

Lecidea toensbergii, the first described sorediate species in *Lecidea sensu stricto*

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A molecular phylogenetic analysis based on the DNA barcode marker (nrITS) of 12 specimens of *Lecidea leucothallina* revealed three monophyletic clades, two apotheciate and one sorediate. The sorediate clade is described as *L. toensbergii*, and the two apotheciate clades are regarded as representing two cryptic species within *L. leucothallina*. Pannarin may be present or absent in the upper cortex in all three clades, and should not be used as a diagnostic character for further separation of taxa in this group.

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Introduction

Lecidea Ach., in the strict sense of Hertel (1995), consists of c. 100 species occurring world-wide and almost exclusively on rock (Hertel & Printzen 2004). All species are apotheciate, i.e., dispersed by ascospores, and vegetative dispersal units are extremely rare. To our knowledge, soralia is reported only once, from a morphotype of *L. atrobrunnea* (Lam. & DC.) Schaer. ssp. *atrobrunnea* from North America (Hertel & Printzen 2004).

Lecidea leucothallina Arnold was described from Austria, and is known from the Alps, the Scandinavian mountains, Iceland, Greenland, Newfoundland, Quebec, and western North America (Hertel 1975, 1995; Consortium of North American Lichen Herbaria 2018, GBIF Secretariat 2018). According to Hertel (1995), the Japanese *L. subleucothallina* Inoue is probably a synonym of this species. Hertel (1995) accepted three varieties of *L. leucothallina*: var. *discrepans* Rambold & Hertel, var. *kujalae* (Räsänen) Hertel. [= *Lecidea kujalae* Räsänen], and var. *leucothallina*. The varieties were based exclusively on chemical characters: hypostictic acid (tentatively) and stictic acid in var. *discrepans*; pannarin in the thallus and in the apothecial pruina in var. *kujalae*; and pannarin in the apothecial pruina (only) in var. *leucothallina*. The three varieties are all reported from the Nordic countries (Hertel 1995), but are regarded as synonyms in the current Nordic checklist (Nordin et al. 2018).

During a project on sorediate, saxicolous, crustose lichens in Norway, funded by the Norwegian Biodiversity Information Centre (www.biodiversity.no), we collected several sorediate specimens that otherwise resemble *L. leucothallina*. In order to decide if soralia occur sporadically in populations of *L. leucothallina* or are diagnostic for a related taxon, we sequenced three sorediate and nine apotheciate specimens of *L. leucothallina* for the fungal DNA barcode marker (the nuclear ribosomal internal transcribed spacer, nrITS).

Material and Methods

Six sorediate collections of *L. leucothallina* from Norway were examined and DNA was successfully extracted from three of them (Table 1, as *Lecidea toensbergii*). In addition, we extracted DNA from nine apotheciate specimens of *L. leucothallina*, five of var. *kujalae* and four of var. *leucothallina* (Table 1, as *L. leucothallina*). We downloaded nrITS sequences from GenBank of one specimen of *L. obliquata* Nyl. and two specimens of *Lecidea silacea* (Hoffm.) Ach. Those two species were selected after having performed a BLAST search for the most similar species in GenBank, and *L. obliquata* was used for rooting the tree. There were no sequences of *L. leucothallina* in GenBank. We also examined morphologically and chemically an isotype of *L. leucothallina* (O), an isotype of var. *discrepans* (O), and a Japanese specimen assumedly representing *L. subleucothallina* (borrowed from TNS).

The DNA sequences with GenBank Accession Numbers MG968256 – MG968261 were produced at the Canadian Centre for DNA Barcoding (CCDB; www.ccdb.ca) for our OLICh project (nhm2.uio.no/olich) at the Norwegian Barcode of Life project (NorBOL; www.norbol.org). The sequences MG973068 – MG973073 were produced at the DNA lab of the Natural History Museum, University of Oslo (O). At O, DNA extraction, PCR amplification, PCR purification, and cycle sequencing were performed as described by Bendiksy & Timdal (2013). The primer pair ITS5/ITS4 (White et al. 1990) were used for amplification and sequencing the nrITS marker (ITS1 + 5.8S + ITS2) at both labs.

