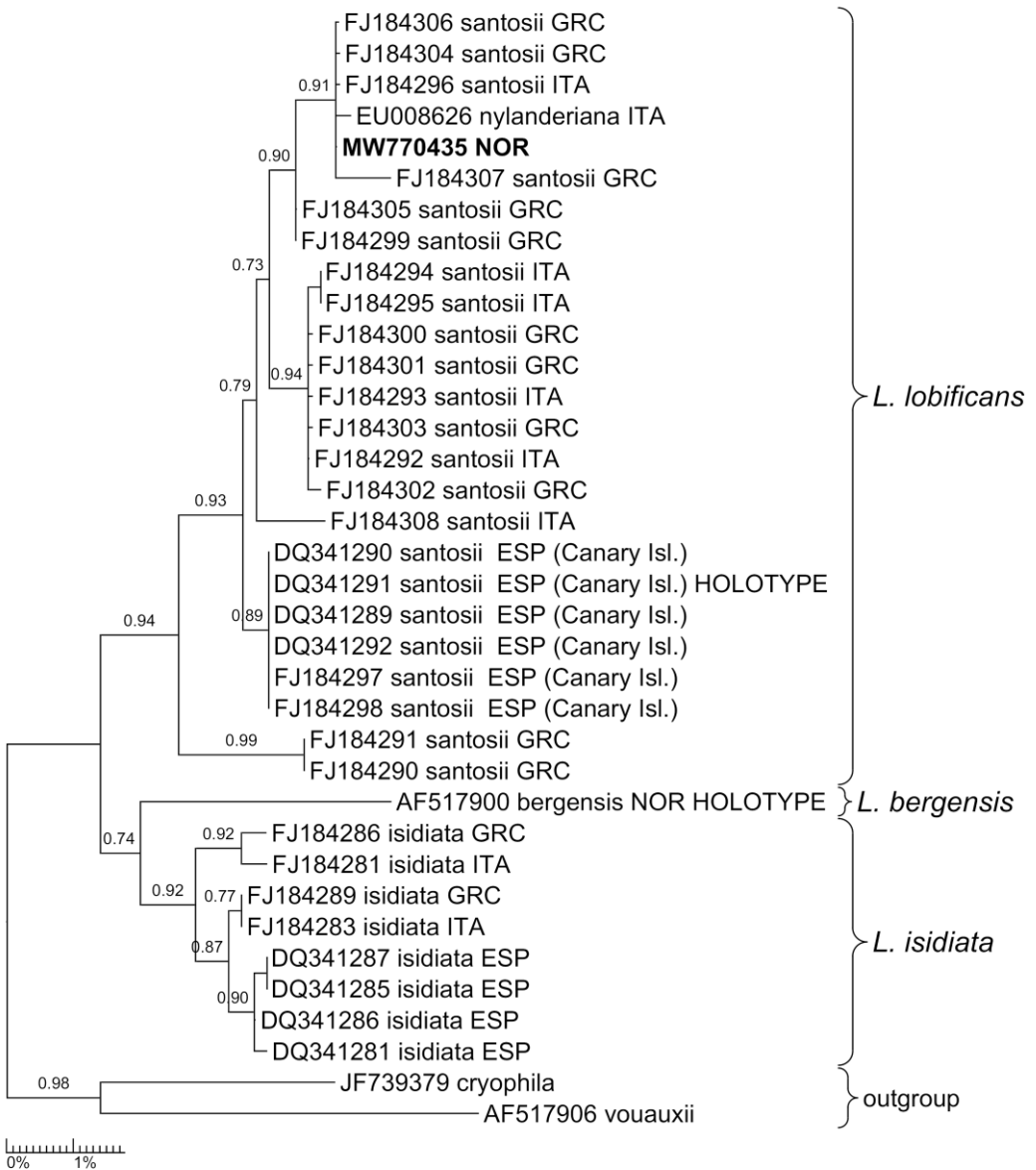
During general sequencing of Norwegian lichens in the OLICH project (Marthinsen et al. 2019; a subproject under the Norwegian Barcode of Life, [www.norbol.org](http://www.norbol.org)) for contributing to the international DNA barcode database BOLD ([www.boldsystems.org](http://www.boldsystems.org)), we have discovered two *Lepraria* species that are new to Northern Europe. This paper presents the discovery.

