Aspects of photobiont selectivity in lichens

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1. Introduction

1.1. The Lichen Symbiosis

Lichens represent one of the most successful symbioses in nature, which includes the capacity to survive extreme environmental conditions. Lichens represent a symbiosis of at least a photobiont (eukaryotic alga or cyanobacteria) and a mycobiont (fungus). The photobiont uses sunlight to assimilate CO_2 and resulting energy-rich compounds are used by the mycobiont to produce a complex morphology. Unlike the mycelia of other fungal life forms, the vegetative bodies of lichens are therefore often exposed at the substrates surfaces. In some areas, lichens are the predominant and pioneering form of life, and it has been estimated that lichens cover up to 10% of the Earth's land surface. The evolutionary success of this life form is underlined by the fact that one fifth of all fungal species undergoes the lichenized life style. Many of the lichenized fungi belong to major lineages in the evolution of ascomycetes, indicating a substantial and early radiation of the lichen symbiosis (Lutzoni et al. 2001). Due to this radiation, that gave rise to app. 18000 species in the symbiotic

stage, lichens are perfect organisms to study the evolution of symbiont associations.

The diversity of lichen symbioses is also demonstrated by their enormous phenotypic variation. Lichen symbioses are organized as thalli (a multicellular plant body lacking apical meristems and typical plant parts) in which fungal and algal parts are more or less organized as functional layers. Roughly, a few basic thallus types can be distinguished (Grube & Hawksworth 2007). About 55% of the lichen fungi form simple (eg. crustose) thalli, ca. 20% form squamulose or placodioid thalli, and ca. 25% form more morphologically advanced foliose or fruticose thalli (Honegger 2008).

Although many lichen fungi can be grown *in vitro* without their symbiotic partner they occur (almost) exclusively in their symbiotic form in nature, that is, together with their algal partners. Despite the dualistic nature of lichens has first been discovered by de Bary and Schwendener in the second half of the 19th century (see Honegger 2000), only few lichenologists have given more interest to the photobiont until now. This is certainly due to the shape-giving morphology of the fungal partners, which are by definition the name-giving partners in the symbiosis. The diversity of the photobionts is therefore still little known. About 40 genera of algae have been reported as photobionts for lichens, and this number has practically been left unchanged since the early 20th century (see

Tschermak-Woess 1988, Büdel 1992). The status of photobiont species has been unclear in many cases. This lack of knowledge can be attributed to the fact that in most cases time-consuming culturing of the photobiont was necessary for an exact identification. Nowadays molecular methods provide a possibility to study the photobiont relationships using DNA sequences that can be retrieved by PCR directly from the total DNA extract of the thalli. Substantial progress has been achieved therefore in the understanding of symbiont selectivity and photobiont relationships in lichens since the 1990ies.

The goal of this investigation is an analysis of specific aspects in patterns of symbiont selectivity in selected lichens. How many species of photobionts can be accepted as partners in lichens? Do these species belong to the same lineage or can different lineages of algae contribute to the diversity of accepted partners. Is there only one algal partner in the lichen thallus or several? How does the mode of propagation contribute to photobiont diversity? Are there shifts in the symbiont selectivity patterns across geographic or climatic gradients? These are some of the questions that will be addressed in the following.

1.2. Lichen photobionts

Lichen photobionts belong to two kingdoms: the green algae, which represent the majority of all photobionts, and the cyanobacteria, which are present in only about 10% of the lichens as the primary photobiont. The most common photobiont genera in lichens are the green algae *Trebouxia* (Trebouxiophyceae) and *Trentepohlia* (Ulvophyceae) and the bacterial genus *Nostoc* (Cyanobacteria). Other genera that are related to these common genera occur as well but less frequently (Friedl & Büdel 2008). *Trebouxia* sp. represents the most common photobiont containing numerous and partly still undescribed species. Sixteen species of *Trebouxia* have been analysed by Friedl (1989). The morphological distinction of photobionts is quite difficult since, in most cases, time consuming culturing is necessary for an exact identification. Due to the fact that most phenotypic characters are ultra structural and difficult to study, the knowledge about the diversity of the photobionts still lags far behind the knowledge of the mycobiont.

Specificity of photobiont associations varies considerably in different lichens. General patterns of photobiont association were summarized for major evolutionary radiations of lichens (Tschermak-Woess 1988, Rambold et al. 1998, Miadlikowska et al. 2006). Major mycobiont lineages show clear preferences for certain photobionts. For

example, within the Lecanoromycetes *Trebouxia* represents the most common photobiont whereas particular lineages specialized on other algal genera: The Cladoniinae associate with *Asterochloris* and *Trentepohlia* is typical for Graphidales. *Trentepohlia* is also the predominant photobiont of the second large main lineage of lichens, the Arthoniales.

The knowledge about photobionts in selected species of lichens has increased within the last decade, due to the application of standard molecular phylogenetic approaches. Especially algae placed in the class Trebouxiophyceae are know better studied. This class includes widespread and common aerial/terrestrial algae, with some primarily lichenized genera, *Trebouxia*, *Asterochloris*, and few others (e.g. Beck et al. 1998, 2002; Dahlkild 2001, Helms et al. 2001, Nelsen & Gargas 2008, 2009, Opanowicz & Grube 2004, Yahr et al. 2004).

Recent research found varying degrees of specificity for algal symbionts by lichen mycobionts. Widespread crustose lichens may be generally much less restricted in their choice of *Trebouxia* species. The saxicolous, crustose *Lecanora rupicola* with a broad ecological amplitude associates with several distinct lineages/species of *Trebouxia* (Blaha et al. 2006). The greatest diversity was detected in the Mediterranean area, which may host a number of yet undescribed photobiont species. Species with narrower ecological amplitude usually also have a quite restricted photobiont range. The latter is often correlated with more complex thallus

structures of lichens, but this is not always the case (Schaper 2003), and geographic distance might also play a role (Nelsen & Gargas 2009). These observations raise a fundamental question of lichen ecology: to what extent is ecological adaptivity determined by photobiont selectivity, or by other fungal characters. Low levels of selectivity for the photobiont can be advantageous for lichens that which are widespread and colonize in different habitats (e.g. *Protoparmeliopsis muralis*, Guzow-Krzeminska 2006).

On the other hand, a restricted number of photobiont species belonging to the genus *Trebouxia* was observed in morphologically advanced foliose lichens in the Physciaceae (Dahlkild et al. 2001, Helms 2003). Similar high selectivity is found in fruticose lichens of the Parmeliaceae (Kroken & Taylor 2000, Opanowicz & Grube 2004). Yet, foliose lichens are not always restricted to particular strains of photobionts, as different algal species have been isolated from thalli of *Parmelia* and *Umbilicaria* species (Friedl 1989, Romeike et al. 2002) and from the strictly sterile genus Thamnolia (Nelson & Gargas 2009).

1.3. Sexual versus asexual Reproduction

In many lichen symbioses the partners propagate independently and the symbiotic life-cycle starts with *de novo* establishment of a symbiosis, after the independently distributed partners have encountered and recognized each other. The timely association with an appropriate partner species is therefore essential for establishing successful symbiotic phenotypes. The competitive advantage of fitter combinations necessarily leads to higher specialization of partnerships, especially in the obligate symbioses. However, the degree of specialization is limited by the availability of suitable partners in the environment. The alternative evolutionary strategy of lower partner specificity increases the likelihood of finding a partner and could be advantageous if varying environmental conditions modify the fitness parameters of the symbiotic phenotypes.

In some cases the problems of partner availability are avoided by mechanisms to disperse symbiotic partners jointly. Also recognized as “vertical transmission”, this propagation mode can be particularly advantageous for rapid colonization of newly available habitats (Poelt 1963). The transmission from parent to offspring which captures an effective symbiont lineage could further lead to co-evolving partnerships

(Douglas 1998). Joint propagation of symbiotic partners is known from diverse symbioses (e.g. Krueger et al. 1996, Poulsen & Boomsma 2005, Sharp et al. 2007). On the other hand, the joint dispersal of ecologically specialized partners can constrain the establishment of symbioses to narrow ecological niches that can be geographically disjunct. Lichenized algae can be distributed together with their fungal partners in mitotically produced organs either produced directly on the vegetative thallus or in specialized regions thereof.

Asexual propagules of diverse types can be distinguished by their morphology. Types with a stratified anatomical structure resembling that of the mature thallus are called isidia. The often occur as small protractions of the surface of the thallus and may be cylindrical, globular, brachiate (branched) or lobula (lobe-like), and occur 20-30% of foliose and fruticose lichens have isidia. Soredia and are more simply organized and consist of few algal cells and enwrapping hyphal elements, which often have hydrophobic surfaces. Some lichen genera, such as *Lepraria*, have given up the production of meiotic fungal spores completely and seem to evolve asexually a surprising diversity of species that disperse symbiont jointly in soredia.

In other lineages, sexual and asexual species can be closely related (Du Rietz 1924), in which the asexual lineage reproduces by joint transmission of symbionts. Provided that these species share direct

common ancestry they are termed ‘species pairs’ in lichenology (Poelt 1970; for a somewhat different meaning of this term in ichthyology see Taylor 1999). Closely related sexual and asexual species, in particular species pairs with complex morphology, often differ in their distributional ranges (Poelt 1963, 1970). The usually wider distribution of the sterile species suggests the colonizing success with vertical symbiont transmission.

1.4. The question of free-living *Trebouxia*

Until recently, it was repeatedly put forward that species of the most common lichen-forming algal genus *Trebouxia* do not occur in free-living stages (Ahmadjian 2002). Consequently fungal spores would need to capture algae from either pre-established lichens or from lichen propagules that contain both symbionts. However, recent evidence leads to doubts of this hypothesis. Free-living trebouxioid lichen algae are perhaps inconspicuous because macroscopically observable colonies are not developed. Instead, they are likely short-living in form of single cells or arranged in few-celled aggregates and present in mixed algal consortia. Initial colonization by lichens of abandoned glass pieces in Antarctica suggest that mycobionts acquisited their photobionts from free-living

stages (Schroeter and Sancho 1996). Further, free-living lichen algae were detected on marble monuments using culture-independent molecular approaches (Capitelli et al. 2007). Studies on sexually reproducing pionieer lichens (Beck et al. 1998) that colonize smooth barks also suggest that free-living *Trebouxia* exists. Nevertheless, available lichen-forming *Trebouxia* species seem to be scattered and locally rare. In cultures lichenized photobionts differ from non-lichenized algae by clearly thinner cell walls (Brunnauer, personal communication) suggesting that their survival in free-living stage, i.e. without the fungal partner, could be rather limited.

Vertical symbiont transmission should have clear consequences on the population genetic structure of symbiotic populations. We hypothesized that the sterile species should display a more distinct linkage of symbionts and a restricted range of photobionts in comparison with the sexual species. As the latter may randomly associate with locally available compatible photobiont strains to resynthesize symbiotic associations, more variation could be expected and algal diversity should be higher in populations of the sexual lichen species.

Within the major lineages of lichens photobiont choice can vary considerably. The observed patterns suggest that evolutionary diversification of lichens could be associated with pronounced photobiont switches. For example, the placement of the morphologically diverse

Gomphillaceae and Asterothyriaceae (both with coccale green algae) in Ostropales (with mostly filamentous green algae) by Lücking et al. (2004) suggests a photobiont switch as a key evolutionary event that preceded the diversification of that lichen group. Substantial photobiont variation may also be present in genera of lichens, although it is still not always clear if the genera are monophyletic. One of the most "promiscuitive" genera is certainly Verrucaria, which even includes the unique association with a brown alga (Sanders et al. 2005). However, the relationships among Verrucaria species are still unsettled. Similar applies to the large genus Arthonia (500 species), in which most morphological groups associate filamentous trentepohlioid algae, but some also with coccale green algae, while others are apparently saprobic. Also, Chaenotheca is a case of high variation of photobionts (Tibell 2001), with 4 green algal genera (coccal Dictyochloropsis, Stichococcus, Trebouxia and filamentous Trentepohlia) being involved. The two central European Petractis species differ remarkable in their photobionts. One species (P. clausa) associates with cyanobacteria (Scytonema) whereas the related P. hypoleuca has a Trentepohlia photobiont.

Apart from the ability to choose among different groups of photobionts, lichen fungi of the same genus can also differ in their selectivity for photobionts. Lecanora conizaeoides selects only one algal

lineage, but Lecanora rupicola can associate with diverse lineages in the genus Trebouxia.

1.5. Selectivity and Specificity

The specificity of a species is defined by the possible pool of partners from which a species is able to select its symbiotic partner. It is determined by inner factors, such as genetic constraints. Culture experiments have shown that different fungal–algal pairs show quite a different efficiency in interaction with each other (Schaper & Ott, 2003). However, a mycobiont that is principally able to form symbiotic structures with more than one algal species may prefer only a subset of these symbionts under natural conditions, depending on the ecological factors. However, unrelated species may have different preferences in the same habitat (Doering & Piercey-Normore (2009).

Selectivity is defined as the frequency of a specific association as it can be observed in nature. In *Cladonia subtenuis* (Yahr et al. 2006) it was shown that the mycobiont shows an ecological specialization for photobionts in different environments. A low specificity leads to a high possibility to adapt to new, changing environments, whereas a high specificity can lead to a very well balanced metabolic interplay between the symbionts that can be particularly advantageous in a specific

environment to favour rapid production of dispersal units. Even though a combination might be optimal in one habitat it might thus represent just one of the possible combinations. A fungus, with a rather low specificity could also form temporary associations and select for better combinations of symbionts during ontogeny. The frequency of any combination may thus be determined by the success of the algal-fungal combination under the given conditions rather than by the mere availability of the partners.

1.6. Questions and Hypotheses

To gain insight in the diversity of lichenized *Trebouxia*, sequences from selected lichens have been included in a phylogenetic analysis to show an overall *Trebouxia* phylogeny. Sequences provided by Helms have been included in the analysis, in order to test the 4 major clades (A; I, G, S) proposed by Helms (2003) and the results are discussed in chapter 3.