The sequences were edited and aligned using Geneious 6.1.8 (www.geneious.com) and its built-in alignment tool ClustalW. The alignment was inspected manually, but no adjustments were made. We analysed and summarized the data with parsimony and Bayesian phylogenetic methods. For the parsimony analyses we used NONA (Goloboff 1999) in combination with WinClada 1.0 (Nixon 1999), applying the heuristic search option with 2000 replicates and maximum trees set to 10 000 and otherwise default settings. We performed parsimony jack-knifing (JK) with 2000 replicates and otherwise default setting. We saved the 50 % majority rule tree with jack-knife values on the supported branches.

For the Bayesian phylogenetic analyses we used MrBayes 3.2.6 (Ronquist et al. 2011) with the partitioning scheme set according to the output of PartitionFinder 2 (Lanfear et al. 2016). PartitionFinder 2 identified K80 as the best model of substitution for all three partitions (ITS1, 5.8S, and ITS2), under the following configuration settings: linked branch lengths, the greedy search scheme, the Bayesian information criterion as selection metric, and by examining only models that are implemented in MrBayes 3.2.6. We determined posterior probabilities (PP) by running one cold and three heated chains for 1 000 000 generations in parallel mode, saving trees every 1000th generation. At that point the average standard deviation of split frequencies had fallen below 0.007. The first 20 % of the trees were discarded as burn-in, and the remaining trees were summarized as a Bayesian 50 % majority rule (MR) consensus tree with PP calculated for each clade.

All specimens were examined by thin-layer chromatography (TLC), performed in accordance with the methods of Culberson (1972) as modified by Menlove (1974) and Culberson & Johnson (1982). In addition, detached areolae and apothecia were observed under the dissecting microscope in a drop of paraphenylenediamine dissolved in ethanol (PD) in order to locate pannarin, which gives an orange reaction in the lichen tissue. Amyloid reactions in the medulla was examined in a modified Lugol's solution (water replaced by 50 % lactic acid) following pretreatment in 10 % KOH (K), the KI reaction.

Table 1. Specimens used in this study with voucher information and GenBank accession numbers for the nrITS sequences. Sequences provided for this work are in bold.

Specimen	Country, Province	Voucher ID	GenBank ID
<i>Lecidea leucothallina</i> 1	Norway, Oppland	BG L-96170	MG968256
<i>Lecidea leucothallina</i> 2	Norway, Hedmark	O L-179956	MG973068
<i>Lecidea leucothallina</i> 3	Norway, Nordland	O L-163673	MG968258
<i>Lecidea leucothallina</i> 4	Norway, Sør-Trøndelag	O L-179641	MG973069
<i>Lecidea leucothallina</i> 5	Norway, Nordland	O L-183838	MG973070
<i>Lecidea leucothallina</i> 6	Norway, Nordland	O L-163621	MG968259
<i>Lecidea leucothallina</i> 7	Norway, Møre og Romsdal	O L-165815	MG968260
<i>Lecidea leucothallina</i> 8	Norway, Nord-Trøndelag	O L-173935	MG973071
<i>Lecidea leucothallina</i> 9	Norway, Nordland	O L-174205	MG973072
<i>Lecidea obhuridata</i>	Austria, Osttirol	Türk 42730	GU074423
<i>Lecidea silacea</i> 1	Austria, Tirol	Türk 42156	GU074424
<i>Lecidea silacea</i> 2	–	AFTOL 1368	HQ650629
<i>Lecidea toensbergii</i> 1	Norway, Oppland	O L-181870	MG973073
<i>Lecidea toensbergii</i> 2	Norway, Møre og Romsdal	O L-165806	MG968257
<i>Lecidea toensbergii</i> 3	Norway, Oppland	BG L-95936	MG968261

