

## The new species *Lecanora bicinctoidea*, its position and considerations about phenotypic evolution in the *Lecanora rupicola* group

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**Abstract:** A phylogenetic analysis of the *Lecanora rupicola* group based on combined nITS rDNA and  $\beta$ -tubulin sequences and a combined dataset of ITS,  $\beta$ -tubulin and partial sequences of polyketide synthase genes reveals a previously unrecognized species, which here is introduced under the name *Lecanora bicinctoidea*. The new species is a sister group of the *L. swartzii* complex (including *L. swartzii* and *L. lojkaeana*), which is characterized by eucorticate ascomata, and a morphological diversity that includes also a dwarf-fruticose lineage. The preferential occurrence on vertical to overhanging siliceous rocks corresponds more closely to *L. swartzii*. A detailed investigation of phenotypic characters reveals that the new species differs from the superficially similar morphospecies *L. bicincta* in several ways, such as a thallus of comparatively small areoles and broadly sessile ascomata and the development of an amphithecial cortex devoid of algal remnants (i.e. an eucortex). *L. bicinctoidea* contains methyl 3 $\alpha$ -hydroxy-4-O-demethylbarbate, a chemical compound not known from other members of the *L. rupicola* group. We also discuss the importance of eucortex formation as one of several factors that are involved in the evolution of substrate-detached thallus structures.

**Key words:**  $\beta$ -tubulin, ITS, KS-domain, *Lecanora rupicola* group, lichenized ascomycota, phylogeny, secondary metabolites

### INTRODUCTION

*Lecanora* is one of the large genera of lichenized ascomycetes, with an estimated number of ca. 300 species (Kirk et al 2001). It is well accepted among lichenologists that the genus is heterogeneous (see also Grube et al 2004), but comprehensive molecular work on the phylogeny of the Lecanoraceae is still in preliminary stages and hampered by the size of the genus. Previous attempts of subgeneric classification

resulted in the description of more or less accepted “satellite” genera of unclear relation to the core of *Lecanora*, while only few studies so far provided phylogenetic hypotheses on the monophyly of such groups. A good case example for a monophyletic group in *Lecanora* is represented by the *Lecanora rupicola* group (Grube et al 2004), which is characterized phenotypically by the presence of the chromone sordidone. One of the species of this group, *L. swartzii*, is interesting in the context of thallus growth-form evolution because it contains a dwarf-fruticose subspecies. It therefore was discussed also as an example for the evolution of growth forms in lichens (Poelt 1989).

Grube et al (2004) also confirmed that the *Lecanora rupicola* group includes not only the saxicolous taxa monographed by Leuckert and Poelt (1989) but also corticolous taxa of the *Lecanora carpinea* group. Moreover an additional sterile species, *Lecanora rouxii*, previously treated under a different name in the genus *Lepraria*, was added. The study however also questioned previous concepts of species recognition in the genus and showed that the polymorphic species such as *L. rupicola* and *L. bicincta* are not supported by ITS data alone.

*L. rupicola* and *L. bicincta* are distinguished by the presence of an apically pigmented parathecium that is formed in the latter. Both morphospecies are otherwise morphologically diverse, and therefore Leuckert and Poelt (1989) attempted an infraspecific classification primarily using characters of secondary chemistry. Only in cases of clearly deviating morphotypes, subspecific entities were based on morphology (e.g. *L. swartzii* ssp. *nuorensis*). Similar chemotypes were found in both morphospecies and numerous specimens were labeled by Josef Poelt as *L. bicincta* s.l. and *L. rupicola* s.l. in the herbarium at the Institute of Plant Sciences in Graz, where the major part of his substantial collections of this group is curated. One of these samples deviated from typical *L. bicincta* by somewhat smaller thallus areoles with narrowly sinuous margins, and we were interested whether this morphotype could be shown to be distinct by molecular data. Using the information on the label we located this morphotype from the original collecting locality and subjected this material to a molecular analysis. In this study we show that this morphotype is indeed distinct from the unresolved bulk of *L. rupicola/bicincta* species complex and we describe it

Accepted for publication 18 October 2006.

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as an additional and distinct species in the *L. rupicola* group.

#### MATERIAL AND METHODS

Lichen material, morphology and anatomy.—Lichen material for this study is deposited in the herbarium GZU and material used for sequencing is listed (TABLE I). Further data about the specimens used in this study can be obtained from the authors on request.

Handmade transversal sections (using Gillette razorblades) and squash preparations were examined in water with a Zeiss Axioscope compound microscope. The estimated values of the quantitative characters are given as:  $(x - SD) - \bar{x} - (x + SD) - (\max)$ , where  $x$  is the arithmetic sample mean,  $SD$  the corresponding standard deviation of the sample and  $\max$  is the highest measured value. For the ascomata and the areoles the maximum values were taken only from healthy, apparently uninfected areoles. Habitus photographs were taken with a digital camera (Nikon Coolpix 4500). For the analysis of the different excipulum types, handmade sections were treated with 10% KOH to dissolve the secondary compounds. Afterward the sections were stained with the fluorescent brightener calcofluor white (excitation wavelength 440 nm, emission wavelength 500–520 nm) and rinsed with 10% KOH. The sections were viewed in an epifluorescence compound microscope (Zeiss Axioscope) with filter set 9 (Zeiss, Vienna) and photographed with a digital camera (Nikon Coolpix 5000). Identification of chemical compounds was performed with HP-TLC according to Arup et al (1993) and critical compounds were analyzed by J. Elix (Canberra) with HPLC of extracts from selected samples.

