Lichenologist **33**(1): 73–86 (2001)
doi:10.1006/lich.2000.0298, available online at http://www.idealibrary.com on IDEAL®

Identification of photobionts from the lichen family *Physciaceae* using algal-specific ITS rDNA sequencing

Gert HELMS, Thomas FRIEDL, Gerhard RAMBOLD and Helmut MAYRHOFER

Abstract: The identity of photobionts from 20 species of the *Physciaceae* from different habitats and geographical regions has been determined by ITS rDNA sequence comparisons in order to estimate the diversity of photobionts within that lichen group, to detect patterns of specificity of mycobionts towards their photobionts and as a part of an ongoing study to investigate possible parallel cladogenesis of both symbionts. Algal-specific PCR primers have been used to determine the ITS rDNA sequences from DNA extractions of dried lichens that were up to 5 years old. Direct comparisons and phylogenetic analyses allowed the assignment of *Physciaceae* photobionts to four distinct clades in the photobiont ITS rDNA phylogeny. The results indicate a diversity within the genus *Trebouxia* Puymaly and *Physciaceae* photobionts that is higher than expected on the basis of morphology alone. *Physciaceae* photobionts belonged to 12 different ITS lineages of which nine could unambiguously be assigned to six morphospecies of *Trebouxia*. The identity of the remaining three sequences was not clarified; they may represent new species. Specificity at the generic level was low as a whole range of photobiont species were found within a genus of *Physciaceae* and different ranges were detected. The photobionts of *Physcia* (Schreb.) Michaux were closely related and represented one morphospecies of *Trebouxia*, whereas the algal partners of *Buellia* De Not and *Rinodina* (Ach.) S. Gray were in distant lineages of the ITS phylogeny and from several *Trebouxia* morphospecies. Photobiont variation within a genus of *Physciaceae* may be due to phylogeny, geographical distance or because photobionts from neighbouring lichens were taken ('algal sharing'). At the species level *Physciaceae* mycobionts seem to be rather selective and contained photobionts that were very closely related within one morphospecies of *Trebouxia*.

Introduction

The diversity of photobionts in any taxonomic group of lichens has been poorly studied so far. Records of photobionts that have been identified at the species level are limited as they refer mainly to distantly related mycobiont taxa and only few examples per group have been studied [see the lists of photobiont taxa in Tschermak-Woess (1988) and Ahmadjian (1993)]. In a recent compilation of the present knowledge of photobionts from various groups of the Lecanorales, it was suggested that the photobionts may be important markers of evolutionary relationships and, therefore, their identification should become a prerequisite in systematic studies of lichens. The capability of lichen fungi to select an appropriate algal partner from a variety of algal taxa common in subaerial habitats may be an important phylogenetic trait (Rambold *et al.* 1998). The taxonomic level at which photobionts may serve as markers of evolutionary relationships of lichens is, however, unknown. According to current taxonomic concepts, *Physciaceae* is a family of the

G. Helms and T. Friedl: Experimentelle Phykologie und Sammlung von Algenkulturen, Albrecht-von-Haller-Institut, Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany.
G. Rambold: Lehrstuhl Pflanzensystematik, Universität Bayreuth, Universitätsstraße 30, D-95448 Bayreuth, Germany.
H. Mayrhofer: Institut für Botanik, Karl-Franzens-Universität Graz, Holteigasse 6, A-8010 Graz, Austria.

0024–2829/01/010073+14 $35.00/0

Lecanorales (Ascomycota) characterized by a variety of ascospore characters (Hafellner *et al.* 1979; Tehler 1996). The *Physciaceae* exhibits a great variety of different growth forms, its species are distributed world-wide in various kinds of habitats and it is very likely that this family of ascomycetes represents a group of evolutionarily closely related fungi. In an ongoing study of the *Physciaceae*, we will address general questions of symbiotic interactions concerning photobiont diversity. The present study of photobionts is part of an investigation of a possible parallel cladogenesis between both bionts in the *Physciaceae*. We address the question whether there is a single algal species or a whole spectrum of suitable photobionts associated with certain genera or species of lichen mycobionts. Photobionts may also be indicative of geographical distribution patterns or certain habitats. The findings from our study may eventually lead to more general conclusions regarding the specificity of lichen fungi towards their algal partners when more studies of photobionts from other natural groups of lichens become available.

As in many groups of lichens, the information about the photobionts of the *Physciaceae* is scant and scattered. Ahmadjian (1960) first reported *Trebouxia impressa* Ahmadjian from *Physcia stellaris* (L.) Nyl., and later, Hildreth & Ahmadjian (1981) recorded *T. higginsiae* (Hildreth & Ahmadjian) Gärtner from *Buellia straminea* Tuck. Using axenic cultures and ITS rDNA sequence data, Beck *et al.* (1998) recently identified the photobionts from various specimens of *Phaeophyscia orbicularis* (Neck.) Moberg, *Physcia adscendens* (Fr.) H. Olivier and *P. stellaris* as different strains of *T. impressa*. Although morphologically identical, the algal partners of *Phaeophyscia orbicularis* and *Physcia adscendens* were different at the sequence level indicating that the actual diversity of lichen photobionts might be higher than expected from their few morphological traits. A high resolution for the differentiation of *Trebouxia* spp., even below the species level (e.g., strain identification), can be obtained using ITS rDNA sequences (Beck *et al.* 1998; Friedl *et al.* 2000). For *Trebouxia* spp. an increasing number of ITS rDNA sequences is available from culture strains (Bhattacharya *et al.* 1996; Beck *et al.* 1998; Beck 1999; Friedl *et al.* 2000) to which newly determined ITS sequences can be compared.

Identification of lichen photobionts at the species level requires pure algal cultures. However, to establish such cultures is time-consuming and often impossible with herbarium specimens of lichens, or when the algal growth proved recalcitrant upon separation from the fungal partner. Green algal-specific PCR primers that bind to conserved flanking regions of the ITS rDNA, for example, at the 3′-end of the 18S rDNA, may allow the specific amplification of lichen photobiont sequences without the need to culture. Therefore, we designed PCR primers from an alignment of published and unpublished 18S rDNA sequences of lichen green algae and corresponding sequences from ascomycetes. Using these primers, we successfully selectively amplified and sequenced photobiont rDNAs even from herbarium material which was up to five years old. Sequencing of algal ITS rDNA from total DNA extractions of lichens is a fast and reliable method for the identification of photobionts. In this study, algal ITS rDNA was amplified and sequenced from 20 species of the *Physciaceae* from different habitats and geographic regions in order to obtain a preliminary estimate of the diversity of photobionts within the group.

Materials and Methods

Lichen taxa and availability of photobiont ITS rDNA sequences

Photobiont ITS rDNA sequences were determined from the following lichen specimens. The ITS sequences are available from the Genbank/EBI data base under the accession numbers as given in brackets after the specimen description. ***Amandinea punctata* (Hoffm.) Coppins & Scheid.**: Italy: *Prov. di Livorno*: Isola d'Elba, on pebbles, iv 1998, *D. Triebel & G. Rambold* 6146 (M) (AJ293780).—***Anaptychia ciliaris* Körb. ex A. Massal.**: Germany: *Bayern*: Pullach, on