## Material and Methods

*Fieldwork:* The material was collected on collecting trips for general studies of Norwegian lichens. Elevation data given for the collection localities are derived from Kartverket (2021), based on field GPS data (datum WGS84).

*Morphology:* The material was studied using dissecting microscopes. The herbarium photographs were made by a DSLR camera (Nikon D800E) and a macro lens (Micro-Nikkor 55 mm f/2.8) attached to a bellows and assembled by focus stacking software (CombineZP).

*Lichen chemistry:* Lichen secondary metabolites were investigated using thin-layer chromatography (TLC) according to the methods of Culberson & Kristinsson (1970), Culberson (1972), and Culberson & Johnsen (1982), using glass plates in all three solvent systems (A, B', C) allowing for the detection and identification of fatty acids. For the identification of the fatty acids toensbergianic acid and roccellic acid, samples from the BG-isotype of *Lepraria sylvicola* Orange (containing both substances) and of *Cliostomum griffithii* (Sm.) Coppins (Tønsberg 18723; BG-L-23421, duplicate) containing roccellic acid according to, e.g., Gilbert & Fox (2009), were used as references. The two fatty acids angardianic and roccellic acid cannot be separated by TLC (Leuckert et al. 1995), and are therefore referred to collectively. Helpful, diagrammatic chromatograms for the identification of



**Figure 1.** Maximum likelihood analysis of the GenBank nrITS accessions of the *Lepraria lobificans* clade, its sister clade (*L. bergensis* and *L. isidiata*), and the Norwegian specimen (MW770435), with *L. cryophila* and *L. vouauxii* as the outgroup. SH-test support values > 0.7 are given above branches.

fatty acids, including those mentioned above, are given by Bayerová et al. (2005), Slavíková-Bayerová & Orange (2006), and Slavíková-Bayerová & Fehrer (2007).

*DNA extraction, PCR, and sequencing:* All steps from DNA extractions and PCR amplification through sequencing and editing of the two sequences produced for this study were performed at the Canadian Centre for DNA Barcoding (<http://www.ccdb.ca>), using the primer pair ITS1-F/ITS4.

*Molecular analyses:* All nrITS sequences of *Lepraria* (including those named as *Lepruloma*) in GenBank (318) were downloaded on 2020-11-25 and, together with our two *Lepraria* sequences plus two sequences of *Stereocaulon paschale* (L.) Hoffm. for the outgroup, included in the construction of a phylogenetic tree under the maximum likelihood criterion. We used SATé ver. 2.2.7 (Liu et al. 2012) with default settings (MAFFT [Katoh et al. 2005, Katoh & Toh 2008] as aligner, Muscle [Edgar 2004] as merger, FastTree [Price et al. 2010] as tree estimator, Shimodaira-Hasegawa test [SH test] for tree evaluation, and GTR+G20 as substitution model) and 10 iterations after last improvement of the likelihood score. Based on that tree, a subset of sequences was selected for the construction of a smaller phylogenetic tree. The subset contained (1) the highly supported clade in the first tree (SH-test support value 0.997) consisting of all (23) *L. lobificans* (named *L. santosii* Argüello & A. Crespo in GenBank and in our Fig. 1) sequences and the single *L. nylanderiana* Kümmerl. & Leuckert sequence plus our sequence MW770435, (2) that clade's sister clade which consisted of *L. bergensis* Tønberg and *L. isidiata* (Llimona) Llimona & A. Crespo, and (3) one representative each of *L. cryophila* Lendemer and *L. vouauxii* (Hue) R.C. Harris as the outgroup. The subset was aligned by ClustalW (Thompson et al. 1994), trimmed at the ends to the nrITS marker and de-gapped in BioEdit (Hall 1999), and then re-run by SATé under the same settings. The 'best tree' was edited in TreeGraph 2 (Stöver & Müller 2010). Genetic distances between the Norwegian sequences and those from GenBank were obtained by performing local BLASTn searches against the downloaded sequences.

