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A phylogenetic study of the *Lecanora rupicola* group (*Lecanoraceae*, *Ascomycota*)

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A molecular phylogeny of the *Lecanora rupicola* group is presented, based on ITS sequence analyses. The study includes saxicolous and corticolous members of the *Lecanora rupicola* group as well as other *Lecanora* species with pruinose apothecia. A phylogenetic hypothesis for species in *Lecanora* s. lat. and various other genera in *Lecanoraceae*, based on an alignment-free distance estimation technique, shows that the *Lecanora rupicola* group forms a monophyletic clade within *Lecanoraceae*. Affinities to the core group of *Lecanora* are not well supported, likewise the monophyly of *Lecanora* s. str. with other species groups in *Lecanora*, such as the lobate taxa (and *Rhizoplaca*) is not supported. A more detailed analysis involving *Lecanora* species with pruinose apothecial discs was carried out with model-based Bayesian Markov chain Monte Carlo (B/MCMC) tree sampling. The results suggest the monophyly of the *Lecanora* species that are characterized by the presence of chromones. Corticolous as well as saxicolous species are included. *Lepraria flavescens* is closely related to the *Lecanora swartzii* subgroup, and the new name *Lecanora rouxii* nom. nov. is introduced for that species. Other *Lecanora* species with pruinose discs are not closely related to the *Lecanora rupicola* group.

INTRODUCTION

The classification of lichenized genera, as it evolved during the past two centuries, was guided primarily by morphological and chemical characters. This was straightforward since, in contrast to the mycelia of most non-lichenized fungi, lichen thalli provide a number of morphological characters that are easily observable. A higher number of thallus characters is usually found in lichens with a more complicated organization, i.e. with foliose or fruticose growth. This contributed to a present-day situation with numerous well-delimited foliose and fruticose genera, whereas some large crustose genera (e.g. *Arthonia*, *Buellia*, *Caloplaca*, *Lecanora*), are insufficiently circumscribed and understood using anachronistic concepts. Most lichenologists agree that such large crustose genera are heterogeneous, but due to the low number of synapomorphic characters only a few, sometimes monotypic, genera have been split from the large complexes.

Lecanora, a representing the largest order of lichenized fungi, *Lecanorales*, is a perfect example of a large

and heterogeneous crustose genus. Due to the size of the genus (ca 300 spp.; Kirk *et al.* 2001) it is understandable that there exists no recent monograph of the whole genus, except for the posthumous publication of Motyka (1995, 1996a, b, c), which has been rejected as a nomenclatural work. Certain morphologically defined groups, have, however, been studied in detail, for example subgenus *Placodium* (Poelt 1958), the *Lecanora rupicola* group (Leuckert & Poelt 1989), the *Lecanora dispersa* group (Poelt, Leuckert & Roux 1995), corticolous species with pruinose discs (Lumbsch *et al.* 1997), species with a dark hypothecium (Lumbsch, Guderley & Elix 1996), or regional monographs of the *Lecanora subfusca* group (Brodo 1984, Miyawaki 1988, Lumbsch 1994, Jüriado 1998, Guderley 1999). The delimitation and relationships of these groups to others in the genus, could not be resolved by these studies, and requires molecular phylogenetic approaches.

Phylogenetic analyses in *Lecanora* were previously carried out by Arup & Grube (1998, 2000). These studies indicated that certain genera that were segregated from the huge core genus *Lecanora* due to their deviating growth forms, form monophyletic groups

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within *Lecanora*. For example, *Arctopeltis* and *Rhizoplaca* were split from *Lecanora* only due to their foliose growth form, yet the phylogenetic position of these genera within *Lecanora* is supported by secondary compounds: *Arctopeltis* containing chlorinated lichexanthones is placed within the *Lecanora dispersa* group, which is usually rich in xanthones; and *Rhizoplaca*, possessing usnic acid, is related to lobate *Lecanora* species which share this compound. While these two genera are traditionally accepted as well-circumscribed segregates, other species groups are maintained in *Lecanora*.

Beside the xanthone-rich *Lecanora dispersa* group, or various usnic acid containing groups, the *L. rupicola* group is chemically distinct in *Lecanora*. All species assigned to this group are characterized by apothecial discs covered by a conspicuous whitish to yellowish pruina, which is composed of sordidone (Huneck & Santesson 1969, Devlin *et al.* 1971). Members of this group comprise saxicolous crustose lichens, and an earlier monographic study accepted four species (Leuckert & Poelt 1989). Only two of these were included in the previous molecular studies of *Lecanora* by Arup & Grube (1998, 2000).

Sordidone is a unique chromone, which usually occurs together with trace amounts of the dechlorinated accessory compound eugenitol. Chromones are only known from a few other lichens which are not closely related to *Lecanora*, for example *Siphula* and *Haematomma* of the *Lecanorales*, and the *Roccellaceae* of the *Arthoniales*. Certain chromone derivatives are uncommon products also in few non-lichenized fungi (Turner & Aldridge 1983, Fujimoto *et al.* 2003, Lin *et al.* 2003), but so far, sordidone is only known from *Lecanora* and one species assigned to *Lepraria*. Apart from the *Lecanora rupicola* group (*sensu* Leuckert & Poelt 1989), certain bark-inhabiting *Lecanora* species also contain sordidone. The corticolous species with pruinose discs were recently revised by Lumbsch *et al.* (1997), who accepted four species which contain sordidone. One of these, *L. carpinea*, was included by Arup & Grube (1998) who confirmed that this species is closely related to the saxicolous taxa of the *L. rupicola* group.

