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Are widespread morphospecies from the *Lecanora dispersa* group (lichen-forming Ascomycota) monophyletic?

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Abstract. To evaluate the current delimitation of broadly distributed morphospecies from the *Lecanora dispersa* group, the nuclear ribosomal internal transcribed spacer region (ITS1, 5.8S and ITS2) was analyzed phylogenetically and compared to phenotypic data variation within and among species. Phylogenetic relationships among 34 individuals representing eight species from the *L. dispersa* group, collected mainly from Poland and other European countries, were inferred using two types of Bayesian analyses (with and without *a priori* alignments), maximum likelihood and maximum parsimony approaches. The highest phylogenetic resolution and the largest number of significantly supported internodes resulted from the Bayesian analysis without *a priori* alignment. Inferred phylogenies confirmed a broader delimitation of the *L. dispersa* group, to include four additional lobate taxa: *L. contractula*, *L. pruinosa*, *L. reuteri*, and *L. thuleana* (= *Arctopeltis thuleana*). *Lecanora crenulata, L. dispersa*, *L. reuterii*, and the core of *L. albescens* and *L. semipallida* were all found to be monophyletic with high support (by at least one phylogenetic analysis) except the first species. Based on the ITS region, phenotypically similar individuals, thought to belong to one monophyletic group, were found to belong to multiple distantly related groups (e.g., members of *L. albescens* and *L. hagenii*), suggesting that morphological, anatomical and chemical characters may not be consistent in predicting species boundaries within the *L. dispersa* group. Potential undescribed species were found within phenotypically defined *L. albescens* and *L. semipallida.* Phylo-taxonomic studies of the *L. dispersa* group with more loci and a more extensive taxon sampling are urgently needed.

Keywords. Bayesian analyses, ITS, molecular phylogenetics, morphology, secondary compounds, systematics.

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Lecanora Ach. represents one of the largest genera of lichenized ascomycetes and traditionally includes species that are characterized by hyaline, non-septate ascospores, *Lecanora*-type asci, the presence of thalline apothecial margins (lecanorine apothecia),

and predominantly crustose, rarely lobate, thalli containing green-algal photobionts (species of *Trebouxia* and related genera). According to a recent estimation, the genus comprises ca. 552 species (Kirk et al. 2008).

Within the genus *Lecanora*, several groups have been recognized at various taxonomic levels (e.g., Eigler 1969; Motyka 1995, 1996). Only selected groups have been critically studied, such as the subgenus *Placodium* with its numerous sections (Poelt 1958; Ryan 1989a,b, 1998; Ryan & Nash 1993, 1997). Species outside this subgenus were traditionally recognized as members of informal groups based on morphological, anatomical and chemical characters: *L. dispersa* group, *L. polytropa* group, *L. rupicola* group, *L. subfusca* group and *L. varia* group (including *L. saligna* and related taxa). Except for the *L. polytropa* group (see Śliwa & Flakus 2011), the taxonomy of each group was revised (e.g., Brodo 1984; Dickhäuser et al. 1995; Fröberg 1997; Guderley 1999; Laundon 2003a,b; Leuckert & Poelt 1989; Lumbsch 1994; Lumbsch et al. 1997; Martinez & Aragón 2004; Poelt et al. 1995; Printzen 2001; Śliwa 2007a,b; Śliwa & Wetmore 2000; van den Boom & Brand 2008). Subgenus *Placodium*, the *L. rupicola* and *L. varia* groups were circumscribed using phylogenetic inferences based on the nuclear ribosomal internal transcribed spacer (ITS) region (Arup & Grube 1998; Grube et al. 2004; Pérez-Ortega et al. 2010).

The *Lecanora dispersa* group was traditionally characterized by thalli generally growing within rocks (endolithic) or bark (endophloedic) or rarely on the surface of these substrates, apothecia with mostly white thalline margins, and the presence of xanthones (sometimes accompanied by minor quantities of pannarin or gyrophoric acid) or the lack of detectable lichen products by thin layer chromatography (TLC). This group, currently comprising 29 species (Śliwa, unpubl.), is considered one of the most taxonomically challenging species complexes within *Lecanora* (Laundon 2003b).

Despite the fact that many members of this group, including the type species *L. dispersa* (Pers.) Sommerf., are very common and have worldwide distributions, their taxonomy was poorly understood and nomenclature not well-established for decades. Several studies focused on regional revisions or selected species: Poelt et al. (1995) on species of the Eastern Alps, Fröberg (1989, 1997) on species from Southern Sweden, Śliwa and Olech (2002) and Castello (2003) on several Antarctic species. Laundon (2003b) briefly discussed the entire *L. dispersa* group and provided suggestions for some taxonomic and nomenclatural changes, as well as a detailed description of one neglected member of the complex, *L. zosterae,* in the British Isles.

Several basic nomenclatural corrections and taxonomic changes within the *L. dispersa* group were provided by Śliwa (2006, 2007b), and Śliwa and Hawksworth (2006), followed by a comprehensive monographic study of 19 species from this group in North America (Śliwa 2007a). Based on combinations of diagnostic features of epithecial granules, paraphyses, amphithecial cortices, ascospores, and specific secondary compounds, Śliwa (2007a) classified these species into four operational subgroups: the *L. dispersa* group *s. str.*, the *L. semipallida* group, the *L. crenulata* group, and the *L. hagenii* group. Recently, two new species, *L. schofieldii* Brodo and *L. antiqua* J.R. Laundon, were added to the *L. dispersa* group (Brodo 2010; Laundon 2010).

