

A contribution to the taxonomy of the genus *Rinodina* (*Physciaceae*, lichenized Ascomycotina) using combined ITS and mtSSU rDNA data

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Abstract: To test the phylogenetic position of phenotypically peculiar species in the *Physciaceae* we generated 47 new sequences (26 of nrITS region and 21 of mtSSU rDNA) from 19 crustose taxa of *Physciaceae* mainly from the genus *Rinodina*. Phylogenetic analysis confirmed the *Buellia* and *Physcia* groups. The analysis revealed a considerable variability of characters traditionally used for classification, especially in the delimitation of the genera *Buellia* and *Rinodina*. While ascus types agree well with the distinction of the *Buellia* and *Physcia* groups, none of the other traditional characters, including excipulum type and ascospore thickening, were consistent within subclades of the *Physcia* group. We suggest that both excipulum type and ascospore characters are rather dynamic in the evolution of *Rinodina* species and only appear consistent in morphologically more complex foliose and fruticose groups, which are characterized by thallus characters not present in the crustose groups. Two recent taxonomic changes are supported by molecular characters: *Endohyalina insularis* (syn. '*Rinodina insularis*') and *Rinodina lindingeri* (syn. '*Buellia lindingeri*'). In addition *Rinodina parvula* (syn. '*Buellia parvula*') is reinstated. New records for *Endohyalina brandii*, *E. diederichii*, *E. insularis* and *Rinodina albana* are presented.

Key words: *Buellia*, *Endohyalina*, molecular phylogeny

Introduction

Comprising *c.* 265 species (Kirk *et al.* 2008), the cosmopolitan genus *Rinodina* (Ach.) Gray is widely distributed in both Hemispheres from polar to tropical latitudes. Detailed taxonomic treatments are available for saxicolous *Rinodina* species from Europe (e.g. Mayrhofer & Poelt 1979; Mayrhofer 1984a; Giralt 2001), Africa (e.g. Mayrhofer 1984a; Matzer & Mayrhofer 1996), Asia (e.g. Mayrhofer 1984a), Australasia (e.g. Mayrhofer 1983, 1984b; Kaschik 2006), and for epiphytic *Rinodina* species in Europe (e.g. Ropin & Mayrhofer 1993, 1995; Giralt & Mayrhofer 1994, 1995; Giralt & Matzer 1994) and North America (e.g. Sheard &

Mayrhofer 2002). Other treatments focus on a single species or a species group on a world-wide scale (e.g., Mayrhofer *et al.* 1990; Matzer & Mayrhofer 1994; Matzer *et al.* 1998; Mayrhofer *et al.* 2001). Identification keys to *Rinodina* species were provided for Great Britain and Ireland (Giavarini *et al.* 2009), south-western Germany (Wirth 1995), Scandinavia (Mayrhofer & Moberg 2002), the Iberian Peninsula (Giralt 2001), the extended Sonora Desert region (Sheard 2004) and Russia (Kotlov 2008).

Rinodina species usually have crustose thalli, lecanorine apothecia, 2-celled brown ascospores with inner wall thickenings and *Lecanora*-type asci. The most important character complexes for determining *Rinodina* species are those of the proper excipula, the ascospores and the asci. The different phenotypes of these characters were classified as categories or "types" (Zahlbruckner 1926; Poelt & Mayrhofer 1979; Rambold *et al.* 1994). The correlation of ascus types with the other categories agreed with a concept of

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two main groups in *Physciaceae* Zahlbr., i.e. the *Physcia* group (including *Rinodina* and also the foliose genera *Physcia* (Schreb.) Michx., *Phaeophyscia* Moberg, *Physconia* Poelt among others) and the *Buellia* group (including *Buellia* De Not., *Diploicia* A. Massal., *Dirinaria* (Tuck.) Clem., *Pyxine* Fr., *Santessonia* Hale & Vobis among others). However, there are some interesting exceptions which combine characters of *Buellia* and *Rinodina*, for example, *Buellia lindingeri* Erichsen, *Buellia parvula* (Mayrhofer & Poelt) Mayrhofer & Scheid. and *Rinodina insularis* (Arnold) Hafellner.