In this contribution we present a phylogenetic analysis of *Lecanora* species with pruinose discs to test whether they form monophyletic groups with other *Lecanoraceae*, and whether any sordidone-lacking species are closely related to the *Lecanora rupicola* group.

MATERIAL AND METHODS

Specimens

Lichen material for this study (Table 1) was borrowed from the herbarium GZU, from the private herbarium of Ulf Arup, and one specimen also of herbarium TSB.

DNA extraction, amplification, and sequencing

Total DNA was extracted from individual thalli according to a modified CTAB method (Cubero *et al.* 1999) or using the DNeasy Plant Mini Kit (Quiagen, Vienna). DNA-extracts were used for PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA. Alternatively to DNA-extraction, we also used algal-free sections from the lichen medulla for direct amplification in some cases. Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). 50 µl PCR mix (10 mM Tris pH 8.3/50 mM KCl/1.5 mM MgCl₂/50 µg gelatine) contained 1.25 U polymerase (Taq DNA polymerase, Amersham), 0.2 mM of each of the four dNTPs, 0.5 µM of each primer and *ca* 10–50 ng genomic DNA. Products were cleaned using QIAquick PCR Purification Kit (Qiagen). Both complementary strands were sequenced using the BigDye Terminator Ready Reaction Kit (Applied Biosystems, Vienna) according to the manufacturers instructions. Sequences were run on an ABI 310 automated sequencer (Applied Biosystems) and assembled with AutoAssembler (Applied Biosystems).

Table 1 presents a list of specimens for which we sequenced the ITS rDNA. For the following species we retrieved sequences from GenBank: *Biatore helvola* AJ247557, *Biatore subduplex* AJ247540, *Lecanora albescens* AF070033, *L. allophana* AF159939, *L. carpinea* AF070020, *L. garovaglii* AF189718, *L. muralis* AF070015, *L. pruinosa* AF070018, *Lepraria flavescens* AF517889, *Protoparmelia badia* AF070023, *Pyrrhospora quernei* AF517930, *Rhizoplaca chrysoleuca* AF159940, and *R. peltata* AF159925.

The alignment for the B/MCMC analysis was produced using a linear Hidden Markov Model (HMM) implemented in the software SAM (Hughey & Krogh 1996; <http://www.cse.ucsc.edu/research/compbio/sam.html>).

Phylogenetic analyses

To assess the relationship of the *Lecanora rupicola* group in a larger phylogenetic context, we constructed an alignment that included representatives of other genera within *Lecanoraceae*. Ambiguous alignment positions needed to be discarded from further analyses. Data exclusion can be done empirically or using described algorithms (Castresana 2000, Löytynoja & Milinkovitch 2001), which also reduces the information content of the data set. One alternative to data exclusion is a recoding of ambiguous alignment portions (Wheeler 1999, Lutzoni *et al.* 2000) for use in parsimony analyses. However, a maximum likelihood distance method that retains the full information of sequences is also available. The program Stalign (Thorne, Kishino & Felsenstein 1991, Thorne & Kishino 1992, Thorne & Churchill 1995) does not require an alignment, it calculates pairwise maximum likelihood distances among all possible pairs of

Table 1. Specimens sequenced for this study, together with information on their origin, and GenBank accession nos.