Despite continuous progress toward resolving the taxonomic diversity of the *L. dispersa* complex, relationships among species remained largely ambiguous. Arup & Grube (1998) reconstructed the relationships among lobate *Lecanora* species (subgenus *Placodium*) in a broad phylogenetic context within the genus, including selected representatives from the *L. dispersa* group. Crustose members of the *L. dispersa* group (*L. albescens* (Hoffm.) Flörke, *L. flotoviana* auct., and *L. perpruinosa* Fröberg) composed a moderately supported clade together with lobate taxa (the monotypic genus *Arctopeltis thuleana* Poelt, *L. contractula* Nyl., *L. pruinosa* Chaub., and *L. reuteri* Schaer.), within the lineage of xanthone-containing *Lecanora* representatives.

The main goals of this study were to evaluate the current delimitation of selected morphospecies within the *Lecanora dispersa* group and to reconstruct relationships among them using phylogenetic inferences (Bayesian, maximum

likelihood and maximum parsimony inferences) based on DNA sequences from the ITS region. We sequenced the ITS of 34 individuals from the five most widespread species of the complex (*L. albescens*, *L. crenulata* Hook., *L. dispersa* (Pers.) Sommerf., *L. hagenii* (Ach.) Ach., *L. semipallida* H. Magn.), single individuals from three other species (*L. andrewii* B. de Lesd., *L. pruinosa*, *L. reuteri*) and added nine ITS sequences from GenBank representing members of the *L. dispersa* group. The ITS data were complemented with 15 phenotypic characters for the maximum parsimony analysis.

Material and Methods

Taxon sampling. For this study, fresh specimens of members of the *Lecanora dispersa* group were collected mainly in Europe (Poland, Slovakia, Estonia, Hungary, and Belgium) in 2009 (**Table 1**). The entire ITS region (ITS1, 5.8S and ITS2) was sequenced for 34 specimens representing eight putative *Lecanora* species. These sequences were complemented by nine GenBank accessioned sequences, representing six *Lecanora* species, *A. thuleana* and one uncultured *Lecanora* clone from the *L. dispersa* group. For the phylogenetic analyses, we selected members of the *L. subfusca* group (*L. allophana* and *L. epibryon*) as the outgroup taxa based on the phylogenetic study of Arup and Grube (1998). A total of 43 new ITS sequences (when all sequenced clones were included) were generated for this study (**Table 1**).

DNA isolation, sequencing and sequence alignment. Genomic DNA was isolated from specimens using a modified protocol based on Zolan and Pukkila (1986) with 2% sodium dodecyl sulphate (SDS) as extraction buffer. Isolated DNA was resuspended in sterile water and stored at −20°C. Symmetric polymerase chain reactions (PCR) were prepared for a 25.0 µL final volume containing 13.85 µL of sterile double-distilled water, 2.5 µL of 10 × Taq polymerase reaction buffer, 2.5 µL of 2.5 mM dNTPs, 0.15 µL of Red Hot® DNA Polymerase from ABgene® (ABgene Inc., Rochester, New York, USA), 1.25 µL for each of the 10 µM primers ITS1F or NS24R and ITS4 primers (Gardes & Bruns 1993; Miadlikowska et al. 2003; White et al. 1990), 2.5 µL of 10 mg/ml bovine serum albumin (BSA; BioLabs) and 1 µL of template genomic DNA (1:10 dilution). PCR was performed under the following conditions: one cycle of 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C. A final extension step of 10 min at 72°C was added, after which the samples were kept at 4°C. Due to the presence of multiple peaks in some initial chromatograms, cloning was required for PCR products of three samples (**Table 1**) and was performed with the TOPO TA Cloning® Kit (Invitrogen™, life technologies, Carlsbad, CA, USA) following the manufacturer's protocol. Eight clones per sample were selected for sequencing. Amplified PCR products were purified with the QIAquick PCR purification Kit (Qiagen, Valencia, CA, USA) or Exo-SAP (Exonuclease I and Shrimp Alkaline Phosphatase, USB Corporation, Cleveland, Ohio, USA) prior to automated sequencing using BigDye Terminator v3.1 (ABI PRISM, Perkin-Elmer Biosystems, Wellesley). Sequencing reaction conditions followed Miadlikowska et al. (2003).

Sequence fragments were subjected to BLAST searches to confirm the identity of each sequence fragments. They were assembled and edited by hand using the software package Sequencher™ 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned manually with MacClade 4.07 (Maddison & Maddison 2003).

Phenotypic data. Fifteen morphological, anatomical and chemical characters were scored as described in Śliwa (2007a) for each specimen collected for this study. Specimens that were not seen (i.e., vouchers for sequences taken from GenBank) were not scored. The fifteen characters included in the morphological data matrix (**Fig. 1**) are listed in **Table 2**.

Phylogenetic analyses. Phylogenetic analyses were performed on the 55-OTU dataset using Bayesian approach as implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) and BAli-Phy (Suchard & Redelings 2006) as well as bootstrap analyses using the maximum likelihood (ML) optimization criterion as implemented in RAxML-VI-HPC (Stamatakis 2006a), and maximum parsimony (MP) optimization criterion as implemented in PAUP* 4.0b10 (Swofford 2003).

