Charles University

Faculty of Science

Study programme: Botany

Mgr. Lucie Vančurová

Photobiont dynamics of *Stereocaulon* lichens

Dynamika fotobiontů ve stélkách lišejníků rodu *Stereocaulon*

Ph.D. Thesis

Supervisor: Doc. Mgr. Pavel Škaloud, Ph.D.

Prague, 2022

Author's declaration

I hereby declare that I have written thesis independently using listed references. I have submitted neither this thesis nor its parts to acquire any other academic degree.

Prohlášení autorky

Čestně prohlašuji, že jsem nepředložila práci ani její části k získání jiného nebo stejného akademického titulu a že jsem práci zpracovala samostatně za použití citované literatury.

In Prague Lucie Vančurová

Acknowledgments

Pavel Škaloud, thank you very much for supervising me during the entire time of my studies. Thanks for your enthusiasm, advice, support, and understanding.

Ondřej Peksa, thank you very much for the idea of studying *Stereocaulon* and for providing the lichenological point of view on our research.

Lucia Muggia, thank you for your hospitality during my stays in Graz and for introducing me to new methods in the lab.

Jiří Malíček, thank you for your cooperation and nice company during the sampling tours.

Veronika Kalníková, thank you for your courage to merge our researches despite knowing me for a long time.

Lenka Flašková, thank you for your advice and assistance in the molecular lab.

Co-authors of the papers, thank you for your great ideas and fantastic cooperation.

My dear family, thank you for your support and patience.

Members of phycological and lichenological groups, thank you for inspirative discussions and sharing your knowledge.

Special thanks go to numerous colleagues, friends, and my parents for collecting lichen samples used in this work.

Funding of this work was provided by Charles University Science Foundation projects GAUK (grant nos. 570313 and 946417) and Primus Research Programme of Charles University (grant no. SCI/13).

Papers included in the thesis

This thesis is based on the following papers:

I. **Vančurová, L.**, Peksa, O., Němcová, Y. & Škaloud, P. (2015) *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain. *Phytotaxa* 219: 118–132. https://doi.org/10.11646/phytotaxa.219.2.2

II. **Vančurová, L.**, Muggia, L., Peksa, O., Řídká, T. & Škaloud, P. (2018) The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota). *Molecular Ecology* 27: 3016–3033. https://doi.org/10.1111/mec.14764

III. **Vančurová, L.**, Kalníková, V., Peksa, O., Škvorová, Z., Malíček, J., Moya, P., Chytrý, K., Černajová, I. & Škaloud, P. (2020) Symbiosis between river and dry lands: Phycobiont dynamics on river gravel bars. *Algal Research* 51: 102062. https://doi.org/10.1016/j.algal.2020.102062

IV. **Vančurová, L.**, Malíček, J., Steinová, J. & Škaloud, P. (2021) Choosing the right life partner: ecological drivers of lichen symbiosis. *Frontiers in Microbiology* 12: 769304. https://doi.org/10.3389/fmicb.2021.769304

Authors' contributions:

I. L.V. designed the study and conducted the sampling, L.V. and O.P. cultivated algae, P.Š. made the microscopical examination, Y.N. conducted TEM, L.V. performed laboratory work and phylogenetic analyses, L.V. and P.Š. wrote the manuscript and produced the figures with contribution from Y.N. and O.P.

II. L.V., P.Š., and O.P. designed the study. L.V., O.P., L.M., and P.Š. conducted fieldwork and collected specimens. L.V. and T.Ř. performed laboratory work with contributions from P.Š., L.M., and O.P. P.Š. and L.V. analyzed the data. L.V., O.P., L.M., and P.Š. wrote the manuscript.

III. L.V., V.K., O.P., and P.Š. designed the study; L.V., V.K., and J.M. conducted fieldwork and collected specimens; L.V., Z.Š., and P.M. performed laboratory work; L.V., V.K., P.M., K. Ch., and P.Š. analyzed the data; all authors participated in writing the manuscript.

IV. L.V., J.M., and P.Š. designed the study. L.V. and J.M. conducted the sampling and the laboratory work. J.M. and J.S. determined the samples. L.V., J.S., and P.Š. conducted the phylogenetic and the statistical analyses. L.V. and J.M. wrote the manuscript and produced the figures with contributions from J.S. and P.Š.

On behalf of all the co-authors, I declare the keynote participation of Lucie Vančurová in completing the research and writing the papers, as described above.

In Prague Pavel Škaloud

Abstract

Lichens are an iconic example of symbiosis. They are widespread throughout the world. In some ecosystems, lichens are dominant autotrophs, sometimes one of the few living organisms capable of surviving local conditions. They grow on a variety of substrata, including artificial surfaces. Great diversity of their life strategies is related to the diversity of symbiotic partners forming the lichen thalli, which remains largely unresearched. Lichens as sessile organisms often have to face a changing environment or adapt to conditions at new localities where their propagules can spread. We have chosen the widespread genus *Stereocaulon* as a model system for studying symbiotic relationships in lichens and the connection of this relationships with environmental conditions.

The main goals of this thesis were (1) to uncover the diversity of phycobionts (i.e., green algal photobionts) associated with *Stereocaulon* within the whole genus and particular species-level lineages; (2) to identify environmental factors affecting the distribution of phycobionts and their relationships with mycobionts on a global and local scale; and (3) to inspect the possibility of sharing phycobiont pool with other lichens and soil. For this purpose, we used phylogenetic analyses (ITS rDNA, 18S rDNA, rbcL, and actin type I gene), Illumina metabarcoding (ITS2 rDNA), light, confocal and transmission electron microscopy, phytosociological sampling, modeling of climatic niches (Hutchinsonian niche concept) and various statistical methods.

The phycobionts recovered from *Stereocaulon* thalli belong to more than 40 species-level lines within five trebouxiophycean genera (*Asterochloris*, *Chloroidium*, *Vulcanochloris*, *Coccomyxa*, and *Elliptochloris*) and one unknown trebouxiophycean lineage. We described *Vulcanochloris* as a new genus with three species and *Stereocaulon canariense*, its mycobiont, as a new species endemic to the Canary Islands.

Phycobiont distribution was driven primarily by mycobiont identity and *vice versa*. The diversity of phycobionts associated with *Stereocaulon* was exceptionally high. However, the individual species-level lineages of mycobionts differed considerably in the number of species and taxonomical range of their phycobionts. Mycobionts associating with more species-level lineages of phycobionts had broader climatic niches than the more specialized ones. The same held for phycobionts having more mycobionts. Among the climatic factors influencing the distribution of phycobionts, temperature seems to be the main driver globally. We also observed this pattern on a smaller geografical scale, in the Mediterranean and Macaronesian regions.

The composition of the phycobiont community was gradually changing during the succession at a locality. Some members of a lichen community can share their phycobiont pool, while others do not share phycobionts. The soil seems to be an insufficient source of phycobionts due to little overlap between the soil and the lichen algal community.

Abstrakt

Lišejníky jsou ikonickým příkladem symbiózy. Jsou rozšířeny po celém světě, v některých ekosystémech jsou dominantními autotrofy, někde jedny z mála živých organismů, které dokáží místní podmínky přežít. Rostou na celé řadě substrátů, včetně povrchů vytvořených člověkem. Velká rozmanitost jejich životních strategií souvisí s diverzitou symbiotických partnerů, kteří lišejníkovou stélku tvoří. Tato diverzita dosud z velké části nebyla prozkoumána. Lišejníky jako sesilní organismy také musí často čelit velmi proměnlivému prostředí, případně se přizpůsobit novým podmínkám, do kterých se mohou rozšířit jejich propagule. Jako modelový systém pro studium symbiotických vztahů v lišejnících a souvislostí těchto vztahů s vlastnostmi okolního prostředí jsme si vybrali široce rozšířený rod *Stereocaulon*.

Hlavními cíli této práce bylo (1) zmapovat diverzitu fotobiontů ve stélkách lišejníků rodu *Stereocaulon* v rámci celého rodu i jednotlivých druhů; (2) identifikovat faktory prostředí ovlivňující distribuci fotobiontů a jejich vztahy s mykobionty v globálním a místním měřítku; a (3) prozkoumat možnost sdílení fotobiontů s jinými lišejníky a půdou. Za tímto účelem jsme v jednotlivých studiích, ze kterých tato práce sestává, využili fylogenetické analýzy ITS rDNA, 18S rDNA, *rbcL* a genu pro aktin typu I, Illumina metabarcoding (ITS2 rDNA), světelnou, konfokální a transmisní elektronovou mikroskopii, fytocenologické snímkování, modelování klimatických nik a různé statistické metody.

Fotobionti nalezení ve stélkách lišejníků rodu *Stereocaulon* patří do více než 40 linií na úrovni druhu v rámci pěti trebouxiofytních rodů (*Asterochloris*, *Chloroidium*, *Vulcanochloris*, *Coccomyxa* a *Elliptochloris*) a jedné neznámé trebouxiofytní linie. *Vulcanochloris* jsme popsali jako nový rod se třemi druhy a *Stereocaulon canariense*, jeho mykobionta, jako nový endemický druh na Kanárských ostrovech.

Pro distribuci fotobiontů je klíčová identita mykobionta a naopak. Zatímco rod *Stereocaulon* jako celek vyniká velkou diverzitou svých fotobiontů, jednotlivé druhy mykobionta se počtem druhů a taxonomickou příslušností fotobiontů značně liší. Mykobionti schopní spolupracovat s více druhy fotobiontů mají širší klimatické niky, to samé platí i naopak pro fotobionty mající větší množství mykobiontů. Mezi klimatickými faktory ovlivňujícími rozšíření fotobiontů se jako klíčová ukázala teplota. To platí v celosvětovém měřítku, ale pozorovali jsme tuto závislost i v rámci Mediteránu a Makaronésie.

Složení společenstva fotobiontů se mění během sukcese na lokalitě. Zdrojem fotobiontů pro lišejníky mohou být některé druhy dalších lišejníků ve společenstvu, zatímco jiné fotobionty nesdílejí. Půda se neukázala být dostatečným zdrojem fotobiontů vzhledem k malému překryvu mezi společenstvem půdních a lišejníkových řas.

Table of contents

Abstract 7

Introduction 10

Aims of the study 17

Key results and discussion 18

Conclusions 27

References 28

Original papers

- Paper I 39
- Paper II 61
- Paper III 93
- Paper IV 123

1. Introduction

1.1. Lichen symbiosis

Lichens are a textbook example of a symbiotic system; however, the fundamental question "What is lichen" is still present. The traditional view of partnership between a single photosynthetic photobiont (green algae or cyanobacterium) and a single heterotrophic mycobiont (fungus) is considered too simplistic. Lichens are microecosystems involving fungi, algae, bacteria, and other microorganisms (Grimm *et al.* 2021; Grube *et al.* 2009). Cystobasidiomycete yeasts, as members of these microecosystems, have been introduced as an essential part of lichen thalli (Černajová & Škaloud 2019; Spribille *et al.* 2016); however, additional studies discovered lichens lacking these organisms (Lendemer *et al.* 2019). In any case, lichenization results in a novel morphology not known from the separate bionts (Lücking *et al.* 2017).

The mycobiont is traditionally considered the main part of lichen, giving its the shape and name (Hawksworth 2015; but see Magain *et al.* 2012). Most lichenized fungi rank among Ascomycota, especially class Lecaronomycetes (Figure 1; Schoch *et al.* 2009). Within Lecanoromycetes, more than 95% of species are lichenized (Kirk *et al.* 2008). However, lichenized species occur also in other groups, such as Basisiomycota (Gasulla *et al.* 2020). Lichenization evolved many times (between 20 and 30 independent lichenization events; Lücking *et al.* 2017). However, only a few of these events led to considerable radiation. The whole class of Lecanoromycetes originates from a single lichenization event (Lücking *et al.* 2017).

Photobionts provide carbohydrate products of photosynthesis. They are a diverse group of autotrophic organisms involving cyanobacteria (cyanobionts) as well as eukaryotic algae (phycobionts). About 50 genera of photobionts were recovered from lichen thalli (Sanders & Masumoto 2021). Nearly all rank among Chlorophyta, essentially classes Trebouxiophyceae and Ulvophyceae. Nevertheless, molecular phylogenetic data have shown that these symbiotic relationships evolved multiple times independently (Leliaert *et al.* 2012). The most common photobiont species comprise green algae *Trebouxia*, *Trentepohlia*, and cyanobacterium *Nostoc* (Friedl & Büdel 2008; Tschermak-Woess 1988b). A whole range of photobiont genera is also known as free-living algae (Darienko *et al.* 2010) or symbionts of other organisms, including animals, protists, and plants (Paracer & Ahmadjian 2000).

The relationship between phycobiont and mycobiont is traditionally considered as mutualism; however, the slow growth of the algae in a lichenized state could indicate an uneven relationship, sometimes called controlled parasitism (Ahmadjian 1993). One of the most critical steps in fungal symbioses involves penetration by the fungus into the living tissues of the other association members (Chethana *et al.* 2021 and references therein).

There are various forms of contact between the main lichen symbionts. Haustoria, mycobiont fibers penetrating directly into algal cells, are the most common type (Honegger 1986). Another type is represented by flattened hyphae surrounding photobiont cells, called appressoria (Chethana *et al.* 2021). Haustoria and appressoria may occur at the same time. The degree of haustoria penetration may depend on the environmental conditions. Some photobiont cells (e.g., *Myrmecia*, *Coccomyxa*) haustoria do not penetrate because of the composition of their cell walls. Non-specialized hyphae may also mediate the contact (Ahmadjian 1987).

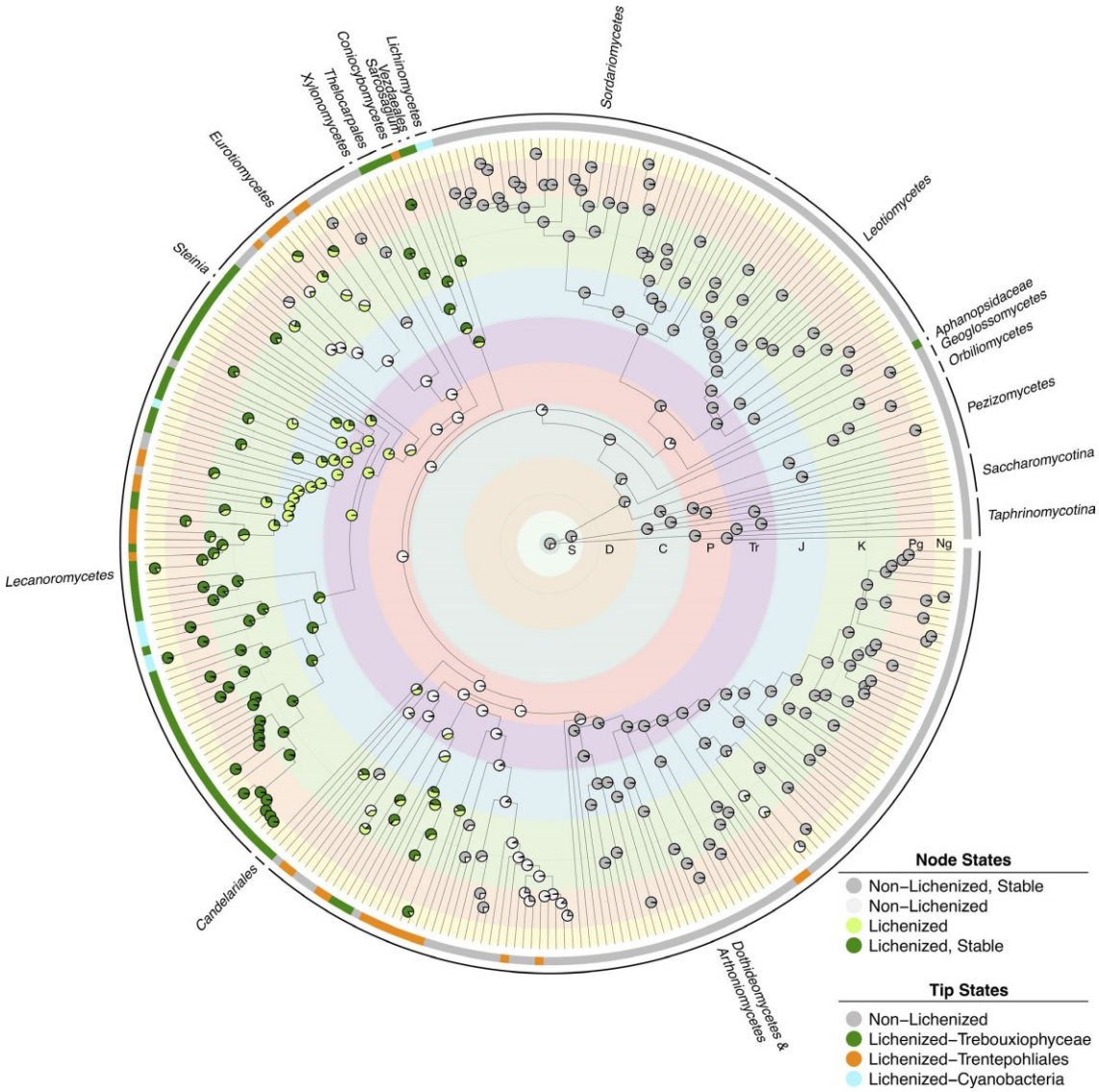


FIGURE 1. Lichenization within Ascomycota. Tip states are colored according to photobiont type (if any), and nodes shaded proportionally to the marginal ancestral state reconstruction. Shaded rings underlying the phylogeny indicate geological time periods, and dashed lines occur in 100 Myr intervals. Geological periods are shaded and abbreviated as: Neogene (Ng), Paleogene (Pg), Cretaceous (K), Jurassic (J), Triassic (T), Permian (P), Carboniferous (C), Devonian (D), Silurian (S) Reprinted from Nelsen *et al.* (2020).

1.1.1 Specificity and selectivity

Despite the rapid increase in knowledge of phycobiont diversity using molecular methods, the number of known lichen taxa exceeds the number of photobiont taxa by two orders of magnitude (Lücking *et al.* 2017; Sanders & Masumoto 2021). Most of the mycobionts co-operate typically with several lineages of one algal genus. For instance, Muggia *et al.* (2014) explored genus *Tephromela* with a whole range of *Trebouxia* lineages as phycobionts, Leavitt *et al.* (2013) studied genera *Xanthoparmelia* and (2016) *Rhizoplaca* with phycobionts of the same genus, Nyati *et al.* (2013) investigated genera *Xanthoria* and *Xanthomendoza* also with several species of *Trebouxia*. Repeatedly the high intrageneric diversity of *Asterochloris* phycobionts in lichens *Cladonia* (Bačkor *et al.* 2010; Beiggi & Piercey-Normore 2007; Piercey-Normore & DePriest 2001; Pino-Bodas & Stenroos 2020; Škaloud & Peksa 2010; Yahr *et al.* 2004) and *Lepraria* (Kosecka *et al.* 2021; Nelsen & Gargas 2006, 2008; Peksa & Škaloud 2011; Škaloud & Peksa 2010) was observed. Basidiolichen *Omphalina* interacts with only one *Coccomyxa* species (Zoller & Lutzoni 2003).

Contrastingly, more diversified phycobionts were discovered in a microlichen *Micarea* associating with two genera from the class Trebouxiophyceae, *Coccomyxa,* and *Elliptochloris* (Yahr *et al.* 2015); endolithic lichen *Bagliettoa* with three trebouxiophycean genera, *Asterochloris*, *Diplosphaera*, and *Trebouxia* (Thüs *et al.* 2011); lichenicolous lichen *Diploschistes muscorum* with three trebouxiophycean genera, *Asterochloris*, *Symbiochloris*, and *Trebouxia* (Wedin *et al.* 2015); *Verrucaria nigrescens* with four genera, *Chloroidium*, *Dilabifilum*, *Diplosphaera*, and *Trebouxia* (Thüs *et al.* 2011; Vaiglová 2017; Voytsekhovich & Beck 2015); and *Chaenotheca* with *Trebouxia*, *Trentepohlia*, *Symbiochloris*, and *Tritostichococcus* (Tibell 2001). *Sticta* associates (besides cyanobacteria) with trebouxiophycean algae, including *Haveochlorella*, *Chloroidium*, *Symbiochloris*, and *Elliptochloris* (Lindgren *et al.* 2020).

The degree of specificity and selectivity of both partners influences the selection of a suitable photobiont. The concept of selectivity and especially specificity vary among different studies (Helms 2003). In this text, we use the term specificity in the sense of a limited taxonomic range of acceptable partners. In contrast, selectivity is defined as giving a preference to a certain group of partners (Rambold *et al.* 1998). For example, the symbiotic partner may be in relation to the other partner non-specific as well as highly selective if it can coexist with various unrelated partners, but in most cases, it is observed in association with the representatives of only one group (Rambold et al. 1998; Yahr et al. 2004).

Low selectivity may be an adaptive strategy under extreme climatic conditions or for colonization of the newly emerged substrata, as demonstrated from the examples of Antarctic lichens (Engelen *et al.* 2010; Romeike *et al.* 2002; Wirtz *et al.* 2003) or lichens

growing on anthropogenic substrata (Guzow-Krzemińska 2006; Muggia *et al.* 2013). The lichens growing at localities spoiled by toxic substances frequently show lower specificity and incorporate in their thalli unusual phycobionts adapted to such conditions (Bačkor *et al.* 2010; Beck 2002; Osyczka *et al.* 2020).

The specificity and selectivity can be related to the mode of reproduction of the lichen (Otálora *et al.* 2010; Piercey-Normore 2009; Werth & Scheidegger 2012; Yahr *et al.* 2004). Vegetative diaspore (mainly soredia and isidia, possibly also thallus fragments) should ensure that both symbionts stay together for dispersal. Conversely, sexually reproducing mycobionts spread by spores independently and must resynthesize the thallus with a compatible photobiont (relichenization; Figure 2; Krishnamurthy & Upreti 2001). Sexually reproducing *Cladonia* species showed low specificity interacting with several unrelated *Asterochloris* lineages, unlike vegetatively dispersing *Cladonia* mycobionts. In Europe, they were associated with only two closely related phycobiont species regardless of the environmental conditions (Steinová *et al.* 2019).

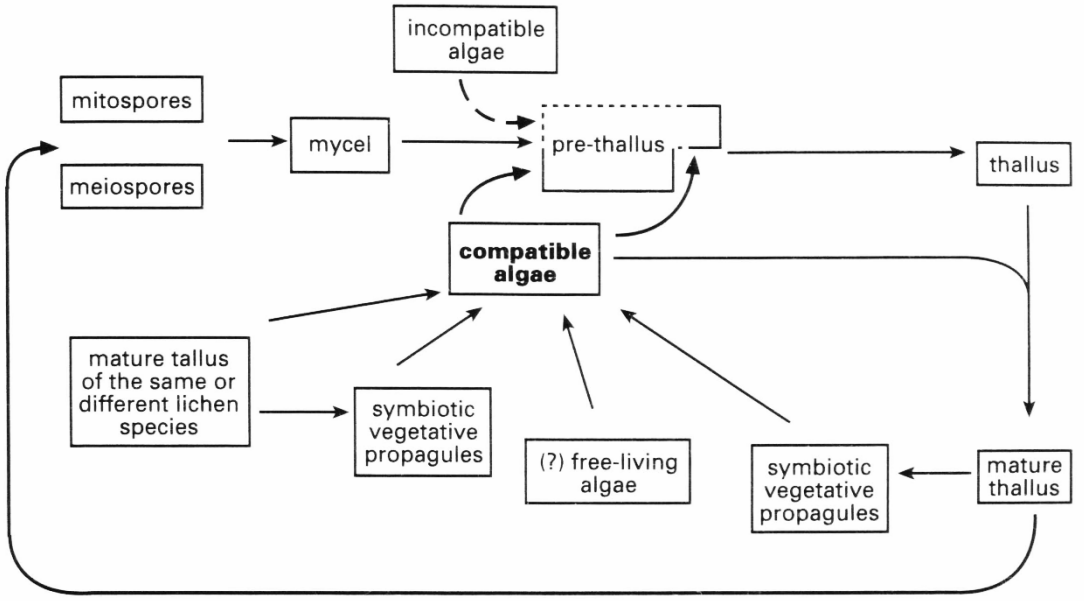


FIGURE 2. A model of relichenization in the reproductive cycle of lichenized ascomycetes. The germinating fungal spore forms a mycelium. After contact with a potential photobiont, both partners establish an inconspicuous non-stratified crust (prethallus). This structure can also be formed with incompatible algae, but compatible photobiont is essential for further development. Free-living algae or lichenized propagules (from the same or other lichen species) can be potential photobiont sources. Reprinted from Beck *et al.* (1998).

1.1.2. Algal plurality

Traditionally, one photobiont has been considered to form a symbiotic relationship with one mycobiont, but recent studies have confirmed the presence of multiple photobiont genotypes in a single thallus (i.e., algal plurality) of various lichens. This phenomenon has been detected by culture-dependent methods (Muggia *et al.* 2011), Sanger sequencing of

tiny thallus fragments (Bačkor *et al.* 2010), using highly specific primers (Casano *et al.* 2011), or methods based on gel electrophoresis of DNA fragments such as RFLP (restriction fragment length polymorphism; Piercey-Normore 2006) or SSCP (single-strand conformation polymorphism; Muggia *et al.* 2013). Difficulties with Sanger sequencing can indicate algal plurality in itself (Paul *et al.* 2018); however, scientific papers usually do not report such failed attempts (Moya *et al.* 2021).

Nowadays, the intrathalline diversity of algae (and other organisms) is studied predominantly using high-throughput sequencing (Onuț-Brännström *et al.* 2018; Park *et al.* 2015). Various lichen species differ significantly in the occurrence of this phenomenon (Dal Grande *et al.* 2017; Smith *et al.* 2020) and the species pool of accessory photobionts, despite growing at the same locality (Molins *et al.* 2018). Leavitt et al. (2015) hypothesized that lichens nonspecific towards their photobionts exhibit algal plurality more probably. Interestingly, there could be significant differences in intrathalline algal diversity within a single thallus. Trebouxiophycean and ulvophycean algae in the thallus of *Cladonia squamosa* in Antarctica shows vertical (from top of the thallus to the ground) and horizontal (from the center of the "colony" to the margin) stratification. The same applies to the fungi and bacteria in these thalli (Noh *et al.* 2020).

Trebouxia phycobionts TR1 and TR9 coexisting in *Ramalina farinacea* thalli in the Mediterranean and Macaronesian regions showed diverse physiological responses to light intensity, temperature, and oxidative stress (Casano *et al.* 2011; Del Hoyo *et al.* 2011) as well as different morpho-physiological strategies against the lead exposure (Álvarez *et al.* 2012). The proportion of both lineages within the thallus varies according to the geographical origin of the sample. Similar patterns were reported for other lichens (Molins *et al.* 2020).

Mostly, the phycobiont determined by Sanger sequencing is consistent with the predominant phycobiont according to high-throughput sequencing (Molins *et al.* 2018; Paul *et al.* 2018). However, due to several method limitations, the number of sequence reads may not perfectly reflect the amount of particular alga (or other organisms) in the sample (Moya *et al.* 2021).

Algal plurality has been observed in other groups of symbiotic organisms as well. One of the best-studied examples is the endosymbiosis between corals and dinoflagellates of the genus *Symbiodinium*. Several studies have proved an algal plurality (occurrence of multiple *Symbiodinium* lineages within the individual host; Baker 2003; Baums *et al.* 2014) in a minor part of studied samples. It is not clear whether the methods used in these studies overestimate or underestimate the occurrence of the minor strains (LaJeunesse & Thornhill 2011). It is important to note that this phenomenon's ecological role and temporal stability remain unknown. Undoubtedly, particular lineages of *Symbiodinium*

show distinct ecological preferences (Baker 2003; Pettay *et al.* 2015; Rowan 2004). Some seem to be well adapted to higher temperatures and irradiance (Iglesias-Prieto *et al.* 2004). However, although juveniles maintain several strains or switch them frequently (Byler *et al.* 2013), the capacity of adult corals for photobiont switching is most likely limited (Baums *et al.* 2014; Byler *et al.* 2013; Iglesias-Prieto *et al.* 2004). An entire diversity of possible symbionts, which varies among different areas, could influence the ability of corals to maintain or switch various algae (Baums *et al.* 2014).

1.2. *Stereocaulon* as a model symbiotic system

Stereocaulon (Lecanorales, Ascomycota) is a genus of lichenized fungi with extensive geographical distribution, from polar regions (Melechin 2015; Smith & Øvstedal 1991) to the tropics (Fritz-Sheridan & Coxson 1988). These lichens occupy localities in a wide range of altitudes and tolerate submersion (Sadowsky *et al.* 2012), arid climate (Singh *et al.* 2013), or toxic substrata (Medeiros *et al.* 2014; Purvis & Halls 1996). Some species rank among the pioneer lichens growing in harsh conditions on newly formed substrata, thereby playing an appreciable role in weathering rocks, including lava flows (Meunier *et al.* 2014; Stretch & Viles 2002). Other species are essential members of communities in areas where the succession of higher plants is blocked by hostile climate or disturbance (Durán *et al.* 2021; Heindel *et al.* 2019). These ecological features make genus *Stereocaulon* a suitable model system for investigating patterns of symbiotic relationships and environmental conditions.

The *Stereocaulon* mycobionts associate with green trebouxiophycean phycobiont providing products of photosynthesis and, in some cases, cyanobiont providing nitrogen by fixing atmospheric nitrogen (Heindel *et al.* 2019; Huss-Danell 1979; Lücking *et al.* 2009). *Rhizonema*, *Stigonema*, *Nostoc*, and other cyanobacteria (Dal Forno *et al.* 2021; Lamb 1951; Lavoie *et al.* 2020; de Oliveira Torres 2021) are located in cephalodia (small gall-like structures) of *Stereocaulon*. Oksanen *et al.* (2004) hypothesized that cyanobacterial toxins reduce reindeer grazing on *Stereocaulon*.

Already Ahmadjian (1960) mentioned A*sterochloris glomerata* as phycobiont of *Stereocaulon*. However, the diversity of its phycobionts has been investigated on a small number of samples in the past (Bačkor *et al.* 2010; Nelsen & Gargas 2006; Peksa & Škaloud 2011; Piercey-Normore & DePriest 2001; Škaloud & Peksa 2010). These studies revealed several lineages of *Asterochloris* as phycobionts of *Stereocaulon*. In one case, Beck (2002) found another trebouxioid alga *Chloroidium*, as phycobiont of *Stereocaulon nanodes*.

The genus *Stereocaulon* includes about 140 species (Lücking *et al.* 2017). Their thallus (Figure 3) is mostly dimorphic, consisting of a crustose primary thallus and a fruticose secondary thallus. In most species, the primary thallus composed of basal granules or

squamules (phyllocladia) tightly attached to the substrate disappears early in development. The fruticose secondary thallus, or pseudopodetium, arises from the primary thallus by the elongation of thalline tissue into often richly branched stalks. Pseudopodetia support persistent phyllocladia (Högnabba 2006; Lamb 1951). Crustose *Stereocaulon* species hold a basal position of the phylogeny published by Högnabba *et al.* (2014). They concluded that the crustose growth form is the plesiomorphic feature of the genus.

FIGURE 3. Thalli of *Stereocaulon* spp.

Högnabba (2006) and Spribille *et al.* (2010) mentioned the necessity of taxonomical revision of the genus *Stereocaulon* based on molecular data. Their studies showed a discrepancy between *Stereocaulon* morphospecies and phylogenetical lineages. Therefore, we should pay attention to mycobiont identity.

2. Aims of the study

The main goals of this thesis were (1) to determine the diversity of phycobionts associated with the lichen-forming genus *Stereocaulon* within the entire genus and species-level lineages; (2) to identify the environmental factors influencing the distribution of phycobionts and their relationships with mycobionts at the global and local scale; and (3) to inspect the possibility of sharing the phycobionts with other lichens and soil.

3. Key results and discussion

3.1. Diversity of phycobionts associated with Stereocaulon

The phycobionts associated with Stereocaulon belong to more than 40 species-level lineages within five trebouxiophycean genera (*Asterochloris, Chloroidium, Vulcanochloris, Coccomyxa,* and *Elliptochloris*) and one unknown trebouxiophycean lineage (URa28; Table 1). Brunner & Honegger (1985) identified a phycobiont of *Stereocaulon* morphologically as *Pseudochlorella*; however, Sanders & Masumoto (2021) assigned this observation rather to *Chloroidium*, which was not known at that time.

TABLE 1. List of phycobiont species-level lineages revealed in assotiation with *Stereocaulon* mycobionts based on molecular data. Clade affiliations: clade 8, clade 12 sensu Škaloud & Peksa (2010), S1, S3 sensu Nelsen & Gargas (2006), *Asterochloris* aff. *italiana*, StA1, StA3– StA8, StC1, StC2 sensu Vančurová *et al.* (2018), StA9 sensu Vančurová *et al.* (2020), StA10 sensu Vančurová *et al.* (2021), Bol4, Bol7, P2, clade A14 sensu Kosecka *et al.* (2021), URa28 sensu Ruprecht *et al.* (2014).

Phycobiont species-level lineage	**Geographic origin of samples**	**References**
Asterochloris antarctica	Iceland	(Vančurová *et al.* 2018)
Asterochloris echinata	Switzerland	(Vančurová *et al.* 2020)
Asterochloris excentrica	USA	(Škaloud & Peksa 2010)
Asterochloris glomerata	Iceland, Argentina, Finland, Czech Republic, Madeira (Portugal), Faroe Islands (Denmark), Slovakia, USA, Switzerland	(Bačkor *et al.* 2010; Piercey-Normore & DePriest 2001; Vančurová *et al.* 2018, 2020)
Asterochloris irregularis	Iceland, Japan, Russia, Czech Republic, USA, Greenland (Denmark), Faroe Islands (Denmark), Austria, Slovakia, Switzerland	(Nelsen & Gargas 2006; Piercey-Normore & DePriest 2001; Škaloud & Peksa 2010; Vančurová *et al.* 2018, 2020)
Asterochloris italiana	Argentina, Madeira (Portugal), Germany, Faroe Islands (Denmark), Switzerland, Canary Islands (Spain)	(Vančurová *et al.* 2018, 2020, 2021)
Asterochloris leprarii	Switzerland	(Vančurová *et al.* 2020)
Asterochloris lobophora	Georgia, Russia, Slovakia, Switzerland	(Bačkor *et al.* 2010; Vančurová *et al.* 2018, 2020)
Asterochloris mediterranea	Canary Islands (Spain), Bolivia	(Kosecka *et al.* 2021; Vančurová *et al.* 2018, 2020)

Phycobiont species-level lineage	**Geographic origin of samples**	**References**
Asterochloris phycobiontica	Austria, Switzerland	(Vančurová *et al.* 2018, 2020)
Asterochloris pseudoirregularis	Faroe Islands (Denmark), Iceland, USA, Austria, Canada, Antarctica	(Nelsen & Gargas 2006; de Oliveira Torres 2021; Vančurová *et al.* 2018)
Asterochloris stereocaulonicola	Argentina, Iceland, Italy, Slovakia, Antarctica	(Kim *et al.* 2020; Vančurová *et al.* 2018)
Asterochloris woessiae	Madeira (Portugal), Faroe Islands (Denmark), Canary Islands (Spain)	(Škaloud *et al.* 2015; Vančurová *et al.* 2018, 2020)
Asterochloris clade 12	USA, Mexico, Czech Republic, Switzerland	(Nelsen & Gargas 2006; Vančurová *et al.* 2018, 2020)
Asterochloris clade 8	Canada, Czech Republic, Switzerland	(Vančurová *et al.* 2018, 2020)
Asterochloris S1	Costa Rica, Bolivia	(Kosecka *et al.* 2021; Nelsen & Gargas 2006)
Asterochloris S3	USA	(Nelsen & Gargas 2006)
Asterochloris aff. *italiana*	Madeira (Portugal), Sweden, Switzerland	(Vančurová *et al.* 2018, 2020, 2021)
Asterochloris StA1	Faroe Islands (Denmark)	(Vančurová *et al.* 2018)
Asterochloris StA3	Tanzania, Switzerland	(Vančurová *et al.* 2018, 2020)
Asterochloris StA4	Sweden, USA, Greenland (Denmark), Switzerland	(Nelsen & Gargas 2006; Vančurová *et al.* 2018, 2020)
Asterochloris StA5	Georgia, Austria, Canada, Switzerland	(Vančurová *et al.* 2018, 2020)
Asterochloris StA6	Argentina	(Vančurová *et al.* 2018)
Asterochloris StA7	Argentina	(Vančurová *et al.* 2018)
Asterochloris StA8	Costa Rica, Venezuela	(Vančurová *et al.* 2018)
Asterochloris StA9	Switzerland	(Vančurová *et al.* 2020)
Asterochloris StA10	Madeira (Portugal)	(Vančurová *et al.* 2021)
Asterochloris Bol4	Bolivia	(Kosecka *et al.* 2021)
Asterochloris Bol7	Bolivia	(Kosecka *et al.* 2021)

Phycobiont species-level lineage	**Geographic origin of samples**	**References**
Asterochloris P2	Bolivia	(Kosecka *et al.* 2021)
Asterochloris clade A14	Panama	(Vančurová *et al.* 2018)
other *Asterochloris*	Tanzania, Argentina	(Vančurová *et al.* 2018)
Chloroidium ellipsoideum	New Zealand, Czech Republic, Italy, Madeira (Portugal), Canary Islands (Spain)	(Vančurová *et al.* 2018, 2021)
Chloroidium lichenum A	Czech Republic, Madeira (Portugal), Canary Islands (Spain), Guatemala, Venezuela, New Zealand, Tanzania	(Vančurová *et al.* 2018, 2021)
Chloroidium lichenum B (*C. angustoellipsoideum*)	Madeira (Portugal), Guatemala, Czech Republic, Canary Islands (Spain)	(Vančurová *et al.* 2018, 2021)
Chloroidium lichenum C (StC1)	Georgia, New Zealand	(Vančurová *et al.* 2018)
Chloroidium lichenum C (StC2)	Czech Republic	(Vančurová *et al.* 2018)
Chloroidium saccharophilum	Austria	(Beck 2002)
other *Chloroidium*	Costa Rica, Venezuela, Guatemala	(Vančurová *et al.* 2018)
Vulcanochloris canariensis	Canary Islands (Spain), Bolivia	(Kosecka *et al.* 2021; Vančurová *et al.* 2015, 2021)
Vulcanochloris guanchorum	Canary Islands (Spain)	(Vančurová *et al.* 2015, 2021)
Vulcanochloris symbiotica	Canary Islands (Spain)	(Vančurová *et al.* 2015, 2021)
Coccomyxa viridis	Switzerland	(Vančurová *et al.* 2020)
Elliptochloris sp.	Switzerland	(Vančurová *et al.* 2020)
Trebouxiophyceae URa28	Switzerland	(Vančurová *et al.* 2020)

Most of the listed species-level lineages belong to *Asterochloris*, which is also associated with most of the studied *Stereocaulon* species-level lineages (Vančurová *et al.* 2018). The discovery of new *Asterochloris* lines is continuing outside Europe, but also in Europe

(Kosecka *et al.* 2021; Moya *et al.* 2015). However, not all species-level lineages recovered from other lichens associate with *Stereocaulon* and *vice versa*. From the relatively large number of published sequences of phycobionts associated with *Cladonia* and *Lepraria*, we can infer reciprocal specificity and selectivity patterns (Škaloud *et al.* 2015; but see Vančurová *et al.* 2020).

Chloroidium is a common free-living trebouxiophycean alga (Darienko *et al.* 2010, 2018; Pangallo *et al.* 2015). Before separating from genus *Chlorella*, it was rarely reported in lichens as *Chlorella ellipsoidea* or *C. saccharophila* (Beck 2002; Tschermak-Woess 1948, 1978, 1988a). Since that time, *Chloroidium* phycobionts have been reported more frequently (Darienko *et al.* 2018; Lindgren *et al.* 2020; Sanders *et al.* 2016; Voytsekhovich & Beck 2015). Several species-level lineages associated with *Stereocaulon*, the most common were *Chloroidium lichenum* A and *Chloroidium ellipsoideum* (Vančurová *et al.* 2018, 2021). After the last taxonomic revision, *C. lichenum* became a paraphyletic species (Darienko *et al.* 2018). Authors divided this species into three clades, monophyletic A and B and polyphyletic C. Therefore, we consider this species to be four species-level lineages (two of them corresponding with clades A and B) according to species delimitation analyses (Vančurová *et al.* 2018).

We described the genus *Vulcanochloris* (sister to *Asterochloris*) as a phycobiont of *Stereocaulon vesuvianum* from La Palma island (Vančurová *et al.* 2015). However, subsequent molecular analyses of mycobiont revealed *S. vesuvianum* polyphyletic (Vančurová *et al.* 2018). Moreover, its three lineages differ in their favor phycobiont and geographical distribution. We recently described species-level lineage OTU52 associated with *Vulcanochloris* as a new species *Stereocaulon canariense* (probably endemic to the Canary Islands; Vančurová *et al.* 2021). Besides volcanic substrata, *Vulcanochloris* occurs in other lichens on calcareous substrata in the Mediterranean (Moya *et al.* 2017, 2020, 2021) and outside this region (Ruprecht *et al.* 2014; Vaiglová 2017), frequently as accessory phycobiont.

Phycobiont genera and species associated with *Stereocaulon* differ in their climatic requirements, both in terms of their optima and width of the climatic niches. We proved the positive correlation between the width of the climatic niche and the number of acceptable symbiotic partners for both *Stereocaulon* phycobionts and mycobionts (Vančurová *et al.* 2018). They may also differ in the affinity to the substrate of various pH (Bačkor *et al.* 2010; Piercey-Normore & DePriest 2001; Steinová *et al.* 2019) or containing potentially toxic substances (Beck 2002; Vančurová *et al.* 2018).

3.2. Phycobiont community patterns along the environmental gradients

Singh et al. (2017) proposed the theory, that phycobionts as well as mycobionts have more symbiotic partners in colder climates based on data involving *Protoparmelia* lichens with their *Trebouxia* symbionts. Using our data, we can compare samples of three abundant mycobiont species-level lineages from boreal and alpine climates of the northern hemisphere (OTU10, OTU35, and OTU47) with three species-level lineages from the Mediterranean and Macaronesian region (*S. vesuvianum* sensu stricto (OTU11), *S. azoreum* (OTU13), and *S. canariense* (OTU52)). The number of symbiotic partners of *Stereocaulon* seems to be similar in cold and warm climates (Figure 4); however, our data were not sampled for the purpose of this question.

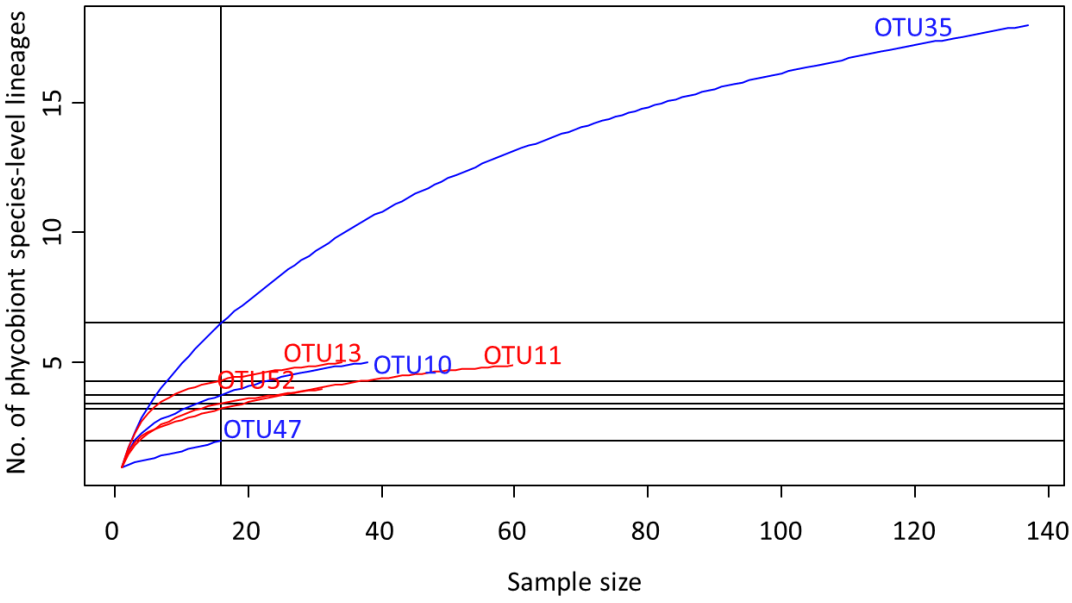


FIGURE 4. Rarefaction curves for six selected mycobiont species-level lineages. A vertical line is drawn at the smallest sample size in the data set with horizontal lines for the rarefied number of species-level lineages of associated phycobionts. Blue lines: samples from boreal and alpine climates of the northern hemisphere. Red lines: samples from the Mediterranean and Macaronesian regions.

On a smaller geographic scale, we investigated phycobiont community patterns in Macaronesia and the Mediterranean region. We analyzed relationships of the three most abundant mycobionts in our dataset (*Stereocaulon vesuvianum*, *Stereocaulon azoreum*, and *Cladonia rangiformis*) and their phycobionts and revealed temperature as a critical factor determining the phycobiont selection in that area in concordance with several previous studies (Molins *et al.* 2020; Rolshausen *et al.* 2020; Vančurová *et al.* 2018). In all combinations of mycobionts and their phycobionts, we found patterns resembling parallel symbiont turnover zones along temperature gradient described recently by Rolshausen *et al.* (2020). Several studies described the gradual change of phycobionts along the altitudinal gradient (Gasulla *et al.* 2020; Vargas Castillo & Beck 2012). However, we found

temperature (in particular annual mean temperature, mean temperature of the wettest quarter, and mean temperature of the coldest quarter) as more important than altitude.

Some species of *Stereocaulon* rank among the pioneer lichens growing on newly emerged substrata, such as river gravel bars. We investigated phycobiont communities along the successional gradient in these dynamic and heterogeneous habitats. The communities of the early-vegetation stages consisted of a few species-level lineages, mainly *Asterochloris phycobiontica* and *Asterochloris* StA5. In more developed stages, these two species-level lineages were gradually substituted by many other lineages. The species richness of the *Stereocaulon* phycobionts was positively correlated with species richness of surrounding lichens (other than *Stereocaulon*). We proposed several explanations of these patterns, such as colonization by locally rare but more specialized phycobionts, increasing microhabitat heterogeneity, or changes in the soil (Vančurová *et al.* 2020). Although the *Asterochloris* phycobionts are generally slow-growing (Elshobary *et al.* 2015), it may be possible that early- and late-successional species differ in their growth rate in the same way as, e.g., benthic diatoms during the succession after disturbation (McCormick & Stevenson 1991).

3.3. Photobiont sharing

Unlike studies examining photobionts of a particular taxonomic group of lichens, Beck (Beck 1999; Beck *et al.* 1998, 2002) made several photobiont inventories of all lichens from spatially defined localities. On a small piece of tree bark, some lichens (*Lecidella elaeochroma* and *Xanthoria parietina*) shared their photobionts, but others not, despite their affinity to a single *Trebouxia* species (but significantly different genotypes; Beck *et al.* 1998). In a saxicolous community (a boulder about 0,3 m^2), they found an array of photobionts: *Trebouxia asymmetrica* (shared by two lichen genera), *Asterochloris irregularis*, *Myrmecia*, and *Nostoc* (Beck *et al.* 2002).

Rikkinen *et al.* (2002) proposed the concept of photobiont mediated guilds based on cyanolichen data. They divided *Nostoc* cyanobionts and associated lichens into two guilds (epiphytic "*Nephroma* guild" and predominantly terricolous "*Peltigera* guild") and described a complex system of cyanobiont sharing with a central role of vegetatively dispersing species ("core species") providing cyanobionts to other members of the community ("fringe species"). *Nostoc* cyanobionts of bipartite (associating solely with cyanobacteria) and tripartite (associating simultaneously with green algae and cyanobacteria) *Nephroma* lichens split into two groups (Fedrowitz *et al.* 2012).

We sampled *Stereocaulon* and two related lichens *Cladonia* and *Lepraria* (Lücking 2019) in the Mediterranean and Macaronesian regions. All six *Stereocaulon* species present in the area showed quite a high level of specificity or selectivity on the algal genus level.

Conversely, on the species level, they have a similar affinity to two or more algal lineages (mostly varied in their distribution according to the climatic gradients as described above). *Stereocaulon* species-level lineages OTU3, OTU13 (*S. azoreum*), and OTU22 associating with the genus *Asterochloris* have the potential to share phycobionts with *Cladonia* and *Lepraria*. Indeed, almost all *Asterochloris* lineages in the study area were shared by different mycobiont genera.

On the level of a particular locality, the number of *Asterochloris* species varied. In some cases, all samples shared one species, but in other localities, each sample contained a different species of *Asterochloris*. We investigated several localities where *Stereocaulon vesuvianum* (OTU11) and *Cladonia* lichens grow in close contact creating dense lichen cushion. Even in these cases, the mycobionts associated with different phycobiont species (Vančurová *et al.* 2021).

Only three of six investigated corticolous lichens (*Hypogymnia physodes*, *Hypogymnia tubulosa*, *Lecanora pulicaris*, *Parmelia sulcata*, *Physcia adscendens*, and *Pseudevernia furfuracea*) growing on tree trunks shared their *Trebouxia* phycobionts. The others hosted another *Trebouxia* species-level lineages. Twelve of 14 *Trebouxia* OTUs were associated exclusively with one mycobiont species (Mark *et al.* 2020). From two to ten *Trebouxia* species-level lineages per study plot (9 m^2) were identified associated with saxicolous lichen communities (of two-or-more fungal morphospecies) in central Europe. Up to 12 lichen morphospecies shared the same *Trebouxia* species-level lineage within a single study plot (Peksa *et al.* in press).

Škvorová *et al.* (in press) sampled all *Cladonia* species within study plots (each of 100 m^2 area) across Europe. The network of *Cladonia* and *Asterochloris* split into four modules with many interactions within the modules. A few widespread mycobionts mediated the interactions between modules. Mainly, multiple phycobiont species-level lineages occurred on a single study plot. One photobiont usually predominated in the locality and was shared by most mycobionts, but sometimes the division into modules also manifested itself within the locality.

In *Stereocaulon*, the worldwide network could be divided into three modules: (1) *Vulcanochloris*, (2) *Chloroidium* with *A. stereocaulonicola*, and (3) rest of *Asterochloris* phycobionts. *Asterochloris* and *Chloroidium* modules were connected mainly by OTU10 mycobiont. Widely distributed species-level lineage OTU10 is unselective but specific towards particular *Asterochloris* lineages. However, at specific localities (anthropogenic, volcanic, or tropical), it was associated with *Chloroidium* spp. (Vančurová *et al.* 2018).

The samples of *Stereocaulon vesuvianum* (OTU11) or *Stereocaulon canariense* (OTU52) associated with *Asterochloris mediterranea* (the most common phycobiont of *Cladonia* and *Lepraria* in the area) are rare but not necessarily irrelevant. In some of these cases, we

found common phycobionts (*Chloroidium* and *Vulcanochloris*, respectively) in the same thallus. Could this be a sign of sharing photobionts with *Asterochloris*-associated mycobionts? One sample of *Stereocaulon canariense* from Tenerife (960 m a.s.l) suggests using this alternative photobiont in rare cases if their preferred photobiont does not occur in the area or can not cope with the local climate (Vančurová *et al.* 2021).

Based on Illumina metabarcoding data, we hypothesized accessory phycobionts to play a role in phycobiont sharing within the community (Vančurová *et al.* 2020). Two *Stereocaulon* species-level lineages shared their intrathaline algae, changing their status between predominant and accessory phycobiont. Conversely, the soil did not seem to be a sufficient source of lichen algae. We did not obtain DNA sequences of surrounding lichens and their photobionts, but we can assume that surrounding *Cladonia* spp. are a potential source of *Asterochloris* phycobionts (Piercey-Normore & DePriest 2001; e.g., Pino-Bodas & Stenroos 2020; Škaloud & Peksa 2010; Yahr *et al.* 2004). Nevertheless, our unpublished data show that the phycobiont pool of *Stereocaulon* and *Cladonia* at a particular locality can be separated despite compatibility of the partners. An array of *Trebouxia*-associated lichens was noticed (e.g., *Lecanora*, *Acarospora*, *Rhizocarpon*; Sanders & Masumoto 2021 and references therein); however, this phycobiont did not associate with *Stereocaulon* samples. There also occurred several species of *Placynthiella*. Voytsekhovich *et al.* (2011) proved the presence of several phycobionts in the thalli of these lichens. One of them, *Elliptochloris*, was associated with *Stereocaulon* in the study area. Besides *Stereocaulon*, *Peltigera* spp. with cyanobionts were the most covering lichens.