*DNA extraction, amplification and sequencing.*—For molecular analyses the thalli first were checked for well developed areoles that did not show any externally visible contaminations by other organisms. Total DNA was extracted from individual thalli, according to a modified CTAB method (Cubero et al 1999) or using the DNeasy Plant Mini Kit (QIAGEN, Vienna) following the manufacturer's instructions, with the exception that the DNA was eluted in sterile water. Primers used for PCR of the nuclear ribosomal ITS region were ITS1F (Gardes and Bruns 1993) and ITS4 (White et al 1990), those for  $\beta$ -tubulin were bt2a and bt2b (Glass and Donaldson 1995) and those for the KS-domain of polyketide synthase genes were LC1 and LC2C (Bingle et al 1999). Fifty  $\mu$ L PCR mix (10 mM Tris pH8.3, 50 mM KCl/1.5 mM MgCl<sub>2</sub>/50  $\mu$ g gelatine) contained 1.25 units of *Taq* DNA Polymerase (Amersham Pharmacia Biotech Inc.), 0.2 mM of each of the four dNTPs, 0.5  $\mu$ M of each primer and ca. 10–50 ng genomic DNA. Products were cleaned with QIAquick PCR Purification Kit (QIAGEN, Vienna). Both complementary strands were sequenced with the BigDyeTerminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Vienna) according to the manufacturer's instructions. Sequences were run on an ABI 310 automated sequencer (Applied Biosystems, Vienna) and raw data were assembled with AutoAssembler (Applied Biosystems, Vienna). To assess the relationship within the *Lecanora rupicola* group, we

constructed an alignment that included selected species of the group by using the Clustal algorithm as implemented in BioEdit (<http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html>). The alignment was improved manually and group I intron sequences at the end of the SSU rDNA gene (at position 1506, Gargas et al 1995) as well as ambiguously aligned positions were removed. The sequences are submitted to EMBL/GenBank, and a list of sequenced taxa and sequences retrieved from GenBank for comparisons together with their GenBank accession numbers is presented (TABLE I).

*Phylogenetic analysis.*—The phylogenetic hypothesis was constructed with a Bayesian approach as implemented in the program MrBayes v3.1. (Huelsenbeck and Ronquist 2001). Because the efficiency of a Bayesian analysis is sensitive to the model parameters we obtained the appropriate model of nucleotide substitution for each gene with the MrModeltest 2.2. (by J.A.A. Nylander, available at <http://www.ebc.uu.se/systzoo/staff/nylander.html>). According to the Akaike information criterion (AIC), the symmetrical model SYM+ $\Gamma$  (Zharkikh 1994) with estimation of site specific rates and assuming a discrete gamma distribution within a class was suggested as the optimal model for  $\beta$ -tubulin and the KS-domain of putative polyketide synthase (PKS) genes and the general time reversible substitution model GTR+ $\Gamma$  (Rodriguez et al 1990) assuming a discrete gamma distribution for ITS. To improve the resolution we combined ITS and  $\beta$ -tubulin for a larger dataset, and ITS,  $\beta$ -tubulin and PKS for selected taxa. Before combining, separately analysed datasets revealed no conflicts in the tree topologies. We also performed a partition homogeneity test as implemented in PAUP\*4.08b (Swofford 2002), which showed that the partitions are not incongruent ( $P$  value = 0.01). The appropriate models (see above) were set for each partition in the combined dataset. In both phylogenies the Markov chain Monte Carlo (MCMC) analysis was run 3 000 000 generations, with four chains starting from a random tree, and using the temperature of 0.1. Every 100th tree was sampled, while the first 10 000 generations were discarded as burn-in. The parsimony analyses were performed with PAUP\*4.08b (Swofford 2002), using 1000 replicates. Bootstrap values higher than 75% are indicated as numbers above branches (FIGS. 1, 2). As outgroup we chose *Lecanora macrocyclos* and *L. allophana* (FIG. 1) and *L. carpinea* (FIG. 2), respectively.

#### RESULTS

We obtained 65 new sequences of three loci (23 new for ITS, 33 for  $\beta$ -tubulin and nine for the KS-domain) for this study. The combined data matrix of ITS and  $\beta$ -tubulin contained 1009 positions. Of these 620 characters were constant, 141 variable characters were uninformative, and 248 nucleotide positions were informative. A total of 1662 positions were used for the analysis of the three-gene dataset. Of these 1179 characters were constant, 349 variable characters were

TABLE I. Specimens used in the current study with locality data and GenBank accession numbers. Newly obtained sequences are in boldface.