bark of *Acer pseudoplatanus* at roadside, x 1994, *T. Friedl* (AJ293770).—***A. runcinata* (With.) J. R. Laundon**: Italy: *Prov. di Livorno*: Isola d'Elba, NE-exposed coastal rocks, iv 1998, *D. Triebel & G. Rambold* 6162 (M) (AJ2937821).—***Buellia elegans* Poelt & Sfulzer**: Austria: *Osttirol*: Obermauern, over S-exposed limestone slope, limestone slate, ix 1996, *U. Trinkaus* 439, *J. Prügger & H. Mayrhofer* (GZU) (AJ293782).—***B. georgei* Trinkaus, H. Mayrhofer & Elix**: Australia: *Western Australia*: Lancelin, on soil over limestone plates, xii 1996, *U. Trinkaus* 356a (GZU) (AJ293783).—***B. zoharyi* Galun**: Spain: *Islas Canarias*: Lanzarote, S. of Haria, on soil, viii 1996, *U. Trinkaus* 450 & *M. Grube* (GZU) (AJ293784).—***Dimelaena oreina* (Ach.) Norman**: Austria: *Steiermark*, Tegitschgraben S Voitsberg, xi 1998, *H. Mayrhofer* 13.737 & *U. Arup* (GZU) (AJ293785).—***Phaeophyscia orbicularis* Necker (Moberg)**: Germany: *Mecklenburg-Vorpommern*: on bark of *Prunus* sp., viii 1998, *M. Schultz* (AJ293786).—***Physcia adscendens* (Fr.) H. Olivier**: Germany: *Baden-Württemberg*: Müllheim, on bark of *Malus domesticus*, iv 1996, *P. Dornes* (AJ293774).—Germany: *Rheinland Pfalz*: Kaiserlautern, on concrete wall, iv 1998, *F. Kauff* (AJ293773).—Germany: *Baden-Württemberg*: Müllheim, on bark, vi 1998, *P. Dornes* (AJ293772).—Germany: *Bayern*: München, Franz-Schrank-Strasse, on bark of *Fraxinus* sp., iv 1999, *G. Helms* (AJ293771).—***P. aipolia* (Ehrh. ex Humb.) Fürnr.**: [Pa1] USA: *Iowa*: Iowa City, Coralville reservoir, on bark, ix 1998, *T. Friedl* (AJ293776).—[Pa2] Germany: *Bayern*: Pullach, on bark of *Acer pseudoplatanus* at roadside, x 1994, *T. Friedl* (AJ293775).—***P. semipinnata* (J. F. Gmel.) Moberg:** Spain: *Prov. Murcia*: Sierra de la Pila, peak of Mt Pila, on *Quercus ilex*, xi 1996, *P. Dornes* (AJ293787).—***P. stellaris***: [Ps1] Germany: *Baden-Württemberg*: Müllheim, on bark, ix 1998, *P. Dornes* (AJ293777).—[Ps2] Germany: *Bayern*: München, Botanical Garden, on bark of *Fraxinus* sp., iv 1999, *G. Helms* (AJ293778).—***P. tenella* (Scop.) DC.**: Germany: *Rheinland-Pffalz*: Kaiserslautern, on bark of *Populus canadensis*. viii 1998, *P. Dornes* (AJ293788).—***Physconia perisidiosa* (Erichsen) Moberg**: Germany: *Bayern*: Pullach, on bark of *Acer pseudoplatanus*, at roadside, x 1994, *T. Friedl* (AJ293779).—***Rinodina atrocinerea* (Hook.) Körb.**: Austria: *Steiermark*, Teigitschgraben S Voitsberg, xi 1998, *H. Mayrhofer* 13.740 & *U. Arup* (GZU) (AJ293791).—***R. capensis* Hampe**: Austria: *Kärnten*: Gailtaler Alpen, Paternion, on bark of *Tilia cordata*, xi 1998, *H. Mayrhofer* 13.723 & *B. Pichorner* (GZU) (AJ293793).—***R. milvina* (Wahlenb.) Th. Fr.**: Finland: *Nylandia*: Sibbo/Sipoo, Kalkstrand, on coastal rocks, viii 1997, *H. Mayrhofer* 13.702 (GZU) (AJ293794).—***R. tunicata* H. Mayrhofer & Poelt**: Greece: *Crete*: Nomós Chanion, *c.* 1 km S Imbros on the road to Hora Sfakion; E-exp. on limestone, v 1997, *H. Mayrhofer* 13.749 & *R. Ertl* (GZU) (AJ293789).—***Rinodina* sp.** Costa Rica: *Guanacaste*: Pacific coast at Puerto Vieja W Brasilito, on rocks, April 1991, *H. Mayrhofer* 9943 & *E. Hierzer* (GZU) (AJ293792).—***Rinodinella controversa* (A. Massal.) H. Mayrhofer & Poelt**: Greece: *Crete*: Nomós Chanion, *c.* 1 km S Imbros on the road to Hora Sfakion, E-exp. on limestone, v 1997, *H. Mayrhofer* 13.747 & *R. Ertl* (GZU) (AJ293790).

DNA extraction, PCR, Sequencing

DNA was isolated from fresh material or herbarium material up to 5 years old. Small fragments of thalli or single apothecia were ground with a micropestle in a 1·5 ml reaction tube placed in an aluminium block pre-cooled in liquid nitrogen. DNA extraction was then performed with the DNeasy Plant Mini Kit (Qiagen) with extraction buffers as recommended by the manufacturer. Total lichen DNA was dissolved in 100 µl TE buffer. A set of green-algal specific 5'-PCR primers was used in combination with non-specific 3'-PCR primers (Table 1). The most sucessful primer combination was AL1500bf and LR3. PCR reactions were performed with 1 µl of the dissolved total lichen DNA in a 50 µl reaction volume containing a reaction mix of 0·2 mM of each of the four dNTPs, 2 mM $MgCl_2$, 4% DMSO, 0·2 µM of each PCR primer, and 1 unit Goldstar Taq-polymerase in Goldstar reaction buffer (Eurogentec). After an initial denaturing step at 95°C for 5 min, 33 cycles of denaturing at 94°C for 40 sec, annealing at 50°C for 40 sec, and extension at 70°C for 120 sec with an increment of 5 sec after each cycle, were performed followed by a final extension at 72°C for 10 min. PCR products were cleaned with High Pure® PCR Product Purification Kit (Roche) and sequenced directly. However, when PCR yielded double bands (see Results and Discussion), each band was extracted from the agarose gels and cloned using the pGEM-T Vector-System I (Promega) and competent DH 5α cells of *Escherichia coli*. Plasmid DNA was isolated using Wizard Plus SV Minipreps (Promega). For cycle sequencing reactions CY-5-labelled primers (Table 1) were used with the Thermo Sequenase Sequencing kit with 7-deaza-dGTP (Amersham) and reactions were run on a ALFexpress II automated sequencer (Pharmacia).

Phylogenetic analyses

The photobiont ITS rDNA sequences determined in this study were compared to available photobiont sequences (see Table 2). The ITS sequences were manually aligned with the aid of secondary structure models (Mai & Coleman 1997; Coleman *et al.* 1998; An *et al.* 1999; Friedl, unpubl.) using the multiple sequence alignment editor SeqEdit (RDP, Maidak *et al.* 1997), and the program SeqPup (Gilbert 1995). The ITS rDNA alignment is available from TF and from TreeBase (http://herbaria.harvard.edu/treebase/; accession no. SN561). Ambiguous positions within the alignment and the 5·8 S rDNA, i.e. positions 1–71, 206, 211, 243–245, 256, 257, 271, 311–467, 494–503, 593–608, and 705–730 compared to the ITS photobiont sequence of *Anaptychia ciliaris* (accession no. AJ293770), were excluded prior to the phylogenetic analyses. The ITS data set contained 438 sequence positions with

TABLE 1. *PCR and sequencing primers used in this paper to amplify and sequence photobiont ITS rDNAs from total DNA extractions of the* Physciaceae.

Primer	Sequence 5′→3′	Position	Reference	Comment
Al1500af	GCGCGCTACACTGATGC	1464–1480*/18S	This study	Green-algal-specific 5′ PCR
Al1500bf	GATGCATTCAACGAGCCTA	1476–1494*/18S	This study	Green-algal-specific 5′ PCR
Al1700f	CCCACCTAGAGGAAGGAG	1737–1754*/18S	This study	Green-algal-specific 5′ PCR
Al1729	AACCCTCCCACYTAGAGGAGGGAG	1731–1754*/18S	This study	Green-algal-specific 5′ PCR
ITS4	TCCTCCGCTTATTGATATGC	738–757‡/26S	White *et al.* (1990)	Unspecific 3′ PCR and ITS sequencing, reverse
LR1850	CCTCACGGTACTTGTTC	306–322§/26S	Friedl (1996)	Unspecific 3′ PCR
LR3	CCGTGTTTCAAGACGGG	590–606§/26S	Friedl & Rokitta (1997)	Unspecific 3′ PCR
1800f	ACCTGCGGAAGGATCATT	25–42‡/18S	Friedl (1996)	ITS sequencing, forward
ITS2N	TGCCTGCGTTCTTCATC	352–368‡/5·8S	Beck *et al.* (1998)	ITS sequencing, reverse
ITS3N	GATGAAGAACGCAGCGA	352–368‡/5·8S	Beck *et al.* (1998)	ITS sequencing, forward

*Sequence positions compared to the 18S rDNA of *Trebouxia asymmetrica* SAG 48.88 (Z21553).
‡Sequence positions compared to the ITS rDNA of *T. asymmetrica* SAG 48.88 (AJ249565).
§Sequence positions compared to the 26S rDNA of *T. asymmetrica* SAG 48.88 (Z95380).

TABLE 2. *Species and strains of* Trebouxia *used in this paper, their GenBank accession numbers and corresponding references of their ITS rDNA sequences*

Trebouxia species	Strain number*	Isolated from lichen	Accession number of ITS rDNA sequence	Reference
T. arboricola	SAG 219-1a	Unknown	Z68705	Bhattacharya *et al.* (1996)
	92.011C3‡	*Pleurosticta acetabulum*	Z68703	Bhattacharya *et al.* (1996)
	M-96.025C1§	*Xanthoria parietina*	AJ007387	Beck *et al.* 1998
T. asymmetrica	SAG 48.88	*Diploschistes diacapsis*	AJ249565	Friedl *et al.* 2000
T. gelatinosa	86.108B2‡	*Flavoparmelia caperata*	Z68697	Bhattacharya *et al.* 1996
T. gigantea	UTEX 2231	*Caloplaca cerina*	AJ249577	Friedl *et al.* 2000
T. higginsiae	UTEX 2232	*Buellia straminea*	AJ249574	Friedl *et al.* 2000
T. impressa	M-96.012A1§	*Physcia adscendens*	AJ007384	Beck *et al.* 1998
	M-96.027D1§	*Physcia adscendens*	AJ007383	Beck *et al.* 1998
	M-96.026D4§	*Phaeophyscia orbicularis*	AJ007386	Beck *et al.* 1998
	87.017E1‡	*Parmelina carporrhizans*	AJ249570	Friedl *et al.* 2000
T. incrustata	UTEX 784	*Lecanora dispersa*	AJ293795	This study
T. jamesii	M-97.017A2§	*Lecidea silacea*	AF128270	Beck 1999
	86.132E1‡	*Hypogymnia physodes*	Z68700	Bhattacharya *et al.* 1996
	86.156C3‡	*Parmelia saxatilis*	Z68701	Bhattacharya *et al.* 1996
T. usneae	UTEX 2235	*Usnea filipendula*	AJ249573	Friedl *et al.* 2000
Trebouxia sp.	87.019A1‡	*Parmotrema tinctorum*	Z68702	Bhattacharya *et al.* 1996
Trebouxia sp.	98.003B2‡	*Neofuscelia pulla*	AJ249572	Friedl *et al.* 2000

*SAG, culture collection of algae at the University of Göttingen (http://www.gwdg.de/~epsag/phykologia/epsag.html); UTEX, culture collection at the University of Austin, Texas (http://www.esb.utexaas.edu/jchen/index.html); ‡private culture collection of T.F. at Göttingen University; §private culture collection of A. Beck (Beck *et al.* 1998).