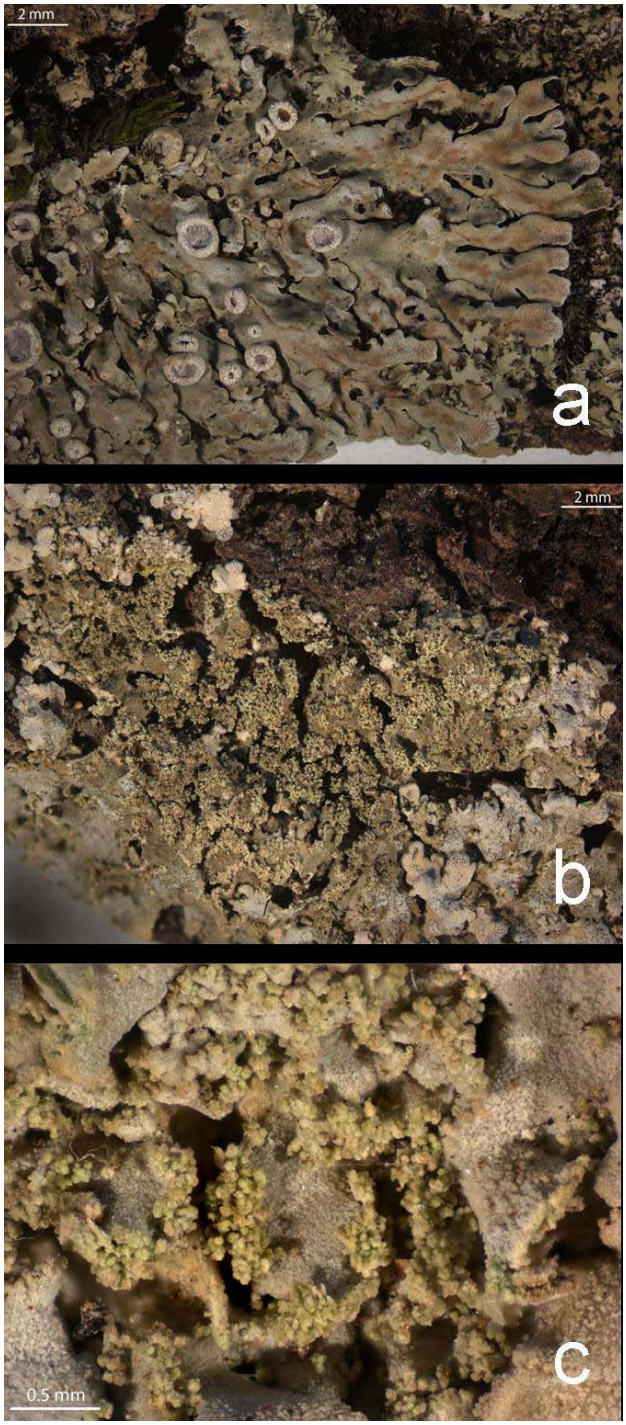

Figure 1.1. The studied species: a) habitus of *Physconia distorta* (bar = 2 mm), b) habitus of *Physconia grisea* (bar = 2 mm), c) soredia of *P. grisea* (bar = 0,5 mm).

To assess algal specificity in lichens with a different degree of symbiont selectivity data have been included from three more lichen species (Figure 1.2.) in chapter 4. The results clearly show that patterns of photobiont selectivity vary strongly among lichens species. In this chapter pairwise mismatch distributions are used to illustrate patterns of photobiont associations. These mismatch distributions are then compared with the phylogenetic distribution of the photobionts from the same data and the genetic diversity of the two species that are discussed in chapter 5. Mismatch distributions are traditionally used to estimate demographic history of populations, but as I will show they might be useful also for understanding symbiotic association patterns.

Differences between the species that differ in their propagation mode should ideally also be reflected by dissimilar population genetic structures of both symbionts. To test this hypothesis with exemplary species the population structure of both symbionts following a North-South gradient was investigated. This was used to assess population structure of both symbionts and symbiont selectivity patterns. The results of the analysis are discussed in chapter 6.

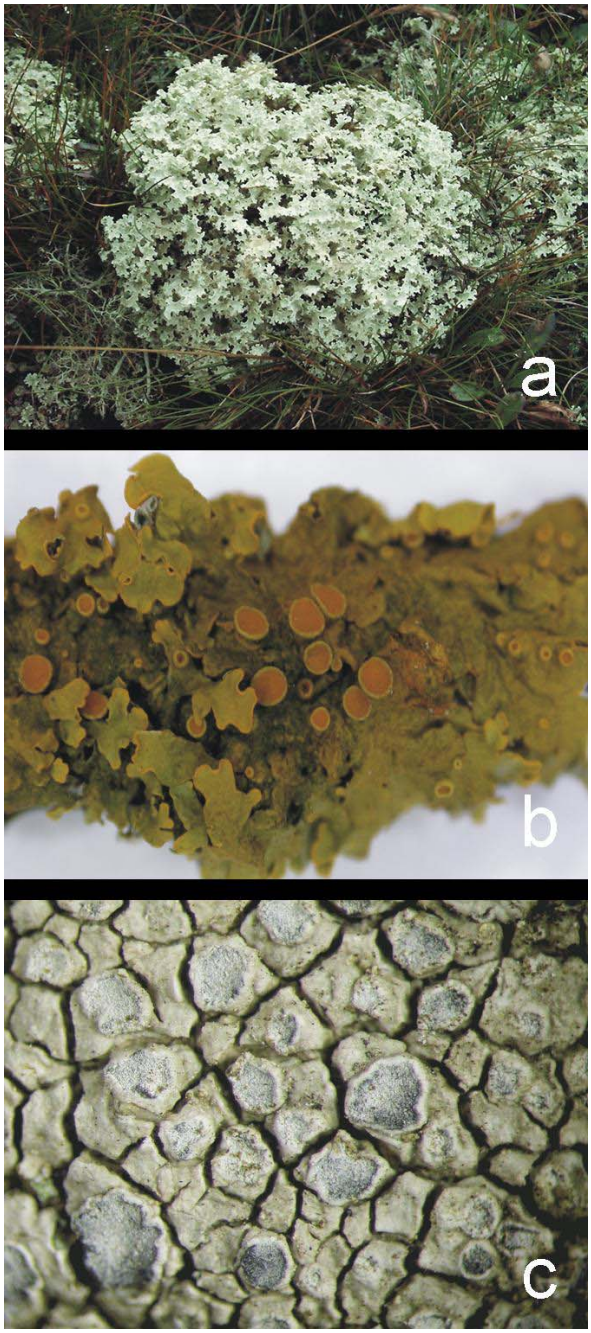

Figure 1.2. Pictures of lichens analysed in chapter 4: habitus of a) *Flavocetraria nivalis* (M. Opanowicz) b) *Xanthoria parietina* and c) *Lecanora rupicola* (J. Blaha).

2. Material and Methods

2.1. Selected species pair

To test the hypothesis that lichens with a dissimilar mode of propagation show differences in population genetic structure, symbiont association patterns in populations of two closely related lichen species were studied. Because there are cases of doubtful taxonomic ranking of sterile and fertile morphs as species pairs (e.g. Lohtander et al. 1998), we selected the following closely related, but clearly separate species *Physconia distorta* and *P. grisea* (Physciaceae). The widely distributed bark-inhabiting foliose lichens are distinct according to morphology and phylogenetic data (Cubero et al. 2004). *P. distorta* is fertile and propagates via ascospores (fig. 1.1a; p.16), whereas *P. grisea* distributes both symbionts jointly in soredia (fig. 1.1.b, c; p.16); only very rarely ascomata were observed). Initial analyses showed that he selected lichens have a similarly high degree of specifity to algal lineages.

2.2. Sampling

Since it is quite difficult to delimit the extent of a population in lichens, a population was arbitrarily regarded as a number of co-occurring thalli of a lichen species in a given area of approximately 100 x 100 m. In each sampling plot, consisting of one or more neighbouring trees, 7-15 thalli were collected. We sampled along a gradient from the Alps to South Italy (Italian sampling plots, see Fig. 2.1; including samples collected by Lucia Muggia). Additionally I included one population from Sweden (collected by Åsa Dahlkild) and one from Norway (collected by Jarle W. Bjerke) for *P. distorta* and one sampling plot from Poland (collected by Martin Kukwa) for *P. grisea* in the analysis to cover diversity of a wider geographic range. Prior to DNA extraction the lichens were checked for visible contaminations by other fungi. Species were determined according to standard references (Wirth 1995).

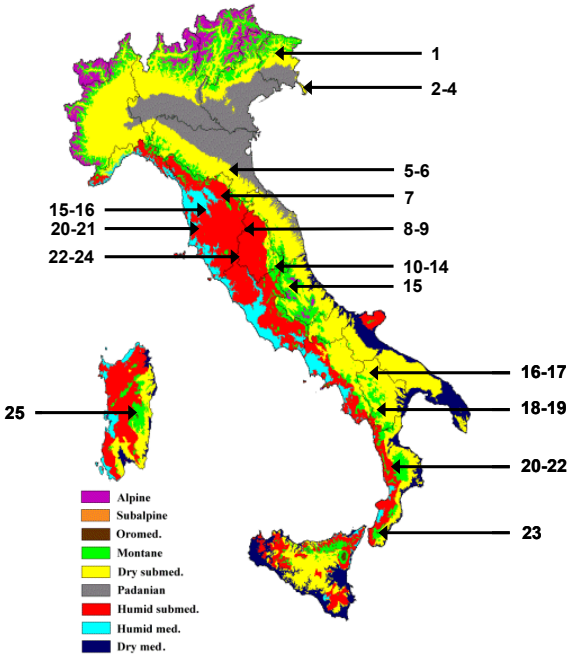

Figure 2.1. Map of Italy showing the sampling plots.

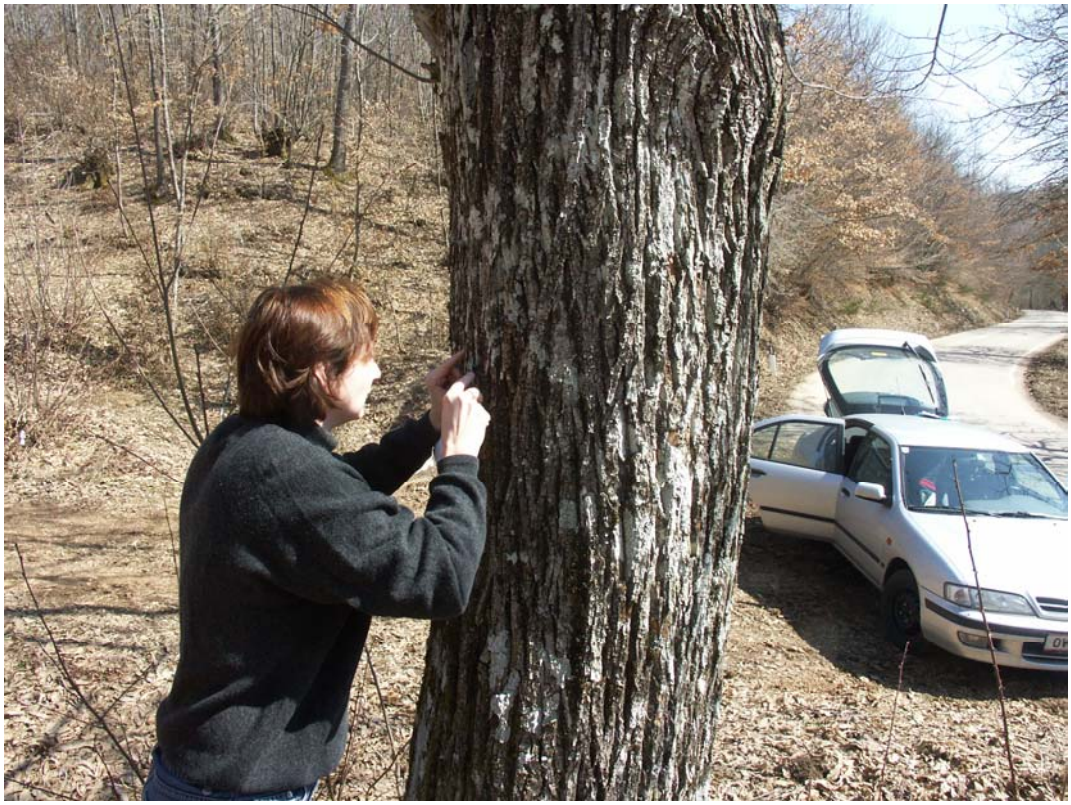

Figure 2.2. Sampling in Italy.

2.3. Molecular Analysis

A total DNA extraction of lichens, including fungal as well as algal DNA, followed a modified CTAB method (Grube 2005). For PCR amplification using an ABI 2700 Cycler (Applera, Vienna) we used the fungal-specific primer pair ITS1F (Gardes & Bruns 1993) and ITS4 (White et al 1990) and in a separate amplification the algal-specific primers IT1T and ITS4T (Kroken & Taylor 2000). After initial denaturation at 95°C for 3min, six touchdown cycles with annealing temperatures decreasing from 54°C for fungal template (56°C for algal template) to 48°C for fungal template (50 °C for algal template) were carried out, followed by 35 cycles (94°C/30s, 48°C/30s, 72°C/155s) and terminating after a final elongation at 72°C for 7 min.

Fifty microliters of PCR cocktail for the amplifications of the fungal ITS region consisted of 5 µl / 10x PCR Buffer Buffer (Qiagen, Vienna), 1.25 units Taq DNA polymerase (Qiagen, Vienna),10 µl / 5x Q Solution (Qiagen, Vienna), 0.2 mM of each of the four dNTPs, 0.5 µl of each primer and ca. 10-50 ng DNA. For the amplifications of the algal ITS region we used the same amount of Taq DNA Polymerase from another supplier (Amersham Pharmacia Biotech Inc.) and appropriate buffer conditions. Products were cleaned using the QIAquick PCR Purification Kit (Qiagen, Vienna). Both complementary strands were sequenced using the ABI Big

Dye Terminator kit (Applera, Vienna). Sequencing reactions were separated on an ABI 3730xl Sequencer and assembled using AutoAssembler (Applera, Vienna). Sequencing of the fungal ITS region was done using the primer pair ITS1-LM (Myllys et al 1999) and ITS2-KL (Lohtander et al 1998).

2.4. Additional Data included for the analysis

For the chapters 3 to 5 sequences provided by Gert Helms (Göttingen; Helms 2003) were included in the analysis. For chapter 3 sequences contributed by Rodrigo Reis (Curitiba, Brazil), representing tropical samples, were included to improve the picture of tropical lineages. For chapter 3 and 4 additional sequences for *Flavocetraria nivalis*, *Lecanora rupicola* and *Xanthoria parietina* (Figure 1.2., p. 18) were obtained from GenBank (http://www.ncbi.nlm.nih.gov/).

2.4. Analysis of the sequences

The sequences were initially aligned using the Clustal algorithm as implemented in BioEdit (Hall 1999) and optimized by eye. Gene diversities and mismatch distributions were calculated with Arlequin 3.01 (Excoffier et al 2005). Haplotype networks have been produced using TCS (Clement et al. 2000). The mismatch distribution and gene diversity was calculated with Arlequin (Schneider et al. 2000).