Species	Locality, Collector (Herbarium)	Genbank no. ITS	Genbank no. $\beta$ -tubulin	Genbank no. KS
<i>Lecanora allophana</i>	Austria, Styria, Teichalpe, U. Arup L98005 (priv. herb.).	AF159939	<b>DQ451638</b>	—
<i>L. bicincta</i>	Austria, Styria, Steir. Randgebirge, Rennfeld, 500 m S of Pischkalm, Mötschmoar, J. Blaha B267 (GZU).	<b>DQ451659</b>	<b>DQ451622</b>	—
<i>L. bicincta</i>	Spain, Catalonia, N of Lleida, Pirineos centrales, between Cabdella and Ponta de Sallente, J. Blaha B334 (GZU).	<b>DQ451664</b>	<b>DQ451628</b>	<b>DQ451643</b>
<i>L. bicincta</i>	Australia, A.C.Territory, Brindabella Range, summit of Mt Franklin, 45 km WSW of Canberra, U. Trinkaus 102 (GZU), isolate EB36.	AY541264	<b>DQ451634</b>	—
<i>L. bicincta</i>	Australia, A.C.Territory, U. Trinkaus 109 (GZU), isolate EB38.	AY541263	<b>DQ451620</b>	—
<i>L. bicincta</i>	Pakistan, Northwestern Himalaya, J. Poelt (GZU), isolate EB82.	AY541242	<b>DQ451625</b>	—
<i>L. bicincta</i>	Norway, Knutshø, M. Grube MG100 (GZU).	AY398707	<b>DQ451635</b>	AY398714
<i>L. bicinctoidea</i>	Austria, Styria, Seetaler Alpen, Zirbitzkogel, 200 m SW of Großen Winterleitensees, J. Blaha B277 (GZU).	<b>DQ451652</b>	<b>DQ451610</b>	—
<i>L. bicincoidea</i>	Austria, Styria, Seetaler Alpen, Zirbitzkogel, steep rocky faces along the way to the Ochsenboden, J. Blaha B282 (GZU).	<b>DQ451651</b>	<b>DQ451609</b>	—
<i>L. bicinctoidea</i>	Austria, Styria, Seetaler Alpen, Zirbitzkogel, steep rocky faces along the way to the Ochsenboden, J. Blaha B284 (GZU).	<b>DQ451650</b>	<b>DQ451608</b>	<b>DQ451640</b>
<i>L. bicinctoidea</i>	Austria, Styria, Seckauer Tauern, Gaalgraben, Krugspitze, 50 m N of the Krugsee, J. Blaha B321 (GZU).	<b>DQ451653</b>	<b>DQ451611</b>	<b>DQ451639</b>
<i>L. bicinctoidea</i>	Austria, Styria, Stuhleck, M. Grube EB26 (GZU).	<b>DQ451649</b>	<b>DQ451607</b>	—
<i>L. bicinctoidea</i>	Austria, Styria, Handalpe, M. Grube EB31 (GZU).	<b>DQ451648</b>	<b>DQ451606</b>	—
<i>L. carpinea</i>	Slovenia, Vojsko, J. Prügger 62808 (GZU), isolate EB29.	AY398710	<b>DQ451617</b>	AY398715
<i>L. lojkaeana</i>	Norwegen, Sjøbo, M. Grube MG101 (GZU).	AY398709	<b>DQ451616</b>	AY398720
<i>L. macrocyclos</i>	Sweden. Skåne, U. Arup L97368 (priv. herb.).	AF159933	<b>DQ451631</b>	—
<i>L. rupicola</i>	Austria, Styria, Seckauer Tauern, Grafenalm next to Maria Schnee, J. Blaha B261 (GZU).	<b>DQ451660</b>	<b>DQ451623</b>	—
<i>L. rupicola</i>	Austria, Styria, Steir. Randgebirge, Rennfeld, 700 m W of Gamshütte, J. Blaha B270 (GZU).	<b>DQ451662</b>	<b>DQ451626</b>	<b>DQ451644</b>
<i>L. rupicola</i>	Austria, Styria, Seetaler Alpen, Zirbitzkogel, steep rocky faces along the way to the Ochsenboden, J. Blaha B288 (GZU).	<b>DQ451661</b>	<b>DQ451624</b>	<b>DQ451645</b>
<i>L. rupicola</i>	France, Corsica, Cole de vergio, J. Blaha B313 (GZU).	<b>DQ451658</b>	<b>DQ451621</b>	<b>DQ451646</b>
<i>L. rupicola</i>	Spain, Catalonia, N of Lleida, Pirineos centrales, Valle de Boi, NP Aigües Tortes, J. Blaha B328 (GZU).	<b>DQ451668</b>	<b>DQ451633</b>	<b>DQ451647</b>
<i>L. rupicola</i>	Spain, Aragonia, N of Sabinanigo, Balneario de Panticosa, along GR11 to Embalse de Bachimana Bajo, J. Blaha B329 (GZU).	<b>DQ451665</b>	<b>DQ451629</b>	—
<i>L. rupicola</i>	Spain, Catalonia, N of Lleida, Pirineos centrales, between Cabdella and Ponta de Sallente, J. Blaha B333 (GZU).	<b>DQ451669</b>	<b>DQ451636</b>	—