Species	Number	Locality	Herbarium	GenBank no.
<i>Bryonora castanea</i>	u193	Austria, Carinthia (<i>Hafellner 33126</i>)	GZU	AY541238
<i>Carbonea vitellinaria</i>	m32321	Austria, Styria (<i>Tuerk 32321</i>)	GZU	AY541239
<i>Lecanora albella</i>	eb46	Austria, Styria (<i>Hafellner 51629</i>)	GZU	AY541240
<i>L. albella</i>	eb56	Austria, Styria (<i>Hafellner 51518</i>)	GZU	AY541241
<i>L. bicincta</i>	eb82	Pakistan, Northwestern Himalaya (<i>Poelt</i>)	GZU	AY541242
<i>L. bicincta</i>	m9	Australia, Australian Capital Territory (<i>Lumbsch</i>)	GZU	AY541243
<i>L. bicincta</i>	m23	Austria, Styria (<i>Hafellner 43027</i>)	GZU	AY541244
<i>L. bicincta</i>	eb38	Australia, Australian Capital Territory (<i>Trinkaus 109</i>)	GZU	AY541263
<i>L. bicincta</i>	eb36	Australia, Australian Capital Territory (<i>Trinkaus 102</i>)	GZU	AY541264
<i>L. caesiurubella</i>	eb19	Mexico, Baja California Sur (<i>Hafellner 44894</i>)	GZU	AY541245
<i>L. carpinea</i>	eb63	Austria, Carinthia (<i>Poelt</i>)	GZU	AY541246
<i>L. carpinea</i>	eb18	Slovenia, Trnovski gozd (<i>Prügger 61232</i>)	GZU	AY541247
<i>L. carpinea</i>	eb11	Austria, Styria (<i>Hafellner 49178</i>)	GZU	AY541248
<i>L. carpinea</i>	eb80	Austria, Styria (<i>Mayrhofer 13987</i>)	GZU	AY541249
<i>L. cateileia</i>	eb70	Canada, British Columbia (<i>Goward & Poelt</i>)	GZU	AY541250
<i>L. epibryon</i>	m120	Austria, Styria (<i>Wilfling 1289</i>)	GZU	AY541251
<i>L. farinacea</i>	eb37	Australia, Australian Capital Territory (<i>Trinkaus 115</i>)	GZU	AY541261
<i>L. farinacea</i>	eb43	Australia, Australian Capital Territory (<i>Trinkaus 113</i>)	GZU	AY541262
<i>L. horiza</i>	u332	Italy, Puglia (<i>Nimis & Tretiach 23103</i>)	TSB	AY541252
<i>L. intumescens</i>	eb4	Austria, Carinthia (<i>Zeiner 453</i>)	GZU	AY541253
<i>L. intumescens</i>	eb52	Austria, Styria (<i>Hafellner 51153</i>)	GZU	AY541254
<i>L. leptyroides</i>	eb15	Slovenia, Trnovski gozd (<i>Prügger 65224</i>)	GZU	AY541255
<i>L. lojkaeana</i>	jb	Norway, Hordaland (<i>Grube</i>)	GZU	AY541256
<i>L. rupicola</i>	eb98	Austria, Styria (<i>Baloch</i>)	GZU	AY541257
<i>L. rupicola</i>	eb32	Greece, Crete (<i>Grube</i>)	GZU	AY541258
<i>L. rupicola</i>	eb27	France, Haute Languedoc (<i>Grube</i>)	GZU	AY541259
<i>L. rupicola</i>	eb65	Greece, Crete (<i>Grube</i>)	GZU	AY541265
<i>L. rupicola</i>	eb66	Greece, Crete (<i>Grube</i>)	GZU	AY541266
<i>L. rupicola</i> subsp. <i>sulphurata</i>	eb71	Turkey, prov. Izmir (<i>Lumbsch</i>)	GZU	AY541260
<i>L. subcarnea</i>	u274	Sweden, Västergötland (<i>Arup L97580</i>)	herb. Arup	AY541267
<i>L. subcarpineae</i>	eb14	Slovenia, Pohorje (<i>Batič, Koch & Mayrhofer</i>)	GZU	AY541268
<i>L. subcarpineae</i>	eb5	Italy, Emilia Romagna (<i>Nimis, Poelt & Tretiach 95/451</i>)	GZU	AY541269
<i>L. subcarpineae</i>	eb6	Slovenia, Trnovski gozd (<i>Prügger 6/16/29</i>)	GZU	AY541270
<i>L. swartzii</i>	eb100	Austria, Styria (<i>Baloch</i>)	GZU	AY541271
<i>L. swartzii</i> subsp. <i>caulescens</i>	eb34	Austria, Styria (<i>Grube</i>)	GZU	AY541272
<i>L. swartzii</i> subsp. <i>nylanderi</i>	eb75	Austria, Styria (<i>Obermayer 2621</i>)	GZU	AY541273
<i>Lecidella carpathica</i>	u339	Austria, Styria (<i>Arup L97005</i>)	herb. Arup	AY541274
<i>L. elaeochroma</i>	u340	Austria, Styria (<i>Arup L98156</i>)	herb. Arup	AY541275
<i>Protoparmelia picea</i>	u227	Sweden, Bohuslän (<i>Arup L97388</i>)	herb. Arup	AY541276
<i>Scoliciosporum umbrinum</i>	m2873	Austria, Styria (<i>Wilfling 2873</i>)	GZU	AY541277
<i>Tephromela armeniaca</i>	u267	Italy, South Tyrol (<i>Arup L97797</i>)	herb. Arup	AY541278
<i>T. atra</i>	u222	Sweden, Skåne (<i>Arup L97376</i>)	herb. Arup	AY541279

sequences. The resulting distance matrix, containing the distances and their standard deviations, is subjected to tree inference using the program Modfitch35, which is a modified version of the Fitch program (Felsenstein 1989), included in the Statalign package. Branch supports can also be assessed by randomization with Statalign, which is called a pseudobootstrap approach by Thorne & Kishino (1992). The support values obtained by this approach are rather conservative when compared to traditional bootstrap techniques.

The phylogenetic hypothesis for *Lecanora*-species with pruinose discs was constructed using a Bayesian approach as implemented in the program MrBayes (Huelsenbeck & Ronquist 2001). The general time reversible substitution model (Rodriguez *et al.* 1990) with estimation of invariant sites and assuming a discrete gamma distribution with four rate categories (GTR+I+ Γ) was used for likelihood calculations. The nucleotide substitution model was selected using

a likelihood ratio test (Huelsenbeck & Crandall 1997) with the program MrModeltest (Nylander 2002), a simplified version of Modeltest v3.06 (Posada & Crandall 1998). For other parameters default settings were used. The Markov Chain Monte Carlo (MCMC) analysis was run for 2 000 000 generations, with 12 chains starting from a random tree, and using the default temperature of 0.2. Every hundredth tree was sampled, while the first 100 000 generations were discarded as burn-in. A consensus phylogram showing mean branch lengths was calculated with the *sumt* command in MrBayes. Phylogenetic trees were drawn using the program Treeview (Page 1996).