On newly emerged substrata without surrounding lichens, sexually reproducing mycobionts can benefit from the ability to associate with common free-living algae, such as *Symbiochloris symbiontica* in the case of *Lecania naegelii* on bare and smooth tree bark (Beck *et al.* 1998), *Diplosphaera/Stichococcus* in the case of *Verrucaria nigrescens* in a limestone mine (Vaiglová 2017), and *Chloroidium* spp. in the case of *Stereocaulon* (Vančurová *et al.* 2018).

Nevertheless, using so-called algal switching, vegetatively dispersing lichens can associate with locally adapted algae (Piercey-Normore & DePriest 2001). *Parmotrema tinctorum* associated with various *Trebouxia* algae from its surroundings, sometimes with more than one genotype in a single thallus. The substrate contamination by its soredia was controlled by sequencing of fungal DNA (Ohmura *et al.* 2019). The algal switching has been repeatedly speculated in mature lichen thallus; however, transplantation experiments with *Psora decipiens* associating with *Myrmecia* did not support this idea. Algal layers of thalli transplanted to semi-arid warm climate or to alpine climate disappeared and were not replaced. Lichens transplanted to mild climates mostly survived with algae from their origin (Williams *et al.* 2017).

Finally, some lichens obtain their algae from thalli of other lichens as parasites. *Diploschistes muscorum* begins as a parasite infecting thallus of *Cladonia symphycarpa* and later develops an independent *Diploschistes* lichen thallus. Wedin *et al.* (2015) observed gradual change of phycobiont composition during this process: (1) *Asterochloris mediterranea* originated from host lichen *Cladonia*, (2) combination of *Asterochloris* and *Trebouxia*, (3) combination of *Symbiochloris* and *Trebouxia* and (4) only *Trebouxia*. Noh *et al.* (2020) revealed, besides other algal and fungal sequences, from thalli of *Cladonia squamosa*, also *Ochrolechia frigida* with its *Trebouxia* symbiont. This lichen (or the algal-free stage of the mycobiont) is growing on various organic substrata and disturbing tissues of the host. In case of parasited lichens, *Ochrolechia* can also gain their phycobionts (Gaßmann & Ott 2000).

4. Conclusions

In general, the diversity of *Stereocaulon* phycobionts involving five trebouxiophycean genera (*Asterochloris*, *Chloroidium*, *Vulcanochloris*, *Coccomyxa*, and *Elliptochloris*) and one unknown trebouxiophycean lineage is exceptionally high. However, particular mycobionts and phycobionts differed in the taxonomical range of acceptable partners and their geographic distribution. We identified the symbiotic partner as the main driver of the distribution of mycobionts and phycobionts. We proved the positive correlation between the width of the climatic niche and the number of acceptable symbiotic partners for both phycobionts and mycobionts.

Phycobiont genera and species differed in their climatic optima. Among the climatic factors, the temperature played a key role. We observed phycobiont turnover zones along the temperature gradient on the example of three mycobionts and contrasting pairs of associated phycobionts. Moreover, the diversity of phycobionts was shifting along the gradient of vegetation succession. The phycobiont communities of the early-successional stages involved relatively few species-level lineages. The observed phycobiont species richness mostly increased in the following stages, while other phycobiont species-level lineages gradually substituted that typical for early-successional stages.

We inspected the possibility of sharing phycobionts within the lichen community and between lichens and soil. In some cases, *Stereocaulon* species-level lineages associated with *Asterochloris* shared their phycobionts with other lichens in the community, such as *Cladonia* and *Lepraria*. However, it was not a rule. Conversely, few *Stereocaulon* species-level lineages were able to associate with various algal genera. Therefore, despite close physical contact, they did not share their phycobionts with lichens associating with other phycobiont genera. It holds for the predominant phycobiont. But, we found multiple phycobiont species (or genera) in a single *Stereocaulon* thallus. We hypothesized the role of additional algae as a possible source of phycobionts for other lichens in a community. According to our data, the soil was an insufficient source of lichen algae.

To sum up, we have achieved the objectives of this thesis, but it can also pose the basis for future research.

5. References

Ahmadjian, V. (1960) Some New and Interesting Species of *Trebouxia*, a Genus of Lichenized Algae. *American Journal of Botany* 47: 677. https://doi.org/10.2307/2439519

Ahmadjian, V. (1987) Coevolution in Lichens. *Annals of The New York Academy of Sciences* 503: 307–315.

Ahmadjian, V. (1993) *The Lichen Symbiosis*. John Wiley and Sons, Inc., New York. 250 pp.

Álvarez, R., del Hoyo, A., García-Breijo, F., Reig-Armiñana, J., del Campo, E.M., Guéra, A., Barreno, E. & Casano, L.M. (2012) Different strategies to achieve Pb-tolerance by the two *Trebouxia* algae coexisting in the lichen *Ramalina farinacea*. *Journal of Plant Physiology* 169: 1797–1806. https://doi.org/10.1016/j.jplph.2012.07.005

Bačkor, M., Peksa, O., Škaloud, P. & Bačkorová, M. (2010) Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. *Ecotoxicology and environmental safety* 73: 603–12. https://doi.org/10.1016/j.ecoenv.2009.11.002

Baker, A.C. (2003) Flexibility and specificity in coral-algal symbiosis: Diversity, ccology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics* 34: 661–689. https://doi.org/10.1146/132417

Baums, I.B., Devlin-Durante, M.K. & Lajeunesse, T.C. (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *Molecular Ecology* 23: 4203–4215. https://doi.org/10.1111/mec.12788

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

Beck, A. (2002) Selektivität der Symbionten schwermetalltoleranter Flechten. Ludwig-Maxmilians-Universität München

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.

Beck, A., Kasalicky, T. & Rambold, G. (2002) Myco-photobiontal selection in a Mediterranean cryptogam community with *Fulgensia fulgida*. *New Phytologist* 153: 317–326.

Beiggi, S. & Piercey-Normore, M.D. (2007) Evolution of ITS Ribosomal RNA Secondary Structures in Fungal and Algal Symbionts of Selected Species of *Cladonia* sect. *Cladonia*. *Journal of Molecular Evolution* 64: 528–542. https://doi.org/10.1007/s00239-006-0115-x

Brunner, U. & Honegger, R. (1985) Chemical and ultrastructural studies on the distribution of sporopolleninlike biopolymers in six genera of lichen phycobionts. *Canadian Journal of Botany* 63: 2221–2230. https://doi.org/10.1139/b85-315

Byler, K.A., Carmi-Veal, M., Fine, M. & Goulet, T.L. (2013) Multiple Symbiont Acquisition Strategies as an Adaptive Mechanism in the Coral *Stylophora pistillata*. *PLoS ONE* 8: 1–7. https://doi.org/10.1371/journal.pone.0059596

Casano, L.M., del Campo, E.M., García-Breijo, F.J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., Guéra, A. & Barreno-Rodríguez, E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental microbiology* 13: 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x

Černajová, I. & Škaloud, P. (2019) The first survey of Cystobasidiomycete yeasts in the lichen genus *Cladonia*; with the description of *Lichenozyma pisutiana* gen. nov., sp. nov. *Fungal Biology* 123: 625–637. https://doi.org/10.1016/j.funbio.2019.05.006

Chethana, K.W.T., Jayawardena, R.S., Chen, Y.-J., Konta, S., Tibpromma, S., Phukhamsakda, C., Abeywickrama, P.D., Samarakoon, M.C., Senwanna, C., Mapook, A., Tang, X., Gomdola, D., Marasinghe, D.S., Padaruth, O.D., Balasuriya, A., Xu, J., Lumyong, S. & Hyde, K.D. (2021) Appressorial interactions with host and their evolution. *Fungal Diversity* 110: 75–107. https://doi.org/10.1007/s13225-021-00487-5

Dal Forno, M., Lawrey, J.D., Sikaroodi, M., Gillevet, P.M., Schuettpelz, E. & Lücking, R. (2021) Extensive photobiont sharing in a rapidly radiating cyanolichen clade. *Molecular Ecology* 30: 1755–1776. https://doi.org/10.1111/mec.15700

Dal Grande, F., Rolshausen, G., Divakar, P.K., Crespo, A., Grande, F.D., Otte, J., Schleuning, M. & Schmitt, I. (2017) Environment and host identity structure communities of green algal symbionts in lichens. *New Phytologist* 217: 277–289. https://doi.org/10.1111/nph.14770

Darienko, T., Gustavs, L., Mudimu, O., Menendez, C.R., Schumann, R., Karsten, U., Friedl, T. & Pröschold, T. (2010) *Chloroidium*, a common terrestrial coccoid green alga previously assigned to *Chlorella* (Trebouxiophyceae, Chlorophyta). *European Journal of Phycology* 45: 79–95.

Darienko, T., Lukešová, A. & Pröschold, T. (2018) The polyphasic approach revealed new species of *Chloroidium* (Trebouxiophyceae, Chlorophyta). *Phytotaxa* 372: 51–66. https://doi.org/10.11646/phytotaxa.372.1.4

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Durán, J., Rodríguez, A., Heiðmarsson, S., Lehmann, J.R.K., del Moral, Á., Garrido-Benavent, I. & De los Ríos, A. (2021) Cryptogamic cover determines soil attributes and functioning in polar terrestrial ecosystems. *Science of The Total Environment* 762: 143169. https://doi.org/10.1016/j.scitotenv.2020.143169

Elshobary, M.E., Osman, M.E.H., Abushady, A.M. & Piercey-Normore, M.D. (2015) Comparison of Lichen-Forming Cyanobacterial and Green Algal Photobionts with Free-Living Algae. *Cryptogamie, Algologie* 36: 81–100. https://doi.org/10.7872/crya.v36.iss1.2015.81

Engelen, A., Convey, P. & Ott, S. (2010) Life history strategy of *Lepraria borealis* at an Antarctic inland site, Coal Nunatak. *The Lichenologist* 42: 339–346. https://doi.org/10.1017/S0024282909990600

Fedrowitz, K., Kaasalainen, U. & Rikkinen, J. (2012) Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. *Ecology and evolution* 2: 2291–303. https://doi.org/10.1002/ece3.343

Friedl, T. & Büdel, B. (2008) Photobionts. *In*: T. H. Nash III (ed.) *Lichen Biology*. Cambridge University Press, New York, pp. 9–26.

Fritz-Sheridan, R.P. & Coxson, D.S. (1988) Nitrogen Fixation on a Tropical Volcano, La Soufrière (Guadeloupe): The Interaction of Temperature, Moisture, and Light with Net Photosynthesis and Nitrogenase Activity in *Stereocaulon virgatum* and Response to Periods of Insolation Shock. *The Lichenologist* 20: 63–81. https://doi.org/10.1017/S002428298800009X

Gaßmann, A. & Ott, S. (2000) Growth Strategy and the Gradual Symbiotic Interactions of the Lichen *Ochrolechia frigida*. *Plant Biology* 2: 368–378. https://doi.org/10.1055/s-2000-3711

Gasulla, F., Barrasa, J.M., Casano, L.M. & del Campo, E.M. (2020) Symbiont composition of the basidiolichen *Lichenomphalia meridionalis* varies with altitude in the Iberian Peninsula. *The Lichenologist* 52: 17–26. https://doi.org/10.1017/S002428291900046X

Grimm, M., Grube, M., Schiefelbein, U., Zühlke, D., Bernhardt, J. & Riedel, K. (2021) The Lichens' Microbiota, Still a Mystery? *Frontiers in Microbiology* 12: 1–25. https://doi.org/10.3389/fmicb.2021.623839

Grube, M., Cardinale, M., de Castro, J.V., Müller, H. & Berg, G. (2009) Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *The ISME Journal* 3: 1105–1115. https://doi.org/10.1038/ismej.2009.63

Guindon, S. & Gascuel, O. (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology* 52: 696–704. https://doi.org/10.1080/10635150390235520

Guzow-Krzemińska, B. (2006) Photobiont flexibility in the lichen *Protoparmeliopsis muralis* as revealed by ITS rDNA analyses. *The Lichenologist* 38: 469–476.

Hawksworth, D.L. (2015) Lichenization: The Origins of a Fungal Life-Style. *Recent Advances in Lichenology*. Springer India, New Delhi, pp. 1–10.

Heindel, R.C., Governali, F.C., Spickard, A.M. & Virginia, R.A. (2019) The Role of Biological Soil Crusts in Nitrogen Cycling and Soil Stabilization in Kangerlussuaq, West Greenland. *Ecosystems* 22: 243–256. https://doi.org/10.1007/s10021-018-0267-8

Helms, G. (2003) Taxonomy and Symbiosis in Associations of Physciaceae and *Trebouxia*. Georg-August Universität Göttingen

Högnabba, F. (2006) Molecular phylogeny of the genus *Stereocaulon* (Stereocaulaceae, lichenized ascomycetes). *Mycological research* 110: 1080–1092. https://doi.org/10.1016/j.mycres.2006.04.013

Högnabba, F., Pino-Bodas, R., Nordin, A., Myllys, L. & Stenroos, S. (2014) Phylogenetic position of the crustose *Stereocaulon* species. *The Lichenologist* 46: 103–114. https://doi.org/10.1017/S002428291300073X

Honegger, R. (1986) Ultrastructural studies in lichens. I. Haustorial types and their frequencies in a range of lichens with trebouxioid photobionts. *New Phytologist* 103: 785–795. https://doi.org/10.1111/j.1469-8137.1986.tb00853.x

Del Hoyo, A., Álvarez, R., Del Campo, E.M., Gasulla, F., Barreno, E. & Casano, L.M. (2011) Oxidative stress induces distinct physiological responses in the two *Trebouxia* phycobionts of the lichen *Ramalina farinacea*. *Annals of Botany* 107: 109–118. https://doi.org/10.1093/aob/mcq206

Huss-Danell, K. (1979) The Cephalodia and their Nitrogenase Activity in the Lichen *Stereocaulon paschale*. *Zeitschrift für Pflanzenphysiologie* 95: 431–440. https://doi.org/10.1016/S0044-328X(79)80214-7

Iglesias-Prieto, R., Beltrán, V.H., LaJeunesse, T.C., Reyes-Bonilla, H. & Thomé, P.E. (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings. Biological sciences / The Royal Society* 271: 1757–1763. https://doi.org/10.1098/rspb.2004.2757

Kim, J.I., Kim, Y.J., Nam, S.W., So, J.E., Hong, S.G., Choi, H.G. & Shin, W. (2020) Taxonomic study of three new Antarctic *Asterochloris* (Trebouxiophyceae) based on morphological and molecular data. *Algae* 35: 17–32. https://doi.org/10.4490/algae.2020.35.2.23

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's dictionary of the fungi*. CABI. 784 pp.

Kosecka, M., Guzow-Krzemińska, B., Černajová, I., Škaloud, P., Jabłońska, A. & Kukwa, M. (2021) New lineages of photobionts in Bolivian lichens expand our knowledge on habitat preferences and distribution of *Asterochloris* algae. *Scientific Reports* 11: 8701. https://doi.org/10.1038/s41598-021-88110-0

Krishnamurthy, K. V. & Upreti, D.K. (2001) Reproductive Biology of Lichens. *Reproductive Biology of Plants*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 127–147.

LaJeunesse, T.C. & Thornhill, D.J. (2011) Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through psbA non-coding region genotyping. *PLoS ONE* 6. https://doi.org/10.1371/journal.pone.0029013

Lamb, I.M. (1951) On the morphology, phylogeny, and taxonomy of the lichen genus *Stereocaulon*. *Canadian Journal of Botany* 29: 522–584. https://doi.org/10.1139/b51-045

Lavoie, C., Renaudin, M., McMullin, R.T., Gagnon, J., Roy, C., Beaulieu, M.-E., Bellenger, J.P. & Villarreal, J.C. (2020) Extremely low genetic diversity of *Stigonema* associated with *Stereocaulon* in eastern Canada. *The Bryologist* 123: 188–203. https://doi.org/10.1639/0007-2745-123.2.188

Leavitt, S.D., Kraichak, E., Nelsen, M.P., Altermann, S., Divakar, P.K. & Alors, D. (2015) Fungal specificity and selectivity for algae play a major role in determining lichen partnerships

across diverse ecogeographic regions in the lichen-forming family Parmeliaceae (Ascomycota). *Molecular Ecology* 24: 3779–3797. https://doi.org/10.1111/mec.13271

Leavitt, S.D., Kraichak, E., Vondrák, J., Nelsen, M.P., Sohrabi, M., Perez-Ortega, S., St Clair, L.L. & Thorsten Lumbsch, H. (2016) Cryptic diversity and symbiont interactions in rock-posy lichens. *Molecular Phylogenetics and Evolution* 99: 261–274. https://doi.org/10.1016/j.ympev.2016.03.030

Leavitt, S.D., Nelsen, M.P., Lumbsch, H.T., Johnson, L. a. & St. Clair, L.L. (2013) Symbiont flexibility in subalpine rock shield lichen communities in the Southwestern USA. *The Bryologist* 116: 149–161. https://doi.org/10.1639/0007-2745-116.2.149

Leliaert, F., Smith, D.R., Moreau, H., Herron, M.D., Verbruggen, H., Delwiche, C.F. & De Clerck, O. (2012) Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* 31: 1–46. https://doi.org/10.1080/07352689.2011.615705

Lendemer, J.C., Keepers, K.G., Tripp, E.A., Pogoda, C.S., McCain, C.M. & Kane, N.C. (2019) A taxonomically broad metagenomic survey of 339 species spanning 57 families suggests cystobasidiomycete yeasts are not ubiquitous across all lichens. *American Journal of Botany* 106: 1090–1095. https://doi.org/10.1002/ajb2.1339

Lindgren, H., Moncada, B., Lücking, R., Magain, N., Simon, A., Goffinet, B., Sérusiaux, E., Nelsen, M.P., Mercado-Díaz, J.A., Widhelm, T.J. & Lumbsch, H.T. (2020) Cophylogenetic patterns in algal symbionts correlate with repeated symbiont switches during diversification and geographic expansion of lichen-forming fungi in the genus *Sticta* (Ascomycota, Peltigeraceae). *Molecular Phylogenetics and Evolution* 150: 106860. https://doi.org/10.1016/j.ympev.2020.106860

Lücking, R. (2019) Stop the Abuse of Time! Strict Temporal Banding is not the Future of Rank-Based Classifications in Fungi (Including Lichens) and Other Organisms. *Critical Reviews in Plant Sciences* 38: 199–253. https://doi.org/10.1080/07352689.2019.1650517

Lücking, R., Hodkinson, B.P. & Leavitt, S.D. (2017) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* 119: 361. https://doi.org/10.1639/0007-2745-119.4.361

Lücking, R., Lawrey, J.D., Sikaroodi, M., Gillevet, P.M., Chaves, J.L., Sipman, H.J.M. & Bungartz, F. (2009) Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. *American journal of botany* 96: 1409–18. https://doi.org/10.3732/ajb.0800258

Magain, N., Goffinet, B. & Sérusiaux, E. (2012) Further photomorphs in the lichen family Lobariaceae from Reunion (Mascarene archipelago) with notes on the phylogeny of *Dendriscocaulon* cyanomorphs. *The Bryologist* 115: 243–254. https://doi.org/10.1639/0007-2745-115.2.243

Mark, K., Laanisto, L., Bueno, C.G., Niinemets, Ü., Keller, C. & Scheidegger, C. (2020) Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *New Phytologist* 227: 1362–1375. https://doi.org/10.1111/nph.16475

McCormick, P. V. & Stevenson, R.J. (1991) Mechanisms of Benthic Algal Succession in Lotic Environments. *Ecology* 72: 1835–1848. https://doi.org/10.2307/1940982

Medeiros, I.D., Fryday, A.M. & Rajakaruna, N. (2014) Additional lichen records and mineralogical data from metal-contaminated sites in Maine. *Rhodora* 116: 323–347. https://doi.org/10.3119/13-26

Melechin, A. (2015) Records of new and rare lichens for Svalbard. *Graphis Scripta* 27: 56–58.

Meunier, J.D., Kirman, S., Strasberg, D., Grauby, O. & Dussouillez, P. (2014) Incipient weathering by *Stereocaulon vulcani* at Réunion volcanic island. *Chemical Geology* 382: 123–131. https://doi.org/10.1016/j.chemgeo.2014.05.033

Molins, A., Chiva, S., Calatayud, Á., Marco, F., García-Breijo, F., Reig-Armiñana, J., Carrasco, P. & Moya, P. (2020) Multidisciplinary approach to describe *Trebouxia* diversity within lichenized fungi *Buellia zoharyi* from the Canary Islands. *Symbiosis* 82: 19–34. https://doi.org/10.1007/s13199-020-00722-8

Molins, A., Moya, P., García-Breijo, F.J., Reig-Armiñana, J. & Barreno, E. (2018) Molecular and morphological diversity of *Trebouxia* microalgae in sphaerothallioid *Circinaria* spp. lichens. T. Mock (ed.). *Journal of Phycology* 54: 494–504. https://doi.org/10.1111/jpy.12751

Moya, P., Chiva, S., Molins, A., Garrido-Benavent, I. & Barreno, E. (2021) Unravelling the Symbiotic Microalgal Diversity in *Buellia zoharyi* (Lichenized Ascomycota) from the Iberian Peninsula and Balearic Islands Using DNA Metabarcoding. *Diversity* 13: 220. https://doi.org/10.3390/d13060220

Moya, P., Molins, A., Chiva, S., Bastida, J. & Barreno, E. (2020) Symbiotic microalgal diversity within lichenicolous lichens and crustose hosts on Iberian Peninsula gypsum biocrusts. *Scientific Reports* 10: 14060. https://doi.org/10.1038/s41598-020-71046-2

Moya, P., Molins, A., Martinez-Alberola, F., Muggia, L. & Barreno, E. (2017) Unexpected associated microalgal diversity in the lichen *Ramalina farinacea* is uncovered by pyrosequencing analyses. *PLoS ONE* 12: 1–21. https://doi.org/10.1371/journal.pone.0175091

Moya, P., Škaloud, P., Chiva, S., García-Breijo, F.J., Reig-Armiñana, J., Vančurová, L. & Barreno, E. (2015) Molecular phylogeny and ultrastructure of the lichen microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands ecosystems. *International journal of systematic and evolutionary microbiology* 65: 1838–54. https://doi.org/10.1099/ijs.0.000185

Muggia, L., Baloch, E., Stabentheiner, E., Grube, M. & Wedin, M. (2011) Photobiont association and genetic diversity of the optionally lichenized fungus *Schizoxylon albescens*. *FEMS Microbiology Ecology* 75: 255–272. https://doi.org/10.1111/j.1574-6941.2010.01002.x

Muggia, L., Perez-Ortega, S., Kopun, T., Zellnig, G. & Grube, M. (2014) Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Annals of Botany* 114: 463–475. https://doi.org/10.1093/aob/mcu146

Muggia, L., Vančurová, L., Škaloud, P., Peksa, O., Wedin, M. & Grube, M. (2013) The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS microbiology ecology* 85: 313–23. https://doi.org/10.1111/1574-6941.12120

Nelsen, M.P. & Gargas, A. (2006) Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta: Trebouxiophyceae). *The Lichenologist* 38: 435–440. https://doi.org/10.1017/S0024282906005779

Nelsen, M.P. & Gargas, A. (2008) Dissociation and horizontal transmission of codispersing lichen symbionts in the genus *Lepraria* (Lecanorales: Stereocaulaceae). *The New phytologist* 177: 264–275. https://doi.org/10.1111/j.1469-8137.2007.02241.x

Nelsen, M.P., Lücking, R., Boyce, C.K., Lumbsch, H.T. & Ree, R.H. (2020) No support for the emergence of lichens prior to the evolution of vascular plants. *Geobiology* 18: 3–13. https://doi.org/10.1111/gbi.12369

Noh, H.-J., Lee, Y.M., Park, C.H., Lee, H.K., Cho, J.-C. & Hong, S.G. (2020) Microbiome in *Cladonia squamosa* Is Vertically Stratified According to Microclimatic Conditions. *Frontiers in Microbiology* 11: 1–13. https://doi.org/10.3389/fmicb.2020.00268

Nyati, S., Bhattacharya, D., Werth, S. & Honegger, R. (2013) Phylogenetic analysis of LSU and SSU rDNA group I introns of lichen photobionts associated with the genera *Xanthoria* and *Xanthomendoza* (Teloschistaceae, lichenized Ascomycetes). *Journal of phycology* 49: 1154–1166. https://doi.org/10.1111/jpy.12126

Ohmura, Y., Takeshita, S. & Kawachi, M. (2019) Photobiont diversity within populations of a vegetatively reproducing lichen, *Parmotrema tinctorum*, can be generated by photobiont switching. *Symbiosis* 77: 59–72. https://doi.org/10.1007/s13199-018-0572-1

Oksanen, I., Jokela, J., Fewer, D.P., Wahlsten, M., Rikkinen, J. & Sivonen, K. (2004) Discovery of Rare and Highly Toxic Microcystins from Lichen-Associated Cyanobacterium *Nostoc* sp. Strain IO-102-I. *Applied and Environmental Microbiology* 70: 5756–5763. https://doi.org/10.1128/AEM.70.10.5756-5763.2004

de Oliveira Torres, V. (2021) Variações morfológicas, químicas e genéticas de *Stereocaulon alpinum* Laurer (Stereocaulaceae) e seus fotobiontes da região sul da América do Sul e Antártica Marítima. Universidade Federal de Mato Grosso do Sul

Onuţ-Brännström, I., Benjamin, M., Scofield, D.G., Heiðmarsson, S., Andersson, M.G.I., Lindström, E.S. & Johannesson, H. (2018) Sharing of photobionts in sympatric populations of *Thamnolia* and *Cetraria* lichens: evidence from high-throughput sequencing. *Scientific Reports 2018* 8: 4406. https://doi.org/10.1038/s41598-018-22470-y

Osyczka, P., Lenart-Boroń, A., Boroń, P. & Rola, K. (2020) Lichen-forming fungi in postindustrial habitats involve alternative photobionts. *Mycologia* 00: 1–13. https://doi.org/10.1080/00275514.2020.1813486

Otálora, M.A.G., Martínez, I., O'Brien, H., Molina, M.C., Aragón, G. & Lutzoni, F. (2010) Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution* 56: 1089–1095. https://doi.org/10.1016/j.ympev.2010.05.013

Pangallo, D., Bučková, M., Kraková, L., Puškárová, A., Šaková, N., Grivalský, T., Chovanová, K. & Zemánková, M. (2015) Biodeterioration of epoxy resin: a microbial survey through culture-independent and culture-dependent approaches. *Environmental microbiology* 17: 462–79. https://doi.org/10.1111/1462-2920.12523

Paracer, S. & Ahmadjian, V. (2000) *Symbiosis: An Introduction to Biological Associations*. 2nd edition. Oxford University Press, Inc., New York. 291 pp.

Park, C.H., Kim, K.M., Elvebakk, A., Kim, O.-S., Jeong, G. & Hong, S.G. (2015) Algal and Fungal Diversity in Antarctic Lichens. *Journal of Eukaryotic Microbiology* 62: 196–205. https://doi.org/10.1111/jeu.12159

Paul, F., Otte, J., Schmitt, I. & Dal Grande, F. (2018) Comparing Sanger sequencing and high-throughput metabarcoding for inferring photobiont diversity in lichens. *Scientific Reports* 8: 8624. https://doi.org/10.1038/s41598-018-26947-8

Peksa, O., Gebouská, T., Škvorová, Z., Vančurová, L. & Škaloud, P. Photobiont-mediated guilds in green algal lichens – an insight into the life of terrestrial symbiotic communities. *FEMS Microbiology Ecology* in press.

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology* 20: 3936–48. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Pettay, D.T., Wham, D.C., Smith, R.T., Iglesias-Prieto, R. & LaJeunesse, T.C. (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proceedings of the National Academy of Sciences of the United States of America* 112: 7513–7518. https://doi.org/10.1073/pnas.1502283112

Piercey-Normore, M.D. (2009) Vegetatively reproducing fungi in three genera of the Parmeliaceae share divergent algal partners. *The Bryologist* 112: 773–785. https://doi.org/10.1639/0007-2745-112.4.773

Piercey-Normore, M.D. & DePriest, P.T. (2001) Algal Switching among Lichen Symbioses. *American Journal of Botany* 88: 1490–1498. https://doi.org/10.2307/3558457

Piercey-Normore, M.D. (2006) The lichen-forming ascomycete *Evernia mesomorpha* associates with multiple genotypes of *Trebouxia jamesii*. *New Phytologist* 169: 331–344. https://doi.org/10.1111/j.1469-8137.2005.01576.x

Pino-Bodas, R. & Stenroos, S. (2020) Global Biodiversity Patterns of the Photobionts Associated with the Genus *Cladonia* (Lecanorales, Ascomycota). *Microbial Ecology*. https://doi.org/10.1007/s00248-020-01633-3

Purvis, O.W. & Halls, C. (1996) A Review of Lichens in Metal-Enriched Environments. *The Lichenologist* 28: 571. https://doi.org/10.1017/S0024282996000758

Rambold, G., Friedl, T. & Beck, A. (1998) Photobionts in Lichens: Possible Indicators of Phylogenetic Relationships? *Bryologist, The* 101: 392–397.

Rikkinen, J., Oksanen, I. & Lohtander, K. (2002) Lichen guilds share related cyanobacterial symbionts. *Science* 297: 357. https://doi.org/10.1126/science.1072961

Rolshausen, G., Hallman, U., Grande, F.D., Otte, J., Knudsen, K. & Schmitt, I. (2020) Expanding the mutualistic niche: parallel symbiont turnover along climatic gradients. *Proceedings of the Royal Society B: Biological Sciences* 287: 20192311. https://doi.org/10.1098/rspb.2019.2311

Romeike, J., Friedl, T., Helms, G. & Ott, S. (2002) Genetic Diversity of Algal and Fungal Partners in Four Species of *Umbilicaria* (Lichenized Ascomycetes) Along a Transect of the Antarctic Peninsula. *Molecular Biology* 19: 1209–1217.

Rowan, R. (2004) Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* 430: 742. https://doi.org/10.1038/430742a

Ruprecht, U., Brunauer, G. & Türk, R. (2014) High photobiont diversity in the common European soil crust lichen *Psora decipiens*. *Biodiversity and conservation* 23: 1771–1785. https://doi.org/10.1007/s10531-014-0662-1

Sadowsky, A., Hussner, A. & Ott, S. (2012) Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane, Norway. *Nova Hedwigia* 94: 323–334. https://doi.org/10.1127/0029-5035/2012/0014

Sanders, W.B. & Masumoto, H. (2021) Lichen algae: the photosynthetic partners in lichen symbioses. *The Lichenologist* 53: 347–393. https://doi.org/10.1017/S0024282921000335

Sanders, W.B., Pérez-Ortega, S., Nelsen, M.P., Lücking, R. & de los Ríos, A. (2016) *Heveochlorella* (Trebouxiophyceae): a little-known genus of unicellular green algae outside the Trebouxiales emerges unexpectedly as a major clade of lichen photobionts in foliicolous communities. O. DeClerck (ed.). *Journal of Phycology* 52: 840–853. https://doi.org/10.1111/jpy.12446

Schoch, C.L., Sung, G.H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., Sybren De Hoog, G., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lücking, R., Büdel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D.M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P.S., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F. & Spatafora, J.W. (2009) The Ascomycota Tree of Life: A Phylum-wide Phylogeny Clarifies the Origin and Evolution of Fundamental Reproductive and Ecological Traits. *Systematic Biology* 58: 224–239. https://doi.org/10.1093/sysbio/syp020

Singh, G., Dal Grande, F., Divakar, P.K., Otte, J., Crespo, A. & Schmitt, I. (2017) Fungal–algal association patterns in lichen symbiosis linked to macroclimate. *New Phytologist* 214: 317–329. https://doi.org/10.1111/nph.14366

Singh, R., Ranjan, S., Nayaka, S., Pathre, U. V. & Shirke, P. a. (2013) Functional characteristics of a fruticose type of lichen, *Stereocaulon foliolosum* Nyl. in response to light and water stress. *Acta Physiologiae Plantarum* 35: 1605–1615. https://doi.org/10.1007/s11738-012-1203-8

Škaloud, P. & Peksa, O. (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris*. *Molecular Phylogenetics and Evolution* 54: 36–46. https://doi.org/10.1016/j.ympev.2009.09.035

Škaloud, P., Steinová, J., Řídká, T., Vančurová, L. & Peksa, O. (2015) Assembling the challenging puzzle of algal biodiversity: species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 51: 507–527. https://doi.org/10.1111/jpy.12295

Škvorová, Z., Černajová, I., Steinová, J., Peksa, O., Moya, P. & Škaloud, P. Promiscuity in lichens follows clear rules: partner switching in *Cladonia* is regulated by climatic factors and soil chemistry. *Frontiers in Microbiology* in press. https://doi.org/10.3389/fmicb.2021.781585

Smith, H.B., Dal Grande, F., Muggia, L., Keuler, R., Divakar, P.K., Grewe, F., Schmitt, I., Lumbsch, H.T. & Leavitt, S.D. (2020) Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis. *Symbiosis* 82: 133–147. https://doi.org/10.1007/s13199-020-00699-4

Smith, R.I.L. & Øvstedal, D.O. (1991) The lichen genus *Stereocaulon* in Antarctica and South Georgia. *Polar Biology* 11. https://doi.org/10.1007/BF00234271

Spribille, T., Pérez-Ortega, S., Tønsberg, T. & Schirokauer, D. (2010) Lichens and lichenicolous fungi of the Klondike Gold Rush National Historic Park, Alaska, in a global biodiversity context. *The Bryologist* 113: 439–515. https://doi.org/10.1639/0007-2745-113.3.439

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C., Schneider, K., Stabentheiner, E., Toome-Heller, M., Thor, G., Mayrhofer, H., Johannesson, H. & McCutcheon, J.P. (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492. https://doi.org/10.1126/science.aaf8287

Steinová, J., Škaloud, P., Yahr, R., Bestová, H. & Muggia, L. (2019) Reproductive and dispersal strategies shape the diversity of mycobiont-photobiont association in *Cladonia* lichens. *Molecular Phylogenetics and Evolution* 134: 226–237. https://doi.org/10.1016/j.ympev.2019.02.014

Stretch, R.. & Viles, H.. (2002) The nature and rate of weathering by lichens on lava flows on Lanzarote. *Geomorphology* 47: 87–94. https://doi.org/10.1016/S0169-555X(02)00143-5

Thüs, H., Muggia, L., Pérez-Ortega, S., Favero-Longo, S.E., Joneson, S., Brien, H.O., Nelsen, M.P., Grube, M., Friedl, T., Brodie, J., Andrew, C.J., Lutzoni, F. & Gueidan, C. (2011) Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota). *European Journal of Phycology* 46: 399–415. https://doi.org/10.1080/09670262.2011.629788

Tibell, L. (2001) Photobiont Association and Molecular Phylogeny of the Lichen Genus *Chaenotheca*. *The Bryologist* 104: 191–198.

Tschermak-Woess, E. (1948) Über wenig bekannte und neue Flechtengonidien. I. *Chlorella ellipsoidea* Gerneck, als neue Flechtenalge. *Österreichische botanische Zeitschrift* 95: 341–343.

Tschermak-Woess, E. (1978) Über den *Chlorella*-Phycobionten von *Trapelia coarctata*. *Plant Systematics and Evolution* 130: 253–263.

Tschermak-Woess, E. (1988a) New and known taxa of *Chlorella* (Chlorophyceae): Occurrence as lichen phycobionts and observations on living dictyosomes. *Plant Systematics and Evolution* 159: 123–139.

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

Vaiglová, Z. (2017) Dynamics of a lichen symbiosis. Charles University

Vančurová, L., Kalníková, V., Peksa, O., Škvorová, Z., Malíček, J., Moya, P., Chytrý, K., Černajová, I. & Škaloud, P. (2020) Symbiosis between river and dry lands: Phycobiont dynamics on river gravel bars. *Algal Research* 51: 102062. https://doi.org/10.1016/j.algal.2020.102062

Vančurová, L., Malíček, J., Steinová, J. & Škaloud, P. (2021) Choosing the Right Life Partner: Ecological Drivers of Lichen Symbiosis. *Frontiers in Microbiology* 12: 769304. https://doi.org/10.3389/fmicb.2021.769304

Vančurová, L., Muggia, L., Peksa, O., Řídká, T. & Škaloud, P. (2018) The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota). *Molecular Ecology* 27: 3016–3033.

https://doi.org/10.1111/mec.14764

Vančurová, L., Peksa, O., Němcová, Y. & Škaloud, P. (2015) *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain. *Phytotaxa* 219: 118–132. https://doi.org/10.11646/phytotaxa.219.2.2

Vargas Castillo, R. & Beck, A. (2012) Photobiont selectivity and specificity in *Caloplaca* species in a fog-induced community in the Atacama Desert, northern Chile. *Fungal Biology* 116: 665–676. https://doi.org/10.1016/j.funbio.2012.04.001

Voytsekhovich, A. & Beck, A. (2015) Lichen photobionts of the rocky outcrops of Karadag massif (Crimean Peninsula). *Symbiosis* 66: 1–16. https://doi.org/10.1007/s13199-015-0346-y

Voytsekhovich, A., Dymytrova, L. & Nadyeina, O. (2011) Photobiont composition of some taxa of the genera *Micarea* and *Placynthiella* (Lecanoromycetes, lichenized Ascomycota) from Ukraine. *Folia Cryptogamica Estonica* 48: 135–148.

Wedin, M., Maier, S., Fernandez-Brime, S., Cronholm, B., Westberg, M. & Grube, M. (2015) Microbiome change by symbiotic invasion in lichens. *Environmental Microbiology* 18: 1428–1439. https://doi.org/10.1111/1462-2920.13032

Werth, S. & Scheidegger, C. (2012) Congruent Genetic Structure in the Lichen-Forming Fungus *Lobaria pulmonaria* and Its Green-Algal Photobiont. *Molecular Plant-Microbe Interactions* 25: 220–230.

Williams, L., Colesie, C., Ullmann, A., Westberg, M., Wedin, M. & Büdel, B. (2017) Lichen acclimation to changing environments: Photobiont switching vs. climate-specific uniqueness in *Psora decipiens*. *Ecology and Evolution* 7: 2560–2574. https://doi.org/10.1002/ece3.2809

Wirtz, N., Lumbsch, H.T., Green, T.G.A., Türk, R., Pintado, A., Sancho, L. & Schroeter, B. (2003) Lichen fungi have low cyanobiont selectivity in maritime Antarctica. *New Phytologist* 160: 177–183. https://doi.org/10.1046/j.1469-8137.2003.00859.x

Yahr, R., Florence, A., Škaloud, P. & Voytsekhovich, A. (2015) Molecular and morphological diversity in photobionts associated with *Micarea* s. str (Lecanorales, Ascomycota). *The Lichenologist* 47: 403–414. https://doi.org/10.1017/S0024282915000341

Yahr, R., Vilgalys, R. & Depriest, P.T. (2004) Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. *Molecular Ecology* 13: 3367–3378. https://doi.org/10.1111/j.1365-294X.2004.02350.x

Zoller, S. & Lutzoni, F. (2003) Slow algae, fast fungi: Exceptionally high nucleotide substitution rate differences between lichenized fungi *Omphalina* and their symbiotic green algae *Coccomyxa*. *Molecular Phylogenetics and Evolution* 29: 629–640. https://doi.org/10.1016/S1055-7903(03)00215-X

6. Original papers

Vulcanochloris (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain

Lucie Vančurová[1]*, Ondřej Peksa[2], Yvonne Němcová[1] & Pavel Škaloud[1]

[1]*Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Prague 2, Czech Republic*

[2]*The West Bohemian Museum in Pilsen, Kopeckého sady 2, 301 00 Plzeň, Czech Republic*

** Corresponding author (E-mail: lucie.vancurova@natur.cuni.cz)*

Abstract

This paper describes a new genus of lichen photobionts, *Vulcanochloris*, with three newly proposed species, *V. canariensis*, *V. guanchorum* and *V. symbiotica*. These algae have been discovered as photobionts of lichen *Stereocaulon vesuvianum* growing on slopes of volcanos and lava fields on La Palma, Canary Islands, Spain. Particular species, as well as the newly proposed genus, are delimited based on ITS rDNA, 18S rDNA and *rbcL* sequences, chloroplast morphology, and ultrastructural features. Phylogenetic analyses infer the genus *Vulcanochloris* as a member of Trebouxiophycean order Trebouxiales, in a sister relationship with the genus *Asterochloris*. Our data point to the similar lifestyle and morphology of these two genera; however, *Vulcanochloris* can be well distinguished by a unique formation of spherical incisions within the pyrenoid. Mycobiont specificity and geographical distribution of the newly proposed genus is further discussed.

Introduction

The class Trebouxiophyceae, originally circumscribed by ultrastructural features as Pleurastrophyceae, is currently defined phylogenetically, predominantly by a similarity in 18S rDNA sequence data. As presently conceived, the class comprises single-celled, colonial and multicellular algae living mainly in freshwater or terrestrial habitats (Leliaert *et al.* 2012). Many members of this class are able to make symbiotic relationships. For example, species of genera *Elliptochloris* Tschermak-Woess (1980b: 71) and *Chlorella* Beyerinck (1890: 758) have been reported as symbionts of invertebrates (Letsch *et al.* 2009, Hoshina *et al.* 2010). The class is generally known to comprise the majority of eukaryotic lichen symbionts, *i.e.*, the phycobionts. The genera *Trebouxia* Puymaly (1924: 109), *Asterochloris* Tschermak-Woess (1980a: 291), *Coccomyxa* Schmidle (1901: 23) and *Myrmecia* Printz (1921: 13) are among the most common photobionts worldwide (Friedl & Büdel 2008, Tschermak-Woess 1988). However, due to a simple morphology and small cell sizes, diversity of Trebouxiophycean algae is still poorly understood. Indeed, a number of new species and genera are still being discovered (Hoshina *et al.* 2010, Neustupa *et al.* 2011, 2013, Gaysina *et al.* 2013). Many findings of new taxa could be expected also among lichen photobionts, mainly among "*Chlorella*-like" lichenized algae (Friedl & Bhattacharya 2002, Nyati *et al.* 2007, Thüs *et al.* 2011).

The Canary Islands are famous for their extraordinary diversity of vascular plants. Among the free-living algae attention was almost exclusively paid to marine representatives (Bouza *et al.* 2006, García-Jiménez *et al.* 2008, Cassano *et al.* 2012). The diversity of lichenized algae has been studied only marginally, as a part of the studies investigating the photobiont diversity of *Tephromela atra* (Hudson 1762: 445) Hafellner (1983: No. 297) (Muggia *et al.* 2010), *Ramalina farinacea* (Linnaeus 1753: 1146) Acharius (1810: 606) (Casano *et al.* 2011, Campo *et al.* 2013), *Lecanora rupicola* (Linnaeus 1767: 132) Zahlbruckner (1928: 525), *L. carpinea* (Linnaeus 1753: 1141) Vainio (1888: 23) (Blaha *et al.* 2006) and *Parmotrema pseudotinctorum* (Abbayes 1951: 973) Hale (1974: 338) (Molins *et al.* 2013). The Canary Islands are known to host a high diversity of lichens and lichenicolous organisms. The most recent checklist lists more than 1600 species for an area of just 7447 km² (Hernández Padrón & Pérez-Vargas 2010). One of the most abundant lichens of Canary Islands, *Stereocaulon vesuvianum* Persoon (1810: 19), has been subjected to a study investigating its role in rock weathering processes (Stretch & Viles 2002). However, no study has been performed to explore the photobiont diversity in this remarkable lichen species, so far.

During our recent investigation of lichen symbionts on slopes of volcanos and lava fields on La Palma, Canary Islands, we discovered a new photobiont lineage in several thalli of *Stereocaulon vesuvianum*. The main goal of this study is to describe this lineage as a new genus of Trebouxiophycean algae, *Vulcanochloris*, and to characterize its three newly proposed species, *Vulcanochloris canariensis*, *V. guanchorum* and *V. symbiotica*.

Material and Methods

The material was collected in October 2011 and May 2013 on La Palma (Canary Islands, Spain) on volcanos, lava fields and lava flows (Table 1). Photobionts were isolated by the thallus fragment method (Ahmadjian 1993) and cultivated as described in Peksa & Škaloud (2008).

For transmission electron microscopy (TEM) investigations, the samples were fixed for 2 h at 5°C in 2% glutaraldehyde in 0.05 M phosphate buffer. Then, they were post-fixed for 2 h in 1% OsO_4 in 0.05 M phosphate buffer and for 12 h at 5 °C in 1% uranyl acetate solution. Then, the samples were dehydrated through an ethanol series and finally, they were embedded in Spurr's medium via isobutanol. Ultrathin sections, cut with a diamond knife were post-stained with lead citrate and examined using a JEOL 1011 transmission electron microscope.

Total genomic DNA was extracted from fragments of thalli following the modified CTAB protocol (Cubero *et al.* 1999), with minor modifications. Three molecular markers were amplified by PCR: nuclear ITS and 18S rDNA, and chloroplast *rbcL*. The internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) was amplified using the algal-specific primer nr-SSU-1780-5' (5'-CTG CGG AAG GAT CAT TGA TTC-3'; Piercey-Normore & DePriest 2001) and a universal primer ITS4-3' (5'-TCC TCC GCT TAT TGA TAT GC-3'; White *et al.* 1990). PCR amplification of the algal ITS began with an initial denaturation at 94 °C for 5 min, and was followed by 35 cycles of denaturing at 94 °C for 1 min, annealing at 50 °C for 1 min and elongation at 72 °C for 2 min, with a final extension at 72 °C for 10 min. The amplification of *rbcL* region was performed as described by Thüs *et al.* (2011) using primers PRASF1-5' (5'-ATG GTT CCA CAA ACA GAA AC-3') and PRASR1-3' (5'-TTG TCA ATA GTA TCA AAT TC-3'; Sherwood *et al.* 2000) or a-ch-rbcL-203- 5'-MPN-5' (5'-GAA TCW TCW ACW GGW ACT TGG ACW AC-3') and a-ch-rbcL-991-3'-MPN-3' (5'-CCT TCT ART TTA CCW ACA AC-3'; Nelsen *et al.* 2011). The amplification of 18S rDNA was performed as described by Thüs *et al.* (2011) using primers 18S F- 5' (5'-AAC CTG GTT GAT CCT GCC AGT-3'; Katana *et al.* 2001) and newly designed 1650R-Astero-3' (5'-TCA CCA GCA CGT CCA AT-3') for first part of 18S rDNA region; and primers Al 1500af-5' (5'-GCG CGC TAC ACT GAT GC-3'; Helms *et al.* 2001) and ITS4-3' (5'-TCC TCC GCT TAT TGA TAT GC-3'; White *et al.* 1990) for second part of 18S rDNA region. PCR reactions were performed in a volume of 20 µL with Red Taq Polymerase (Sigma) as described by Peksa & Škaloud (2011) or with My Taq Polymerase (11.8 µL sterile Milli-Q Water, 4 µL 5x My Taq PCR buffer (Bioline), 0.5 µL of primers (25 $pM.mL^{-1}$), 0.2 µL My Taq HS DNA Polymerase (Bioline) (1 $U.mL^{-1}$), 3 µL of DNA (not quantified)). The PCR products were purified and sequenced at Macrogen in Seoul, Korea. The newly obtained sequences of the ITS rDNA, 18S rDNA and *rbcL* regions were deposited in GenBank with the accession numbers KR952309–KR952331.

Sequences of the *rbcL* gene were selected primarily based on the dataset of Fučíková *et al.* (2014b), and based on BLAST searches of our newly collected sequences. The outgroup was composed of ten representatives of order Chlorellales, which appears to be outside the Trebouxiophyceae according to recent research (Fučíková *et al.* 2014a). Alignment was produced manually by using MEGA6 (Tamura *et al.* 2013).

TABLE 1. Localities of specimens of *Stereocaulon vesuvianum* from La Palma, Canary Islands, Spain.

Specimen	Locality	Substrate	Elevation	GPS	Date	GenBank accession		
						*rbc*L	SSU rDNA	ITS rDNA
L1616	Volcán de San Antonio	lava stone	about 630	28.481944° N, 17.849444° W	16/10/2011	KR952309		KR952317
L1617	Volcán de San Antonio	lava stone	about 500	28.481389° N, 17.845556° W	16/10/2011			KR952318
L1618	Volcán de San Antonio	lava stone	about 500	28.481389° N, 17.845556° W	16/10/2011	KR952310	KR952314	KR952319
L1620	Volcán Teneguía	lava stone	about 330	28.473056° N, 17.847222° W	16/10/2011	KR952311	KR952315	KR952320
A72	Volcán de San Antonio	top of the volcano	589	28.485500° N, 17.849917° W	17/5/2013			KR952321
A73	Volcán de San Antonio	top of the volcano	589	28.485500° N, 17.849917° W	17/5/2013			KR952322
A74	Volcán de San Antonio	lava stone	575	28.487167° N, 17.849139° W	17/5/2013			KR952323
A75	Volcán de San Antonio	lava stone	550	28.486511° N, 17.849786° W	17/5/2013			KR952324
A77	foothill of Volcán de San Antonio	lava stone	399	28.477694° N, 17.850361° W	17/5/2013			KR952325
A78	Volcán Teneguía	lava	396	28.474722° N, 17.851028° W	17/5/2013			KR952326
A80	foothill of Volcán Teneguía	lava stone	188	28.464139° N, 17.845333° W	17/5/2013			KR952327
A97	3 km to the East of El Paso	rock on edge of lava field	849	28.653167° N, 17.851194° W	19/5/2013			KR952328
A98	3 km to the East of El Paso	lava field	860	28.652800° N, 17.851200° W	19/5/2013	KR952312		KR952329
A104	2.5 km to the north-east Puerto de Naos	lava field	400	28.604722° N, 17.895389° W	20/5/2013	KR952313	KR952316	KR952330
A105	2.5 km to the north-east Puerto de Naos	lava field	463	28.598806° N, 17.89338° W	20/5/2013			KR952331

Sequences of 18S rDNA were selected primarily based on the dataset of Škaloud *et al.* 2015. Ingroup comprises 36 sequences of representatives of Trebouxiales order, including three newly obtained sequences. *Lobosphaera incisa* (AY762602) was selected as outgroup. The sequences were aligned using MAFFT version 7 software (Katoh & Standley 2013) under the Q-INS-I strategy. The ITS rDNA data set consisted of 31 sequences: 15 newly obtained sequences from La Palma, three highly similar sequences from NCBI and 13 representatives of main lineages of *Asterochlosis* genus. Alignment was produced manually according to the secondary structures of ITS2 of *Asterochloris* (Škaloud & Peksa 2010) by using MEGA6 (Tamura *et al.* 2013).

The phylogenetic trees were inferred with Bayesian Inference (BI) by using MrBayes v. 3.2.2 (Huelsenbeck & Ronquist 2001), maximum likelihood (ML) analysis using GARLI v. 2.0 (Zwickl 2006), and maximum parsimony (MP) analysis using PAUP v. 4.0b10 (Swofford 2003), respectively. BI and ML analysis were carried out on a partitioned dataset to differentiate among individual *rbcL* codon positions or ITS1, 5.8 S and ITS2 rDNA regions. Substitution models were selected using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Guindon & Gascuel 2003, Darriba *et al.* 2012): for the first *rbcL* codon position TIM1+I+Γ (gamma shape 0.8380), second *rbcL* codon position TVMef+I+Γ (gamma shape 0.3960), third *rbcL* codon position TVM+I+Γ (gamma shape 0.9800), 18S rDNA TrNef+I, ITS1 TrNef+Γ (gamma shape 1.0700), 5.8S JC, ITS2 TPM3+Γ (gamma shape 0.2540). ML analysis was carried out using default settings, five search replicates, and the automatic termination set at 10^5 generations. The MP analysis was performed using heuristic searches with 1000 random sequence addition replicates and random addition of sequences (the number was limited to 10^4 for each replicate). ML and MP bootstrap support values were obtained from 100 and 1000 bootstrap replicates, respectively. Only one search replicate was applied for the ML bootstrapping.