TABLE I. Continued

Species	Locality, Collector (Herbarium)	Genbank no. ITS	Genbank no. $\beta$ -tubulin	Genbank no. KS
<i>L. rupicola</i>	Spain, Catalonia, N of Lleida, Cabdella, Refugio de Colomina, NP Aigües Tortes, J. Blaha B357 (GZU).	<b>DQ451670</b>	<b>DQ451637</b>	—
<i>L. rupicola</i>	Austria, Styria, Handalpe, M. Grube MG4 (GZU).	<b>DQ451666</b>	<b>DQ451630</b>	—
<i>L. rupicola</i>	Austria, Salzburg, Obertauern, E. Baloch MG111 (GZU).	<b>DQ451667</b>	<b>DQ451632</b>	—
<i>L. rupicola</i>	Poland, "Rutka" nature reserve in Suwalki Landscape Park, Suwalki Lake Distrikt, NE Poland, M. Opanowicz MOPA78 (GZU).	<b>DQ451663</b>	<b>DQ451627</b>	—
<i>L. subcarpineae</i>	Italy, 1995, P.L. Nimis 95/451 (GZU), isolate EB5.	AY541269	<b>DQ451618</b>	—
<i>L. subcarpineae</i>	Slovenia 1995, J. Pügger 65311 (GZU), isolate EB13.	<b>DQ451657</b>	<b>DQ451619</b>	—
<i>L. swartzii</i>	Austria, Styria, Fischbacher Alpen, Teufelstein, J. Blaha B306 (GZU).	<b>DQ451656</b>	<b>DQ451615</b>	DQ451641
<i>L. swartzii</i>	Austria, Styria, Handalpe, E. Baloch EB24 (GZU).	<b>DQ451655</b>	<b>DQ451614</b>	—
<i>L. swartzii</i> ssp. <i>caulescens</i>	Austria, Styria, Handalpe, M. Grube EB34 (GZU).	<b>AY541272</b>	<b>DQ451613</b>	—
<i>L. swartzii</i>	Norway, Soebo, M. Grube MG102 (GZU).	<b>DQ451654</b>	<b>DQ451612</b>	DQ451642

uninformative and 134 nucleotide positions were informative.

The majority rule consensus tree of the combined ITS/ $\beta$ -tubulin dataset (FIG. 1) was calculated from 580 002 trees, the average log-likelihood of the tree sample is  $-4264.91$  (arithmetic mean). The likelihood parameters in the sample had these average values (variance) for the ITS partition: rate matrix  $r(\text{GT}) = 0.083$  (0),  $r(\text{CT}) = 0.393$  ( $\pm 0.001$ ),  $r(\text{CG}) = 0.113$  (0),  $r(\text{AT}) = 0.128$  (0),  $r(\text{AG}) = 0.188$  (0),  $r(\text{AC}) = 0.094$  ( $\pm 0.003$ ), gamma shape parameter  $\alpha = 0.413$  ( $\pm 0.005$ ); and these for the  $\beta$ -tubulin partition: rate matrix  $r(\text{GT}) = 0.092$  (0),  $r(\text{CT}) = 0.397$  ( $\pm 0.001$ ),  $r(\text{CG}) = 0.084$  (0),  $r(\text{AT}) = 0.164$  (0),  $r(\text{AG}) = 0.180$  (0),  $r(\text{AC}) = 0.092$  (0), and the gamma shape parameter  $\alpha = 2.431$  ( $\pm 0.030$ ). Estimated base frequencies of the GTR model for the ITS dataset are:  $\pi(\text{A}) = 0.221$  (0),  $\pi(\text{C}) = 0.272$  (0),  $\pi(\text{G}) = 0.260$  (0),  $\pi(\text{T}) = 0.246$  (0).

The majority rule consensus tree of the combined ITS,  $\beta$ -tubulin and KS dataset (FIG. 2) was calculated from 580 002 trees, the average log-likelihood of the tree sample is  $-4023.08$  (arithmetic mean). The likelihood parameters in the sample had these average values (variance) for the ITS partition: rate matrix  $r(\text{GT}) = 0.067$  (0),  $r(\text{CT}) = 0.426$  ( $\pm 0.001$ ),  $r(\text{CG}) = 0.127$  (0),  $r(\text{AT}) = 0.050$  (0),  $r(\text{AG}) = 0.214$  ( $\pm 0.001$ ),  $r(\text{AC}) = 0.117$  (0), gamma shape parameter  $\alpha = 1.280$  ( $\pm 0.004$ ); and these for the  $\beta$ -tubulin partition: rate matrix  $r(\text{GT}) = 0.075$  ( $\pm 0.001$ ),  $r(\text{CT}) = 0.412$  ( $\pm 0.004$ ),  $r(\text{CG}) = 0.163$  ( $\pm 0.002$ ),  $r(\text{AT}) = 0.095$  ( $\pm 0.001$ ),  $r(\text{AG}) = 0.201$  ( $\pm 0.002$ ),  $r(\text{AC}) = 0.053$  ( $\pm 0.001$ ), gamma shape parameter  $\alpha = 1.368$  ( $\pm 0.009$ ); and these for the KS partition: rate matrix  $r(\text{GT}) = 0.048$  (0),  $r(\text{CT}) = 0.513$  ( $\pm 0.003$ ),  $r(\text{CG}) =$

$0.068$  (0),  $r(\text{AT}) = 0.019$  (0),  $r(\text{AG}) = 0.277$  ( $\pm 0.002$ ),  $r(\text{AC}) = 0.075$  (0) and the gamma shape parameter  $\alpha = 0.240$  ( $\pm 0.086$ ). Estimated base frequencies of the GTR model for the ITS dataset are:  $\pi(\text{A}) = 0.223$  (0),  $\pi(\text{C}) = 0.262$  (0),  $\pi(\text{G}) = 0.262$  (0),  $\pi(\text{T}) = 0.251$  (0).

The combined Bayesian analysis of ITS and  $\beta$ -tubulin (FIG. 1) resulted in four distinct, well supported clades (posterior probability 100%) within the *Lecanora rupicola* group. Clade A comprises the two closely related species *L. rupicola* and *L. bicincta*, yet the two species are not resolved. Representing the *Lecanora swartzii* subgroup, Clade B includes the new species *L. bicinctoidea* and its sister Clade C comprises *L. swartzii* as well as the sorediate *L. lojkaeana*. The *L. carpineae* subgroup with the corticolous *L. carpineae* and *L. subcarpineae* (Clade D) is the sister group of the saxicolous species. The phylogeny of three combined genes (ITS,  $\beta$ -tubulin, KS-domain of putative polyketide synthase genes) of selected taxa (FIG. 2), with *L. carpineae* as outgroup, shows a similar topology.