We conducted Bayesian analysis on the ITS data set (PP1), divided into three partitions (ITS1, 5.8S,

Table 1. Voucher information (including country, region, substrate and date when specimens were collected, collector name, and collection number) for *Lecanora* specimens used for this study. All specimens are deposited in KRAM. GenBank accession numbers are provided for sequences generated for this study, whereas GenBank identification (GI) numbers are listed for sequences obtained from GenBank. *Lecanora dispersa* 8A–D, *L. hagenii* A–C, and *L. semipallida* 4A–E, represent different clones within each of the tree individuals.

Taxon	Voucher	GenBank GI/Accession
Arctopeltis thuleana = *L. thuleana*		11878043
Lecanora albescens 1	POLAND, Kraków, limestone, Jan. 2009, *Śliwa 4086*	JQ993715
L. albescens 2	SLOVAKIA, Stara Lubovnia, Feb. 2009, *Śliwa 4105*	JQ993716
L. albescens 3	POLAND, Kraków, concrete, Jan. 2009, *Śliwa 4090*	JQ993717
L. albescens 4	POLAND, Rzeszów, concrete, Feb. 2009, *Śliwa 4096*	JQ993718
L. albescens 5	BELGIUM, Brussels, concrete, Jan. 2009, *Safin*	JQ993719
L. albescens 6	ESTONIA, Tartu, concrete, Jan. 2009, *Jûrîado*	JQ993720
L. albescens 7	POLAND, Poznań, concrete, Aug. 2008, *Zarabska*	JQ993721
L. albescens 8	POLAND, Olsztyn, concrete, Jan. 2009, *Kubiak*	JQ993726
L. albescens 9	POLAND, Olsztyn, concrete, Jan. 2009, *Kubiak*	JQ993727
L. albescens 1		6524435
L. andrewii	ESTONIA, Höralaid, rock, July 2007, *Suija*	JQ993722
L. contractuala		6524434
L. crenulata 1	POLAND, Pieniny Mts, limestone, Feb. 2009, *Śliwa 4111*	JQ993723
L. crenulata 2	SLOVAKIA, Stara Lubovnia, limstone, Feb. 2009, *Śliwa 4106*	JQ993724
L. crenulata 3	POLAND, Kraków, limestone, Jan. 2009, *Śliwa 4087*	JQ993725
L. dispersa 2	POLAND, Szczecin, concrete, Jan. 2009, *Wieczorek*	JQ993728
L. dispersa 6	HUNGARY, Budapest, wood, Jan. 2009, *Lőkös*	JQ993729
L. dispersa 8A	HUNGARY, Budapest, bark, Jan. 2009, *Lőkös*	JQ993730
L. dispersa 8B		JQ993731
L. dispersa 8C		JQ993732
L. dispersa 8D		JQ993733
L. dispersa 9	HUNGARY, Budapest, rock, Jan. 2009, *Lőkös*	JQ993734
L. dispersa = *L. semipallida*		162950873
L. flotoviana = *L. semipallida*		6524436
L. hagenii 2	HUNGARY, Budapest, bark, Jan. 2009, *Lőkös*	JQ993735
L. hagenii 3A	IRAN, Gorgan, bark, Oct. 2007, *Sohrabi*	JQ993736
L. hagenii 3B		JQ993738
L. hagenii 3C		JQ993737
L. hagenii 6	HUNGARY, Budapest, wood, Jan. 2009, *Lőkös*	JQ993739
L. perpruinosa		6524427
L. pruinosa		6524420
L. pruinosa 1	POLAND, Pieniny Mts, limestone, Feb. 2009, *Śliwa 4112*	JQ993740
L. reuteri		6524428
L. reuteri 1	POLAND, Pieniny Mts, limestone, Feb. 2009, *Śliwa 4113*	JQ993741
L. semipallida 1	POLAND, Kotlina Żywiecka basin, rock, Sept. 2005, *Wilk 3898*	JQ993742
L. semipallida 2	POLAND, Olsztyn, concrete, Jan. 2009, *Kubiak*	JQ993746
L. semipallida 3	POLAND, Kotlina Żywiecka basin, rock, Sept. 2005, *Wilk 3884*	JQ993747
L. semipallida 4A	POLAND, Śnieżka Mt., concrete, Sept. 2008, *Śliwa 4080*	JQ993752
L. semipallida 4B		JQ993748
L. semipallida 4C		JQ993750
L. semipallida 4D		JQ993751
L. semipallida 4E		JQ993749
L. semipallida 5	POLAND, Rzeszów, concrete, Feb. 2009, *Śliwa 4095*	JQ993753
L. semipallida 6	POLAND, Lublin, concrete, Nov. 2008, *Wójciak*	JQ993754
L. semipallida 7	SLOVAKIA, Gerlachov, concrete, Feb. 2009, *Śliwa 4102*	JQ993755

Table 1. Continued

Taxon	Voucher	GenBank GI/Accession
L. semipallida 8	Slovakia, Tatrzańska Łomnica, concrete, Aug. 2005, *Śliwa 3512*	JQ993756
L. semipallida 9	Hungary, Siófok, Sóstó, concrete, Jan. 2009, *Lőkös*	JQ993757
L. semipallida 10	Estonia, Tõrvandi, concrete, Jan. 2009, *Jůrîado*	JQ993743
L. semipallida 11	Poland, Kielce, concrete, Feb. 2009, *Łubek*	JQ993744
L. semipallida 12	Peru, Cañon del Colca, Nov. 2008, *Flakus 10096*	JQ993745
Uncultured *Lecanora* clone		239923347
Outgroup		
L. allophana		6524433
L. allophana		6524416
L. epibryon		47088201