RESULTS

The tree for *Lecanoraceae* based on the alignment-free maximum likelihood distance approach is represented in Fig. 1 as a circular tree. Not all genera are supported

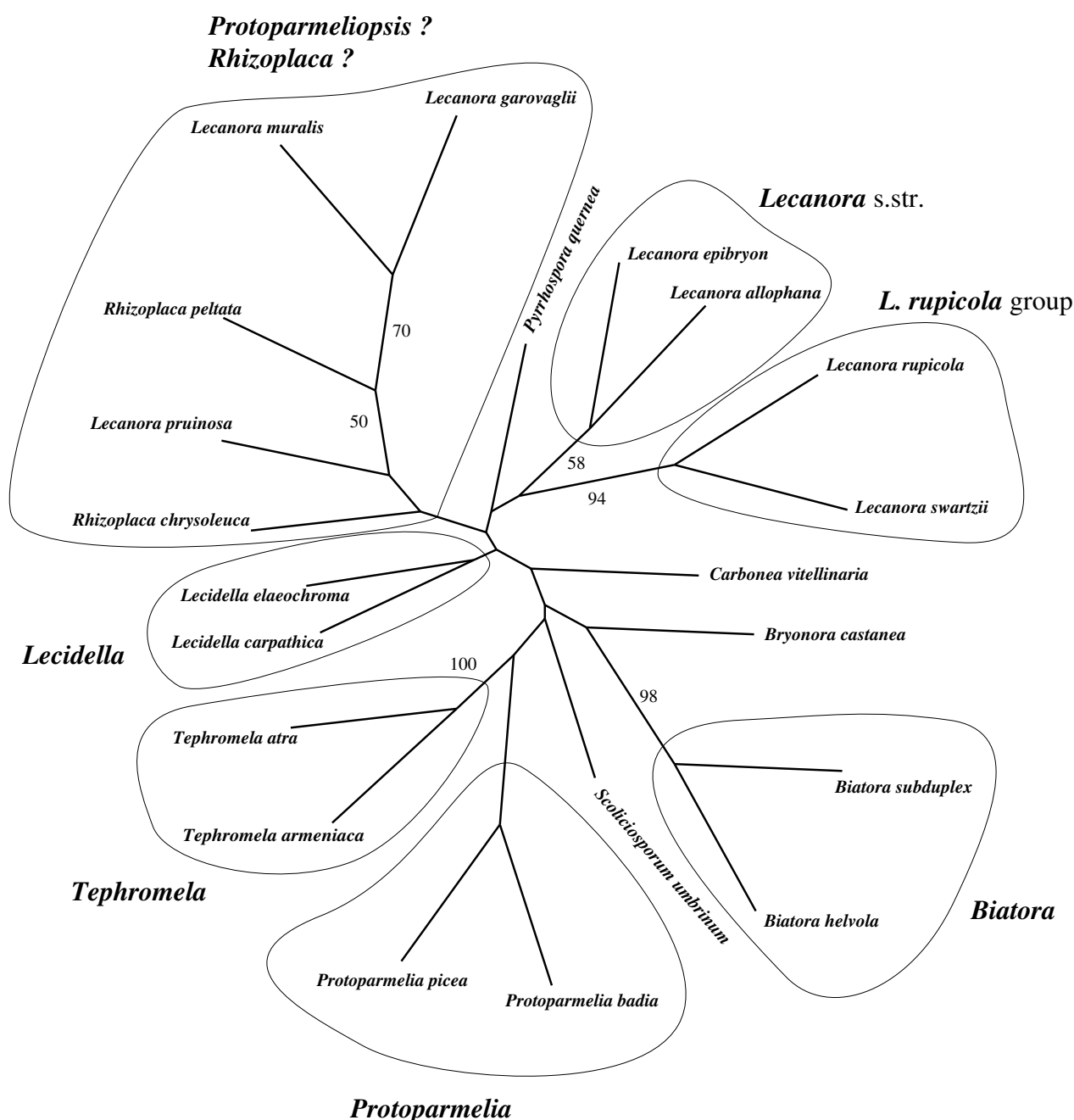


Fig. 1. Maximum likelihood distance tree obtained by an alignment free approach using the program Statalign. Pseudobootstrap values above 50 % are indicated at branches.

by pseudobootstrap support in Statalign, however, it should be noted that the support values are rather conservative in comparison with 'normal' bootstrap techniques (Thorne & Kishino 1992). *Protoparmelia*, *Biatorella* and the *Lecanora rupicola* group are well-supported by pseudobootstrap values. *Lecanora s. str.* is supported only by 58 %. Lobate species of *Lecanora* group together. The *Lecanora muralis* group with *L. muralis* and *L. garovaglii* are supported by a pseudobootstrap of 70 %. *Rhizoplaca peltata*, *Lecanora pruinosa* and *R. chrysoleuca* are basal to this assemblage. However, all these lobate species are clearly distinct from *Lecanora s. str.* and from the *Lecanora rupicola* group. The two species of *Lecidella* form one

clade in the tree but receive a pseudobootstrap support of less than 50 %; the same is true for *Tephromela*. *Pyrrhospora*, *Carbonea*, *Scoliciosporum* and *Bryonora* which have no clear affiliation to other genera.