To compare alternative phylogenetic topologies, the one-tailed Shimodaira-Hasegawa nonparametric tests (SH tests; Shimodaira & Hasegawa 1999). For the tests, ML trees were calculated with specified topological constraints using GARLI v. 2.0. Thereafter, trees with topological constraints were compared with the optimal topology using the SH test statistics, inferred with the RELL bootstrap option, as implemented in PAUP v. 4.0b10.

Results

Phylum **Chlorophyta**

Class **Trebouxiophyceae**

Order **Trebouxiales**

Family **Trebouxiaceae**

Vulcanochloris Vančurová, Peksa, Němcová *et* Škaloud, *gen. nov.*

Vegetative cells spherical, rarely oval or oviform. Cell wall thin, seldom a flat local thickening of the cell wall can be observed. Rarely, the cell wall is slightly thickened along its entire surface. Cells contain a single asteroid chloroplast, with a distinct pyrenoid in its centre. The pyrenoid often contains one to several spherical incisions. Prior to aplano- and zoosporogenesis, the chloroplast flattens and assumes a parietal position. Asexual reproduction by 16–128 aplanospores or 64–128 zoospores. Zoospores naked, with two apical flagella and a simple basal chloroplast; stigma not observed. Mature aplanospores and zoospores liberated by rupturing of the mother cell wall. Lichen photobiont, so far found only in thalli of *Stereocaulon vesuvianum*. Morphologically similar to *Asterochloris*, from which it differs by the presence of spherical incisions in the pyrenoid matrix.

Type species:—*Vulcanochloris canariensis*, *sp. nov.* (see below)

Etymology:—From "Vulcanus" (L), Roman god of fire, and "chloris" (Gr.), meaning greenish-yellow. The name indicates that this algal genus was originally reported from a volcanic substrate.

Chloroplast morphology and ultrastructure:—The chloroplast is centrally located, axial, with variously arranged lobes reaching the cell periphery. Several chloroplast types can be recognized, as follows: i) a deeply lobed type, characterized by long lobes emerging directly from the thin chloroplast layer spreading around the pyrenoid ("Tieflappig Typ" *sensu* Gärtner 1985b; Figs. 1A, B); ii) a shallowly lobed type, which is similar to the previous type but differs in that the chloroplast lobes are shorter, emerging from the central mass of the chloroplast layer ("Normaltyp" *sensu* Gärtner 1985b; Figs. 1C, D); iii) a crenulate type, characterized by a central, massive chloroplast with a regularly nodulated surface ("Crenulater Typ" *sensu* Gärtner 1985a; Figs. 1E, F); and vi) an echinate type, distinguished by numerous thin radial lobes emerging uniformly from the central mass of the chloroplast layer (Figs. 1G, H). In the late ontogenetic stages, specifically prior to zoo- or aplanosporogenesis, the chloroplast transforms into the parietal type, with smooth, never lobed margins, which is followed by its division into numerous parts in preparation for asexual reproduction (Fig. 1I).

Large, distinct pyrenoid lies in the chloroplast centre (Figs. 1A, C, E, G). The pyrenoid is usually spherical, rarely irregularly elongated, surrounded by a high number of small starch grains (Figs. 1C, J). The pyrenoid is irregularly transversed by inclusions bearing a close structural resemblance to the chloroplast thylakoids (Figs. 1K, M). In some cases, the incisions are clearly lined by membranes (Figs. 1K, M). No pyrenoglobuli are associated with the thylakoid-like inclusions in the centre of the pyrenoid matrix. Instead, they are developed at the pyrenoid periphery (Figs. 1K, M). One to several electron-lucent, spherical to elongated regions are frequently formed within the pyrenoid matrix (Figs. 1N, O). Rarely, these regions may be associated with several pyrenoglobuli (Fig. 1N). Occasionally, a higher number (more than 8) of these electron-lucent regions are formed within the pyrenoid matrix (Fig. 1P). These regions probably correspond to spherical pyrenoid incisions observed in a light microscope (Figs. 1E, G, J).

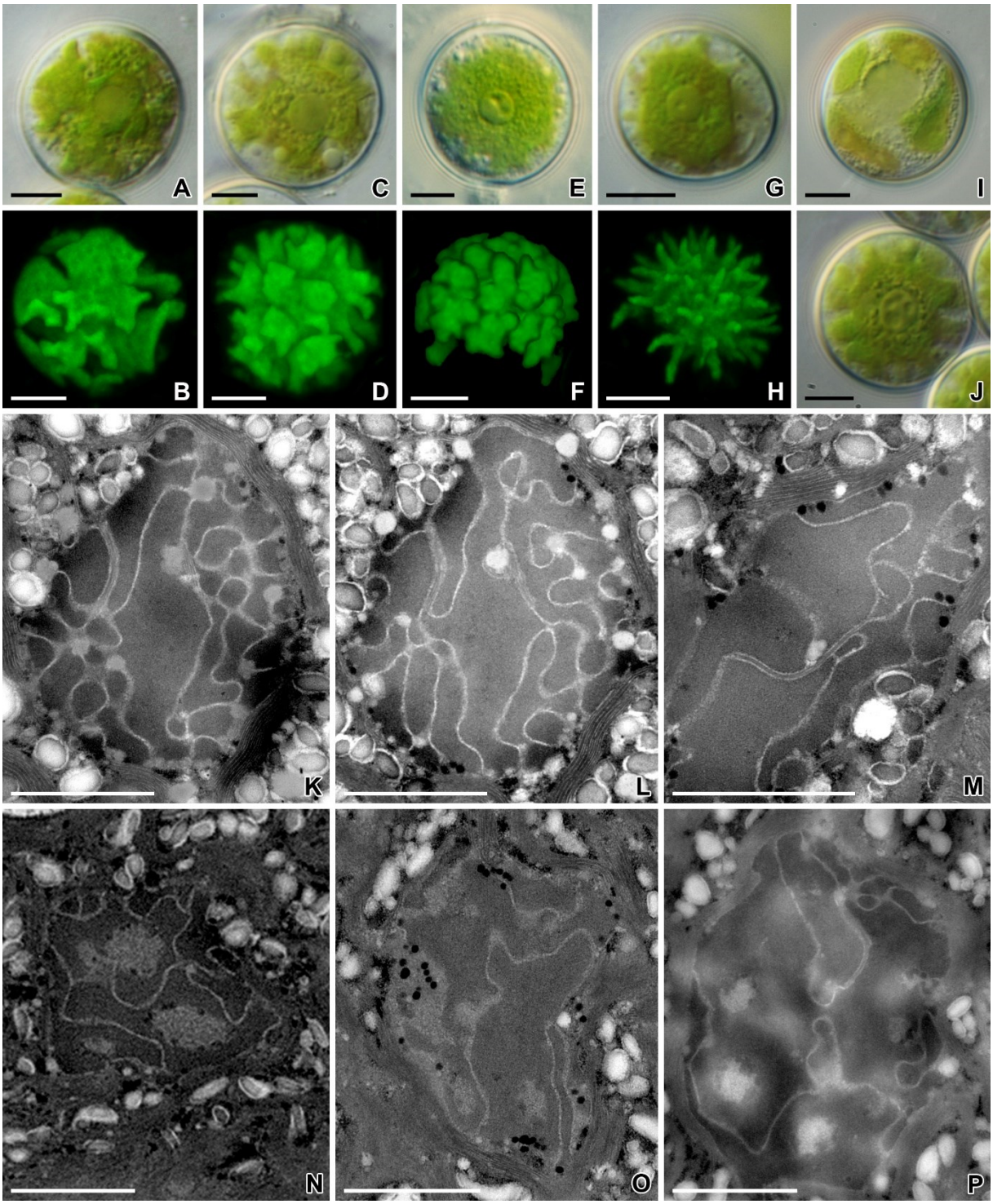


FIGURE 1. *Vulcanochloris canariensis*, *gen. et sp. nov.*. Chloroplast morphology and ultrastructure. A, B. A deeply lobed type of chloroplast. C, D. Shallowly lobed type of chloroplast. E, F. Crenulate type of chloroplast. G, H. Echinate type of chloroplast. I. Parietal type of chloroplast. J. High number of small starch grains surrounding the pyrenoid. K–M. Pyrenoid irregularly transversed by inclusions bearing a close structural resemblance to the chloroplast thylakoids. N, O. One to several electron-lucent, spherical to elongated regions frequently formed within the pyrenoid matrix. P. Higher number (more than 8) of the electron-lucent regions formed within the pyrenoid matrix. Scale bars = 5 μm (A–J); 1 μm (K–P).

Molecular analyses:—To evaluate both the phylogenetic position and genetic diversity of *Vulcanochloris*, we sequenced *rbcL* gene, 18S rDNA and ITS rDNA spacer for several isolates. The

rbcL data set consists of 70 Trebouxiophycean taxa, with 1139 characters. All phylogenetic analyses (BI, ML, MP) resolved *Vulcanochloris* as a distinct clade within Trebouxiales, with full statistical support (Fig. 2). In addition, sister position of genera *Vulcanochloris* and *Asterochloris* was highly supported, as well, with a moderate to very strong support for the monophyly of the latter genus. To further evaluate the reciprocal monophyly of *Vulcanochloris* and *Asterochloris*, we performed several Shimodaira-Hasegawa nonparametric tests (SH tests) comparing the best tree with four optimal trees constrained for *Asterochloris* paraphyly. The four topological constraints each represented one of the paraphyletic trees obtained by the ML botstrapping, as follows: i) monophyly of *Vulcanochloris*, *A. erici*, *A. magna* and *A. phycobiontica*, ii) monophyly of *Vulcanochloris*, *A. erici* and *A. phycobiontica*, iii) monophyly of *Vulcanochloris* and *A. erici*, iv) monophyly of *Vulcanochloris*, *A. erici* and *A. magna*. Tree comparisons indicated that paraphyly of *Asterochloris* was a significantly worse interpretation of these data ($p < 0.001$, -ln for monophyly: 22,873.5, -ln for paraphyly: i) 22,905.5, ii) 22,902.9, iii) 22,893.4, iv) 22,893.4), supporting the reciprocal monophyly of genera *Asterochloris* and *Vulcanochloris*.

18S rDNA data set consisted of 37 sequences with 1776 characters, including three newly obtained *Vulcanochloris* sequences. Bayesian inference of the 18S rDNA and *rbcL* data yielded similar tree topologies, resolving *Vulcanochloris*, *Asterochloris*, *Trebouxia*, and *Myrmecia* as well-defined, distinct genera. In the 18S rDNA analysis (see Supplementary File 1), a clade of environmental sequences from soil samples (Lesaulnier *et al.* 2008) was additionally inferred. Comparison with other 18S rDNA sequences showed that six *Asterochloris* strains and two *Vulcanochloris* samples (A104, L1618) contained IB3 group I introns at position 516 relative to the *E. coli* coding region. The exon SSU rDNA sequences of samples A104 and L1618 were completely identical.

ITS rDNA data set consisted of 31 sequences with 502 characters, including 15 newly obtained *Vulcanochloris* sequences, 13 *Asterochloris* sequences selected to encompass the entire diversity of this genus, and 3 additional sequences retrieved by BLAST searches at NCBI. The BI, ML, and MP phylogenetic analyses inferred from the ITS rDNA sequences resulted in highly similar phylogenetic trees, recognizing *Asterochloris* and *Vulcanochloris* as two distinct lineages, with full statistical support (Fig. 3). Newly obtained *Vulcanochloris* sequences formed three distinct lineages, here referred to as *V. symbiotica* sp. nov., *V. canariensis* sp. nov., and *V. guanchorum* sp. nov. *V. symbiotica* represents the most common lineage, containing 80% of all investigated isolates. This species was detected in all investigated localities. The second lineage, *V. canariensis*, consisted of two, genetically distinct isolates, A98 and L1620. The third lineage consisted of *V. guanchorum* isolate A104, and genetically identical sequence deposited in GenBank as "Chlorophyta sp. URa22" (KF907692). Finally, two additional sequences retrieved from GenBank as "*Asterochloris* sp. URa17" (KF907645, KF907671) were found to be members of the genus *Vulcanochloris*. However, their phylogenetic position, as well as the relationship among the three *Vulcanochloris* lineages, remain unresolved, though the *rbcL* and SSU rDNA phylogenetic analyses point to the close relationship of *V. guanchorum* and *V. symbiotica*.

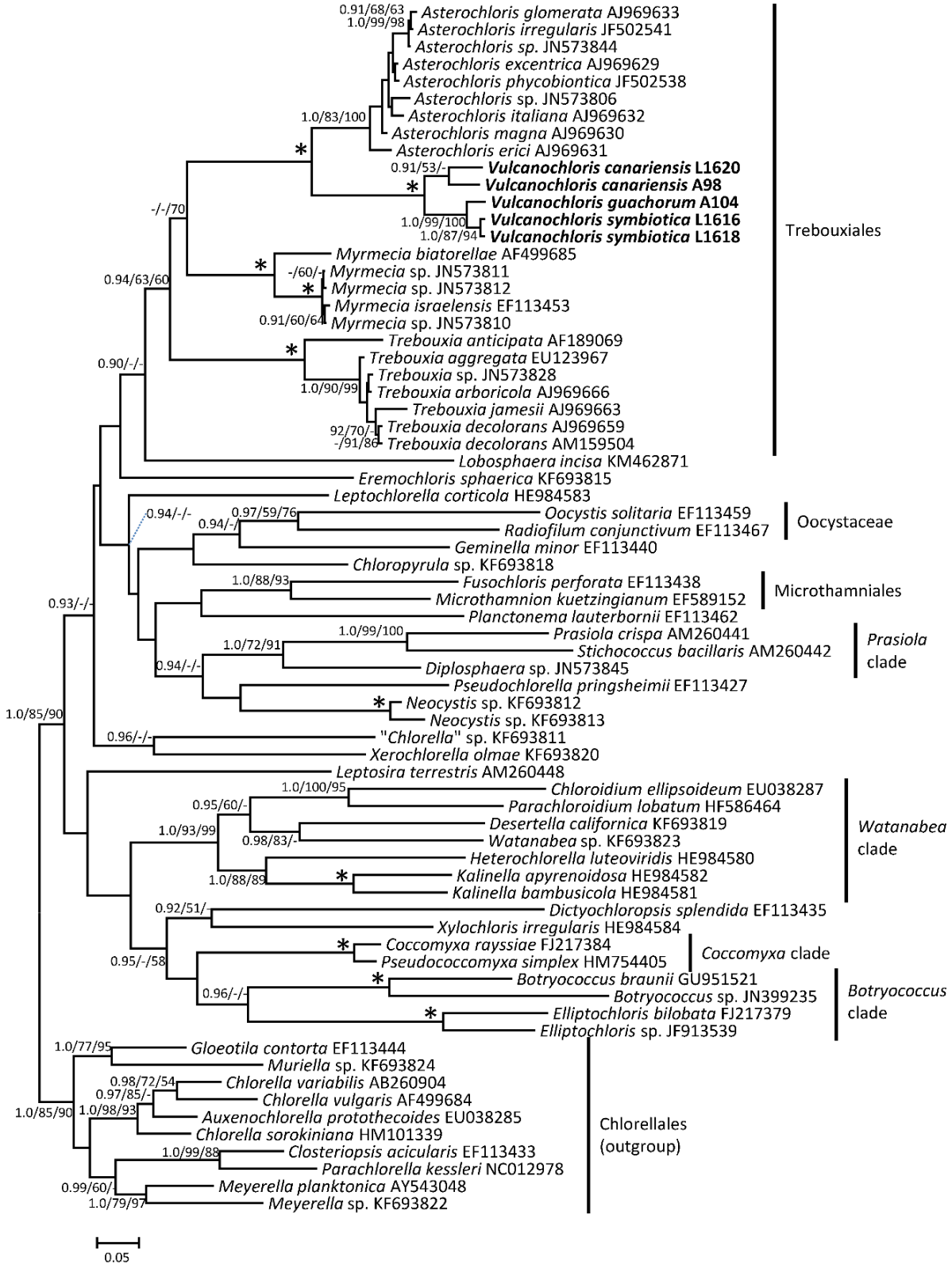


FIGURE 2. Bayesian analysis based on the *rbcL* dataset. Values at the nodes indicate statistical support estimated by three methods—MrBayes posterior-node probability (left), maximum-likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Asterisk represents full support. Scale bar shows the estimated number of substitutions per site. Newly sequenced strains are marked in bold.

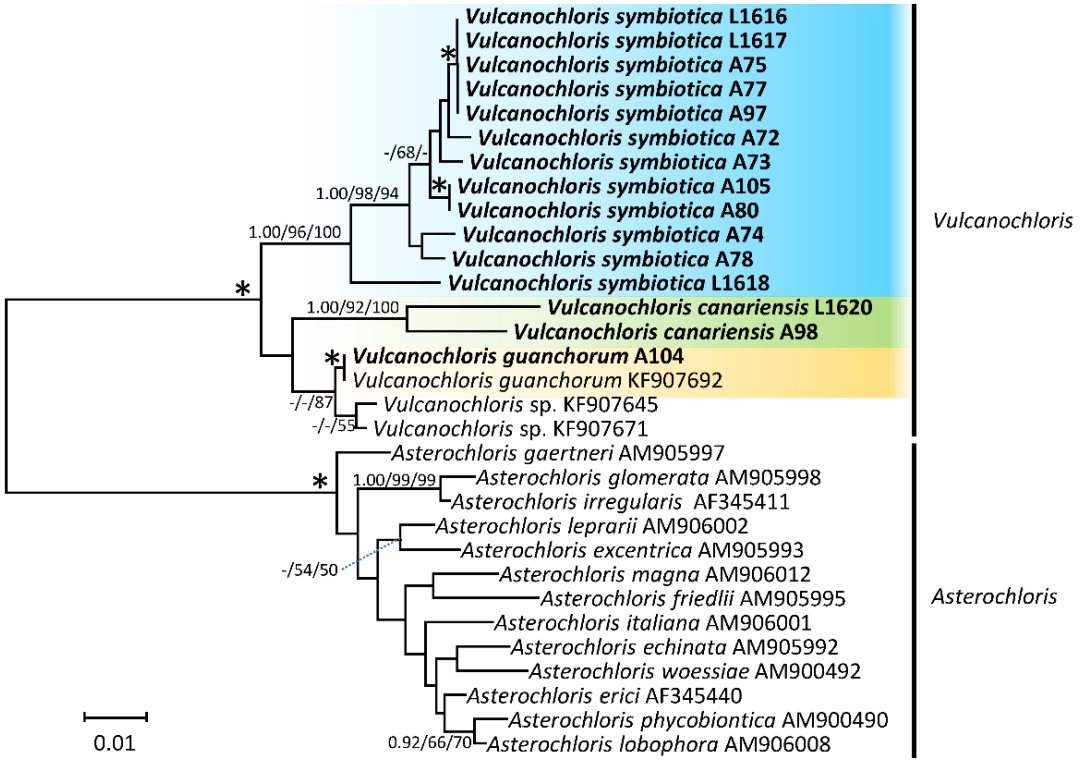


FIGURE 3. Bayesian analysis based on the ITS rDNA dataset. Values at the nodes indicate statistical support estimated by three methods—MrBayes posterior-node probability (left), maximum-likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Asterisk represents full support. Scale bar shows the estimated number of substitutions per site. Newly sequenced strains are marked in bold.

Above-mentioned genetic investigation, as well as detailed morphological analyses of all the studied *Vulcanochloris* strains, revealed the existence of three distinct species. Descriptions of these new taxa are provided below.

Vulcanochloris canariensis Vančurová, Peksa, Němcová *et* Škaloud, *sp. nov.*

Vegetative cells spherical or oval, up to 21 µm in diameter (Figs. 4A–C). Cell wall thin, seldom a flat local thickening of the cell wall (up to 3 µm thick) can be distinguished (Fig. 4B). Very rarely, the cell wall is slightly thickened along its entire surface. Chloroplast in young cells assumes the central position with several lobes spreading towards the cell's periphery (Fig. 4A). Mature cells exhibit a crenulate chloroplast, characterized by a central, massive chloroplast with a regularly nodulated surface (Figs. 4B–E). Rarely, the shallowly lobed chloroplast has been observed as well (Figs. 4F, G). The chloroplast contains one distinct, centrally positioned pyrenoid, frequently containing one to several spherical incisions (Figs. 4A–C, H). A number of small starch grains are distributed around the pyrenoid (Fig. 4C). Asexual reproduction by 16–64 aplanospores formed in spherical or ellipsoidal sporangia, often bearing a local cell wall thickening (Fig. 4I). Zoospores not observed.

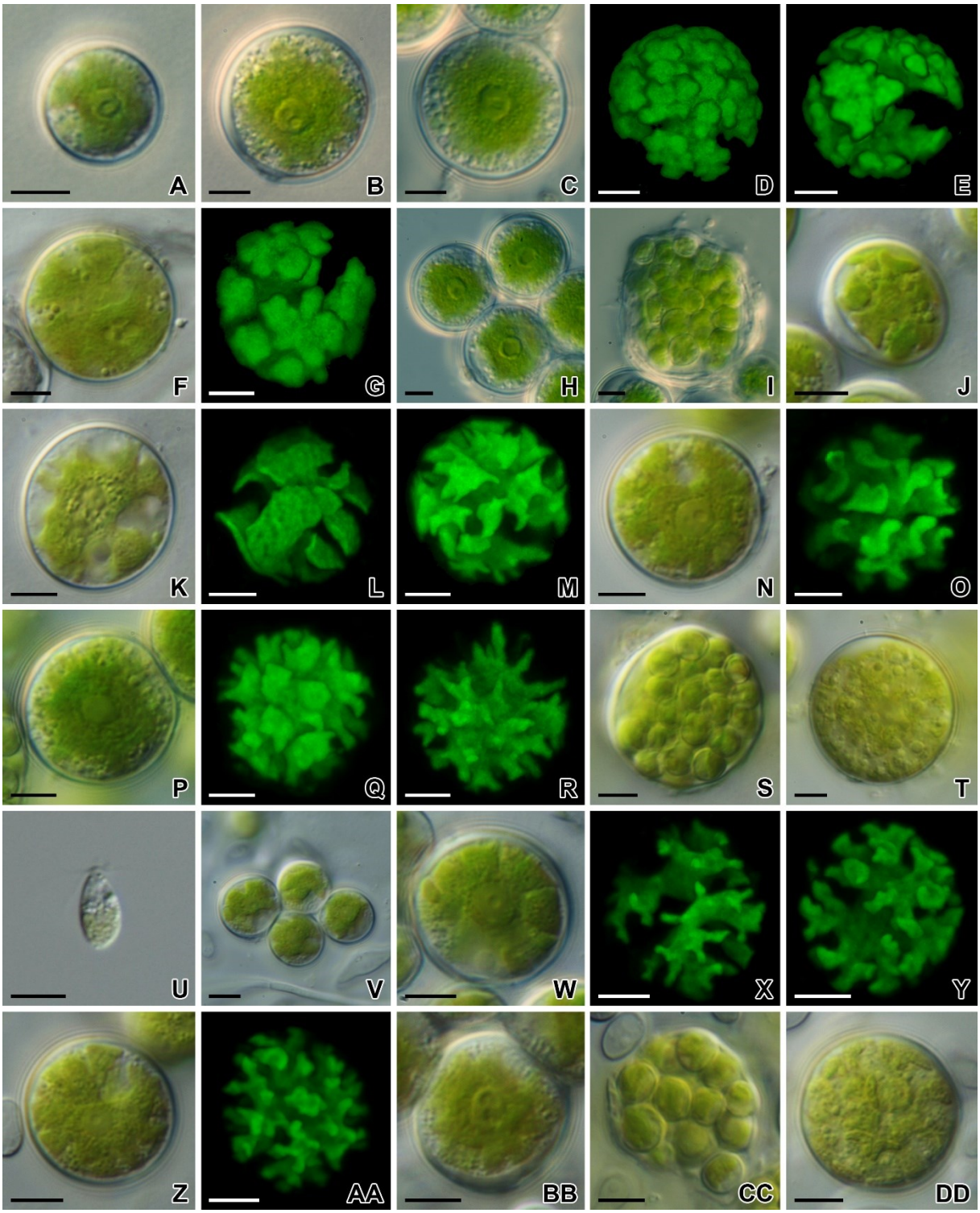


FIGURE 4. *Vulcanochloris*, *gen. nov*. Morphology in a light or a confocal microscope. A–I. *Vulcanochloris canariensis*, *sp. nov*. A–C. Spherical or oval vegetative cells. B–E. Crenulate chloroplast. F–G. Shallowly lobed chloroplast. A–C, H. One distinct, centrally positioned pyrenoid, frequently containing one to several spherical incisions. I. Asexual reproduction by 16–64 aplanospores formed in spherical or ellipsoidal sporangia. J–U. *Vulcanochloris symbiotica sp. nov*. J, K, N. Spherical, oval and oviform vegetative cells. K–M. Deeply lobed chloroplast. N, O. Shallowly lobed chloroplast. P, Q. Crenulate chloroplast. R. Echinate chloroplast. K, N, P. One distinct pyrenoid located in its centre. N. One to several spherical incisions in pyrenoid. S, T. Asexual reproduction by 32 aplanospores or 128 zoospores produced in spherical or ellipsoidal sporangia. U. Zoospores drop-shaped, naked, with two apical flagella and a simple basal chloroplast. V–DD. *Vulcanochloris guanchorum*, *sp. nov*. V, W.

Spherical, occasionally oval vegetative cells. V. Chloroplast in young cells in the central position with several lobes spreading towards the cells periphery. W–Y. Deeply lobed chloroplast. Z–AA. Shallowly lobed chloroplast. BB. One distinct, centrally positioned pyrenoid, often containing one to several spherical incisions. CC, DD. Asexual reproduction by 16–32 aplanospores or 64–128 zoospores produced in spherical or ellipsoidal sporangia. Scale bars = 5 μm.

Type:—SPAIN. Santa Cruz de Tenerife: La Palma, slope of Volcán Teneguía, 28.473056° N, 17.847222° W, 330 m a. s. l., *L. Vančurová, 16 October 2011* (holotype: CAUP!, cryopreserved photobiont cells isolated from the specimen L1620, deposited in the Culture Collection of Algae of the Charles University in Prague as the item TYPE-H 1016). Reference strain: CAUP H 1016.

Habitat:—In thalli of *Stereocaulon vesuvianum* growing on basalt lava stones and rocks.

Etymology:—The specific epitheton reflects the place of origin of all known samples (Canary Islands).

Vulcanochloris symbiotica Vančurová, Peksa, Němcová *et* Škaloud, *sp. nov.*

Vegetative cells usually spherical, occasionally oval and oviform, up to 18 μm in diameter (Figs. 4J, K, N). Cell wall thin, seldom a flat local thickening of the cell wall (up to 3 μm thick) can be distinguished (Fig. 4J). Very rarely, the cell wall is slightly thickened along its entire surface. Chloroplast in young cells assumes the central position with several lobes spreading towards the cell's periphery (Fig. 4J). Mature cells exhibit a broad range of chloroplast types, with a deeply lobed form being the mostly frequently observed (Figs. 4K–M). In addition, the shallowly lobed (Figs. 4N, O), crenulate (Figs. 4P, Q) and echinate chloroplast (Fig. 4R) is observed as well. Lobes of the deeply lobed chloroplast are not simply terminated, but extended to either irregular plates (Fig. 4L) or branched projections (Fig. 4M). The chloroplast contains one distinct pyrenoid located in its centre (Figs. 4K, N, P). The pyrenoid occasionally contains one to several spherical incisions (Fig. 4N). A number of small starch grains are distributed around the pyrenoid (Fig. 4K). Asexual reproduction by 32 aplanospores or 128 zoospores produced in spherical or ellipsoidal sporangia with diameters up to 22 μm (Figs. 4S, T). Zoospores drop-shaped, naked, with two apical flagella and a simple basal chloroplast, 7.0–7.5 μm long and 3–4 μm wide (Fig. 4U).

Type:—SPAIN. Santa Cruz de Tenerife: La Palma, top of Volcán de San Antonio, 28.485500° N, 17.849917° W, 589 m a.s.l., *L. Vančurová & J. Malíček, 17 May 2013* (holotype: CAUP!, cryopreserved photobiont cells isolated from the specimen A72, deposited in the Culture Collection of Algae of the Charles University in Prague as the item TYPE-H 1017). Reference strain: CAUP H 1017

Habitat:— In thalli of *Stereocaulon vesuvianum* growing on basalt lava stones and rocks.

Etymology:—The specific epitheton reflects symbiotic lifestyle of this alga.

Vulcanochloris guanchorum Vančurová, Peksa, Němcová *et* Škaloud, *sp. nov.*

Vegetative cells spherical, occasionally oval, up to 20 μm in diameter (Figs. 4V, W). Cell wall thin, seldom a flat local thickening of the cell wall (up to 3.5 μm thick) can be distinguished. Very rarely, the cell wall is slightly thickened along its entire surface. Chloroplast in young cells assumes the central position with several lobes spreading towards the cell's periphery (Fig. 4V). Mature cells exhibit either a deeply lobed (Figs. 4W–Y) or a shallowly lobed chloroplast (Figs. 4Z, AA), simply terminated at their ends. The chloroplast contains one distinct, centrally positioned pyrenoid, often containing one to several spherical incisions (Fig. 4BB). A number of small starch grains are distributed around the pyrenoid. Asexual reproduction by 16–32 aplanospores or 64–128 zoospores produced in spherical or ellipsoidal sporangia (Figs. 4CC, DD). Zoospores drop-shaped, naked, with two apical flagella and a simple basal chloroplast, ca 6.5 μm long and 3 μm wide.

Type:—SPAIN. Santa Cruz de Tenerife: La Palma, lava field of Volcán de San Juan, 2.5 km to the north-east Puerto de Naos, 28.604722° N, 17.895389° W, 400 m a.s.l., *L. Vančurová & J. Malíček, 20 May 2013* (holotype: CAUP!, cryopreserved photobiont cells isolated from the specimen A104, deposited in the Culture Collection of Algae of the Charles University in Prague as the item TYPE-H 1018). Reference strain: CAUP H 1018

Habitat:—In thalli of *Stereocaulon vesuvianum* growing on basalt lava stones and rocks.

Etymology:—The species is named after the Guanches, aboriginal Berber inhabitants of the Canary Islands.

Discussion

Recent phylogenetic studies provide a wide evidence of unsuspected, often morphologically cryptic, diversity in coccoid green algae (Leliaert *et al.* 2014). In the green algal class Trebouxiophyceae, numerous genus-level revisions, transfers, splits, and new taxa descriptions have been published during the last 15 years (Neustupa *et al.* 2011, 2013, Gaysina *et al.* 2013, Fučíková *et al.* 2014b). In this paper, we are adding a new piece to the puzzle of understanding the real diversity within the Trebouxiophyceae, by describing a new genus of coccoid green algae, *Vulcanochloris*.

According to our *rbcL* phylogenetic analysis, *Vulcanochloris* comprises a distinct genus within the order Trebouxiales (Fig. 2), in a sister position to the genus *Asterochloris*. These findings are in accordance with our 18S rDNA phylogenetic analysis (see Supplementary File 1), indicating a close relationship of these two genera. Nevertheless, this genetic similarity does not contradict resolving *Asterochloris* and *Vulcanochloris* as two distinct genera, since the exon 18S rDNA sequences were shown to evolve extremely slowly in *Asterochloris* (Škaloud *et al.* 2015). Morphologically, these two genera are highly similar, specifically in the formation of axial, lobed chloroplast type, the chloroplast transformation prior to sporogenesis, and production of a high number of daughter cells (aplanospores). This similarity could be even

conceptualized as the ground to regard the newly discovered lineage as a new, yet distinct, species within the genus *Asterochloris*. However, a specific pyrenoid structure (see below) and substantial genetic divergence of ITS rDNA sequences (Fig. 3) warrants describing *Vulcanochloris* as a distinct genus.

The presence and structure of pyrenoids represents an important feature in delimitation of green algal genera and species (Ettl & Gärtner 1995, Pröschold & Leliaert 2007). In addition, the ultrastructure of pyrenoids has been shown to be phylogenetically informative. Before the application of molecular techniques, pyrenoid ultrastructure has been used as one of the most important features to trace the evolutionary history of coccoid green algae. For example, in the genus *Chlorella*, pyrenoid ultrastructure has been applied to separate the species into several evolutionary coherent groups (Ikeda & Takeda 1995, Kalina & Punčochářová 1987). Later, molecular phylogenetic investigations corroborated this separation, showing the polyphyletic origin of *Chlorella* species (Huss *et al.* 1999). According to the present knowledge, the traditionally conceived genus *Chlorella* forms at least 10 particular lineages corresponding to different genera (Škaloud *et al.* 2014). In fact, many of these lineages were previously shown to differ ultrastructurally. For example, whereas true *Chorella* species possess a pyrenoid bisected by a pair of thylakoids, pyrenoids of *Heterochlorella* Neustupa *et al.* (2009: 167) and *Chloroidium* (Krüger 1906: 94) Darienko *et al.* (2010: 189) are bisected by up to four stacked thylakoids or by many single undulating thylakoids, respectively (Ikeda & Takeda 1995, Němcová & Kalina 2000).

Similarly, a substantial variability in pyrenoid ultrastructure has been documented in the genus *Trebouxia* (Fisher & Lang 1971), a close relative of *Vulcanochloris*. According to the arrangements and forms of thylakoid lamellae within the pyrenoid matrix, Friedl (1989) separated particular *Trebouxia* species into the eight natural groups. Later investigations showed a large congruence of the ultrastructural and molecular data (Helms 2003, Nyati 2006). Indeed, three groups of species have been even shown to form a separate genus *Asterochloris* (Škaloud & Peksa 2010). The pyrenoid ultrastructure found in *Vulcanochloris* cannot be assigned to any of the pyrenoid types previously described by Friedl (1989). The most prominent feature distinguishing *Vulcanochloris* from all other investigated taxa is the presence of electron-lucent, spherical to elongated regions formed within the pyrenoid matrix. To our knowledge, this pyrenoid ultrastructure, as well as the formation of spherical pyrenoid incisions observed in light microscope, was never reported for any other green algal taxa.

The genetic diversity within the genus *Vulcanochloris* is very high, fully comparable with the sister genus *Asterochloris* actually comprising 13 distinct species (Škaloud *et al.* 2015). Just for illustration, ITS rDNA sequences of *Vulcanochloris* from La Palma (Canary Islands, Spain) diverged each other 0.02–5.07%. Considering this substantial genetic diversity and morphological differentiation of particular lineages, we proposed the description of three

species within the newly described genus *Vulcanochloris*. *Vulcanochloris symbiotica* is the most variable in the chloroplast morphology. Deeply lobed form is the most frequently observed. Furthermore, the shallowly lobed, crenulate and echinate chloroplast is observed as well. Lobes of the deeply lobed chloroplast are not simply terminated, but extended to either irregular plates or branched projections. On the contrary, lobes of chloroplasts of *V. guanchorum* are simply terminated at their ends. Finally, *V. canariensis* possess exclusively crenulate chloroplast. Chloroplast morphology has been recognized as one of the most important features to distinguish species within the related genera *Asterochloris* and *Trebouxia* (Helms 2003, Škaloud & Peksa 2010, Škaloud *et al.* 2015). Therefore, we consider the combination of the above-mentioned morphological differences with molecular data as a gold standard to delimit species boundaries in the newly proposed genus *Vulcanochloris*.

The species of *Vulcanochloris* belong to a few green algae described directly from lichens and known only in a lichenized form, similarly to two of the most recently described phycobionts—the sister genus *Asterochloris* represented by *A. phycobiontica* Tschermak-Woess (1980a: 291), and *Elliptochloris bilobata* Tschermak-Woess (1980b: 71). Interestingly, *Asterochloris* was several times recorded from *Stereocaulon* Hoffmann (1796: 128), including *S. vesuvianum*, which is the exclusive mycobiont of *Vulcanochloris* algae (Nelsen & Gargas 2006, Peksa & Škaloud 2011). Such sharing of the same mycobiont is certainly enabled by the close relationship between both algal genera. On the Canary Islands, the association of *S. vesuvianum* with *Vulcanochloris* instead of *Asterochloris* could represent a local adaptation to the harsh conditions on the lava stones and rocks. Besides *Stereocaulon*, *Asterochloris* is associated with many other taxa of lichen-forming fungi (Škaloud & Peksa 2010). Therefore, we consider the high specificity of *Vulcanochloris* algae to *Stereocaulon* as not definitive. Interestingly, some thalli of *Parmotrema pseudotinctorum* from the island of La Palma were associated with the phycobiont clone PAL4.11, closely related to *Asterochloris* (Molins *et al.* 2013). This clone could, in fact, very probably represent the newly proposed genus *Vulcanochloris*. However, since the authors used the *psb*A gene sequences to characterize genetically the phycobiont clones, we cannot compare their findings with our data, and thus confirm the presence of *Vulcanochloris* in *Parmotrema* lichens.

We report here a common occurrence of *Vulcanochloris* on La Palma. However, one lineage closely related to *V. guanchorum* was recently discovered at limestone localities in Germany and Sweden (Ruprecht *et al.* 2014), rejecting a putative endemic nature of *Vulcanochloris* on the Canary Islands. Although the biogeography of microorganisms has become a highly discussed topic (Caron 2009), investigations dealing with the biogeography of symbiotic protists are still very scarce. The population studies on lichenized *Trebouxia* species indicated that the distribution of particular genotypes is particularly shaped by either climatic factors (Fernández-Mendoza *et al.* 2011) or distribution patterns of mycobiont partners (Buckley *et al.* 2014). The single study dealing with the biogeography of *Asterochloris* photobionts indicated generally cosmopolitan distribution of species

(Řídká *et al.* 2014). The existence of two lineages endemic to India has been explained by specific climatic or habitat preferences rather than by the historic factors. Accordingly, we expect that occurrence of *Vulcanochloris* is similarly associated with specific conditions common on La Palma, but rare in other areas.

Future work should therefore include follow-up investigations designed to evaluate whether *Vulcanochloris* occurs on volcanic localities in the rest of the world, and whether it associates exclusively with *Stereocaulon vesuvianum*, with other mycobionts, or even occurs as a free-living alga.

Acknowledgements

This work was supported by the Charles University Science Foundation project GAUK 570313. We are very grateful to J. Malíček, J. Vančurová and L. Vančura to their kind help during field work. We thank J. Váňa for assistance with Latin grammar, Lenka Flašková for help with molecular work and two anonymous reviewers for their valuable comments on the original version of the manuscript.

References

Abbayes, H. des (1951) Lichens récoltes en Guinee Francaise et en Cote d'Ivoire (Mission H. des Abbayes, 1948). IV.--Parmeliacees. *Bulletin de l'Institut Fondamental d' Afrique Noire* 13: 965–977.

Ahmadjian, V. (1993) *The Lichen Symbiosis*. John Wiley and Sons, Inc., New York, 250 pp.

Acharius, E. (1810) *Lichenographia Universalis*. Göttingen, 696 pp. http://dx.doi.org/10.5962/bhl.title.79418

Beijerinck, M.W. (1890) Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen. *Botanische Zeitung* 47: 725–739, 741–754, 757–768, 781–785.

Blaha, J., Baloch, E. & Grube, M. (2006) High photobiont diversity associated with the euryoecious lichen-forming ascomycete *Lecanora rupicola* (Lecanoraceae , Ascomycota). *Biological Journal of the Linnean Society* 88: 283–293. http://dx.doi.org/10.1111/j.1095-8312.2006.00640.x

Bouza, N., Caujape-Castells, J., Gonzalez-Perez, M.A. & Sosa, P.A. (2006) Genetic structure of natural populations in the red algae *Gelidium Canariense* (Gelidiales, Rhodophyta) investigated by random amplified polymorphic DNA (RAPD) Markers. *Journal of Phycology* 42: 304–311. http://dx.doi.org/10.1111/j.1529-8817.2006.00201.x

Buckley, H.L., Rafat, A., Ridden, J.D., Cruickshank, R.H., Ridgway, H.J. & Paterson, A.M. (2014) Phylogenetic congruence of lichenised fungi and algae is affected by spatial scale and taxonomic diversity. *PeerJ* 2: e573. http://dx.doi.org/10.7717/peerj.573

Campo, E.M., Catalá, S., Gimeno, J., del Hoyo, A., Martínez-Alberola, F., Casano, L.M., Grube, M. & Barreno-Rodríguez, E. (2013) The genetic structure of the cosmopolitan three-partner lichen *Ramalina farinacea* evidences the concerted diversification of symbionts. *FEMS Microbiology Ecology* 83: 310–323. http://dx.doi.org/10.1111/j.1574-6941.2012.01474.x

Caron, D. A. (2009) Past presidents address: Protistan biogeography: Why all the fuss? *Journal of Eukaryotic Microbiology* 56: 105–112. http://dx.doi.org/10.1111/j.1550-7408.2008.00381.x

Casano, L.M., del Campo, E.M., García-Breijo, F.J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., Guéra, A. & Barreno-Rodríguez, E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental microbiology* 13: 806–818. http://dx.doi.org/10.1111/j.1462-2920.2010.02386.x

Cassano, V., Metti, Y., Millar, A.J.K., Gil-Rodríguez, M.C., Sentíes, A., Díaz-Larrea, J., Oliveira, M.C. & Fujii, M.T. (2012) Redefining the taxonomic status of *Laurencia dendroidea* (Ceramiales, Rhodophyta) from Brazil and the Canary Islands. *European Journal of Phycology* 47: 67–81. http://dx.doi.org/10.1080/09670262.2011.647334

Cubero, O.F., Crespo, A., Fatehi, J. & Bridge, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* 216: 243–249. http://dx.doi.org/10.1007/BF01084401

Darienko, T., Gustavs, L., Mudimu, O., Menendez, C.R., Schumann, R., Karsten, U., Friedl, T. & Pröschold, T. (2010) *Chloroidium*, a common terrestrial coccoid green alga previously assigned to *Chlorella* (Trebouxiophyceae, Chlorophyta). *European Journal of Phycology* 45: 79–95. http://dx.doi.org/ 10.1080/09670260903362820

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. http://dx.doi.org/10.1038/nmeth.2109

Ettl, H. & Gärtner, G. (1995) *Syllabus der Boden-, Luft- und Flechtenalgen*. Gustav Fischer Verlag, Stuttgart, 721 pp.

Fernández-Mendoza, F., Domaschke, S., García, M.A., Jordan, P., Martín, M.P. & Printzen, C. (2011) Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. *Molecular Ecology* 20: 1208–1232. http://dx.doi.org/10.1111/j.1365-294X.2010.04993.x

Fisher, K.A. & Lang, N.J. (1971) Ultrastructure of the pyrenoid of *Trebouxia* in *Ramalina menziesii* Tuck. *Journal of Phycology* 7: 25–37. http://dx.doi.org/10.1111/j.1529-8817.1971.tb01475.x

Friedl, T. (1989) Comparative ultrastructure of pyrenoids in *Trebouxia* (Microthamniales, Chlorophyta). *Plant Systematics and Evolution* 164: 145–159. http://dx.doi.org/10.1007/BF00940435

Friedl, T. & Bhattacharya, D. (2002) Origin and evolution of green lichen algae. *In*: J. Seckbach (Ed.) *Symbiosis: Mechanisms and Model Systems*. Kluwer Academic Publishers, Dordrecht, pp. 343–357. http://dx.doi.org/10.1007/0-306-48173-1_21

Friedl, T. & Büdel, B. (2008) Photobionts. *In*: Nash III, T.H. (Ed.) *Lichen Biology*. Cambridge University Press, New York, pp. 9–26. http://dx.doi.org/10.1017/CBO9780511790478.003

Fučíková, K., Leliaert, F., Cooper, E.D., Škaloud, P., D'Hondt, S., De Clerck, O., Gurgel, C.F.D., Lewis, L.A., Lewis, P.O., Lopez-Bautista, J.M., Delwiche, C.F. & Verbruggen, H. (2014)a) New phylogenetic hypotheses for the core Chlorophyta based on chloroplast sequence data. *Frontiers in Ecology and Evolution* 2: 1–12. http://dx.doi.org/10.3389/fevo.2014.00063

Fučíková, K., Lewis, P.O. & Lewis, L.A. (2014b) Widespread desert affiliation of trebouxiophycean algae (Trebouxiophyceae, Chlorophyta) including discovery of three new desert genera. *Phycological Research* 62: 294–305. http://dx.doi.org/10.1111/pre.12062

García-Jiménez, P., Geraldino, P.J.L., Boo, S.M. & Robaina, R.R. (2008) Red alga *Grateloupia imbricata* (Halymeniaceae), a species introduced into the Canary Islands. *Phycological Research* 56: 166–171. http://dx.doi.org/10.1111/j.1440-1835.2008.00498.x

Gärtner, G. (1985a) Die Gattung *Trebouxia* Puymaly (Chlorellales, Chlorophyceae). *Algological Studies* 41: 495–548.

Gärtner, G. (1985b) Taxonomische Probleme bei den Flechtenalgengattungen *Trebouxia* und *Pseudotrebouxia* (Chlorophyceae, Chlorellales). *Phyton* 25: 101–111.

Gaysina, L., Němcová, Y., Škaloud, P., Ševčíková, T. & Eliáš, M. (2013) *Chloropyrula uraliensis gen. et sp. nov.* (Trebouxiophyceae, Chlorophyta), a new green coccoid alga with a unique ultrastructure, isolated from soil in South Urals. *Journal of Systematics and Evolution* 51: 476–484. http://dx.doi.org/10.1111/jse.12014

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704. http://dx.doi.org/10.1080/10635150390235520

Hafellner, J. (1983) *Tephromela atra* (Huds.) Hafellner. *In*: Kalb K. (Ed.) *Lichenes Neotropici 7*. Selbstverl, no. 251–300.

Hale, M.E. (1974) New combinations in the lichen genus *Parmotrema* Massalongo. *Phytologia* 28: 334–339.

Helms, G. (2003) *Taxonomy and symbiosis in associations of Physciaceae and* Trebouxia. Georg-August Universität Göttingen, Göttingen, 156 pp.

Helms, G., Friedl, T., Rambold, G. & Mayrhofer, H. (2001) Identification of photobionts from the lichen family Physciaceae using algal-specific ITS rDNA sequencing. *The Lichenologist* 33: 73–86. http://dx.doi.org/10.1006/lich.2000.0298

Hernández Padrón, C.E. & Pérez Vargas, I. (2010) Lichenes, lichenicolous fungi. *In*: Arechavaleta Hernández, M., Rodríguez Núñez, S., Zurita Pérez, N. & García Ramírez, A. (Coords.) *Lista de especies silvestres de Canarias. Hongos, plantas y animales terrestres. 2009*. Gobierno de Canarias, Santa Cruz de Tenerife, pp. 71–105.

Hoffmann, G.F. (1796) *Deutschlands Flora oder botanisches Taschenbuch. Zweyter Theil für das Jahr 1795. Cryptogamie*. B.I.I. Palm, Erlangen, 200 pp.

Hoshina, R., Iwataki, M. & Imamura, N. (2010) *Chlorella variabilis* and *Micractinium reisseri sp. nov.* (Chlorellaceae, Trebouxiophyceae): Redescription of the endosymbiotic green algae of *Paramecium bursaria* (Peniculia, Oligohymenophorea) in the 120th year. *Phycological Research* 58: 188–201. http://dx.doi.org/10.1111/j.1440-1835.2010.00579.x

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755. http://dx.doi.org/10.1093/bioinformatics/17.8.754

Hudson, W. (1762) *Flora anglica*. London, 536 pp.

Huss, V.A.R., Frank, C., Hartmann, E.C., Hirmer, M., Kloboucek, A., Seidel, B.M., Wenzeler, P. & Kessler, E. (1999) Biochemical taxonomy and molecular phylogeny of genus *Chlorella* sensu lato (Chlorophyta). *Journal of Phycology* 35: 587–598. http://dx.doi.org/10.1046/j.1529-8817.1999.3530587.x

Ikeda, T. & Takeda, H. (1995) Species-specific differences of pyrenoids in *Chlorella* (Chlorophyta). *Journal of Phycology* 31: 813–818. http://dx.doi.org/10.1111/j.0022-3646.1995.00813.x

Kalina, T. & Punčochářová, M. (1987) Taxonomy of the subfamily Scotiellocystoideae Fott 1976 (Chlorellaceae, Chlorophyceae). *Algological Studies* 45: 473–521.

Katana, A., Kwiatowski, J., Spalik, K., Zakryś, B., Szalacha, E. & Szymańska, H. (2001) Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. *Journal of Phycology* 37: 443–451. http://dx.doi.org/10.1046/j.1529-8817.2001.037003443.x

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. http://dx.doi.org/10.1093/molbev/mst010

Krüger, W. (1906) Beiträge zur Kenntnis der Organismen des Saftflusses (sog. Scheimflusses) der Laubbaum. II. Über zwei aus Saftflussen rein gezüchtete Algen. *Beitrage Physiologie und Morphologie niederer Organismen* 4: 69–116.

Leliaert, F., Smith, D.R., Moreau, H., Herron, M.D., Verbruggen, H., Delwiche, C.F. & De Clerck, O. (2012) Phylogeny and molecular evolution of the Green Algae. *Critical Reviews in Plant Sciences* 31: 1–46. http://dx.doi.org/10.1080/07352689.2011.615705

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J.M., Zuccarello, G.C. & Clerck, O. De (2014) DNA-based species delimitation in algae DNA-based species delimitation in algae. *European Journal of Phycology* 49: 179–196. http://dx.doi.org/10.1080/09670262.2014.904524

Lesaulnier, C., Papamichail, D., McCorkle, S., Ollivier, B., Skiena, S., Taghavi, S., Zak, D. & Van Der Lelie, D. (2008) Elevated atmospheric CO_2 affects soil microbial diversity associated with trembling aspen. *Environmental Microbiology* 10: 926–941. http://dx.doi.org/10.1111/j.1462-2920.2007.01512.x

Letsch, M.R., Muller-Parker, G., Friedl, T. & Lewis, L.A. (2009) *Elliptochloris marina sp. nov.* (Trebouxiophyceae, Chlorophyta), symbiotic green alga of the temperate Pacific Sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* (Anthozoa, Cnidaria). *Journal of Phycology* 45: 1127–1135. http://dx.doi.org/10.1111/j.1529-8817.2009.00727.x

Linnaeus, C. (1753) *Species Plantarum*. Laurentius Salvius, Stockholm, 1200 pp.http://dx.doi.org/10.5962/bhl.title.669

Linnaeus, C. (1767) *Mantissa Plantarum*. Salvii, Stockholm, 144 pp. http://dx.doi.org/10.5962/bhl.title.69083

Molins, A., García-Breijo, F.J., Reig-Armiñana, J., del Campo, E.M., Casano, L.M. & Barreno, E. (2013) Coexistence of different intrathalline symbiotic algae and bacterial biofilms in the foliose Canarian lichen *Parmotrema pseudotinctorum*. *Vieraea: Folia Scientarum Biologicarum Canariensium* 41: 349–370.

Muggia, L., Zellnig, G., Rabensteiner, J. & Grube, M. (2010) Morphological and phylogenetic study of algal partners associated with the lichen-forming fungus *Tephromela atra* from the Mediterranean region. *Symbiosis* 51: 149–160. http://dx.doi.org/10.1007/s13199-010-0060-8

Nelsen, M.P. & Gargas, A. (2006) Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta : Trebouxiophyceae). *The Lichenologist* 38: 435–440. http://dx.doi.org/10.1017/S0024282906005779

Nelsen, M.P., Plata, E.R., Andrew, C.J., Lücking, R. & Lumbsch, H.T. (2011) Phylogenetic Diversity of Trentepohlialean Algae Associated with Lichen-Forming Fungi. *Journal of Phycology* 47: 282–290. http://dx.doi.org/10.1017/S0024282906005779

Němcová, Y. & Kalina, T. (2000) Cell wall development, microfibril and pyrenoid structure in type strains of *Chlorella vulgaris*, *C. kessleri*, *C. sorokiniana* compared with *C. luteoviridis* (Trebouxiophyceae, Chlorophyta). *Algological studies* 100: 95–105.