In addition to the known secondary compounds of the members of the group (i.e. atranorin, arthothelin, eugenitol, psoromic acid, sordidone and thiophanic acid) the chemical analysis of *L. bicinctoidea* revealed a new compound in the group, the depside methyl  $3\alpha$ -hydroxy-4-O-demethylbarbatate.

#### TAXONOMY

***Lecanora bicinctoidea*** Blaha & Grube sp. nov.  
FIGS. 3–4, 8

A specie simillima, *Lecanora bicincta*, differt ascomatibus maioribus sessilibusque et areolis minoribus substantiam methyl  $3\alpha$ -hydroxy-4-O-demethylbarbatatum continentibus.

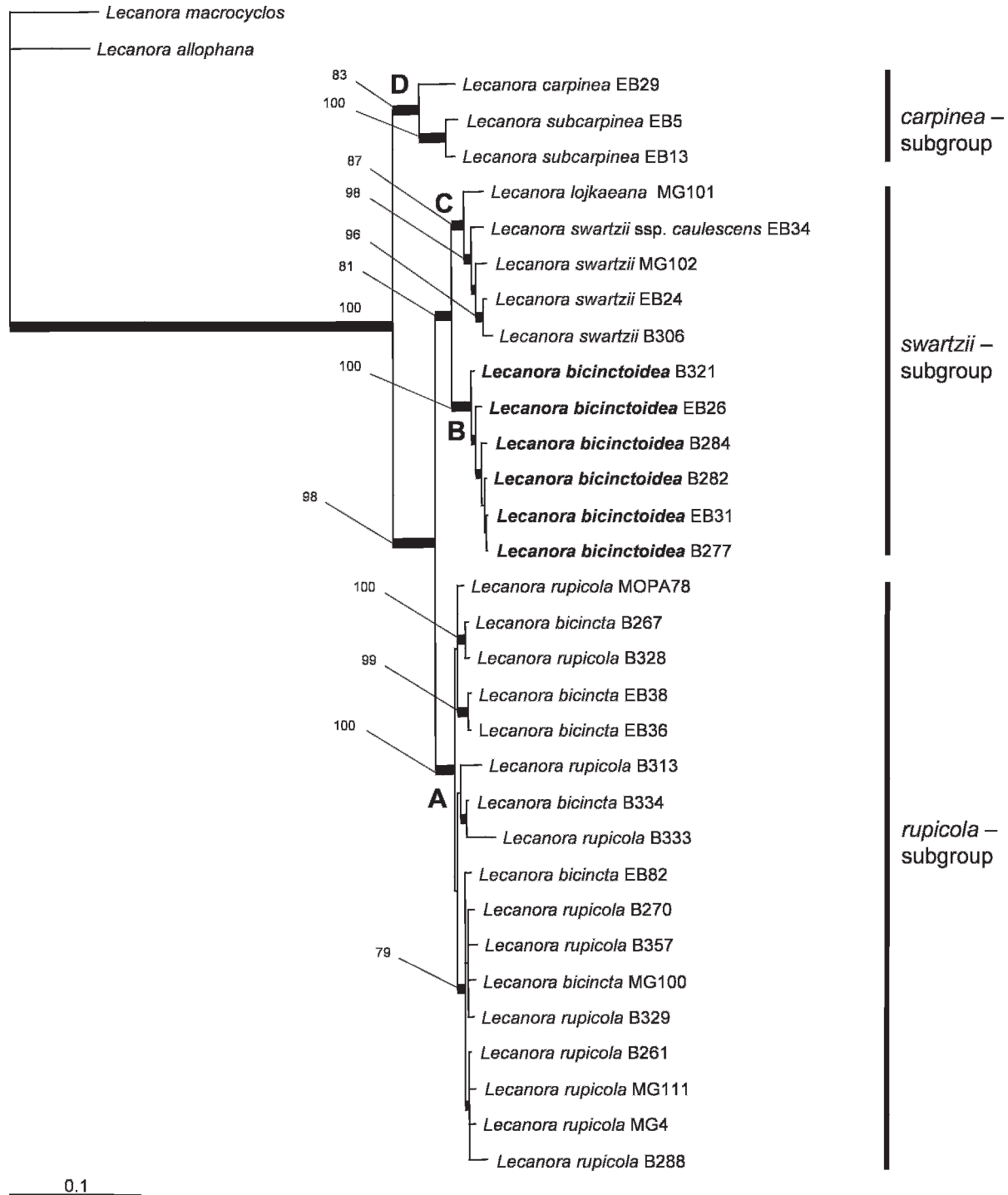


FIG. 1. Phylogenetic tree based on a combined dataset of ITS and  $\beta$ -tubulin. Posterior probabilities equal to or greater than 95% are represented by thick lines. Parsimony bootstrap values  $\geq 75\%$  are indicated by numbers.