## Results

*Lepraria lobificans:* The phylogenetic reconstruction is shown in Fig. 1. The alignment was 575 bp long and contained 79 variable sites. Our nrITS sequence of *L. lobificans* (MW770435) occurred in a highly supported clade (SH-test support value 0.94) with the 23 GenBank sequences of *L. lobificans* (including the holotype of *L. santosii*) and the single sequence of *L. nylanderiana*. Our local BLASTn search showed a 96.0–100 % similarity (0–20 nucleotide substitutions, 0–3 gaps, 512–559 bp alignment) with the sequences of *L. lobificans* and *L. nylanderiana* in GenBank.

*Lepraria sylvicola:* No separate phylogenetic reconstruction was made for this species, but in the first reconstruction of the whole genus (not shown), our nrITS sequence (MW770436) was recovered in a highly supported clade (SH-test support value: 1.0) with the six sequences of *L. sylvicola* obtained from GenBank (all from the UK; including the holotype). Our local BLASTn search showed a 99.8–100 % similarity (0 nucleotide substitutions, 0–1 gap, 561–595 bp alignment) with the sequences of *L. sylvicola* deposited in GenBank.

## Discussion

The phylogenetic analyses and BLASTn searches clearly show that our material belong in *L. lobificans* (syn. *L. santosii*) and *L. sylvicola*, and the two species are hence here reported as new to Northern Europe.



**Figure 2.** *Lepraria lobificans*, Norway (BG-L-98010). Scale bar = 2 mm. Photo E. Timdal.

According to type studies by Lendemer (2013), *Lepraria lobificans* Nyl. is the correct name for *L. santosii* and the species previously known as *L. lobificans* should be named *L. finkii* (de Lesd.) R.C. Harris. This name change has not been implemented in the Nordic checklist (Nordin et al. 2021), where the name *L. lobificans* is used for the common Nordic *L. finkii*.

From the position in the phylogeny, the GenBank sequence of *L. nylanderiana* (EU008626) belongs in *L. lobificans*, and the identity of the voucher specimen should be checked.

## The Species

### *Lepraria lobificans* Nyl.

Fig. 2

New to Northern Europe.

*Description:* Descriptions of the species (as *L. santosii*) are given by Crespo et al. (2006) and Tretiach et al. (2009).

*Chemistry:* Atranorin, angardianic/roccellic acid, and protocetraric acid (by TLC).

*Ecology and distribution:* The species is known from one locality in SW Norway (Fig. 3), where it occurred on W-facing, slightly overhanging rock walls on the western side of the island Bømlo, close to and facing the North Sea.

According to Tretiach et al. (2009), it is widespread and locally common from sea level to c. 1000 m altitude throughout the Mediterranean-Macaronesian region. There is also a record from



**Figure 3.** Collection site for *Lepraria lobificans* (arrow) and close-ups of the population represented by BG-L-105286. Photos T. Tønsberg 2021-03-16.

Yunnan, China, in GBIF. The current report from western Norway represents a significant range extension, both geographically and ecologically.

*Comments:* The Norwegian material agrees with the descriptions of Crespo et al. (2006) and Tretiach et al. (2009) in all important characters such as the whitish colour of the upper surface, the forming of colonies of confluent/aggregated rosettes with distinctly raised margins (which in the Norwegian material may be developed already in thalli as small as to 0.4 mm), and the hypothallus hyphae being K<sup>-</sup> (anthraquinones absent). Tretiach et al. (2009) recognized seven chemotypes within the species on the basis of permutations of fumarprotocetraric acid, protocetraric acid, roccellic acid, stictic + constictic acid, zeorin, and an unknown UV<sup>+</sup> vivid pink compound (all chemotypes contain atranorin). Our specimen belongs in their chemotype 5.