and ITS2), using the software package BAli-Phy (Suchard & Redelings 2006). The analysis was performed on a non-censored alignment sampled from the joint posterior distribution of phylogeny, alignment, and model parameter. This approach takes into account alignment uncertainty by integrating over space all alignments in proportion to their posterior probability (Gaya et al. 2011, B2 analysis; Redelings & Suchard 2005). The alignment for 5.8S sequences was held constant, while the alignment for ITS1 and ITS2 was allowed to vary through the Markov chain Monte Carlo (MCMC) process. The substitution model parameters for each partition were Tamura-Nei (Tamura & Nei 1993) with gwF formulation (Goldman & Whelan 2002) and Dirichlet-distributed rate variation with 3 categories (Gaya et al. 2011, B2 analysis), except that the ITS1 and ITS2 partitions were constrained to have the same parameters. The relative substitution rate for each partition was allowed to vary independently. The alignment algorithm for the ITS1 and ITS2 partitions followed the RS07 indel model with the same parameters. The ratio of the indel rate to the substitution rate was estimated to be 0.0904 according to the posterior median, with a 95% Bayesian credible interval (BCI) of 0.0651, 0.124. The posterior median estimate of the mean indel length was 2.66 with a 95% BCI of 2.18, 3.34. We sampled

Table 2. List of morphological and chemical characters scored for the specimens of the *Lecanora dispersa* group and the outgroup included in this study.

1. Thallus: 0 = crustose; 1 = rosulate.
2. Apothecial margin: 0 = continuous; 1 = discontinuous (cracked or undulate).
3. Apothecial pruina: 0 = absent; 1 = present.
4. Apothecial cortex: 0 = conglutinated hyphae to amorphous structure; 1 = prosoplectenchyma; 2 = paraplectenchyma.
5. Cortical granules: 0 = sparse; 1 = abundant.
6. Epithecial granules and their visibility in polarized light: 0 = absent; 1 = present, positive; 2 = present, negative.
7. Epithecial granules and their solubility in K (25% water solution of KOH): 0 = absent; 1 = present, insoluble; 2 = present, soluble.
8. Epithecial granules and their solubility in N (65% nitric acid): 0 = absent; 1 = present, insoluble; 2 = present, soluble.
9. Paraphyses: 0 = unbranched, free in K; 1 = branched, coherent in K.
10. Ascospores: 0 = broadly ellipsoid (mean length to width ratio [Q] = 1.2–1.8); 1 = ellipsoid (Q=1.9–2.3) to narrowly ellipsoid (Q=2.4–3.5).
11. Chloroxanthones: 0 = absent; 1 = present.
12. Methylated chloroxanthones: 0 = absent; 1 = present.
13. Pannarin: 0 = absent; 1 = present.
14. Atranorin: 0 = absent; 1 = present.
15. Substratum: 0 = corticolous or musciclous; 1 = saxicolous.

100,000 iterations from each of eight MCMC chains, and discarded the first 45,000 iterations as burnin. The Potential Scale Reduction Factor (PSRF) based on the width of 80% credible intervals was ≤ 1.014 for all variables, which suggests that different MCMC chains did not get trapped in different local optima. The effective sample size (ESS) based on the combined samples was greater than 3488 for all scalar variables, and was greater than 400 for all splits when treating support for each split as a binary variable. The average standard deviation of split frequencies (ASDSF) was 0.012, suggesting that enough samples were obtained. The maximum standard deviation of split frequencies (MSDSF) was 0.051.

For Bayesian phylogenetic analyses with MrBayes (PP2), separate models of evolution were estimated for the, ITS1, 5.8S and ITS2 regions using the Akaike Information Criterion (AIC) as implemented in MrModeltest 2.3 (Nylander 2004). A six-rate parameter model for nucleotide substitutions (GTR+I+Γ, Rodríguez et al. 1990) with a gamma distribution approximated with four categories, and a proportion of invariable sites was used for all data partitions except the 5.8S, for which a two-parameter model with a proportion of invariable sites (K80+I, Kimura 1980) was implemented. Bayesian analyses were performed with four independent chains for 50,000,000 generations, sampling every 500th tree. Two independent Bayesian runs were conducted to ensure that stationarity was reached and the runs converged at the same log-likelihood level (verified by eye and with the AWTY [Are We There Yet?] option; Nylander et al. 2008; Wilgenbusch et al. 2004). After discarding the burn-in, the last 60,000 trees of each run were used to calculate a 50% majority-rule consensus tree.