The current generic concept of *Rinodina* s. lat. is polymorphic with respect to ascospore pigmentation and internal wall formation (e.g., Hafellner *et al.* 1979; Mayrhofer 1982; Kaschik 2006). Initial phylogenetic analyses of the *Physciaceae* suggested that differences in these characters do not reflect larger lineages in *Rinodina*, but might rather be specific at species level or below (Grube & Arup 2001; Helms *et al.* 2003; Kaschik 2006). These studies also raise the hypothesis that *Rinodina* is a paraphyletic genus, with foliose and fruticose lineages probably originating from various crustose ancestors. All of these investigations, however, relied on ITS data only, which limits the resolution of deeper branches. MtSSU and nrITS rDNA sites were used for studying the natural relationships between the lichen families *Physciaceae* and *Caliciaceae* Chevall. (Wedin *et al.* 2002). These analyses include only three *Rinodina* species which were close to the foliose genera *Physcia* and *Anaptychia* Körb. within the *Physcia* group. Helms *et al.* (2003) included six *Rinodina* species in their phylogeny of selected *Physciaceae* and twenty species in their phylogeny of all *Physciaceae* using ITS data only. Variation among the non-coding ITS regions is useful for delimitations at species level (Myllys *et al.* 2001; Kaschik 2006), whereas the mtSSU rDNA sequence is a more conserved gene locus and allows the investigation of relationships between genera (Crespo *et al.* 2001; Wedin *et al.* 2002). A combined phylogenetic analysis using both nrITS and mtSSU rDNA genes should be valuable in distinguishing

genera or species groups. The aim of this study is to test the phylogenetic importance of the main traits which are used for the delimitation of *Rinodina* species as well as to check the phylogenetic positions of some 'extraneous' *Rinodina* and *Buellia* species (*Buellia lindingeri*, *Buellia parvula* and *Rinodina insularis*) using nrITS and mtSSU rDNA molecular data.

Materials and Methods

Taxon sampling

Lichen material used for sequencing is listed in Table 1 and is deposited in GZU or KW. Most samples selected for molecular analyses were collected during 2005–2007 in Austria, Ukraine, Russia, Crete and Sweden. Other sequences for our analysis were downloaded from GenBank NCBI (Table 2). In addition the following public herbaria and private collections have contributed with the loan of specimens: G, GZU, M, SZU, hb. Groner, hb. Vězda and hb. Zimmermann.

DNA extraction, PCR amplification, DNA sequencing and sequence alignment

Lichen specimens were carefully checked for any externally visible infections from other organisms. Total DNA was extracted from apothecia using a modified CTAB method as described by Cubero *et al.* (1999). DNA extracts were used for PCR amplification of the ITS regions of the nuclear rDNA and SSU region of the mitochondrial gene. Primers used for PCR of the nuclear ribosomal ITS region were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), those for mitochondrial SSU gene were mtSSU1 and mtSSU3R (Zoller *et al.* 1999). The PCR amplification was carried out with Applied Biosystems Gene Amp PCR System 2400. Products were cleaned with QIAquick PCR Purification Kit (QIAGEN, Vienna) according to the manufacturer's protocol. The PCR products were then sequenced with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied, Vienna) according to the manufacturer's instructions. Sequences were run on an ABI 310 automated sequencer (Applied, Vienna).

Sequences obtained of both strands were assembled and edited using AutoAssembler Software (Applied Biosystems, Vienna), which we used for the alignment in BioEdit (<http://jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html>) together with sequences downloaded from GenBank NCBI. Alignment was automatically carried out using the Clustal algorithm with standard settings in BioEdit and then improved manually. Data sets of nrITS and mtSSU rDNA were combined in BioEdit and consisted of 1237 sites from 44 taxa.

TABLE 1. List of specimens and GenBank accession numbers of the new nrITS and mtSSU rDNA sequences generated for the study

Species	Locality	Collection reference number	GenBank accession number	
			nrITS	mtSSU
<i>Amandinea punctata</i>	Ukraine, Donetsk Upland	GZU 000272563	GU553286	GU553306
<i>Buellia erubescens</i>	Russia, Komi	KW 63381	GU553289	GU553307
<i>B. schaereri</i>	Austria, Styria	GZU 000272658	GU553288	GU553308
<i>Diplotomma alboatrum</i>	Ukraine, Donetsk Upland	GZU 000272564	GU553287	GU553309
<i>Rinodina alba</i>	Greece, Crete	GZU 000272655	GU553290	GU553310
<i>R. albana</i>	Austria, Carinthia	GZU 000272651	GU553297	
<i>R. bischoffii</i>	Ukraine, Donetsk Upland	KW 63380	GU553291	GU553311
<i>R. calcareae</i>	Greece, Crete	GZU 000272654	GU553292	GU553312
<i>R. capensis</i>	Austria, Styria	GZU 000272663	GU553293	GU553313
<i>R. exigua</i>	Sweden, Östergötland	GZU 000272652	GU553294	GU553314
<i>R. glauca</i>	Austria, Styria	GZU 000272662	GU553295	GU553315
<i>R. immersa</i>	Ukraine, Carpathians	GZU000272656	GU553296	
<i>R. lecanorina</i>	Greece, Crete	GZU 000272660	GU553298	GU553316
<i>R. milvina</i>	Ukraine, Donetsk Upland	KW 63379	GU553299	GU553317
<i>R. mniaraea</i> var. <i>mniaraeiza</i>	Austria, Carinthia	GZU 000272664	GU553300	
<i>R. oleae</i>	Sweden, Skåne	GZU 000272565	GU553301	GU553318
<i>R. pyrina</i>	Sweden, Östergötland	GZU 000272653	GU553302	GU553319
<i>R. septentrionalis</i>	Russia, Komi	GZU 000272561	GU553303	GU553320
<i>R. sophodes</i>	Austria, Styria	GZU 000272661	GU553304	GU553321
<i>R. teichophila</i>	Greece, Crete	GZU 000272659	GU553305	GU553322