Neustupa, J., Němcová, Y., Eliáš, M. & Škaloud, P. (2009) *Kalinella bambusicola gen. et sp. nov.* (Trebouxiophyceae, Chlorophyta), a novel coccoid *Chlorella*-like subaerial alga from Southeast Asia. *Phycological Research* 57: 159–169. http://dx.doi.org/10.1111/j.1440-1835.2009.00534.x

Neustupa, J., Eliáš, M., Škaloud, P., Němcová, Y. & Šejnohová, L. (2011) *Xylochloris irregularis gen. et sp. nov.* (Trebouxiophyceae, Chlorophyta), a novel subaerial coccoid green alga. *Phycologia* 50: 57–66. http://dx.doi.org/10.2216/08-64.1

Neustupa, J., Němcová, Y., Veselá, J., Steinová, J. & Škaloud, P. (2013) *Parachloroidium gen. nov.* (Trebouxiophyceae, Chlorophyta), a novel genus of coccoid green algae from subaerial corticolous biofilms. *Phycologia* 52: 411–421. http://dx.doi.org/10.2216/13

Nyati, S. (2006) Photobiont diversity in Teloschistaceae (Lecanoromycetes). Universität Zürich, Zurich, 130 pp.

Nyati, S., Beck, A. & Honegger, R. (2007) Fine structure and phylogeny of green algal photobionts in the microfilamentous genus *Psoroglaena* (Verrucariaceae, lichen-forming ascomycetes). *Plant Biology* 9: 390–399. http://dx.doi.org/10.1055/s-2006-924654

Peksa, O. & Škaloud, P. (2008) Changes in chloroplast structure in lichenized algae. *Symbiosis* 46: 153–160.

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology* 20: 3936–48. http://dx.doi.org/10.1111/j.1365-294X.2011.05168.x

Persoon, C.H. (1810) Novae lichenum species. *Annalen der Wetterauischen Gesellschaft für die Gesammte Naturkunde* 2: 9–20.

Piercey-Normore, M.D. & DePriest, P.T. (2001) Algal Switching among Lichen Symbioses. *American Journal of Botany* 88: 1490–1498. http://dx.doi.org/10.2307/3558457

Printz, H. (1921) Subaërial algae from South Africa. *Det Kongelige Norske Videnskabers Selskabs Skrifter* 1920: 1–41, I–XIV.

Pröschold, T. & Leliaert, F. (2007) Systematics of the green algae: conflict of classic and modern approaches. *In*: Brodie J. & Lewis, J. (Eds.) *Unravelling the algae: the past, present, and future of algal systematics*. CRC Press, Inc., Boca Raton, pp. 123–153.

Puymaly, A. de (1924) Le *Chlorococcum humicola* (Nag.) Rabenh. *Revue Algologique* 1: 107–114.

Řídká, T., Peksa, O., Rai, H. & Upreti, D.K. (2014) Photobiont diversity in Indian *Cladonia* lichens, with special emphasis on the geographical patterns. *In*: Rai, H. & Upreti, D.K. (Eds.) *Terricolous lichens in India*. Springer, New York, pp. 53–71. http://dx.doi.org/10.1007/978-1-4614-8736-4_4

Ruprecht, U., Brunauer, G. & Türk, R. (2014) High photobiont diversity in the common European soil crust lichen *Psora decipiens*. *Biodiversity and Conservation* 23: 1771–1785. http://dx.doi.org/10.1007/s10531-014-0662-1

Schmidle, W. (1901) Über drei Algengenera. *Berichte der deutschen botanischen Gessellschaft* 19: 10–24.

Sherwood, A.R., Garbary, D.J. & Sheath, R.G. (2000) Assessing the phylogenetic position of the Prasiolales (Chlorophyta) using rbcL and 18S rRNA gene sequence data. *Phycologia* 39: 139–146. http://dx.doi.org/10.2216/i0031-8884-39-2-139.1

Shimodaira, H., Hasegawa, M., 1999. Likelihood-Based Tests of Topologies in Phylogenetics. Mol. Biol. Evol. 16, 1114–1116.

Škaloud, P. & Peksa, O. (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris*. *Molecular Phylogenetics and Evolution* 54: 36–46. http://dx.doi.org/10.1016/j.ympev.2009.09.035

Škaloud, P., Němcová, Y., Pytela, J., Bogdanov, N.I., Bock, C. & Pickinpaugh, S.H. (2014) *Planktochlorella nurekis gen. et sp. nov.* (Trebouxiophyceae, Chlorophyta), a novel coccoid green alga carrying significant biotechnological potential. *Fottea* 14: 53–62. http://dx.doi.org/10.5507/fot.2014.004

Škaloud, P., Steinová, J., Řídká, T., Vančurová, L. & Peksa, O. (2015) Assembling the challenging puzzle of algal biodiversity: Species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 51: 507–527. http://dx.doi.org/10.1111/jpy.12295

Stretch, R. & Viles, H. (2002) The nature and rate of weathering by lichens on lava flows on Lanzarote. *Geomorphology* 47: 87–94. http://dx.doi.org/10.1016/S0169-555X(02)00143-5

Swofford, D.L. (2003) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods*).

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. http://dx.doi.org/10.1093/molbev/msm092

Thüs, H., Muggia, L., Pérez-Ortega, S., Favero-Longo, S.E., Joneson, S., Brien, H.O., Nelsen, M.P., Grube, M., Friedl, T., Brodie, J., Andrew, C.J., Lutzoni, F. & Gueidan, C. (2011) Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota). *European Journal of Phycology* 46: 399–415. http://dx.doi.org/10.1080/09670262.2011.629788

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

Tschermak-Woess, E. (1980b) *Elliptochloris bilobata*, *gen. et spec. nov.*, the phycobiont of *Catolechia wahlenbergii*. *Plant Systematics and Evolution* 136: 62–72.

Tschermak-Woess, E. (1988) The algal partner. *In*: Galun, M. (Ed.) *CRC Handbook of Lichenology*. CRC Press, Inc., Boca Raton, pp. 39–92.

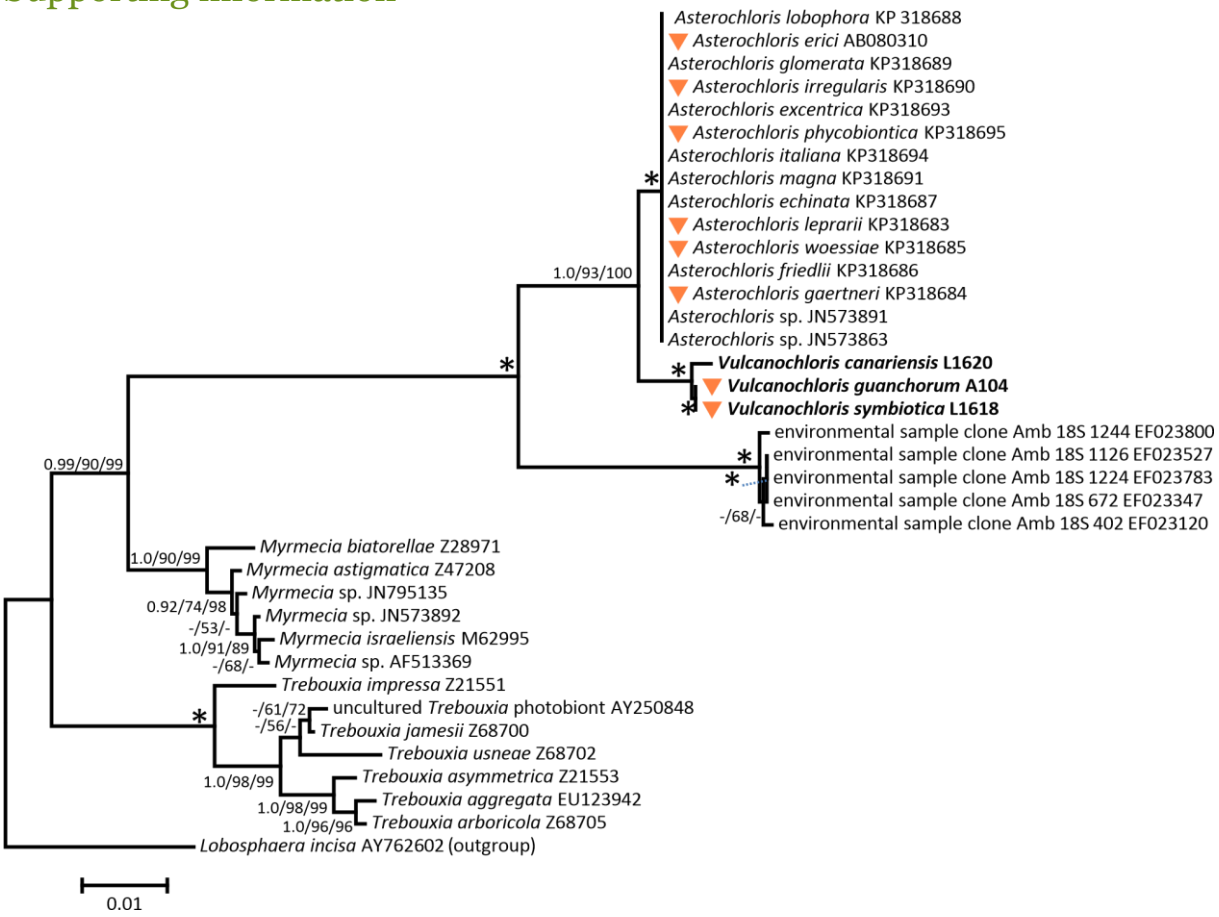
Vainio, E.A. (1888) Notulae de synonymia lichenum. *Meddelanden af Societas pro Fauna et Flora Fennica* 14: 20–30.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315–322.

Zahlbruckner, A. (1928) *Catalogus Lichenum Universalis 5*. Bornträger, Leipzig, 814 pp.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. The University of Texas, Austin, 115 pp.

Supporting information



SUPPLEMENTARY FILE 1. Bayesian analysis based on the 18S rDNA dataset. Values at the nodes indicate statistical support estimated by three methods—MrBayes posterior-node probability (left), maximum-likelihood bootstrap (middle), and maximum parsimony bootstrap (right). Asterisk represents full support. Scale bar shows the estimated number of substitutions per site. Newly sequenced strains are marked in bold. Those sequences containing the IB3 group I introns are marked by a triangle.

The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota)

Lucie Vančurová[1]*, Lucia Muggia[2], Ondřej Peksa[3], Tereza Řídká[1] & Pavel Škaloud[1]

[1]*Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Prague 2, Czech Republic*

[2]*University of Trieste, Department of Life Sciences, via Giorgieri 10, 34127 Trieste, Italy*

[3]*The West Bohemian Museum in Pilsen, Kopeckého sady 2, 301 00 Plzeň, Czech Republic*

** Corresponding author (E-mail: lucie.vancurova@natur.cuni.cz)*

Abstract

Symbiosis plays a fundamental role in nature. Lichens are among the best known, globally distributed symbiotic systems whose ecology is shaped by the requirements of all symbionts forming the holobiont. The widespread lichen-forming fungal genus *Stereocaulon* provides a suitable model to study the ecology of microscopic green algal symbionts (i.e., phycobionts) within the lichen symbiosis. We analyzed 282 *Stereocaulon* specimens, collected in diverse habitats worldwide, using the algal ITS rDNA and actin gene sequences and fungal ITS rDNA sequences. Phylogenetic analyses revealed a great diversity among the predominant phycobionts. The algal genus *Asterochloris* (Trebouxiophyceae) was recovered in most sampled thalli, but two additional genera, *Vulcanochloris* and *Chloroidium*, were also found. We used variation-partitioning analyses to investigate the effects of climatic conditions, substrate/habitat characteristic, spatial distribution, and mycobionts on phycobiont distribution. Analogically, we examined the effects of climate, substrate/habitat, spatial distribution, and phycobionts on mycobiont distribution. According to our analyses, the distribution of phycobionts is primarily driven by mycobionts and *vice versa*. Specificity and selectivity of both partners, as well as their ecological requirements and the width of their niches vary significantly among the species-level lineages. We demonstrated that species-level lineages, which accept more symbiotic partners, have wider climatic niches, overlapping with the niches of their partners. Furthermore, the survival of lichens on substrata with high concentrations of heavy metals appears to be supported by their association with toxicity-tolerant phycobionts. In general, low specificity towards phycobionts allows the host to associate with ecologically diversified algae, thereby, broadening its ecological amplitude.

Keywords: symbiosis, lichen, phycobiont, ecological niches, diversity, specificity

Introduction

A number of invertebrates, such as sea anemones, corals, and platyhelminths, as well as protists, have evolved mutualistic associations with photosynthetic partners. They provide photoassimilates to the hosts, enabling them to colonize habitats where they would normally not survive (Paracer & Ahmadjian 2000). Lichens are an iconic example of symbiotic systems, composed of various heterotrophic and autotrophic partners. The exclusive presence of multiple autotrophic and heterotrophic organisms gives rise to a thallus with a typical phenotype and a characteristic combination of secondary compounds (Spribille *et al.* 2016). Lichens are found in a wide range of terrestrial environments throughout the world. In some ecosystems, lichens are the dominant autotrophs (Romeike *et al.* 2002).

Approximately 100 species within 40 genera of green algae and cyanobacteria have been reported for the more than 20,000 species of mycobionts (Kirk *et al.* 2008). The most common photobionts comprise the green algal genera *Trebouxia* and *Trentepohlia* and the cyanobacterium *Nostoc* (Friedl & Büdel 2008; Tschermak-Woess 1988b). The degree of specificity and selectivity that both the fungal and algal partners show for each other is crucial for the development of the lichen thallus. The term specificity delimits the taxonomic range of acceptable partners, whereas selectivity refers to the preference for a certain group of partners (Rambold *et al.* 1998; Yahr *et al.* 2004, 2006). Most mycobiont species associate with several lineages of a single algal genus, frequently *Trebouxia* (Casano *et al.* 2011; Helms *et al.* 2001; Leavitt *et al.* 2013, 2015, 2016; Muggia *et al.* 2014; Nyati *et al.* 2013; Singh *et al.* 2017). Zoller and Lutzoni (2003) studied the interaction of basidiolichen *Omphalina* with only one species of the genus *Coccomyxa*. The phycobiont diversity of the lichen-forming fungal genera *Cladonia* (Bačkor *et al.* 2010; Beiggi & Piercey-Normore 2007; Piercey-Normore & DePriest 2001; Škaloud & Peksa 2010; Yahr *et al.* 2004) and *Lepraria* (Nelsen & Gargas 2006, 2008; Peksa & Škaloud 2011; Škaloud & Peksa 2010), which are closely related to the genus *Stereocaulon*, has also been described. Both mycobiont genera, *Cladonia* and *Lepraria*, associate with a wide range of *Asterochloris* species, which require diverse ecological conditions (Peksa & Škaloud 2011; Škaloud *et al.* 2015). In contrast, more diversified phycobionts in the microlichen genus *Micarea* were found to associate with two genera, *Coccomyxa* and *Elliptochloris* (Trebouxiophyceae; Yahr, Florence, Škaloud, & Voytsekhovich, 2015). A much broader range of potential photobiont partners was observed for species of the family Verrucariaceae, where the mycobionts associate with phycobionts of nine genera in five orders of the Chlorophyta and one genus in Xanthophyceae (Thüs *et al.* 2011).

Stereocaulon (Lecanorales, Ascomycota) is a widely distributed, ecologically successful lichen-forming genus, comprising mycobiont species with a broad ecological requirements and extensive geographical distribution, sometimes associating with both phycobionts and cyanobionts (the latter located in particular structures known as cephalodia; Lücking et al., 2009). *Stereocaulon* lichens occur in highly diverse environments, from polar (Seo *et al.* 2008) to tropical regions (Ismed *et al.* 2012), at different altitudes, and frequently on metal-rich substrata (Medeiros *et al.* 2014; Purvis &

Halls 1996). Some species of this genus also tolerate submersion (Sadowsky *et al.* 2012), as well as drought (Singh *et al.* 2013). *Stereocaulon* ranks among the pioneer lichens that grow in harsh conditions on bare substrata, such as lava flows and relatively exposed siliceous blocks, thereby, contributing to their weathering (Meunier *et al.* 2014; Stretch & Viles 2002). Multiple lineages of *Asterochloris* are associated with diverse *Stereocaulon* species (Nelsen & Gargas, 2008; Peksa & Škaloud, 2011). *Chloroidium* was found in *Stereocaulon nanodes* (Beck, 2002), and members of the newly described genus *Vulcanochloris* are the phycobionts of *Stereocaulon vesuvianum* (Vančurová *et al.* 2015).

Previous ecological studies on lichen phycobionts focused mainly on the type of growth substrate (Bačkor *et al.* 2010; Leavitt *et al.* 2013; Muggia *et al.* 2014). Several studies have investigated the effects of various climatic conditions (Dal Grande *et al.* 2017; Fernández-Mendoza *et al.* 2011; Leavitt *et al.* 2016; Marini *et al.* 2011; Peksa & Škaloud 2011; Singh *et al.* 2017). Recently, Rolshausen et al. (2017) described mutualist-mediated climatic niche expansion. Moreover, global climate change events have also been discussed in association with lichen phycobionts. Aptroot and van Herk (2007) considered the genus *Trentepohlia*, whose members prefers warm and humid climates, to be an indicator of climate change in temperate zones. Most analogous studies, which considered the effects of temperature on coral-algae symbiosis, showed that the preferences for certain photobionts are key factors in the distribution of the host (Howells *et al.* 2012).

Since host distribution may be greatly influenced by the requirements of the photobionts, the aim of our work was to determine the phycobiont (i.e., green eukaryotic photobiont) diversity of *Stereocaulon* lichens and the association between this diversity and environmental conditions. This study represents the first investigation aimed at understanding the effects of climatic conditions, substrate/habitat types, spatial structure, and symbiotic partner (mycobiont) on the diversity of lichen phycobionts on a global scale. We applied both phylogenetic and statistical analyses to numerous *Stereocaulon* specimens collected in diverse habitats worldwide to address the following questions: 1) What is the diversity of phycobionts associated with the lichen-forming genus *Stereocaulon* within the entire genus and species-level lineages?; 2) Which environmental factors influence the global distribution of phycobionts?; 3) Do phycobionts and mycobionts exhibit reciprocal specificity/selectivity, and how does this affect the width of their climatic niches?

Material and Methods

Taxon sampling

A total of 282 *Stereocaulon* specimens belonging to 20 fungal morphospecies (of 130 – 140 known morphospecies; Högnabba, 2006) collected all over the world (Figure 1, Table S1), were analyzed. The following data were collected for the lichen samples: type of substrate, habitat, GPS coordinates, and altitude. The sampling sites represented various habitats and diverse types of substrates and were located at an altitude 17–4500 m (Table S1). The sampling was carried out in 2008–2016, and attempts were made for the sampling to be as comprehensive as possible

concerning both the *Stereocaulon* morphospecies and their ecology. The mycobiont morphospecies were identified using standard morphological and chemical analyses. Chemical analyses involved thin-layer chromatography (TLC) on Merck silica gel 60 F254 pre-coated glass plates in solvent systems A, B, and C according to Orange et al. (2001). Lichen specimens were deposited in the herbaria GZU, PL, PRA, and PRC (herbaria acronyms follow Index Herbariorum; Thiers 2016), and the private herbarium of J. Malíček.

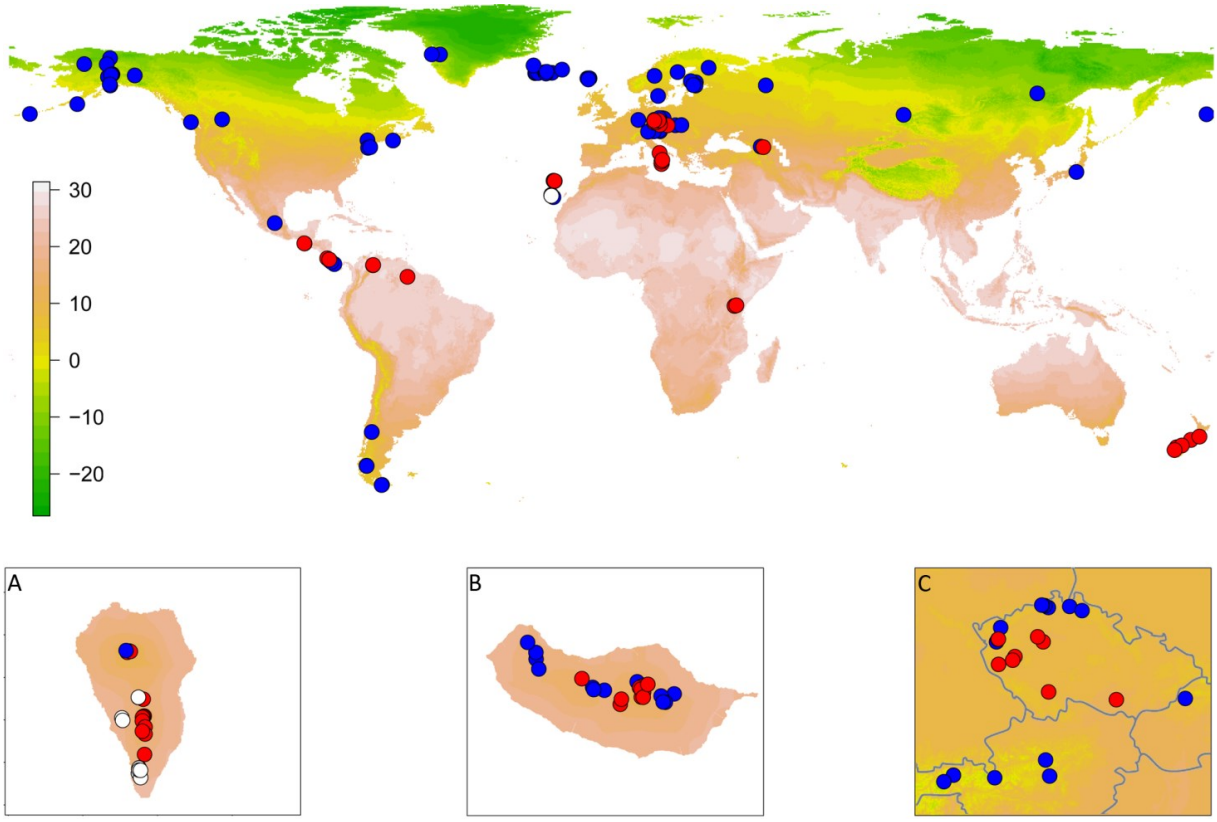


FIGURE 1. Distribution of phycobionts associating with the lichen-forming fungal genus *Stereocaulon*. Blue dots – *Asterochloris*, red dots – *Chloroidium*, white dots – *Vulcanochloris*. Legend – annual mean temperature gradient. Magnified cut-outs – A, La Palma (Canary Islands), B, Madeira, C, Czech Republic.

Phycobiont isolation, DNA extraction, amplification, and sequencing

DNA was extracted from phycobiont cultures or directly from lichen thalli (total lichen DNA). Phycobionts were isolated using the thallus fragment method (Ahmadjian 1993) and cultivated as described in Peksa and Škaloud (2008). Lichen thalli were examined under a dissecting microscope and washed before DNA extraction to prevent contamination by soredia from other lichens. DNA was extracted from thallus fragments following the CTAB protocol (Cubero *et al.* 1999). The algal and fungal nuclear internal transcribed spacer (ITS, ITS1-5.8S-ITS2 rDNA) and the algal actin type I gene (including one complete exon and two introns located at codon positions 206 and 248; Weber & Kabsch 1994) were PCR amplified using primers listed in Table S2. The PCR conditions were as follows: an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturing at

94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 2 min, with a final extension step at 72 °C for 10 min. The actin type I locus was amplified as described by Peksa and Škaloud (2011) using four algal-specific primer pairs (Table S2). All PCR amplifications were performed in a volume of 20 μl with Red Taq Polymerase (Sigma) as described by Peksa and Škaloud (2011) or with My Taq Polymerase. Negative controls, without DNA template, were included in every PCR run to eliminate false-positive results caused by contaminants in the reagents. The PCR products were purified and sequenced using the same primers with an Applied Biosystems (Seoul, Korea) automated sequencer (ABI 3730XL) at Macrogen in Seoul, Korea. The newly obtained sequences of the ITS rDNA and actin type I regions were deposited in GenBank under accession numbers MH382116–MH382150 and MH414969–MH415451 (Table S1).

Sequence alignment and DNA analyses

Individual sequence alignments were prepared separately for *Asterochloris* and *Chloroidium* because they present considerable sequence divergence at the ITS locus. In addition, the sequences obtained for *Asterochloris* were analyzed as a single locus dataset for the ITS rDNA (data not shown) and as a concatenated dataset of ITS rDNA and actin type I loci. The *Vulcanochloris* samples utilized in this study were derived from the recent analysis of Vančurová et al. (2015), and therefore no new phylogenetic inference is presented here. Alignment of ITS rDNA sequences of *Stereocaulon* mycobionts was prepared.

The *Asterochloris* ITS rDNA dataset consisted of 220 sequences: 168 newly obtained sequences from *Stereocaulon* specimens and one newly obtained sequence from *Cladonia*, 19 previously published sequences from *Stereocaulon*, and 32 sequences from other lichens retrieved from GenBank. The actin type I dataset consisted of 74 sequences: 31 newly obtained sequences from *Stereocaulon* specimens, 11 previously published sequences from *Stereocaulon*, and 32 sequences from other lichens. When selecting the available sequences from GenBank, the care was taken to include all known *Asterochloris* species as well as other previously published *Asterochloris* species-level lineages. The alignment was produced by MAFFT v.7 software (Katoh & Standley 2013) under the Q-INS-I strategy and manually edited according to the published secondary structures of ITS2 (Škaloud & Peksa, 2010) using MEGA6 (Tamura *et al.* 2013). The actin type I sequences were aligned using MAFFT v.7 software (Katoh & Standley, 2013) under the Q-INS-I strategy. After deleting identical sequences, the resulting concatenated alignment comprised 71 samples represented by 71 ITS rDNA (Appendix S1) and 66 actin type I sequences (Appendix S2); missing actin data were replaced with question marks.

The *Chloroidium* ITS rDNA dataset comprised 111 sequences: 80 newly obtained sequences from *Stereocaulon* specimens and 31 representative sequences from all known free-living *Chloroidium* species (Table S3). The alignment was produced by MAFFT v.7 software (Katoh & Standley 2013) under the Q-INS-I strategy and manually edited using MEGA6 (Tamura *et al.* 2013) according to the ITS2 secondary structures constructed by RNAfold WebServer (Gruber *et al.* 2008) with default

settings. After removing identical sequences, the resulting alignment comprised 45 sequences (Appendix S3).

The *Stereocaulon* mycobiont ITS rDNA dataset consisted of 335 sequences: 234 newly obtained sequences from our *Stereocaulon* specimens and 88 previously published sequences. The alignment was produced using MAFFT v.7 software (Katoh & Standley 2013) under the Q-INS-I strategy. After removing identical sequences, the resulting alignment comprised 195 sequences (not presented).

Phylogenetic trees were inferred with Bayesian Inference (BI) using MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001), maximum likelihood (ML) analysis using GARLI v.2.0 (Zwickl 2006), and maximum parsimony (MP) analysis using PAUP v.4.0b10 (Swofford 2003). BI and ML analyses were carried out on a partitioned dataset to differentiate among ITS1, 5.8 S and ITS2 rDNA, actin intron 206, actin intron 248, and actin exon regions. Substitution models (Table S4) were selected using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Darriba *et al.* 2012; Guindon & Gascuel 2003). ML analysis was carried out using default settings, five search replicates, and the automatic termination set at 5 million generations. The MP analysis was performed using heuristic searches with 1000 random sequence addition replicates and random addition of sequences (the number was limited to 104 per replicate). ML and MP bootstrap support values were obtained from 100 and 1000 bootstrap replicates, respectively. Only one search replicate was applied for ML bootstrapping.

Species-level lineages delimitation

We performed three species delimitation analyses (GMYC, bPTP, ABGD) to estimate putative species boundaries in the *Asterochloris, Chloroidium* and *Stereocaulon* (mycobiont) datasets. Since the presence of identical sequences may result in artifactual species trees (Hoef-Emden 2012), we merged all identical sequences in our dataset. First, we performed the Bayesian analyses with BEAST 1.8.2 (Drummond *et al.* 2012) to obtain ultrametric trees under the assumption of uncorrelated lognormal relaxed molecular clock. For each of the alignment partitions, the most appropriate substitution model (Table S4) was estimated using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Darriba *et al.* 2012; Guindon & Gascuel 2003). The analyses were performed under the constant population size coalescent as the tree prior and Ucld mean prior was set to exponential distribution with mean 10 and initial value 1. Five MCMC analyses were run for 30 million generations, sampling every 10,000 generations. The outputs were diagnosed for convergence using TRACER v. 1.7 (Rambaut *et al.* 2018) and the five tree files were merged using the burn-in set to 3 million generations (all ESS values of the merged dataset were above 900). Consensus tree was generated using TreeAnnotator 1.8.2.

The GMYC analysis was performed on ultrametric consensus tree under the single-threshold model, using the SPLITS package (Monaghan *et al.* 2009) in R 3.3.0 (R Core Team 2017). The bPTP analysis was also performed on ultrametric consensus tree, using the bPTP web Server

(http://species.h-its.org/ptp/). The analysis was run for 200,000 generations, using 0.3 burn-in and 100 thinning. Both ML and Bayesian solutions were examined. Finally, the ABGD analysis was performed on the concatenated alignment, using the ABGD web server (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). Genetic distances were calculated using the K80 model, and the model parameters were set to Pmin 0.001, Pmax 0.01, Steps 10, and Nb bins 20. Separate analyses were run under varying relative gap width values (0.1, 0.3, 0.5, 0.8, 1.0) to assess the consistency of the inferred groups.

A name was assigned to the recovered lineages of *Asterochloris* and *Chloroidium* using (1) the original name given to the lineage when it was first published (e.g., A9); (2) the name of a known species that had been formally described in previous phylogenetic studies; (3) the names *A. aff. irregularis*, *A. aff. italiana* and *C. aff. ellipsoideum* indicating affinity to that species, or (4) the nomenclature StA1–StA8 (*Asterochloris*) and StC1 and StC2 (*Chloroidium*) to indicate lineages identified as new and not yet formally described. For the algal species-level lineages containing only one sample we used the name of that sample (e.g., sample A504). For the purpose of this study, the species-level lineages of *Stereocaulon* mycobiont were named OTU1–OTU57. The taxonomic revision of *Stereocaulon* is not the aim of this study, therefore the species names (*S. vesuvianum*, *S. azoreum* and *S. nanodes*) were assigned to the lineages only when their identity was obvious.

Variation partitioning

From the entire dataset of 282 *Stereocaulon* specimens 35 were excluded due to the lack of mycobiont sequences and 6 due to the insufficient substrate/habitat data, resulting to a dataset of 241 samples. The relative effects of climate, substrate/habitat, geographical distance, and the symbiotic partner, on the variance in photobiont as well as mycobiont diversity were analyzed by variation partitioning in redundancy analysis, using the *varpart* function in the vegan package (Oksanen *et al.* 2017). The phylogenetic distances of phycobionts or mycobionts were used as a response variable, coded as the first 10 PcoA axes. Climatic data were obtained from the CHELSA Bioclim database (Karger *et al.* 2017) at a resolution of 2.5 arc minutes. At each sampling site, climatic data were obtained by applying a 5km buffer to limit the effects of spatial bias. The 19 environmental variables were condensed into principal component variables (PCs). The Broken-stick distribution (Jackson 1993) was used to select which principal components to include in variation partitioning analysis, using the *bstick* function in the vegan package (Oksanen *et al.* 2017). Accordingly, PC1–PC4 were selected. Analogically, the presence/absence matrix of 12 substrate/habitat variables (Table S5) were transformed into principal component variables. Again, PC1–PC4 were selected by the Broken-stick distribution. Geographical distance values (latitude and longitude) were transformed to the principal coordinates of neighbor matrices (PCNM) vectors representing the geographical distances at various spatial scales (Borcard *et al.* 2004). PCNM vectors were calculated based on the pairwise geographical distances obtained by the *distGPS*

function in the BoSSA package (Lefeuvre 2018). The first 100 PCNM were used fort the analysis. All analyses were performed in R (R Core Team 2017).

Niche hypervolumes

The climatic niche of the most abundant species-level lineages of phycobionts and mycobionts and three genera of phycobionts were represented using the Hutchinsonian niche concept that describes a species niche as an n-dimensional hypervolume, where the dimensions are environmental variables (Hutchinson 1957). In the present study, these environmental dimensions were described based on a 19 Bioclim variables (Karger *et al.* 2017). The climatic hypervolumes were constructed by multivariate kernel density estimation (Blonder *et al.* 2014). First, we performed the PCA analysis of 19 Bioclim variables to reduce the total number of predictors. First two PCA axes (explaining 65% of the total variance) were then selected to calculate hypervolumes for each species-level lineages and genera. The boundaries of the kernel density estimates were delineated by the probability threshold, using the 0.85 quantile value. To project the niche spaces of particular lineages, hypervolume contours were plotted based on 5000 random background points, using the alphahull contour type and alpha smoothing value 0.55. The analyses were performed in R, using the hypervolume (Blonder *et al.* 2014) and alphahull (Pateiro-Lopez & Rodriguez-Casal 2016) packages.

The relationship between specificity towards the symbiotic partner and width of climatic niche were inspected as correlation between the number of accepted partners and size of climatic hypervolume. Since the number of samples of particular species-level lineages varied significantly, the number of accepted species-level lineages of symbiotic partners were down-sampled to the smallest sample size in the data set, which is 15 samples for the seven most abundant lineages of mycobiont and 11 for eight most abundant lineages of phycobiont (Figure S1). The rarefaction was performed using *rarefy* function in vegan package (Oksanen *et al.* 2017). The linear regression was performed separately for the mycobiont and phycobiont species-level lineages. Since the parametric regression analyses can be significantly biased in small sample sizes, we performed the Bayesian linear regression instead. We constructed a regression model where we modelled the number of accepted species-level lineages (X_i) as $X_i \sim Normal\ (\mu_i,\sigma)$, where $\underline{\mu_i}$ was determined as $a + b * hypervolume_i$ (a = intercept, b = slope of the regression line) and σ as the variance of the residuals. The priors were set as follows: $a \sim$ Normal (0, 0.001), $b \sim$ Normal (0, 0.001), $\sigma \sim$ Uniform (0, 100). We ran three chains of the model for 1,000,000 iterations, discarding the initial 100,000 as burnin. We fit the regression model in program JAGS 4.2.0 (Plummer 2003) through the R2JAGS package in R.

Results

Molecular sequence data and phylogenetic analysis

In total, we generated 518 new sequences, which were deposited in GenBank under accession numbers MH382116–MH382150 and MH414969–MH415451 (Table S1), and the alignments have been deposited as Appendix S1-S3.

Based their ITS rDNA sequence analysis, the phycobionts in *Stereocaulon* belong to three genera: *Asterochloris*, *Chloroidium*, and *Vulcanochloris*. *Asterochloris* and *Vulcanochloris* are closely related genera within the order Trebouxiales, whereas *Chloroidium* belongs to the unrelated *Watanabea* clade within the same class, Trebouxiophyceae.

The phylogenetic hypothesis resulting from Bayesian analysis of the ITS rDNA and actin type I sequences of *Asterochloris* is shown in Figure S2. We recovered phylogenetic relationships congruent with those obtained in previous studies (Moya *et al.* 2015; Peksa & Škaloud 2011; Škaloud *et al.* 2015). According to three DNA species delimitation analyses (GMYC, bPTP, and ABGD), putative species boundaries in *Asterochloris* dataset were estimated. The species were delimited based on the consensus of these analyses, leading to the delimitation of 39 species clusters. We recovered sequences from *Stereocaulon* thalli in 27 lineages, 10 of which (lineages *Asterochloris aff. irregularis*, *A. aff. italiana*, and StA1-StA8) are new highly resolved lineages in *Asterochloris*. The majority of the new lineages exclusively comprise newly obtained sequences, whereas others include previously published sequences with unresolved positions in *Asterochloris* phylogenetic analyses in previous studies (Cordeiro *et al.* 2005; Moya *et al.* 2015; Peksa & Škaloud 2011; Piercey-Normore & DePriest 2001; Škaloud & Peksa 2010). Nine of the *Asterochloris* lineages could be assigned to formally described species, namely, *A. erici*, *A. excentrica*, *A. glomerata*, *A. italiana*, *A. irregularis*, *A. lobophora*, *A. mediterranea*, *A. phycobiontica*, and *A. woessiae*. The most frequently occurring phycobionts belonged to the species *A. irregularis*, accounting for 32% of *Asterochloris* phycobionts of *Stereocaulon*. The phylogenetic backbone sustains the three main clades, clades A–C, *sensu* Škaloud and Peksa (2010). Even though the phycobionts of *Stereocaulon* were recovered in all three *Asterochloris* clades, they differed in the abundance of *Stereocaulon* sequences; clade B includes only 16 of these sequences, whereas 103 and 68 sequences were recovered within clades A and C, respectively.

A phylogram resulting from Bayesian analysis of ITS rDNA sequences of *Chloroidium* is shown in Figure S3. The phylogenetic relationships are congruent with those identified by Darienko (2010). According to the three DNA species delimitation analyses (GMYC, bPTP, and ABGD) putative species boundaries in *Chloroidium* dataset were estimated. The species were delimited based on the consensus of these analyses, leading to the delimitation of 12 species clusters. The *Chloroidium* phycobionts analyzed were clustered into nine lineages. Two of the lineages could be placed in formally described species, *C. ellipsoideum* and *C. angustoellipsoideum*, whereas StC1, StC2, and *C. aff. ellipsoideum* are new lineages in *Chloroidium* (clade StC2 contains one new and one previously published sequence). Three of the nine lineages also include free-living algae. The most

frequently occurring phycobionts belong to the species *C. aff. ellipsoideum*, accounting for 54% of *Chloroidium* phycobionts of *Stereocaulon*. In contrast, representatives of *C. saccharophilum* and *C. engadiensis* were not found to be phycobionts.

The *Vulcanochloris* dataset was previously analyzed by Vančurová *et al.* (2015). The phycobionts belonging to this genus were recovered in 15 *Stereocaulon* thalli. All identified ITS sequences were detected in three species: *V. canariensis*, *V. symbiotica*, and *V. guanchorum*.

In several cases, we recovered more than one phycobiont genotype from a single lichen thallus (either by direct sequencing of total DNA or by genotyping multiple cultures isolated from a single thallus). Representatives of *Chloroidium ellipsoideum* and *C. angustoellipsoideum* were detected simultaneously four times (samples VancurovaA421, VancurovaLV5, VancurovaOP1118, and VancurovaOP1083; up to six sequences from a single lichen sample). Three sequences of sample VancurovaOP1077 (Vancurova1077, VancurovaOP1077.1, and VancurovaOP1077.2) correspond to three divergent *C. ellipsoideum* genotypes. Moreover, the sequences VancurovaKO25.1 and VancurovaKO25.2 classified into two genera, *Asterochloris* and *Chloroidium,* respectively.

In contrast, multiple sequences from a single sample were often identical; VancurovaL1248 (direct from thallus) and DS1.1 (from culture) represent *Asterochloris irregularis*, and sequences L952 (direct from thallus) and CAB.1 and CAB.2 (from culture) are from the same genotype of *C. ellipsoideum*.

The phylogenetic hypothesis resulting from Bayesian analysis of the ITS rDNA sequences of *Stereocaulon* mycobionts (not shown) is largely congruent with that identified by Högnabba (2006). Many morphospecies in both our and Högnabba's phylogram were paraphyletic, but some lineages clearly correspond with morphospecies. According to three DNA species delimitation analyses (GMYC, bPTP, and ABGD), putative species boundaries in the *Stereocaulon* mycobiont dataset were estimated. The species were delimited based on the consensus of different analyses, leading to the delimitation of 57 species clusters. We recovered sequences from *Stereocaulon* thalli in 30 lineages. The most frequently occurring mycobionts belonged to OTU10, which corresponds with species *S. vesuvianum/S. arcticum*, accounting for 24% of samples.

The associations between phycobiont, mycobiont, and environmental conditions

To identify the factors that shape the symbiotic partner distribution of *Stereocaulon* lichens, we performed variation-partitioning analyses (Figure 2, Figure 3). We analyzed the relative contributions of climate, habitat/substrate, geographical distance, and symbiotic partner to phycobiont and mycobiont distribution.

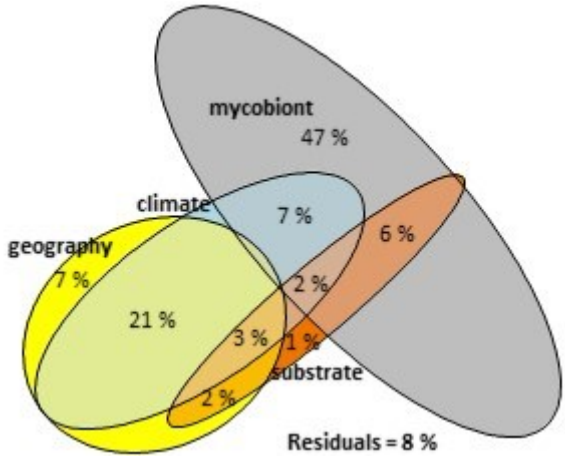


FIGURE 2. Venn's diagram showing the variation in distribution of phycobionts associated with the lichen-forming fungal genus *Stereocaulon* explained by effects of climate, substrate/habitat, geographical distance, and the mycobiont.

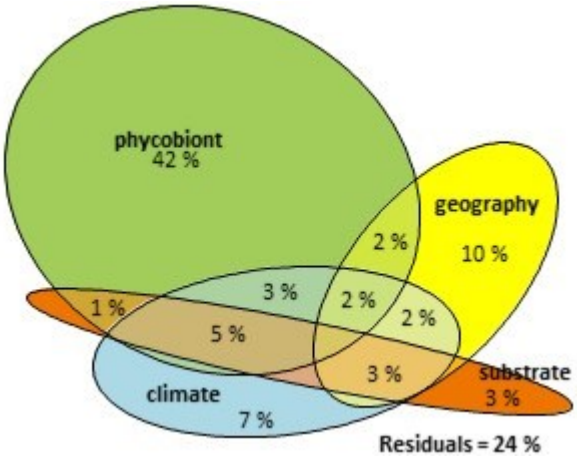


FIGURE 3. Venn's diagram showing the variation in distribution of *Stereocaulon* mycobionts explained by effects of climate, substrate/habitat, geographical distance, and the phycobiont.

Among the phycobionts, climatic conditions, substrate and habitat, geographical distance, and the symbiotic partner (i.e., mycobiont) explained 92% of the variation (Figure 2). The largest proportion of the variation was explained by the mycobiont (47% independent effect and 22% in combination with other variables). Several algal species-level lineages showed specificity towards a single mycobiont OTU (algal-fungal pairs StA1-OTU10 and *V. symbiotica*-OTU52; Figure 4). Others were not specific towards a single mycobiont, but co-operate in most cases with one fungal species-level lineage (i.e., it is selective towards symbiotic partner). For example, OTU47 accepts three algal species-level lineages, but prefers *A. irregularis* (Figure 4). Geographical distance independently explained 7% of the variability, while 33% was shared with other variables. The variables associated with substrate and habitat independently explained 1% of the variability, while 13% was shared with other variables. The climatic conditions explained 33% of the variability shared with other variables (21% with geography), but explained nothing independently.

Climatic conditions, substrate and habitat, geographical distance, and the symbiotic partner (i.e., phycobiont) explained 76% of the variation in the phylogeny of mycobionts. The greatest proportion of the variation (42% independent effect, 15% shared with other variables) was explained by the symbiotic partner, analogically. Although all largely represented mycobiont species-level lineages co-operate with several species of phycobionts, at the level of algal genera they are mostly specific (Figure 4). Geographical distance was the second most important variable, which independently explained 10% of the variability, while 11% was shared with other variables. Besides worldwide distributed mycobionts (especially OTU10), species-level lineages with limited distribution were also identified. For example, OTU52 was found only on La Palma island (Canary Islands), OTU11 as well as OTU13 (*S. azoreum*) in the Mediterranean region, and OTU47 in the Circumboreal region. The climatic variables independently explained 7% of the variability, while

16% was shared with other variables. The fourth variable, substrate and habitat characteristics, accounted for only a small proportion of the variation (3% of the independent effect, 10% in combination with other variables).

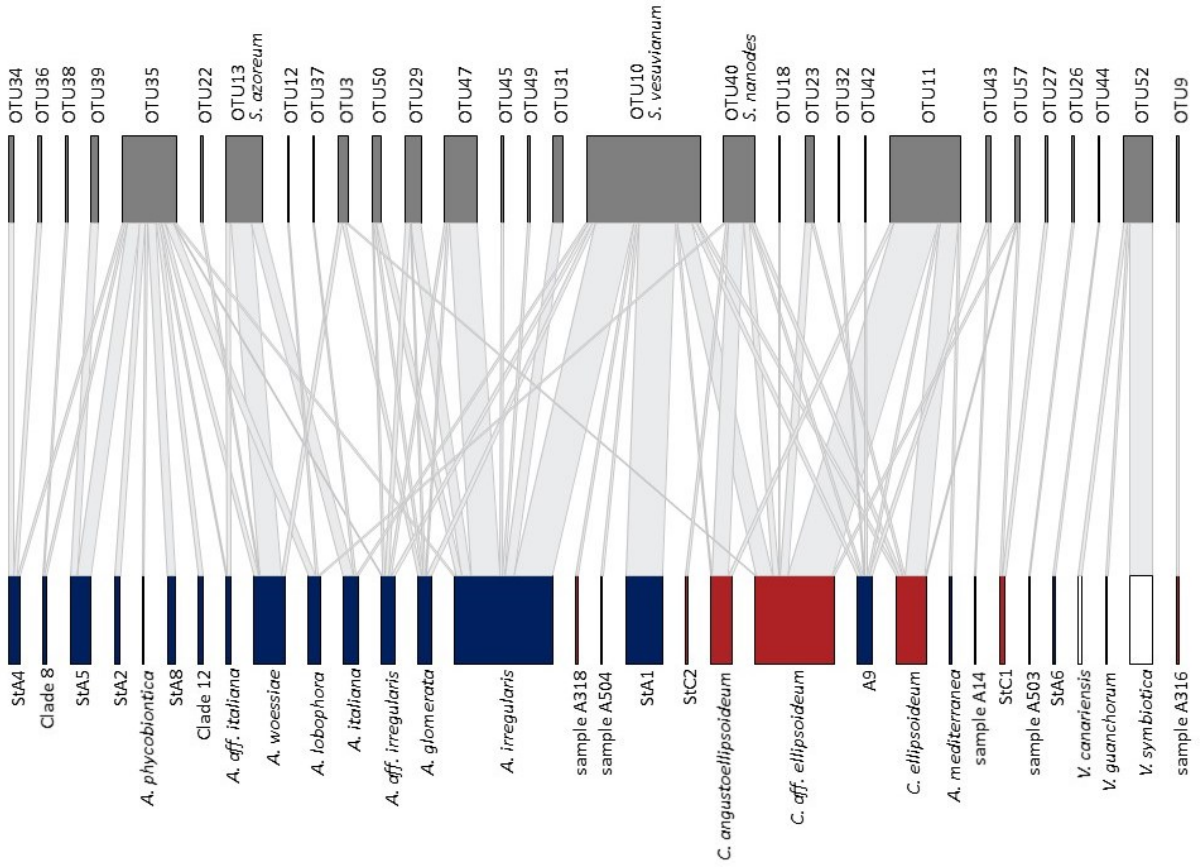


FIGURE 4. Interaction network structure between lichen mycobiont species-level lineages in the genus *Stereocaulon* and phycobiont species-level lineages. The width of the links is proportional to the number of specimens forming the association.

Climatic niches and specificity between the symbiotic partners

We constructed two-dimensional (PC1-PC2 explaining 65.3% variation of climatic variables) hypervolumes for seven most abundant fungal species-level lineages, three algal genera, and the eight most abundant algal species level lineages.

Among the algal genera (Figure 5A), *Asterochloris* and *Chloroidium* have relatively wide niches, unlike *Vulcanochloris*. The climatic data suggest that *Asterochloris* prefers humid climates, *Vulcanochloris* tolerates extremely dry conditions, and *Chloroidium* accepts a wide range of humidity levels (Figure 6A). We also detected obvious differences in precipitation seasonality (Figure 6B): *Asterochloris* occurs in conditions with the most stable precipitation levels, whereas *Chloroidium* accepts highly variable precipitation levels. *Asterochloris* seems to be the most psychrophilic of the three genera, unlike *Vulcanochloris*, which likely prefers relatively elevated temperatures. Lastly, *Chloroidium* phycobionts were found at an annual mean temperature above

o °C (Figure 6C). Only one exception of this rule was observed: sample VancurovaA35 was found at a location with an annual mean temperature of -2 °C.

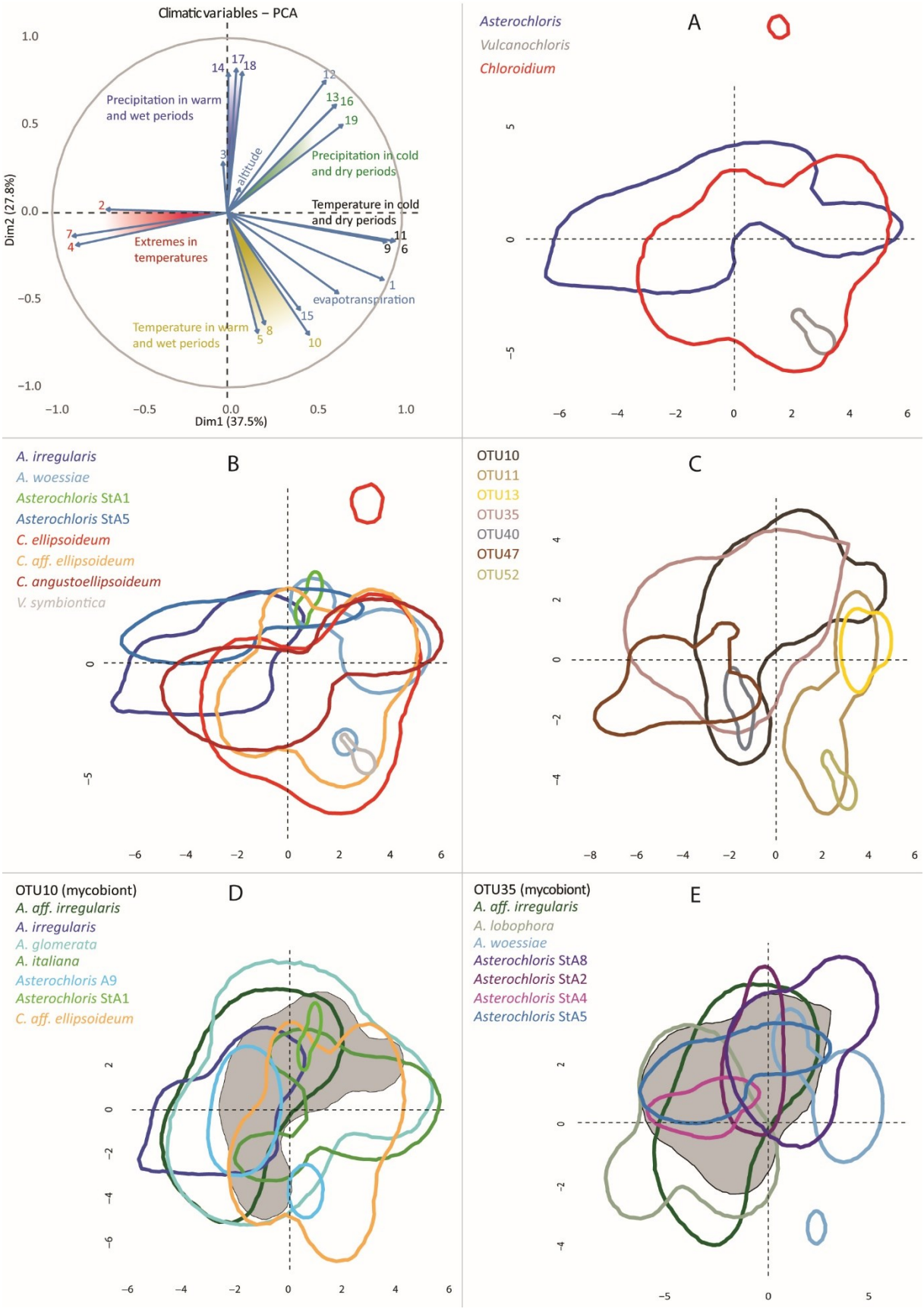


FIGURE 5. Climatic niche hypervolumes for A, algal genera *Asterochloris*, *Vulcanochloris* and *Chloroidium*, B) 8 most abundant algal species-level lineages (phycobionts), C) 7 most abundant fungal species-level lineages (mycobionts), D) fungal OTU10 (grey filled) with its 7 most abundant (of total 11) associating phycobionts, E) fungal OTU35 (grey filled) with its 7 most abundant (of total 12) associating phycobionts based on climatic PC1–PC2 axes (explaining 65% of variation). Climatic variables: 1 = annual mean temperature, 2 = mean diurnal range, 3 = isothermality, 4 = temperature seasonality, 5 = max temperature of warmest month, 6 = min temperature of coldest month, 7 = temperature annual range, 8 = mean temperature of wettest quarter, 9 = mean temperature of driest quarter, 10 = mean temperature of warmest quarter, 11 = mean temperature of coldest quarter, 12 = annual precipitation, 13 = precipitation of wettest month, 14 = precipitation of driest month, 15 = precipitation seasonality, 16 = precipitation of wettest quarter, 17 = precipitation of driest quarter, 18 = precipitation of warmest quarter, 19 = precipitation of coldest quarter (Karger *et al.* 2017).