The phylogenetic hypothesis was established using a Bayesian approach as implemented in the program MrBayes 3.1.2 (Huelsenbeck &

Ronquist 2003, Ronquist *et al*. 2005). The General Time Reversible substitution model with estimation of invariant sites and assuming a gamma distribution with four categories (GTR+I+G) was used for likelihood calculations. The optimal nucleotide substitution model was found before with the program MrModeltest 3.7 (written by J.A.A. Nylander and available at http://morphobank.ebc.uu.se/mrbayes/) using the Akaike Information Criterion (AIC). For other parameters the default settings were used. The Markov Chain Monte Carlo (MCMC) algorithm was run for two million generations, with 6 chains starting from a random tree and using the default temperature of 0.2. Every 10th trees were sampled while the first 1000 generations were discarded as burn-in. The consensus phylograms based on the mean branch lengths were calculated with the command *sumt* in MrBayes (see MrBayes 3.1 Manual, Ronquist *et al*. 2005). The phylogenetic trees were drawn with the program TreeView (Page 1996).

3. Diversity of *Trebouxia* in lichens

3.1. Results of investigations on the overall photobiont diversity

In this chapter, sequences of photobionts from different lichens were analysed together with newly generated data in this thesis, published sequences from Genbank, and sequences from the theses of Gert Helms (2003) and Rodrigo Reis (2005). The latter data were generated in our laboratory. This broader sampling was used to present a comprehensive picture of genetic diversity of trebouxioid photobionts. I followed the four clades of Helms namely A, I, G and S and calculated each of the clades in a separate tree.

The analysis of the clade A is depicted in figure 3.1. (p. 29). This tree includes also the samples from *Xanthoria parietina* and *Lecanora rupicola*. The photobionts of *X. parietina* group within two clades, whereas *L. rupicola* can be found in 4 different clades within clade A. These clades seem to represent quite distinct representatives in the tree, some of which cannot be assigned to a described species. The subclades from Helms that are confirmed by the present trees are A9a, A11a, A3a, A6a and A10a. The clades A1, A2, A4, A7 and A8 the subdivision in subclades can not be confirmed.

The phylogenetic analysis of the clade I included (figure 3.2. p. 30) sequences from *Physconia grisea, Physconia distorta* and *Lecanora rupicola.* Some samples from the Southern Hemisphere were included as well. The samples from *Physconia* cluster within different parts of clade I1 where also some of the samples from *L. rupicola* can be found. The samples from the Southern Hemisphere built a new, distinct clade within the I4 clade. The clades that can be confirmed by the analysis are I1d, I1g, I1j, I1n, I1e, I1c, I1a, I1b, I1k and I6a. *T. impressa* and *T. potteri* can be found within the I1a clade as shown in Helms (2003).

Clade G shown in figure 3.3 (p. 31) includes particularly many samples from the (sub)tropical regions of the Southern Hemisphere. With the analysis of the enlarged dataset more variation in the G6 clade can be found. In clade G5a only fruticose samples can be found and in clade G3a-d all the *Ramalina* samples from Restinga cluster together. Furthermore it can be shown that all Physciaceae cluster within clade G9a. The clades that can be confirmed by the analysis of the extended dataset are G2a, G1a, G5a, G9a and G7a.

The enlarged analysis of clade S, is shown in figure 3.4. (p. 32). Sequences from *Flavocetraria nivalis* and selected sequences of *L. rupicola* have been included in the dataset. *L. rupicola* clusters in different parts of clade S2, namely in clade S2b, S2c, S2d and S2a. The photobionts of *F. nivalis* can be found in cluster S2b and S2f and a new sister clade. The clades that can be confirmed by the analysis are S2b, S2f, S2c, S2d, S2e and S2a.

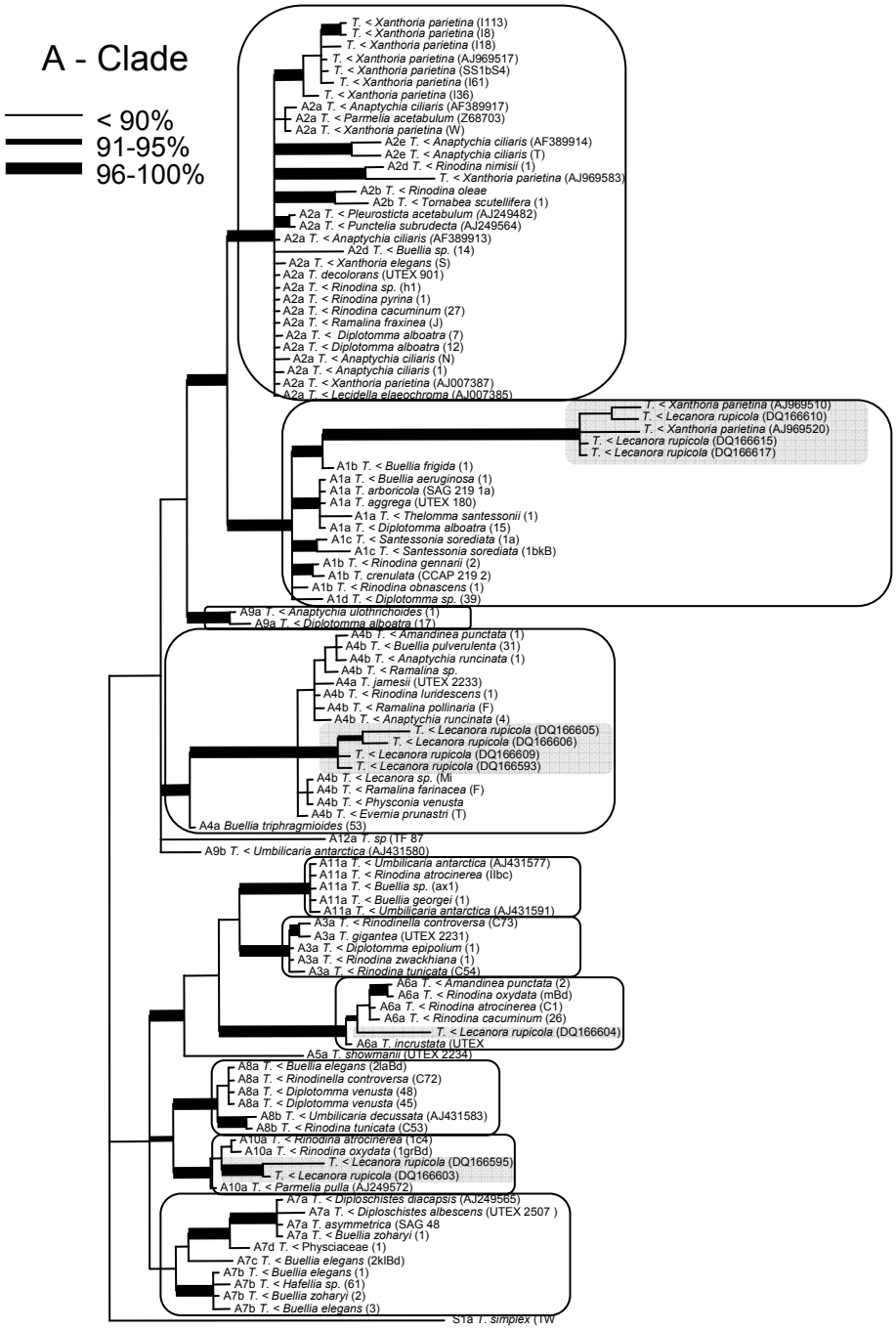

Figure 3.1. Phylogenetic tree of clade A analysed through Bayesian phylogenetic analyses. Posterior probabilities equal or more than 95% are indicated by thickened branches.

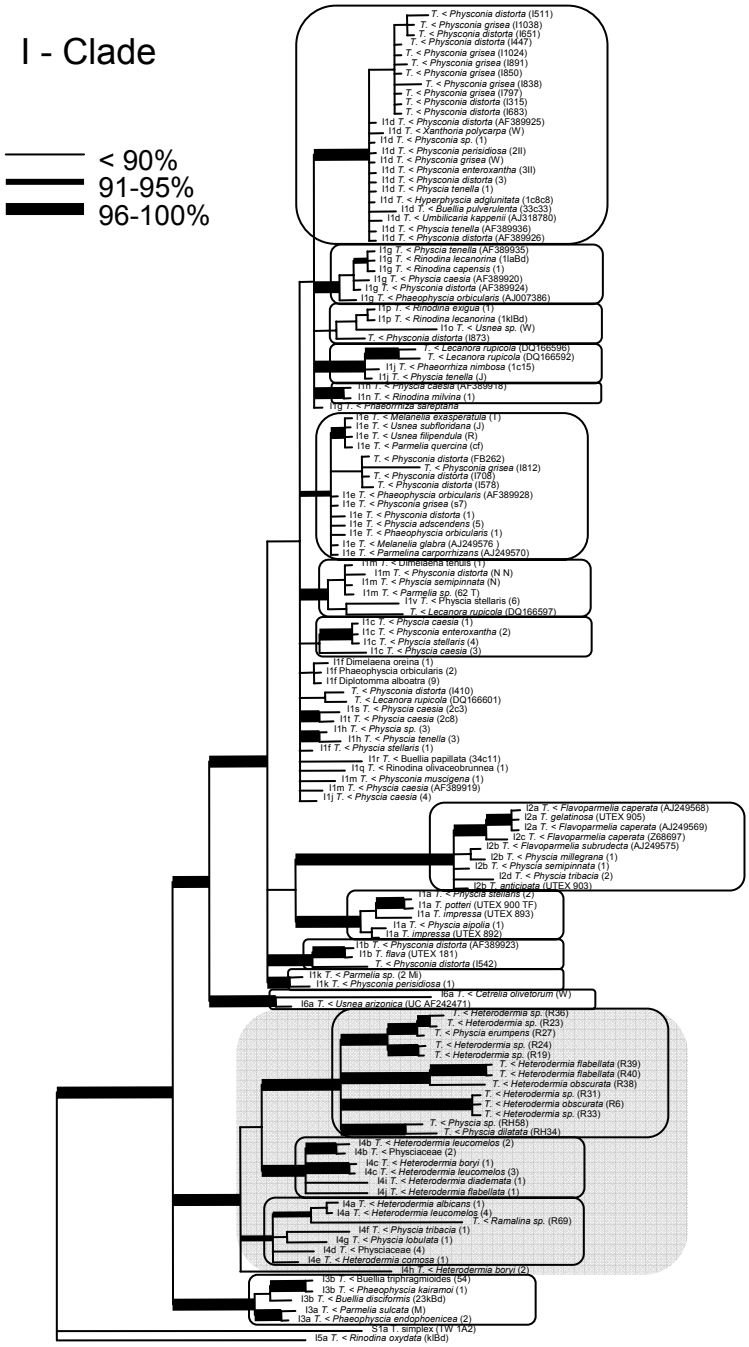

Figure 3.2. Phylogenetic tree of clade I analysed through Bayesian phylogenetic analyses. Posterior probabilities equal or more than 95% are indicated by thickened branches.

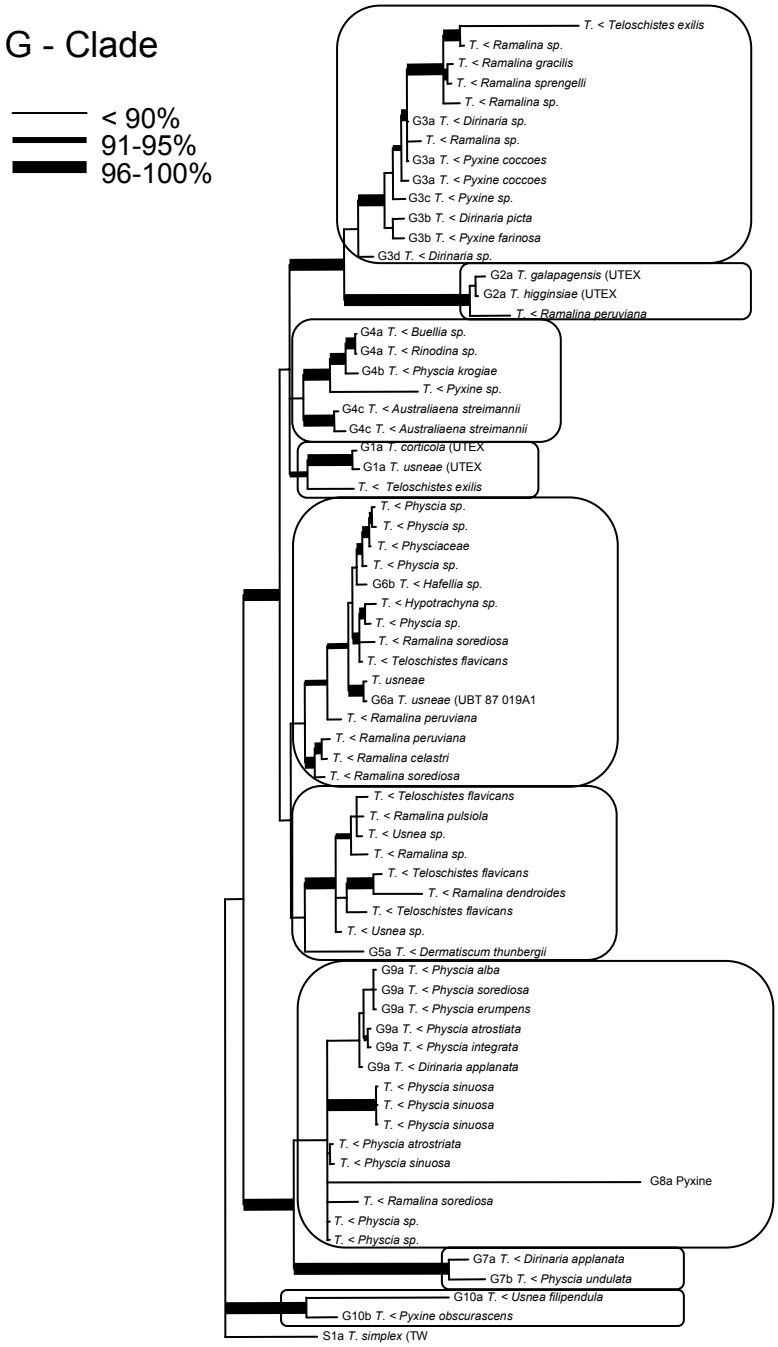

Figure 3.3. Phylogenetic tree of clade G analysed by Bayesian phylogenetic analyses. Posterior probabilities equal or more than 95% are indicated by thickened branches.