Phylogenetic analysis

Based on the combined data matrix, a parsimony tree was constructed in PAUP*4.08b (Swofford 2002), using a heuristic search with 1000 replicates of random sequence additions. Seventy-three ambiguous positions were excluded from the data matrix, gaps were treated as missing values. The phylogram was constructed with the Bayesian approach as implemented in the MrBayes 3.0b4. (Huelsenbeck & Ronquist 2001). Support values obtained in this analysis are regarded as significant when exceeding 0.95. The trees were drawn using TREE-VIEW (Page 1996). As in a previous study, we chose *Rhizocarpon geographicum* (L.) DC. as a suitable out-group (Grube & Arup 2001).

Morphology and chemistry

The material was examined using a stereo- and high power microscopy. Thin-layer chromatography (TLC) was employed using standard methods (e.g. Orange *et al.* 2001).

Results

We generated 47 new sequences (26 of nrITS region and 21 of mtSSU rDNA) from 19 crustose species of the *Physciaceae* (mainly from the genus *Rinodina*) (Table 1).

The species analysed are split into two groups, which correspond to the previously recognized *Buellia* and *Physcia* groups (Fig. 1, Grube & Arup 2001; Helms *et al.* 2003; Kaschik 2006). Genetic variation is poorly resolved in the *Buellia*-group with our markers, therefore we concentrate on the *Physcia* group in the following discussion. Our combined analysis shows that there are several more or less well supported units of *Rinodina* species within the *Physcia* group. These units of *Rinodina* species also include other genera such as *Physcia*, *Phaeophyscia*, *Physconia*, *Phaeorrhiza* H. Mayrhofer & Poelt and *Rinodinella* H. Mayrhofer & Poelt. Except for the last two genera, these are characterized by a more complex morphological organization, such as foliose thalli with more phenotypic characters of taxonomic value than found in the crustose groups.

The monophyletic and well-supported Clade A includes only the three species *Rinodina exigua* (Ach.) Gray, *R. confragosa* (Ach.) Körb. and *R. capensis* Hampe. All these species contain atranorin and have ascospores of the *Physcia*-type.

TABLE 2. List of species and accession numbers of sequences downloaded from GenBank

Species	GenBank accession number	
	nrITS	mtSSU
<i>Endohyalina insularis</i> *	DQ849302	
<i>Phaeophyscia ciliata</i>	EF582752	EF582803
<i>Phaeorrhiza sareptana</i> var. <i>sphaerocarpa</i>	AF250801	AY143421
<i>Physcia aipolia</i>	DQ782836	DQ912290
<i>Physconia distorta</i>	EF582763	EF582813
<i>Rhizocarpon</i> <i>geographicum</i>	AF483619	AF483187
<i>Rinodina archaea</i>	DQ849292	
<i>R. atrocineria</i>	AF540544	
<i>R. confragosa</i>	AF250808	
<i>R. interpolata</i>	AF250809	
<i>R. lindingeri</i> **	AF250789	AY143419
<i>R. luridata</i>	DQ849304	
<i>R. luridescens</i>	AJ544183	
<i>R. obnascens</i>	AJ544185	
<i>R. olivaceobrunnea</i>	AF540547	
<i>R. orculata</i>	DQ849309	
<i>R. oxydata</i>	AF540548	
<i>R. parvula</i> ***	AF540545	
<i>R. plana</i>	AF250812	AY143425
<i>R. roscida</i>	DQ849317	
<i>R. tunicata</i>	AF540551	
<i>R. turfacea</i>	AF224362	
<i>R. zwackhiana</i>	AF540552	
<i>Rinodinella controversa</i>	AJ421423	

*as *Rinodina insularis*, **as *Buellia lindingeri*, ***as *Rinodina lecanorina*

The moderately supported Clade B is nesting *Physcia aipolia* (Ehrh. ex Humb.) Fűrnr. basal with *Rinodina alba* Metzler ex Arnold and *R. atrocineria* (Hook.) Körb. The last two are related silicicolous species with ascospores of the *Pachysporaria*-type. The other *Rinodina* species in Clade B are corticolous, except for the siliceous *R. interpolata* (Stirt.) Sheard and the muscicolous *R. olivaceobrunnea* C. W. Dodge & G. E. Baker, and most have *Physcia*-type ascospores, except for *R. orculata* Poelt & M. Steiner and *R. archaea* (Ach.) Arnold which possess the related *Physconia*-type ascospores. A highly supported monophyletic subclade includes *R. orculata*, *R. interpolata*, *R. archaea* and *R. olivaceobrunnea*. These are species with varying ecology.