249/194 variable/parsimony informative sites. The data were subjected to maximum parsimony, distance (neighbour-joining and minimum evolution), and maximum likelihood analyses following Friedl *et al.* (2000) except that weighted maximum parsimony and distance analyses were done using PAUP* V4·0b3a (Swofford 2000).

Results and Discussion

Molecular identification of photobionts

Photobiont ITS rDNA sequences from *Physciaceae* were identified either by direct comparisons with available ITS sequences from isolated strains of *Trebouxia* spp. (Table 2) or by phylogenetic analyses including available sequences. Identity or almost identity of five photobiont ITS sequences with available sequences from cultured strains of *Trebouxia* spp. allowed the unequivocal assignment of *Physciaceae* photobionts to a known species of *Trebouxia*. Among the photobiont ITS sequences from *Physcia adscendens*, *P. tenella* and *Phaeophyscia orbicularis*, and the ITS rDNAs of two cultured strains identified as *T. impressa* (M-96.012A2 and M-96.027D1), only four sequence positions were different. The *Physcia semipinnata* photobiont ITS was almost identical with the corresponding sequence of a cultured strain of *T. gelatinosa* (86.108B2); differences being found only in one highly variable region of ITS-1. The identity of the *Anaptychia ciliaris* photobiont ITS sequence as *T. arboricola* Puymaly was unambiguous because it differed in only four positions from the corresponding sequences of two culture strains (92.011C3 and M-96.025C1) identified as *T. arboricola*. However, the *A. ciliaris* photobiont sequence and the ITS rDNA of another strain of *T. arboricola*, SAG 219-1a, differed at 43 positions. In addition, strain SAG 219-1a contained a 28 nucleotide long insertion in ITS-1 lacking in the corresponding sequences from *A. ciliaris* and other *T. arboricola* strains. Three *Physciaceae*

photobiont ITS rDNA sequences were found to be identical or almost identical with corresponding sequences from cultures that represent authentic strains which have been used to characterize species of *Trebouxia*. The photobiont ITS sequences from *Buellia zoharyi* and *Rinodina atrocinerea* were identical with the ITS rDNAs of *Trebouxia asymmetrica* Friedl & Gärtner (culture strain SAG 48.88) and *T. incrustata* Ahmadjian ex Gärtner (culture strain UTEX 784) respectively. The photobiont from *Rinodinella controversa* was assigned to *T. gigantea* (Hildreth & Ahmadjian) Gärtner since its ITS rDNA was identical except for one position with the corresponding sequence from culture strain UTEX 2231.

The identity of other *Physciaceae* photobiont ITS sequences became clear only in phylogenetic trees because these sequences differed greatly from available *Trebouxia* ITS sequences. In the ITS phylogenies, most of the *Physciaceae* photobiont sequences formed distinct clades together with the available sequences (Fig. 1). Five clades were resolved in the ITS phylogenies and the monophyletic origin of each of these clades was well supported in bootstrap tests (clades I–V, Fig. 1). Pairwise genetic distances between these ITS clades (average 0·321) were considerably higher than within clades (average 0·140). Interestingly, the average pairwise genetic distance within clade IV (0·264) was much higher than within other clades (ranging from 0·037 within clade II to 0·009 within clade I) which may be due to an accelerated rate of mutational changes among sequences from that clade. Alternatively, clade IV may contain multiple clades which so far have been only poorly sampled. Relationships among the clades were unresolved except for the close relationship of clade II with clade III, i.e. *T. impressa* with *T. gelatinosa* Ahmadjian ex Archibald. These relationships have already been shown in analyses of ITS rDNAs from cultured *Trebouxia* strains (Friedl *et al.* 2000). Each clade in the ITS phylogeny corresponded to a single morphospecies (clades II, III and V) or encompassed several morphospecies (clades I and IV).

Species of *Trebouxia* have so far been described using only morphological features and, therefore, are called morphospecies here. However, there are also synapomorphic ITS rDNA sequence characters which allow characterization of species of *Trebouxia* at the molecular level (see below). Apart from *T. asymmetrica* and *T. incrustata* each morphospecies in Fig. 1 is represented by two or more ITS genotypes. *Trebouxia gigantea* consisted of two distinct lineages, but their monophyly is only supported by low bootstrap values. *Trebouxia jamesii* (Hildreth & Ahmadjian) Gärtner is represented by three distinct lineages. Within *T. impressa*, at least three distinct lineages can be resolved. One lineage is formed by the nearly identical algal sequences from *Phaeophyscia orbicularis*, *Physcia adscendens* and *P. tenella*. A second lineage within *T. impressa* is constituted by the photobiont ITS sequences from *Dimelaena oreina*, *Physcia aipolia* [thallus Pa2], *P. stellaris*, *Physconia perisidiosa*, and strain 87.017E1 (Fig. 1). A monophyletic origin of that lineage is resolved in all three types of phylogenetic analyses, but with low bootstrap support. A third lineage of *T. impressa* may be formed by the close relationship among the photobionts from another specimen of *Physcia aipolia* [thallus Pa1], *Rinodina capensis*, *R. milvina* and a cultured strain of *T. impressa* isolated from *Phaeophyscia orbicularis* (M-96.026D4), but these relationships are not supported in bootstrap tests using maximum parsimony. The photobiont from a *P. aipolia* specimen [Pa1] and *R. capensis* may be very closely related because their ITS sequences differ at only a single position.

Highly variable regions in *Trebouxia* ITS sequences revealed important synapomorphies which unequivocally identify *Physciaceae* photobionts and clearly separate species of *Trebouxia* at the molecular level. These variable sequence regions had to be excluded from phylogenetic analyses due to considerable length variations (Fig. 2). A particularly long insertion in ITS-1 (between positions 118 and 119 in the photobiont ITS of *Anaptychia ciliaris*, Fig. 2) is shared by the *Physcia semipinnata* algal