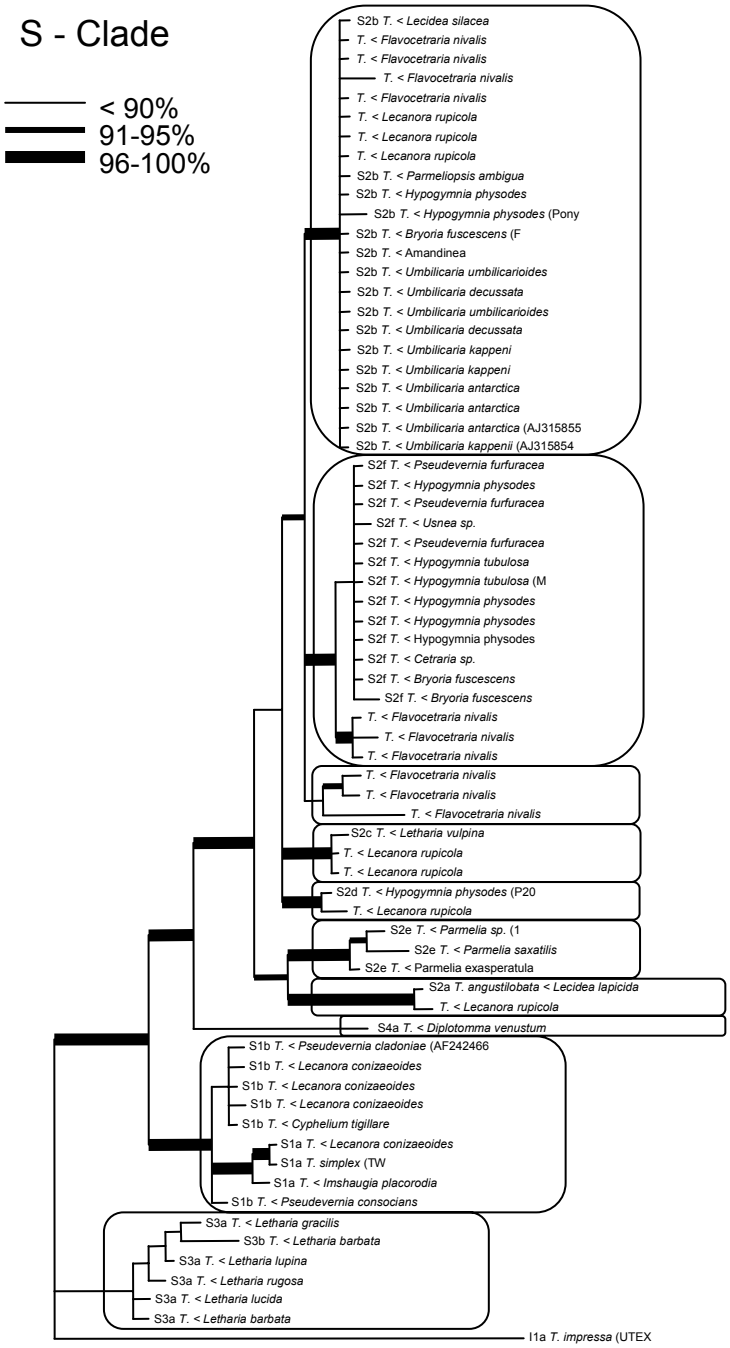

Figure 3.4. Phylogenetic tree of clade S analysed through Bayesian phylogenetic analyses. Posterior probabilities equal or more than 95% are indicated by thickened branches.

3.1. Discussion on the overall photobiont diversity

The photobiont sequences of *L. rupicola*, according to Blaha et al. (2006), are found in six different clades. Four of these clades cluster within clade A and can be found also in my analysis of clade A and are represented by informal taxa sp1 (A6), sp2 (A4), sp3 (A2) and *Trebouxia incrustata* (A10) clade. Interestingly, lineage sp1, which belongs to clade A6 according to the analysis of Blaha et al., turns out in clade A10a in my analysis. Species 2 can be confirmed within clade A4. Sp3 which originally belonged to clade A2 (Blaha et al.2006) can be found in A1 in this analysis. The species *incrustata* can be confirmed in its position within clade A10. Interestingly all clades where the *L. rupicola* samples are found (highlighted in grey within figure 3.1. p. 29) show extremely long branches and seem to be quite distinct from all other species. Despite this fact the samples from sp3 group together with two photobionts from X*. parietina*. These samples represent different geographic origins and lichens of saxicolous and epiphytic origin. In contrast to the results of Nyati (2006) no differentiation between saxicolous and epiphytic samples of *X. parietina* was found. All other samples cluster within one part of clade 2, showing the high photobiont specificity of *X. parietina*. The photobiont of *Tephromela atra* which has been morphologically investigated by Muggia et al. (2008) would cluster in clade A1. In all 3 analyses (Blaha 2006, Helms 2003, Muggia 2007) as well as in my analysis this species

represents a sisterclade to clade A2 which contains *T. arboricola*. This is also well supported by the morphological analysis of Muggia et al. (2008). The authors conclude, from the fact that it has pyrenoids of the arboricola type as well as of the gigantean type, that it represents a new distinct species which is supported by my phylogenetic analysis. However the conclusion that this photobiont strain is strictly adapted to the climatic conditions of the Mediterranean area has to be rejected by my analysis, because one of the *X. parietina* samples originates from the UK. For the overall phylogeny of clade A of Helms only 4 of the defined clades can be confirmed showing that there is still a lot of diversity and probably a lot of cryptic species to be discovered.

In clade I (figure 3.2. p. 30) samples of *L. rupicola* can be found within the *impressa/flava/potteri* clade, in the subclade I1j. All photobionts of the analysed *Physconia* samples can also be found in the clade I1. The clade I1 is confirmed in my analysis. The I2 clade clusters within the I1 clade and is in my analysis a sister clade to clade I1a which contains the strains of *T. potteri* and *T. impressa. T.flava* represents the sisterclade I1b. The overall phylogeny of this *impressa/flava/potteri* clade is confirmed but within this clade there seems to be still a lot of variation. Interestingly these three species represent 3 clearly distinct morphospecies (Friedl 1989). All threes species share the same thylakoid arrangement, the same pyrenoid type (impressa type) and the same cell type. *T. potteri* differs to

T.impressa and *T. flava* in the chloroplast type. T. flava differs to *T. potteri* and *T.impressa* in the autospore formation (Friedl 1989). However especially T. potteri and T. impressa show now clear separation in the analysis of the ITS-sequences. So the overall phylogeny of the *Trebouxia* Tree suggests that there might be a lot of cryptic species.

Interestingly the samples of tropical origin from Reis that have been included in clade I form a new “tropical” clade within clade I4, including only samples from *Heterodermia* and *Physcia*. This is well supported by the fact that within the tree in Helms (2003) of clade I the I4 subclade also represents only tropical samples. The I4 and the I3 clades are confirmed by the analysis of extended dataset but the subclades of I4 seem to be intermixed in the new analysis.

The remainder of the tropical samples sampled by Rodrigo Reis all cluster within clade G (figure 3.3. p. 31). Almost all of the samples that have been included in the analysis by Helms are tropical samples as well and he suggests in his thesis that clade G represents a primarily tropical group of *Trebouxia* species as counterpart to the clades A, I and S, which are more commonly found in temperate to cold regions. This is supported by the fact that all samples, which originate from non-tropical climates, are in clade I, except for the subclade I4.

All samples from *Ramalina sp.* that originate from the “Restinga” cluster within clade G3, which includes lichens collected in coastal

Restinga habitats of SE Brazil. Within clade G6a-b an extremely high diversity can be found, which might result in some new subclades when further sequences can be included in the analysis. Within what is defined as clade G5a only fruticose samples of tropical origin cluster together. The tropical samples of Physciacea and some *Ramalina* samples form a clade which supports clade G9a of Helm (2003). All main clades within clade G are supported by the analysis of the extended dataset although splits into additional clades as also supposed by Helms are confirmed.

Within clade S (figure 3.4. p. 32) again samples of photobionts from *L. rupicola* can be found showing once more the low specificity for photobionts of this fungus. The samples from *Flavocetraria nivalis* that have been included in the analysis all cluster within the clades S2b and S2f and a third new clade showing the high specificity for photobionts of this fungus. Samples of L. rupicola cluster within what is described by Kroken and Taylor (2000) as *T. "vulpinae".* This photobionts originate all exclusively from *Letharia vulpina.* The photobionts from all other *Letharia* species forms the species complex of *T. "letharii"* (Kroken and Taylor 2000). This species complex represents according to Kroken and Taylor (2000) six phylogenetic species and in my analysis they built clade S3a-b. Apart from clade S1 where no clear delimitation between S1a and S1b can be found, all subclades of clade S are confirmed by my analysis.

4. Assessment of specificity/selectivity in lichens

4.1. Results of the investigations on the variation of trebouxioid lichens in selected lichens

In this chapter I present data on different patterns of photobiont selectivity in lichens. For this purpose the trebouxioid photobionts of five lichens were investigated: *Flavocetraria nivalis*, *Lecanora rupicola*, *Xanthoria parietina*, *Physconia distorta* and *Physconia grisea.* Except for *L. rupicola*, these lichens represent foliose growth types. Their ecology is, however, diverse: *Flavocetraria nivalis* is typically found on acidic ground in windswept heathlands of higher altitudes in the Alps or rarely at lowland habitats in Poland and S-Sweden. *Lecanora rupicola* usually grows on mineral-rich siliceous rocks with a broad ecological amplitude. *Xanthoria parietina* can be found on the bark of deciduous trees as well as on rocks up to higher altitudes. *Physconia distorta* and *Physconia grisea* may be present in habitats similar to *X. parietina*, that is, often on single-standing deciduous trees with a nutrient-rich bark. Fig. 4.1. shows the phylogenetic position of the *Trebouxia* sequences of these lichens that were analysed together with other sequences from the NCBI GenBank are shown in one tree. The photobionts of *L. rupicola* cluster in 6 different clades of *Trebouxia*. The

photobionts belong to the *T. incrustata*, the *T. impressa*, the *T. simplex* clade and three new clades, one of them mentioned in Helms et al. (2001). However the photobionts of *F. nivalis* and *X. parietina* are found only in one clade within *Trebouxia*. They belong to *T. simplex* and *T. decolorans* respectively. The photobionts of *P. distorta* and *P. grisea* show a slightly wider range of photobionts in this tree, they belong to the *T. impressa/flava/potteri* clade. The two species represent closely related sexual and asexual species. Despite their different reproductive strategies they show a similar degree of selectivity for their photobiont and they share the same photobionts.

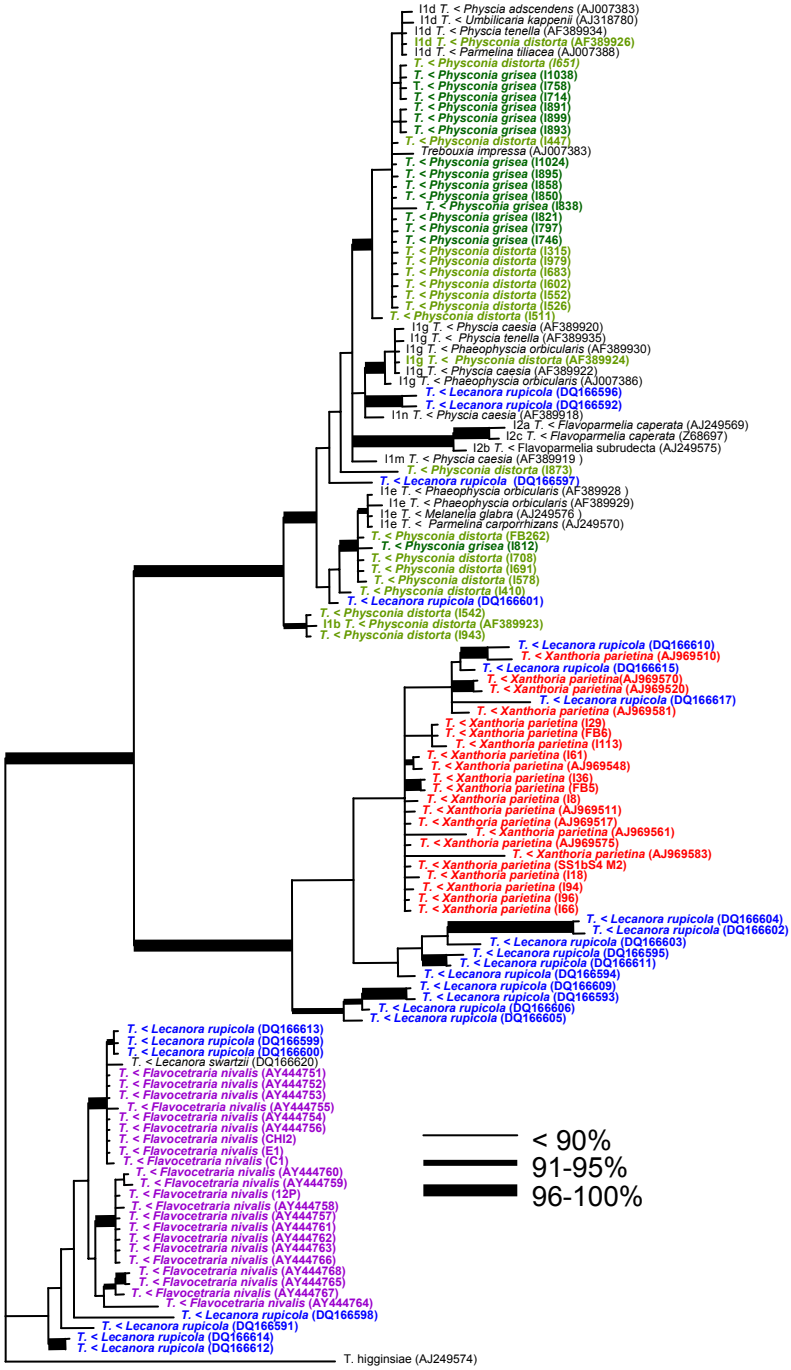

Figure 4.1. Phylogenetic tree of photobionts of selected lichens (species marked in different colours). Bayesian phylogenetic analyses. Posterior probabilities equal or more than 95% are indicated by thickened branches. Clades are named according to Helms (2003).

Lichen species	Mismatch observed mean
Lecanora rupicola	43,20
Xanthoria parietina	20,18
Flavocetraria nivalis	7,97
Physconia distorta	6,17
Physconia grisea	2,86

Table 4.1. Mismatch observed mean in selected lichens

The results from the mismatch distribution this are clearly supported by the mismatch observed mean which can be seen in Table 4.1 *L. rupicola* has a significantly higher mean indicating diverse photobionts. Compared to this the differences within *X. parietina* are only half as much. Looking at the values for the two *Physconia* species it can be seen that there is a significant difference in the mismatch observed mean whereas no difference can be found looking at the genetic difference. The mismatch observed mean seems to be quite sensitive to the sampling whereas the mismatch distribution curve itself remains very stable. This can be confirmed when the full dataset for *P. distorta* and *P. grisea* (391 sequences for *P. distorta* and 174 Sequences for *P. grisea*; data not shown) the mismatch mean values for P. distorta increases significantly whereas it decreases slightly but the distribution curves stay the same. Even if all sequences of *P. distorta* and *P. grisea* are included in one

dataset, the mismatch distribution remains the same and no evidence for population separation can be found. Because they share the same pool of photobionts which make them a very good model system for studying differences in symbiont selectivity in sexually and asexually reproducing lichen symbiosis as will be discussed in chapter 5. The sequences of the sexually reproducing species show a larger range of differences. They are also more widely distributed over the tree. Despite the genetic diversity, representing the probability that two randomly chosen haplotypes are the same, no significant differences between the two species can be seen.