The Bayesian analysis included additional *Lecanora* species with pruinose apothecial discs, as well as *Pyrrhospora quernea* and *Lecidella elaeochroma* as members of other genera, while *Protoparmelia badia* was used as the outgroup taxon. The likelihood parameters in the sample had the following average values (\pm one standard deviation): rate matrix $r(\text{GT})=1.000$ (± 0), $r(\text{CT})=7.980$ (± 2.811), $r(\text{CG})=1.515$ (± 0.151), $r(\text{AT})=2.733$ (± 0.452), $r(\text{AG})=4.266$ (± 0.952), $r(\text{AC})=2.113$ (± 0.317), base frequencies $\pi(\text{A})=0.204$

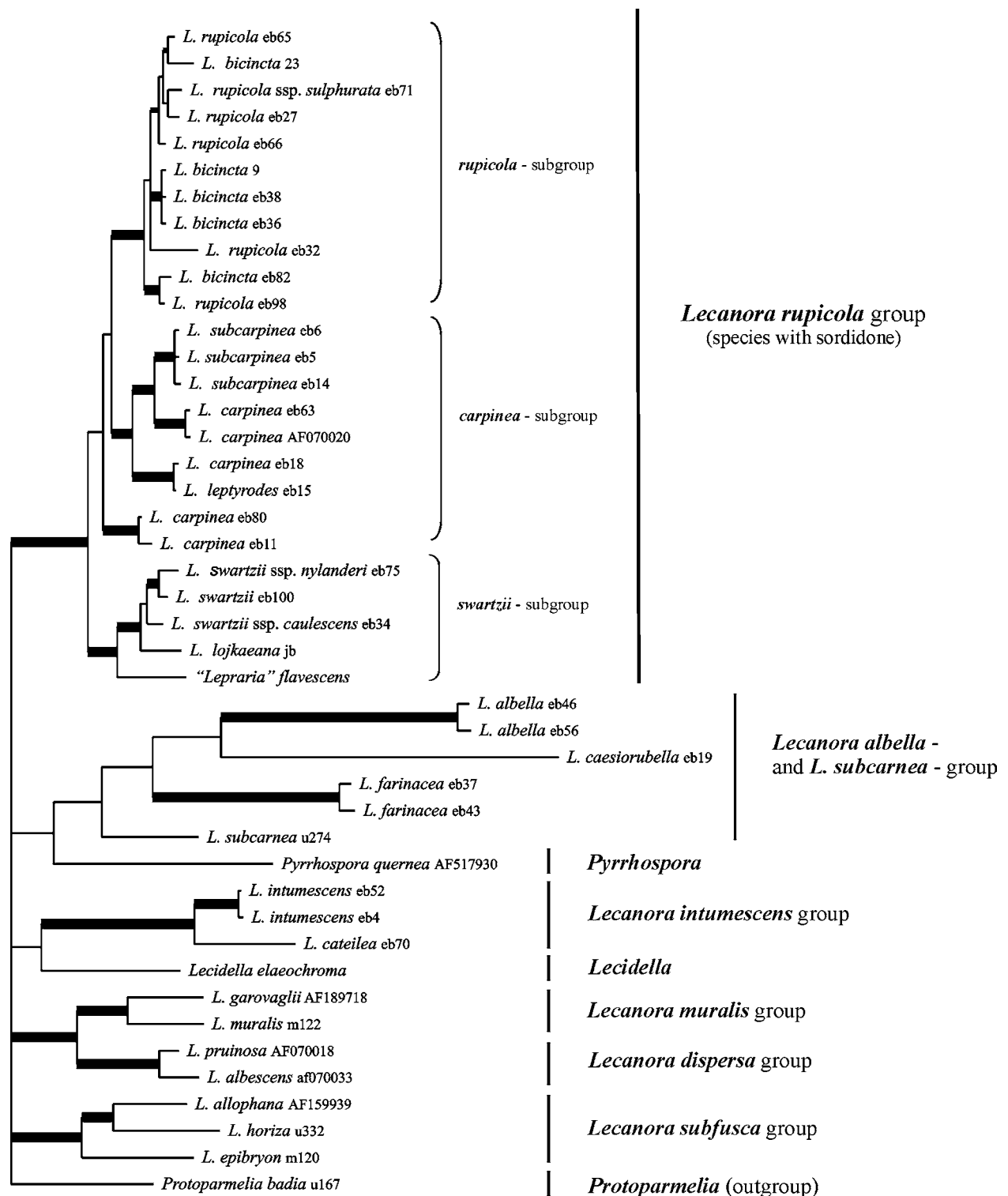


Fig. 2. 50 % Majority-rule consensus tree based on 19 001 trees from a B/MCMC tree sampling procedure. Posterior probability supports are indicated in thickness of the internodes: — < 90 %, — 90–94 %, — 95–100 %.

(± 0), $\pi(C)=0.295$ (± 0), $\pi(G)=0.265$ (± 0), $\pi(T)=0.236$ (± 0), gamma shape parameter $\alpha=0.736$ (± 0.033), and the proportion of invariable site $p(\text{invar})=0.202$ (± 0.005). The majority-rule consensus tree of 19 001 sampled trees is shown in Fig. 2.