The monophyletic and highly supported Clade C includes *Phaeophyscia ciliata* (Hoffm.) Moberg and *Rinodinella controversa* (A. Massal.) H. Mayrhofer & Poelt. Three subclades can be recognized, the first of these consists of calcareous taxa such as *Rinodinella controversa*, *Rinodina tunicata* H. Mayrhofer & Poelt, *R. immersa* (Körb.) Arnold, *R. bischoffii* (Hepp) A. Massal., *R. zwackhiana* (Kremp.) Körb. and interestingly the muscicolous *R. roscida* (Sommerf.) Arnold. The second subclade includes species with *Teichophila*-type ascospores (*R. teichophila* (Nyl.) Arnold and *R. albana* (A. Massal.) A. Massal.). The third subclade includes species occurring on calcareous rocks with *Bicincta*-type ascospores, such as *R. luridata* (Körb.) H. Mayrhofer *et al.* and *R. lecanorina* (A. Massal.) A. Massal.

Clade D is distinguished here for pragmatic reasons although it is not monophyletic. It includes species of *Rinodina* with ambiguous relationships as well as *Physconia distorta* (With.) J. R. Laundon and *Phaeorrhiza sareptana* (Tomin) H. Mayrhofer & Poelt. A well-supported monophyletic subclade comprises *R. luridescens* (Anzi) Arnold together with the morphologically peculiar species *Rinodina lindingeri* (Erichsen) Giralt & van den Boom and *R. parvula* H. Mayrhofer & Poelt. All species in this clade have different types of excipulum and ascospores and occur on different substrata. However, the relationships of this clade to other lineages in *Rinodina* including Clade C remain poorly resolved.

Clade E consists of the two species, *Rinodina oleae* Bagl. with *Dirinaria*-type ascospores and *R. pyrina* (Ach.) Arnold with *Physconia*-type ascospores.

The monophyletic Clade F includes species with *Milvina*-type ascospores such as *Rinodina obnascens* (Nyl.) H. Olivier, *R. milvina* (Wahlenb.) Th. Fr., and the type species *R. sophodes* (Ach.) A. Massal., as well as those with *Physcia*-type ascospores of the related corticolous species pair *R. glauca* Ropin and *R. septentrionalis* Malme.

The species analysed of the *Buellia* group belong to the genera *Amandinea* M. Choisy,