*Thallus* crustose, cream-white to white, areoles small 0.4–0.6–0.8(1.0) mm diam, flat to slightly convex, areole edges sinuous, surface  $\pm$  smooth; soredia absent; *prothallus* distinct, black;

*Ascomata* 0.7–1.0–1.3(1.7) mm diam, round, broadly sessile, arising singly to juxtaposed, thalline exciple entire

and slightly sinuous; disks blue-gray to blue, flat to convex, densely gray to white pruinose; *epithecium* dark olivaceous brown, interspersed with crystals dissolving in K; *hymenium* hyaline, 60–75–90(100)  $\mu$ m tall; *paraphyses* 2  $\mu$ m thick, sparsely branched, apices not or slightly thickened, 2–3  $\mu$ m. *Asci* 8-spored, ca. 15  $\mu$ m broad, *Lecanora*-type; *ascospores*

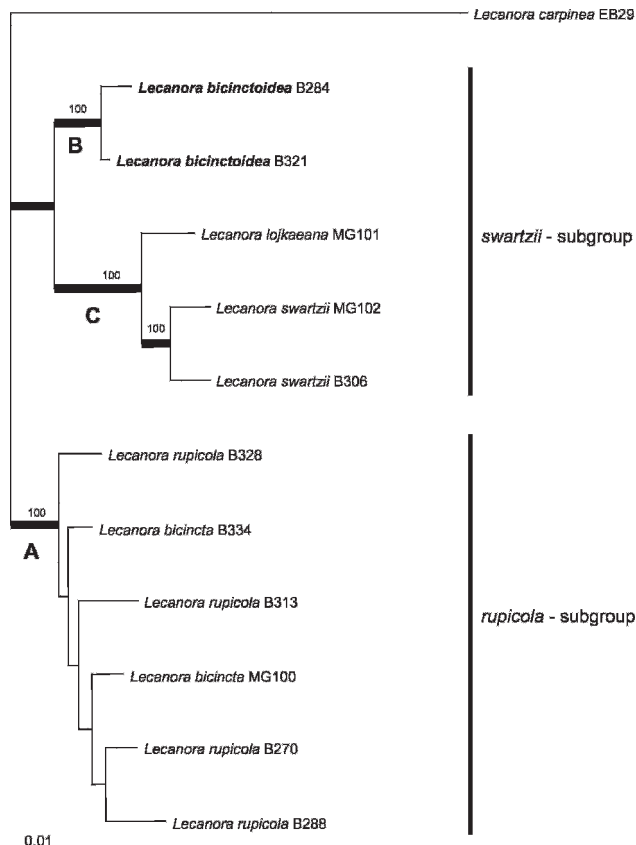


FIG. 2. Phylogenetic hypothesis based on a combined dataset of ITS,  $\beta$ -tubulin and the KS domain of polyketide synthase genes. Posterior probabilities equal to or greater than 95% are represented by thick lines. Parsimony bootstrap values  $\geq 75\%$  are indicated by numbers.

hyaline, simple, ellipsoid, wall thick,  $10.2\text{--}11.6\text{--}13(15) \times 5.7\text{--}6.6\text{--}7.5(8)$ ; *conidiomata* with dark brown to black ostium, conidia filiform,  $(18)25(29) \times 1 \mu\text{m}$ .

**Chemistry.** Thallus K  $\pm$  pale yellow, C–, apothecial disk Pd–, K–, C+ yellow to orange. Arthrothelin, atranorin, chloroatranorin, eugenitol, isoarthothelin, methyl  $3\alpha$ -hydroxy-4-O-demethylbarbatate, psoromic acid (facultative), sordidone, thiophanic acid (facultative).

**Etymology.** The epithet *bicinctoidea* is based on the similarity of the new species and *Lecanora bicincta*; both share a more or less distinct black margin in the upper part of the ascomata between the hymenium and the thalline excipulum.

**Ecology and distribution.** *Lecanora bicinctoidea* grows on vertical to overhanging siliceous rocks. So far the species is known from various mountain ranges in Styria, Austria, (Fischbacher Alpen, Gurktaler Alpen, Seckauer Tauern, Seetaler Alpen, Steirisches Randgebirge) and Italy (Nimis personal communication).