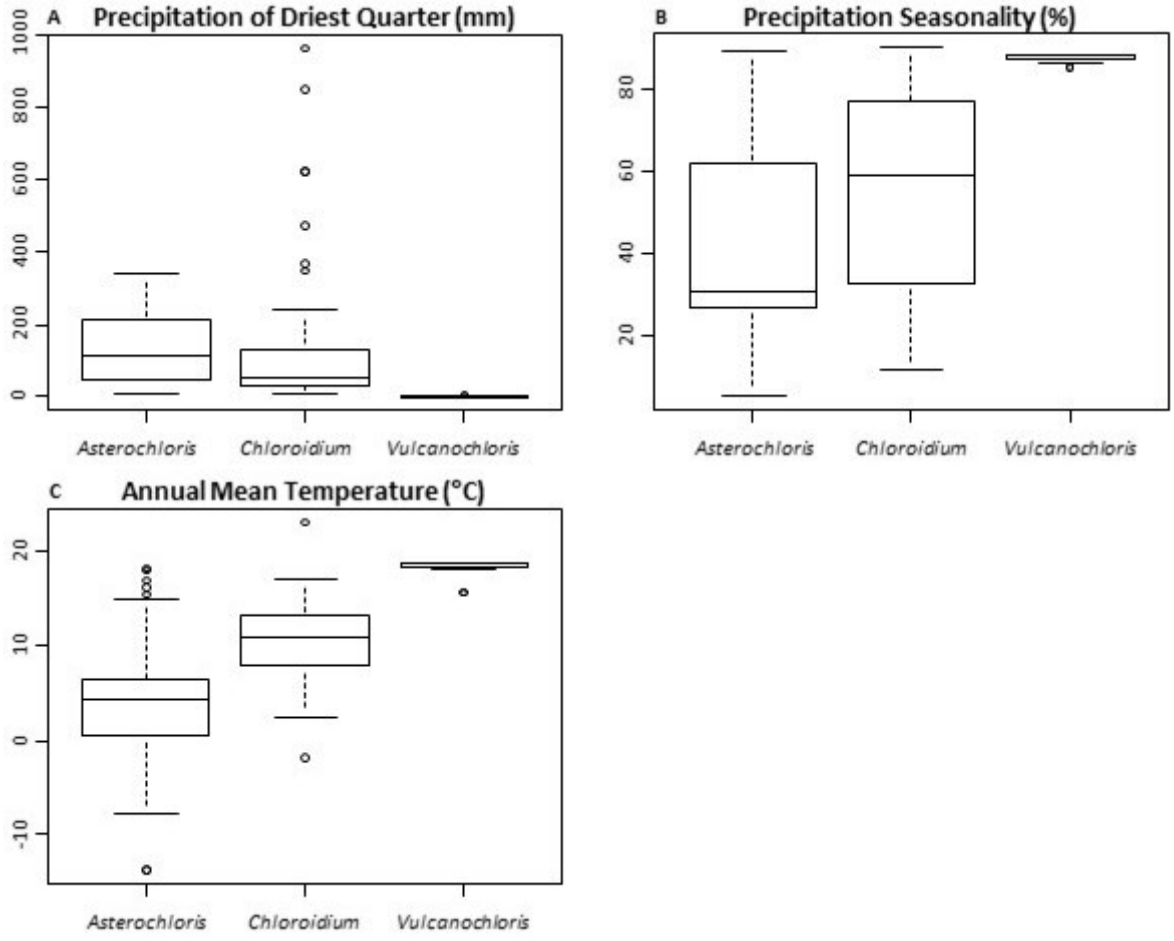


FIGURE 6. Differences in the distribution of three phycobiont genera associated with the lichen-forming fungal genus *Stereocaulon* along the gradient of A, precipitation of driest quarter, B, precipitation seasonality, C, annual mean temperature.

As represented on the plot of the hypervolumes of the eight most abundant phycobionts (Figure 5B), the climatic niche of the genus *Asterochloris* is composed of quite distinct niches of species-level lineages. The climatic data suggest that algal species-level lineages are quite heterogeneous in terms of temperature preference (Figure 7). Interestingly, *Asterochloris italiana* and *A. woessiae* appear to be relatively thermophilic within the generally psychrophilic genus. These two lineages

also occur in more stable climates, as distinct from lineage StA5 and *A. irregularis*, which seem to tolerate considerable temperature seasonality (Figure 5B; Figure S4).

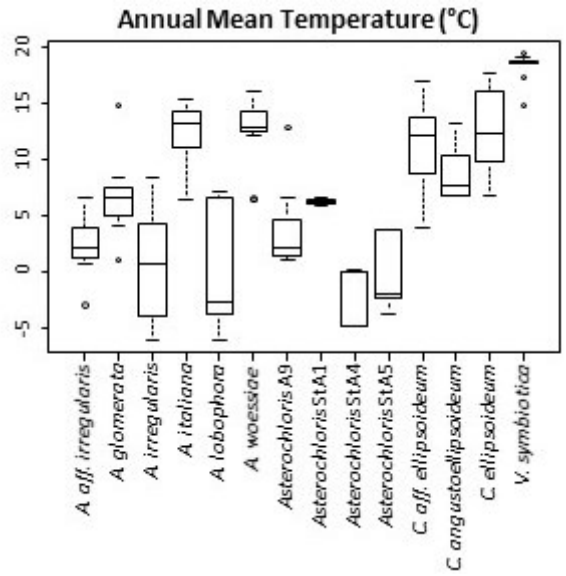


FIGURE 7. Differences in the distribution of 14 most abundant (≥5 specimens) phycobiont species-level lineages associated with the lichen-forming fungal genus *Stereocaulon* along the gradient of annual mean temperature.

The seven most abundant mycobiont species-level lineages could be divided into specialists or generalists with narrow or broad climatic niches, respectively (Figure 5C). They also differ in their specificity towards their algal partner (3–12 algal partners within the entire dataset and 2.8–8.6 algal partners after down-sampling to the smallest sample size in the data set). The similar pattern was also observed within the eight most abundant phycobiont species-level lineages, which co-operate with 1–8 fungal partners (1–4.59 fungal partners after the down-sampling). The hypothesis that species with wide niches corroborate with more symbiotic partners was confirmed using the Bayesian linear regression for fungal as well as algal species-level lineages (Figure 8; Figure S5, S6). For two fungal species-level lineages with the widest climatic niches (OTU10 and OTU35) and the most algal partners, plots combined fungal hypervolume with hypervolumes of their phycobionts were produced (Figure 5D, E).

Discussion

Phycobiont diversity

This study provides insights into the genetic diversity and ecological requirements of phycobionts associated with the lichen-forming fungal genus *Stereocaulon* worldwide. In *Stereocaulon* the main phycobiont genus is *Asterochloris*, for which we recovered 27 lineages (Figure S2), while the second and the third most prevalent genera are *Chloroidium* and *Vulcanochloris*, respectively.

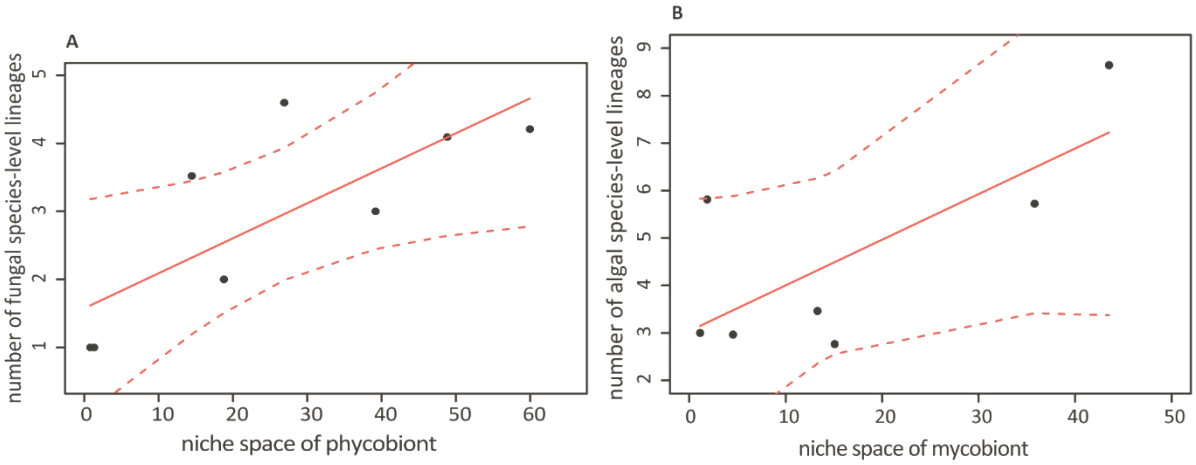


FIGURE 8. A, Bayesian linear regression of algal niche space (hypervolume) as a predictor of the number of accepted species-level fungal lineages. B, Bayesian linear regression of fungal niche space (hypervolume) as a predictor of the number of accepted species-level algal lineages. Dashed lines show the 95% CRI around the regression line.

The phycobiont diversity observed here in *Stereocaulon* appears to be exceptional, especially in terms of the number of algal genera. Lichens generally associate with multiple lineages belonging to a single photobiont genus. Indeed, a wide range of *Trebouxia* lineages are phycobionts of species belonging to various lichen genera, e.g., *Protoparmelia*, *Rhizoplaca*, *Tephromela, Xanthoparmelia, Xanthoria*, and *Xanthomendoza* (Leavitt *et al.* 2013, 2016; Muggia *et al.* 2013a, 2018; Nyati *et al.* 2013), and high infrageneric diversity of *Asterochloris* phycobionts has also been observed in species of *Cladonia* (Bačkor *et al.* 2010; Beiggi & Piercey-Normore 2007; Piercey-Normore & DePriest 2001; Škaloud & Peksa 2010; Yahr *et al.* 2004) and *Lepraria* (Nelsen & Gargas 2006, 2008; Peksa & Škaloud 2011; Škaloud & Peksa 2010). Interestingly, only a few other lichens, which have crustose growth and (generally) a poorly developed cortex (Helms 2003; Thüs *et al.* 2011), are known to build their thalli with phycobionts belonging to different Trebouxiophycean genera (*Lepraria borealis*, Engelen, Convey, & Ott, 2010; *Micarea*, Yahr *et al.*, Yahr et al., 2015; *Bagliettoa* and *Verrucaria nigrescens*, Thüs et al., 2011, Voytsekhovich & Beck, 2015; *Diploschistes muscorum*, Wedin et al., 2015). In contrast, the *Stereocaulon* species considered in this study have complex dimorphic thalli and a well-developed cortex (crustose species of *Stereocaulon* were not included).

Our results also expand upon the known diversity of *Chloroidium* in lichens, as three novel lineages were here identified (Figure S3) and four samples from Central America probably represent still undescribed species within *Chloroidium*. Also Sanders *et al.* (2016) recently presented a new lineage of phycobiont sister to the *Chloroidium* clade from the lichen *Bapalmuia lineata*, which grows on leaves in Panama, which suggest Central America to host an unexplored diverse group of symbiotic algae. In general, the genus *Chloroidium* has rarely been reported in lichens (Beck 2002), being known only from the genera *Trapelia* (Beck 2002; Tschermak-Woess 1948, 1978), *Psilolechia*, *Lecidea* (Beck 2002), *Bacidia* (Tschermak-Woess 1988a), *Verrucaria* (Voytsekhovich

& Beck 2015), *Galidea*, and *Gomphillus* (Sanders *et al.* 2016). Unfortunately, most of these reports cannot be compared with our results, because the studies were based mainly on morphology, and little molecular data were published. Only the recent work of Sanders *et al.* (2016) offers rbcL sequences comparable to those generated by our group. The sequence of the phycobiont of *Galidea* (KX235274; Sanders *et al.* 2016) is identical to the rbcL sequence (not shown) of our sample VancurovaO24 collected in New Zealand (*Chloroidium aff. ellipsoideum*), and the phycobiont of *Gomphillus* (KX235269) appears to be a member of the StC2 lineage. However, as the rbcL marker generally shows lower resolution than the ITS rDNA, we did not further analyze it here. Although previously overlooked, the co-occurrence of several phycobionts in individual lichen thalli (i.e., algal plurality) is a relative common phenomenon (Bačkor *et al.* 2010; Moya *et al.* 2017; Muggia *et al.* 2011, 2014; Onuţ-Brännström *et al.* 2018; Park *et al.* 2015; Voytsekhovich & Beck 2015). We also obtained evidence for algal plurality in several *Stereocaulon* samples, which strengthens the potential of this lichen genus as a suitable model for high-throughput sequencing studies.

The phycobiont diversity in *Stereocaulon* should not be regarded only from a taxonomic or systematic point of view, instead it also extends to the different ecological requirements of the phycobionts involved (see below). As demonstrated for *Ramalina farinacea* (Álvarez *et al.* 2012; Casano *et al.* 2011; Del Hoyo *et al.* 2011), also in *Stereocaulon* the co-occurrence of phycobionts with diverse physiological responses could be an effective adaptive strategy for the successful, pioneering colonization of habitats.

In contrast to lichen symbioses, algal plurality for coral ecosystems has been explored in greater detail. Several studies have suggested the co-occurrence of multiple *Symbiodinium* lineages within individual hosts (Baker 2003; Baums *et al.* 2014). Particular lineages of *Symbiodinium* show distinct ecological preferences (Baker 2003; Pettay *et al.* 2015; Rowan 2004), and some are well-adapted to high temperatures and irradiance (Iglesias-Prieto *et al.* 2004). The ability of corals to maintain or switch various algae could be influenced by the diversity of possible symbionts, which varies among areas (Baums *et al.* 2014). However, although juvenile corals maintain several strains, or switch strains frequently (Byler *et al.* 2013), the capacity of adult corals to switch photobionts is rather limited (Baums *et al.* 2014; Byler *et al.* 2013; Iglesias-Prieto *et al.* 2004). It is therefore necessary to clarify whether the aforementioned phycobiont co-occurrences in the *Stereocaulon* species are as stable as that of the pair *Trebouxia jamesii*/*Trebouxia* TR9 found in *Ramalina farinacea*, or whether they represent only transitional phases of algal switching (Wedin *et al.* 2015).

Ecology and distribution of phycobionts

Our results suggest that amount and seasonality of precipitation may be key factors affecting the distribution of the three phycobiont genera (Figure 6A, B). According to climatic data, the distribution of *Vulcanochloris* as a phycobiont of *Stereocaulon* is restricted to areas with precipitation during the driest quarter, ranging from 3 to 6 mm. *Chloroidium* occurs in areas with a broad range of precipitation during the driest quarter (4 - 960 mm), whereas *Asterochloris* is

distributed in areas with precipitation in the driest quarter ranging from 6 to 316 mm. In terms of temperature variables (Figure 6C), *Vulcanochloris* appears to be the most thermophilic phycobiont (annual mean temperature up to 19.8 °C) of *Stereocaulon*. The overwhelming majority of *Chloroidium* phycobionts are distributed in areas with an annual mean temperature above 0 °C. While most of the *Asterochloris* species are rather psychrophilic, *A. italiana* and *A. woessiae* prefer annual mean temperatures above 5 °C and 10 °C, respectively (Figure 7). Our results complement the finding of Peksa and Škaloud (2011) who showed that the genus *Lepraria* harbors *A. woessiae* phycobionts at low altitudes in central and southeastern Europe. Another thermophilic lineage of this genus is *A. mediterranea*, which is, however, represented by only one sample in our dataset. The distribution of this species is concentrated in the Mediterranean region (Moya *et al.* 2015). It is not clear whether *Asterochloris* distribution is restricted by low temperatures or if the reduced amount of liquid water prevents its distribution in polar regions (Engelen *et al.* 2010; Park *et al.* 2015). An example of the joint influence of temperature and humidity is as follows: in samples from Alaska and Greenland, at very low temperatures (year mean < -5 °C), *Asterochloris* phycobionts tolerate very low precipitation (as low as 239 mm/y in total).

Still it is not possible to distinguish between the climatic preferences of *Vulcanochloris* and the mycobiont OTU52 because of their reciprocal specificity (Figure 4). In case of two other phycobiont genera, these problems have not occurred because of more acceptable species-level lineages of mycobionts, including several common ones. Tolerance of *Vulcanochloris* towards extreme drought should be confirmed by culture experiments and compared with that of *Asterochloris* (e. g., Gasulla et al., 2013) or other trebouxiophycean phycobionts (Candotto-Carniel *et al.* 2016; Centeno *et al.* 2016).

The distribution of *Stereocaulon* species follows a pattern that is highly influenced by the type of substrate. Areas of soil and exposed bedrock rich in heavy metals are geologically and ecologically dissimilar from the surrounding areas and may become “edaphic islands” harboring distinct flora, including lichens (Medeiros *et al.* 2014). Many of the analyzed samples came from metal-rich substrates, which in our dataset are dominated by *Chloroidium* phycobionts seemingly tolerating toxic substances (e.g., heavy metals; Beck, 2002). Indeed, many free-living *Chloroidium* species occur in anthropogenic habitats (Darienko *et al.* 2010; Hallmann *et al.* 2016; Pangallo *et al.* 2015). Also, when copper (Cu) concentrations are high in the thalli of *Lecidea inops*, the photobiont *Asterochloris irregularis* can be replaced by either *Trebouxia* or *Chloroidium* species (Beck, 2002). Heavy-metals (Cu, Zn, Pb, Cd, and Ni) levels vary according to the growth morphology of the lichens (Bačkor *et al.* 2010; Rola *et al.* 2016). Significantly higher levels are found in crustose and dimorphic lichens (such as *Cladonia* species), characterized by the presence of thallus parts that strongly adhere to the substrate. In *Stereocaulon*, which also commonly exhibit dimorphic growth, *Chloroidium* was observed in morphospecies with shorter pseudopodetia (ascending thallus branches) and thalli that strongly attach to rocks (*S. nanodes* and *S. vesuvianum*). Thus, the survival of these lichens on substrates bearing high concentrations of heavy metals seems to rely

on their association with toxicity tolerant *Chloroidium* phycobionts. Being volcanic rocks also metal-rich substrates many *Stereocaulon* species abundant on them (Abrams *et al.* 1996; Cutler *et al.* 2008; Stretch & Viles 2002) principally associate with *Chloroidium* and *Vulcanochloris* phycobionts (Figure 9).

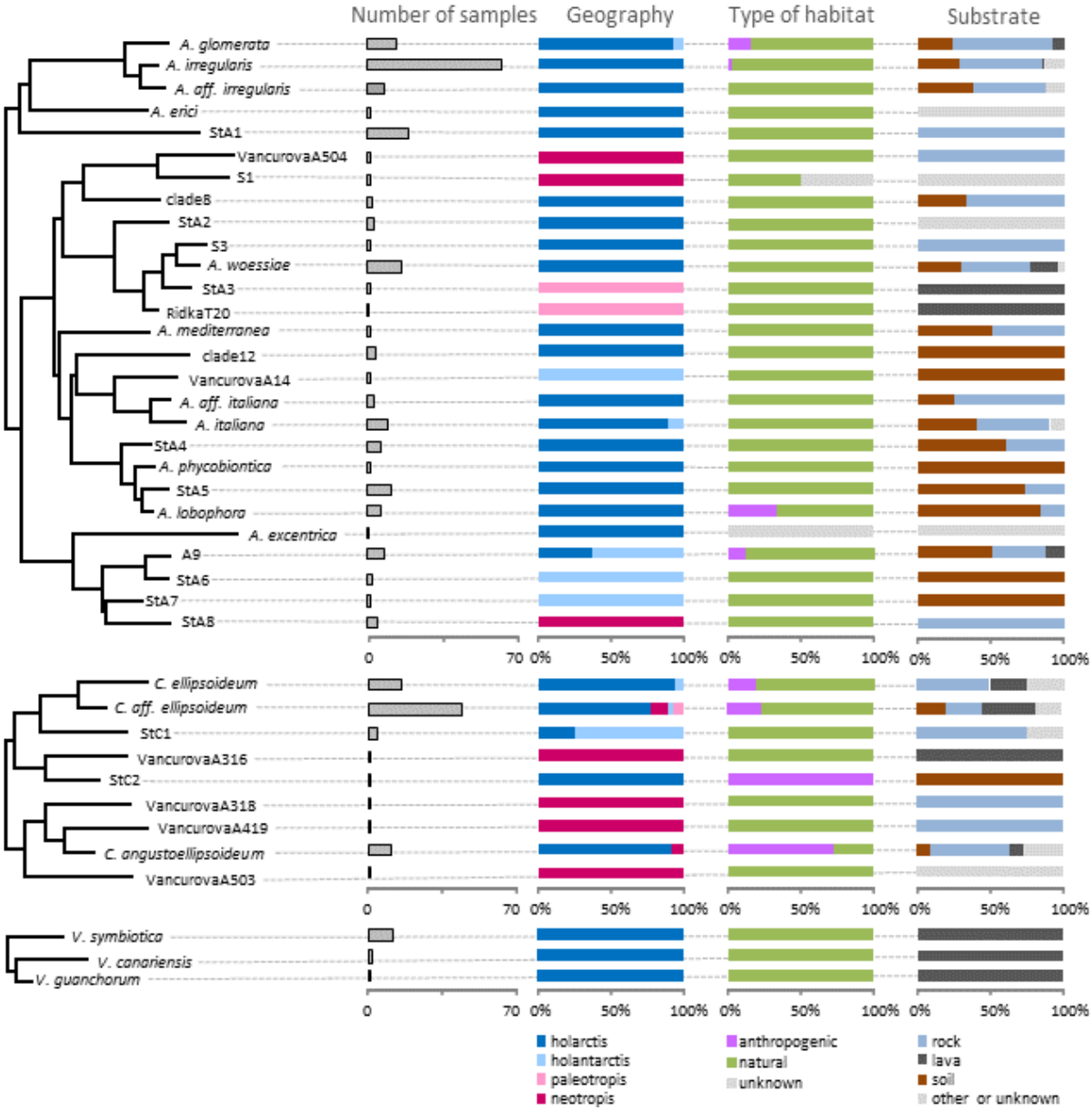


Figure 9. Visualized abundance, geographic and habitat, and substrate distribution of phycobionts associating with the lichen-forming genus *Stereocaulon*. From left to right: phylogenetic hypotheses based on ITS rDNA + actin type I gene (*Asterochloris*) or sole ITS rDNA sequences (other two genera); barcharts showing the absolute phycobiont abundances; proportional abundances in phytogeographical regions; proportional abundances in habitats; and proportional abundances on substrates.

In contrast, *Asterochloris* species rarely associated with *Stereocaulon* species colonizing metal-rich substrates. On volcanic rocks, mainly *Asterochloris woessiae* was recovered. Previously, this phycobiont species was recovered in lichens from a former industrial sedimentation basin (Bačkor *et al.* 2010) strongly polluted by heavy metals, with excess concentrations of metals, such as Fe,

Mn, Zn, Al, and Cd (Kovář 2004), which suggests its toxicity tolerance. The high pH values characterizing volcanic rocks may be a further factor influencing the distribution of *Vulcanochloris* and *Asterochloris woessiae* on this substrate (Peksa & Škaloud 2011; Škaloud *et al.* 2015).

The ecological and distribution patterns of phycobionts of *Stereocaulon* is also determined by geographic distance (7% independent effect, 33% in combination with other variables; Figure 3) and confirm the results of Yahr et al. (2006), who found that phycobiont variability at lower taxonomic levels is dependent on geography.

The distribution of *Asterochloris irregularis* is limited to the Northern Hemisphere (Figure 9), where it widely occurs in eight *Stereocaulon* mycobiont species-level lineages (Figure 4) and many other lichens (according to the NCBI database and our unpublished data). We hypothesize that this psychrophilic alga could not survive in the tropical climatic zone. The same could also be expected for lineages StA4 and StA5, which are also psychrophilic and have only been found in the Northern Hemisphere to date. In agreement with this hypothesis, several studies (Fernández-Mendoza *et al.* 2011; Lindblom & Sochting 2013) reveal distinct genotypes of phycobionts specific to either southern or northern polar/boreal regions. In contrast, some other green algae, such as *Klebsormidium* (Streptophyta) (Ryšánek *et al.* 2016), exhibit a great dispersal capacity, and several genotypes were found in both southern and northern polar regions. It seems that the scarcity of areas with climatic conditions suitable for *Asterochloris irregularis* limits its dispersal in the Southern Hemisphere.

The finding that the distribution of some lineages is limited to certain geographic areas might be biased by undersampling (Ryšánek *et al.* 2015). Some *Vulcanochloris* species were recently found in the Czech Republic (Vaiglová 2017) as phycobionts of *Protoblastenia rupestris*, and in Germany and Sweden likely as free-living soil algae (Ruprecht *et al.* 2014). *Vulcanochloris* can easily be mistaken for *Asterochloris* because differences in chloroplast morphology (Vančurová *et al.* 2015) might be blurred by the lichenized state (Peksa & Škaloud 2008). It is likely that various *Vulcanochloris* species occur also in other areas as phycobionts of *Stereocaulon*.

Most protist lineages are widespread, whereas some others are locally highly abundant, but globally rare (Ryšánek *et al.* 2015). In addition to *Vulcanochloris*, the *Asterochloris* lineage StA1 also exhibited this pattern: it was quite abundant at all sampling sites on Faroe Islands (73% of all samples) but absent elsewhere. Moreover, Leavitt et al. (2016) found multiple *Trebouxia* species-level lineages at all studied sites, but their abundance varied considerably.

Specificity and selectivity of lichen associations

The majority of the *Stereocaulon* mycobiont species-level lineages appear to be specific towards phycobiont genera (i.e., one mycobiont species accepts exclusively one algal genus): most of them choose *Asterochloris* photobionts, while OTU52 takes *Vulcanochloris* (Figure 4). The fungal species-level lineages accept mostly more than one congeneric algal species, but prefers one of them

(i.e., mycobionts are selective towards individual algal species; Rambold et al., 1998; Yahr et al., 2004, 2006).

The terms specificity and selectivity are also used in the opposite case, towards the host (Finney *et al.* 2010). A general comparison of present, previously published, and unpublished data (containing mainly sequences of *Cladonia* and *Lepraria* phycobionts), shows that six *Asterochloris* lineages (*A. irregularis*, StA1, StA2, StA4, StA5, and A9) are selective and three are specific (*A. excentrica*, StA6, StA8) towards *Stereocaulon* mycobionts.

Since mutualistic symbiosis can affect niche width (Duffy & Johnson 2017; Gerz *et al.* 2018), we wondered if the number of accepted algal partners correlates with the climatic niche width of the mycobiont. This holds true in the case of both mycobiont and photobionts (Figure 8): our results show that the less specific phycobionts which associated with many fungal partners have also the widest distribution.

Interestingly, fungal OTU40 (*S. nanodes*) accepts six photobiont species-level lineages within two algal genera and seems to have quite small climatic niche. This can be explained by: i) most of our samples came from anthropogenic habitats (as indicated by substrate and habitat variables including plastic, metal, mine and sludgebed). A similar behavior was observed in the mycobiont *Protoparmeliopsis muralis*, which shows lower selectivity for the photobiont on anthropogenic substrates than in natural habitats (Guzow-Krzemińska 2006; Muggia *et al.* 2013b); ii) OTU40 is the *Stereocaulon* species living in the tightest connection to the substrate within the *Stereocaulon* species included in this study. This factor is considered to decrease mycobiont specificity towards the phycobionts (Helms 2003).

One example of both unspecific and unselective fungal species level lineage is OTU35, which accepts 12 *Asterochloris* species-level lineages. In addition, *S. vesuvianum* (OTU10) accepts 11 algal species-level lineages belonging to *Asterochloris* and *Chloroidium*. Both OTU35 and OTU10 have a wide climatic niche (Figure 5D, E), which overlaps in various parts with niches of their phycobionts. Probably, they select the best adapted phycobionts in particular localities (see above). A similar pattern was described by Rolshausen et al. (2017) on the example of *Lasallia pustulata*. This generalist mycobiont associated with one *Trebouxia* species in the majority of its climatic niche, but on the periphery, it chooses more specialized phycobionts.

The ability to associate with numerous symbiotic partners adapted to various ecological conditions (including extreme drought or wide temperature ranges), likely represent an effective, adaptive strategy to cope with changing climate conditions (Aptroot & van Herk 2007; Baums *et al.* 2014; Colesie *et al.* 2017; Matos *et al.* 2015).

Conclusions

This study highlighted the exceptional diversity of predominant phycobionts, including the three trebouxioid genera, *Asterochloris*, *Chloroidium*, and *Vulcanochloris*, and several dozens of their

species, associated with the lichen-forming fungal genus *Stereocaulon*. Certain mycobiont species accept one to 12 phycobiont species-level lineages, belonging to one or two genera. Our results also provide further evidence of the co-occurrence of algal plurality in individual lichen thalli, which strengthens the suitability of the *Stereocaulon* symbiotic systems as models for future research based on high-throughput sequencing.

The distribution of both phycobionts and mycobionts of *Stereocaulon* lichens was almost completely explained by climatic conditions, habitat or substrate variables, geographical distance, and symbiotic partners. The symbiotic partner was identified as the best explanatory variable. Among the phycobionts, certain genera, as well as species level lineages, tolerate dissimilar temperature and precipitation levels, and climate seasonality. Several phycobionts occurred on specific substrates, for example lava flows or anthropogenic substrates, and seem to tolerate toxic substances.

The mycobiont genus *Stereocaulon* is highly unspecific towards phycobionts, but comprises numerous species-level lineages, which vary significantly in the number of acceptable phycobionts. The same is applicable for the specificity and selectivity of phycobionts towards mycobionts. The positive correlation between the width of climatic niche and number of acceptable symbiotic partners were proven for both phycobionts and mycobionts. The ability to associate with numerous symbiotic partners adapted to various ecological conditions, as well as the ability to associate with free-living algae (*Chloroidium*), could represent an effective adaptive strategy.

Acknowledgements

We are grateful to everyone who helped us during this study, especially Magdalena Oset for identifying some *Stereocaulon* specimens, J. Vančurová, J. Vondrák, J. Malíček, J. Steinová, S. Werth, D. Svoboda, Z. Palice, F. Bouda, W. Obermayer, D. Trauber, V. Kalníková, V. Fanta, K. Vančura, L. Vančura, J. Pilátová, and O. Koukol for collecting material and/or help with field work, and L. Flašková, B. Klug, and S. Kraker for help with molecular work. Martin Grube is thanked for hosting LV in the lab. We thank a lot the tree anonymous reviewers, who contributed to the quality of the study by their useful suggestions. This study was supported by the grant no. GP13-39185P from the Czech Science Foundation and the Primus Research Programme of Charles University no. SCI/13. Part off the laboratory work was conducted during the cooperation with the Karl-Franzen University Graz during the OEAD 2010-2011 project action entitled “Symbiotic selection arenas in lichens”.

Data accessibility

DNA sequences are available in GenBank under Accession nos MH382116–MH382150 and MH414969–MH415451. DNA alignments are available as Appendix S1–S3.

Author Contributions

L.V., P.Š., and O.P. designed the study. L.V., O.P., L.M., and P.Š. conducted fieldwork and collected specimens. L.V. and T.Ř. performed laboratory work with contributions from P.Š., L.M., and O.P. P.Š. and L.V. analyzed the data. L.V., O.P., L.M., and P.Š. wrote the manuscript.

References

Abrams, M., Bianchi, R. & Pieri, D. (1996) Revised Mapping of Lava Flows on Mount Etna , Sicily. *Photogrammetic Engineering and Remote sensing* 62: 1353–1359.

Ahmadjian, V. (1993) *The Lichen Symbiosis*. John Wiley and Sons, Inc., New York. 250 pp.

Álvarez, R., del Hoyo, A., García-Breijo, F., Reig-Armiñana, J., del Campo, E.M., Guéra, A., Barreno, E. & Casano, L.M. (2012) Different strategies to achieve Pb-tolerance by the two *Trebouxia* algae coexisting in the lichen *Ramalina farinacea*. *Journal of Plant Physiology* 169: 1797–1806. https://doi.org/10.1016/j.jplph.2012.07.005

Aptroot, A. & van Herk, C.M. (2007) Further evidence of the effects of global warming on lichens, particularly those with *Trentepohlia* phycobionts. *Environmental Pollution* 146: 293–298. https://doi.org/10.1016/j.envpol.2006.03.018

Bačkor, M., Peksa, O., Škaloud, P. & Bačkorová, M. (2010) Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. *Ecotoxicology and environmental safety* 73: 603–12. https://doi.org/10.1016/j.ecoenv.2009.11.002

Baker, A.C. (2003) Flexibility and specificity in coral-algal symbiosis: Diversity, ccology, and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics* 34: 661–689. https://doi.org/10.1146/132417

Baums, I.B., Devlin-Durante, M.K. & Lajeunesse, T.C. (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *Molecular Ecology* 23: 4203–4215. https://doi.org/10.1111/mec.12788

Beck, A. (2002) Selektivität der Symbionten schwermetalltoleranter Flechten. Ludwig-Maxmilians-Universität München

Beiggi, S. & Piercey-Normore, M.D. (2007) Evolution of ITS Ribosomal RNA Secondary Structures in Fungal and Algal Symbionts of Selected Species of *Cladonia* sect. *Cladonia*. *Journal of Molecular Evolution* 64: 528–542. https://doi.org/10.1007/s00239-006-0115-x

Blonder, B., Lamanna, C., Violle, C. & Enquist, B.J. (2014) The n-dimensional hypervolume. *Global Ecology and Biogeography* 23: 595–609. https://doi.org/10.1111/geb.12146

Borcard, D., Legendre, P., Avois-Jacquet, C. & Tuomisto, H. (2004) Dissecting the spatial structure of ecological data at multiple scales. *Ecology* 85: 1826–1832. https://doi.org/10.1890/03-3111

Byler, K.A., Carmi-Veal, M., Fine, M. & Goulet, T.L. (2013) Multiple Symbiont Acquisition Strategies as an Adaptive Mechanism in the Coral *Stylophora pistillata*. *PLoS ONE* 8: 1–7. https://doi.org/10.1371/journal.pone.0059596

Candotto-Carniel, F., Gerdol, M., Montagner, A., Banchi, E., De Moro, G., Manfrin, C., Muggia, L., Pallavicini, A. & Tretiach, M. (2016) New features of desiccation tolerance in the lichen photobiont *Trebouxia gelatinosa* are revealed by a transcriptomic approach. *Plant Molecular Biology* 91: 319–339. https://doi.org/10.1007/s11103-016-0468-5

Casano, L.M., del Campo, E.M., García-Breijo, F.J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., Guéra, A. & Barreno-Rodríguez, E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental microbiology* 13: 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x

Centeno, D.C., Hell, A.F., Braga, M.R., del Campo, E.M. & Casano, L.M. (2016) Contrasting strategies used by lichen microalgae to cope with desiccation-rehydration stress revealed by metabolite profiling and cell wall analysis. *Environmental Microbiology* 18: 1546–1560. https://doi.org/10.1111/1462-2920.13249

Colesie, C., Büdel, B., Hurry, V. & Green, T.G.A. (2017) Can Antarctic lichens acclimatize to changes in temperature? *Global Change Biology*: 1123–1135. https://doi.org/10.1111/gcb.13984

Cordeiro, L.M.C., Reis, R. a, Cruz, L.M., Stocker-Wörgötter, E., Grube, M. & Iacomini, M. (2005) Molecular studies of photobionts of selected lichens from the coastal vegetation of Brazil. *FEMS microbiology ecology* 54: 381–90. https://doi.org/10.1016/j.femsec.2005.05.003

Cubero, O.F., Crespo, A., Fatehi, J. & Bridge, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* 216: 243–249. https://doi.org/10.1007/BF01084401

Cutler, N.A., Belyea, L.R. & Dugmore, A.J. (2008) Spatial patterns of microsite colonisation on two young lava flows on Mount Hekla, Iceland. *Journal of Vegetation Science* 19: 277–286. https://doi.org/10.3170/2008-8-18371

Dal Grande, F., Rolshausen, G., Divakar, P.K., Crespo, A., Grande, F.D., Otte, J., Schleuning, M. & Schmitt, I. (2017) Environment and host identity structure communities of green algal symbionts in lichens. *New Phytologist* 217: 277–289. https://doi.org/10.1111/nph.14770

Darienko, T., Gustavs, L., Mudimu, O., Menendez, C.R., Schumann, R., Karsten, U., Friedl, T. & Pröschold, T. (2010) *Chloroidium*, a common terrestrial coccoid green alga previously assigned to *Chlorella* (Trebouxiophyceae, Chlorophyta). *European Journal of Phycology* 45: 79–95.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973. https://doi.org/10.1093/molbev/mss075

Duffy, K.J. & Johnson, S.D. (2017) Specialized mutualisms may constrain the geographical distribution of flowering plants. *Proceedings of the Royal Society B: Biological Sciences* 284: 20171841. https://doi.org/10.1098/rspb.2017.1841

Engelen, A., Convey, P. & Ott, S. (2010) Life history strategy of Lepraria borealis at an Antarctic inland site, Coal Nunatak. *The Lichenologist* 42: 339–346. https://doi.org/10.1017/S0024282909990600

Fernández-Mendoza, F., Domaschke, S., García, M.A., Jordan, P., Martín, M.P. & Printzen, C. (2011) Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. *Molecular Ecology* 20: 1208–1232. https://doi.org/10.1111/j.1365-294X.2010.04993.x

Finney, J.C., Pettay, D.T., Sampayo, E.M., Warner, M.E., Oxenford, H.A. & LaJeunesse, T.C. (2010) The Relative Significance of Host–Habitat, Depth, and Geography on the Ecology, Endemism, and Speciation of Coral Endosymbionts in the Genus *Symbiodinium*. *Microbial Ecology* 60: 250–263. https://doi.org/10.1007/s00248-010-9681-y

Friedl, T. & Büdel, B. (2008) Photobionts. *In*: T. H. Nash III (ed.) *Lichen Biology*. Cambridge University Press, New York, pp. 9–26.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

Gasulla, F., Jain, R., Barreno, E., Guéra, A., Balbuena, T.S., Thelen, J.J. & Oliver, M.J. (2013) The response of *Asterochloris erici* (Ahmadjian) Skaloud et Peksa to desiccation: A proteomic approach. *Plant, Cell and Environment* 36: 1363–1378. https://doi.org/10.1111/pce.12065

Gerz, M., Guillermo Bueno, C., Ozinga, W.A., Zobel, M. & Moora, M. (2018) Niche differentiation and expansion of plant species are associated with mycorrhizal symbiosis. *Journal of Ecology* 106: 254–264. https://doi.org/10.1111/1365-2745.12873

Gruber, A.R., Lorenz, R., Bernhart, S.H., Neuböck, R. & Hofacker, I.L. (2008) The Vienna RNA websuite. *Nucleic acids research* 36: 70–74. https://doi.org/10.1093/nar/gkn188

Guindon, S. & Gascuel, O. (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology* 52: 696–704. https://doi.org/10.1080/10635150390235520

Guzow-Krzemińska, B. (2006) Photobiont flexibility in the lichen *Protoparmeliopsis muralis* as revealed by ITS rDNA analyses. *The Lichenologist* 38: 469–476.

Hallmann, C., Hoppert, M., Mudimu, O. & Friedl, T. (2016) Biodiversity of green algae covering artificial hard substrate surfaces in a suburban environment: a case study using molecular approaches. *Journal of Phycology* 52: 732–744. https://doi.org/10.1111/jpy.12437

Helms, G. (2003) Taxonomy and Symbiosis in Associations of Physciaceae and *Trebouxia*. Georg-August Universität Göttingen

Helms, G., Friedl, T., Rambold, G. & Mayrhofer, H. (2001) Identification of photobionts from the lichen family Physciaceae using algal-specific ITS rDNA sequencing. *The Lichenologist* 33: 73–86. https://doi.org/10.1006/lich.2000.0298

Hoef-Emden, K. (2012) Pitfalls of establishing DNA barcoding systems in protists: The cryptophyceae as a test case. *PLoS ONE* 7. https://doi.org/10.1371/journal.pone.0043652

Högnabba, F. (2006) Molecular phylogeny of the genus *Stereocaulon* (Stereocaulaceae , lichenized ascomycetes). *Mycological research* 110: 1080–1092. https://doi.org/10.1016/j.mycres.2006.04.013

Howells, E.J., Beltran, V.H., Larsen, N.W., Bay, L.K., Willis, B.L. & van Oppen, M.J.H. (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change* 2: 116–120.

Del Hoyo, A., Álvarez, R., Del Campo, E.M., Gasulla, F., Barreno, E. & Casano, L.M. (2011) Oxidative stress induces distinct physiological responses in the two *Trebouxia* phycobionts of the lichen *Ramalina farinacea*. *Annals of Botany* 107: 109–118. https://doi.org/10.1093/aob/mcq206

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hutchinson, G.E. (1957) Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology* 22: 415–427. https://doi.org/10.1101/SQB.1957.022.01.039

Iglesias-Prieto, R., Beltrán, V.H., LaJeunesse, T.C., Reyes-Bonilla, H. & Thomé, P.E. (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings. Biological sciences/The Royal Society* 271: 1757–1763. https://doi.org/10.1098/rspb.2004.2757

Ismed, F., Lohézic-Le Dévéhat, F., Delalande, O., Sinbandhit, S., Bakhtiar, A. & Boustie, J. (2012) Lobarin from the Sumatran lichen, *Stereocaulon halei*. *Fitoterapia* 83: 1693–8. https://doi.org/10.1016/j.fitote.2012.09.025

Jackson, D.A. (1993) Stopping Rules in Principal Components Analysis: A Comparison of Heuristical and Statistical Approaches. *Ecology* 74: 2204–2214. https://doi.org/10.2307/1939574

Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P. & Kessler, M. (2017) Climatologies at high resolution for the earth's land surface areas. *Scientific Data* 4: 170122. https://doi.org/10.1038/sdata.2017.122

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–80. https://doi.org/10.1093/molbev/mst010

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth & Bisby's dictionary of the fungi*. CABI. 784 pp.

Kovář, P. (2004) Industrial deposits of abandoned sedimentation basins – technology of the origin and vegetation. *In*: P. Kovář (ed.) *Natural Recovery of Human-Made Deposits in Landscape (Biotic Interactions and Ore/Ash-Slag Artificial Ecosystems)*. Academia, Prague, pp. 15–29.

Leavitt, S.D., Kraichak, E., Nelsen, M.P., Altermann, S., Divakar, P.K. & Alors, D. (2015) Fungal specificity and selectivity for algae play a major role in determining lichen partnerships across diverse ecogeographic regions in the lichen-forming family Parmeliaceae (Ascomycota). *Molecular Ecology* 24: 3779–3797. https://doi.org/10.1111/mec.13271

Leavitt, S.D., Kraichak, E., Vondrak, J., Nelsen, M.P., Sohrabi, M., Perez-Ortega, S., St Clair, L.L. & Thorsten Lumbsch, H. (2016) Cryptic diversity and symbiont interactions in rock-posy lichens. *Molecular Phylogenetics and Evolution* 99: 261–274. https://doi.org/10.1016/j.ympev.2016.03.030

Leavitt, S.D., Nelsen, M.P., Lumbsch, H.T., Johnson, L. a. & St. Clair, L.L. (2013) Symbiont flexibility in

subalpine rock shield lichen communities in the Southwestern USA. *The Bryologist* 116: 149–161. https://doi.org/10.1639/0007-2745-116.2.149

Lefeuvre, P. (2018) BoSSA: A Bunch of Structure and Sequence Analysis.

Lindblom, L. & Sochting, U. (2013) Genetic diversity of the photobiont of the bipolar lichen-forming ascomycete Xanthomendoza borealis. *Herzogia* 26: 307–322.

Lücking, R., Lawrey, J.D., Sikaroodi, M., Gillevet, P.M., Chaves, J.L., Sipman, H.J.M. & Bungartz, F. (2009) Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. *American journal of botany* 96: 1409–18. https://doi.org/10.3732/ajb.0800258

Marini, L., Nascimbene, J. & Nimis, P.L. (2011) Science of the Total Environment Large-scale patterns of epiphytic lichen species richness : Photobiont-dependent response to climate and forest structure. *Science of the Total Environment, The* 409: 4381–4386. https://doi.org/10.1016/j.scitotenv.2011.07.010

Matos, P., Pinho, P., Aragón, G., Martínez, I., Nunes, A., Soares, A.M.V.M. & Branquinho, C. (2015) Lichen traits responding to aridity. *Journal of Ecology* 103: 451–458. https://doi.org/10.1111/1365-2745.12364

Medeiros, I.D., Fryday, A.M. & Rajakaruna, N. (2014) Additional lichen records and mineralogical data from metal-contaminated sites in Maine. *Rhodora* 116: 323–347. https://doi.org/10.3119/13-26

Meunier, J.D., Kirman, S., Strasberg, D., Grauby, O. & Dussouillez, P. (2014) Incipient weathering by *Stereocaulon vulcani* at Réunion volcanic island. *Chemical Geology* 382: 123–131. https://doi.org/10.1016/j.chemgeo.2014.05.033

Monaghan, M.T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D.J.G., Lees, D.C., Ranaivosolo, R., Eggleton, P., Barraclough, T.G. & Vogler, A.P. (2009) Accelerated Species Inventory on Madagascar Using Coalescent-Based Models of Species Delineation. *Systematic Biology* 58: 298–311. https://doi.org/10.1093/sysbio/syp027

Moya, P., Molins, A., Martinez-Alberola, F., Muggia, L. & Barreno, E. (2017) Unexpected associated microalgal diversity in the lichen *Ramalina farinacea* is uncovered by pyrosequencing analyses. *PLoS ONE* 12: 1–21. https://doi.org/10.1371/journal.pone.0175091

Moya, P., Škaloud, P., Chiva, S., García-Breijo, F.J., Reig-Armiñana, J., Vančurová, L. & Barreno, E. (2015) Molecular phylogeny and ultrastructure of the lichen microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands ecosystems. *International journal of systematic and evolutionary microbiology* 65: 1838–54. https://doi.org/10.1099/ijs.0.000185

Muggia, L., Baloch, E., Stabentheiner, E., Grube, M. & Wedin, M. (2011) Photobiont association and genetic diversity of the optionally lichenized fungus *Schizoxylon albescens*. *FEMS Microbiology Ecology* 75: 255–272. https://doi.org/10.1111/j.1574-6941.2010.01002.x

Muggia, L., Klug, B., Berg, G. & Grube, M. (2013a) Localization of bacteria in lichens from Alpine soil crusts by fluorescence in situ hybridization. *Applied Soil Ecology* 68: 20–25. https://doi.org/10.1016/j.apsoil.2013.03.008

Muggia, L., Leavitt, S.D. & Barreno, E. (2018) The hidden diversity of lichenised Trebouxiophyceae (Chlorophyta). *Phycologia* 57: xxx–xxx.

Muggia, L., Perez-Ortega, S., Kopun, T., Zellnig, G. & Grube, M. (2014) Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Annals of Botany* 114: 463–475. https://doi.org/10.1093/aob/mcu146

Muggia, L., Vančurová, L., Škaloud, P., Peksa, O., Wedin, M. & Grube, M. (2013b) The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS microbiology ecology* 85: 313–23. https://doi.org/10.1111/1574-6941.12120

Nelsen, M.P. & Gargas, A. (2006) Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta: Trebouxiophyceae). *The Lichenologist* 38: 435–440. https://doi.org/10.1017/S0024282906005779

Nelsen, M.P. & Gargas, A. (2008) Dissociation and horizontal transmission of codispersing lichen symbionts in the genus *Lepraria* (Lecanorales: Stereocaulaceae). *The New phytologist* 177: 264–275. https://doi.org/10.1111/j.1469-8137.2007.02241.x

Nyati, S., Bhattacharya, D., Werth, S. & Honegger, R. (2013) Phylogenetic analysis of LSU and SSU rDNA group I introns of lichen photobionts associated with the genera *Xanthoria* and *Xanthomendoza* (Teloschistaceae, lichenized Ascomycetes). *Journal of phycology* 49: 1154–1166. https://doi.org/10.1111/jpy.12126

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2017) vegan: Community Ecology Package.

Onuț-Brännström, I., Benjamin, M., Scofield, D.G., Heiðmarsson, S., Andersson, M.G.I., Lindström, E.S. & Johannesson, H. (2018) Sharing of photobionts in sympatric populations of *Thamnolia* and *Cetraria* lichens: evidence from high-throughput sequencing. *Scientific Reports 2018* 8: 4406. https://doi.org/10.1038/s41598-018-22470-y

Orange, A., James, P.W. & White, F.J. (2001) *Microchemical Methods for the Identification of Lichens*. British Lichen Society

Pangallo, D., Bučková, M., Kraková, L., Puškárová, A., Šaková, N., Grivalský, T., Chovanová, K. & Zemánková, M. (2015) Biodeterioration of epoxy resin: a microbial survey through culture-independent and culture-dependent approaches. *Environmental microbiology* 17: 462–79. https://doi.org/10.1111/1462-2920.12523

Paracer, S. & Ahmadjian, V. (2000) *Symbiosis: An Introduction to Biological Associations*. 2nd edition. Oxford University Press, Inc., New York. 291 pp. p.

Park, C.H., Kim, K.M., Elvebakk, A., Kim, O.-S., Jeong, G. & Hong, S.G. (2015) Algal and Fungal Diversity in Antarctic Lichens. *Journal of Eukaryotic Microbiology* 62: 196–205. https://doi.org/10.1111/jeu.12159

Pateiro-Lopez, B. & Rodriguez-Casal, A. (2016) alphahull: Generalization of the Convex Hull of a Sample of Points in the Plane.

Peksa, O. & Škaloud, P. (2008) Changes in chloroplast structure in lichenized algae. *Symbiosis* 46: 153–160.

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology* 20: 3936–48. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Pettay, D.T., Wham, D.C., Smith, R.T., Iglesias-Prieto, R. & LaJeunesse, T.C. (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proceedings of the National Academy of Sciences of the United States of America* 112: 7513–7518. https://doi.org/10.1073/pnas.1502283112

Piercey-Normore, M.D. & DePriest, P.T. (2001) Algal Switching among Lichen Symbioses. *American Journal of Botany* 88: 1490–1498. https://doi.org/10.2307/3558457

Plummer, M. (2003) JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling.

Purvis, O.W. & Halls, C. (1996) A Review of Lichens in Metal-Enriched Environments. *The Lichenologist* 28: 571. https://doi.org/10.1017/S0024282996000758

R Core Team (2017) R: A Language and Environment for Statistical Computing.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Tracer.

Rambold, G., Friedl, T. & Beck, A. (1998) Photobionts in Lichens: Possible Indicators of Phylogenetic Relationships? *Bryologist, The* 101: 392–397.

Rola, K., Osyczka, P. & Kafel, A. (2016) Different Heavy Metal Accumulation Strategies of Epilithic Lichens Colonising Artificial Post-Smelting Wastes. *Archives of Environmental Contamination and Toxicology* 70: 418–428. https://doi.org/10.1007/s00244-015-0180-5

Rolshausen, G., Dal Grande, F., Sadowska-Deś, A.D., Otte, J. & Schmitt, I. (2017) Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions. *Ecography*: 1–12. https://doi.org/10.1111/ecog.03457

Romeike, J., Friedl, T., Helms, G. & Ott, S. (2002) Genetic Diversity of Algal and Fungal Partners in Four Species of *Umbilicaria* (Lichenized Ascomycetes) Along a Transect of the Antarctic Peninsula. *Molecular Biology* 19: 1209–1217.