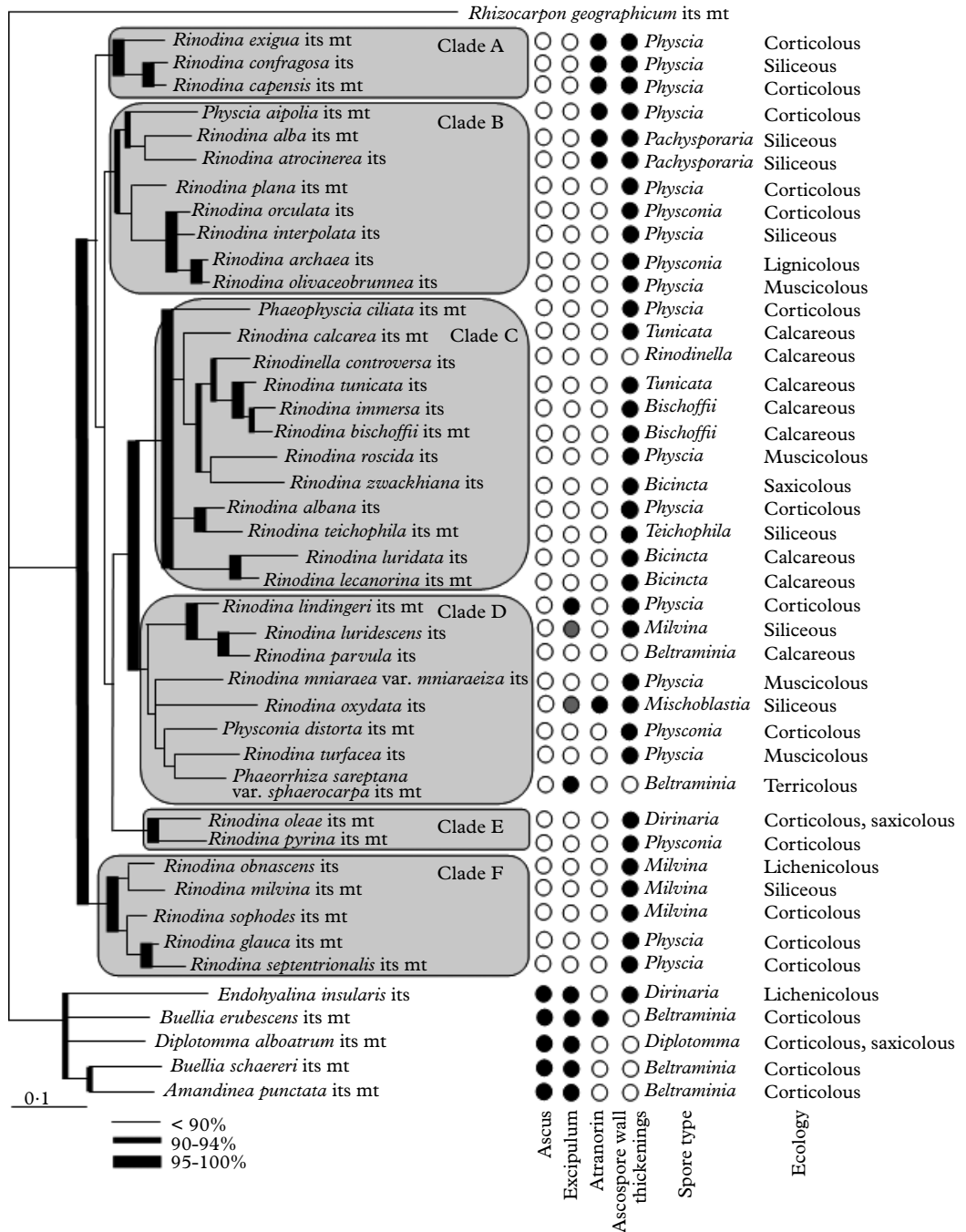


FIG. 1. Bayesian consensus nrITS and mtSSU rDNA phylogeny of selected *Physciaceae* compared with phenotypical and ecological characters. Ascus type: ● *Bacidia*-type, ○ *Lecanora*-type; excipulum: ● lecideine, ○ lecanorine; atronarin and ascospore thickenings: ● present, ○ absent.

Buellia, *Diplotomma* Flot. and *Endohyalina* Marbach. The phenotypically unusual *Endohyalina insularis*, previously suggested to belong to *Rinodina*, is distinct from the latter genus and is clearly a member of the *Buellia* group (Fig. 1).

Taxonomy

Endohyalina insularis (Arnold) Giralt, van den Boom & Elix

Mycological Progress 9: 44 (2010).—*Buellia saxatilis* f. *insularis* Arnold, *Verh. zool.-bot. Ges. Wien* 46: 119 (1896).—*Rinodina insularis* (Arnold) Hafellner, *Beih. Nova Hedwigia* 62: 87 (1979); type: South Tirol, Plan (Südtirol, Gröden, Augitporphyr oder plan gegen das Sellajoch), Aug. 1895, leg. Arnold (M—holotype).

The phylogenetic analysis based on nrITS and mtSSU sequence data indicates that *Endohyalina insularis* is not monophyletic with other species of *Rinodina*, but groups in *Buellia* s. lat. (Fig. 1), as already shown by Kaschik (2006 as '*Rinodina*' *insularis*). Morphologically, *Endohyalina insularis* has lecideine apothecia, *Dirinaria*-type ascospores and *Bacidia*-type asci similar to other representatives of *Buellia* s. lat. As a lichenicolous lichen, the species grows on lichens belonging to the *Lecanora rupicola* (L.) Zahlbr. group (Rambold *et al.* 1994). The species was described as *Buellia saxatilis* f. *insularis* by Arnold (1896). The combination with *Rinodina* was mainly based on the ascospore wall thickenings (Hafellner 1979), while it was speculated that lichenicolous *Rinodina* species might easily loose algae in their excipula. Supported by ascus and excipulum structure, ascospore characters, as well as molecular data, we accept this species as *Endohyalina insularis*. The genus *Endohyalina* was described by Marbach (2000).

The species is widely distributed. In Europe it has been found in the British Isles, France, Greece, Italy, Austria, Romania (e.g., Hafellner 1979; Mayrhofer & Poelt 1979; Mayrhofer 1984a; Triebel *et al.* 1991; Calatayud *et al.* 1995; Giralt 2001; Hitch 2006), in Macaronesia (e.g. Hafellner 1995),

and in Turkey (Halici *et al.* 2005). Samples from North America thus far originate from Arizona and British Columbia (Triebel *et al.* 1991; Sheard 2004) and the species is also found in the Southern Hemisphere in Australia, New Zealand and Chile (Triebel *et al.* 1991; Mayrhofer & Lambauer 2004; Kaschik 2006; Hafellner & Mayrhofer 2007).