Lecanora species containing sordidone form a monophyletic group with 100 % posterior probability (*Lecanora rupicola* group clade, Fig. 2). Within this

clade, the *L. swartzii* subgroup sensu Leuckert & Poelt (1989) is well supported. The *Lecanora swartzii* clade consists of *L. swartzii*, including the two subspecies *nylanderi* and *caulescens*, the sorediate *L. lojkaeana* and the sterile *Lepraria flavescens*, which has a basal position to the other species. A sister group to this assemblage are corticolous and saxicolous species (posterior probability 77%). At the basis are two

specimens of *Lecanora carpinea*, while the other corticolous taxa form a sister assemblage to the *L. rupicola*/*L. bicincta* complex. Specimens of the corticolous *L. subcarpinea*, *L. carpinea* and *L. leptyroides* form a highly supported group (100% pp). The *L. rupicola*/*L. bicincta* complex receives a posterior probability of 100%, but the relationships within this complex remain unresolved. Hence, the traditional morphological concept, which distinguishes *L. rupicola* from *L. bicincta*, is not supported by ITS data.

Other corticolous and saxicolous species with pruinose discs do not form a monophyletic group with species of the *Lecanora rupicola* group. Especially the species of the former *Lecanora pallida* group (*Lecanora caesiorubella*, *L. albelli*, and *L. subcarnea*) that have been discussed to be close to *L. rupicola* and *L. carpinea* (Choisy 1929, Eigler 1969) form a separate clade together with the pantropical *L. farinacea*. The position of two representatives of related genera of *Lecanora*, *Pyrrhospora quernea* and *Lecidella elaeochroma*, is poorly supported by posterior probabilities and morphology.

In the distance analysis and the Bayesian analysis, the included *Lecanora* species are not supported as a monophyletic group. While *Lecanora s. str.* (here with *L. epibryon*, *L. allophana* and *L. horiza*) and the *Lecanora rupicola* group are supported as groups and seem closer to each other, lobate *Lecanoras* appear more distant, and group together with *Rhizoplaca* species. The position of *Rhizoplaca* species within *Lecanora* has also been shown by Arup & Grube (2000) by parsimony analyses.

DISCUSSION

Relationships in Lecanora s. lat.

The trees presented here show the heterogeneity of *Lecanora s. lat.* Some morphologically distinct segregates of the large genus, such as *Tephromela* and others, are now widely accepted and supported (Fig. 1). Other groups which are still maintained in *Lecanora*, however, appear to be more distinct from the core group of *Lecanora* than morphological data alone would suggest. This is the case for groups with lobate *Lecanora* species, which were formerly classified as subgenus *Placodium* (Poelt 1958). The name *Protoparmeliopsis* was introduced for *Lecanora muralis* (a member of *Placodium*) by Choisy (1929), but never accepted by other authors. Later, Hafellner (1984) suggested taking up this name if the *Lecanora muralis* group were to be ranked at genus level. If *Protoparmeliopsis* were accepted in a broader sense, it might also include other usnic acid-containing *Lecanora* species, e.g. the *Lecanora polytropa* group, which contains both lobate and crustose members (e.g. *L. concolor*, *L. disperso-areolata*, *L. intricata*, *L. polytropa*). On the other hand, it might be considered better to accept the older name *Rhizoplaca* as a genus name also for certain

groups of lobate *Lecanora* species, but a solution of this question should also consider data from another genetic locus.

While usnic acid-containing monophyletic groups in *Lecanora* are diverse in morphology, some primarily crustose lineages are distinct from each other in their secondary compound patterns. The rank of the *Lecanora dispersa* group, where chlorinated xanthenes are found as the major constituents, is a matter for future debate. It forms a moderately supported group with the clades of lobate and usnic acid containing *Lecanora* species (Arup & Grube 1998). Here, we focus on a group characterized by the presence of a chemical compound, which is not found in other lichens.