Results

Twelve nrITS sequences were generated (Table 1). The alignment was 518 base pairs long and the ingroup contained 19 parsimony informative characters. The parsimony and the Bayesian analyses produced congruent 50 % majority rules trees. The jack-knife percent values (JK) of the former analysis and the posterior probability values (PP) of the latter are superimposed on the maximum parsimony 50 % majority rule consensus tree in Fig. 1. In the former analysis, the tree length was 112 steps, the Consistency Index (CI) was 92, and the Retention Index (RI) was 92. We regard clade support of at least 60 % JK and at least 0.9 PP as significant. Using *L. silacea* as outgroup, *Lecidea leucothallina* s. lat. (including both apotheciate and sorediate morphotypes, the latter called *L. toensbergii* in Fig. 1) fell out as monophyletic with maximum support (JK/PP: 100/1). Within that clade, three subclades fell out as monophyletic with significant branch support: Two subclades (A and C) of apotheciate specimens with support values 98/1 and 97/1, respectively, and one subclade (B) of sorediate specimens with support values 83/1 (Fig. 1).

The PD reaction was positive in the apothecial pruina and negative in the thallus medulla in all specimens of *L. leucothallina* (apotheciate and sorediate), except for var. *discrepans* (see below). In the sorediate specimens, there was a positive reaction in the soralia. The reaction in the upper cortex varied from negative (PD–) via faintly to distinctly positive (PD+). Both negative and positive reactions in the upper cortex were found in all three subclades of *L. leucothallina* s. lat.

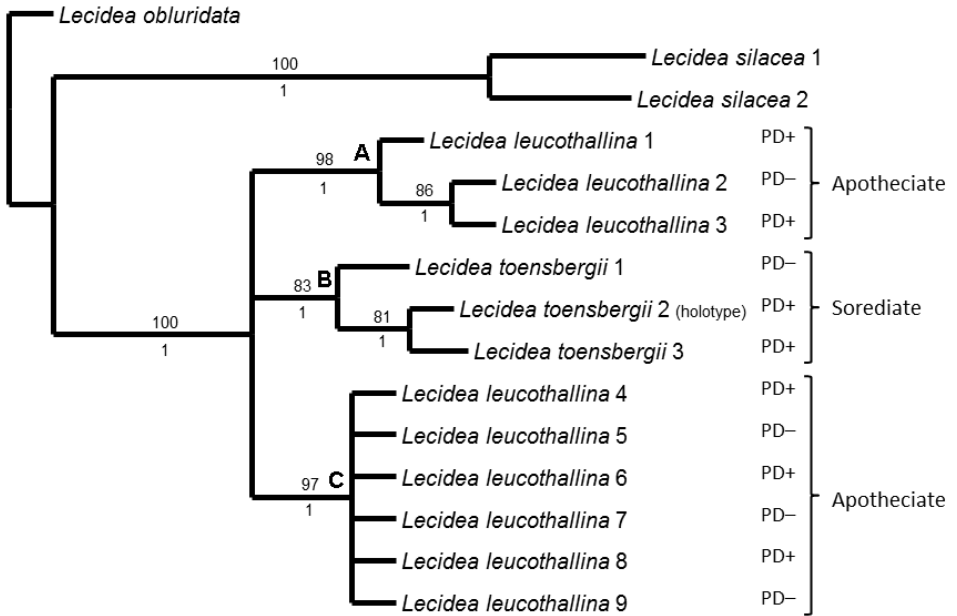


Figure 1. Maximum parsimony 50 % majority rule consensus tree based on an alignment of nrITS sequences of 12 accessions of *Lecidea leucothallina* and *Lecidea toensbergii*, with three accessions of *Lecidea obluridata* and *Lecidea silacea* for rooting and outgroup (see Table 1). Parsimony jack-knife support values are shown above the branches and Bayesian posterior probabilities below. With indication of the PD reaction in the upper cortex.

(Fig. 1). The TLC results confirmed the presence of pannarin in all PD+ specimens (except var. *discrepans*).

The isotype of *Lecidea leucothallina* gave a PD+ orange reaction in the apothecial pruina only, and did not contain lichen substances in the thallus (TLC).

The isotype of var. *discrepans* was K+ yellow and PD+ orange in the medulla, and contained stictic acid (major) and norstictic acid (trace) by TLC; no hypostictic acid, nor pannarin, was detected. The medulla was distinctly KI+ blue. The thallus was more brown coloured than what is usually seen in *Lecidea leucothallina*, and the apothecia were epruinose.