Selected specimen examined. Greece: Crete: Monophatsi, Asterusi Gebirge, bei Kapetaniana, 1942, Rechinger (M, associated with Tephromela atra).

Endohyalina brandii Giralt, van den Boom & Elix

Mycological Progress 9: 41 (2010).

This species is similar to *Endohyalina insularis* and obligately lichenicolous on *Aspicilia intermutans* (Nyl.) Arnold. Apart from growing on *Lecanora rupicola*, *E. insularis* is distinguished by the larger, initially immersed apothecia, the larger ascospores and the darker hypothecium. The new species was previously known only from Tenerife and El Hierro in the Canary Islands (Giralt *et al.* 2010b). We here report the first specimens from Europe (Iberian Peninsula: Spain) and La Palma, another island in the Canary Islands.

Other specimens examined. Spain: Prov. Barcelona: Sierra de Montseny, Hänge NE der Paßhöhe des Coll Formich, 1983, Hafellner 17322 (GZU). Prov. Tarragona: Sierra de Prades, NW-geneigte Hänge SW ober dem Monasterio de Poblet, an der Straße nach Prades, 1983, Hafellner 17489 (GZU). Canary Islands: La Palma: Caldera de Taburiente, Taburiente, am Westrand des Schotterfeldes "La Playa", 1991, Mayrhofer 10643 & Matzer (GZU).

Endohyalina diederichii Giralt, van den Boom & Elix

Mycological Progress 9: 42 (2010).

This species is obligately lichenicolous on the genus *Ramalina* Ach. It is characterized by a densely inspersioned hymenium with abundant oil droplets and fusiform ascospores. It is closely related to *E. ericina* (Nyl.) Giralt *et al.* but differs in its lichenicolous habit and its smaller apothecia. It is known only from Lanzarote, Fuerteventura, El Hierro and

Tenerife in the Canary Islands (Giralt *et al.* 2010b).

Other specimen examined. Canary Islands: La Gomera: La Fortaleza, 1983, Rambold 1709 (M).

Rinodina albana (A. Massal.) A. Massal.

Ric. Auton. Lich. Crost.: 15 (1852).—*Hagenia albana* A. Massal., *Verh. zool.-bot. Ges. Wien* 1: 221 (1851); type: locality not indicated, hb. Massalongo (VER—lectotype).

Rinodina albana is characterized by a cortex consisting of hyaline hyphae with globose lumina, apothecia with a brown disc, distinct proper margin visible as a ring within the thick thalline margin, and large ascospores of the *Teichophila*-type (Sheard & Mayrhofer 2002) with lumina, if well developed, mainly of the *Physcia*-type. A torus is not visible. The surface of the ascospores is microrugulate and already visible at magnification $\times 400$. The hymenia often do not include any good ascospores, and where ascospores are present, the asci usually do not contain a complement of eight fully developed ascospores. Ropin & Mayrhofer (1993), Giralt & Mayrhofer (1995) and Giralt (2001) referred the ascospores to the *Physcia*-type. There are frequent conidiomata around the base of the apothecia containing bacilliform conidia. *Rinodina albana* resembles *R. trevisanii* (Hepp) Körb. and *R. exigua*. *Rinodina trevisanii* is characterized by ascospores of the *Physconia*-type with a distinct torus (Mayrhofer & Sheard 2007). *Rinodina exigua* differs in containing at least traces of atranorin in a much weaker developed apothecial cortex and in having *Physcia*-type ascospores with a distinct torus (Ropin & Mayrhofer 1993). The phylogenetic analysis supports the close relationship of the corticolous *R. albana* to the saxicolous *R. teichophila*. The ascospores of the latter species and its relatives in the Southern Hemisphere were discussed by Matzer & Mayrhofer (1994). *Rinodina albana* is a rare species occurring mainly on the bark of deciduous trees. It is reported from Central Europe (Austria, Czech Republic, France, Germany, Romania, Slovakia, Slovenia, Switzerland) and southern Europe (Italy, Montenegro, Spain) by

Ropin & Mayrhofer (1993) and Giralt & Mayrhofer (1995).