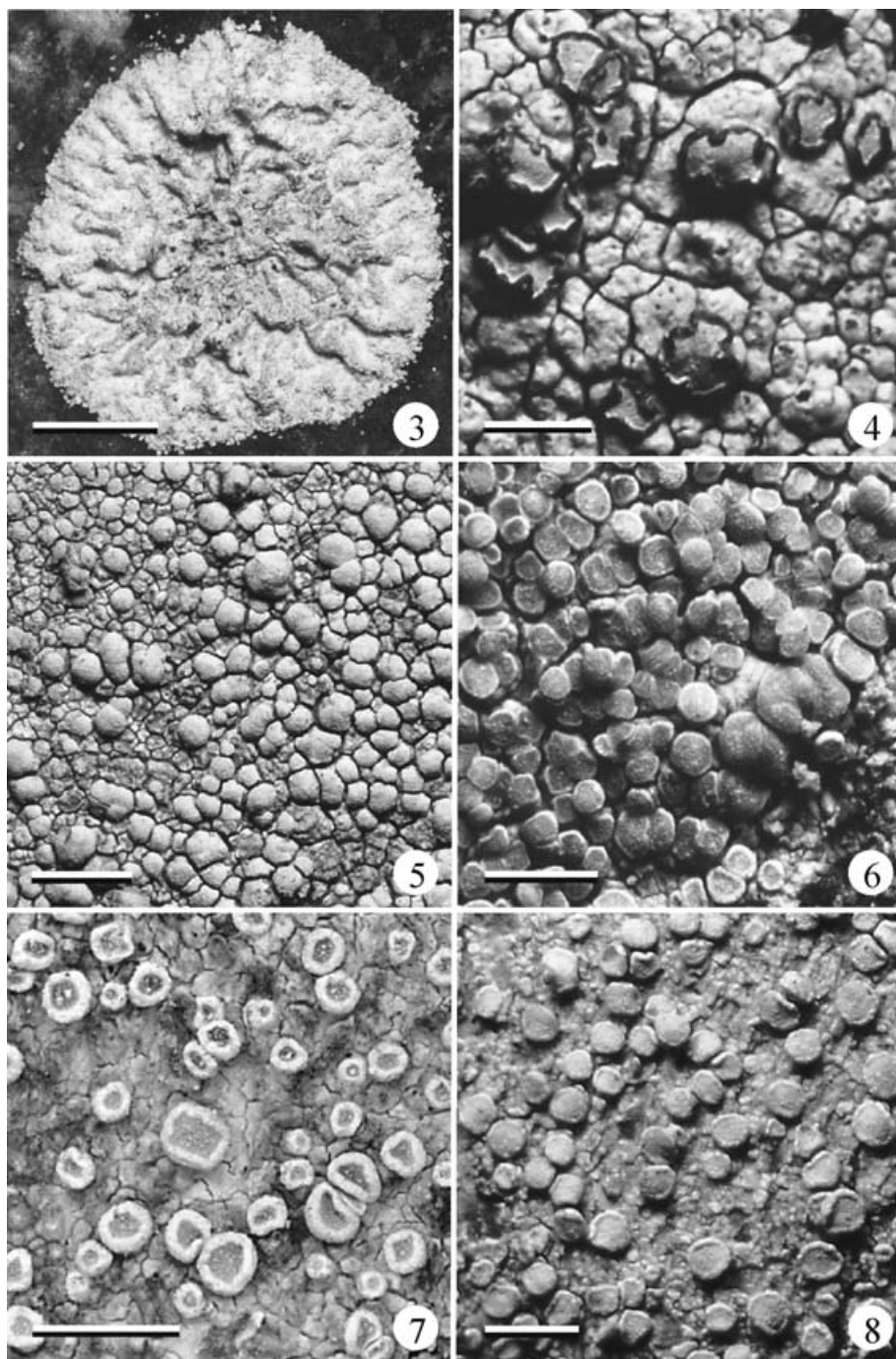
The sordidone-containing *Lecanora rupicola* group is clearly supported as a group in our trees with 100% posterior probability and 94% pseudobootstrap support. This unites all *Lecanora* species with sordidone in a monophyletic group. The genus name *Glaucumaria* was already introduced by Choisy (1929) for *Lecanora rupicola*, and later also used in Hafellner (1984). However, we hesitate to accept this name at a particular taxonomic rank before further studies clarify its relationships to other *Lecanoraceae*. The *Lecanora rupicola* group includes corticolous and saxicolous species, which mostly have a crustose growth habit, with continuous to areolate thalli. Only *L. swartzii* subsp. *caulescens* has a more or less fruticose growth.

Besides sordidone and the accessory compound eugenitol, all species also contain atranorin. Most saxicolous taxa produce roccellic acid, and in some of them different xanthenes of the thiophanic acid series and arthothelin series are present.

Relationships within the Lecanora rupicola group

The Bayesian analysis revealed the relationships within the *Lecanora rupicola* group and supports the concept of Leuckert & Poelt (1989), who divided the *Lecanora rupicola* group in two subgroups: the *swartzii*- and the *rupicola*-subgroup. The *swartzii*-subgroup is characterized by ascomatal margins, which possess an algal-free, strongly conglutinate eucortex and a more or less loose medullar plectenchyma in inner parts.

Substantial variation of growth form is present in the *Lecanora swartzii* subgroup. *L. swartzii s. str.* is a strictly crustose species, whereas ssp. *caulescens* is characterized by an almost shrubby habit. This is connected with the development of a true cortex (eucortex) of the thalli, which extends from the cortex of the apothecial margin. Leuckert & Poelt (1989) thought that chemical characters indicate that the subspecies *caulescens* emerged from ssp. *swartzii*. We have no support for this hypothesis and at present, it is also unclear at what rank *L. swartzii ssp. caulescens* should be classified. The single specimen of the sterile *L. lojkaeana* is clearly positioned within the *L. swartzii* subgroup, which agrees with data from secondary chemistry (Leuckert & Poelt 1989).



Figs 3–8. General appearance of species of the *Lecanora rupicola* group. **Fig. 3.** *L. rouxii* (Austria, *Arup* L98280). Bar = 2 mm. **Fig. 4.** *L. bicincta* (Austria, *Arup* L97020). Bar = 1 mm. **Fig. 5.** *L. rupicola* (Sweden, *Arup* s.n.). Bar = 2 mm. **Fig. 6.** *L. carpinea* (Sweden, *Arup* s.n.). Bar = 2 mm. **Fig. 7.** *L. subcarpinea* (Slovenia, *Arup* L97611). Bar = 2 mm. **Fig. 8.** *L. leptyrodes* (Sweden, *Arup* s.n.). Bar = 2 mm.