Rowan, R. (2004) Coral bleaching: thermal adaptation in reef coral symbionts. *Nature* 430: 742. https://doi.org/10.1038/430742a

Ruprecht, U., Brunauer, G. & Türk, R. (2014) High photobiont diversity in the common European soil crust lichen *Psora decipiens*. *Biodiversity and conservation* 23: 1771–1785. https://doi.org/10.1007/s10531-014-0662-1

Ryšánek, D., Elster, J., Kováčik, L. & Škaloud, P. (2016) Diversity and dispersal capacities of a terrestrial algal genus *Klebsormidium* (Streptophyta) in polar regions. *FEMS Microbiology Ecology* 92: 1–9. https://doi.org/10.1093/femsec/fiw039

Ryšánek, D., Hrčková, K. & Škaloud, P. (2015) Global ubiquity and local endemism of free-living terrestrial protists: phylogeographic assessment of the streptophyte alga *Klebsormidium*. *Environmental Microbiology* 17: 689–698. https://doi.org/10.1111/1462-2920.12501

Sadowsky, A., Hussner, A. & Ott, S. (2012) Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane, Norway. *Nova Hedwigia* 94: 323–334. https://doi.org/10.1127/0029-5035/2012/0014

Sanders, W.B., Pérez-Ortega, S., Nelsen, M.P., Lücking, R. & de los Ríos, A. (2016) *Heveochlorella* (Trebouxiophyceae): a little-known genus of unicellular green algae outside the Trebouxiales emerges unexpectedly as a major clade of lichen photobionts in foliicolous communities. O. DeClerck (ed.). *Journal of Phycology* 52: 840–853. https://doi.org/10.1111/jpy.12446

Seo, C., Sohn, J.H., Park, S.M., Yim, J.H., Lee, H.K. & Oh, H. (2008) Usimines A-C, bioactive usnic acid derivatives from the Antarctic lichen *Stereocaulon alpinum*. *Journal of natural products* 71: 710–2. https://doi.org/10.1021/np070464b

Singh, G., Dal Grande, F., Divakar, P.K., Otte, J., Crespo, A. & Schmitt, I. (2017) Fungal–algal association patterns in lichen symbiosis linked to macroclimate. *New Phytologist* 214: 317–329. https://doi.org/10.1111/nph.14366

Singh, R., Ranjan, S., Nayaka, S., Pathre, U. V. & Shirke, P. a. (2013) Functional characteristics of a fruticose type of lichen, *Stereocaulon foliolosum* Nyl. in response to light and water stress. *Acta Physiologiae Plantarum* 35: 1605–1615. https://doi.org/10.1007/s11738-012-1203-8

Škaloud, P. & Peksa, O. (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris*. *Molecular Phylogenetics and Evolution* 54: 36–46. https://doi.org/10.1016/j.ympev.2009.09.035

Škaloud, P., Steinová, J., Řídká, T., Vančurová, L. & Peksa, O. (2015) Assembling the challenging puzzle of algal biodiversity: species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 51: 507–527. https://doi.org/10.1111/jpy.12295

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C., Schneider, K., Stabentheiner, E., Toome-Heller, M., Thor, G., Mayrhofer, H., Johannesson, H. & McCutcheon, J.P. (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492. https://doi.org/10.1126/science.aaf8287

Stretch, R.. & Viles, H.. (2002) The nature and rate of weathering by lichens on lava flows on Lanzarote. *Geomorphology* 47: 87–94. https://doi.org/10.1016/S0169-555X(02)00143-5

Swofford, D.L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular biology and evolution* 30: 2725–2729. https://doi.org/10.1093/molbev/msm092

Thiers, B. (2016) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgum.nybg.org/science/ih/

Thüs, H., Muggia, L., Pérez-Ortega, S., Favero-Longo, S.E., Joneson, S., Brien, H.O., Nelsen, M.P., Grube, M., Friedl, T., Brodie, J., Andrew, C.J., Lutzoni, F. & Gueidan, C. (2011) Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota). *European Journal of Phycology* 46: 399–415. https://doi.org/10.1080/09670262.2011.629788

Tschermak-Woess, E. (1948) Über wenig bekannte und neue Flechtengonidien I. *Chlorella ellipsoidea* Gerneck, als neue Flechtenalge. *Österreichische botanische Zeitschrift* 95: 341–343.

Tschermak-Woess, E. (1978) Über den *Chlorella*-Phycobionten von *Trapelia coarctata*. *Plant Systematics and Evolution* 130: 253–263.

Tschermak-Woess, E. (1988a) New and known taxa of *Chlorella* (Chlorophyceae): Occurrence as lichen phycobionts and observations on living dictyosomes. *Plant Systematics and Evolution* 159: 123–139.

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

Vaiglová, Z. (2017) Dynamics of a lichen symbiosis. Charles University

Vančurová, L., Peksa, O., Němcová, Y. & Škaloud, P. (2015) *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain. *Phytotaxa* 219: 118–132. https://doi.org/10.11646/phytotaxa.219.2.2

Voytsekhovich, A. & Beck, A. (2015) Lichen photobionts of the rocky outcrops of Karadag massif (Crimean Peninsula). *Symbiosis* 66: 1–16. https://doi.org/10.1007/s13199-015-0346-y

Weber, K. & Kabsch, W. (1994) Intron Positions in Actin Genes Seem Unrelated To the Secondary Structure of the Protein. *Embo Journal* 13: 1280–1286.

Wedin, M., Maier, S., Fernandez-Brime, S., Cronholm, B., Westberg, M. & Grube, M. (2015) Microbiome change by symbiotic invasion in lichens. *Environmental Microbiology* 18: 1428–1439. https://doi.org/10.1111/1462-2920.13032

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

Yahr, R., Florence, A., Škaloud, P. & Voytsekhovich, A. (2015) Molecular and morphological diversity in photobionts associated with *Micarea* s. str (Lecanorales , Ascomycota). *The Lichenologist* 47: 403–414. https://doi.org/10.1017/S0024282915000341

Yahr, R., Vilgalys, R. & Depriest, P.T. (2004) Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. *Molecular Ecology* 13: 3367–3378. https://doi.org/10.1111/j.1365-294X.2004.02350.x

Yahr, R., Vilgalys, R. & DePriest, P.T. (2006) Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *The New phytologist* 171: 847–60. https://doi.org/10.1111/j.1469-8137.2006.01792.x

Zoller, S. & Lutzoni, F. (2003) Slow algae, fast fungi: Exceptionally high nucleotide substitution rate differences between lichenized fungi *Omphalina* and their symbiotic green algae *Coccomyxa*. *Molecular Phylogenetics and Evolution* 29: 629–640. https://doi.org/10.1016/S1055-7903(03)00215-X

Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin

Supporting information

Additional supporting information may be found in the online version of this article.

TABLE S1. Details of the material, the area of collection and GenBank accession numbers of *Stereocaulon* samples (newly obtained in bold).

TABLE S2. Primers used in this study.

TABLE S3. Details of the area of collection and GenBank accession numbers of free-living *Chloroidium* cultures (previously published and retrieved from GenBank).

TABLE S4. Substitution models selected for each partition of *Asterochloris*, *Chloroidium* and *Stereocaulon* (mycobiont) datasets using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Guindon & Gascuel 2003, Darriba *et al.* 2012).

TABLE S5. Presence/absence matrix of 12 substrate/habitat variables (substrates: soil, rock, lava, plastic and metal; habitat characteristics: mine, sludge bed, occurrence of moss, other plants or forest, closeness of road or river).

FIGURE S1. Rarefaction curves for 8 algal and 7 fungal most abundant species-level lineages. Vertical line is drawn at smallest sample size in the data set with horizontal lines for the rarefied number of species-level lineages of associated mycobionts/phycobionts.

FIGURE S2. Phylogenetic hypothesis of *Asterochloris* resulting from the Bayesian analysis of combined ITS rDNA and actin type I sequences. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold. Sequences of phycobionts associated with the lichen-forming fungal genus *Stereocaulon* are marked in black. Sequences of phycobionts of other lichens are marked in grey. Clade affiliations: clade 8, clade 9, clade A, clade B, clade C sensu Škaloud & Peksa (2010), A4, A9, A11 sensu Peksa & Škaloud (2011), URa14 sensu Ruprecht *et al.* (2014), I1, I2 sensu Řídká *et al.* (2014), S1, S3 sensu Nelsen & Gargas (2006). *A. aff. irregularis*, *A. aff. italiana* and StA1 – StA8 lineages were identified as new in present study.

FIGURE S3. Phylogenetic hypothesis of *Chloroidium* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold. Sequences of phycobionts associated with the lichen-forming fungal genus *Stereocaulon* are marked in black. Sequences of free-living algae are marked in green. *C. aff. ellipsoideum*, StC1 and StC2 lineages were identified as new in present study.

FIGURE S4. Differences in the distribution of 14 most abundant (≥5 specimens) phycobiont species-level lineages (on the left) associated with the lichen-forming fungal genus *Stereocaulon* and 12 most abundant (≥5 specimens) mycobiont species-level lineages of *Stereocaulon* (on the right) along the gradient of annual mean temperature, temperature annual range, annual precipitation, and precipitation of driest quarter.

FIGURE S5. Trace and density plots of Markov Chain Monte Carlo samples for four parameters: intercept (a) and slope (b) or regression line, deviance and variance of the residuals (sigma). Slope of the regression line (density of b) was significantly different from 0 (95% confidence interval (CI) 0.025, 0.096).

FIGURE S6. Trace and density plots of Markov Chain Monte Carlo samples for four parameters: intercept (a) and slope (b) or regression line, deviance and variance of the residuals (sigma). Slope of

the regression line (density of b) was clearly shifted, but not significantly different from 0 (95% confidence interval (CI) -0.024, 0.213).

APPENDIX S1. *Asterochloris* ITS rDNA alignment after deleting identical sequences (71 unique sequences). Missing data were replaced with questions marks.

APPENDIX S2. *Asterochloris* actin type I alignment after deleting identical sequences (66 unique sequences). Missing data were replaced with questions marks.

APPENDIX S3. *Chloroidium* ITS rDNA alignment after deleting identical sequences (45 unique sequences). Missing data were replaced with questions marks.

The Supplementary Material for this article can be found online at:
https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14764

Symbiosis between river and dry lands: Phycobiont dynamics on river gravel bars

Lucie Vančurová[1*], Veronika Kalníková[2], Ondřej Peksa[3], Zuzana Škvorová[1], Jiří Malíček[4], Patricia Moya[5], Kryštof Chytrý[2], Ivana Černajová[1] & Pavel Škaloud[1]

[1]Charles University, Faculty of Science, Department of Botany, Benátská 2, 128 01 Prague 2, Czech Republic

[2]Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

[3]The West Bohemian Museum in Pilsen, Kopeckého sady 2, 301 00 Plzeň, Czech Republic

[4]The Czech Academy of Sciences, Institute of Botany, Zámek 1, 252 43 Průhonice, Czech Republic

[5]Dpto. Botánica, ICBIBE Fac. CC. Biológicas, Universitat de València, Dr. Moliner, 50. 46100-Burjassot, Valencia, Spain

** Corresponding author (E-mail: lucie.vancurova@natur.cuni.cz)*

Abstract

River gravel bars are dynamic and heterogeneous habitats straddling the transition between aquatic and terrestrial environments. Periodic flooding, low nutrient concentrations, frost, lack of stable sites, drought, and ground surface heat significantly influence the biota of these habitats. Mutualistic symbiosis may be a successful strategy for organisms to survive and proliferate under such harsh conditions. The lichen genus *Stereocaulon* was selected as a model symbiotic system from among the organisms living on river gravel bars. The goal of the current study was to determine the effect of this dynamic environment on phycobiont (i.e., green eukaryotic photobiont) community structure. We analyzed 147 *Stereocaulon* specimens collected in the Swiss Alps using Sanger sequencing (fungal internal transcribed spacer (ITS) rDNA, algal ITS rDNA, and algal actin type I gene) and analyzed 8 selected thalli and 12 soil samples using Illumina metabarcoding (ITS2 rDNA). Phytosociological sampling was performed for all 13 study plots. Our analyses of communities of phycobionts, lichens, bryophytes, and vascular plants indicated a gradual change in the phycobiont community along a successional gradient. The particularly large phycobiont diversity associated with *Stereocaulon* mycobionts included algae, here reported as phycobionts for the first time. Each of the two *Stereocaulon* mycobiont operational taxonomic units had a distinct pool of predominant phycobionts. The thalli selected for Illumina metabarcoding contained a wide range of additional algae, i.e., they showed algal plurality.

Keywords: specificity, lichen phycobiont, succession, community composition, metabarcoding, algal plurality

Introduction

River gravel bars are dynamic and heterogeneous habitats that occur in a range of ecosystems, from glacial floodplains and wide alpine river valleys to the piedmont (Hohensinner *et al.* 2018; Malard *et al.* 2006; Montgomery & Buffington 1998; Tockner *et al.* 2006). Periodic flooding, together with variations in the speed and intensity of the water current, create richly braided rivers with a mosaic of channels, pools, bars, and islands (Junk *et al.* 1989; Tockner *et al.* 2000; Ward *et al.* 2002). The destruction and reformation of river gravel bars by floods result in wide structural changes and cyclic vegetation succession. The early successional vegetation type, together with subsequent vegetation types (mainly shrubs), form a mosaic of microhabitat conditions that are determined according to the disturbance levels (Gilvear *et al.* 2008; Müller 1996; Pettit & Froend 2001; Prach *et al.* 2016; Tockner *et al.* 2000; Wellstein *et al.* 2003). Gravel bars in alpine zones significantly contribute to the regional diversity of the alpine environment (Tockner & Malard 2003). The early- to mid-successional stands are often occupied by relatively diverse communities of vascular plants, which are characterized by high species richness and evenness, and relatively low vegetation cover. In later successional stages, the evenness and species richness decrease as organic matter and nutrients accumulate, and competition from established dominant species increases, as demonstrated in many studies (Corenblit *et al.* 2009; Kalníková *et al.* 2018; Prach *et al.* 2014).

Glacier-fed alpine rivers are highly influenced by daily flooding from the melting glaciers, which makes their conditions even more extreme (e.g., Milner and Petts 1994; Tockner *et al.* 2000; Malard *et al.* 2006). Low nutrient concentrations in surficial substrates represent the most limiting environmental factor in glacial floodplains. Recently deglaciated terrain is characterized by bare soils, which do not contain any organic matter and initially lack a soil seed bank. Additionally, frost, lack of stable sites, drought, and ground surface heat significantly influence life in these habitats (Marcante *et al.* 2014; Stöcklin & Bäumler 1996; Tockner *et al.* 2006). To help survive and persist in these conditions, common characteristics of gravel bar species include high diaspore dispersibility, fast growth, tolerance of disturbance, clonal growth, and the ability to grow on nutrient-poor soils (Ellenberg & Leuschner 2010; Jeník 1955; Karrenberg *et al.* 2003; Muotka & Virtanen 1995; Stöcklin 1999; Vitt *et al.* 1986).

Under the harsh conditions of river gravel bars, mutualistic symbiosis may be a successful strategy for organisms to survive and proliferate (Doty *et al.* 2016; Moran 2007). Since lichens represent one of the oldest known and most recognizable examples of mutualistic symbiosis in stressful conditions (Seckbach & Grube 2010), the lichen genus *Stereocaulon* was selected as a model symbiotic system from among the organisms living on river gravel bars. *Stereocaulon* is a widespread and ecologically successful pioneer lichen to be able to grow under harsh conditions on newly formed substrates (Meunier *et al.* 2014; Stretch & Viles 2002). Moreover, previous studies confirmed its ability to survive episodic submersion (Sadowsky *et al.* 2012), even though it is not aquatic. Lichens are complex symbiotic systems, composed of various heterotrophic and autotrophic organisms. The presence of these various autotrophic and heterotrophic symbionts

gives rise to a thallus with a typical phenotype (Spribille *et al.* 2016). The *Stereocaulon* mycobionts are associated with green algal symbionts (i.e., phycobionts) and sometimes with additional cyanobionts located in specialized structures (Lavoie *et al.* 2020; Lücking *et al.* 2009). Recently, an exceptionally high diversity of phycobionts was discovered to be associated with *Stereocaulon*, including three ecologically diversified trebouxiophycean genera, *Asterochloris*, *Vulcanochloris,* and *Chloroidium* (Vančurová *et al.* 2015, 2018).

The ecological amplitude of the lichen mycobiont may be influenced by its specificity for the phycobionts (Rolshausen *et al.* 2017; Vančurová *et al.* 2018). Symbiotic interactions vary along environmental gradients (Godschalx *et al.* 2019) and could be affected by stressful environments (Engelen *et al.* 2010; Romeike *et al.* 2002). Therefore, the goal of our study was to determine patterns in phycobiont diversity of *Stereocaulon* along a gradient of vegetation succession. Sanger sequencing of all 147 samples and Illumina metabarcoding of 8 selected thalli were applied to *Stereocaulon* specimens collected from 13 study plots to address the following questions: (1) is phycobiont diversity influenced by succession?; (2) how specific is *Stereocaulon* towards its phycobionts on river gravel bars?; and (3) does *Stereocaulon* growing on gravel bars exhibit algal plurality?

Material and methods

Study area and field sampling

The sampling was carried out in August 2017. Four localities, all situated on river gravel bars of glacial floodplains (1995–2070 m a.s.l.), were sampled across three glacial valleys: Morteratsch locality in the Morteratsch valley, Roseg I and Roseg II localities in the Roseg valley in the Bernina range, and Lonza locality in the Lötschental valley of the Lonza River in the Bernese Alps (a map is shown in Fig. S1). 13 vegetation plots (4 m × 4 m) were investigated. Study plots in each locality represented three successional stages (Burga *et al.* 2010): (1) early stage (herbaceous early-successional scattered vegetation characterized by stands of alpine and scree-related herbs), (2) moderate (sparse scrub vegetation with willow species and *Myricaria germanica*), and (3) developed (stands with scattered trees of *Larix decidua* and scrubs of *Juniperus communis* subsp. *nana*), with the exception of the Roseg II locality where only the first and second stages were present (Tables 1, S1). Photographs of the study plots at different successional stages are given in Fig. S2.

Coordinates of each plot were recorded using a portable GPS (WGS-84 coordination system). The elevation of the gravel bar (as a distance from its highest point to the actual water level) and distance from the river were measured. One soil sample per plot was taken. All lichen, bryophyte and vascular plant taxa within the vegetation plots were recorded, with lichens and bryophytes collected from soil and stones. The cover of each species according to the extended Braun-Blanquet cover scale (Westhoff & van der Maarel 1978) and the total vegetation cover and the cover of each layer (tree, shrub, herb, moss, and lichen) were estimated in each plot. Vegetation plot data are listed in the Table S1. Ellenberg indicator values (Ellenberg *et al.* 1991) and indicator values for bryophytes

(Hill *et al.* 1999, 2007) were calculated. On each plot, a minimum of 10 *Stereocaulon* samples was collected; for each sample the type of substrate was noted. Only one morphospecies of *Stereocaulon* (*S. alpinum*) was found in the study area. Lichen morphospecies were identified in the field, as well as in the laboratory using standard microscopic and chemical methods, including spot tests and thin-layer chromatography (TLC). *Stereocaulon* vouchers were deposited in the Herbarium of Charles University in Prague (PRC) and vouchers of accompanying lichens in the personal herbarium of J. Malíček. Vascular plants and bryophytes unidentified in the field were collected for laboratory determination. All records for the vegetation plots were stored in the Gravel bar vegetation database – ID: EU-00-025 (Kalníková & Kudrnovsky 2017), which is included in the European Vegetation Archive (Chytrý *et al.* 2016). Nomenclature follows Euro+Med PlantBase (2006–2019) for vascular plants, Hill *et al.* (2006) for mosses, Grolle & Long (2000) for liverworts, and Nimis *et al.* (2018) for lichens.

Table 1 Location of study plots

Plot number	Successional stage	Locality	Altitude (m)	River distance (m)	Height above river (m)	GPS coordinates	
1	1	Morteratsch	2070	10.0	0.8	46.4308528	9.9357028
2	2	Morteratsch	2018	35.0	2.5	46.4305556	9.9350000
3	3	Morteratsch	2026	240.0	13.0	46.4332500	9.9332500
4	1	RosegI	2034	15.0	0.5	46.4253611	9.8604444
5	1	RosegI	2031	2.0	0.7	46.4250278	9.8602500
6	2	RosegI	2040	75.0	1.0	46.4238333	9.8590278
7	3	RosegI	2050	20.0	2.5	46.4215556	9.8586111
8	2	RosegII	2012	10.0	1.5	46.4341111	9.8649722
9	1	RosegII	1997	2.5	0.4	46.4376944	9.8702778
10	1	Lonza	1995	0.3	1.0	46.4459722	7.8999167
11	1	Lonza	2027	4.0	1.0	46.4473056	7.9040556
12	2	Lonza	2003	16.0	0.9	46.4463333	7.9000833
13	3	Lonza	2007	200.0	10.0	46.4465000	7.8997778

DNA extraction, amplification, and Sanger sequencing

DNA was extracted from lichen thalli (total lichen DNA). Lichen thalli were examined under a dissecting microscope and washed with water before DNA extraction to remove possible surface contamination. Total genomic DNA was isolated from thallus fragments following the CTAB

protocol (Cubero *et al.* 1999). Both algal and fungal nuclear internal transcribed spacers (ITS rDNA) and the algal actin type I gene (including one complete exon and two introns located at codon positions 206 and 248; Weber and Kabsch 1994) were PCR amplified using primers listed in Table 2. PCRs were performed as described in Vančurová *et al.* (2018). All PCRs were performed in a volume of 20 µl using Red Taq Polymerase (Sigma) as described by Peksa and Škaloud (2011) or with My Taq Polymerase. Negative controls, without DNA template, were included in every PCR run to eliminate false-positive results caused by contaminants in the reagents. The PCR products were sequenced using the same primers at Macrogen in Amsterdam, Netherlands. The newly obtained sequences were deposited in GenBank under accession numbers MT066249–MT066395, MT076321–MT076465, and MT093213–MT093219 (Table S2).

Table 2 Primers used in this study

Name	Sequence		Reference
nr-SSU-1780-5'	5'-CTG CGG AAG GAT CAT TGA TTC-3'	algal ITS region, algal-specific	Piercey-Normore & DePriest 2001
ITS1-F-5'	5'- CTT GGT CAT TTA GAG GAA GTA A -3'	fungal ITS region, fungal-specific	Gardes & Bruns 1993
ITS4-3'	5'-TCC TCC GCT TAT TGA TAT GC-3'	algal and fungal ITS region, universal	White *et al.* 1990
ActinF2 Astero-5'	5'-AGC GCG GGT ACA GCT TCA C-3'	actin type I locus, algal specific	Škaloud & Peksa, 2010
ActinR2 Astero-3'	5'-CAG CAC TTC AGG GCA GCG GAA-3'	actin type I locus, algal specific	Škaloud & Peksa, 2010
1378-Chlorophyta	5'-TTG CCT TGT CAG GTT GAT TCC GG-3'	Illumina sequencing of ITS2	this study
5.8F-Chlorophyta	5'-GAA TTC CGT GAA CCA TCG AAT CTT T-3'	Illumina sequencing of ITS2	this study

Sequence alignment and DNA analyses

Asterochloris datasets were analyzed both as a single locus for the ITS rDNA (data not shown) and as a concatenated dataset of ITS rDNA and actin type I loci. The *Asterochloris* ITS rDNA dataset consisted of 202 sequences (142 newly obtained and 60 previously published) from *Stereocaulon* and other lichens retrieved from GenBank. The actin type I dataset consisted of 67 sequences (7 newly obtained and 60 previously published). Actin type I locus was sequenced primarily in those samples where unique ITS rDNA barcodes were obtained, to increase the phylogenetic resolution. Since (1) ITS rDNA and actin type I topologies are highly congruent, and (2) the samples with identical ITS rDNA barcodes generally show identical actin type I locus sequences, actin type I sequences were not obtained for all studied strains. The alignment was automatically performed by

MAFFT v.7 software (Katoh & Standley 2013) under the Q-INS-I strategy and manually edited according to the published secondary structures of ITS2 rDNA (Škaloud & Peksa 2010) using MEGA v.6 (Tamura *et al.* 2013). The actin type I sequences were aligned using MAFFT v.7 software (Katoh & Standley 2013) under the Q-INS-I strategy. After deleting identical sequences, the resulting concatenated alignment comprised 64 samples represented by unique ITS rDNA and actin type I sequences.

The ITS rDNA dataset of the *Stereocaulon* mycobiont comprised 171 sequences: 145 newly obtained sequences and 26 representative sequences selected to cover all the main clades 1–8 published by Högnabba (2006). The alignment was automatically performed by MAFFT v.7 software (Katoh & Standley 2013) under the Q-INS-I strategy. After removing identical sequences, the resulting alignment comprised 48 sequences. All DNA alignments are freely available on Mendeley Data: http://dx.doi.org/10.17632/jchg5h3t5k.1.

Phylogenetic relationships were inferred with the Bayesian Inference (BI) carried out in MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001), maximum likelihood (ML) analysis implemented in GARLI v.2.0 (Zwickl 2006), and maximum parsimony (MP) analysis using PAUP v.4.0b10 (Swofford 2003). BI and ML analyses were carried out on a dataset partitioned into ITS1, 5.8 S and ITS2 rDNA, actin intron 206, actin intron 248, and actin exon regions. The best-fit substitution models (Table S3) were selected using the Bayesian information criterion (BIC) implemented in JModelTest2 (Darriba *et al.* 2012; Guindon & Gascuel 2003). ML analysis was carried out using default settings, five search replicates, with an automatic termination set at 5 million generations. The MP analysis was performed using heuristic searches with 1000 random sequence addition replicates and random addition of sequences (the number was limited to 10^4 per replicate). ML and MP bootstrap support values were obtained from 100 and 1000 bootstrap replicates, respectively. Only one search replicate was applied for ML bootstrapping.

Ecological community analyses

From a total of 147 samples with successfully sequenced phycobionts, two were excluded due to the absence of a mycobiont sequence. Since the mycobiont identity affects the phycobiont diversity (Vančurová *et al.* 2018), four samples belonging to the minority species-level lineage (OTU2) were also excluded. Thus, statistical analyses were carried out using 141 members of the prevailing mycobiont species-level lineage (OTU35) and their phycobionts. Since the number of samples per plot varied, it was impossible to perform analyses requiring an equal number of samples per plot with the original dataset. Therefore, the number of phycobiont species-level lineages was rarefied to the smallest sample size in the data set, i.e. five samples (Fig. S3). After excluding study plot no. 11 (with the sample size of 5), the smallest sample size in the data set increased to 10 samples. The rarefaction was performed using the *rarefy* function in *vegan* R package (Oksanen *et al.* 2019).

To visualize phycobiont diversity in the context of surrounding vegetation, an ordination model (non-metric multidimensional scaling; NMDS) was computed as available in vegan package of R (Oksanen *et al*. 2019). The input dataset included vascular plants, bryophytes and lichens, whose cover values were converted to percentage and further log transformed. Afterwards, four variables (i.e., successional stage, number of lichen species, number of phycobiont species rarefied to sample size 5 and proportion of locally common phycobionts) were passively fitted using the function *envfit* of vegan R package. *Asterochloris* StA5 and *A. phycobiontica* were considered as locally common phycobionts.

Thereafter, the relationship between species richness of *Stereocaulon alpinum* OTU35 phycobionts and overall lichen species richness was investigated in order to determine if there was a correlation between the number of phycobiont species-level lineages and number of lichen species. The linear regression was performed separately for the dataset including all plots and the dataset restricted to plots with sample size ≥ 10. Since the parametric regression analyses can be significantly biased in small sample sizes, the Bayesian linear regression was used and the number of phycobionts modeled as a function of lichen species richness.

The gradual change of phycobiont community composition was inspected as a correlation between the proportion of the two most abundant phycobiont species-level lineages ((number of *Asterochloris phycobiontica* samples + number of StA5 lineage samples)/number of all samples) and the successional stage (coded 1, 2 and 3). The proportion of the most abundant phycobionts was modeled as a function of the successional stage. The program JAGS v. 4.2.0 (Plummer 2003) through the *R2JAGS* package (Su & Yajima 2015) in R was used to fit all regression models.

The vegetation plot data were stored in Turboveg for Windows v.2 database (Hennekens & Schaminée 2001) and further managed with JUICE software (Tichý 2002) and in the R environment (R Core Team 2017) with the help of the *vegan* R package (Oksanen *et al.* 2019).

Bipartite association networks were produced using *bipartite* R package (Dormann *et al.* 2008).

Illumina metabarcoding of algal communities in selected lichen thalli and soil samples

In order to describe algal plurality in *Stereocaulon* thalli, Illumina metabarcoding was performed. Eight thalli (four assigned to mycobiont OTU35 and four to OTU2) were examined. The samples which showed difficulties with Sanger sequencing of predominant phycobiont, a probable/possible sign of the algal plurality, were selected (Paul *et al.* 2018). Therefore, the evaluation of the frequency of this phenomenon is beyond the scope of this study. The samples were rehydrated with Milli-Q sterile water one day before being processed and stored in a growth chamber at 20°C under a 12h/12h light/dark cycle (15 μmol/m²/s). Thalli were cleaned under a stereomicroscope to remove soil particles and then superficially sterilized following Arnold et al. (2009). Fragments from

different parts of each thallus were randomly excised and pooled together (0.1 mg). Total genomic DNA was isolated and purified using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany).

Soil samples, one from each study plot, were sieved to remove contamination. Total genomic DNA was isolated and purified using the Soil DNA Isolation Plus Kit® (Norgen Biotek Corp.), following the manufacturer's instructions. Since the soil sample from plot number 7 was not processed successfully, only 12 soil samples were analyzed in the next steps.

Chlorophyta algal communities associated with the eight thalli and 12 soil samples were assayed using Illumina high-throughput sequencing of ITS2 of the rRNA operon, proposed as a universal barcode across eukaryotic kingdoms (Coleman 2009). High-coverage PCR primers at conserved sites were designed using a customized database for the algal phylum Chlorophyta (Table 2).

Amplicons for Illumina MiSeq sequencing were generated from nested PCR: in the first PCR the forward 1378-Chlorophyta (newly designed; Table 2) and the reverse ITS4 primers (White *et al.* 1990) were used and 27 amplification cycles were run, in the second PCR three replicates were amplified using the primers 5.8F-Chlorophyta (newly designed; Table 2) and ITS4 modified with Illumina overhang adaptors (forward overhang: 5´-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3'; reverse overhang: 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3' and 22 amplification cycles were run. These three replicates were then pooled together. PCR reactions were performed as described in Moya *et al.* (2017): All PCRs (25 µl) contained 2.5 µl of 10X buffer, 0.4 µM primers, 0.2 mM dNTPs, and 0.6 u/µl of ExTaq (Takara, Shiga, Japan), and sterile Milli-Q water was used to bring to correct volume. The PCR conditions were 1 cycle of 95°C for 2 min; 27 or 22 number of cycles (as described above) of 94°C for 30 s, 56°C for 45 s, and 72°C for 1 min; and a final extension of 72°C for 5 min.

PCR products were purified using AMPure XP beads (Beckman Coulter). Indexing PCR and addition of Nextera sequence adapters were performed using Nextera XT Index kit (Illumina Inc., San Diego, CA, USA) following the protocol for Illumina L library preparation. Finally, a second purification round was carried out using AMPure XP beads. Libraries were then quantified and pooled together. The libraries were sequenced on Illumina MiSeq platform using the MiSeq Reagent Kit v3 (paired end 2x 300 bp), at STAB Vida, Lisbon, Portugal and Genomics Core Facility at the University of Valencia, Spain.

Bioinformatics analyses

Quality control analysis of the Illumina MiSeq paired-end reads was performed using the FastQC v.0.11.8. Raw reads were processed using Quantitative Insights Into Microbial Ecology 2 (QIIME2 v.2018.11; Bolyen *et al.* 2018). Demultiplexed paired-end sequence reads were pre-processed using DADA2 (Callahan *et al.* 2016), a package integrated into Qiime2 that accounts for quality filtering, denoising, joining paired ends, and removal of chimeric sequences. The first 20 bp were trimmed from forward and reverse reads before merging to remove adaptors. In order to remove lower

quality bases, amplicon sequence variants (ASVs) were truncated at position 210 based on the FastQC reports during this step.

Subsequent analyses were based on the ASV table, which contained the count for each unique sequence in each sample. Only ASVs with frequency ≥100 were further analyzed. BLAST searches were used to confirm the sequence identity. Exclusively algal sequences were further analyzed. A phylogenetic tree (Fig. S4) was inferred with Bayesian Inference (BI) using MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001) as described above. Euler diagrams were produced using *eulerr* R package (Larsson 2019).

Results

Species composition in the study plots

In total, 88 vascular plant taxa, 19 bryophyte taxa, and 45 lichen taxa were recorded within the 13 study plots. Table S4 contains a summary of the species richness for individual plots.

Diversity of phycobionts and mycobionts

To address the overall diversity of the *Stereocaulon* mycobionts and their phycobionts in the study area, a phylogenetic analysis of the internal transcribed spacer (ITS) rDNA loci of both partners was performed. A phylogram resulting from the Bayesian analysis of the ITS rDNA sequences of the *Stereocaulon* mycobionts is shown in Fig. 1. The majority of the recovered mycobiont sequences formed a well-supported lineage delimited as operational taxonomic unit 35 (OTU35) by Vančurová *et al.* (2018). Five sequences matched the distantly related OTU2 (sister to DQ396973 and DQ396974), despite the morphological similarity of all the studied samples. Both OTU35 and OTU2 fall into Group 8b *sensu* Högnabba (2006).

The predominant phycobiont (i.e., the most abundant alga within a particular thallus; Paul *et al.* 2018) detected in 97% of the *Stereocaulon* samples belonged to the genus *Asterochloris*, while only five specimens represented other trebouxiophycean algae. In the case of these five specimens (coded A574, A574.1, A633, A634, and A634V) the identity of the phycobionts was confirmed by a Blast search against the GenBank database. Significant matches from 99% to 87% were obtained for A574 as *Coccomyxa viridis* HG973000, A574.1 as *Elliptochloris reniformis* LT560354, and A633, A634, and A634V as uncultured Trebouxiophyceae FJ554399. These latter three sequences formed a well-supported clade with the more distantly related sequence KF907701 (86% sequence similarity; Fig. S4), which was previously assigned to clade URa28 (Ruprecht *et al.* 2014). These sequences are referred to by this nomenclature hereafter.

The phylogenetic hypothesis resulting from the Bayesian analysis of the ITS rDNA and actin type I sequences of *Asterochloris* (Fig. 2) was congruent with that of previous studies (Gauslaa *et al.* 2013; Moya *et al.* 2015; Peksa & Škaloud 2011; Škaloud *et al.* 2015; Vančurová *et al.* 2018). The species boundaries delimited by Vančurová et al. (2018) and the nomenclature used, *ibidem,* were

maintained. A total of 14 lineages, including one novel lineage, here referred to as StA9, were recorded. Eleven of these lineages were previously determined as phycobionts of *Stereocaulon* (Vančurová *et al.* 2018 and references therein); namely, *A. glomerata*, *A. irregularis*, *A. italiana*, *A. lobophora*, *A. phycobiontica*, *A.* aff. *italiana*, *Asterochloris* clade 8, *Asterochloris* clade 12, *Asterochloris* StA3, *Asterochloris* StA4, and *Asterochloris* StA5.

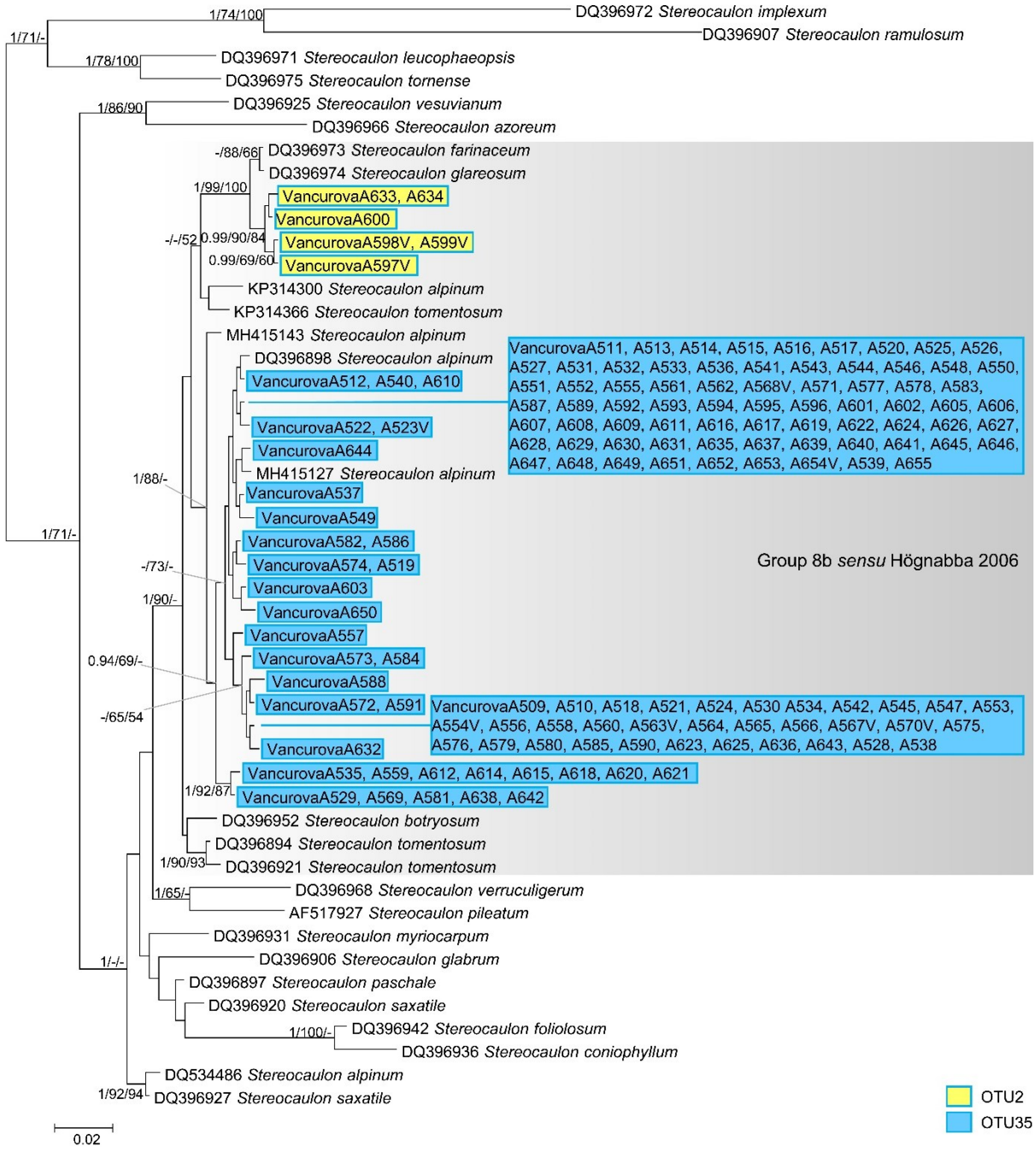


Fig. 1 Unrooted phylogenetic hypothesis of *Stereocaulon* resulting from the Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are in boxes. All new sequences belong to Group 8b *sensu* Högnabba (2006), as marked

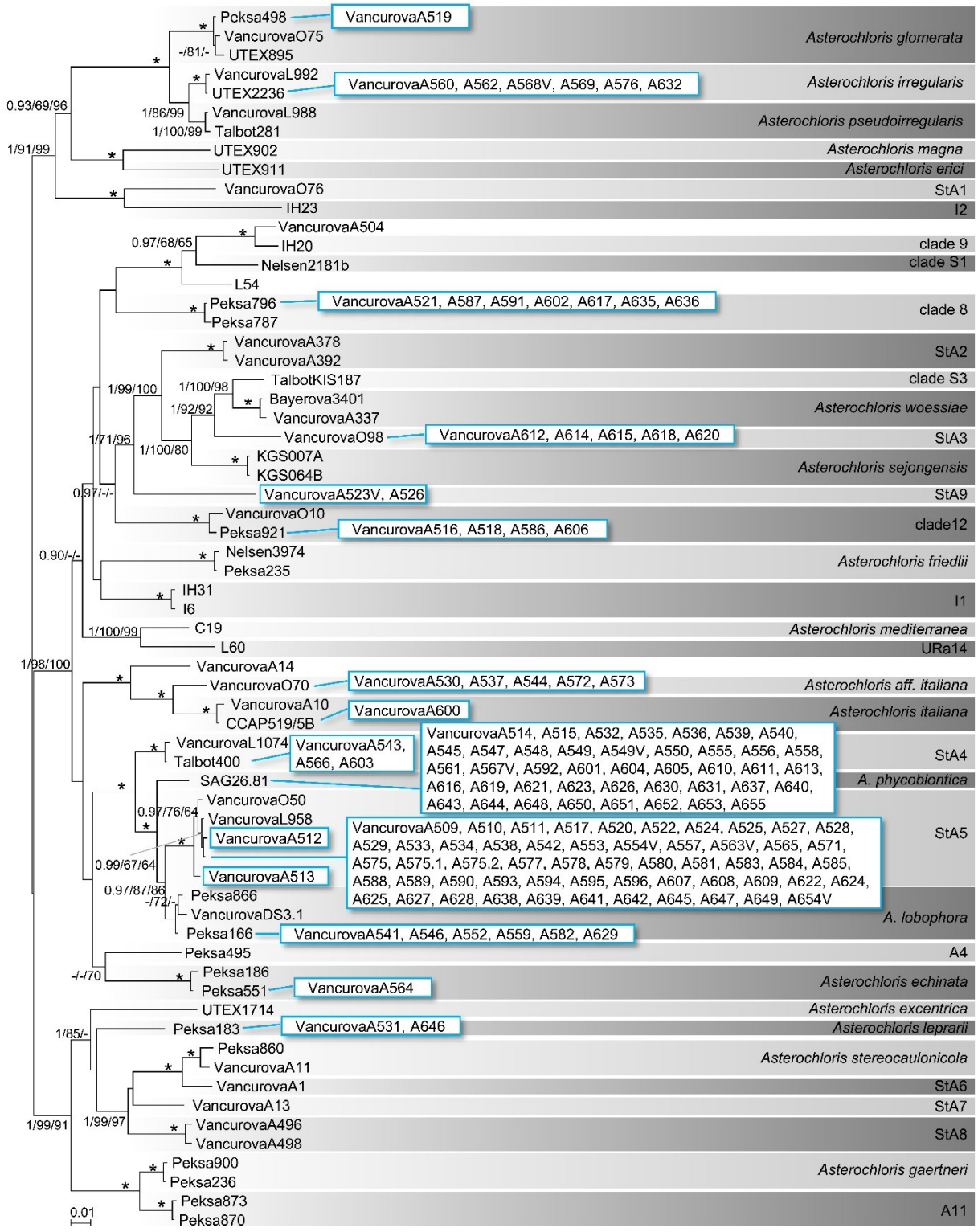


Fig. 2 Unrooted phylogenetic hypothesis of *Asterochloris* resulting from the Bayesian analysis of combined ITS rDNA and actin type I sequences. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are in boxes. Clade affiliations: clade 8, clade 9 *sensu* Škaloud and Peksa (2010), A4, A11 *sensu* Peksa and Škaloud (2011), URa14 *sensu* Ruprecht et al. (2014), I1, I2 *sensu* Řídká et al. (2014), S1, S3 *sensu* Nelsen and Gargas (2006), *A. aff. italiana* and StA1 – StA8 *sensu* Vančurová et al. (2018). StA9 lineage was identified as new in present study. Table S5 contains accession numbers of reference sequences retrieved from GenBank

Two of these 14 lineages (*A. echinata* and *A. leprarii*) were found in association with a *Stereocaulon* mycobiont for the first time in this study. The most frequently occurring phycobionts were linked with the lineages *Asterochloris* StA5 and *A. phycobiontica*.

Phycobiont community structure and its changes along a successional gradient

Stereocaulon alpinum OTU2 (n=4) was only sampled in two study plots (nos. 8 and 11). One sample was assigned to *A. italiana* and three were assigned to the trebouxiophycean lineage URa28. None was shared with the dominant *Stereocaulon* mycobiont (OTU35) in the study area (Fig. 3).

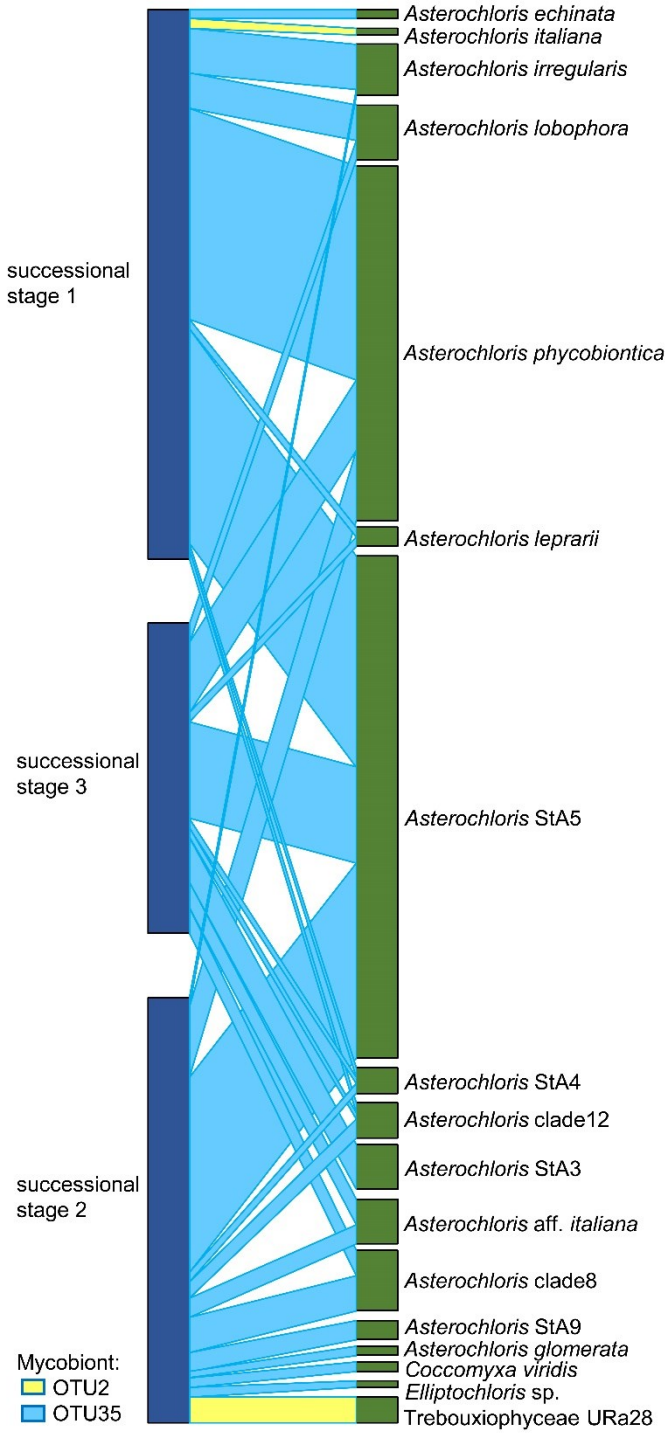


Fig. 3 Bipartite association network between successional stages and phycobiont species-level lineages. The width of the links is proportional to the number of specimens forming the association

Stereocaulon alpinum OTU35 (n = 141) was associated with 15 distinct species-level lineages of the phycobionts in the study area: 13 lineages of *Asterochloris*, 1 *Coccomyxa,* and 1 *Elliptochloris*. Phycobionts from two to six species-level lineages were recorded in each of the 13 study plots (Fig. S5). When the sample size was reduced to five, 2.0–3.7 phycobiont species per plot were expected. For a sample size of 10 (excluding plot 11, which only had five samples), 2.8–5.3 phycobiont species per plot were expected (Fig. S3; Table 1).

To visualize the phycobiont diversity in the context of the surrounding vegetation (vascular plants, bryophytes, and lichens), an ordination model was computed. The non-metric multidimensional scaling (NMDS) ordination (stress value 0.132) mostly reflected the successional gradient (Fig. 4). The fitted variable of succession (fit in the ordination: $r^2 = 0.3689$) was positively correlated with number of lichens ($r^2 = 0.7273$) and the number of phycobiont species ($r^2 = 0.1872$); in contrast it was strongly negatively correlated with the proportion of locally common phycobionts ($r^2 = 0.1988$).

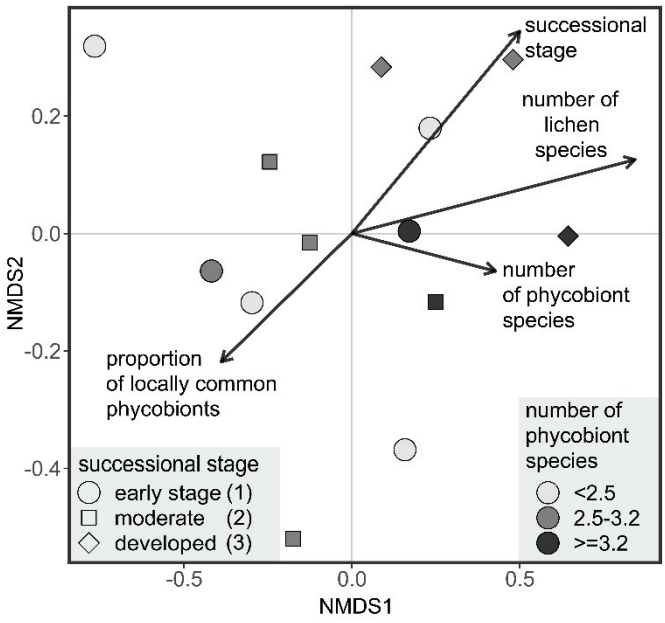


Fig. 4 Ordination diagram of non-metric multidimensional scaling (NMDS). The stress of the model is 0.132. The shape of the symbols represents the successional stage and their color the three levels of number of phycobiont species. Four variables (successional stage, number of lichen species, number of phycobiont species and proportion of locally common phycobionts) were passively superimposed onto the ordination plot

These results were supported by the Bayesian linear regression: the species richness of the *S. alpinum* OTU35 phycobionts significantly increased with the species richness of all the lichens recorded at a study plot (Fig. 5), while the proportion of the two most abundant phycobiont species-level lineages (*A. phycobiontica* and *Asterochloris* StA5) significantly decreased with increasing successional stage in favor of other species recovered at a lower frequency (Fig. 6). The phycobiont communities of the early-successional stages were relatively species-poor and mostly consisted of

species that were generally abundant in the study area. With ongoing succession, the number of locally rare phycobiont species increased, together with the total number of phycobiont and lichen species.