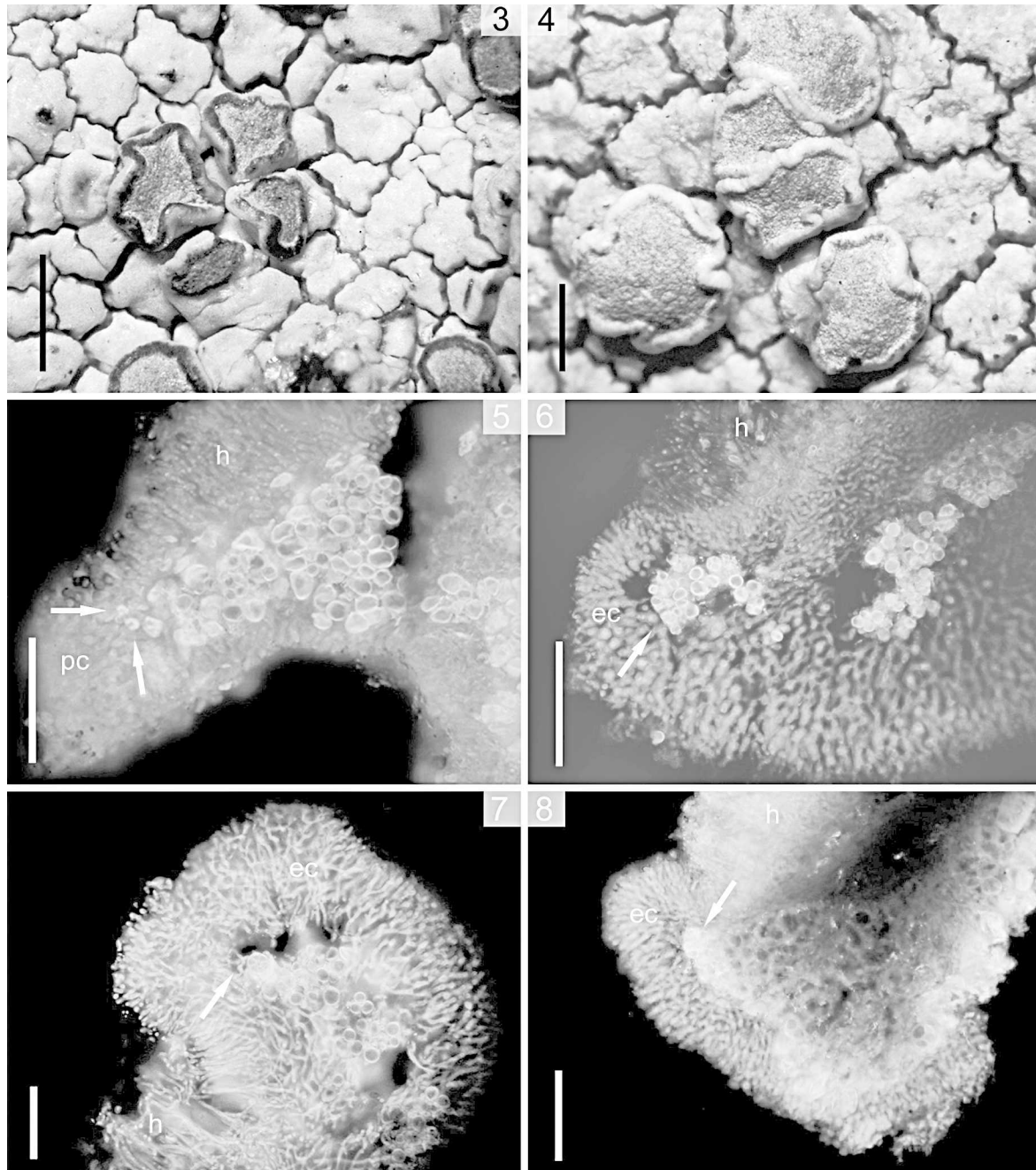
**HOLOTYPE:** AUSTRIA. STYRIA: Steirisches Randgebirge, Koralpe, Handalpe, hiking trail from the Weinebene to the Moserkogel, NW Handalpe, ca. 1800 m, siliceous rocks, ‘Öfen’, 28-IX-2002; J. Blaha (GZU).

**ADDITIONAL SPECIMENS EXAMINED:** AUSTRIA. STYRIA: Seetaler Alpen, Zirbitzkogel, 200 m SW of Großer Winterleitensee; J. Blaha B277 (GZU). Seetaler Alpen, Zirbitzkogel, steep rocky faces on way to the Ochsenboden; J. Blaha B282, B284 (GZU). Seckauer Tauern, Gaalgraben, Krugspitze, 50 m N of the Krugsee; J. Blaha B321 (GZU). Fischbacher Alpen, Stuhleck; M. Grube EB26 (GZU). Steirisches Randgebirge, Koralpe, Handalpe; M. Grube EB31 (GZU). Steirisches Randgebirge, Koralpe, Handalpe; E. Baloch EB111 (GZU). Gurktaler Alpen, Frauenalpe, J. Blaha B451 (GZU). Seckauer Alpen, Hochreichart, B. Emmerer, H. Komposch, J. Blaha B437, B438 (GZU).

#### DISCUSSION

The broad morphological spectrum within the species of the well circumscribed *Lecanora rupicola* group (Grube et al 2004) complicates the recognition of distinct species, especially in crustose samples assigned to the closely related *L. rupicola* and *L. bicincta*. The traditional distinguishing character between the two species is the apical dark parathecium, which should be developed only in *L. bicincta*. This character seems to agree with ecological preferences: *L. rupicola* is more frequently found at lower altitudes, whereas *L. bicincta* prefers, at least in the Alps, higher altitudes (Hafellner unpublished data). However the distinguishing morphological character is not always clearly developed. We suspect that there are modifying factors, in correlation with the local ecological conditions (e.g. sun exposure). Also our data from additional gene loci cannot well resolve *L. bicincta* and *L. rupicola*. However, because multiple separable morpho- and chemotypes can occur at the same locality (Grube unpublished data), we assume that several closely related, yet independent, lineages could be present in this complex.

*Lecanora bicinctoidea* (FIGS. 3, 4) is clearly distinguished from the poorly resolved *Lecanora bicincta/rupicola* complex by molecular, morphological and chemical data. *L. bicinctoidea* contains, in addition to sordidone, eugenitol and atranorin, the depside methyl  $3\alpha$ -hydroxy-4-O-demethylbarbatate, which is not known from other members of the *L. rupicola* group. This  $\beta$ -orcinol derivate is biosynthetically closely related to atranorin, methyl barbatate and methyl 4-O-demethylbarbatate. The reduction of atranorin at  $\alpha$ -C3 results in methyl  $3\alpha$ -hydroxy-4-O-demethylbarbatate. It is not common in lichenized ascomycetes and first was documented in *Oropogon loxensis* (Culberson and Culberson 1978). The compound was detected both by HP-TLC (according to



FIGS. 3–8. Habitus of *Lecanora bicinctoidea* (EB111) with an apically black parathecium. 4. Habitus of *Lecanora bicinctoidea* (B282) with poorly pigmented parathecium. 5. Excipulum of *Lecanora bicincta* (B340). 6. Excipulum of *Lecanora carpinea* (Hafellner 24669). 7. Excipulum of *Lecanora swartzii* (Poelt). 8. Excipulum of *Lecanora bicinctoidea* (EB26). Arrows indicate algal cells, eucortex (ec), phenocortex (pc), hymenium (h). Bars 3–4 = 1 mm, 5–8 = 50  $\mu$ m.