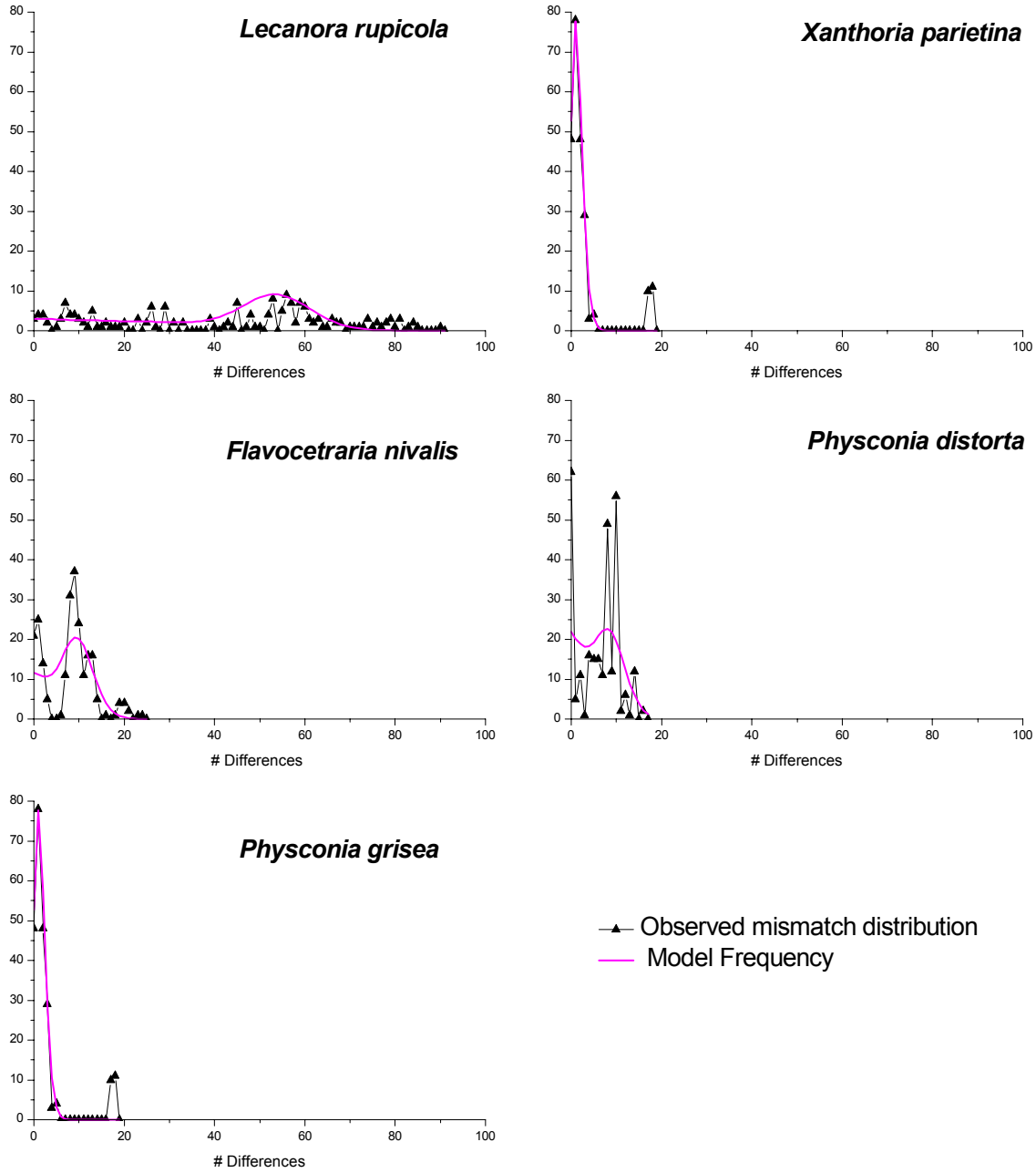

Figure C.2.2. Mismatch distributions curves of selected lichens

The mismatch distribution curves shown in Fig. 4.2 illustrate that the photobionts of *L. rupicola* scatter more or less equally over the whole range whereas in *X. parietina* and *F. nivalis* one clear maximum representing one clade can be seen which is comparable to the pattern found in *P. distorta* and *P. grisea*. This result is reconfirmed in a similar mismatch distribution curve if the full dataset with all photobionts for the last two species is in included in the dataset.

4.2. Discussion on the variation of trebouxioid lichens in selected lichens

The underlying reasons for a high selectivity of lichen mycobionts for particular photobionts are still poorly understood. Bubrick and Galun (1980) and Bubrick et al. (1985) detected a phycobiont-binding protein in *Xanthoria parietina* which could serve for recognition of photobiont types. It is unclear whether such proteins also exist in lichens species with low selectivity for their photobionts. In these species, a photobiont species can generally be selected from a more diverse "pool of locally available algae" (Beck et al. 1998, 2002).

Species of various genera select different photobionts in parts of their geographic distribution. The diversity and phylogenetic position of photobionts in the widespread saxicolous, crustose lichen species *Lecanora rupicola* s. lat. was investigated by Blaha et al (2006). The algal

partners of this lichen species complex belong to at least 3 distinct lineages in the genus *Trebouxia*. Irrespective of the different algal partners, all lichen thalli abundantly developed ascomata. Apparently *L. rupicola* maintains full fecundity with a low degree of selectivity for photobionts. This capability could be a reason for the presence of this lichen-forming species in ample ecological situations represented by its wide geographic range. A comparable pattern is represented by *Tephromela atra* (Muggia et al. 2007), which could, however be a complex of nascent or morphologically hard to distinguish species. Alpine specimens not only differed in their photobiont species from Mediterranean samples, but they also represented an own lineage within the mycobiont species complex. *Protoparmeliopsis muralis* is a further species with rather wide ecological variation (Guzow-Krzemińska 2006). This species also selects a wide range of photobionts but in this case variation is found at the same site in different individuals.

Other crustose species with narrower ecological niches seem to be more restrictive for their photobionts. *in vitro* reassociation experiments by Schaper (2003) indicated that symbiotic stages of the crustose *Fulgensia fulgida* are rapidly formed with their genuine photobionts, whereas formations of lichen associations are clearly delayed in resynthesis experiments when other photobionts. This agrees somehow with the old data of Ahmadjian et al. (1980) and Ahmadjian & Jacobs (1981) who

observed that mycobionts of *Cladonia cristatella* and *Rhizoplaca chrysoleuca* form well differentiated thalli only with the appropriate algae.

A higher degree of selectivity was found in so far studied foliose and fruticose species. *Flavoparmelia nivalis* and *Letharia vulpina*, respectively, accept only subset of photobionts lineages in the *Trebouxia simplex* complex, whereas *Xanthoria parietina* associates with *Trebouxia decolorans* only. Possibly, the high photobiont selectivity in various Teloschistaceae could be genetically fixed. Both *Xanthoria* and *Fulgensia* were shown to have high selectivity, and there are also data from *Caloplaca* species which confirm this pattern (Muggia et al 2007).

These observations raise the fundamental question whether high selectivity for their symbionts constrains the ecology of lichen fungi. Temperate *Physconia* species have a rather narrow range of suitable photobionts, and all of the algal strains found by us cluster in the *Trebouxia impressa/flava/potteri* clade. The delimitation of species in this clade is hardly settled and ultrastructural observations (e.g. Friedl 1989) do not agree with separate monophyletic lineages.

Lichen mycobionts may take up suitable algae either from free-living *Trebouxia*. According to some studies (Bubrick et al. 1984; Mukhtar et al. 1994; Sanders 2005), free-living *Trebouxia* strains seem to be commonly available, although this possibility has been rejected previously by others (e.g. Ahmadjian 1988). Alternatively lichens might be able to

obtain photobionts from neighbouring lichen thalli. This selection for locally adapted algae could make it easier to adjust quickly to a newly found environment, somehow recalling the hypothesis of Habitat Adapted Symbiosis, proposed by Rodriguez et al. 2008. The results from *Lecanora rupicola* and *Tephromela atra* support this hypothesis for both lichens. The case in *P. distorta* and *P. grisea*, seems to be more complex as these species have a rather narrow general range of photobiont variation, but locally switches among photobiont strains seem to be common. It is possible that the joint dispersal of the symbionts in the asexually reproducing species ensures the survival of the propagules or the resulting lichen initial until a vital or appropriate photobiont cells can be found.

Some lichens seem to select one specific photobiont strain even if related strains are available. It was shown for *Hypogymnia physodes* and *Lecanora conizaeoides* that despite the fact that they share the same habitats they do not share the same photobionts (Hauck et al. 2007). Samples, which grow next to each other, associate with different but closely related strains of *Trebouxia*.

5. Role of propagation mode on selectivity

5.1. Results of the investigations in vertical transmission in lichens

Symbiotic associations in closely related lichens of the genus *Physconia*, which differ in their propagation mode, have been investigated to analyze the impact of vertical transmission in lichens on the symbiotic association patterns.

Thirty-nine populations were investigated for this study (27 of *P. distorta*, 12 of *P. grisea*). From 7-15 thalli of the respective species from each population I sequenced both mycobiont and photobiont ITS regions. A total of 1066 new sequences were generated (539 of the photobiont, 527 of the mycobiont ITS). Representative sequences have been submitted to GenBank under the numbers EU795052 – EU795082. The majority-rule consensus tree of the algal ITS dataset (Fig. 5.1) was calculated from 398000 trees. The likelihood parameters in the sample had the following average values (variance) for the ITS partition: rate matrix r(GT) = 0.067 (± 0), r(CT) = 0.417 (± 0.002), r(CG) = 0.042 (± 0), r(AT) = 0.162 (± 0), r(AG) = 0.204 (± 0.001), r(AC) =0.105 (± 0), gamma shape parameter α = 0.206 (± 0.001).

Figure 5.1. Phylogenetic tree of *Physconia* photobionts as assessed by Bayesian phylogenetic analyses. Posterior probabilities equal or more than 95% are indicated by thickened branches.

An analysis of the diversity of photobionts in both species was performed to assess whether the species have a similiar symbiont specificity. Photobionts of *Physconia* belong to a monophyletic group that includes the described species *Trebouxia impressa, T. flava and T. potteri* (Fig. 4.1). Within this complex, specific lineages of clade I1 are selected. The same genotypes were found in both lichen (mycobiont) species, without evidence for the preference of certain algal genotypes in each fungal species.

The diversity of photobiont genotypes was variable in the populations under study. Algal gene diversity was highest in populations of *P. grisea* from Friuli-Venezia-Giulia, Italy (0.868) and in populations of *P. distorta* from Calabria, Italy (0.944). Moreover, the photobiont genotypic variation within populations varies considerably. Two populations of the sterile *Physconia grisea* show no variation in fungal genotypes (contrasting a diversity of algal genotypes of 0.571 and 0.868, respectively). In comparison with photobiont gene diversity in populations of *Physconia distorta*, photobiont gene diversity is not significantly lower in *P. grisea* (Figure 5.1.).

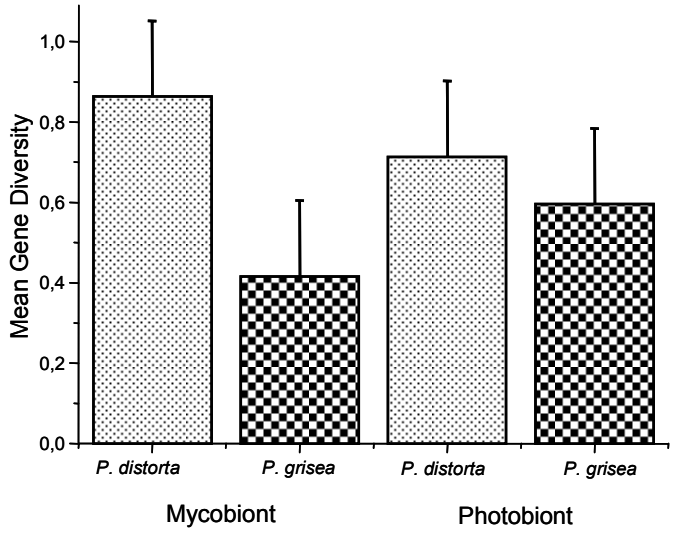

Figure 5.1. Comparison of mycobiont and photobiont diversity in populations of the two analysed species.

The situation is however clearly different, when the diversity of fungal genotypes in populations of these species is compared. Gene diversity in *P. grisea* is less than half of the estimate for the sexual *P. distorta*. If there would be a strict vertical transmission of photobionts, the drop of gene diversity in the sterile species should be similar in mycobiont and photobiont. Despite the lower fungal diversity, *P. grisea* associates with almost as many photobiont genotypes as the fertile *P. distorta*. Two populations of *P. grisea* did not vary with respect to mycobiont genotypes, but both populations comprised more then one photobiont genotype.

5.1. Discussion of vertical transmission in lichens

The clade to which the *Physconia* photobionts belong comprises photobionts of diverse lichens of different major groups belong (including photobionts of temperate species in other genera of Physciaceae such as *Heterodermia*, *Physcia* and *Rinodina*).The photobiont genotypes have extremely wide geographic ranges, from Germany to Finland and Spain. Other species of the I-clade (Helms G 2003) such as *Trebouxia anticipata* and *T. gelatinosa* were not found as photobionts of *Physconia*. No apparent preferences of either *P. grisea* or *P. distorta* are observed within this clade.

A shift in the frequencies of certain genotypes towards the warmer southern part of our gradient was not apparent. Temperate *Physconia* species share a rather narrow range of suitable photobionts, but similar ITS-genotypes across their geographic range. This agrees with an overall higher photobiont selectivity found in foliose and shrubby lichen species (e.g. Kroken & Taylor 2000, Opanowicz & Grube 2004, but see an exception in *Umbilicaria* species from maritime Antarctica, Romeike et al. 2002). Nonetheless considerable diversity of photobiont genotypes occurs in populations of both studied lichens. Individual populations may differ

significantly in their photobiont gene diversity, but a clear correlation with ecological conditions has not been observed.