Selected specimens examined. Austria: Kärnten: Gailtaler Alpen, Gemeinde Paternion, Anwesen Farchen nördlich Kreuzen, *Fagus sylvatica*, 2000, Mayrhofer 13976 & Pichorner (GZU). *Salzburg:* Tennengau, Vorderwiestal, alte Wiestalstraße, bei Almdurchbruch, 550 m, 1985, Blieberger & Türk (SZU). *Vorarlberg:* Bregenzerwald, Hittisau, von Hinterberg nach Gfreren, *Fraxinus excelsior*, 1992, Pfefferkorn 8525 (SZU).—**Montenegro:** Lovćen: Ivanova Korita, on bark of *Fagus sylvatica*, 2004, Mayrhofer 18277, Nedović, Knežević & Jakić (GZU).—**Slovenia:** Triglav National Park: Bohinj area, Voje valley, c. 3.5 km N from Stara Fužina, *Juglans regia*, 2002, Batič & Mrak (GZU).—**Switzerland:** Bern: Gemeinde Hasliberg, *Acer platanoides*, 1998, Dietrich 12082 (G).—*Glarus:* Gemeinde Elm, *Acer pseudoplatanus*, 1997, Roth 11707 (G). *Graubünden:* Gemeinde Rueun, *Fraxinus excelsior*, 1998, Dietrich 12090 (G). *Schwyz:* Muotatal, Mittst Weid, Waldrand unterhalb Prugelstraße, *Fraxinus excelsior*, 1997, Groner 1843 (Groner). *St. Gallen:* Gemeinde Walenstadt, Walenstadtberg, *Ulmus glabra*, Roth 11705 (G). *Tessin:* Gemeinde Malvaglia, Caslou, *Juglans regia*, 1995, Keller 2108 (G). *Waadt:* Gemeinde Chateau-d'Oex, Lac de l'Hongrin, *Fraxinus excelsior*, 1996, Groner 11697 (G). *Wallis:* Gemeinde Kippel, Haslä, *Fraxinus excelsior*, 1997, Scheidegger 11695 (G).

Rinodina lindingeri (Erichsen) Giralt & van den Boom

Bryologist 113: 101 (2010).—*Buellia lindingeri* Erichsen, *Hedwigia* 66: 281 (1926); type: Macaronesia, Canary Islands, Tenerife, Mesa Gallardina near Laguna, on *Agave americana*, 750 m, 1917, L. Lindinger (HB—holotype).

In the present phylogenetic study *Rinodina lindingeri* belongs in the *Physcia*-group, and is closely related to *R. luridescens* and *R. parvula* (Fig. 1). This confirms previous ITS data, where *Rinodina lindingeri* was not supported in *Buellia* s. lat. (Grube & Arup 2001; Molina *et al.* 2002; Wedin *et al.* 2002; Helms *et al.* 2003; Kaschik 2006, all as *Buellia lindingeri*). The species has lecideine to pseudolecanorine apothecia, *Physcia*-type ascospores and *Lecanora*-type asci (Giralt & Matzer 1994; Rambold *et al.* 1994; Giralt *et al.* 2010a). The species was described as *Buellia lindingeri* by Erichsen (1926) with material from Macaronesia and all subsequent records have been from this region (Giralt & Matzer 1994; Giralt *et al.* 2010a).

Selected specimen examined. **Canary Islands:** *Fuerteventura*: Morro de la Cruz, c. 600 m, 2006, Zimmermann (hb. Zimmermann).

Rinodina parvula H. Mayrhofer & Poelt

Bibl. Lich. 12: 138 (1979); type: Macedonia, Sar planina, Rudoka, Popova sapka W Tetovo, nge W der Bergstation der Bergbahn, ± 2000 m alt., 8 July 1977, Poelt (GZU—holotype).

In the phylogenetic study *Rinodina parvula* is nested within the *Physcia* group, close to *R. luridescens* (Fig. 1), confirming previously published ITS trees (Grube & Arup 2001; Helms *et al.* 2003; Kaschik 2006). Despite the general similarity of thalline and apothecial characters and substratum of *R. parvula* and *R. lecanorina* (Fig. 1, Clade C), these two species are clearly distinguished by their types of ascospores, i.e. *Buellia*- and *Bicincta*-type, respectively. Their superficial morphological similarity may be the reason for numerous misidentifications of *R. parvula* as *R. lecanorina*. A specimen of *R. lecanorina* from GZU (Mayrhofer 13120, GenBank AF540545, Helms *et al.* 2003) is here identified as *R. parvula*. We also suspect that the *R. lecanorina* specimen used in Grube & Arup (2001; M283, GenBank AF250810) belongs to *R. parvula*, due to the phylogenetic position close to *R. lindingeri* (as *Buellia lindingeri*) on their respective phylogenetic tree.

Rinodina parvula is characterized by cryptolecanorine to lecanorine apothecia, *Beltraminea*-type ascospores and *Lecanora*-type asci (Rambold *et al.* 1994). The species was described as *Rinodina parvula* by Mayrhofer & Poelt (1979) because of its type of apothecium. The authors emphasized that this species may be more closely related to *Buellia* according to the type of ascospores, and it was noted that this species deserves comparative study with other *Buellia* species (Mayrhofer 1984a). Scheidegger (1993) transferred it to *Buellia* and hypothesized a relationship of this species with *Buellia aethalea* (Ach.) Th. Fr. The relationship with the *Buellia* group is not supported at all by our phylogenetic data and we therefore retain this taxon as *Rinodina parvula*.