In addition to Bayesian posterior probabilities, phylogenetic confidence was estimated using maximum likelihood bootstrap proportions (ML-BS) calculated with 1000 bootstrap pseudoreplicates using RAxML implementing the GTRCAT model with gamma distribution, approximated with four categories (Stamatakis 2006b). For the MP bootstrap analyses (MP-BS), unambiguously aligned sites of the ITS1, 5.8S, and ITS2 were subjected to three step-matrices (one for each data partition) including gaps as the fifth character state. Symmetric step matrices were created as follows: the options SHOW CHARACTER STATUS/FULL DETAILS/HIDE EXCLUDED CHARACTERS from the Data menu in PAUP* were selected. From the resulting table, the "States" column showing all nucleotide states found at each of the unambiguously aligned variable sites was saved as a separate text file. This file was then used as input for STMatrix 3.0 (Lutzoni & Zoller, Dept. of Biology, Duke University; http://www.lutzonilab.net/downloads), which generates a step matrix (in Nexus format) by calculating frequencies of reciprocal changes from one state to another and converting them into costs of changes using the negative natural logarithm of the frequencies (Felsenstein 1981; Wheeler 1990). Phylogenetic signal from ambiguously aligned regions was recovered without violating positional homology using the program INAASE 2.3b. (Lutzoni et al. 2000; http://www.lutzonilab.net/downloads). MP bootstrap analyses were conducted on unambiguously aligned sites of the 55-OTU ITS dataset subjected to three step-matrices generated by STMatrix, twelve recoded INAASE characters with their respective step-matrix, and 15 phenotypic characters. The MP dataset consists of a total of 982 characters, of which 768 characters were excluded (232 ambiguous and 536 constant characters) and 214 variable characters were included (63 parsimony uninformative and 151 parsimony informative characters). All 15 phenotypic characters were unordered and equally weighted (1.0), except three phenotypic characters related to epithecial granules (characters 6, 7, and 8), which were down-weighted (0.3) because they are not independent. Multistate taxa (0&1, **Fig. 1**) were interpreted as polymorphic during the MP search. MP bootstrap analyses (were completed by implementing 1000 bootstrap pseudoreplicates with 20 RAS per bootstrap replicate and saving only 10 trees from each replicate due to the large number of equally most parsimonious trees. The number of RAS per bootstrap replicate was calculated taking into consideration the frequency at which the shortest tree was found during the initial heuristic search when we examined how often the most parsimonious tree was found out of 1000 replicates.

Bootstrap proportions (MP-BS and ML-BS) ≥ 70%, and posterior probability values (PP1, resulting from BAli-Phy, and PP2, resulting from MrBayes

analyses) ≥ 95%, were considered significant. The nexus file for the MP analysis was deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/12681).

Results and Discussion

All phylogenetic analyses (Bayesian, ML and MP) recovered congruent trees for internodes with bootstrap support ≥ 70%, but with a high degree of variation in phylogenetic resolution and support (majority rule consensus tree from BAli-Phy is shown in **Fig. 1**). Among the four searches the highest phylogenetic resolution and the largest number of significantly supported internodes was obtained by using BAli-Phy (i.e., 23 internodes, compare to 14 for ML, 13 for PP2, and 11 for MP). Eight internodes were strongly supported exclusively by the PP1 analysis (≥ 0.95), and one internode by each, the ML and MP (≥ 70%) analyses. Fourteen of the total 34 internodes obtained significant support from at least two of the four different methods we applied for estimating phylogenetic confidence (**Fig. 1**).

The *Lecanora dispersa* group is monophyletic and significantly supported by all inferences (**Fig. 1**) when including four lobate taxa (*L. contractula*, *L. pruinosa*, *L. reuteri* and *Arctopeltis thuleana*), in addition to broadly accepted crustose members. This broader delimitation of the group, has previously been recovered by Arup and Grube (1998) based on ITS data, and was suggested by Feige and Lumbsch (1998) based on ascomata ontogenies (similarities of *L. contractula* and *A. thuleana* with *L. dispersa*). Individuals with lobate or semi-lobate thalli occur also in another species, *L. albescens,* which has always been considered as a member of the *L. dispersa* group. In addition, peltate apothecia similar to those of *A. thuleana* are known from another member of the group, *L. zosterae* (Ach.) Nyl. (not included in our analyses). The lack of a crystalline epithecium in *A. thuleana* and *L. contractula* (Poelt 1983) is another feature supporting a close relationship between these two species and *L. zosterae*. Moreover, the secondary metabolites detected in *L. contractula* (major: 2,5-dichloro-6-*O*-methylnorlichexanthone; minor: 5-chloro-6-*O*-methylnorlichexanthone and 5-chlorolichexanthone; Elix & Crook 1992) and anticipated to be present also in *A. thuleana* due to the same type of reaction with C and K, support their placement in the *L. dispersa* group. *Arctopeltis thuleana*, formerly regarded as a variety of *L. contractula*, represents a monotypic genus introduced by Poelt (1983). Morphological, chemical and developmental similarities between *A. thuleana* and *L. contractula* and other members of the *L. dispersa* group, as well as their placement inside the group (**Fig. 1**), provide strong evidence for reverting *Arctopeltis* to *Lecanora*. Accordingly, the following new combination is necessary: ***Lecanora thuleana*** (Poelt) Śliwa, ***comb. nov.*** (≡ *Arctopeltis thuleana* Poelt, Int. J. Mycol. Lichenol. 1(2): 147, 1983; MycoBank No.: MB 800267).

In this study we confirm, with high confidence, the monophyly of a core *L. albescens* (i.e., except *L. albescens* 5, 8 and 9) recognized here as *L. albescens s.str.*, a core *L. dispersa* (i.e., except *L. dispersa* 9), a core *L. reuteri* and *L. semipallida* (except *L. semipallida* 12) recognized here as *L. semipallida s.l.* because of potential presence of underscribed species. Monophyly of *L. albescens s.str.* obtained high support from all analyses. This species is characterized by a combination of external areolate thallus, often forming distinct rosettes and usually slightly lobate at the margins, and presence of *dispersa*-type granulation in the epithecium (granules are insoluble in K and N and are found throughout the hymenium). *Lecanora albescens* was considered closely related to *L. dispersa* based on similarities in anatomical and chemical characters (Śliwa 2007a). Although their sister relationship received significant support only from PP1, both taxa are part of strongly supported (all but MP-BS) monophyletic group together with non-monophyletic *L. pruinosa*, *L. andrewii* and *L. hagenii* 6.