A significant difference in the gene diversities of the mycobionts of sterile and fertile species was observed (Fig. 4.2). A similar decrease of the genetic diversity is observed in the species pair of *Cavernularia* (Printzen, pers. comm.). My working hypothesis was that this decrease in diversity should be observed as well in the algal partner if vertical transmission is perfectly retaining the original fungal algal associations. If this hypothesis would be valid a significant decrease of photobiont diversity in populations of the sterile lichen would have been observed, similar to the decrease in mycobiont gene diversity. This is clearly not the case and the propagation strategy had no significant effect on the photobiont diversity. It might be argued that rare sexuality in the sterile species could be responsible for the maintenance of photobiont diversity, but it is more likely that switches of the photobiont seem to be frequent in all investigated lichen species. Either the photobionts in the mitotically produced soredia are not necessarily the same as present in the soredia generating thalli, or soredia may acquire different algal strains after dispersal and landing. Microscopic investigation rejects the former hypothesis. There is no evidence so far that free-living algae attach to the developing soredia, which are tightly encaged by branching, hydrophobic hyphae. Furthermore, no observations of epithalline algae on other parts

of the investigated lichen thalli have been made so. On the other hand rather dense occurrence of free-living algae among soredia structures and in initial thallus structures on the bark surface can be found in the vicinity of lichen thalli by staining with Calcofluor white and observation with an epifluorescence stereo microscope (unpublished data). It is likely that these algal consortia also include appropriate strains for re-lichenization. Growth towards algae of mycobionts transplanted to natural habitat has been observed in more detail by Etges & Ott (2001).

It is possible that the algae in the dispersed soredia are not necessarily the same as in the developing lichens, which results from soredial propagation. In contrast to those in soralia, soredia on bark have a looser structure. In the latter hyphae can branch out to contact either the bark substrate or neighbouring algae. It may well be that algae in sheltered microsites of the bark are more vital than the few photobiont cells which were poorly protected while they joined the mycobiont during dispersal. I think that it is most likely that new photobiont-mycobiont combinations occur at this stage of development. I suggest that the main role of the photobiont in soredia is to prolong the survival of the co-propagated fungal hyphae. Depending on the viability of the soredial algae, the soredial fungus can choose between establishing a thallus with the rather few co-propagated alga or with adjacent, and possibly more vital free-living algae. This hypothesis does not exclude the possibility of

vertical transmission of lichen associations by soredia. However, the initial phase of thallus formation is most sensitive to local conditions, and may sometimes vary at the scale of micrometers. It remains to be addressed by future population genetic studies, whether photobiont switching after attachment may also be observed in lichens with more complex and stratified asexual propagules such as isidia or phyllidia, which protect the co-propagated algae more efficiently against various environmental stress factors, during the propagation phase and initial establishment at the landing site. Another point that might be questioned is, if more then one photobiont can be present within the lichen thallus. This has been noticed for example in *Protoparmeliopsis muralis* (Guzow-Krzeminska, personal communication) and other lichens (Schaper 2003) but in these cases this fact was clearly indicated by a double band or ambiguous sequences. In *Physconia* I never found neither double bands nor ambiguous sequences throughout the entire samples. This may have to do with the sampling methods. I routinely used parts of the growing thallus margins, which appear rather homogeneous and likely contain the same photobionts.

However, in many lichens thallus rejuvenation can be observed in old and large thalli. This is usually apparent by small lobes growing out of the thallus centers. If these newly generated lobes are initiated by the re-capture of alga from the environment, this may also provide a possibility for algal switching and heterogeneity within the same fungal individual.

This mode of intrathalline algal switching is perfectly displayed by lichens which can associate with highly different algae to produce photomorphs. For example *Lobaria amplissima* can associate with green algae but also with cyanobacteria. The coralloid cyanobacterial photomorph differs substantially and often arises from the central part of large foliose green algal morphs. Initial observations in old thalli of *Physconia* species seem to confirm this possibility also for intrathallin variation with respect to closely related photobionts, because algae in different rejuvenation lobes had occasionally different green shades and subtle differences in their arrangements. These variation need to be studied in greater detail in the future, but the idea is raised that old lichen thalli could represent a kind of symbiotic arena, where new combinations of algal associations might be tested without harming the already existing and supporting vegetative lichen organism

It has earlier been noticed (Ott 1987), that lichen mycobionts may associate in nature loosely with other photobionts, until they find the appropriate alga to form a typical thallus. Whether such locally optimal algae are part of a pre-existing lichen symbiosis or represent free-living algae is still a matter of debate in lichens as no structures are developed for propagation of algal partners alone. Evidence for the existence of free-living *Trebouxia* increases (Schroeter and Sancho 1996, Sanders 2005, Capitelli et al. 2007), but they apparently do not form large colonies and

are likely ephemeric. One source of free-living algae could be lichens which experience a shift to increased humidity during their life-time, e.g. by a closing vegetation in forests. Under such situations, algal colonies can grow out from the lichen thalli (Grube, unpublished observations). However, the dispersal of such algal colonies has yet to be studied in detail. Another source for algae are faecal pellets of orobatid mites, which frequently graze on lichens. Their droppings were shown to contain viable *Trebouxia* cells (Meier et al. 2002).

The mechanisms behind photobiont specificity are still poorly understood. They can involve phycobiont-binding proteins (Bubrick and Galun 1980, Bubrick et al. 1985, Sacristan et al. 2006) or compounds excreted by the algal cells (J. Meeßen, pers. comm.). It has been shown by re-association experiments that mycobionts can only form well-differentiated thalli with specific algae, whereas other associations resulted in undifferentiated, sorediate thalli (Ahmadjian et al. 1980, Ahmadjian & Jacobs 1981). However, formation of proper thalli can simply be delayed, unless the algae are not too distant from the optimal lineages. Optional vertical transmission likely increases the reproductive success of sterile lichens under a wider range of microclimatic conditions and prevents strict patterns of co-evolution. This aspect may be of significance also in other lichen lineages, such as *Lepraria* (Nelson & Gargas 2008), where

mycobiont species have diverged chemically and ecologically even in the absence of sexuality and with uniform co-dispersal by soredia.

6. Geographic patterns

6.1. Results of the investigations of the geographic patterns

The investigated species of *Physconia* select photobionts from a bulk of closely related lineages that can all be assigned to *Trebouxia impressa/potteri/flava* clade, which is still poorly studied by ultrastructural methods. Other species of the I-clade (Helms 2003) such as *Trebouxia anticipata* and *T. gelatinosa* were not found. Looking at the different gene diversities within the populations (Table 6.1) a considerably variation in diversities from population to population can be found but no clear north south gradient can be observed. However there is correlation between the gene diversity and the altitude (Figure 6.1.).

Sampl. Plot	*Physconia distorta*		*Physconia grisea*	
	Fungus	Algae	Fungus	Algae
A	0,89	0,76	0,60	0,26
B	1,00	0,89	x	x
C	0,93	0,76	0,65	0,90
D	0,83	0,53	0,46	0,63
E	0,62	0,76	0,00	0,57
F	0,65	0,76	0,00	0,87
G	1,00	1,00	x	x
AT	0,77	0,86	x	x
PL	x	x	0,64	0,78
NW	0,44	0,74	x	x
SW	0,86	0,89	x	x

Table 6.1. Mean gene diversities along a gradient from Southern Italy A to Northern Italy G and the Gene Diversities in Austria (AT), Poland (PL), Norway (NW) and Sweden (SW).

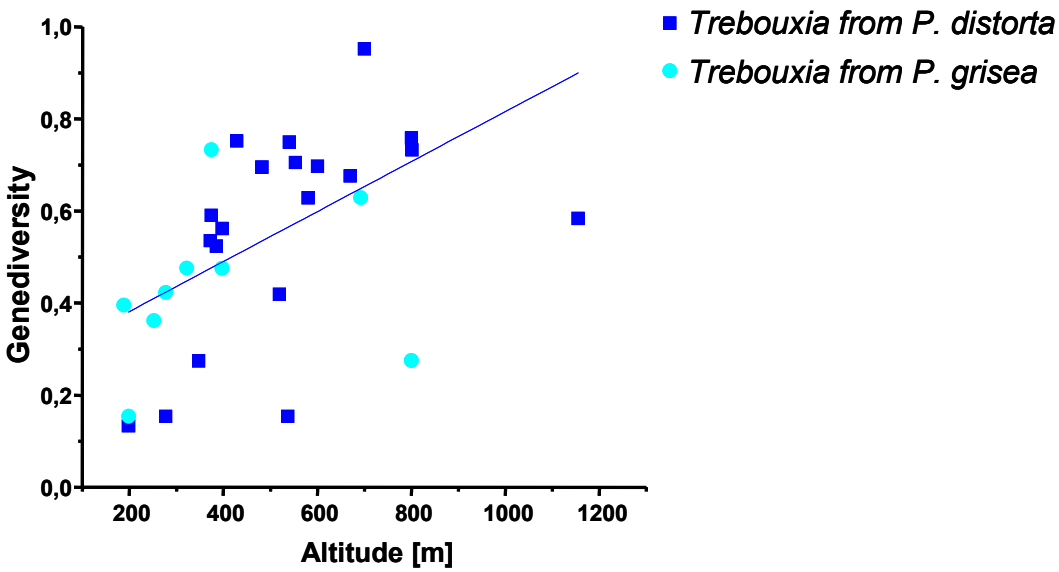

Figure 6.1. Altitude and gene diversity in the Photobionts *of P. grisea* and *P. distorta.*

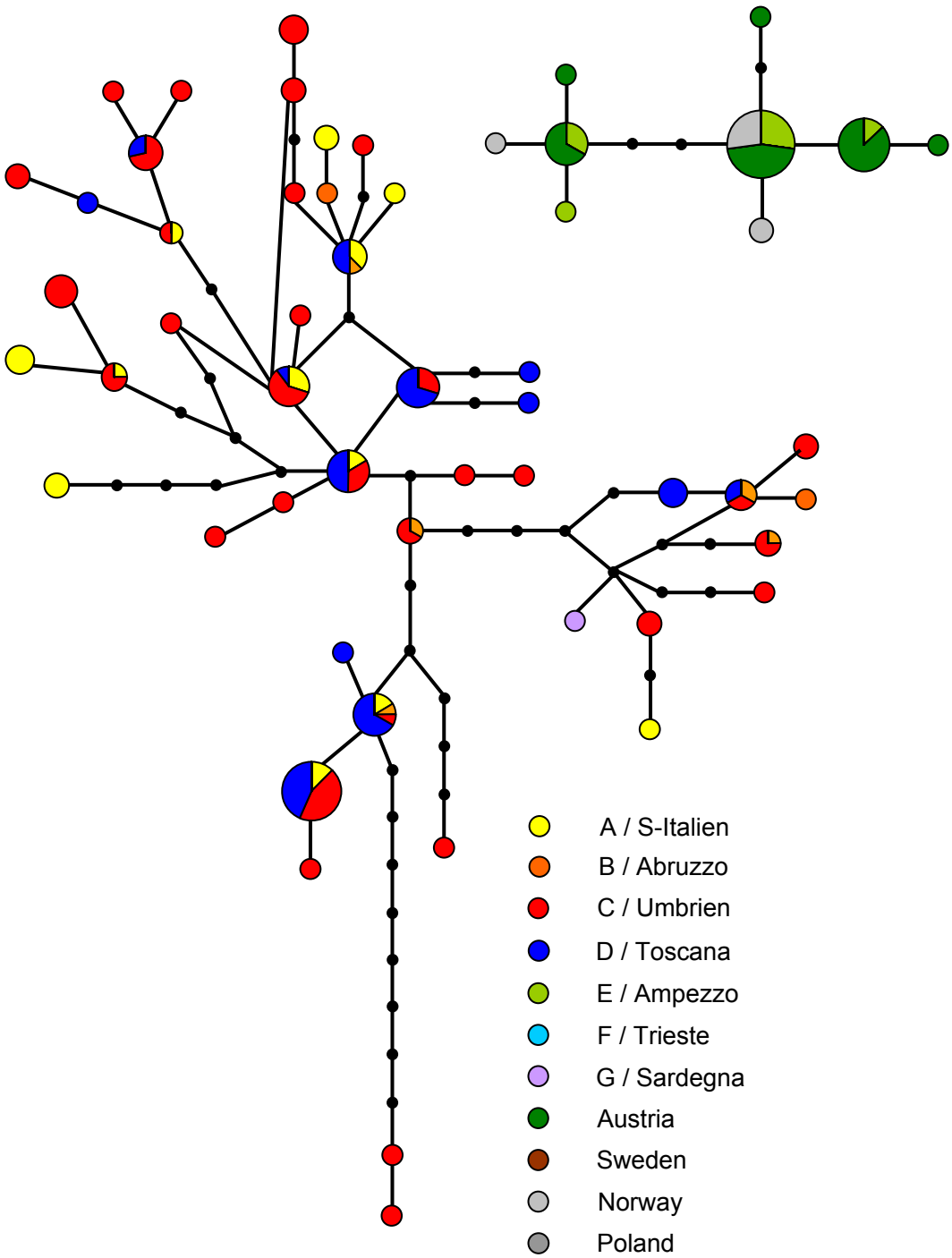

Figure 6.1. Two of the haplotype networks of the mycobiont of *P. distorta.* Size of the nodes refer to the number of sequences and the colours, according to the legend, to the sampling places.

By analyzing the mycobionts with a haplotype network approach we find that the network of *P. distorta* disintegrates into separate smaller networks showing high diversity within this species. We get a “northern” network (Fig. 6.1) where only samples from Austria, Norway and Ampezzo (close to Austria) can be found. Additionally we get a second network that consist of the more “southern” sampled lichens from Southern Italy to Tuscany (Fig. 6.1) and one more intermediate network with samples from all over Italy, one sample from Austria and the Swedish samples (Fig. 6.2).

In contrast to this *P. grisea* is forms only one network and the Polish samples can be found within one part of the haplotype network.