The species was described from Macedonia, but is also known from Austria, Switzerland

(Mayrhofer & Poelt 1979; Mayrhofer 1984a; Hofmann *et al.* 1993; Hafellner *et al.* 2005) and Spain (Navarro-Rosinés & Hladun 1990; Giralt 2001).

Other specimens examined. **Austria:** *Styria*: Grazer Bergland, Jungfernsprung bei der Ruine Gösting, 1996, Mayrhofer 13120 (GZU).—**Italy:** *Südtirol*: Gröden, Unterkofel über St. Ulrich, 1899, Arnold (M).—**Switzerland:** *Tessin*: Adula Alpen, Val Piora, NE-exponierte Abhänge des Föisc über dem Ritomsee, 1984, Mayrhofer 4418 (GZU).—**Slovakia:** *Tatra Magna*: pars Belanské Tatry. in monte Bujačic, 1955, Vězda [hb. Vězda, associated with *Rinodina bischoffii* and *R. castanomela* (Nyl.) Arnold].

Discussion

In the present study we have examined the phylogenetic position of the phenotypically peculiar species *Buellia lindingeri*, *B. parvula* and *Rinodina insularis*, whose systematic positions are controversial (Scheidegger 1993; Helms *et al.* 2003; Kaschik 2006; Giralt *et al.* 2010a, b). Our analysis shows that *Buellia lindingeri* and *B. parvula* belong to the genus *Rinodina*, although the first species has a lecideine excipulum and the second is characterized by *Buellia*-type ascospores. Both taxa are phylogenetically close to *Rinodina luridescens* (Fig. 1, Clade D), which differs in exciple character and spore-type. This clade is particularly interesting as it shows an impressive phenotypic variation among crustose species. Apparently morphological evolution of taxonomically important traits is more dynamic in clades of the *Physcia*-group, which can clearly complicate the consistent application of characters traditionally used for classification. Analogously, *Rinodina insularis* was transferred to the genus *Endohyalina* by Giralt *et al.* (2010b). It is placed with species of the *Buellia*-group in spite of spore wall thickenings, which were previously regarded as characteristic for *Rinodina*.

The combined analysis of the nrITS and mtSSU rDNA confirms the monophyly of *Buellia* and *Physcia* groups as well as the heterogeneity of *Rinodina* (Fig. 1, Grube & Arup 2001; Helms *et al.* 2003; Kaschik 2006). We tried to assess correlations of some anatomical, morphological, chemical

and ecological characters with highly supported monophyletic groups within *Rinodina*. Our analysis shows that there are still no distinctly diagnostic criteria that can be used for clear delimitation of groups within *Rinodina* or *Buellia*. While the ascus type consistently distinguishes the *Buellia* and *Physcia* groups, the excipulum type is highly variable in the *Physcia* group where some of the *Rinodina* species have either lecideine, pseudo-lecanorine or lecanorine apothecia (Fig. 1; see also *Rinodinella* in Grube & Arup 2001). The presence of a hypothecium pigmentation is a constant character in the *Buellia* group, but is present only in the phylogenetically close *Rinodina lindingeri* and *R. luridescens* from the *Physcia* group (Fig. 1, Clade D). The presence of atranorin is correlated with one Clade A (Fig. 1) which joins three *Rinodina* species with *Physcia*-type ascospores, *R. exigua*, *R. confragosa* and *R. capensis*. Atranorin was also found in two other clades (Fig. 1). Ascospore wall thickenings varied considerably among the species studied. The *Physcia* group may also include species without spore wall thickenings such as *Rinodinella controversa*, *Rinodina parvula* and *Phaeorrhiza sareptana*, whereas the *Buellia* group can include species with spore wall thickening, such as *Endohyalina insularis* (and also species traditionally assigned to *Hafellia* spp.). Therefore, spore wall thickenings are not suitable for the delimitation within the *Buellia* and *Physcia* groups. The type of ascospores did not correlate with any of the subclades in the phylogenetic tree presented here, but it was in fact more or less constant in smaller monophyletic groups. They are only useful in providing distinct diagnostic characters. The substratum preferences of the investigated species were also variable in each group (Fig. 1). However, it should be pointed out that the different clades show at least some substratum preferences: Clade A consists of mainly corticolous species, Clades B and F contain mainly corticolous and siliceous and Clade C includes calcareous species. Preliminary results of our ecological analysis are not presented here, but they suggest that other ecological preferences may also be useful in distinguishing distinct clades

within *Rinodina* and *Buellia*. Indeed some mycobiont species appear to demonstrate preferences for particular strains of trebouxioid photobionts, which appear to be better adapted to the habitats where the lichen grows. Classical morphological and anatomical characters should thus be re-evaluated and augmented by other parameters that may eventually prove more useful to arrive at a new generic delimitation within the *Physciaceae*.

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