The Japanese specimen was morphologically and chemically apparently congruent with Nordic specimens of apotheciate *Lecidea leucothallina*, e.g. showing a PD+ (faintly) orange upper cortex (pannarin by TLC) and a PD- medulla.

Discussion

The molecular phylogeny (Fig. 1) indicates that *Lecidea leucothallina* consists of three monophyletic clades, two apotheciate (A and C) and one sorediate (B). We are not able to see any morphologically or chemically distinguishing features between members of the two apotheciate clades and regard them as morphologically cryptic species. The separation of the two varieties var. *kujalae* and var. *leucothallina* based on the presence or absence of pannarin in the thallus (Hertel 1995) does not seem justified as both chemotypes occur in both clades. In the protologue (Inoue 1982), *L. subleucothallina* was distinguished from *L. leucothallina* mainly by the presence of pannarin in the thallus (and the somewhat smaller, but overlapping ascospore size), but our results indicate that this seems equally unjustified. Hence, three species names are available for this collective species, *L. leucothallina* (priority 1879), *L. kujalae* (1943), and *L. subleucothallina* (1982), but it is not known which name belongs to which cryptic species.

The sorediate clade (B) is here regarded as a morphologically distinct species and described as *L. toensbergii* below. Two of the sequenced specimens of *L. toensbergii* (Fig. 1 and Table 1: specimens 1 and 3) were collected in the Jotunheimen Mountains in central South Norway ca. 3 km apart, and ca. 20 km NE of the nearest sequenced *L. leucothallina* (specimen 1). The third *L. toensbergii* (specimen 2) was collected ca. 90 km to the NNW of *L. toensbergii* specimens 1 and 3, and only 100 m from *L. leucothallina* specimen 7. This, and the monophyly of the sorediate clade (Fig. 1), indicates that sorediate morphotypes are not occurring sporadically within the populations of *L. leucothallina*, but rather represent a separate genetic lineage to be found over greater distances in the Norwegian mountains.

Although the phylogenetic analysis indicates that the apotheciate clades (A and C) consists of two distinct species, we refrain from recognizing them as such here because of (1) our uncertainty in how to treat morphologically cryptic species taxonomically and (2) the unknown application of the three available species names.

The amyloid medullary reaction, presence of stictic acid, and the lack of apothecial pruina indicates that var. *discrepans* does not belong in *L. leucothallina*. Rather, we believe it is close to *L. praenubila* Nyl.

Taxonomy

Lecidea toensbergii Haugan & Timdal, sp. nov.

MycoBank: MB824350

Diagnosis: The species differs from *L. leucothallina* in forming soralia.

Type: Norway, Møre og Romsdal: Norddal, W slope of Skarfjellenden, 62.4275°N, 7.6602°E, 870 m alt., siliceous rock in late snow-bed, low alpine heath, 2010, Haugan 9156 (O L-165806, holotype [TLC: pannarin; GenBank: MG968257, nrITS]). Fig. 2.

Description: Thallus lichenized, not parasitic, areolate, effuse, up to more than 10 cm diam.; prothallus black, distinct at the thallus margin and between areolae; areolae isodiametrical, irregular in outline, from angular to rounded, plane to moderately convex, pale grey to pale brown, with a concolorous or paler margin, slightly shiny, epruinose, up to 1.0 mm diam.; soralia marginal on the areolae, rounded, sharply delimited, white to pale grey, PD+ orange; upper cortex 50–70 µm thick, including an up to 15 µm thick epinecral layer, composed of irregularly oriented, thick-walled hyphae with rounded lumina, often containing crystals (not dissolving in K; dissolving in