The position of the sordidone-containing '*Lepraria*' *flavescens* in this subgroup is not surprising. Ekman & Tønsberg (2002) already showed the relationship of this species with *Lecanora*, but have not focused on a more detailed placement within the genus. A new name has to be selected: the epithet '*flavescens*' is not available in *Lecanora*, as there is already a *Lecanora flavescens* (Bagl.) Bagl. 1879 (a synonym of *Lecanora rupicola* ssp. *sulphurata* (Ach.) Leuckert & Poelt 1989 (Nimis 1993). For

this reason the name *Lecanora rouxii* is introduced here*.

* ***Lecanora rouxii*** S. Ekman & Tønsberg, **nom. nov.** Basionym: *Lepraria flavescens* Cl. Roux & Tønsberg, *Graphis Scripta* **13**: 48 (2002). Syn.: *Lepraria flavescens* Clauzade & Cl. Roux, *Bull. Soc. Linn. Provence* **30**: 34 (1977); nom. inval. (Arts. 32.3). Type: **France**: Provence: Vaucluse, Saignon (aud Apt). vajo de Saignon al Rocsa-liere inter Saint-Michel kaj Bel. Air, sur stonmuro (el burdigala kalka grejso) N-orientiga, alt. 400 m, 1976 Clauzade & Cl. Roux (hb. Cl. Roux – holotype; BG-isotype). *Lecanora rouxii* is named in honour of Claude Roux.

Lecanora rouxii (Fig. 3) grows in similar rain-protected habitats as do *L. swartzii* and *L. lojkaeana*, but in contrast to the latter it prefers limestone. This is quite unusual since no other member of this group occurs on limestone. Only *L. bicincta* has been collected from marble once, but on superficially decalcified spots (Wilfling 1998).

The *rupicola*-subgroup in the sense of Leuckert & Poelt (1989) has amphithecia without large intercellular spaces in inner parts and the algal cells are also found in outer parts. Two species are distinguished in this subgroup: *L. bicincta* is characterized by a dark ring lining the outer edge of the hymenium (Fig. 4) and formed by the dark-pigmented apical cells of the parathecium. In *L. rupicola* these cells are hyaline and a dark ring is not present (Fig. 5). In our analysis the clade with members of the morphospecies *Lecanora rupicola* and *L. bicincta* is not resolved, hence their diagnostic morphological characters do not correlate with the ITS phylogeny. We assume that the development of a dark ring, typical for *L. bicincta*, is to some extent influenced by environmental conditions. The distribution of compounds in the lichen thalli has been used by Leuckert & Poelt (1989) for the characterization of subspecies, but molecular support for the evolutionary significance of such compounds needs further studies. Interestingly, three Australian samples of this species complex group together, but additional data are needed to resolve whether Australian material is genetically distinct.

The corticolous members do not form a monophyletic entity, and two specimens of *L. carpineae* are separate from other samples of the taxon. Morphologically these two corticolous *L. carpineae* samples are distinct from other specimens placed in this species, and we assume that these could possibly represent a separate species, yet to be described. *L. carpineae* (Fig. 6) is rich in diverse morphotypes, and may consist of several species which still need to be delimited. Samples of *L. subcarpineae* (Fig. 7) do form a monophyletic group; this species is also well characterized by ascomata, which become quite large (up to 2 mm diam), and by the contents of psoromic acid. *L. leptyroides* (Fig. 8) groups together with one sample of *L. carpineae*. Also in this case the position of *L. carpineae* could be due to the variation in this species and because only one sample of *L. leptyroides* was included. Morphologically, *L. leptyroides* is easily distinguished by the characteristic, thickish apothecial margins, which have a loose, hydrophobic pseudo-cortex.

Other sordidone-containing *Lecanora* species, (e.g. *L. subpallens*) have not yet been analysed because we had no appropriate material for molecular analyses but it is likely that these also belong to the *Lecanora rupicola* group. The same is certainly true for other described subspecific taxa in the group, i.e. *L. rupicola* subsp. *efflorescens*, subsp. *subplanata*, subsp. *arctoa*, and *L. swartzii* subsp. *nuorensis*.

Lecanora species with pruinose discs

Lecanora species with pruinose discs that lack sordidone are clearly excluded from the *Lecanora rupicola* group in our analyses. We did not attempt to include a larger number of taxa in case their placement in other species groups was rather clear from previous publications. For example epihymenial pruinose are rather common in the *Lecanora dispersa* group (Poelt & Leuckert 1995, Arup & Grube 1998). Some species were considered to be more closely related to the *Lecanora rupicola* group, such as the '*Lecanora albella* group' (the *L. pallida* group; circumscribed as species with whitish pruinose discs, lacking large crystals in the margins, and K+ yellow thalli) and species of the closely related '*L. subcarnea* group'; they clearly form a separate group. The same is true for the clade with *L. intumescens* and *L. cateilea*. While we could show that these species groups are distinct from the *Lecanora rupicola* group the clarification of taxonomic ranks in the *Lecanoraceae* requires a marker which is less variable than ITS.

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