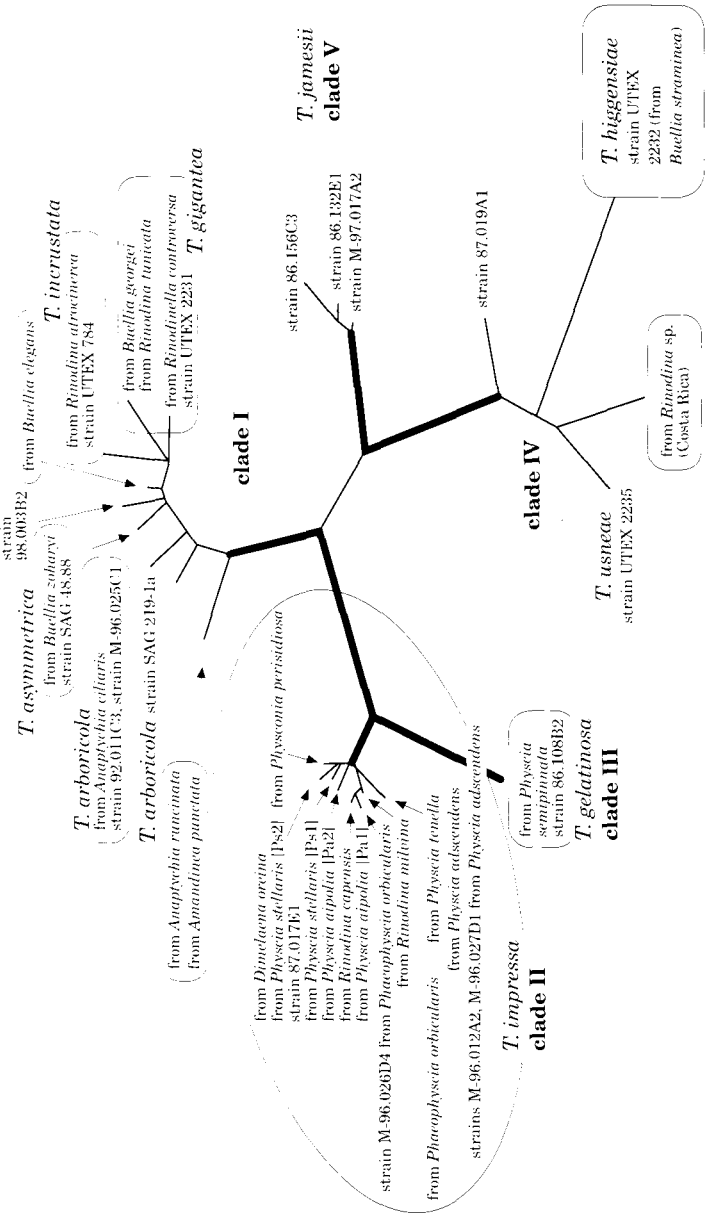
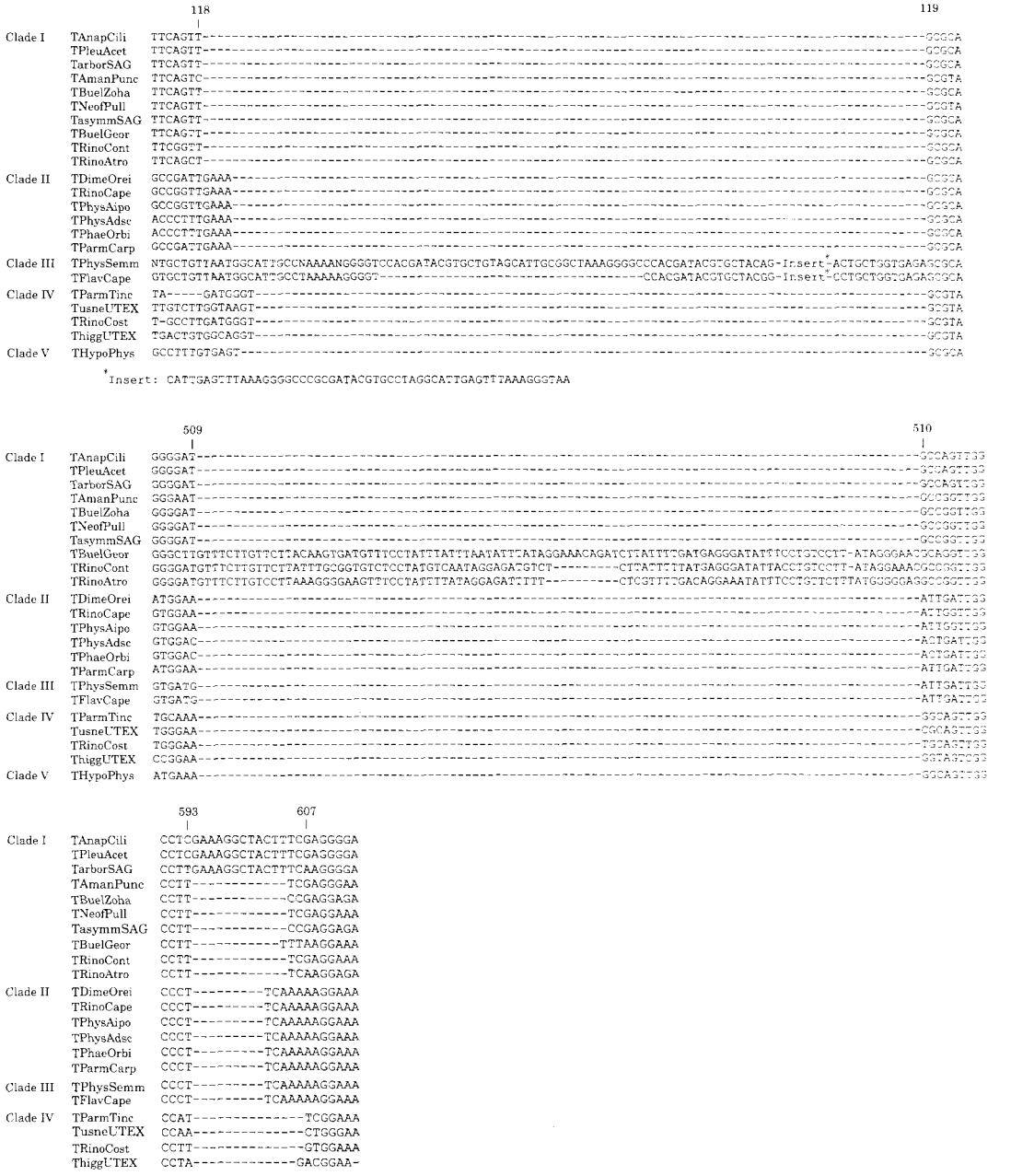


FIG. 1. Unrooted phylogeny of photobiont ITS rDNA sequences from *Physciaceae* and corresponding sequences from *Trebouxia* strains. This tree was inferred with the maximum-likelihood method. Thick lines mark internal nodes whose monophyly was supported in more than 70% of 2000 bootstrap replicates with maximum parsimony, minimum evolution, and neighbour-joining analyses. Where identical or almost identical sequences (see text) for several taxa or strains were determined, one sequence was used for the analyses; the others were simply added to the figure. Names of taxa in larger print indicate photobiont sequences from the *Physciaceae*; circled names represent branches or clades representing *Physciaceae* photobionts.


118
119
Clade I TAnapCili TTCAGTT----------GCGCA
TPleuAcet TTCAGTT----------GCGCA
TarborSAG TTCAGTT----------GCGCA
TAmanPunc TTCAGTC----------GCGTA
TBuelZoha TTCAGTT----------GCGCA
TNeofPull TTCAGTT----------GCGTA
TasymmSAG TTCAGTT----------GCGCA
TBuelGeor TTCAGTT----------GCGCA
TRinoCont TTCGGTT----------GCGCA
TRinoAtro TTCAGCT----------GCGCA
Clade II TDimeOrei GCCGATTGAAA----------GCGCA
TRinoCape GCCGGTTGAAA----------GCGCA
TPhysAipo GCCGGTTGAAA----------GCGCA
TPhysAdsc ACCCTTTGAAA----------GCGCA
TPhaeOrbi ACCCTTTGAAA----------GCGCA
TParmCarp GCCGATTGAAA----------GCGCA
Clade III TPhysSemm NTGCTGTTAATGGCATTGCCNAAAANGGGGTCCACGATACGTGCTGTAGCATTGCGGCTAAAGGGGCCCACGATACGTGCTACAG-Insert*-ACTGCTGGTGAGAGCGCA
TFlavCape GTGCTGTTAATGGCATTGCCTAAAAAGGGGT----------CCACGATACGTGCTACGG-Insert*-CCTGCTGGTGAGAGCGCA
Clade IV TParmTinc TA-----GATGGGT----------GCGTA
TusneUTEX TTGTCTTGGTAAGT----------GCGTA
TRinoCost T-GCCTTGATGGGT----------GCGTA
ThiggUTEX TGACTGTGGCAGGT----------GCGTA
Clade V THypoPhys GCCTTTGTGAGT----------GCGCA
*Insert: CATTGAGTTTAAAGGGGCCCGCGATACGTGCCTAGGCATTGAGTTTAAAGGGTAA
509
510
Clade I TAnapCili GGGGAT----------GCCAGTTGG
TPleuAcet GGGGAT----------GCCAGTTGG
TarborSAG GGGGAT----------GCCAGTTGG
TAmanPunc GGGAAT----------GCCGGTTGG
TBuelZoha GGGGAT----------GCCGGTTGG
TNeofPull GGGGAT----------GCCGGTTGG
TasymmSAG GGGGAT----------GCCGGTTGG
TBuelGeor GGGCTTGTTTCTTGTTCTTACAAGTGATGTTTCCTATTTATTTAATATTTATAGGAAACAGATCTTATTTTGATGAGGGATATTTCCTGTCCTT-ATAGGGAACGCAGGTTGG
TRinoCont GGGGATGTTTCTTGTTCTTATTTGCGGTGTCTCCTATGTCAATAGGAGATGTCT----------CTTATTTTTATGAGGGATATTACCTGTCCTT-ATAGGAAACGCCGGTTGG
TRinoAtro GGGGATGTTTCTTGTCCTTAAAGGGGAAGTTTCCTATTTTATAGGAGATTTTT----------CTCGTTTTGACAGGAAATATTTCCTGTTCTTTATGGGGGAGGCCGGTTGG
Clade II TDimeOrei ATGGAA----------ATTGATTGG
TRinoCape GTGGAA----------ATTGGTTGG
TPhysAipo GTGGAA----------ATTGGTTGG
TPhysAdsc GTGGAC----------ACTGATTGG
TPhaeOrbi GTGGAC----------ACTGATTGG
TParmCarp ATGGAA----------ATTGATTGG
Clade III TPhysSemm GTGATG----------ATTGATTGG
TFlavCape GTGATG----------ATTGATTGG
Clade IV TParmTinc TGCAAA----------GGCAGTTGG
TusneUTEX TGGGAA----------CGCAGTTGG
TRinoCost TGGGAA----------TGCAGTTGG
ThiggUTEX CCGGAA----------GGTAGTCGG
Clade V THypoPhys ATGAAA----------GGCAGTTGG
593
607
Clade I TAnapCili CCTCGAAAGGCTACTTTCGAGGGGA
TPleuAcet CCTCGAAAGGCTACTTTCGAGGGGA
TarborSAG CCTTGAAAGGCTACTTTCAAGGGGA
TAmanPunc CCTT----------TCGAGGGAA
TBuelZoha CCTT----------CCGAGGAGA
TNeofPull CCTT----------TCGAGGAAA
TasymmSAG CCTT----------CCGAGGAGA
TBuelGeor CCTT----------TTTAAGGAAA
TRinoCont CCTT----------TCGAGGAAA
TRinoAtro CCTT----------TCAAGGAGA
Clade II TDimeOrei CCCT---------TCAAAAAGGAAA
TRinoCape CCCT---------TCAAAAAGGAAA
TPhysAipo CCCT---------TCAAAAAGGAAA
TPhysAdsc CCCT---------TCAAAAAGGAAA
TPhaeOrbi CCCT---------TCAAAAAGGAAA
TParmCarp CCCT---------TCAAAAAGGAAA
Clade III TPhysSemm CCCT---------TCAAAAAGGAAA
TFlavCape CCCT---------TCAAAAAGGAAA
Clade IV TParmTinc CCAT----------TCGGAAA
TusneUTEX CCAA----------CTGGGAA
TRinoCost CCTT----------GTGGAAA
ThiggUTEX CCTA----------GACGGAA-
Clade V THypoPhys CCTT----------TCCGGAAA