Although, delimited as monophyletic, *Lecanora dispersa s.str.,* excluding *L. dispersa* 9, received strong phylogenetic support from all analyses except MP-BS (**Fig. 1**). Members of *L. dispersa s.str.* are recognized by having a thallus immersed in the substrate or indistinct and granulose, and epithecial granules (often localized or also found throughout the hymenium), which are insoluble in K and N. The major secondary metabolite, 2,7-dichlorolichexanthone, often co-occurs with minor pannarin that gives a PD+ orange reaction on the

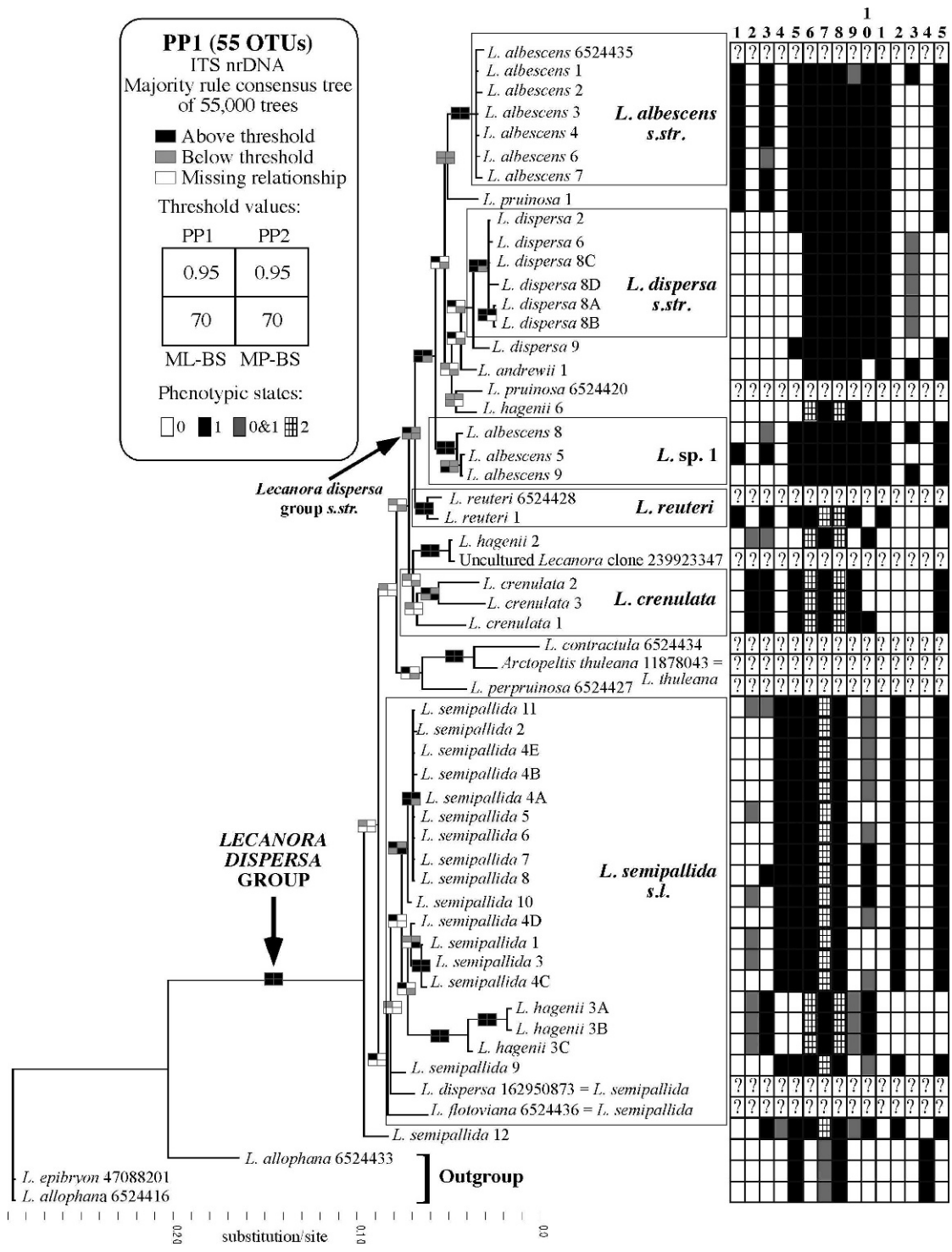

Figure 1. Phylogenetic relationships among 55 individuals representing 12 putative *Lecanora* species from the *L. dispersa* group *s.l.* and three outgroup OTUs (*L. allophana* and *L. epibryon*) resulting from the Bayesian analysis without a priori alignment (BAli-Phy; majority rule consensus tree of 55,000 trees; PP1). Four-cell grids indicate internode support in terms of posterior probability based on Bayesian inferences using BAli-Phy (PP1), or MrBayes (PP2), and in terms of bootstrap frequency (BS) under MP or ML analysis (see text for details). Table to the right shows coded character states for 15 phenotypic characters (see **Table 2**) for each specimen included in the combined MP analysis.

apothecial margin, which is a diagnostic character for many specimens (individuals without detectable secondary metabolites are also frequent). It is one of the most widely distributed and morphologically variable species (especially in terms of size, color and shape of apothecia, as well as thallus habit) of the complex. *Lecanora dispersa* 9, the specimen placed sister to the monophyletic highly supported core of *L. dispersa* (without significant support) has the typical morphology and chemistry of the species *s.str.*, but is the only one collected from a natural limestone substrate, whereas all remaining specimens occurred on concrete, bark and wood.