Chemotype 2 of *Lepraria nivalis* J.R. Laundon (see Leuckert et al. 2004) is chemically similar, but that species is morphologically distinct by the marginal lobes having no raised rim (Tretiach et al. 2009).

*Lepraria finkii* is distinct from *L. lobificans* morphologically by the green to greenish grey thallus with loosely embedded propagules, and chemically by the absence of fatty acids (see Tønsberg 1992).

*Specimens examined:* **Norway.** *Hordaland:* Bømlo, island Bømlo, Hope, 59.6735°N, 5.1706°E, alt. 3 m, saxicolous on W-facing, somewhat overhanging rock wall, 2015-04-28, T. Tønsberg 44766 (BG-L-98010; dupl.: O-L-228352) [GenBank: MW770435]; 2021-03-16, T. Tønsberg 48910 (BG-L-105286).

*Type material of Lepraria santosii examined:* **Canary Islands.** *Tenerife:* Las Mercedes, at road cutting, on soil over basaltic rocks, alt. 850 m, 2005-03-28, A. Crespo (MAF-14003–holotype, BG-L-81984–isotype).

### ***Lepraria sylvicola* Orange**

Fig. 4

New to Northern Europe.

*Description:* Description of the species is given by Slavíková-Bayerová & Orange (2006) based on material from Great Britain and Ireland.

*Chemistry:* Angardianic/roccellic acid, toensbergianic acid, and atranorin (all major); an unidentified fatty acid (solvent A only; trace) (by TLC).

*Ecology and distribution:* In Norway *L. sylvicola* is known only from the island Søla on the coast of southernmost North Norway where it was collected at an altitude of 32 m. The species was found saxicolous in shallow crevice in the north facing wall of a boulder. The material includes two small specimens on separate rock pieces. *Lepraria finkii* is an associate species.

The species was described from the British Isles (Slavíková-Bayerová & Orange 2006) where it is most common on bark, especially that of *Quercus petraea* and has later been reported from France (Kukwa & Diederich 2007) and Luxembourg (Diederich et al. 2009) where it is saxicolous. In GBIF there are also records from Estonia, Spain (no locality given, but judging from the habitat apparently the Canary Islands), and Ukraine.

*Comments:* The morphology of the Norwegian material agrees well with the isotype from Scotland in BG in important characters such as the thallus being powdery, the K<sup>+</sup> purple red subthalline hyphae, and the chemistry including the rather rarely occurring constituent toensbergianic acid. The thalli are small compared with the type material (up to 15 mm diam., vs up to 30 mm in the



**Figure 4.** *Lepraria sylvicola*, Norway (BG-L-99170). Scale bar = 2 mm. Photo E. Timdal.

isotype), thinner (up to 0.16 mm, versus up to 0.32 mm in the BG isotype), and have smaller granules (24–48  $\mu\text{m}$ , versus 40–160  $\mu\text{m}$  according to Slavíková-Bayerová & Orange 2006). We regard these differences to be of no taxonomic value as the thalli in the Norwegian specimens are less mature and, especially, since the nrITS sequence is almost identical with other sequences deposited in GenBank. The colour (assessed 2021-01-09) is pale greenish.

*Specimen examined: Norway. Nordland:* Vega, island Søla N, lower part of N-facing boulder field, 65.67467°N, 11.72629°E, alt. 32 m, saxicolous in shaded crevice in N-facing wall of boulder, 2015-08-11, T. Tønsberg 45800 (BG-L-99170) [GenBank: MW770436].

*Type material of Lepraria sylvicola examined: UK. Scotland:* Westernness (V.C. 97), near Fort William, Loch Linnhe, north-west of Corrychurrachan, National Grid 27/0507.6724, on trunk of *Quercus petraea* in woodland, alt. 10 m, 2004-05-15, A. Orange 15013, duplicate ex NMW (BG-L-101920–isotype).

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