partner and a cultured strain of *T. gelatinosa*, but is lacking in other *Trebouxia* species. A long insertion in a highly variable region in ITS-2 (between positions 509 and 510 of the *A. ciliaris* photobiont ITS) was only shared by *T. gigantea* and *T. incrustata*. Sequence differences in that region distinguish two lineages of *T. gigantea* from one-another and separate these photobionts from *T. incrustata* (Fig. 2). A unique sequence in ITS-2, only found in the *Anaptychia ciliaris* photobiont ITS (positions 593–607, Fig. 2) and all *Trebouxia arboricola* strains, distinguishes *T. arboricola* from other membranes of clade I. The presence of this particular sequence portion in all strains of *T. arboricola* (including strain SAG 219-1a) which is absent in all other *Trebouxia* species strongly suggests the monophyletic origin of that species. However, *T. arboricola* was paraphyletic with other members of clade I in the ITS phylogeny (Fig. 1).

For a few *Physciaceae* taxa, it was impossible to assign unambiguously their photobionts to a certain species of *Trebouxia*. The identity of the *Buellia elegans* photobiont was uncertain. Its common origin with *T. asymmetrica* strain SAG 48.88 and an unidentified strain from *Neofuscelia pulla* (Ach.) Essl. (98.003B2) was supported by maximum parsimony and distance analyses only, but not by maximum likelihood (Fig. 1). The *Anaptychia runcinata* and *Amandinea punctata* photobionts were not closely related to any particular lineage of *Trebouxia* in the phylogenetic analyses; rather they formed an independent lineage within clade I (Fig. 1). *Rinodina* sp. (sample from Costa Rica) appeared as a distinct lineage within clade IV. A close relationship with *T. usneae* (Hildreth & Ahnadjian) Gärtner strain UTEX 2235 was supported by maximum likelihood and neighbour-joining distance analyses, but not in other analyses or bootstrap tests.

Diversity of photobionts within the *Physciaceae*

The diversity of *Physciaceae* photobionts is quite remarkable. *Physciaceae* photobionts are found in four of the five *Trebouxia* ITS clades (Fig. 1) and, therefore, may cover almost all of the known diversity of the genus *Trebouxia*. Five clades were previously resolved in phylogenetic analyses of ITS rDNA sequences from culture strains of *Trebouxia* species (Friedl *et al.* 2000). The algal partners of the *Physciaceae* investigated here belong to a total of 12 different ITS lineages ('ITS genotypes') of which nine represented six morphospecies and three formed distinct lineages that cannot be unambiguously identified. One additional lineage representing a *Physciaceae* photobiont is *T. higginsiae* strain UTEX 2232 that has been isolated from *Buellia straminea* (Hildreth & Ahmadjian 1981). So far *Physciaceae* photobionts have not been found in clade V (corresponding to *T. jamesii*) of the ITS phylogeny. This may be due to the taxon sampling in the present study, but it could also be that

FIG. 2. Alignment of photobiont ITS rDNA sequences from *Physciaceae* and available *Trebouxia* ITS sequences showing only insertions/deletions that define unequivocally certain photobionts (see text). Numbers indicate positions relative to the ITS rDNA sequence of the photobiont from *Anaptychia ciliaris* (Genbank Accession AJ293770). Sequences are arranged in groups that correspond to the five clades of the ITS phylogeny (Fig. 1). Clade I: TAnapCili, from *Anaptychia ciliaris*; TPleuAcet, strain 92.011C3 from *Pleurosticta acetabulum*; TarborSAG, *T. arbicola* strain SAG 219-1a; TAmanPunc, from *Amandinea punctata*; TBuelZoha, from *Buellia zoharyi*; TNeofPull, strain 98.003B2 from *Neofuscelia pulla*; TasymmSAG, *T. asymmetrica* strain SAG 48.88; TBuelGeor, from *Buellia georgei*; TRinoCont, from *Rinodina controversa*; TRinoAtro, from *Rinodina atrocinerea*. Clade II: TDimeOrei, from *Dinelaena oreina*; TRinoCape, from *Rinodina capensis*; TPhysAipo, from *Physcia aipolia*; TPhysAdsc, from *Physcia adscendens*; TPhaeOrbi, from *Phaeophyscia orbicularis*; TParmCarp, strain 87.017E1 from *Parmelina carporrhizans*. Clade III: TPhysSemm, from *Physcia semipinnata*; TFlavCape, strain 86.108B2 from *Flavoparmelia caperata*. Clade IV: TParmTinc, strain 87.019A1 from *Parmotrema tinctorum*; TusneUTEX, *T. usneae* strain UTEX2235; TRinoCost, from *Rinodina* sp. (Costa Rica); ThiggUTEX, *T. higginsiae* strain UTEX 2232. Clade V: THypoPhys, strain 86.132E1 from *Hypogymnia physodes*.

T. jamesii is not a suitable photobiont for *Physciaceae* lichen fungi. Our present taxon sample suggests that *Trebouxia* species from ITS clades I (e.g., *T. arboricola*) and II (*T. impressa*) may be the preferred photobionts of the *Physciaceae*. Our selection of taxa of the *Physciaceae* revealed a remarkably high ratio of photobiont ITS genotypes to lichen species of 12 : 20. Therefore, the actual diversity of lichen photobionts may be much greater than previously estimated on the basis of algal cultures and morphology alone. For example, *Physciaceae* photobionts were not only found in all lineages of clade I as represented by ITS sequences from *Trebouxia* culture strains, but also formed two additional independent lineages within that clade. These new lineages might represent new species of *Trebouxia*. Although much attention needs to be given to molecular characters for a refined taxonomy of *Trebouxia* species, morphological characters still remain essential for their characterization. Therefore, culture strains are required for as many photobiont ITS lineages as possible to test current taxonomic concepts in *Trebouxia*. Different ITS genotypes within a certain clade may also represent different morphologies. Differences in chloroplast morphology were found between two taxa of *Trebouxia* that were very closely related within one clade in ITS phylogenies (Beck 1999).

A photobiont diversity similar to that found in the *Physciaceae* may also be detected in other evolutionarily closely related groups of lichens. For example, from quite a number of species and genera of *Parmeliaceae* the photobionts have been characterized based on morphology and culture strains (Friedl 1989). However, taxon sampling was limited in this earlier study due to the need of fresh lichen material for photobiont cultures. The analysis of ITS rDNAs from cultured *Parmeliaceae* photobionts shows that the algal partners of that family also represent most clades of *Trebouxia* (Bhattacharya *et al.* 1996; Friedl *et al.* 2000). Photobionts from *Pleurosticta acetabulum* (Necker) Elix & Lumbsch and *Neofuscelia pulla* belonged to clade I, those from *Parmelina carporrhizans* (Taylor) Poelt & Vězda to clade II, those from *Flavoparmelia caperta* (L.) Hale to clade III, those from *Parmotrema tinctorum* (Nyl.) Hale to clade IV, and those from *Parmelia saxatilis* (L.) Ach. to clade V.

It is remarkable that all known *Physciaceae* photobionts belong to *Trebouxia*. Since we designed our PCR primers to be specific for green algae and not only for *Trebouxia* species, the results suggest that no green algae other than *Trebouxia* species are photobionts of the *Physciaceae*. This finding suggests that mycobionts at the level of families phylogenetically track a certain genus of photobiont (Rambold *et al.* 1998). There is no record of *Asterochloris* Tschermak-Woess or any other coccoid green algae in the *Physciaceae*. The earlier distinction of *Asterochloris* from *Trebouxia* (Tschermak-Woess 1980) is supported by an expanded analysis of ITS rDNAs from a variety of cultured strains of *Trebouxia* (Friedl, unpublished) which found congruences between morphology and rDNA sequence analyses. *Trebouxia* and *Asterochloris* seem to be indicative of two formerly distinguished major groups of the order Lecanorales.