Lecanora reuteri, represented in the phylogeny by only two sequences, is highly supported as monophyletic. The species differs from most members of the *L. dispersa* group because of its placodioid thallus divided into wide, convex, dense and often curved lobes. The color of the thallus is diagnostic – whitish yellow with a delicate pinkish tint (distinctly ochraceous when wet) and the thallus surface is pruinose and often distinctly scabrid. *Lecanora reuteri* produces detectable xanthones: arthothelin and dichloronorlichexanthone, therefore, the thallus has a positive color reaction with C (orange) and K (pale orange). The morphology and chemistry of *Lecanora reuteri* closely resemble those of *L. pruinosa* (a non-monophyletic species in our phylogeny; **Fig. 1**), however, *L. reuteri* has a considerably smaller and thicker thallus consisting of very convex lobes. They also differ in the type of epithecium and the spot test reaction of the thallus (compare Clauzade & Roux 1985 with Roux 1976). Our phylogeny confirmed with a high confidence a separation of these two species (**Fig. 1**).

A monophyletic *Lecanora semipallida s.l.* is supported only by Bayesian posterior probabilities (PP1) and includes, in addition to specimens collected for this study, two perhaps misidentified sequences from GenBank (*L. dispersa* 162950873 and *L. flotoviana* 6524436; **Fig. 1**; **Table 1**) and one *L. hagenii* specimen (represented by three clones 3A–C). It is possible that *L. dispersa* was identified and the sequence submitted to GenBank (in 2007) prior to the separation of *L. semipallida* from *L. dispersa s.str.* (Śliwa 2007a). As noticed by Śliwa (2007a) during the revisionary work of the *L. dispersa* group, *L. semipallida* has more distinct apothecia and is more abundant in the field, therefore, is more likely to be collected than *L. dispersa*. *Lecanora semipallida* is the correct name for the common and widespread member of the *L. dispersa* group to which the name *L. flotoviana* (auct. non Spreng.) had been misapplied (Śliwa 2007b; the second individual retrieved from GenBank). Diagnostic characters used to delimit *L. semipallida* include the presence of epithecial granules that are soluble in K, and vinetorin (5-chloro-3-*O*-methylnorlichexanthone) resulting in positive spot tests and UV reactions of the apothecial disc. Morphologically, *L. semipallida* is a highly variable species especially in terms of size, shape and coloration of apothecia and, therefore, is likely to be confused with *L. dispersa*. Despite high morphological similarity, ecological preferences (both taxa tend to grow together in calcium-containing substrates), and identical distribution patterns, *L. dispersa* can be distinguished from *L. semipallida* anatomically and phylogenetically (**Fig. 1**). However, the unexpected placement of *L. hagenii* 3 within *L. semipallida* suggests much broader than anticipated morphological and chemical delimitation of the latter species, or presence of multiple taxonomic entities, which cannot be disentangled based on current data.

Although monophyletic, no support was recovered for *Lecanora crenulata* (including *L. crenulata* 1), a species growing on limestone, preferably in natural habitats. It is characterized by an endolithic thallus, slightly or heavily pruinose to scabrose apothecia with prominent and usually thick, cracked (with 3–5 fissures) or crenate, epruinose or pruinose, white margins. Epithecial granules are insoluble in K and soluble in N. Lichen secondary products were not detected in this species. Based on morphology (discontinuous, crenate apothecial margin and pruinose disc) and chemistry (lack of lichen products), *L. crenulata* was assumed to be closely related to *L. hagenii* (Śliwa 2007a). If true, then our phylogeny suggests that *L. hagenii* 2, together with Uncultured *Lecanora* clone 239923347, represent *L. hagenii s. str.* whereas other individuals of *L. hagenii*, as defined morphologically, may represent different taxa (**Fig. 1**).

Lecanora pruinosa, represented by two specimens was found to be non-monophyletic, but it's polyphyly is not supported. The species is characterized by a well-developed, placodioid, white, whitish gray or pale yellow, heavily pruinose thalli, numerous, clustered or scattered apothecia, which cover the middle part of the thallus and never reach the margins. An important diagnostic character for this species is the color reaction of the thallus in response to C (orange or reddish) and sometimes K (yellow) due to the presence of xanthone-type lichen compounds (arthothelin and 2,7-dichloronorlichexanthone; Edwards et al. 2009). *Lecanora pruinosa* resembles *L. reuteri* (see discussion above) and its general appearance can also be similar to another, much more frequent species, *L. albescens*. However, *L albescens* has a far less pruinose thallus and does not react with C.

Delimitation of *Lecanora hagenii* is problematic as members of this morphospecies are distributed across the phylogeny (**Fig. 1**). Some authors consider *L. hagenii* as a corticolous form of *L. dispersa* (Poelt et al. 1995), others note the frequent occurrence of intermediate forms between these two taxa (Laundon 2003b). Before Śliwa (2007a), *L. hagenii* was always assumed to be most closely related to *L. dispersa*, from which it differs morphologically by having a thin, even, regular apothecial margin surrounding a small brown disc and growing on bark and wood (Laundon 2003b). In the revisionary work by Śliwa (2007a), *L. hagenii* was separated from *L. dispersa* based on the following anatomical characters: 1) a lack of *L. dispersa*-type epithecial granules; 2) paraphyses that are simple, thicker, expanded apically and ± free in K; 3) more narrowly ellipsoid spores; and 4) a lack of detectable secondary compounds. Contrary to some authors (e.g. Poelt et al. 1995; Wirth 1995), these two taxa do not differ substantially in their substratum preferences. Although *L. dispersa* is generally saxicolous and *L. hagenii* corticolous, both taxa grow on other substrates, e.g., bark of dust-contaminated trees, other lichens, man-made substrates including concrete, mortar, bricks, asbestos and metal.