Arup et al 1993, R<sub>f</sub>-classes 5/ 5/ 5) and corroborated with HPLC by J. Elix. The ecological requirements of *Lecanora bicinctoidea* are vertical to overhanging siliceous rocks, where it consistently associates with *Trebouxia simplex* (as assessed by algal ITS sequencing, data not shown). This humid and shaded environment corresponds to that of *L. swartzii*

(Leuckert and Poelt 1989), which usually co-occurs at sampling sites in several mountain ranges in Styria investigated so far.

In this study we also included the ketoacyl synthase (KS) domain of polyketide synthase genes (fungal type I PKS) as an additional locus to confirm the distinctiveness of the new species. Polyketide synthase

genes represent a gene family of considerable functional importance in fungi because the translated proteins are essential in the production of secondary metabolites. Grube and Blaha (2003) suggested that paralogy of PKS genes can be detected by phylogenetic incongruency with rDNA data, and Schmitt et al (2005) found that numerous paralogs for nonreducing polyketide synthases exist in lichens. Within *Lecanora* three such paralogs have been discovered (Blaha et al unpublished data). We therefore have taken care to include only orthologous KS-sequences from our samples in this analysis. The PKS paralog included here belongs to a subgroup in Clade V of Schmitt et al (2005), in which all paralogs detected directly from the *Lecanora rupicola* group with the primers LC1 and LC2C (Bingle et al 1999) form a monophyletic group. In addition the presence of an mRNA intron at the same position further corroborates the orthology of this PKS gene. Whereas the functional assignment of the analysed PKS genes to a particular biosynthetic pathway is difficult and would require extensive experimental studies, we are convinced that orthologous PKS genes represent valuable markers for phylogenetic studies within genera.

*Lecanora swartzii* ssp. *caulescens* was discussed before as an example for the evolution of fruticose growth form among crustose relatives. Poelt (1989) found that an eucortex is developed at the upper surface in this dwarf-fruticose lichen. He hypothesized that the early formation of an eucortex is usually connected to the ascomatal ontogeny in this subspecies. Subsequently Poelt somehow generalized this observation and suggested that the proleptic formation of ascomatal eucortices and the delay of ascoma formation could be a principal phenomenon in the evolution of foliose thalli; in this context the eucortex was functionally interpreted as a requirement to stabilize the thallus structure ("exoskeleton", Poelt 1991). However this hypothesis was not developed further (e.g. by comparative ontogenetic studies).

We assume that the exclusion of algal remnants in otherwise gelatinized fungal plectenchyma could promote the evolution of substrate-detached thallus types. Layers in heteromerous thalli may differ in the capacity to increase in size with water uptake. Layers, which consist of a matrix of extracellular polysaccharides (e.g. hymenia, eucortices), usually swell more pronouncedly in water than layers without such a matrix (Blaha unpublished data). As a consequence the repeated swelling and shrinking of thalli under natural conditions of fluctuating hydration likely acts as a mechanic force in a physiologically active and growing thallus, especially when the thallus is fixed with the lower surface to a rigid substrate (such as

rocks). Under these conditions strictly crustose thalli with an eucortex might be regarded as exceptional. It in fact is interesting that growth-form transitions observed in other groups of rock-inhabiting *Lecanora* also correlate with increasingly well developed eucortices, e.g. in the *Lecanora polytropha* group (crustose *L. polytropha* vs. lobate *L. concolor*, Arup and Grube 1998) and in the *L. novomexicana* group (lobate *L. novomexicana* vs. foliose *Rhizoplaca melanophthalma*, Arup and Grube 2000). Similar hygroscopic forces also could influence apothecial functions. It might be hypothesized that the eucorticate margin could compensate for the hygroscopic movements of hymenial parts. Preliminary observations in *L. swartzii* suggest that the eucorticate amphithecial cortex acts like a clamp that keeps hydrated ascomatal sections in shape, whereas removal of the eucortex causes the hymenium to disintegrate from subtending structures (Blaha unpublished data).

Our data show that the *Lecanora swartzii* group including *L. bicinctoidea* contains only species that develop a eucorticate amphithecium, whereas the related strictly crustose *L. rupicola/bicincta* complex is characterized by ascomatal margins with a phenocortex (see FIGS. 5–8). *L. bicinctoidea* is thus a basal crustose lineage in a species group that exhibits an interesting morphological variation, from broadly sessile to distinctly elevated and basally constricted ascomata, and from crustose to dwarf-fruticose thalli.

#### ACKNOWLEDGMENTS

We thank Elisabeth Baloch (Stockholm) for helpful comments. Barbara Fetz and Sigrun Kraker (Graz) are thanked for technical assistance. We acknowledge Jack Elix (Canberra) for analyzing samples by HPLC, Walter Obermayer (Graz) for help with the fluorescence microscopy and Christian Scheuer (Graz) for improving the Latin diagnosis. Financial support by the Austrian Science Foundation FWF (P14610, P16738) to MG is gratefully acknowledged.

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