Specificity of mycobionts towards their algal partners

Photobionts from genera of the *Physciaceae* from which more than one species was studied (i.e. *Anaptychia*, *Buellia*, *Physcia*, and *Rinodina*) belonged to several lineages or clades in the ITS rDNA phylogeny (Fig. 1). This means that a variety of photobionts are suitable algal partners for genera of the *Physciaceae*. Genera of the *Physciaceae* may also express different patterns of specificity towards their photobionts. A rather narrow spectrum of algal partners is found in the genus *Physcia*. Only photobionts from two closely related *Trebouxia* species or clades, *T. gelatinosa* and *T. impressa*, were detected in *Physcia*. Among *Rinodina* species, a broader range of photobionts was found, i.e. algae from three distant ITS

clades (I, II and IV) of *Trebouxia* representing at least three different algal morphospecies (*T. gigantea*, *T. incrustata*, and *T. impressa*) and an additional unidentified ITS genotype (Fig. 1). Similarly, among *Buellia* species, four different algal ITS genotypes from two distant *Trebouxia* clades (clades I and IV) representing at least three morphospecies (*T. asymmetrica*, *T. gigantea* and *T. higginsiae*) were found. From these findings it is tempting to speculate that there may exist a correlation between lichen growth form and different ranges of photobiont variation. Foliose taxa of the *Physciaceae* (e.g. *Physcia*) may be more restrictive in their photobiont choice than crustose ones (e.g., *Buellia*, *Rinodina*). However, this hypothesis needs to be tested more thoroughly by further taxon sampling in the *Physciaceae*.

At the species level less photobiont variation was found in the *Physciaceae*, i.e. the photobionts of different specimens of the same lichen species were very closely related. Within *Physcia adscendens* (from which two photobiont culture strains are available and four specimens were studied here) the photobiont sequences are genetically nearly identical (differences in only four positions); they belong to one lineage of *Trebouxia impressa* in the ITS phylogeny (Fig. 1). In *Physcia aipolia* and *Phaeophyscia orbicularis* the photobionts were from two different ITS lineages, but all were within the same ITS clade representing *T. impressa*. A reason for the presence of closely related photobionts in these three lichen species may be that they both occur in similar lichen communities (i.e. the *Xanthorion parietinae*) or on nitrogen rich tree bark, and, therefore, the same pool of photobionts may have been used by these lichens. More specimens per species of the *Physciaceae* and from a broader range of habitats need to be examined in order to obtain a clearer picture of photobiont specificity patterns.

Photobiont variation within a species or genus of *Physciaceae* may be due to geographical separation. The samples of *Physcia aipolia* [Pa1] and [Pa2], with different photobiont ITS genotypes were from the USA and Germany, respectively. Different photobiont ITS genotypes were also found in *Phaeophyscia orbicularis*. One photobiont was found in a *Phaeophyscia orbicularis* specimen from northern Germany and the other from an algal culture isolated from a lichen specimen taken from southern Germany (Beck *et al.* 1998). That *Physcia semipinnata* had a photobiont species (*T. gelatinosa*) different from that of the other investigated *Physcia* species (*T. impressa*) may be due to *P. semipinnata* being common in the Mediterranean, whereas the other investigated samples of *Physcia* species predominantly occur in central Europe. Differences in photobionts among *Buellia* species may be correlated with the distribution of that lichen genus in both Hemispheres. *Buellia elegans* and *B. zoharyi* were from different localities in the Northern Hemisphere (Austria and the Canary Islands) and their photobionts formed two closely related lineages within clade I. *Buellia georgei* and *B. straminea* occur in distant regions of the Southern Hemisphere (Australia; Trinkaus *et al.* 2001) and the tropical Pacific (Galapagos Islands; Zahlbruckner 1931) respectively; their photobionts were very different from those of the Northern Hemisphere *Buellia* species and were in two different clades (I and IV).

The presence of a particular photobiont in a lichen may depend on whether appropriate algae are available in the habitat. Sources of photobionts other than free-living subaerial algae may be symbiotic propagules of other lichens (for discussion see Beck *et al.* 1998). This may result in *Physciaceae* sharing their photobionts with neighbouring lichens. Two different photobionts were found in *Anaptychia ciliaris* and *A. runcinata*, from two different habitats and geographically distant localities. However, both *Anaptychia* species may share their photobionts with other lichens from their habitats. *Anaptychia* species may not be highly selective, but incorporate suitable algae as they become available from neighbouring lichens. The investigated specimen of *A. ciliaris* was from tree bark with neighbouring thalli of *Xanthoria parietina* and *Pleurosticta acetabulum*.

From the latter two lichens (from another locality) the photobionts have been isolated and their ITS rDNA sequences are available (Beck *et al.* 1998). The *A. ciliaris* photobiont ITS rDNA was almost identical with the corresponding sequences from the photobionts of these lichens. The studied sample of *A. runcinata*, however, was from an exposed coastal rock. Its photobiont ITS rDNA was identical with the photobiont ITS of *Amandinea punctata* from a neighbouring locality on the same island. A similar example was *Rinodina capensis* from the Austrian Alps and *R. milvina* from the coast of Finland. Though their photobionts belong to one lineage of *T. impressa* (Fig. 1), their ITS rDNA sequences differ in six positions and, therefore, both photobionts may be from different sources. *Rinodina capensis* may share its algal partner with *Physcia* species which grow on the bark of trees in the neighbourhood of *R. capensis*. The *R. capensis* photobiont ITS rDNA was almost identical with the corresponding sequence from a *Physcia aipolia* specimen (from another locality) differing in a single base pair. These examples show that it is important to investigate the photobionts from neigbouring lichen thalli of the same locality in order to explain the variation of photobionts within a lichen genus. Further examples of algal sharing among non-closely related lichens have previously been demonstrated by Beck (1999).

A high selectivity towards photobionts due to phylogeny may be present when two neighbouring lichen species from the same locality have two different photobionts. *Dimelaena oreina* and *Rinodina atrocinerea* were from the same locality, but their photobionts were *Trebouxia impressa* and *T. incrustata*, respectively (Fig. 1). The specimens of *Rinodina tunicata* and *Rinodinella controversa* investigated were from the same locality and both occurred on a calcareous substratum, but their photobionts pertain to two different lineages belonging to *T. gigantea* (Fig. 1); their photobiont ITS sequences differed by a total of 55 positions. High selectivity may even lead to identical photobionts in lichens that occur in geographically very distant localities. *Buellia georgei* from Australia and *Rinodina tunicata* from Greece had identical algal ITS rDNA.

These findings suggest that at the generic level, *Physciaceae* mycobionts may accept a whole range of photobionts, whereas at the species level only very closely related ITS genotypes within one *Trebouxia* morphospecies can be found. The patterns of specificity may be due to either phylogeny (as in *Physcia* where two closely related species of *Trebouxia* seem to be the preferred algal partners) or biogeography. Geographical separation may cause two closely related lichen species to have distantly related algal partners. *Physciaceae* with a low specificity may simply take their photobionts from neighbouring lichens of the same habitat.

More than one photobiont in the same lichen thallus

Some lichens may represent triple symbioses, i.e. an association of one mycobiont with two photobionts in the same lichen thallus, or there may exist areas in a lichen thallus where two different photobionts occur. In the *Parmeliaceae* an example is known from *Pleurosticta acetabulum* where two genetically different strains were isolated from the same thallus. They differed by the presence or the absence of a group I intron at the 3′-end of the 18S rDNA and were otherwise identical except for four positions in their ITS rDNAs (Bhattacharya *et al.* 1996). In the *Physciaceae* more than one photobiont in the same thallus may also occur. Our initial experiments with *Rinodina atrocinerea*, *R. tunicata* and *Rinodinella controversa* revealed two photobionts in each of these lichens. PCR assays yielded double bands and the subsequent cloning and sequencing of both bands showed two algal ITS rDNAs belonging to clade I were present. When the size of the lichen sample is reduced to a single apothecium, however, only a single algal ITS was detected and these were the sequences used for phylogenetic analyses (Fig. 1). The second algal ITS may have come either from another lichen which was not seen in the sample because it may have

been overgrown by the lichen under investigation. As an alternative, *R. atrocinerea* and *R. tunicata* may represent triple symbioses, at least in some parts of their thalli. Interestingly, these observations were made only in those members of the *Physciaceae*, which, at the generic level, were found to be little selective in their photobiont choice.