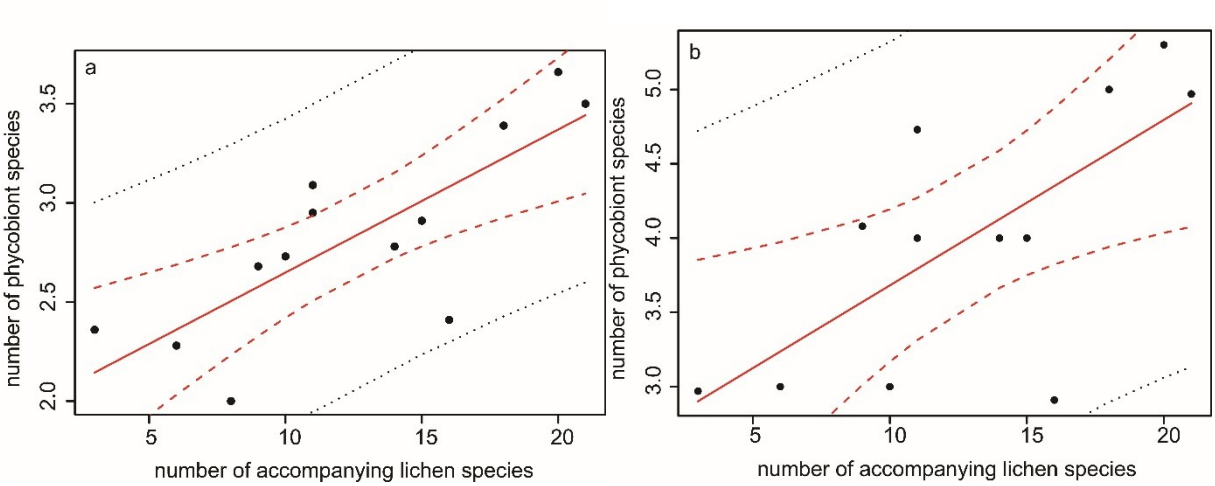


Fig. 5 Bayesian linear regression of number of accompanying lichen species as a predictor of the number of phycobionts associated with mycobiont OTU35 rarefied to **a** sample size of 5, **b** sample size of 10 per plot. Dashed lines show the 95% CRI around the regression line

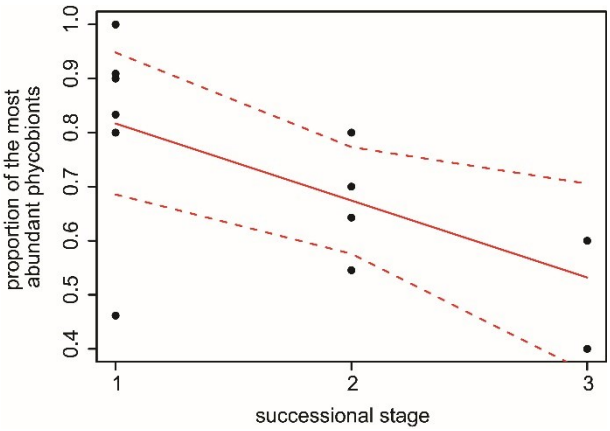


Fig. 6 Bayesian linear regression of number of a successional stage as a predictor of the proportion of the most abundant phycobiont species-level lineages ((number of *Asterochloris phycobiontica* samples + number of StA5 lineage samples)/number of all samples). Dashed lines show the 95% CRI around the regression line

Algal plurality detected using microalgal metabarcoding

Phycobiont diversity within particular lichen thalli (n = 8) was inspected using Illumina metabarcoding. A total of 1,945,186 raw readings were generated, of which 1,240,063 passed the demultiplexing step and quality filter. This represented an average of 155,007 algal reads (minimum = 38,329, maximum = 246,121, median = 179,415) per sample. The filtered metabarcoding dataset consisted of 116 hits (4 to 44 per sample).

The abundances of the recovered algal clades by sample are shown in Fig. S6, with the predominant phycobiont comprising 52.4–98.8% of the readings. Thalli A523M, A554M, A563M, and A570M

(assigned to the mycobiont OTU35) contained various *Asterochloris* species as the predominant phycobiont, which were assigned to lineage StA9 (sample A523M), *A. irregularis* (A563M), and *A. phycobiontica*/StA4/StA5 (A554M and A570M). The species-level lineages StA4, StA5, and *A. phycobiontica* were indistinguishable using the ITS2 rDNA marker. The relative frequency of amplicon sequence variants (ASVs) linked to *Asterochloris* spp. by sample is shown in Fig. S7. Thalli A597M, A598M, A633M, and A634M (assigned to mycobiont OTU2) predominantly contained phycobionts from the trebouxiophycean lineage URa28.

Soil as a potential reservoir for phycobionts

The phycobiont diversity in 12 soil samples was analyzed using Illumina metabarcoding. A total of 1,524,198 raw reads were generated, 876,596 of which passed the demultiplexing step and quality filter. This represented an average of 73,049 algal reads (minimum = 18,255, maximum = 164,643, and median = 63,827) per sample. The filtered metabarcoding dataset consisted of 427 hits (4 to 89 per sample). The phylogenetic hypothesis resulting from the Bayesian analysis of the ITS2 rDNA sequences obtained by the metabarcoding of soil samples, selected lichen samples, and reference sequences from GenBank is shown in Fig. S4. Sequences were recovered in 44 well-supported clades, 27 of which exclusively contained soil algae, 2 exclusively contained phycobionts, and 15 were shared by these two groups. The occurrence of particular clades of soil algae in each study plot is depicted in Fig. S8.

To determine the shared pool of algae between the lichen phycobionts and free-living soil algae, the occurrence of particular algal ITS2 haplotypes was analyzed, including the whole dataset obtained by Sanger sequencing (probably predominant phycobionts; Fig. 7a). Nine of the haplotypes obtained by Sanger sequencing were also found by Illumina sequencing of the soil and lichens. The vast majority of haplotypes were unique for the soil (n = 256) or Illumina lichen (n = 79) datasets. However, 27 haplotypes were shared by the soil and lichens but were not detected by Sanger sequencing using DNA extractions from *Stereocaulon* in the study area. The same analysis that was restricted to haplotypes with a frequency ≥ 1000 (to eliminate possible bias produced by errors from the polymerase chain reaction and sequencing; Huse *et al.* 2010) showed a similar pattern (Fig. 7b).

Discussion

Change of community structure along a successional gradient

Succession on river gravel bars is an important driver of both species composition and diversity. It is well documented in the case of vascular plants (e. g., Gilvear *et al.* 2008; Prach *et al.* 2014), but also applies to microorganisms, such as soil bacteria or mycorrhizal fungi (Li *et al.* 2014; Sheng *et al.* 2017). However, different groups of taxa respond differently to this gradient; for example, vascular plants are known to follow a nested-community pattern, where the highest species

diversity is associated with early- to mid-successional stages, and community diversity declines with ongoing succession (e.g., Walker and del Moral 2003; Corenblit *et al.* 2009; Chytrý *et al.* 2015). This pattern of vascular plant species richness was observed in the current study.

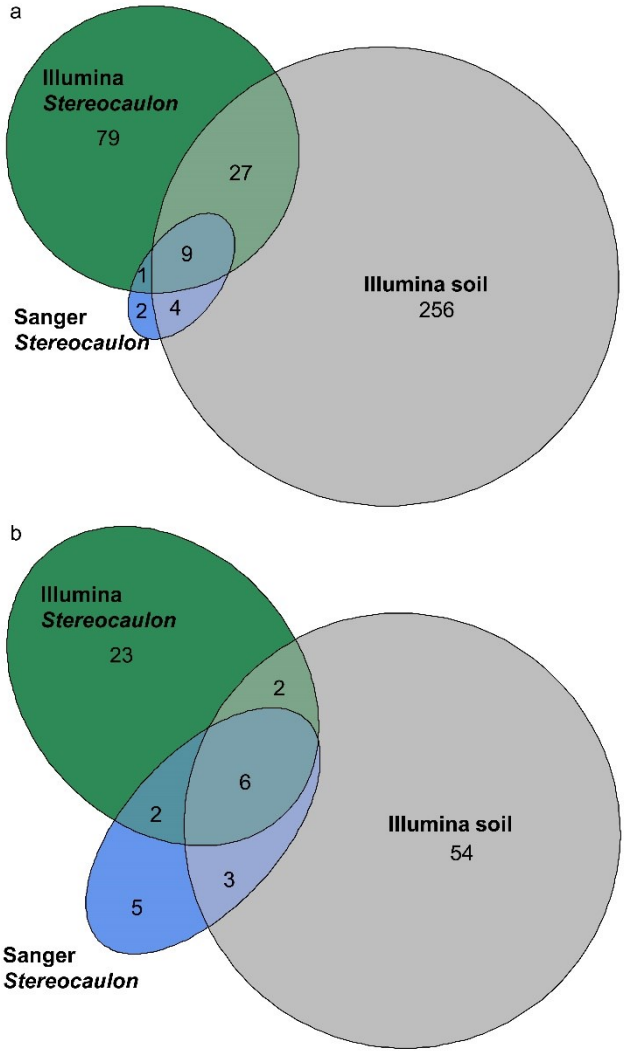


Fig. 7 Euler diagrams depicting sets of algal ITS2 rDNA haplotypes recovered from selected *Stereocaulon* thalli (n = 8) using Illumina metabarcoding, from all *Stereocaulon* samples (n = 147) using Sanger sequencing and from soil samples (n = 12) using Illumina metabarcoding. In case of Illumina metabarcoding sets, only haplotypes with frequency **a** ≥100 and **b** ≥1000 were included

The pattern observed for the phycobiont communities of *Stereocaulon* lichens differed. The phycobiont communities of the early-vegetation stages were composed of relatively few species-level lineages, such as *A. phycobiontica* and *Asterochloris* StA5 (Fig. 3), which are alpine and psychrophilic (Peksa & Škaloud 2011; Vančurová *et al.* 2018). As locally adapted lineages, they are probably common in populations surrounding the study plots. Therefore, newly emerged river gravel bars are easily colonized by the *A. phycobiontica* and *Asterochloris* StA5 lineages. In subsequent stages, the observed species richness of the phycobiont algae mostly increased and these two species-level lineages were gradually substituted by other lineages (Fig. 6). Some of these lineages could be specialized to slightly different microhabitat conditions within particular plots; for example, clades 8, 12, and StA3 tolerate a higher pH (Bačkor *et al.* 2010; Piercey-Normore & DePriest 2001; Steinová *et al.* 2019; Vančurová *et al.* 2018). On the river gravel bars of glacial floodplains, organisms with various substrate optima could coexist due to the heterogeneity of the substrate transported by a river or glacier from distant localities and various substrate layers. In

the study area, acidophilic vascular plants and bryophytes dominated, but the occurrence of basophilic species, such as *Didymodon fallax*, *Lophozia excisa*, *Syntrichia ruralis*, and *Veronica fruticans*, as found in our data, was considered an indication of the basic fractions of the substrate.

On the river gravel bars, the species richness of the phycobionts was positively correlated with that of lichens (Fig. 5). This correlation possibly indicates the phycobionts and mycobionts use similar dispersal strategies while colonizing newly exposed gravel bar stands. However, it could also be connected with other variables, such as changing microhabitat heterogeneity. The species richness of terricolous lichens on glacier forelands in the Alps was positively correlated with the time since deglaciation (Nascimbene *et al.* 2017), analogous to lichen species richness on deglaciated plots in maritime Antarctica (Favero-Longo *et al.* 2012). In both cases, most species, once established, persisted until the oldest successional stages. Beck *et al.* (2019) found two haplotypes of *Stichococcus antarcticus*, a phycobiont of the *Placopsis* lichen in maritime Antarctica, exclusively occurred in areas that had been deglaciated for a long time and had a more developed soil and lichen community. The succession of vegetation causes numerous physical and chemical changes in the soil, as the abundance of organic matter in the soil increases with increasing plant cover. Therefore, the correlation between the species richness of various organisms and successional stages is frequently connected with changing soil characteristics, such as nutrient concentrations (Burga *et al.* 2010; Corenblit *et al.* 2009; Li *et al.* 2014; Sheng *et al.* 2017).

There are two possible sources of phycobionts for the lichens on river gravel bars: algae that continually colonize the gravel bars from surrounding areas or soil algae *in situ* (Dal Grande *et al.* 2012; Fontaine *et al.* 2012; Ohmura *et al.* 2019). Several algal clades were found in both the soil and the lichens (Fig. S4), but the phycobiont pool appeared to be independent of the soil algae; for example, the most frequent ITS2 rDNA haplotype among Sanger sequences (*A. phycobiontica*/StA4/StA5, which were recovered in 69% of all samples) was present in only two soil samples at very low abundances (212 (2.4%) and 135 (3.1%) of algal readings). In addition, other haplotypes were abundant in the lichens and rare in the soil or *vice versa*. Approximately, only ten ITS2 haplotypes belonging to the genera *Asterochloris*, *Elliptochloris,* and clade URa28 were abundant in both soil and lichen thalli. In Fig. 8, a comparison of the relative abundances of the algal clades in soil samples and lichen thalli is presented. Only plots 4, 5, 6, and 8, with soil samples generating > 5000 algal reads and with *Stereocaulon* samples analyzed using Illumina metabarcoding, are displayed. The discrepancy between the communities of soil and lichen algae supports our hypothesis that phycobionts originating from the surrounding area (probably from other lichen populations) colonize the recently emerged plots, without a substantial contribution from the "soil seed bank." However, these results should be perceived as the basis for future research. The number of soil samples was rather limited, and some taxa could have remained undetected (Rippin *et al.* 2018). Nevertheless, the taxonomic composition of the algae occurring on the river gravel bars was comparable to the pool of soil algae detected in the foreland of the Damma glacier in the Swiss Alps (Frey *et al.* 2013). Notably, a significantly different algal community was

found in the early-successional stage, which in that case was represented by bare soil near a receding glacier. The lichen phycobionts, including *Asterochloris*, were reported from the soil in the transitional and developed stages. One of *Asterochloris* sequences recovered by Frey *et al.* (2013) corresponds with the lineage StA9, which was first reported as a lichen phycobiont in the present study.

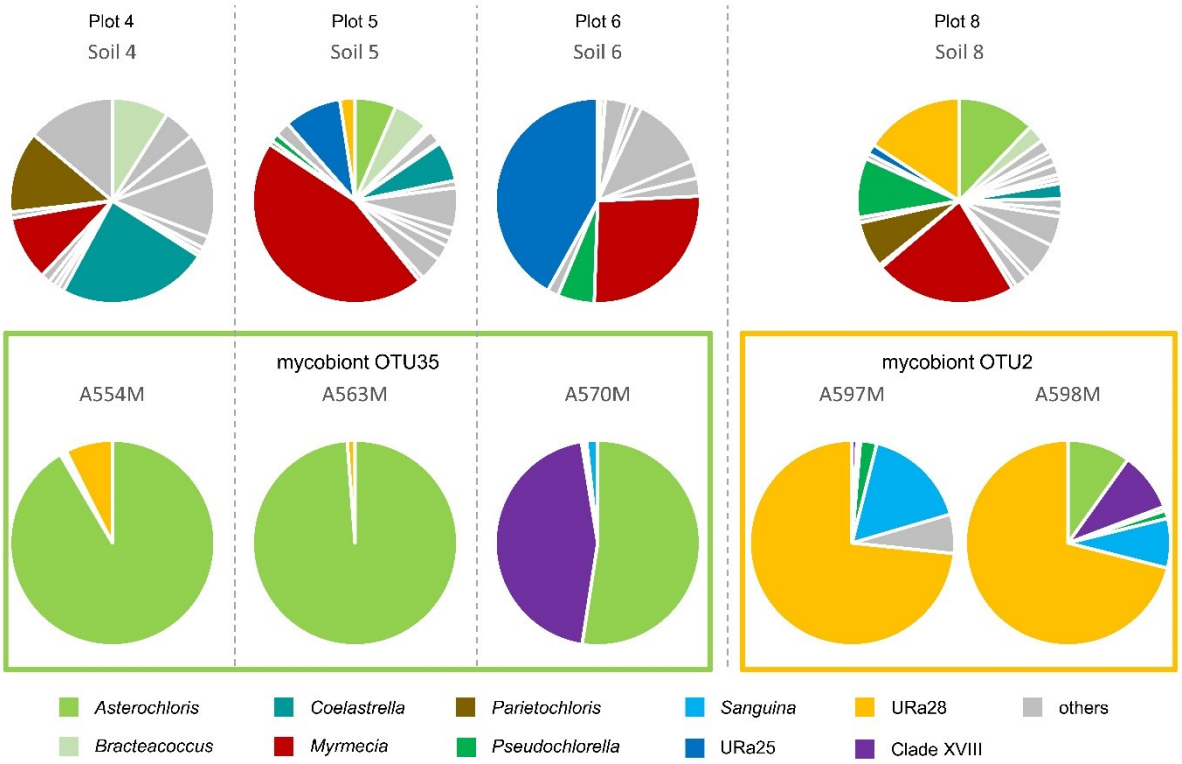


Fig. 8 Relative abundances of algal clades within soil (first line) and *Stereocaulon* (second line) samples. Solely plots 4, 5, 6, and 8 with soil samples generating >5000 algal reads and with *Stereocaulon* samples analyzed using Illumina metabarcoding were displayed. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. (2014). Clade XVIII was identified as new in present study. Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis presented in Fig. S4

A similar pattern was demonstrated for corals, with little overlap between the pool of photosynthetic symbionts in the sediment and the host (Quigley *et al.* 2017); however, Ali *et al.* (2019) demonstrated a significant influence of the sediment on coral symbiosis establishment.

Low specificity as an adaptive strategy

The specificity (i.e., the taxonomic range of acceptable partners; Rambold *et al.* 1998; Yahr *et al.* 2004, 2006) of both mycobionts and phycobionts has been considered a crucial characteristic of lichen interactions. A reduced specificity of symbiotic partners was frequently reported as an advantageous strategy in harsh environments (Engelen *et al.* 2010; Romeike *et al.* 2002).

Both species-level lineages of *Stereocaulon* recorded in the study area (Fig. 1) were morphologically identical and indistinguishable in the field. The overwhelming majority of samples belonged to OTU35, which was reported to have low specificity towards its phycobionts (Vančurová *et al.* 2018).

On river gravel bars, the mycobionts belonging to this lineage frequently associated with algae that are generally known as the phycobionts of *Lepraria* lichens (*A. phycobiontica*, *A. echinata*, and *A. leprarii*; Škaloud *et al.* 2015). Such low specificity (OTU35 was associated with 13 species-level lineages of *Asterochloris* and, in two cases, with representatives of other trebouxiophycean phycobionts) could facilitate the colonization of heterogeneous and harsh habitats, including river gravel bars of glacial floodplains.

On the other hand, *S. alpinum* OTU2 was associated with different phycobionts, despite growing in the same environment as *S. alpinum* OTU35. The OTU2 mycobiont is mostly associated with the trebouxiophycean alga URa28. This alga was previously detected in a soil sample from Canada, either as a soil alga or possibly as a phycobiont of *Stereocaulon* sp., which was recorded in the same location (Hartmann *et al.* 2009).

One of the alternative hypotheses concerning the specificity towards the phycobionts is that lichens tightly attached to the substrate were considered less specific than lichens with a fruticose growth form (Helms 2003; Leavitt *et al.* 2015). However, *S. alpinum* has a well-developed fruticose thallus, and our samples were no exception to this, despite harsh environmental conditions. Within the genus *Stereocaulon*, other mycobiont species were reported with low specificity towards their phycobionts and were able to establish symbiosis with more than one algal genus. The existence of more algal genera associated with *Stereocaulon* lichens, in addition to *Asterochloris*, *Chloroidium* (Beck 2002), *Vulcanochloris* (Vančurová *et al.* 2015, 2018), *Elliptochloris*, *Coccomyxa*, and the clade URa28, could be expected.

Algal plurality

The occurrence of more than one phycobiont species in a single lichen thallus (i.e., algal plurality) was an overlooked phenomenon but revealed to be quite common in lichens (e.g., Bačkor *et al.* 2010; Muggia *et al.* 2013; Moya *et al.* 2017; Onuţ-Brännström *et al.* 2018; Smith *et al.* 2020), and has been documented in *Stereocaulon* (Vančurová *et al.* 2018). However, various lichen species differ in the prevalence of algal plurality. Dal Grande *et al.* (2017) revealed the occurrence of more than one phycobiont species in 49.2% of *Lasallia hispanica* thalli but in only 1.7% of *L. pustulata* thalli. Leavitt *et al.* (2015) proposed the hypothesis that those lichens, which are unspecific towards their phycobionts, more frequently exhibit algal plurality.

Using Illumina metabarcoding, more than one phycobiont species was found in all selected samples from both the mycobiont species-level lineages (Fig. S6). However, these samples were selected because of difficulties with Sanger sequencing, which in itself could indicate algal plurality (Paul *et al.* 2018). Illumina metabarcoding as well as Sanger sequencing uncovered URa28 as the predominant phycobiont of *S. alpinum* OTU2. In most cases, the phycobiont determined by Sanger sequencing corresponded with the predominant phycobiont according to high-throughput sequencing (Molins *et al.* 2018; Paul *et al.* 2018).

Even though the two mycobiont species-level lineages, OTU2 and OTU35, differed in their predominant phycobiont pools, they shared the pool of other intrathalline algae, unlike two *Circinaria* spp. collected at the same location; these shared the predominant phycobiont pool but showed a completely different pool of other intrathalline algae (Molins *et al.* 2018). The *Stereocaulon* OTUs significantly differed in the frequency of intrathalline algae (Fig. S6). Above all, most of the OTU35 thalli (with *Asterochloris* as the predominant phycobiont) contained a small amount of URa28 algae, and most of the OTU2 thalli (with URa28 as the predominant phycobiont) contained a small amount of *Asterochloris*. Several algal clades interacted exclusively with one mycobiont species-level lineage, but their frequency was generally low. A comparable phycobiont pair, *Trebouxia jamesii*/*Trebouxia* sp. TR9, found in the *Ramalina farinacea* lichen, is assumed to physiologically benefit that symbiotic system (Casano *et al.* 2011; Centeno *et al.* 2016). Gasulla *et al.* (2020) found two strains of *Coccomyxa* phycobionts in the thalli of the basidiolichen *Lichenomphalia*; one of the strains was restricted to lower altitudes, one to higher altitudes, and both were present in the thalli growing at intermediate altitudes. Alternatively, the minor phycobionts could occur in thalli without affecting the lichen and may be used as a source of algal symbionts for other lichens in the locality. This hypothesis is in concordance with our results, which point to a lack of symbiotic algae in the soil.

Conclusions

The main goal of this study was to determine the connections between phycobiont diversity and the successional gradient. The diversity of phycobionts on river gravel bars shifted along the successional vegetation gradient, from early-successional herbaceous stages through to the scrub and then young tree stages. The phycobiont communities of the early-successional stages were composed of relatively few species lineages. In subsequent stages, the observed species richness of the phycobionts mostly increased, while the species-level lineages typical for early-successional stages were gradually substituted by others that were probably adapted to the heterogenous microhabitat conditions of the river gravel bars. Moreover, a positive correlation was revealed between the species richness of the phycobionts and that of the accompanying lichens in the locality.

The second question addressed was related to the specificity of *Stereocaulon* lichens towards their phycobionts on river gravel bars. The substantial phycobiont diversity (including 14 *Asterochloris* species-level lineages and three additional trebouxiophycean algae) that was recovered from the river gravel bars suggested low specificity of *Stereocaulon* mycobionts. This range of phycobionts may help them to cope with the heterogeneous and dynamic conditions of the river gravel bars in glacial floodplains.

Finally, algal plurality was examined; more than one phycobiont species was found in samples belonging to both mycobiont OTUs (OTU2 and OTU35). *Asterochloris* phycobionts were recovered as the predominant phycobionts of OTU35, while the trebouxiophycean lineage URa28 was the

predominant phycobiont of OTU2. However, the broad community of other intrathalline algae was shared by both mycobionts.

Besides novel insights into the community structure of symbiotic microorganisms under the harsh and dynamic conditions of river gravel bars, this study presents challenging questions concerning cryptic lichen species and specificity towards the phycobionts, the dispersal of microscopic symbionts, the ecological function of additional intrathalline algae, and an observed discrepancy between the communities of soil and lichen algae.

Author contributions

L.V., V.K., O.P., and P.Š. designed the study; L.V., V.K., and J.M. conducted fieldwork and collected specimens; L.V., Z.Š., and P.M. performed laboratory work; L.V., V.K., P.M., K. Ch., and P.Š. analyzed the data; all authors participated in writing the manuscript and provided final approval of the version to be submitted.

Acknowledgements

We are grateful to Helmut Mayrhofer and Jan Vondrák for identifying of a few critical lichens, Svatava Kubešová for help with bryophyte identification, Vít Grulich and Jiří Danihelka for identifying some vascular plants, and the anonymous reviewers for their valuable comments on the original version of the manuscript. This work was supported by the Charles University Science Foundation project GAUK 946417, the Primus Research Programme of Charles University no. SCI/13 and by the long-term research development project RVO 67985939.

No conflicts, informed consent, or human or animal rights are applicable to this study.

References

Ali, A., Kriefall, N.G., Emery, L.E., Kenkel, C.D., Matz, M. V. & Davies, S.W. (2019) Recruit symbiosis establishment and Symbiodiniaceae composition influenced by adult corals and reef sediment. *Coral Reefs* 38: 405–415. https://doi.org/10.1007/s00338-019-01790-z

Arnold, A.E., Miadlikowska, J., Higgins, K.L., Sarvate, S.D., Gugger, P., Way, A., Hofstetter, V., Kauff, F. & Lutzoni, F. (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Systematic Biology* 58: 283–297. https://doi.org/10.1093/sysbio/syp001

Bačkor, M., Peksa, O., Škaloud, P. & Bačkorová, M. (2010) Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. *Ecotoxicology and environmental safety* 73: 603–12. https://doi.org/10.1016/j.ecoenv.2009.11.002

Beck, A. (2002) Selektivität der Symbionten schwermetalltoleranter Flechten. Ludwig-Maxmilians-Universität München

Beck, A., Bechteler, J., Casanova-Katny, A. & Dzhilyanova, I. (2019) The pioneer lichen *Placopsis* in maritime Antarctica: Genetic diversity of their mycobionts and green algal symbionts, and their correlation with deglaciation time. *Symbiosis* 79: 1–24. https://doi.org/10.1007/s13199-019-00624-4

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C., Ghalith, G.A. Al, Alexander, H., Alm, E.J., Arumugam, M., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Colin, J., Brown, C.T., Callahan, B.J., Mauricio, A., Rodríguez, C., Chase, J., Cope, E., Silva, R. Da, Dorrestein, P.C., Douglas, G.M., Duvallet, C., Edwardson, C.F., Ernst, M., Fouquier, J., Gauglitz, J.M., Gibson, D.L., Gonzalez, A., Huttley, G.A., Janssen, S., Jarmusch, A.K., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Ley, R., Loftfield, E., Marotz, C., Martin, B., Mcdonald, D., Mciver, L.J., Alexey, V., Metcalf, J.L., Morgan, S.C., Morton, J.T. & Naimey, A.T. (2018) QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Preprints*. https://doi.org/10.7287/peerj.preprints.27295

Burga, C.A., Krüsi, B., Egli, M., Wernli, M., Elsener, S., Ziefle, M., Fischer, T. & Mavris, C. (2010) Plant succession and soil development on the foreland of the Morteratsch glacier (Pontresina, Switzerland): straightforward or chaotic? *Flora* 205: 561–576. https://doi.org/10.1016/j.flora.2009.10.001

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13: 581.

Casano, L.M., del Campo, E.M., García-Breijo, F.J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., Guéra, A. & Barreno-Rodríguez, E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental microbiology* 13: 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x

Centeno, D.C., Hell, A.F., Braga, M.R., del Campo, E.M. & Casano, L.M. (2016) Contrasting strategies used by lichen microalgae to cope with desiccation-rehydration stress revealed by metabolite profiling and cell wall analysis. *Environmental Microbiology* 18: 1546–1560. https://doi.org/10.1111/1462-2920.13249

Chytrý, M., Dražil, T., Hájek, M., Kalníková, V., Preislerová, Z., Šibík, J., Ujházy, K., Axmanová, I., Bernátová, D., Blanár, D., Dančák, M., Dřevojan, P., Fajmon, K., Galvánek, D., Hájková, P., Herben, T., Hrivnák, R., Janeček, Š., Janišová, M., Jiráská, Š., Kliment, J., Kochjarová, J., Lepš, J., Leskovjanská, A., Merunková, K., Mládek, J., Slezák, M., Šeffer, J., Šefferová, V., Škodová, I., Uhlířová, J., Ujházyová, M. & Vymazalová, M. (2015) The most species-rich plant communities in the Czech Republic and Slovakia (with new world records). *Preslia* 87: 217–278.

Chytrý, M., Hennekens, S.M., Jiménez-Alfaro, B., Knollová, I., Dengler, J., Jansen, F., Landucci, F., Schaminée, J.H.J., Aćić, S., Agrillo, E., Ambarlı, D., Angelini, P., Apostolova, I., Attorre, F., Berg, C., Bergmeier, E., Biurrun, I., Botta-Dukát, Z., Brisse, H., Campos, J.A., Carlón, L., Čarni, A., Casella, L., Csiky, J., Ćušterevska, R., Dajić Stevanović, Z., Danihelka, J., De Bie, E., de Ruffray, P., De Sanctis, M., Dickoré, W.B., Dimopoulos, P., Dubyna, D., Dziuba, T., Ejrnaes, R., Ermakov, N., Ewald, J., Fanelli, G., Fernández-González, F., FitzPatrick, Ú., Font, X., García-Mijangos, I., Gavilán, R.G., Golub, V., Guarino, R., Haveman, R., Indreica, A., Işık Gürsoy, D., Jandt, U., Janssen, J.A.M., Jiroušek, M., Kącki, Z., Kavgacı, A., Kleikamp, M., Kolomiychuk, V., Krstivojević Ćuk, M., Krstonošić, D., Kuzemko, A., Lenoir, J., Lysenko, T., Marcenò, C., Martynenko, V., Michalcová, D., Moeslund, J.E., Onyshchenko, V., Pedashenko, H., Pérez-Haase, A., Peterka, T., Prokhorov, V., Rašomavičius, V., Rodríguez-Rojo, M.P., Rodwell, J.S., Rogova, T., Ruprecht, E., Rūsiņa, S., Seidler, G., Šibík, J., Šilc, U., Škvorc, Ž., Sopotlieva, D., Stančić, Z., Svenning, J.-C., Swacha, G., Tsiripidis, I., Turtureanu, P.D., Uğurlu, E., Uogintas, D., Valachovič, M., Vashenyak, Y., Vassilev, K., Venanzoni, R., Virtanen, R., Weekes, L., Willner, W., Wohlgemuth, T. & Yamalov, S. (2016) European Vegetation Archive (EVA): an integrated database of European vegetation plots. M. Pärtel (ed.). *Applied Vegetation Science* 19: 173–180. https://doi.org/10.1111/avsc.12191

Coleman, A.W. (2009) Is there a molecular key to the level of "biological species" in eukaryotes? A DNA guide. *Molecular Phylogenetics and Evolution* 50: 197–203. https://doi.org/10.1016/j.ympev.2008.10.008

Corenblit, D., Steiger, J., Gurnell, A.M., Tabacchi, E. & Roques, L. (2009) Control of sediment dynamics by vegetation as a key function driving biogeomorphic succession within fluvial corridors. *Earth Surface Processes and Landforms* 34: 1790–1810. https://doi.org/10.1002/esp.1876

Cubero, O.F., Crespo, A., Fatehi, J. & Bridge, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* 216: 243–249. https://doi.org/10.1007/BF01084401

Dal Grande, F., Rolshausen, G., Divakar, P.K., Crespo, A., Grande, F.D., Otte, J., Schleuning, M. & Schmitt, I. (2017) Environment and host identity structure communities of green algal symbionts

in lichens. *New Phytologist* 217: 277–289. https://doi.org/10.1111/nph.14770

Dal Grande, F., Widmer, I., Wagner, H.H. & Scheidegger, C. (2012) Vertical and horizontal photobiont transmission within populations of a lichen symbiosis. *Molecular Ecology* 21: 3159–72. https://doi.org/10.1111/j.1365-294X.2012.05482.x

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Dormann, C.F., Gruber, B. & Fruend, J. (2008) Introducing the bipartite Package: Analysing Ecological Networks. *R News* 8: 8–11.

Doty, S.L., Sher, A.W., Fleck, N.D., Khorasani, M., Bumgarner, R.E., Khan, Z., Ko, A.W.K., Kim, S.H. & DeLuca, T.H. (2016) Variable nitrogen fixation in wild *Populus*. B. R. Albrectsen (ed.). *PLoS ONE* 11: e0155979. https://doi.org/10.1371/journal.pone.0155979

Ellenberg, H. & Leuschner, C. (2010) *Vegetation Mitteleuropas mit den Alpen*. Eugen Ulmer, Stuttgart. 1334 pp.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W. & Paulissen, D. 1991. (1991) Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* 18: 1–248.

Engelen, A., Convey, P. & Ott, S. (2010) Life history strategy of *Lepraria borealis* at an Antarctic inland site, Coal Nunatak. *The Lichenologist* 42: 339–346. https://doi.org/10.1017/S0024282909990600

Euro+Med (2006–2019) Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/

Favero-Longo, S.E., Worland, M.R., Convey, P., Smith, R.I.L., Piervittori, R., Guglielmin, M. & Cannone, N. (2012) Primary succession of lichen and bryophyte communities following glacial recession on Signy Island, South Orkney Islands, Maritime Antarctic. *Antarctic Science* 24: 323–336. https://doi.org/10.1017/S0954102012000120

Fontaine, K.M., Beck, A., Stocker-Wörgötter, E. & Piercey-Normore, M.D. (2012) Photobiont Relationships and Phylogenetic History of *Dermatocarpon luridum var. luridum* and Related *Dermatocarpon* Species. Plants 1: 39–60. https://doi.org/10.3390/plants1020039

Frey, B., Bühler, L., Schmutz, S., Zumsteg, A. & Furrer, G. (2013) Molecular characterization of phototrophic microorganisms in the forefield of a receding glacier in the Swiss Alps. *Environmental Research Letters* 8: 15033. https://doi.org/10.1088/1748-9326/8/1/015033

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

Gasulla, F., Barrasa, J.M., Casano, L.M. & del Campo, E.M. (2020) Symbiont composition of the basidiolichen *Lichenomphalia meridionalis* varies with altitude in the Iberian Peninsula. *The Lichenologist* 52: 17–26. https://doi.org/10.1017/S002428291900046X

Gauslaa, Y., Bidussi, M., Solhaug, K.A., Asplund, J. & Larsson, P. (2013) Seasonal and spatial variation in carbon based secondary compounds in green algal and cyanobacterial members of the epiphytic lichen genus *Lobaria*. *Phytochemistry* 94: 91–8. https://doi.org/10.1016/j.phytochem.2013.04.003

Gilvear, D., Francis, R., Willby, N. & Gurnell, A.M.A. (2008) Gravel bars: a key habitat of gravel-bed rivers for vegetation. *In*: H. Habersack, H. Piégay, & M. Rinaldi (eds.) *Gravel-bed rivers VI: from process understanding to river restauration*. Elsevier, Amsterdam, pp. 677–700.

Godschalx, A.L., Rodríguez-Castañeda, G. & Rasmann, S. (2019) Contribution of different predator guilds to tritrophic interactions along ecological clines. *Current Opinion in Insect Science* 32: 104–109. https://doi.org/10.1016/j.cois.2019.01.002

Grolle, R. & Long, D.G. (2000) An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. *Journal of Bryology* 22: 103–140. https://doi.org/10.1179/jbr.2000.22.2.103

Guindon, S. & Gascuel, O. (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology* 52: 696–704. https://doi.org/10.1080/10635150390235520

Hartmann, M., Lee, S., Hallam, S.J. & Mohn, W.W. (2009) Bacterial, archaeal and eukaryal community

structures throughout soil horizons of harvested and naturally disturbed forest stands. *Environmental Microbiology* 11: 3045–3062. https://doi.org/10.1111/j.1462-2920.2009.02008.x

Helms, G. (2003) Taxonomy and Symbiosis in Associations of Physciaceae and *Trebouxia*. Georg-August Universität Göttingen

Hennekens, S.M. & Schaminée, J.H.J. (2001) TURBOVEG, a comprehensive data base management system for vegetation data. *Journal of Vegetation Science* 12: 589–591. https://doi.org/10.2307/3237010

Hill, M.O., Bell, N., Bruggeman-Nannenga, M.A., Brugués, M., Cano, M.J., Enroth, J., Flatberg, K.I., Frahm, J.-P., Gallego, M.T., Garilleti, R., Guerra, J., Hedenäs, L., Holyoak, D.T., Ignatov, M.S., Lara, F., Mazimpaka, V., Muñoz, J. & Söderström, L. (2006) An annotated checklist of the mosses of Europe and Macaronesia. *Journal of Bryology* 28: 198–267. https://doi.org/10.1179/174328206X119998

Hill, M.O., Mountford, J.O., Roy, D.B. & Bunce, R.G.H. (1999) *Ellenberg's indicator values for British plants. ECOFACT Volume 2 Technical Annex*. Institute of Terrestrial Ecology

Hill, M.O., Preston, C.D., Bosanquet, S.D.S. & Roy, D.B. (2007) *Bryoatt: Attributes of British and Irish Mosses, Liverworts and Hornworts*. NERC Centre for Ecology & Hydrology & Countryside Council for Wales. 80 pp.

Högnabba, F. (2006) Molecular phylogeny of the genus *Stereocaulon* (Stereocaulaceae, lichenized ascomycetes). *Mycological research* 110: 1080–1092. https://doi.org/10.1016/j.mycres.2006.04.013

Hohensinner, S., Hauer, C. & Muhar, S. (2018) River Morphology, Channelization, and Habitat Restoration. *In*: S. Schmutz & J. Sendzimir (eds.) *Riverine Ecosystem Management*. Springer International Publishing, Cham, pp. 41–65.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics (Oxford, England)* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Huse, S.M., Welch, D.M., Morrison, H.G. & Sogin, M.L. (2010) Ironing out the wrinkles in the rare biosphere through improved OTU clustering. *Environmental Microbiology* 12: 1889–1898. https://doi.org/10.1111/j.1462-2920.2010.02193.x

Jeník, J. (1955) Sukcese rostlin na náplavech řeky Belé v Tatrách (Succession of plants on gravel bars of the Belá River in the Tatra Mountains). *Acta Universitatis Carolinae* 4: 1–59.

Junk, W.J., Bayley, P.B. & Sparks, R.E. (1989) The Flood Pulse Concept in River-Floodplain Systems. *Canadian Journal of Fisheries and Aquatic Sciences* 106: 110–127.

Kalníková, V., Chytrý, K. & Chytrý, M. (2018) Early vegetation succession on gravel bars of Czech Carpathian streams. *Folia Geobotanica* 53: 317–332. https://doi.org/10.1007/s12224-018-9323-6

Kalníková, V. & Kudrnovsky, H. (2017) Gravel Bar Vegetation Database. *Phytocoenologia* 47: 109–110. https://doi.org/10.1127/phyto/2017/0177

Karrenberg, S., Kollmann, J., Edwards, P.J., Gurnell, A.M. & Petts, G.E. (2003) Patterns in woody vegetation along the active zone of a near-natural Alpine river. *Basic and Applied Ecology* 4: 157–166. https://doi.org/10.1078/1439-1791-00123

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–80. https://doi.org/10.1093/molbev/mst010

Larsson, J. (2019) eulerr: Area-Proportional Euler and Venn Diagrams with Ellipses.

Lavoie, C., Renaudin, M., McMullin, R.T., Gagnon, J., Roy, C., Beaulieu, M.-E., Bellenger, J.P. & Villarreal, J.C. (2020) Extremely low genetic diversity of *Stigonema* associated with *Stereocaulon* in eastern Canada. *The Bryologist* 123: 188–203. https://doi.org/10.1639/0007-2745-123.2.188

Leavitt, S.D., Kraichak, E., Nelsen, M.P., Altermann, S., Divakar, P.K. & Alors, D. (2015) Fungal specificity and selectivity for algae play a major role in determining lichen partnerships across diverse ecogeographic regions in the lichen-forming family Parmeliaceae (Ascomycota). *Molecular Ecology* 24: 3779–3797. https://doi.org/10.1111/mec.13271

Li, Y., Wen, H., Chen, L. & Yin, T. (2014) Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. *PLoS ONE* 9. https://doi.org/10.1371/journal.pone.0115024

Lücking, R., Lawrey, J.D., Sikaroodi, M., Gillevet, P.M., Chaves, J.L., Sipman, H.J.M. & Bungartz, F. (2009) Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. *American journal of botany* 96: 1409–18. https://doi.org/10.3732/ajb.0800258

Malard, F., Uehlinger, U., Zah, R. & Tockner, K. (2006) Flood-Pulse and Riverscape Dynamics in a Braided Glacial River. *Ecology* 87: 704–716. https://doi.org/10.1890/04-0889

Marcante, S., Erschbamer, B., Buchner, O. & Neuner, G. (2014) Heat tolerance of early developmental stages of glacier foreland species in the growth chamber and in the field. *Plant Ecology* 215: 747–758. https://doi.org/10.1007/s11258-014-0361-8

Meunier, J.D., Kirman, S., Strasberg, D., Grauby, O. & Dussouillez, P. (2014) Incipient weathering by *Stereocaulon vulcani* at Réunion volcanic island. *Chemical Geology* 382: 123–131. https://doi.org/10.1016/j.chemgeo.2014.05.033

Milner, A.M. & Petts, G.E. (1994) Glacial rivers: physical habitat and ecology. *Freshwater Biology* 32: 295–307. https://doi.org/10.1111/j.1365-2427.1994.tb01127.x

Molins, A., Moya, P., García-Breijo, F.J., Reig-Armiñana, J. & Barreno, E. (2018) Molecular and morphological diversity of *Trebouxia* microalgae in sphaerothallioid *Circinaria* spp. lichens. T. Mock (ed.). *Journal of Phycology* 54: 494–504. https://doi.org/10.1111/jpy.12751

Montgomery, D.R. & Buffington, J.M. (1998) Chanel Processes, Classification, and Response. *In*: R. Naiman & R. Bilby (eds.) *River Ecology and Management*. Springer-Verlag New York, pp. 13–42.

Moran, N.A. (2007) Symbiosis as an adaptive process and source of phenotypic complexity. *Proceedings of the National Academy of Sciences* 104: 8627–8633. https://doi.org/10.1073/pnas.0611659104

Moya, P., Molins, A., Martinez-Alberola, F., Muggia, L. & Barreno, E. (2017) Unexpected associated microalgal diversity in the lichen *Ramalina farinacea* is uncovered by pyrosequencing analyses. *PLoS ONE* 12: 1–21. https://doi.org/10.1371/journal.pone.0175091

Moya, P., Škaloud, P., Chiva, S., García-Breijo, F.J., Reig-Armiñana, J., Vančurová, L. & Barreno, E. (2015) Molecular phylogeny and ultrastructure of the lichen microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands ecosystems. *International journal of systematic and evolutionary microbiology* 65: 1838–54. https://doi.org/10.1099/ijs.0.000185

Muggia, L., Vančurová, L., Škaloud, P., Peksa, O., Wedin, M. & Grube, M. (2013) The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS microbiology ecology* 85: 313–23. https://doi.org/10.1111/1574-6941.12120

Müller, N. (1996) River dynamics and floodplain vegetation and their alterations due to human impact. *Large Rivers* 9: 477–512. https://doi.org/10.1127/lr/9/1996/477

Muotka, T. & Virtanen, R. (1995) The stream as a habitat templet for bryophytes: species' distributions along gradients in disturbance and substratum heterogeneity. *Freshwater Biology* 33: 141–160. https://doi.org/10.1111/j.1365-2427.1995.tb01156.x

Nascimbene, J., Mayrhofer, H., Dainese, M. & Bilovitz, P.O. (2017) Assembly patterns of soil-dwelling lichens after glacier retreat in the European Alps. *Journal of Biogeography* 44: 1393–1404. https://doi.org/10.1111/jbi.12970

Nelsen, M.P. & Gargas, A. (2006) Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta: Trebouxiophyceae). *The Lichenologist* 38: 435–440. https://doi.org/10.1017/S0024282906005779

Nimis, P.L., Hafellner, J., Roux, C., Clerc, P., Mayrhofer, H., Martellos, S. & Bilovitz, P.O. (2018) The lichens of the Alps - An annotated checklist. *MycoKeys* 31: 1–634. https://doi.org/10.3897/mycokeys.31.23658

Ohmura, Y., Takeshita, S. & Kawachi, M. (2019) Photobiont diversity within populations of a vegetatively reproducing lichen, *Parmotrema tinctorum*, can be generated by photobiont switching. *Symbiosis* 77: 59–72. https://doi.org/10.1007/s13199-018-0572-1

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara,

R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2019) vegan: Community Ecology Package.

Onuţ-Brännström, I., Benjamin, M., Scofield, D.G., Heiðmarsson, S., Andersson, M.G.I., Lindström, E.S. & Johannesson, H. (2018) Sharing of photobionts in sympatric populations of *Thamnolia* and *Cetraria* lichens: evidence from high-throughput sequencing. *Scientific Reports 2018* 8: 4406. https://doi.org/10.1038/s41598-018-22470-y

Paul, F., Otte, J., Schmitt, I. & Dal Grande, F. (2018) Comparing Sanger sequencing and high-throughput metabarcoding for inferring photobiont diversity in lichens. *Scientific Reports* 8: 8624. https://doi.org/10.1038/s41598-018-26947-8

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology* 20: 3936–48. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Pettit, N.E. & Froend, R.H. (2001) Variability in flood disturbance and the impact on riparian tree recruitment in two contrasting river systems. *Wetlands Ecology and Management* 9: 13–25. https://doi.org/10.1023/A:1008471100136

Piercey-Normore, M.D. & DePriest, P.T. (2001) Algal Switching among Lichen Symbioses. *American Journal of Botany* 88: 1490–1498. https://doi.org/10.2307/3558457

Plummer, M. (2003) JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Proc Int Workshop Distrib stat Comput 124:125.

Prach, K., Petřík, P., Brož, Z. & Song, J.-S. (2014) Vegetation Succession on River Sediments along the Nakdong River, South Korea. *Folia Geobotanica* 49: 507–519. https://doi.org/10.1007/s12224-014-9195-3

Prach, K., Tichý, L., Lencová, K., Adámek, M., Koutecký, T., Sádlo, J., Bartošová, A., Novák, J., Kovář, P., Jírová, A., Šmilauer, P. & Řehounková, K. (2016) Does succession run towards potential natural vegetation? An analysis across seres. *Journal of Vegetation Science* 27: 515–523. https://doi.org/10.1111/jvs.12383

Quigley, K.M., Bay, L.K. & Willis, B.L. (2017) Temperature and Water Quality-Related Patterns in Sediment-Associated *Symbiodinium* Communities Impact Symbiont Uptake and Fitness of Juveniles in the Genus *Acropora*. *Frontiers in Marine Science* 4. https://doi.org/10.3389/fmars.2017.00401

R Core Team (2017) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org

Rambold, G., Friedl, T. & Beck, A. (1998) Photobionts in Lichens: Possible Indicators of Phylogenetic Relationships? *Bryologist, The* 101: 392–397.

Řídká, T., Peksa, O., Rai, H. & Upreti, D.K. (2014) Photobiont Diversity in Indian *Cladonia* Lichens, with Special Emphasis on the Geographical Patterns. *In*: H. Rai & D. K. Upreti (eds.) *Terricolous Lichens in India*. Springer New York, pp. 53–71.

Rippin, M., Borchhardt, N., Williams, L., Colesie, C., Jung, P., Büdel, B., Karsten, U. & Becker, B. (2018) Genus richness of microalgae and Cyanobacteria in biological soil crusts from Svalbard and Livingston Island: morphological versus molecular approaches. *Polar Biology* 41: 909–923. https://doi.org/10.1007/s00300-018-2252-2

Rolshausen, G., Dal Grande, F., Sadowska-Deś, A.D., Otte, J. & Schmitt, I. (2017) Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions. *Ecography*: 1–12. https://doi.org/10.1111/ecog.03457

Romeike, J., Friedl, T., Helms, G. & Ott, S. (2002) Genetic Diversity of Algal and Fungal Partners in Four Species of *Umbilicaria* (Lichenized Ascomycetes) Along a Transect of the Antarctic Peninsula. *Molecular Biology* 19: 1209–1217.

Ruprecht, U., Brunauer, G. & Türk, R. (2014) High photobiont diversity in the common European soil crust lichen *Psora decipiens*. *Biodiversity and conservation* 23: 1771–1785. https://doi.org/10.1007/s10531-014-0662-1

Sadowsky, A., Hussner, A. & Ott, S. (2012) Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane,

Norway. *Nova Hedwigia* 94: 323–334. https://doi.org/10.1127/0029-5035/2012/0014

Seckbach, J. & Grube, M. eds. (2010) *Symbioses and Stress*. Springer Netherlands, Dordrecht

Sheng, M., Chen, X., Zhang, X., Hamel, C., Cui, X., Chen, J., Chen, H. & Tang, M. (2017) Changes in arbuscular mycorrhizal fungal attributes along a chronosequence of black locust (*Robinia pseudoacacia*) plantations can be attributed to the plantation-induced variation in soil properties. *Science of The Total Environment* 599–600: 273–283. https://doi.org/10.1016/j.scitotenv.2017.04.199

Škaloud, P. & Peksa, O. (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris*. *Molecular Phylogenetics and Evolution* 54: 36–46. https://doi.org/10.1016/j.ympev.2009.09.035

Škaloud, P., Steinová, J., Řídká, T., Vančurová, L. & Peksa, O. (2015) Assembling the challenging puzzle of algal biodiversity: species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 51: 507–527. https://doi.org/10.1111/jpy.12295

Smith, H.B., Dal Grande, F., Muggia, L., Keuler, R., Divakar, P.K., Grewe, F., Schmitt, I., Lumbsch, H.T. & Leavitt, S.D. (2020) Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis. *Symbiosis* 82: 133–147. https://doi.org/10.1007/s13199-020-00699-4

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C., Schneider, K., Stabentheiner, E., Toome-Heller, M., Thor, G., Mayrhofer, H., Johannesson, H. & McCutcheon, J.P. (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492. https://doi.org/10.1126/science.aaf8287

Steinová, J., Škaloud, P., Yahr, R., Bestová, H. & Muggia, L. (2019) Reproductive and dispersal strategies shape the diversity of mycobiont-photobiont association in *Cladonia* lichens. *Molecular Phylogenetics and Evolution* 134: 226–237. https://doi.org/10.1016/j.ympev.2019.02.014

Stöcklin, J. (1999) Differences in life history traits of related Epilobium species: Clonality, seed size and seed number. *Folia Geobotanica* 34: 7–18. https://doi.org/10.1007/BF02803073

Stöcklin, J. & Bäumler, E. (1996) Seed rain, seedling establishment and clonal growth strategies on a glacier foreland. *Journal of Vegetation Science* 7: 45–56. https://doi.org/10.2307/3236415

Stretch, R.. & Viles, H.. (2002) The nature and rate of weathering by lichens on lava flows on Lanzarote. *Geomorphology* 47: 87–94. https://doi.org/10.1016/S0169-555X(02)00143-5

Su, Y.-S. & Yajima, M. (2015) R2jags: Using R to Run "JAGS". R package version 0.5-6, http://CRAN. r-project. org/package= R2jags

Swofford, D.L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular biology and evolution* 30: 2725–2729. https://doi.org/10.1093/molbev/msm092

Tichý, L. (2002) JUICE, software for vegetation classification. *Journal of Vegetation Science* 13: 451–453. https://doi.org/10.1111/j.1654-1103.2002.tb02069.x

Tockner, K. & Malard, F. (2003) Channel Typology. *In*: J. V. Ward & U. Uehlinger (eds.) *Ecology of a Glacial Flood Plain*. Springer Netherlands, Dordrecht, pp. 57–73.

Tockner, K., Malard, F. & Ward, J. V (2000) An extension of the flood pulse concept. *Hydrological Processes* 14: 2861–2883.

Tockner, K., Paetzold, A., Karaus, U., Claret, C. & Zettel, J. (2006) Ecology of Braided Rivers. *In*: G. H. S. Smith, J. L. Best, C. S. Bristow, & G. E. Petts (eds.) *Braided Rivers*. Blackwell Publishing Ltd., Oxford, UK, pp. 339–359.

Vančurová, L., Muggia, L., Peksa, O., Řídká, T. & Škaloud, P. (2018) The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota). *Molecular Ecology* 27: 3016–3033. https://doi.org/10.1111/mec.14764

Vančurová, L., Peksa, O., Němcová, Y. & Škaloud, P. (2015) *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain. *Phytotaxa* 219: 118–132. https://doi.org/10.11646/phytotaxa.219.2.2

Vitt, D.H., Glime, J.M. & Lafarge England, C. (1986) Bryophyte vegetation and habitat gradients of montane streams in western canada. *Hikobia* 9: 367–386.

Walker, L.R. & del Moral, R. (2003) *Primary Succession and Ecosystem Rehabilitation*. Cambridge University Press, Cambridge

Ward, J. V, Tockner, K., Arscott, D.B. & Claret, C. (2002) Riverine landscape diversity. *Freshwater Biology* 47: 517–539. https://doi.org/https://doi.org/10.1046/j.1365-2427.2002.00893.x

Weber, K. & Kabsch, W. (1994) Intron Positions in Actin Genes Seem Unrelated To the Secondary Structure of the Protein. *Embo Journal* 13: 1280–1286.

Wellstein, C., Uehlinger, U. & Zah, R. (2003) Terrestrial Floodplain Vegetation. *In*: J. V. Ward & U. Uehlinger (eds.) *Ecology of a Glacial Flood Plain*. Springer Netherlands, Dordrecht, pp. 109–121.

Westhoff, V. & van der Maarel, E. (1978) The Braun-Blanquet Approach. *In*: R. H. Whittaker (ed.) *Classification of Plant Communities*. Springer Netherlands, Dordrecht, pp. 287–399.