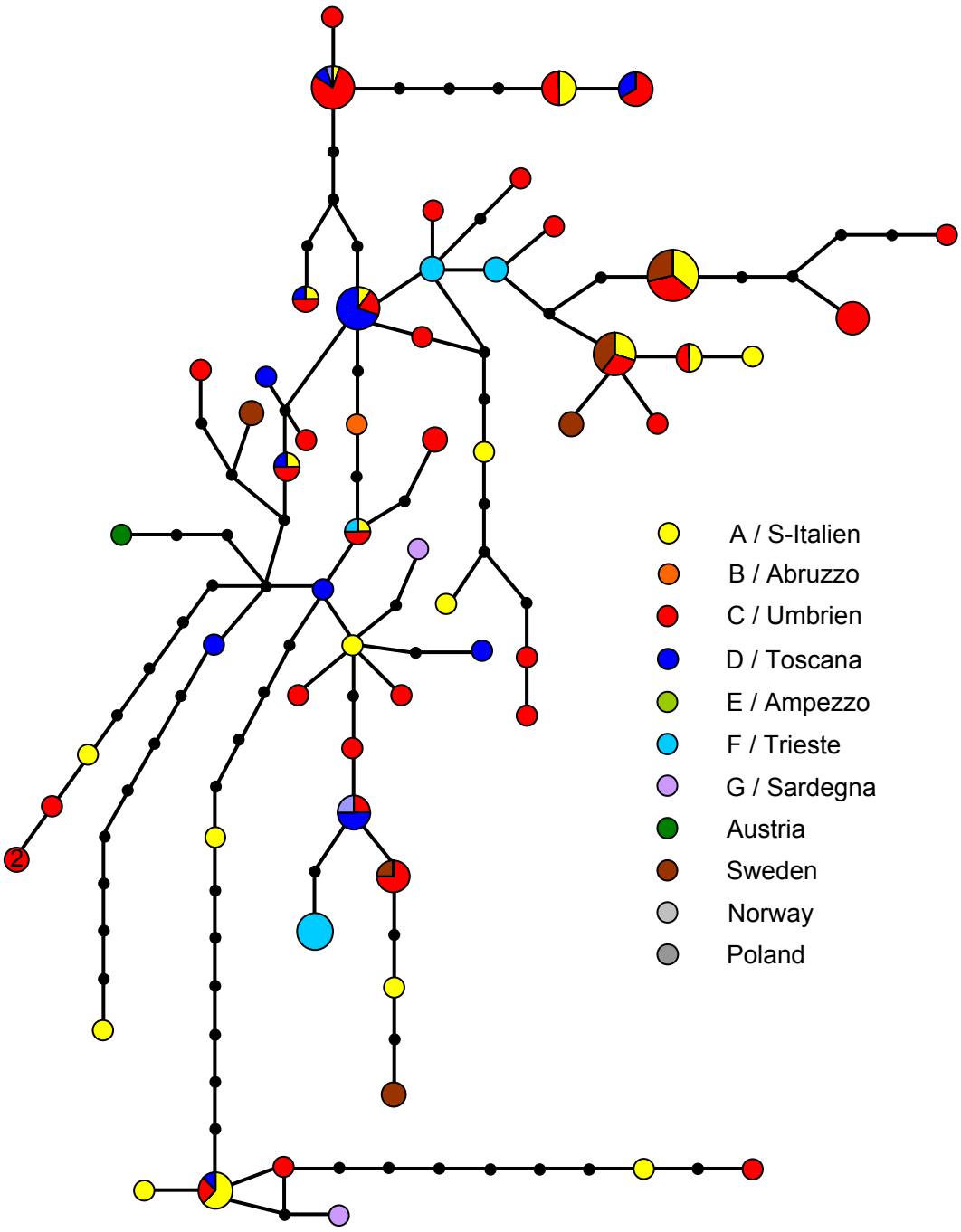

Figure 6.2. Haplotype network of the mycobiont of *P. distorta.* Size of the nodes refer to the number of sequences and the colours, according to the legend, to the sampling places.

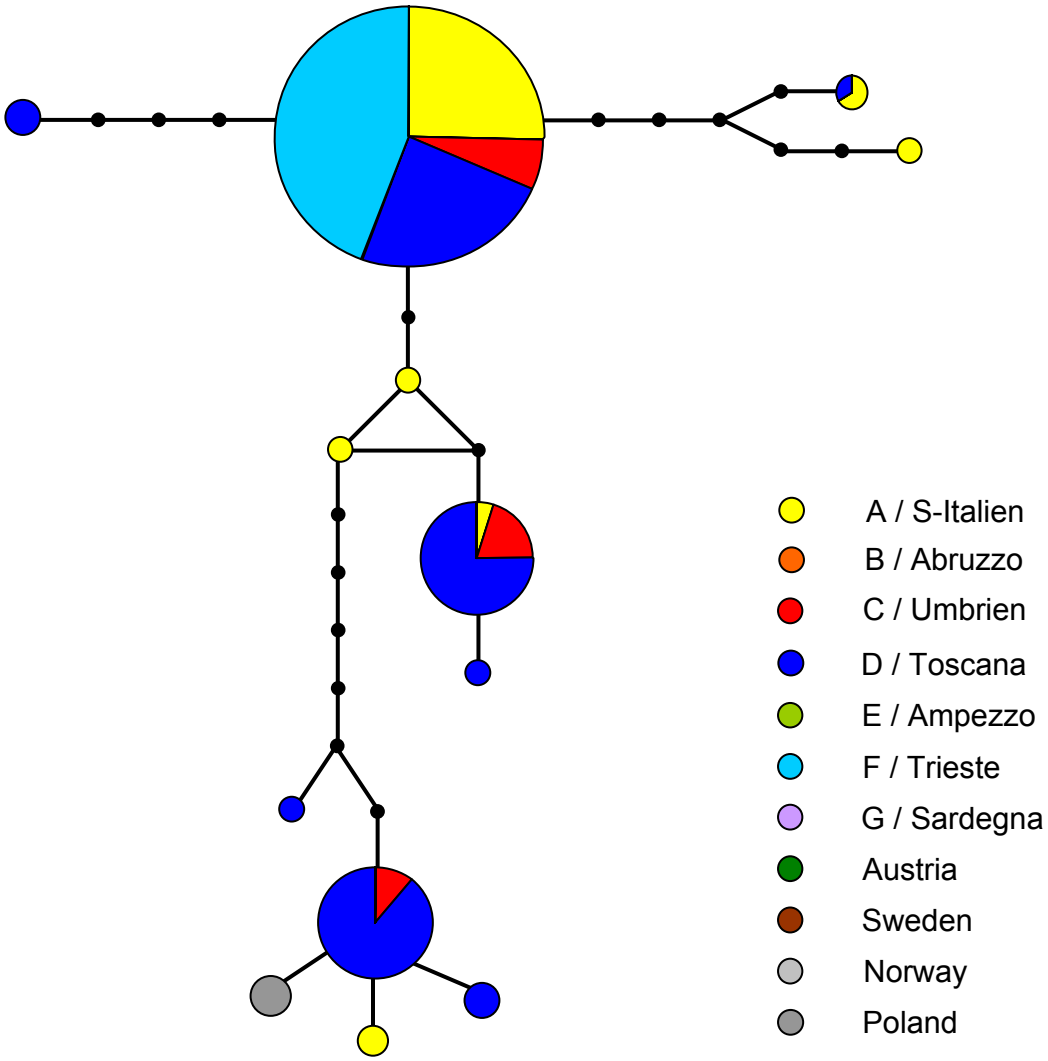

Figure 6.2. Haplotype network of the mycobiont of *P. grisea.* Size of the nodes refer to the number of sequences and the colours, according to the legend, to the sampling places.

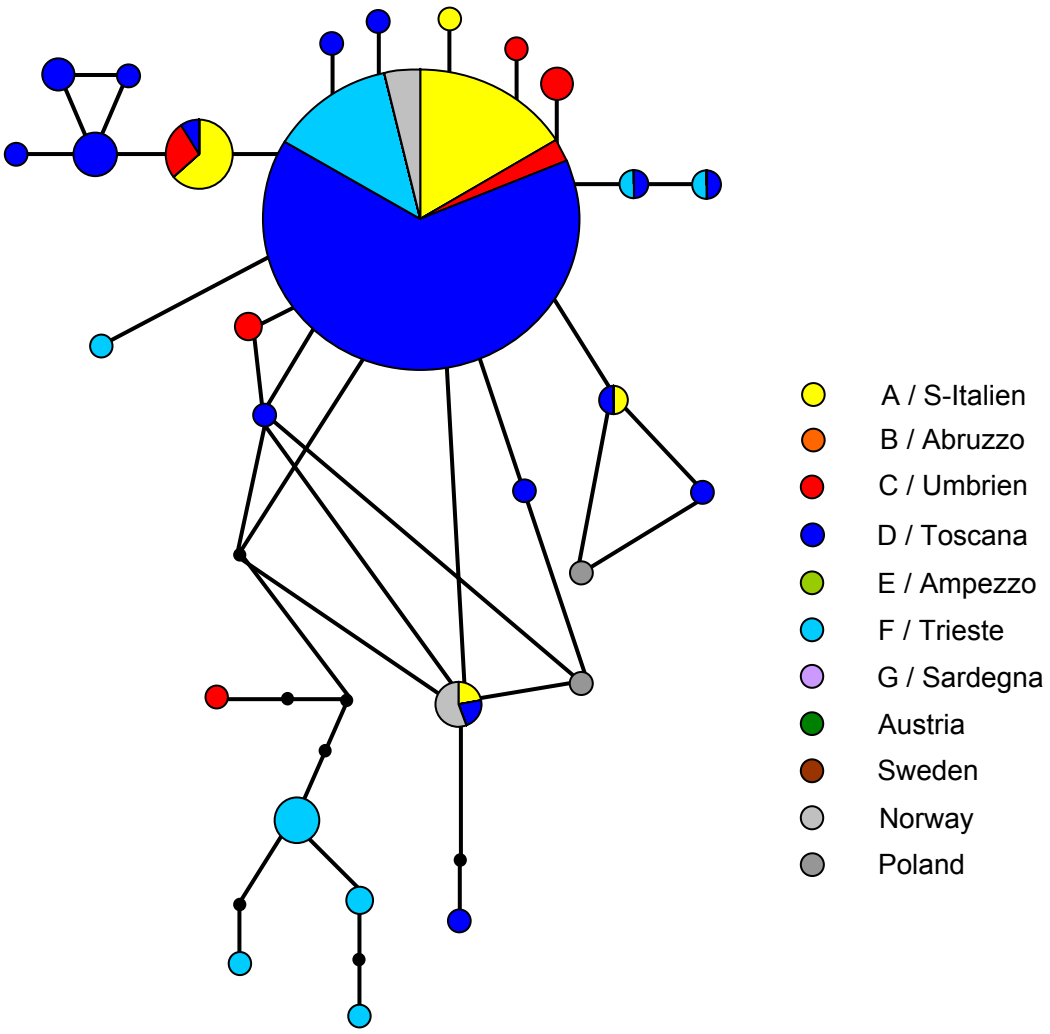

Figure 6.3. Haplotype network of the photobiont of *P. grisea.* Sizes of the nodes refer to the number of sequences and the colours, according to the legend, to the sampling places.

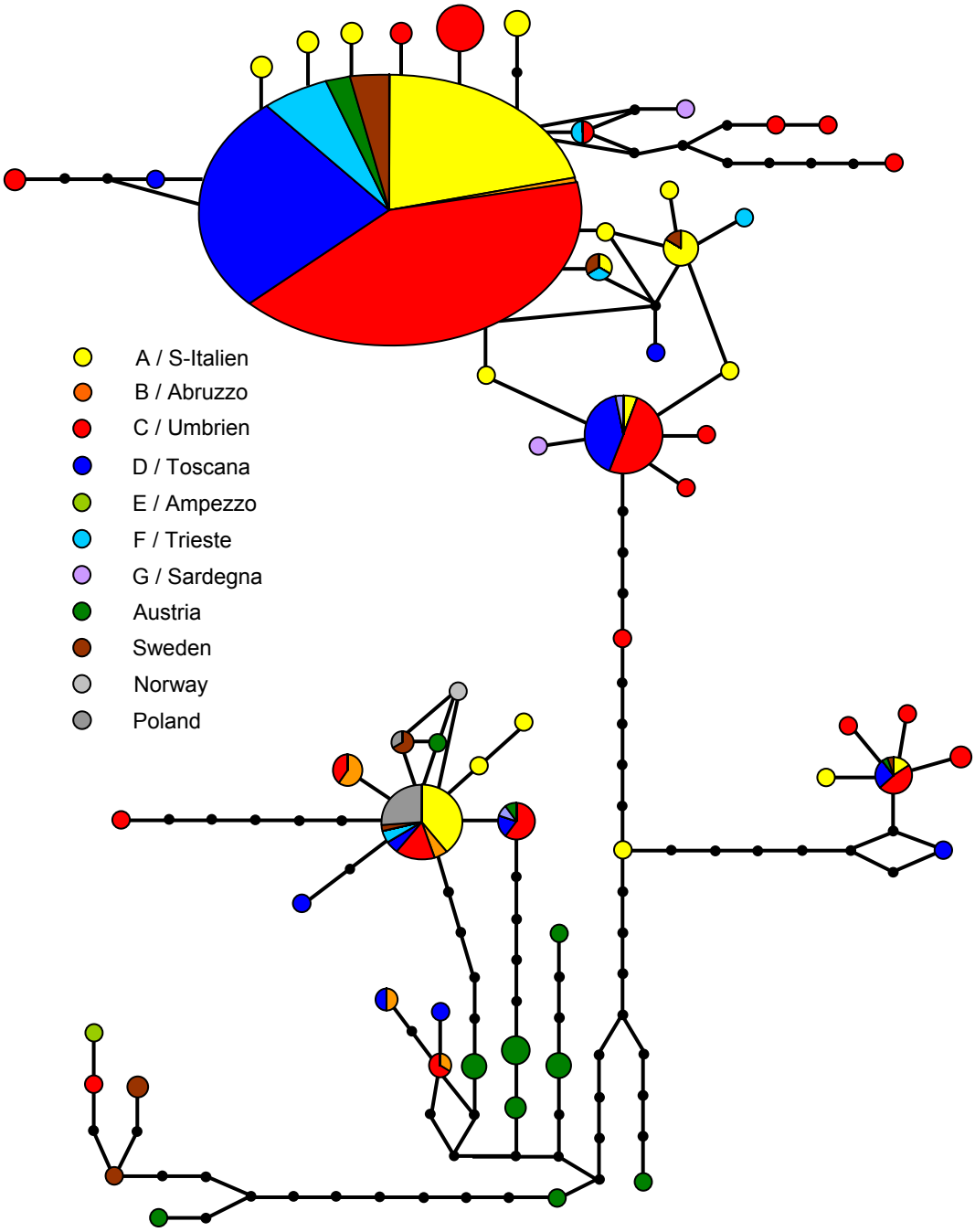

Figure 6.4. Haplotype network of the photobiont of *P. distorta.* Size of the nodes refer to the number of sequences and the colours, according to the legend, to the sampling places.

Rank abundance curves or “Whittaker plot” curves are used by ecologists to display relative species abundance in a give area. The most abundant component is given rank 1, the next abundant the rank 2, etc. The shape of such curves tells us about the evenness of abundance. Here, I use curves to display the abundance of symbiont haplotypes.

These curves (Figure 6.5.) show that *P. distorta* mycobiont haplotype abundance is rather even largely without predominant haplotypes. Only the network of “mycobiont I” has a predominat haplotype. This is clearly different from the algal partners in both species. Here, there is a distinct abundance of few haplotypes. Interestingly, the mycobiont of *P. grisea* have similar rank abundance.