Photobionts and evolutionary relationships of the *Physciaceae*

Photobionts may be important markers of evolutionary relationships in lichens. The capacity to specifically select an appropriate algal partner from a variety of algal taxa in subaerial habitats may be an important phylogenetic trait (Rambold *et al.* 1998). Whether photobionts are indicative of evolutionary relationships among genera and species of the *Physciaceae* can only be adequately addressed when a phylogeny of the *Physciaceae* fungal partners becomes available. For example, *Rinodina* species that are polyphyletic in the algal tree (Fig. 1) are also polyphyletic in the fungal phylogeny (Grube & Arup 2001). *Rinodina* sp. (sample from Costa Rica) may belong to the genus *Pachysporaria* (Malme) M. Choisy, a segregate of the genus *Rinodina* with several taxa in the tropics (Marbach 2000: 344) which is in need of a thorough revision. The close relationships of the photobionts from *Physcia*, *Physconia*, and *Phaeophyscia* [*T. impressa* and *T. gelatinosa* (Fig. 1)], may also support a close relationship of the fungal partners from these lichens. *Anaptychia runcinata* and *A. ciliaris* may not be as closely related within the genus as preliminary mycobiont ITS rDNA sequence analyses suggest (H. Mayrhofer, unpublished) and their photobionts are also distantly related (Fig. 1). Two different major ascus types have been found in the *Physciaceae* (Rambold *et al.* 1994). However, no correlation of photobionts with ascus types has been found so far. Photobionts from lichens with *Bacidia*-type asci, for example, *Amandinea punctata*, *Buellia* species and *Dimaelaena oreina*, were found within the same clades as photobionts from taxa with *Lecanora*-type asci, such as, *Anaptychia*, *Physcia*, and *Rinodina* (Fig. 1). The photobiont ITS sequences from lichens with *Lecanora*-type asci may even be identical with those having *Bacidia*-type asci. The photobiont ITS sequences from *Anaptychia runcinata* and *Rinodina tunicata* (both with *Lecanora*-type asci) were identical with those from *Amandinea punctata* and *Buellia georgei* (both with *Bacidia*-type asci), respectively.

Conclusions and perspectives

Green algal-specific PCR amplification and ITS rDNA sequencing followed by comparisons with ITS sequences already available is a successful and relatively fast method for identifying photobionts. Even small amounts of lichen tissue (e.g., a single apothecium) and even old herbarium material is sufficient to specifically amplify green algal ITS rDNAs from total DNA extractions using the PCR primers shown in Table 1. From the rather limited number of taxa used in this study it can be concluded that genera of the *Physciaceae* may have different ranges of suitable algal partners which possibly depend on the growth form. *Physciaceae* at the species level may be more selective in their choice of photobiont, i.e. only a narrow range of closely related *Trebouxia* ITS genotypes may occur within a species. Using algal-specific PCR we hope to investigate thoroughly photobionts from a broader range (i.e. most of the presently known taxa) of the *Physciaceae*. It must be further tested whether the photobiont varies among specimens of the same species from geographically distant localities and various habitats. It will be highly interesting to map features regarding the photobiont diversity on a phylogeny of *Physciaceae* fungal partners. From the same samples that have been used for photobiont sequencing, we will also determine the ITS sequences of the fungal partners and both independent phylogenies will then be tested for congruities in order to check for a possible parallel cladogenesis.

We thank P. Dornes (Frankfurt) for doing much of the sequencing work and provision of lichen samples, U. Trinkaus (Graz) for provision of specimens, R. Ertl (Graz) for his support in the field, A. Beck (München), D. P. Fewer (Göttingen) for discussion and E. Zufall-Roth for her help in the laboratory. We are also grateful

to Burkhard Büdel (Kaiserslautern) for valuable help and provision of laboratory space at the beginning of this project. This work was supported by grants from the Deutsche Forschungsgemeinschaft to T.F. (FR 905/6-1, 7-1, 7-2) and from the Austrian Science Foundation (FWF project P13389-BIO) to H.M.

References

Ahmadjian, V. (1960) Some new and interesting species of *Trebouxia*, a genus of lichenized algae. *American Journal of Botany* **47:** 677–683.

Ahmadjian, V. (1993) The lichen photobiont—what can it tell us about lichen systematics? *Bryologist* **96:** 310–313.

An, S. S., Friedl, T. & Hegewald, E. (1999) Phylogenetic relationships of *Scenedesmus* and *Scenedesmus*-like coccoid green algae as inferred from ITS-2 rDNA sequence comparisons. *Plant Biology* **1:** 418–428.

Beck, A. (1999) Photobiont inventory of a lichen community growing on heavy-metal-rich rock. *Lichenologist* **31:** 501–510.

Beck, A., Friedl, T. & Rambold, G. (1998) Selectivity of photobiont choice in a defined lichen community: inferences from cultural and molecular studies. *New Phytologist* **139:** 709–720.

Bhattacharya, D., Friedl, T. & Damberger, S. (1996) Nuclear-encoded rDNA group I introns: origin and phylogenetic relationships of insertion site lineages in the green algae. *Molecular Biology and Evolution* **13:** 978–989.

Coleman, A. W., Preparata, R. M., Mehrota, B. & Mai, J. C. (1998) Derivation of the secondary structure of the ITS-1 transcript in Volvocales and its taxonomic correlations. *Protist* **149:** 135–146.

Friedl, T. (1989) *Systematik und Biologie von* Trebouxia *(Microthamniales, Chlorophyta) als Phycobiont der Parmeliaceae (lichenisierte Ascomyceten)*. Inaugural-Dissertation, Universität Bayreuth, Germany.

Friedl, T. (1996) Evolution of the polyphyletic genus *Pleurastrum* (Chlorophyta): inferences from nuclear-encoded DNA sequences and motile cell ultrastructure. *Phycologia* **35:** 456–469.

Friedl, T. & Rokitta, C. (1997) Species relationships in the lichen alga *Trebouxia* (Chlorophyta, Trebouxiophyceae): molecular phylogenetic analyses of nuclear-encoded large subunit rRNA gene sequences. *Symbiosis* **23:** 125–148.

Friedl, T., Besendahl, A., Pfeiffer, P. & Bhattacharya, D. (2000) The distribution of group I introns in lichen algae suggests that lichenization facilitates intron lateral transfer. *Molecular Phylogenetics and Evolution* **14:** 342–352.

Gilbert, D. G. (1995) *SeqPup, V.0.6f.* Bloomington: Indiana University.

Grube, M. & Arup, U. (2001) Morphological and molecular evolution in the *Physciaceae* (Lecanorales, lichenized Ascomycotina), with special emphasis on the genus *Rinodina*. *Lichenologist* **33:** 63–72.

Hafellner, J., Mayrhofer, H. & Poelt, J. (1979) Die Gattungen der Flechtenfamilie *Physciaceae*. *Herzogia* **5:** 39–79.

Hildreth, K. C. & Ahmadjian, V. (1981) A study of *Trebouxia* and *Pseudotrebouxia* isolates from different lichens. *Lichenologist* **13:** 65–86.

Mai, J. C. & Coleman, A. (1997) The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *Journal of Molecular Evolution* **44:** 258–271.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1997) The RDP (Ribosomal Database Project). *Nucleic Acids Research* **25:** 109–111.

Marbach, B. (2000) Corticole und lignicole Arten der Flechtengattung *Buellia* sensu lato in den Subtropen und Tropen. *Bibliotheca Lichenologica* **74:** 1–384.

Rambold, G., Mayrhofer, H. & Matzer, M. (1994) On the ascus types in Physciaceae (Lecanorales). *Plant Systematics and Evolution* **192:** 31–40.

Rambold, G., Friedl, T. & Beck, A. (1998) Photobionts in lichens: possible indicators of phylogenetic relationships? *Bryologist* **101:** 392–397.

Swofford, D. L. (2000) *PAUP: Phylogenetic Analysis Using Parsimony, V4.0b3.* Washington DC: Smithsonian Institution.

Tehler, A. (1996) Systematics, phylogeny and classification. In *Lichen Biology* (T. H. Nash, III, ed.): 217–239. Cambridge: Cambridge University Press.

Trinkaus, U., Mayrhofer, H. & Elix, J. A. (2001) Revision of the *Buellia epigaea*-group (lichenized Ascomycetes, *Physciaceae*) 2. The species in Australia. *Lichenologist* **33:** 47–62.

Tschermak-Woess, E. (1980) *Asterochloris phycobiontica* gen. et spec. nov., der Phycobiont der Flechte *Varicellaria carneonivea*. *Plant Systematics and Evolution* **135:** 279–294.

Tschermak-Woess, E. (1988) The algal partner. In *CRC Handbook of Lichenology* (M. Galun, ed.): 39–92. Florida: CRC Press.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innes, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. San Diego: Academic Press.

Zahlbruckner, A. (1931) *Catalogus lichenum universalis*. Leipzig: Gebrüder Borntraeger.

Accepted for publication 28 *June* 2000

INSTRUCTIONS TO AUTHORS

The Lichenologist is an international journal and welcomes papers and short communications from contributors anywhere in the world on any aspect of lichenology.

Manuscripts must be original, clearly and precisely presented in English and submitted in triplicate exclusively to the Senior Editor: Dr P. D. Crittenden, School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K. A cover sheet should accompany the manuscript including the following information: title and a short running title of not more than 6 words for page headings (not required for Short Communications); name and contact details for the corresponding author (i.e. postal address, telephone and fax numbers, and email address); the total number of pages (text plus references) and numbers of figures and tables. Authors submitting a manuscript do so on the understanding that, if it is accepted, copyright of the paper will be assigned exclusively to the publisher (see Copyright below).