Lecanora andrewii (represented by a single specimen in our tree; **Fig. 1**) is considered to be rare. It is a maritime species characterized by a thallus that is clearly visible, and consists of dispersed to aggregated areoles (especially in the vicinity of apothecia). It has mostly aggregated apothecia with pale brown to reddish brown or blackish epruinose or slightly pruinose discs and margins paler than the thallus and disc, often whitish. The majority of granules in the epithecium are insoluble. Presence of *L. dispersa*-type granules in the epithecium and the frequent presence of pannarin in addition to xanthones (*L. andrewii*: arthothelin and 2,7-dichlorolichexanthone) support a close relationship of this species to *L. dispersa s. str.* as shown on **Fig. 1**, however, without significant support.

Cryptic and semi-cryptic taxonomic entities, including potential undescribed species, were revealed within the *Lecanora dispersa* group. Although monophyletic and strongly supported, *L. albescens* 5, 8, and 9 (*L.* sp. 1; **Fig. 1**) were phylogenetically resolved outside the core of *L. albescens*. No phenotypic trait, currently used within the *L. dispersa* group, supports this monophyletic group. The general morphology of *L. albescens* 5 (collected in Belgium) and *L. albescens* 8 and 9 (collected from a single locality in N Poland) corresponds to typical *L. albescens* (e.g., relatively large and pruinose apothecia), except for their reduced thalli. *Lecanora semipallida* 12, which is placed outside of *L. semipallida s.l.* (**Fig. 1**) was collected in South America and has a unique combination of phenotypic features: yellowish to vivid brown heavily pruinose apothecia (the pruina gives apothecia a bluish appearance), the amphithecial cortex that differs from typical *L. semipallida*, and paraphyses that are simple but not free in K. Collections from a broader geographical range are necessary to circumscribe this potentially new species.

In general, phylogenetic relationships among species within the *L. dispersa* group were poorly supported. A close affiliation of the following species/specimens was, however, inferred with significant support from one or more analyses: *L. albescens s.str.* + *L. dispersa s.str.* + *L. andrewii* + *L. pruinosa* 6524420 and *L. hagenii* 6 + *L.* sp.1. + *L. reuteri*. All these species/specimens (except *L. hagenii* 6) produce chloroxanthones (2,7-dichlorolichexanthone, 2,7-dichloronorlichexanthone, 2,4,5-

trichloronorlichexanthone) as major secondary metabolites and most of them were considered by Śliwa (2007a) as the *L. dispersa* group *s.str.* (**Fig. 1**). *Lecanora pruinosa* and *L. reuteri* are two additional taxa revealed by our phylogeny to be part of this group.

Phylogenetic placements of all remaining species/specimens in the current study are unsettled outside the *Lecanora dispersa* group *s.str.* They are chemically diverse by containing methylated chloroxanthones (5-chloro-3-*O*-methylnorlichexanthone, 2,5-dichloro-6-*O*-methylnorlichexanthone) as major lichen compounds (*L. thuleana, L. contractula,* and *L. semipallida*) or lacking lichen products (*L. crenulata, L. hagenii,* and *L. perpruinosa*). Based on a combination of morphological, anatomical and chemical features, however, they correspond to three groups of taxa within the *L. dispersa* complex: *L. crenulata* group, *L. hagenii* group, and *L. semipallida* group (Śliwa 2007a).

It is very likely that three different clones within *Lecanora hagenii* 3 (A–C) and four within *L. dispersa* 8 (A–D) represent different ITS copies within each individual. Clones of *L. semipallida* 4 (A–E) are intermixed with other individuals and divided into two separate highly supported monophyletic groups. However, the complex genetic structure within *L. semipallida* depicted by relatively long branches and diverse morphology (**Fig. 1**) may also suggest the presence of multiple, genetically different mycobionts in a single thallus of *L. semipallida*, or of multiple taxonomic entities. ITS sequencing of single-spore strains, and more samples in general, are needed for a further taxonomic revision of the *Lecanora dispersa* group.

For this study, incorporating the phylogenetic signal from the ITS region under Bayesian inference without *a priori* alignment (BAli-Phy) was the most phylogenetically powerful method. The resulting phylogeny provided much greater levels of resolution and confidence than all other methods, including MP, which also allowed us to recover signal from ambiguously-aligned regions of the ITS using INAASE (INAASE) and to include phenotypic characters (**Fig. 1**). Future study of the *Lecanora dispersa* group should be based on more characters (other loci), more extensive taxon sampling, including 18 missing species (*L. agardhiana* Ach., *L. antiqua*, *L. expectans* Darb., *L. flowersiana* H. Magn., *L. fugiens* Nyl., *L. invadens* H. Magn., *L. juniperina* Śliwa, *L. mons-nivis* Darb., *L. percrenata* H. Magn., *L. persimilis* (Th. Fr.) Nyl., *L. poeltiana* Clauzade & Cl. Roux, *L. salina* H. Magn., *L. sambuci* (Pers.) Nyl., *L. schofieldii, L. sverdrupiana* Øvst., *L. torrida* Vain., *L. wetmorei* Śliwa, and *L. zosterae*), and more representatives from various localities across the geographical range of each taxon.

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