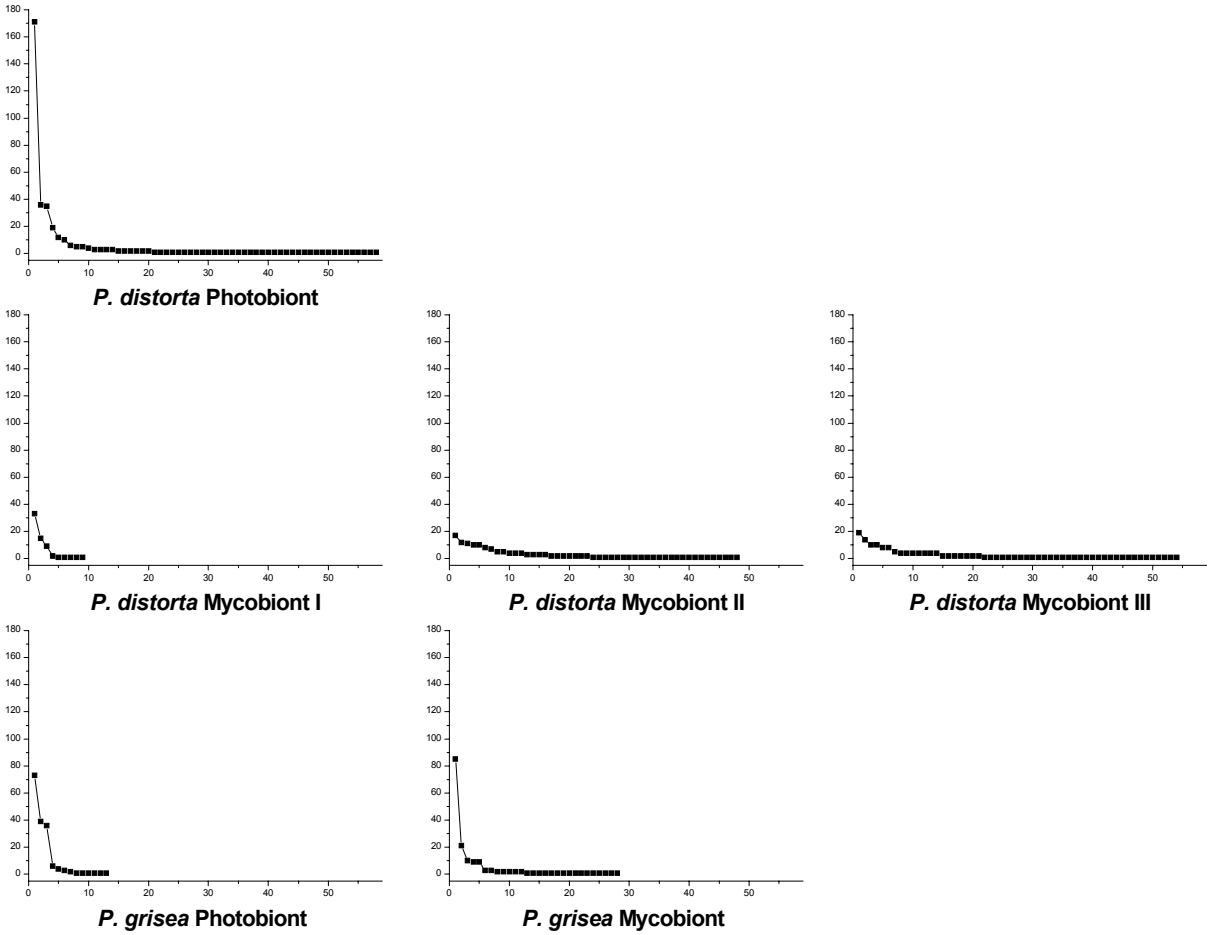

Figure 6.5. Rank abundance curves of *Physconia distorta* and *Physconia grisea*

6.2. Discussion of the geographic patterns

In *P. distorta* and *P. grisea* the photobionts are selected from a distinct clade of closely related photobionts, but within that clade no apparent preferences are seen, and most of the lineages within this clade are also found in other lichens. Both closely related species with different reproducing strategies share the same photobionts. The same photobionts can also be found in *Physconia* species on rocks. The hypothesis that the sorediate species show a more pronounced “coupling” of genotypes than the apotheciate species can be rejected for *Physconia*. The results of the geographic distribution of the ITS genotypes confirm the hypothesis that switches of the photobiont seem to be frequent in the investigated lichen species.

The high genetic diversity of *P. distorta* can not be captured by a single haplotype network (using the 95% parsimony rule in TCS). In the investigated geographic range some differentiation is indicated. I could distinguish a “boreal-alpine” network (Fig. 6.1) in which only samples from Austria, Norway and N-Italy can be found. Additionally I got a “Southern Italian” network including the samples exclusively from Southern Italy and one more “intermediate” network with samples from all over Italy and one sample from Austria and the Swedish samples. In contrast, the single

haplotype network of *P. grisea* (Fig. 6.3) shows that the gene diversity is very low and also the differences between the haplotypes are very small. The Polish samples cluster within one part of the haplotype network. The networks confirm the clear differences of the genetic diversity between the sexually and the asexually reproducing species mycobionts. The photobiont ITS networks of the two lichen species show no evident geographic pattern (Fig. 6.4 & 6.5). In a recent study Sanders (2005) showed that soredia, which are colonizing artificial supports in natural environments, form short extensions of fungal hyphae, but do not develop further. It is plausible that the coccal unicellular growth seems perfectly suited to maximize the spread of the species. Perhaps the reasons for previous debates whether or not lichen-forming *Trebouxia* species are occurring in free-living state are because they do not form conspicuous colonies but occur as maximally spread single cells or few-cell aggregates. In these stages sexuality might be expressed which could likely contribute to the detected diversity of photobiont lineages. The hypothesis that the fertile mycobiont takes algae from local pools which differentiate locally or regionally, whereas the algae of the sterile mycobiont could be transported over larger distances can not be supported by the data. It rather seems, at least according to the ITS sequence data, that all photobiont strains have extremely wide geographic ranges and are widespread in appropriate

habitats. This does not exclude the possibility that physiological adaptation is possible but not detected by lack of ITS variation.

While high diversity of photobionts was observed in *Lecanora rupicola* from the Mediterranean area (Blaha et al. 2006), gene diversities are not detectably higher in samples of *Physconia* from Italy (Table 6.1.). According to our results we can state that the genetic diversity varies considerably from population to population but does not change significantly along the North-South gradient. Interestingly a tendency of higher gene diversity can be found when the altitude is correlated with the diversity of the photobionts (Fig. 6.6) with the highest gene diversity around 800 m. This altitude is more or less the limit of the suitable phorophyte trees, primarily deciduous trees (often single) with rough bark. This pattern can be observed in both lichens.

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8. Appendix

8.1. Sampling Sites

Nr.	Location
I 2	Italy, Umbria, Gavelli, N 42°41´13´´ E 12°54´02´´,alt. 1155m, Acer sp., leg. Sabine Wornik, 21.03.05
I 3	Italy, Umbria, Sant´Anatolia di Narco, N 42°43´59´´ E 12° 50´07´´, alt. 347m Quercus pubescens, leg. Sabine Wornik, 21.03.03
I 4	Italy, Umbria, Monte Gallene, Piede Paterna, N 42°45´22´´ E 12° 50´28´´, alt. 537m Quercus pubescens, leg. Sabine Wornik, 21.03.03
I 5	Italy, Umbria, Meggiano, N 42°48´04´´ E 12°51´37´´, alt. 801m, Quercus pubescens, leg. Sabine Wornik, 21.03.05
I 6	Italy, Umbria, on the way from Maggione to Serreto di Spoleto, N 42°50´09´´ E 12°54´32´´, alt. 580m, Quercus pubescens, leg. Sabine Wornik, 21.03.05
I 7	Italy, Umbria, Monte Peglia Parking area near the Public access to the riserva naturale, N 42°48´00´´ E 12°12´21´´, alt. 800 m, Quercus pubescens, leg. Sabine Wornik, 22.03.05
I 9	Italy, Umbria, Monte Peglia, N 42°46´40´´ E 12°12´13´´, alt. 670 m, Quercus pubescens, leg. Sabine Wornik, 22.03.05
I 11	Italy, Umbria, street from Colonetta di Prodo to Orvieto, N 42°44´56 E 12°10´48, alt. 482 m,Quercus pubescens, leg. Sabine Wornik, 22.03.05
I 12	Italy, Umbria, Road to Orvieto close to Orvieto Scalo, N 42° 44´21´´ E 12°08´49´´, alt. 214m, Quercus pubescens, leg. Sabine Wornik, 22.03.05
I 13	Italy, Umbria,Lago Trasimeno, Boschi di Panicarola, N 43°03´51´´ E 12°07´15, alt. 374m, Quercus pubescens, leg. Sabine Wornik, 22.03.05
I 14	Italy, Umbria, on the road from Magione to Castel Rigone, Col Piccione, N 43°10´54´´ E 12°13´24´´, alt.428m, Quercus pubescens, leg. Sabine Wornik, 22.03.2005
I 16	Italy, Toscana, in the east of Poggibonsi,N 43°28´18´´ E 11°09´45´´, alt.198m, Quercus pubescens, leg. Sabine Wornik, 23.03.05
I 17	Italy, Toscana, San Gimignano, N 43°27´39´´ E 11°02´24´´, alt. 277, Quercus pubescens, leg. Sabine Wornik, 23.03.05
I 18	Italy, Toscana, Castellina in Chianti, N 43°28´51´´ E 11°18´26´´. alt. 600m, Quercus pubescens, leg. Sabine Wornik, 23.03.05
I 19	Italy, Toscana, near Radda in Chianti, Villa La Barone, N 43° 29´13´´ E 11°22´34´´, alt. 519m, Quercus pubescens, leg. Sabine Wornik, 23.03.05
I 20	Italy, Toscana, Lucciana, N 44°01´36´´ E11°06´17´´, alt. 385m, Acer sp., leg. Sabine Wornik, 24.03.05
I 21	Italy, Toscana, Monachino, in front of the osteria, N 44°01´32´´ E 11°02´07´´, alt. 692m, Salix sp., leg. Sabine Wornik, 24.03.05

I 24	Italy, Toscana, Monte Catini Alto, N 43°53´38´´ E 10°47´20´´, alt 252m, Salix sp., leg. Sabine Wornik, 24.03.05
I 25	Italy, Emilia Romagna, Grizzana, N 44°15´27´´ E 11°09´31´´, alt. 540m, Quercus pubescens, leg. Sabine Wornik, 25.03.05
I 26	Italy, Emilia Romagna, Calvenzano, Chiesa di San Apollinare, N 44° 18´08´´ E 11° 08´41´´, alt. 188m, Tilia sp., leg. Sabine Wornik, 25.03.05
I 30	Italy; Friul-Venezia-Giulia, Opicina way to Monrupino, N 45°41´34´´ E 13°47´42´´, alt. 322m, Tilia sp., alt. 322, leg. Sabine Wornik, 29.03.05
I 31	Italy, Friul-Venezia-Giulia, close to Basovizza, on the road from Opicina to Basovizza, N 45°38´44´´ E 13°51´36´´, alt. 372m, Tilia sp., leg. Sabine Wornik, 25.03.05
I 32	Italy, Friul-Venezia-Giulia, close to Basovizza, on the road from Opicina to Basovizza, N 45°37´35´´ E 13°52´15´´, alt. 383m, Quercus pubescens, leg. Sabine Wornik, 25.03.05
I 33	Italy, Friul-Venezia-Giulia, San Lorenzo, Monte Stena, N 45°37´33´´ E13°52´26´´, alt. 397m, Quercus pubescens, leg. Sabine Wornik, 25.03.05
I 34	Italy, Friul-Venezia-Giulia, Ampezzo, Tilia lined street, N 46°24´50´´ E 12°48´14´´, alt. 553m, Tilia sp., leg. Sabine Wornik, 19.06.05
I 35	Italy, Calabria, Cosenza, Rende Università di Cosenza, Orto Botanico, alt. , on Acer sp., leg. D. Puntillo S.Wornik, 05.10.05
I 36	Italy, Calabria, Cosenza, Montalto UFFU 40, Bosco di Mavigliano, N 39°23´11,5 E 16°12´53,8´´, alt. 227m, Quercus pubescens, leg. D. Puntillo & S. Wornik, 05.10.2005
I 37	Italy, Calabria, Cosenza, Montalto UFFU 40, Bosco di Mavigliano, N 39°23´40´´ E 16°13´20´´, alt. 191m, Quercus pubescens, leg. D. Puntillo & S. Wornik, 05.10.2005
I 38	Italy, Basilikata, on the way from Case Pascalicchio to Moliterno, close to Moliterno, N 40°13´04´´ E 15°53´25´´,alt. 815m, on Quercus cerris, leg. S. Wornik, 07.10.05
I 40	Italy, SS 103 from Moliterno to Viggiano, N 40°13´04´´ E 15°55´18´´, alt. 846m, Quercus cerris, leg. S.Wornik, 07.10.05
I 41	Italy, Basilikata, SS 103 from Moliterno to Viggiano, N 40°19´50,7´´ E 15°55´17,9´´, alt. 791m, Quercus pubescens, leg. S. Wornik,07.10.05
I 42	Italy, Basilikata, W of Castel Lagopesole, N 40°48´28,4´´ E 15°43´56,6´´, alt.833m, Quercus, leg. S. Wornik, 09.10.05
I 43	Italy, Basilikata, Melfi, park outside the citygate, N 40°59´35´´ E 15°39´26´´, alt. 533m, Tilia sp., leg. S.Wornik, 09.10.05
I 44	Italy, Aspromonte Massiv, from Piano Zillastro to Plati, N 38°13´36´´ E 16°01´18,9´´, alt. 888m, Quercus pubescens, leg. S. Wornik, 11.10.05
L 1	Italy, Toscana, Firenze, Marranti, on the SS 302, alt. 350m, Tilia sp., leg. Lucia Muggia, 21.03.05, Nr. 36711 Herbarium P.L. Nimis TSB Trieste
L 2	Italy, Toscana, Firenze, Ronta on the SS 302,between Marranti and Borgo San Lorenzo, Costa delle Alpe, Fonte delle Alpe, alt. 800m, Cupressus sp., leg. Lucia Muggia, 21.03.05, Nr. 36699 Herbarium P.L. Nimis TSB Trieste
L 3	Italy, Abruzzo, Pescara, Maiella, on the SS 487,between Caramanico and Santa Eufemia, alt. 600, Quercus pubescens, leg. Lucia Muggia, 19.06.05

N	Norway, Troms, Lenvik, Senja E, Skognesbotnelva, in a Swamp Forest along the river, Map 1433IV, UTM WSG 84 125-127 E 925-926 N, alt. 18-25m, on Alnus incana, leg.det. Jarle W: Bjerke 007/05, 26.05.05
P1	Poland, Pojezierze, Ilawskie, E of Szramowo village, NW of Prabuty Town, between Dziergoń and Liwieniec lakes, N 53°55´00´´ E 19°10´40´´, by the road open area, on Acer pseudoplatanus, 07.08.05, leg M. Kukwa 4563, UGDA
P2	Poland, Pojezierze, Ilawskie, Straszewo Wiszary village, W part of the village, trees along roadside, on Acer platanoides, 11.09.2005, leg Martin Kukwa 4600 UGDA
SW	Sweden, Uppland, Norrtâlje, Rô opposite road-cross towards Beateberg, Acer platanoides, leg. Åsa Dahlkild, 05, Tree1