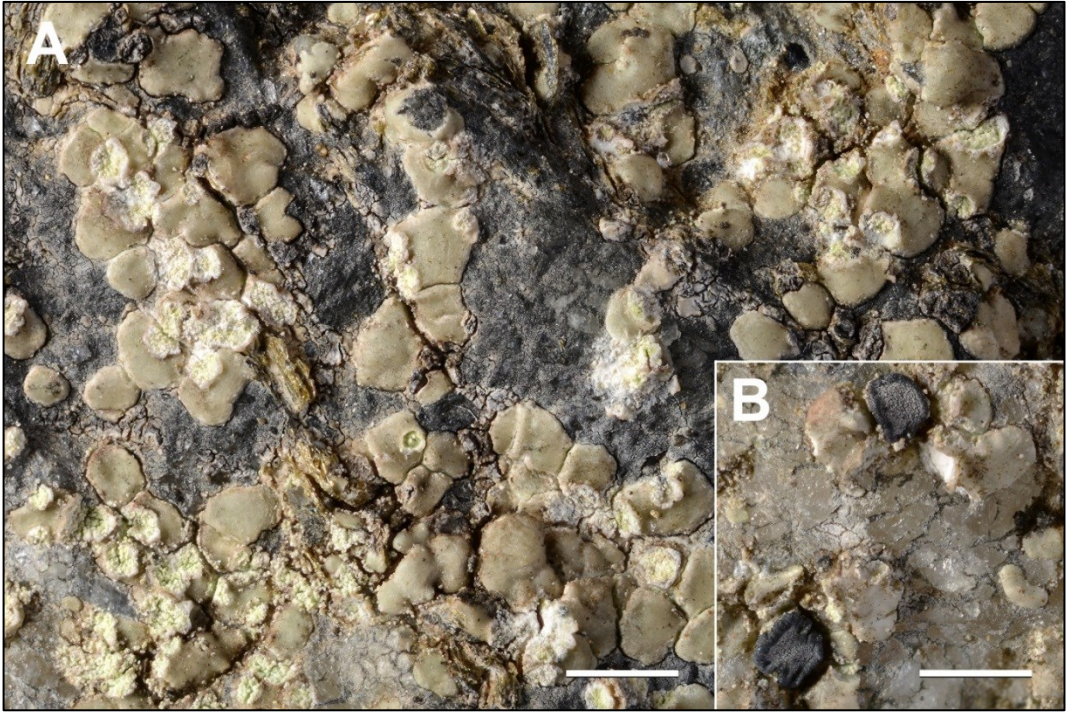


Figure 2. *Lecidea toensbergii*, holotype. A: Sorediate areolae. B: Apothecia. Scale bars: 1 mm.

PD with orange, needle-shaped crystal precipitation) in the stainable layer; medulla not containing crystals, KI–.

Apothecia mostly simple, rounded or with a somewhat flexuous margin, attached marginally to the areolae or to the hypothallus, up to 1.0 mm diam.; disc plane to weakly convex, black, dull, faintly white (PD+ orange) pruinose; margin distinct, persistent, black, not pruinose; excipulum dark brown throughout or slightly paler in inner part, not containing crystals, K–, KI–; hypothecium dark brown, not containing crystals, K–, KI–; hymenium colourless, KI+ blue, 60–80 μ m high; epihymenium dark olivaceous brown, containing crystals (not dissolving in K, PD+ orange); ascus clavate, with a well-developed, weakly amyloid tholus containing a deeply amyloid apical cap and a rudimentary amyloid tube in the upper half (*Lecidea* type); ascospores simple, ellipsoid, 8–12 \times 4–5 μ m (n=10).

Conidiomata not seen.

Chemistry: Pannarin (by TLC). Spot reactions: Upper cortex and soralia PD+ orange or more rarely PD–, medulla PD–, apothecial pruina PD+ orange. All parts K–, C–, KC–, UV–.

Etymology: The species is named in honour of Dr. Tor Tønsberg in appreciation of his significant studies on lichens, in particular on the sorediate species.

Habitat and distribution: *Lecidea toensbergii* is probably common in rocky, alpine habitats where it occurs on vertical to overhanging rock walls and on rocks on the ground under overhangs. The species usually grows in more sheltered and drier habitats than *L. leucothallina*. In high alpine

boulder fields, e.g. in the Jotunheimen Mountains it is apparently abundant on the lower side of rocks and thus often hidden until the rocks are turned over. The species is known from only six localities, all in Norway, but is widely distributed from the mountains of south Norway to subarctic north Norway.