Text. This must be typewritten on A4 (210 × 297 mm) or letter (8·5 × 11 inch) in double spacing with 2·5 cm margins all round. On all points of style concerning text and tables consult recent copies of the journal. Words to be italicized should be either underlined or typed in italics but not both. Complete scientific names (genus, species and authority) must be cited at first mention. Thereafter the generic name may be abbreviated to the initial except at the beginning of a sentence or where the abbreviation might result in confusion with other genera. Authors must return their revised manuscripts with a PC compatible disk. Documents should preferably be in Word 97 or more recent versions. Recent issues should be consulted for layout of new species, new combinations, synonymy and lists of specimens examined. Examples of style are given below. The spelling of locality names in the British Isles and abroad must follow the most recent editions of maps published by the Ordnance Survey and *The Times Atlas of the World*, respectively.

Examples of style employed in

(a) description of new species:

Ramalina jamesii Krog sp. nov.

Thallus saxicola, ascendens vel plus minusve inclinatus . . . . etc.

Typus: Portugal, Madeira, Porto Santo, Pico do Facho, 33°05′N, 16°19′W, on acidic rock, 350 m alt., 7 April 1988, *H. Krog & E. Timdal* 6163 (O—holotypus; BM, UPS—isotypi).

(b) citation of described species or new combinations:

Pyrenopsis furfurea (Nyl.) Th. Fr.

Bot. Notiser **1866:** 58 (1866); type: Scotland, Ben Lawers, 1864, *Jones* (H-NYL 42916—lectotype; BM—isolectotype).

Pyrenopsidium terrigenum (Th. Fr.) Forss., *Nova Acta R. Soc. Scient. Upsal.* ser. 3, **13**(6): 81 (1985).—*Pyrenopsis haematopsis* (Sommerf.) *ß. terrigena* Th. Fr. in Hellbom, *Öfvers K. Vetens Akad. Forh.* **22**(6): 478 (1865); type: Sweden, Lule Lappmark, Skarfi, 1864, *Hellbom* (UPS—holotype).

(c) citation of specimens examined:

Long lists of citations are discouraged. Data should be reproduced as either maps or lists containing only data essential for locating specimens. Complete lists, with the above format, can be deposited with *The Lichenologist* and other appropriate Institutions, and their location noted in the text.

Selected specimens examined. **British Isles:** *Scotland:* **V.C.96**, Easterness: Abernethy Forest, near Forest Lodge, 38/01.16, on *Pinus* lignum, 1975, *Coppins* [2199] *& Rose* (BM, E).—**Germany:** *Bayern:* Allgauer Alpen, 1957, *Schoppel & Poelt* [Poelt, *Lichenes Alpinum* no. 56] (H).—**Australia:** *Tasmania:* Weindorfers Forest, 41°38′S, 145°56′E, 920 m, 1988, *Kantvilas* 68/88 (E); Cox Bight, behind west beach, sea-level, 1985, *J. A. Elix* 20945 (ANUC). *Victoria:* Bellel Creek, *c.* 1800 m, 5 vi 1983, *M. E. Hale* (HO).

Tables. These must be self-explanatory and each presented on separate pages outside the main text. A short title should be provided with any additional information contained in footnotes. Vertical columns should be separated by spacing; vertical lines must be avoided.

Figures. Refer to all **drawings**, **diagrams**, **graphs** and **photographs** as figures. These should be of the highest quality and suitable for direct reproduction. Each figure should be presented on a separate page. Plan figures to appear within a single column (67 mm) or for reproduction across two columns (139 mm).

Drawings, **diagrams** and **graphs** should be submitted in black ink on white card, white paper or tracing film at up to twice the size they will finally appear. Preferred symbols for graphs are ●, ○, ■, □, ▲, △.

Photographs (colour or black and white) should be submitted at the size they will appear. All photographs should be printed on glossy paper; several may be grouped together by mounting on white card with a narrow gap between them.

Subdivisions of figures should be labelled with capital letters, e.g. A, B, C, etc. No letters, arrows, scales, etc. should be done on the original figures; indicate these on mounts or photocopies accompanying each manuscript.

All **legends** for figures should be provided on a separate page to be included with the text of the paper after the references.

References. Citations in the text should take the form: Green & White (1988) or (Brown 1988*a*, *b*, 1989; Smith & Jones 1999). Multiple citations should be ordered chronologically. When papers are by three or more authors, give only the name of the first author followed by *et al.* (e.g. Halonen *et al.* 1998) throughout the text. At the end of the text, list the references alphabetically using the following standard forms:

Culberson, C. F. & Kristinsson, H. (1970*a*) A standardized method for identification of lichen products. *Journal of Chromatography* **46:** 85–93.

Hale, M. E. (1983) *The Biology of Lichens.* 3rd Edn. London: Arnold.

Hawksworth, D. L. (1976) Lichen chemotaxonomy. In *Lichenology: Progress and Problems* (D. H. Brown, D. L. Hawksworth & R. H. Bailey, eds): 139–184. London: Academic Press.

When citing an Academic Press journal, include the digital object identifier (DOI), if noted, from the article's title page. Please note the following examples:

McCarthy, P. M. (2000) Key to the saxicolous taxa of *Porina*. *Lichenologist* **32:** 1–13. doi:10.1006/lich.1999.0213

Fos, S. & Clerc, P. (2000) The lichen genus *Usnea* on *Quercus robur* in Iberian cork-oak forests. *Lichenologist* **32:** 67–88. doi:10.1006/lich.1999.0242

References should be listed in alphabetic sequence with: single authors, by date; two authors, alphabetically, then by date; three or more authors by date only.

Abbreviations, etc. For guidance on units, symbols, chemical nomenclature and abbreviations consult Instructions to authors for the *The New Phytologist*.

Digital Object Identifier. Academic Press assigns a unique digital object identifier (DOI) to every article it publishes. The DOI appears on the title page of the article. It is assigned after the article has been accepted for publication and persists throughout the lifetime of the article. Due to its persistence, it can be used to query Academic Press for information on the article during the production process, to find the article on the Internet through various Web sites, including IDEAL, and to cite the article in academic references. When citing an article in a reference section, it is important to include the article's DOI in the reference as volume and page information is not always available for articles published online. The References section, above, shows samples of DOI included in references. Further information may be found at <http://www.academicpress.com/doi>.

Copyright. Authors submitting a manuscript do so on the understanding that if it is accepted for publication, copyright in the article, including the right to reproduce the article in all forms and media, shall be assigned exclusively to the British Lichen Society. The Copyright Transfer Agreement, which may be copied from the pages immediately preceding these Instructions for Authors or found on the journal home page listed on the back cover, should be signed by the appropriate person(s) and should accompany the original submission of a manuscript to this journal. The transfer of copyright does not take effect until the manuscript is accepted for publication. Copyright in the paper for the United States of America shall be assigned to the Society. It is the policy of the publisher that authors need not obtain permission in the following cases only: (1) to use their original figures or tables in their future works; (2) to make copies of their papers for use in their classroom teaching; and (3) to include their papers as part of their dissertations.

Offprints. In consideration for the assignment of copyright, the Society will supply 50 offprints of each paper or Short Communication but not of Book Reviews. Further offprints may be ordered at extra cost at the proof stage. Reprints must be ordered directly from Academic Press Ltd when the author returns the proofs of their article.

Page charges are not levied, but if funds to cover printing costs are available quicker publication, or that of unusually long papers, can be facilitated. Authors are responsible for the cost of coloured illustrations.

Books dealing with any aspect of lichenology will be reviewed. Publishers wishing to have works reviewed in *The Lichenologist* should send them to Dr B. J. Coppins, Royal Botanic Gardens, Inverleith Row, Edinburgh, EH3 5LR, in the first instance.

Notice: While the Senior Editor and Editors endeavour to check the accuracy of statements in contributions in as far as they are able, it should be emphasized that views expressed in papers in *The Lichenologist* are those of their authors and do not necessarily represent those of the British Lichen Society, Academic Press Ltd, the Senior Editor, or the Editors.

Membership of the British Lichen Society is open to all individuals (not institutions) anywhere in the world interested in any part of lichenology. Subscriptions, due on joining and afterwards on 1 January each year, are: ordinary members, £25; associate members, £18·50; senior associate members, £7·50; junior associate members, £5·00 (US dollar rates are double the sterling rates). Members receive the *Bulletin* of the British Lichen Society and issues of *The Lichenologist* published in the year they join, and afterwards while they remain members of the Society, free of charge. Associate members do not receive *The Lichenologist*. Details of the membership, facilities and activities of the Society and an application form may be found on the British Lichen Society web site at http://www.argonet.co.uk/jmgray/ or obtained from the Secretary (address above).

Membership of the British Lichen Society is open to all individuals (not institutions) anywhere in the world interested in any part of lichenology. Subscriptions, due on joining and afterwards on 1 January each year, are: ordinary members, £25; associate members, £18·50; senior associate members, £7·50; junior associate members, £5·00 (US dollar rates are double the sterling rates). Members receive the *Bulletin* of the British Lichen Society and issues of *The Lichenologist* published in the year they join, and afterwards while they remain members of the Society, free of charge. Associate members do not receive *The Lichenologist*. Details of the membership, facilities and activities of the Society and an application form may be found on the British Lichen Society web site at http://www.argonet.co.uk/jmgray/ or obtained from the Secretary (address above).