Additional specimens examined: Lecidea toensbergii: Norway, Oppland: Lom, Dugurdskampen, 61.6997°N, 8.3877°E, 1521 m alt., on lower side of rocks in alpine boulder-scrée, 2012, Bratli & Haugan 11067 (O L-181870); Lom, Gjuvvasshøi, 61.6741°N, 8.3699°E, 1861 m alt., saxicolous on boulder on polygonal pattern ground in high alpine zone, 2013, Tønsberg 42350 (BG-L-95936); Lom, Breidsæterdalen, N of Krossbu, E and uphill from road, 61.5958°N, 8.0410°E, 1070 m alt., saxicolous on loose rocks on floor under boulder in boulder field, 2013, Tønsberg 42459 (BG-L-95937). *Nordland:* Grane, Børgefjell National Park, Simskardet, N of Simskardelva, 65.2872°N, 13.6415°E, 450 m alt., saxicolous on N-facing, upper part of vertical wall of boulder in open *Betula pubescens* forest in SSW facing slope, 2011, Tønsberg 40982 (BG-L-95728). *Finnmark:* Sør-Varanger, hill W of Storsand, 70.0000°N, 29.3666°E, 35 m alt., 2014, Haugan VAR19-1 (O L-195706).

Lecidea leucothallina, cryptic species 1: **Norway, Hedmark:** Alvdal, Mt Tronfjellet, the old copper mine Gruvvangen, 62.1406°N, 10.5900°E, 770 m alt., on rock in old waste heap, 2012, Rui & Timdal 12718 (O L-179956). *Oppland:* Lom, Breidsæterdalen, N of Krossbu, E and uphill from road, 61.5958°N, 8.0410°E, saxicolous on loose rocks on floor under boulder in boulder field, 2013, Tønsberg 42462a (BG-L-96170). *Nordland:* Saltdal, Junkerdalen, Skaerremjåkka, 66.7504°N, 15.8051°E, 600 m alt., rock outcrop in alpine zone, 2010, Timdal 11612, (O L-163673).

Lecidea leucothallina, cryptic species 2: **Norway, Møre og Romsdal:** Norddal, W slope of Skarfjellenden, 62.4270°N, 7.6622°E, 880 m alt., siliceous rock, low alpine heath, 2010, Haugan 9165 (O L-165815). *Sør-Trøndelag:* Røros, Stortvartz, the site of the old copper mine, 62.6267°N, 11.9700°E, 870 m alt., rock in old stone wall, 2012, Bendiksby et al. 10959 (O L-179641). *Nord-Trøndelag:* Steinkjer, Mikk, Gruvdalen, 63.9653°N, 12.1345°E, 515 m alt., rock outcrop near old copper mine (abandoned in 1783), 2011, Bendiksby et al. 10368 (O L-173935). *Nordland:* Fauske, Sulitjelma, Furuhaugen, old copper mine (in operation between 1896 and 1921), 67.1495°N, 15.9649°E, 290 m alt., rock in slag heap, 2012, Haugan 11278 (O L-183838); Hattfjelldal: north side of Mt Krutvassrøddiken, 65.6916°N, 14.3199°E, 620 m alt., vertical to overhanging rock wall, 2010, Timdal 11560 (O L-163621); Vefsn: E of Tennvatnet, 66.0508°N, 13.6456°E, 390 m alt., siliceous rock outcrop in subalpine birch forest, 2011, Haugan 10628 (O L-174205).

Lecidea leucothallina, unknown cryptic species: **Austria, Tirol:** an Glimmerblöcken der begrasten Höhe beim Kühkamplseck östlich oberhalb Gurgl, 2350 m alt., 2878-08-13, Arnold [Arnold, Lich. Exs. 760] (O L-1318, isotype). **Japan, Pref. Shizuoka:** Honshu, Shizuoka-shi, Mibu-glacial cirque (Kar) of Mt Mibu neighbouring Mt Ainotake 35°64'N, 138°22'E, ca 1800–1850 m alt., 2008-08-19, Inoue 32329 (TNS).

Lecidea leucothallina var. *discrepans*: **Norway, Hordaland:** Ulvik, Umgebung von Finse, sehr lange schneebedeckte, stark verwitterte, etwas erzhaltige kleinere Schieferblöcke im groben Blockschutt am SE-Hang des L. Finsenu, 1300 m alt., 1974-08-25, Hertel 15224 (O L-109232, isotype).

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