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

Yahr, R., Vilgalys, R. & Depriest, P.T. (2004) Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. *Molecular Ecology* 13: 3367–3378. https://doi.org/10.1111/j.1365-294X.2004.02350.x

Yahr, R., Vilgalys, R. & DePriest, P.T. (2006) Geographic variation in algal partners of *Cladonia* subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *The New phytologist* 171: 847–60. https://doi.org/10.1111/j.1469-8137.2006.01792.x

Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin

Supplementary material

Table S1 Species composition of gravel bar vegetation plots. Species are ranked by decreasing frequency of occurrence. The species score is given on the nine-degree Braun-Blanquet cover-abundance scale. All vegetation plots are stored in Gravel bar vegetation database (Kalníková and Kudrnovsky 2017)

Table S2 GenBank accession numbers, phycobiont species-level lineage, mycobiont OTU, substrate and affiliation to study plot of *Stereocaulon* samples

Table S3 Substitution models selected for each partition of *Asterochloris* and *Stereocaulon* (mycobiont) datasets and for algal ITS2 rDNA dataset using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Guindon & Gascuel 2003, Darriba et al. 2012)

Table S4 Environmental and vegetation characteristics of study plots

Table S5 Accession numbers of *Asterochloris* reference sequences retrieved from GenBank

Table S6 Relative abundance of each ASV in each *Stereocaulon* sample

Fig. S1 Location of the four localities distributed across the Swiss Alps: Lonza locality in the Bernese Alps (on the left), Morteratsch valley, Roseg I and Roseg II localities in the Bernina range (on the right). White colored areas indicate glaciers, green colored areas indicate forests. Maps were created using https://opendata.swiss/en

Fig. S2 Photographs of study plots of different succession stages: **a**, **b** successional stage 1 (plots 5 and 9), **c** successional stage 2 (plot 2), **d** successional stage 3 (plot 3)

Fig. S3 Rarefaction curves for 13 study plots. Vertical lines are drawn at sample size of n = 5 and sample size of n = 10

Fig. S4 Unrooted phylogenetic hypothesis resulting from Bayesian analysis of the algal ITS2 rDNA sequences obtained by Illumina metabarcoding of soil samples, selected lichen samples and reference sequences from GenBank. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. (2014). Clades I–XVIII were identified as new in present study. Values at the nodes indicate the statistical supports of Bayesian posterior probability

Fig. S5 Bipartite association network between study plots and phycobiont species-level lineages. The width of the links is proportional to the number of specimens forming the association

Fig. S6 Eight *Stereocaulon* thalli selected for Illumina metabarcoding and abundances of recovered algal clades. A523M, A554M, A563M, A570M belong to mycobiont OTU35. A597M, A598M, A633M, A634M belong to mycobiont OTU2. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. (2014). Clades VII and XVIII were identified as new in present study. Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis presented in Fig. S4. Table S6 describes relative abundance of each ASV in each sample

Fig. S7 Relative abundances of ASVs linked to various *Asterochloris* species. Exclusively samples with *Asterochloris* as a predominant phycobiont (>50% algal reads belonged to *Asterochloris*) were displayed

Fig. S8 Abundances of ASVs linked to algal clades recovered from soil samples. Clade affiliations: URa25, URa28 *sensu* Ruprecht et al. (2014). Clades I–XVIII were identified as new in present study. Amplicon sequence variants (ASVs) were sorted into these clades based on phylogenetic hypothesis presented in Fig. S4

The Supplementary Material for this article can be found online at:
https://ars.els-cdn.com/content/image/1-s2.0-S2211926420306810-mmc1.pdf

Choosing the right life partner: ecological drivers of lichen symbiosis

Lucie Vančurová[1]*, Jiří Malíček[2], Jana Steinová[1], Pavel Škaloud[1]

[1] Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic

[2] Institute of Botany, The Czech Academy of Sciences, Průhonice, Czech Republic

** Corresponding author (E-mail: lucie.vancurova@natur.cuni.cz)*

Abstract

Lichens are an iconic example of symbiotic systems whose ecology is shaped by the requirements of the symbionts. Previous studies suggest that fungal (mycobionts), as well as photosynthesizing (phycobionts or cyanobionts) partners have a specific range of acceptable symbionts that can be chosen according to specific environmental conditions. This study aimed to investigate the effects of climatic conditions and mycobiont identity on phycobiont distribution within the lichen genera *Stereocaulon*, *Cladonia*, and *Lepraria*. The study area comprised the Canary Islands, Madeira, Sicily, and the Aeolian Islands, spanning a wide range of climatic conditions. These islands are known for their unique and diverse fauna and flora; however, lichen phycobionts have remained unstudied in most of these areas. In total, we genetically analyzed 339 lichen samples. The phycobiont pool differed significantly from that outside the studied area. *Asterochloris mediterranea* was identified as the most abundant phycobiont. However, its distribution was limited by climatic constraints. Other species of *Asterochloris* and representatives of the genera *Chloroidium*, *Vulcanochloris*, and *Myrmecia* were also recovered as phycobionts. The selection of symbiotic partners from the local phycobiont pool was driven by mycobiont specificity (i.e., the taxonomic range of acceptable partners) and the environmental conditions, mainly temperature. Interestingly, the dominant fungal species responded differently in their selection of algal symbionts along the environmental gradients. *Cladonia rangiformis* associated with its phycobiont *A. mediterranea* in a broader range of temperatures than *Stereocaulon azoreum*, which favors other *Asterochloris* species along most of the temperature gradient. *S. vesuvianum* associated with *Chloroidium* spp., which also differed in their temperature optima. Finally, we described *Stereocaulon canariense* as a new endemic species ecologically distinct from the other *Stereocaulon* species on the Canary Islands.

Keywords: *Asterochloris mediterranea*, lichen, Macaronesia, phycobiont sharing, specificity, *Stereocaulon canariense*, symbiosis, temperature gradient

Introduction

The Canary Islands, Madeira, Sicily, and the Aeolian Islands are known for their unique and diverse fauna and flora (del Arco Aguilar & Rodríguez Delgado 2018; Giovino *et al.* 2016; Di Gristina *et al.* 2016; Troia 2012). Macaronesia is also known for its many endemic lichens (Sérusiaux *et al.*, 2011; Sparrius *et al.*, 2017; Vondrák *et al.*, 2020) and numerous lichen taxa from this area have been described (van den Boom & Clerc 2015; van den Boom & Ertz 2013; van den Boom & Magain 2020; Crespo *et al.* 2006; Hafellner 2008; Hernández Padrón & Pérez-Vargas 2010; Vondrák *et al.* 2020). A substantial elevation gradient is typical in most of these areas; therefore, the climatic conditions are diverse despite the relatively small area of the islands. The vegetation is clearly divided into altitudinal belts. These belts are asymmetrically developed on the windward (cooler, more humid and rainier, and more climatically diverse) and leeward (warmer and drier) slopes (del Arco Aguilar & Rodríguez Delgado 2018). All these islands, except for Sicily, are of volcanic origin.

Lichens are a classic example of symbiotic systems growing worldwide from polar to tropical regions. Due to their symbiotic nature, they can cope well with harsh conditions and newly formed bare substrates. The genera *Cladonia*, *Lepraria*, and *Stereocaulon* belong to the two closely related families Cladoniaceae (*Cladonia*) and Stereocaulaceae (*Stereocaulon* and *Lepraria*; Lücking, 2019). These mycobionts are known to preferably associate with *Asterochloris* phycobionts (Bačkor *et al.* 2010; Beiggi & Piercey-Normore 2007; Nelsen & Gargas 2006, 2008; Peksa & Škaloud 2011; Piercey-Normore & DePriest 2001; Škaloud & Peksa 2010; Yahr *et al.* 2004). When describing the relationships between phycobionts and mycobionts, the terms specificity and selectivity are frequently used. The specificity delimits the taxonomic range of acceptable partners, whereas selectivity refers to the preference for a particular group of partners (Rambold *et al.* 1998; Yahr *et al.* 2004, 2006). Particular species of *Cladonia*, *Lepraria*, and *Stereocaulon* differ in their preferred *Asterochloris* species-level lineages (i.e., they are selective towards their phycobionts; Peksa and Škaloud, 2011; Škaloud *et al.*, 2015; Steinová *et al.*, 2019; Vančurová *et al.*, 2020). A relatively high number of studies have focused on phycobionts of *Cladonia*, *Lepraria*, and *Stereocaulon*; however, new phycobiont species-level lineages continue being discovered (Kosecka *et al.* 2021; Moya *et al.* 2015; Pino-Bodas & Stenroos 2020; Vančurová *et al.* 2018). In addition, other trebouxiophycean algal genera have been detected as phycobionts of *Stereocaulon* (Beck 2002; Kosecka *et al.* 2021; Vančurová *et al.* 2015, 2018, 2020), *Cladonia* (Ahmadjian & Jacobs 1981; Korchikov *et al.* 2018; Osyczka *et al.* 2020), and *Lepraria* (Engelen *et al.* 2010). The ability to associate with an alternative photobiont (i.e., lower selectivity) can be caused by a stressful environment, as previously shown (Engelen *et al.* 2010; Osyczka *et al.* 2020; Romeike *et al.* 2002).

Since lichen phycobionts in this region have remained almost unresearched (past studies involved only a limited number of samples; Muggia *et al.*, 2014; Leavitt *et al.*, 2015; Moya *et al.*, 2015, 2018; Boluda *et al.*, 2019; Molins *et al.*, 2020), our aim was to supplement the missing information on photobiont diversity in this study area and explore ecological factors shaping symbiotic relationships. Moreover, several studies have documented the phycobionts that are shared by diverse lichen species/genera (Bačkor *et al.*, 2010; Vargas Castillo and Beck, 2012; Peksa *et al.*,

2021), whereas other studies failed to search for the source of lichen phycobionts (Vančurová *et al.*, 2020). Therefore, we also examined the sharing of phycobionts within the lichen community. This is closely related to the level of specificity and selectivity of the symbiotic partners. Hence, the main questions we addressed were what are 1) the patterns of phycobiont diversity within the study area, 2) the shared pool of phycobionts, and 3) the effect of the climatic conditions on the symbiotic relationships.

Material and Methods

Taxon sampling

We analyzed a total of 339 specimens (Supplementary Table 1) belonging to three closely related fungal genera *Cladonia* (n=179), *Lepraria* (n=23), and *Stereocaulon* (n=137) collected between years 2011–2020 on the Canary Islands (El Hierro, La Palma, La Gomera, Tenerife, Gran Canaria, Lanzarote), Madeira, Sicily, and the Aeolian Islands (Vulcano, Salina, Stromboli; Supplementary Figure 1). The sampling sites represented diverse habitats from malpaíses (i.e., barren landscapes) to a laurel forest, various types of substrates, and were located at a range of altitudes 75–2360 m. We identified the mycobiont morphospecies using standard morphological methods, taxonomical keys (e.g., Lamb, 1978; Baruffo *et al.*, 2006; Burgaz and Ahti, 2009) and thin-layer chromatography (TLC) on Merck silica gel 60 F254 pre-coated glass plates in solvent systems A, B, and C, according to Orange *et al.* (2001). For the purpose of describing *Stereocaulon* species, measurements and observations were done in water, except for ascospores and paraphyses observed in KOH. Lichen specimens were deposited in the Herbarium of the Institute of Botany, the Czech Academy of Sciences (PRA), and the private herbarium of J. Malíček.

DNA extraction, amplification, and sequencing

We extracted DNA directly from lichen thalli (total lichen DNA) following the standard CTAB protocol for lichens (Cubero *et al.* 1999). Firstly, we examined thalli under a dissecting microscope and washed them with running water (except for *Lepraria* thalli) before DNA extraction to prevent contamination by soredia from other lichens. We amplified the algal and fungal nuclear internal transcribed spacer (ITS, ITS1-5.8S-ITS2 rDNA) and the algal actin type I gene (including one complete exon and two introns located at codon positions 206 and 248; Weber & Kabsch 1994) using primers listed in Table 1. The PCR conditions were as described in Vančurová *et al.* (2018). Every PCR run included negative controls, without a DNA template. The PCR products were purified using by NucleoMag® NGS Clean-up and Size Select kit (Macherey-Nagel, Duren, Germany) and sequenced using the same primers at Macrogen in Amsterdam, Netherlands. The 507 newly obtained sequences of the ITS rDNA and actin type I regions are available in GenBank under accession numbers OL622077–OL622095 and OL625120–OL625607 (Supplementary Table 1).

Table 1. Primers used in this study.

Name	Sequence	Function	Reference
nr-SSU-1780-5'	5'-CTG CGG AAG GAT CAT TGA TTC-3'	algal ITS region, algal-specific	Piercey-Normore and DePriest, 2001
ITS1-F-5'	5'- CTT GGT CAT TTA GAG GAA GTA A -3'	fungal ITS region, fungal-specific	Gardes and Bruns, 1993
ITS4-3'	5'-TCC TCC GCT TAT TGA TAT GC-3'	algal and fungal ITS region, universal	White *et al.*, 1990
a-nu-act1-0645-5'	5'-GAC AGA GCG TGG KTA CAG-3'	actin type I locus, algal specific	Nelsen and Gargas, 2006
a-nu-act1-0818-3'	5'-TGA ACA GCA CCT CAG GGC A-3'	actin type I locus, algal specific	Nelsen and Gargas, 2006
ActinF2 Astero-5'	5'-AGC GCG GGT ACA GCT TCA C-3'	actin type I locus, algal specific	Škaloud and Peksa, 2010
ActinR2 Astero-3'	5'-CAG CAC TTC AGG GCA GCG GAA-3'	actin type I locus, algal specific	Škaloud and Peksa, 2010
ActinF Astero-5'	5'-GGG TAC AGC TTC AC-3'	actin type I locus, algal specific	Vančurová *et al.*, 2018
ActinR Astero-3'	5'-TGA ACA GCA CTT CAG GGC A-3'	actin type I locus, algal specific	Vančurová *et al.*, 2018
ActinF3 Astero-5'	5'-AGC TTC ACC ACC ACT GCA G-3'	actin type I locus, algal specific	Vančurová *et al.*, 2018
ActinR3 Astero-3'	5'-AGC GGA AKC GCT CGC TGC C-3'	actin type I locus, algal specific	Vančurová *et al.*, 2018

Sequence alignment and DNA analyses

We prepared individual sequence alignments for *Stereocaulon*, *Cladonia*, *Lepraria, Chloroidium*, *Vulcanochloris* (ITS rDNA), and *Asterochloris* (ITS rDNA and actin type I) datasets using MAFFT v.7 software (Katoh & Standley 2013). The sequences obtained for *Asterochloris* were analyzed as a single locus dataset for the ITS rDNA (data not shown) and as a concatenated dataset of ITS rDNA and actin type I loci. In the case of *Cladonia*, we used Gblocks to remove introns from alignment and to eliminate poorly aligned positions (Castresana 2000).

Supplementary Table 2 summarizes a total number of sequences included in particular alignments, newly obtained sequences, previously published sequences originated from the study area, reference sequences retrieved from GenBank, and unique sequences after deleting identical ones. All DNA alignments are freely available on Mendeley Data: http://dx.doi.org/10.17632/428v52svtp.1.

Phylogenetic trees were inferred with Bayesian Inference (BI) using MrBayes v.3.2.7a (Ronquist *et al.* 2012; Ronquist & Huelsenbeck 2003), maximum likelihood (ML) analysis using GARLI v.2.0

(Zwickl 2006), and maximum parsimony (MP) analysis using PAUP v.4.0b10 (Swofford 2003). BI and ML analyses were carried out on a partitioned dataset to differentiate among ITS1, 5.8 S and ITS2 rDNA, actin intron 206, actin intron 248, and actin exon regions. Substitution models (Supplementary Table 3) were selected using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Darriba *et al.* 2012; Guindon & Gascuel 2003). Two parallel MCMC runs, with four chains, were carried out for 10 and 5 million generations for *Asterochloris* and other datasets, respectively. Trees and parameters were sampled every 100 generations. Convergence of the two cold chains was assessed during the run by calculating the average standard deviation of split frequencies (SDSF). The SDSF value between simultaneous runs was < 0.01 in all cases. Finally, the burn-in values were determined using the 'sump' command. ML analysis was carried out using default settings, five search replicates, and the automatic termination set at 5 million generations. The MP analysis was performed using heuristic searches with 1000 random sequence addition replicates and random addition of sequences (the number was limited to 10^4 per replicate). ML and MP bootstrap support values were obtained from 100, and 1000 bootstrap replicates, respectively. Only one search replicate was applied for ML bootstrapping.

We maintained the nomenclature and species boundaries delimited in recent studies (Kosecka *et al.* 2021; Škaloud *et al.* 2015; Vančurová *et al.* 2018, 2020) for *Asterochloris* species and the species boundaries delimited by Vančurová *et al.* (2018) and the nomenclature used therein (OTU1–OTU57) for *Stereocaulon* species-level lineages.

Interaction networks

We depicted the interactions between phycobiont species-level lineages in the genus *Asterochloris* and mycobiont genera and between phycobiont species-level lineages and mycobiont species-level lineages in the genus *Stereocaulon* as interaction networks produced using bipartite package (Dormann *et al.* 2008) in R.

Niche hypervolumes

The climatic niche of the most abundant species-level lineages of phycobionts and *Stereocaulon* mycobionts were represented using the Hutchinsonian niche concept that describes a species niche as an n-dimensional hypervolume, where the dimensions are environmental variables (Hutchinson 1957). In the present study, these environmental dimensions were defined based on 19 Bioclim variables (Karger *et al.* 2017). We constructed the climatic hypervolumes by multivariate kernel density estimation (Blonder *et al.* 2014). First, we performed the PCA analysis of 19 Bioclim variables to reduce the total number of predictors. The first two PCA axes (explaining 82% of the total variance) were then selected to calculate hypervolumes for each species-level lineage and genus. The boundaries of the kernel density estimates were delineated by the probability threshold, using the 0.85 quantile value. To project the niche spaces of particular lineages, hypervolume contours were plotted based on 5,000 random background points, using the alphahull contour type

and alpha smoothing value 0.55. The analyses were performed in R, using the hypervolume (Blonder *et al.* 2014) and alphahull (Pateiro-Lopez & Rodriguez-Casal 2016) packages.

Statistical comparisons of contrasting phycobiont groups

To assess the influence of climatic variables on phycobiont distribution, the contrasting phycobiont groups were compared for each Bioclim variable and altitude using Welch's two-sample t-test. We compared: (1) *Asterochloris mediterranea* (as the most abundant phycobiont in the study area) with other *Asterochloris* spp. in association with all mycobionts, (2) *Asterochloris mediterranea* with other *Asterochloris* spp. in association with *Cladonia rangiformis* (as the most abundant *Cladonia* species in the study area) mycobiont, (3) *Asterochloris mediterranea* with other *Asterochloris* spp. in association with *Stereocaulon azoreum* mycobiont (as the most abundant *Stereocaulon* species in the study area associated with *Asterochloris* spp.), and (4) *Chloroidium ellipsoideum* with *C. lichenum* A (sensu Darienko *et al.*, 2018) in association with *Stereocaulon vesuvianum* (OTU11) mycobiont (as the most abundant *Stereocaulon* species in the study area). We tested phycobionts in pairs with their mycobionts to inspect ecological requirements of the symbiotic system (unlike to separate analyses of symbiotic partners in previous studies, e.g. Vančurová *et al.*, 2018). We could not compare those groups of phycobionts associated with *Lepraria* spp. mycobionts due to the low number of samples. We performed the analyses in R using function t.test.

Results

Diversity of mycobionts

We recovered *Stereocaulon* mycobionts from the study area in six lineages (Figure 1): OTU13 (identified morphologically as *S. azoreum* and clustered with *S. azoreum* sequences), OTU11 (identified morphologically as *S. vesuvianum*), OTU3 (identified morphologically as *S. pileatum* and clustered with *S. pileatum* and *S. octomerellum*), OTU52 (described here as a new species *Stereocaulon canariense*; sister to *S. virgatum* sequence DQ396964), OTU22 (clustered with *S. delisei* and *S. corticatulum*), and OTU23 (clustered with *S. atlanticum* and *S. ramulosum*). The most frequent species in the study area was *Stereocaulon vesuvianum* (47% of the *Stereocaulon* samples).

A Bayesian analysis of the ITS rDNA of *Cladonia* mycobionts resulted in 22 supported *Cladonia* lineages (Supplementary Figure 2). The most recovered *Cladonia* species was *C. rangiformis* (30% of the *Cladonia* samples), followed by *C. humilis* (20% of the *Cladonia* samples). In most cases, phylogenetically separated lineages corresponded to morphologically delimited species; however, the lineage "*Cladonia* sp. 1" contained several morphologically different entities.

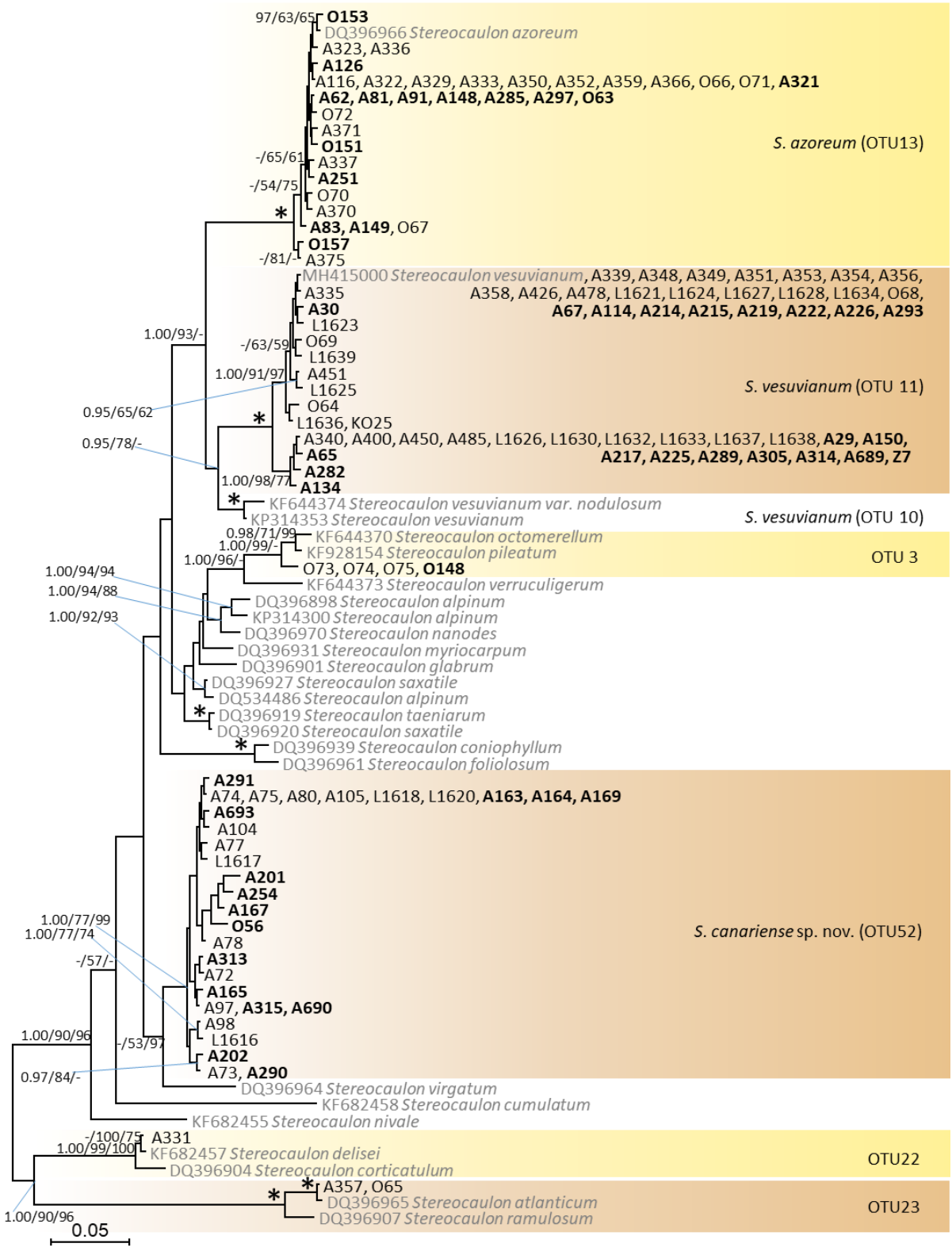


Figure 1. Phylogenetic hypothesis (unrooted tree) of *Stereocaulon* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold. We selected the reference sequences from GenBank (in grey) taking care to cover eight main *Stereocaulon* clades (Högnabba 2006).

It has been previously reported that ITS rDNA may fail to distinguish *Cladonia* species in some cases (Kanz *et al.* 2015; Kelly *et al.* 2011); however, it has been suggested as a *Cladonia* barcode marker at the same time (Pino-Bodas *et al.* 2013).

The phylogenetic hypothesis resulting from the Bayesian analysis of the ITS rDNA sequences of *Lepraria* (Supplementary Figure 3) was congruent with that of previous studies (Crespo *et al.* 2006; Tretiach *et al.* 2009). We recovered *Lepraria* mycobionts from the study area in 5 lineages. The most abundant was *L. santosii* (55% of *Lepraria* samples), originally described from Tenerife (Crespo *et al.* 2006).

Diversity of phycobionts

A phylogram resulting from the Bayesian analysis of ITS rDNA and actin type I sequences of *Asterochloris* is shown in Figure 2. We recorded phycobionts from the study area in eight species-level lineages. The overwhelming majority of samples belonged to *Asterochloris mediterranea* (72% of *Asterochloris* samples). *A. woessiae* and *A. italiana* were also relatively common (16% and 7%, respectively). Other lineages were rather rare. The lineage StA10 is a new, highly resolved lineage in *Asterochloris*.

The phylogenetic hypothesis resulting from the Bayesian analysis of the ITS rDNA sequences of *Chloroidium* (Figure 3) was congruent with that of previous studies (Darienko *et al.* 2018; Vančurová *et al.* 2018). Since *C. lichenum* became a paraphyletic species (divided into three clades: A, B, and C) after the last taxonomic revision (Darienko *et al.* 2018), we considered the two monophyletic clades *C. lichenum* A and *C. lichenum B* as species level-lineages (following species delimitation analyses; Vančurová *et al.* 2018). We recovered *Chloroidium* phycobionts from the study area in three species-level lineages: *C. ellipsoideum* (45% of *Chloroidium* samples), *C. lichenum* A (50% of *Chloroidium* samples), and *C. lichenum* B.

Furthermore, we recovered *Vulcanochloris* phycobionts from the study area in three lineages corresponding to three species (Figure 4): *V. symbiotica* (70% of *Vulcanochloris* samples), *V. guanchorum*, and *V. canariensis*.

Finally, we confirmed the identity of sample A194 by a BLAST search against the GenBank database. The most similar hit (95% sequence identity) was *Myrmecia israliensis* KY981668. Therefore, we labelled this sample as *Myrmecia* sp.

Distribution of phycobionts

In total, we found 15 species-level lineages within four genera of phycobionts, from one to nine on each island (Figure 5). Madeira was identified as the most phycobiont species-rich island in our study area. The number of species-level lineages was highest (4.1) also after rarefaction to seven samples, which was the smallest number of samples per island in the dataset.

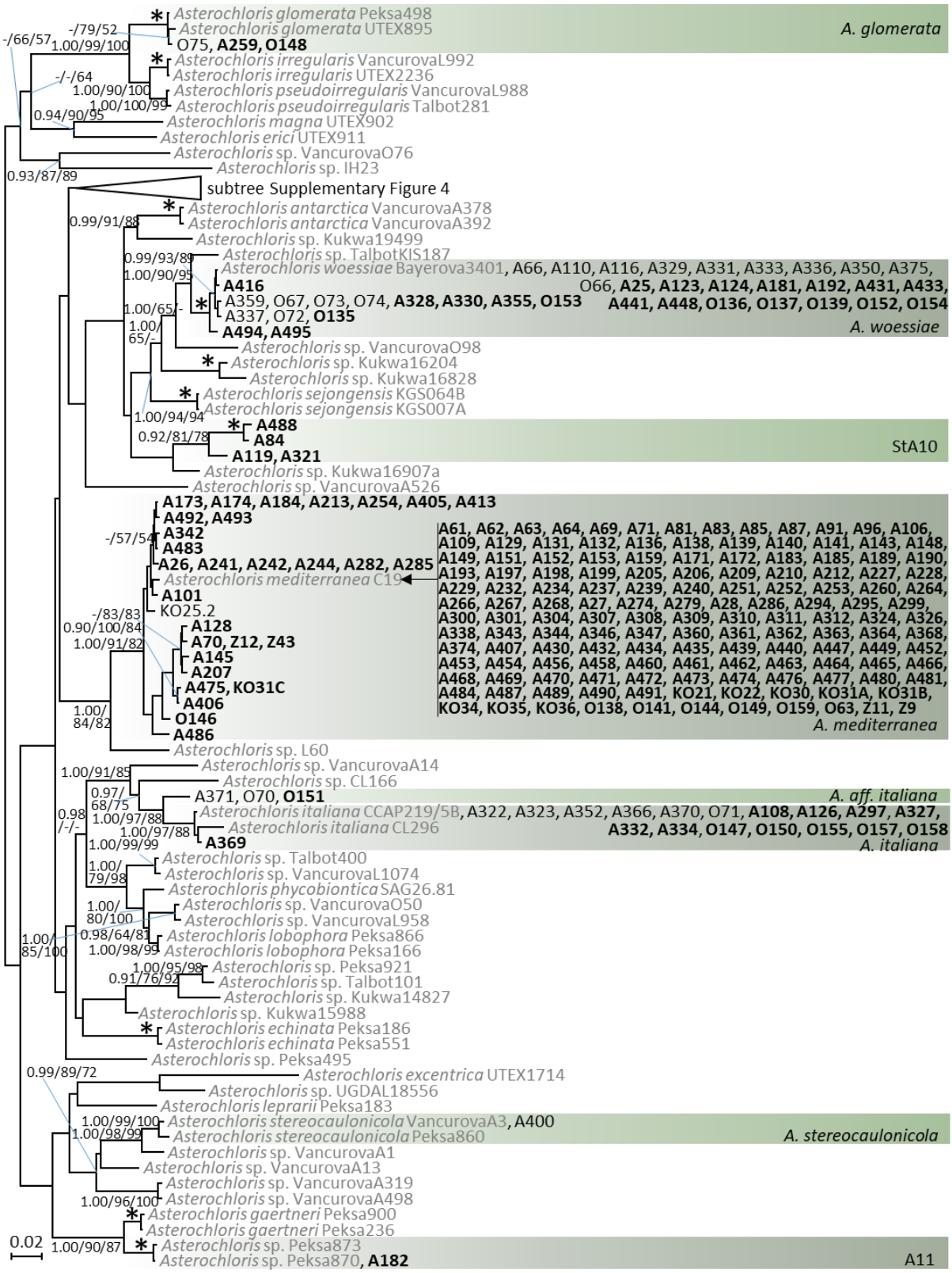


Figure 2. Phylogenetic hypothesis (unrooted tree) of *Asterochloris* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold. We selected the reference sequences from GenBank (in grey; accession numbers are listed in Supplementary Table 4) taking care

to include all known *Asterochloris* species as well as other previously published *Asterochloris* species-level lineages. The collapsed clade is displayed in Supplementary Figure 4.

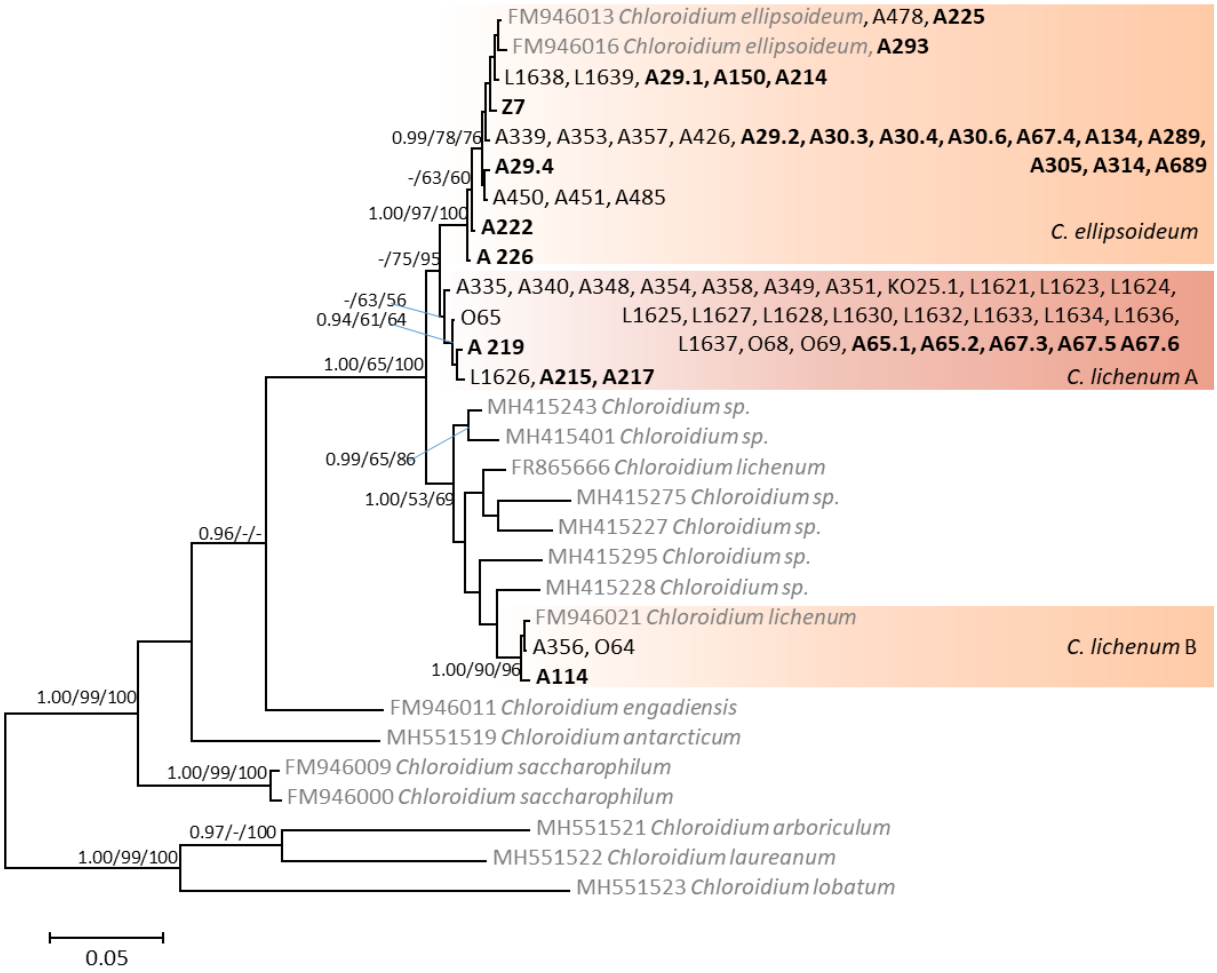


Figure 3. Phylogenetic hypothesis (unrooted tree) of *Chloroidium* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold, reference sequences from GenBank are in grey.

Asterochloris mediterranea represented the most abundant phycobiont in our dataset, was present on all studied islands and formed associations with all three mycobiont genera (Figures 5, 6A). However, its prevalence in the thalli of different genera of mycobionts varied. *Asterochloris mediterranea* was present in 83%, 73% and 28% of the thalli involving *Asterochloris* phycobionts in association with *Cladonia*, *Lepraria*, and *Stereocaulon*, respectively (Figure 6B). *Asterochloris woessiae* and *Asterochloris* StA10 were also shared by all three mycobiont genera and present on multiple islands. On the other hand, *A. italiana* and *A. glomerata* associated solely with representatives of *Stereocaulon* and *Cladonia*.

Chloroidium ellipsoideum associated with *S. vesuvianum* and *Stereocaulon* OTU23 (Figures 5, 6B), was the second most geographically widespread phycobiont, and was present on eight islands (absent on Gran Canaria, Lanzarote, and Isola Salina).

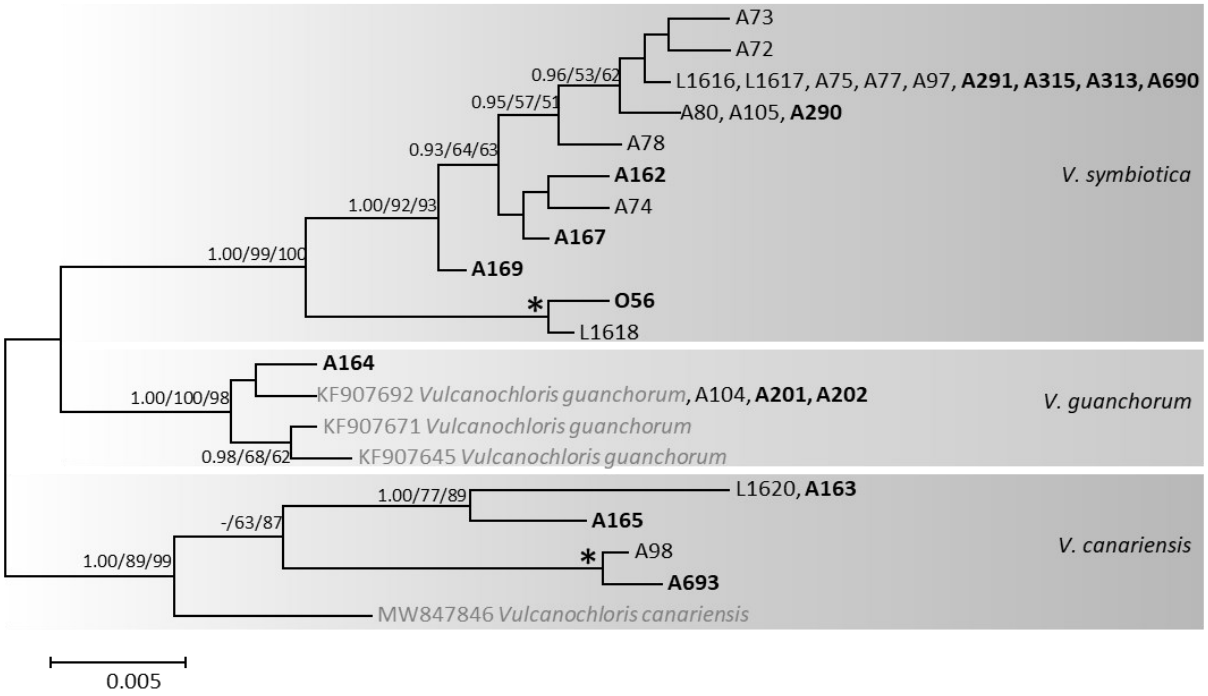


Figure 4. Phylogenetic hypothesis (unrooted tree) of *Vulcanochloris* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold, reference sequences from GenBank are in grey.

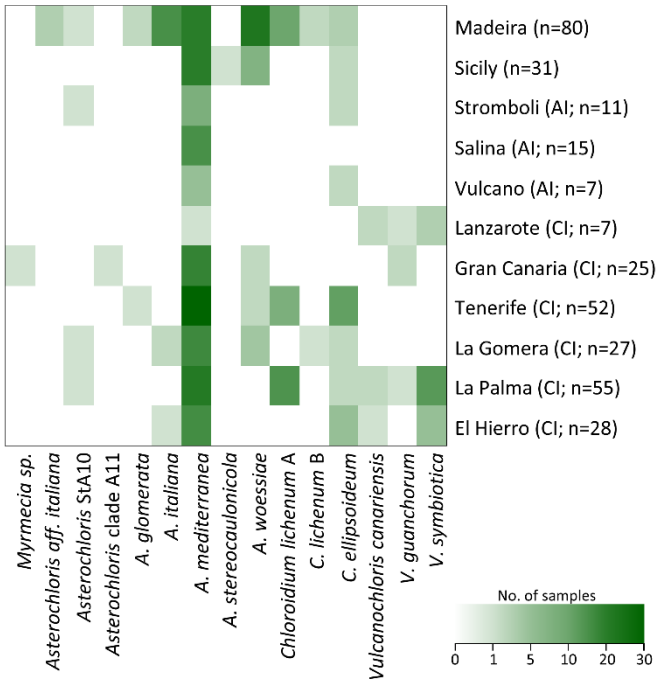


Figure 5. Distribution of phycobiont species-level lineages on particular islands within the study area. Number of samples is indicated in brackets. Abbreviations: AI: Aeolian Islands, CI: Canary Islands.

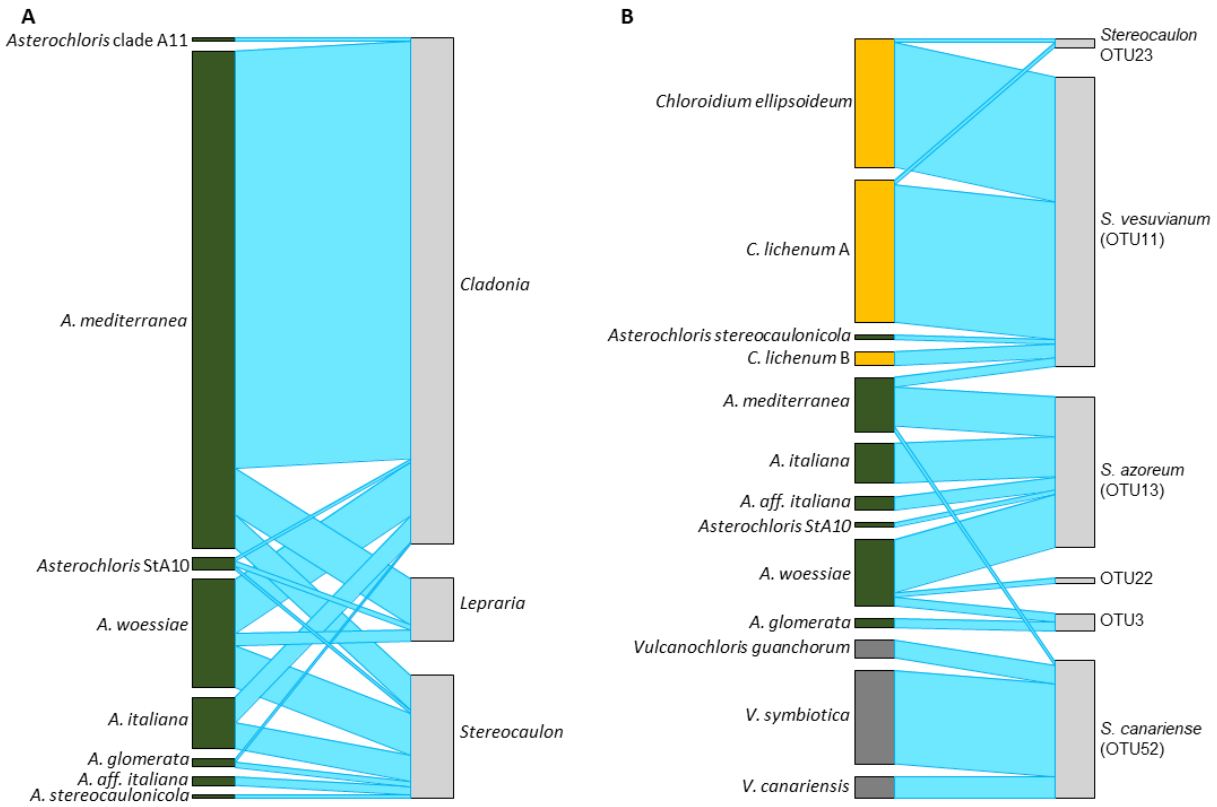


Figure 6. Interaction network structures: **(A)** between phycobiont species-level lineages in the genus *Asterochloris* and mycobiont genera, **(B)** between phycobiont species-level lineages and mycobiont species-level lineages in the genus *Stereocaulon*. The width of the links is proportional to the number of specimens forming the association.

The phycobionts *Myrmecia* sp. (associated with a *Cladonia* mycobiont), *Asterochloris* clade A11 (with *Cladonia*), and *A. stereocaulonicola* (with *Stereocaulon*) were recorded only once in our dataset. The phycobiont *A. aff. italiana* was found in three samples but only on a single island (Madeira), and was associated with a single mycobiont (*Stereocaulon azoreum*).

Vulcanochloris phycobionts were restricted to the Canary Islands, namely La Palma, Lanzarote, El Hierro, and Gran Canaria. On La Palma and Lanzarote, all three *Vulcanochloris* species were present. Interestingly, *Vulcanochloris* phycobionts were found exclusively in association with *Stereocaulon canariense* (Figure 6B).

Climatic niches

We constructed two-dimensional (PC1-PC2, explaining 82.4% of the variation in climatic variables) hypervolumes for the six most abundant algal species-level lineages and the three most abundant *Stereocaulon* mycobiont species-level lineages. Since the climatic characteristics differed considerably between Macaronesia and the Mediterranean region, the hypervolumes split into two isolated parts. Within each of them, there was a gradient of climatic niches along PC1, representing mostly the gradients of temperature and precipitation (Figure 7).

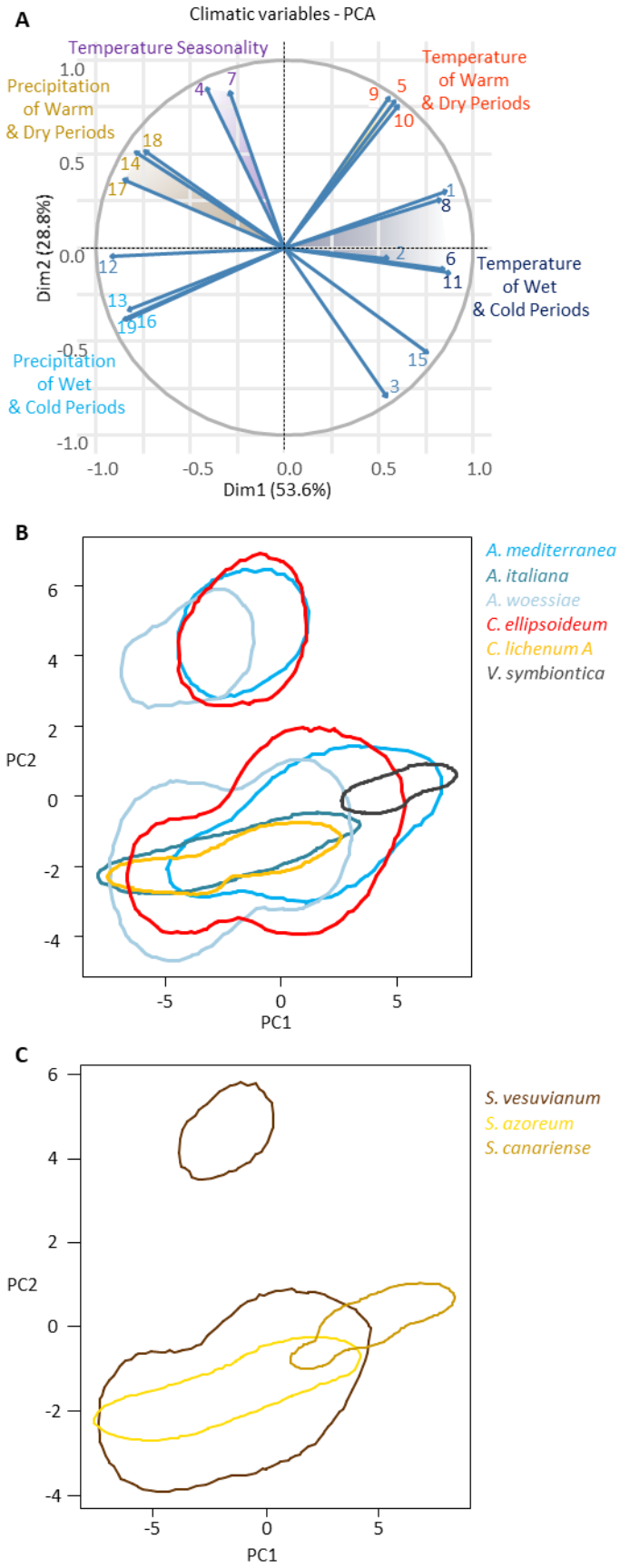


Figure 7. (A) Principal coordinate analysis of 19 Bioclim variables: 1 = annual mean temperature, 2 = mean diurnal range, 3 = isothermality, 4 = temperature seasonality, 5 = max temperature of warmest month, 6 = min temperature of coldest month, 7 = temperature annual range, 8 = mean temperature of wettest quarter, 9 = mean temperature of driest quarter, 10 = mean temperature of warmest quarter, 11 = mean temperature of coldest quarter, 12 = annual precipitation, 13 = precipitation of wettest month, 14 = precipitation of driest month, 15 = precipitation seasonality, 16 = precipitation of wettest quarter, 17 = precipitation of driest quarter, 18 = precipitation of warmest quarter, 19 = precipitation of coldest quarter. Climatic niche hypervolumes for **(B)** six most abundant phycobiont species-level lineages and **(C)** three most abundant *Stereocaulon* mycobiont species-level lineageas.

Among the algal species-level lineages, *Vulcanochloris symbiotica* tolerated the driest and warmest climate. On the other hand, *Asterochloris woessiae*, *A. italiana*, and *Chloroidium lichenum* A were distributed predominantly in the more humid and relatively colder areas. *Asterochloris mediterranea* and *C. ellipsoideum* were widely distributed in warm and dry areas and their climatic niches partly overlapped with that of both sides of the continuum. Climatic niches of *A. mediterranea*, *C. ellipsoideum,* and *A. woessiae* involved the whole range of temperature and precipitation seasonality (corresponding roughly with PC2). Remarkably, the niches of *A. mediterranea* and *C. ellipsoideum* overlapped to a large extent. Similarly, the niches of *A. italiana* and *C. lichenum* A were equivalent.

The climatic hypervolume of *Stereocaulon vesuvianum* was the widest among *Stereocaulon* species. It included the whole range of temperature seasonality in Macaronesia as well as that of the Mediterranean region. The warmest and driest areas with high precipitation seasonality were dominated by *S. canariense*. The climatic niche of *S. azoreum* overlapped with part of that of *S. vesuvianum*. These two species likely differed in microclimatic factors (not included in this study).

Finally, we focused on the niche of the most abundant phycobiont in the study area, *Asterochloris mediterranea*. We inspected the differences between the climatic requirements of this species and those of other *Asterochloris* species associated with either two dominant mycobiont species (*Cladonia rangiformis* and *Stereocaulon azoreum*) or all mycobionts using Welch's t-tests. Similarly, we tested the differences between *Chloroidium ellipsoideum* and *C. lichenum* A. Table 2 summarizes the results of the tests performed for various combinations of phycobionts and mycobionts. The means of three variables showed significant differences ($\alpha = 0.01$) across all combinations: BIO1 – annual mean temperature, BIO8 – mean temperature of the wettest quarter, and BIO11 – mean temperature of the coldest quarter (Figure 8). In contrast, we did not find any significant difference between the groups in BIO3 (Isothermality (BIO2/BIO7)) and BIO4 (Temperature Seasonality) climatic variables.

Interestingly, along the gradient of annual mean temperature, *A. mediterranea* was replaced by other *Asterochloris* species at different temperature levels depending on whether it was associated with *Stereocaulon azoreum* or *Cladonia rangiformis* (the later in about 1°C colder temperature). Furthermore, *Chloroidium ellipsoideum* was replaced by *C. lichenum* A at an annual mean temperature of approximately 13°C (Figure 8).

Table 2. Results of Welch's t-tests (p-values) performed for contrasting pairs of phycobionts in combination with their mycobionts. Bioclim variables: 1 = annual mean temperature, 2 = mean diurnal range, 3 = isothermality, 4 = temperature seasonality, 5 = max temperature of warmest month, 6 = min temperature of coldest month, 7 = temperature annual range, 8 = mean temperature of wettest quarter, 9 = mean temperature of driest quarter, 10 = mean temperature of warmest quarter, 11 = mean temperature of coldest quarter, 12 = annual precipitation, 13 = precipitation of wettest month, 14 = precipitation of driest month, 15 = precipitation seasonality, 16 = precipitation of wettest quarter, 17 = precipitation of driest quarter, 18 = precipitation of warmest quarter, 19 = precipitation of coldest quarter.

climatic variable	***Asterochloris mediterranea* with other *Asterochloris* spp. in association with all mycobionts**	***Asterochloris mediterranea* with other *Asterochloris* spp. in association with *Cladonia rangiformis***	***Asterochloris mediterranea* with other *Asterochloris* spp. in association with *Stereocaulon azoreum***	***Chloroidium ellipsoideum* with *C. lichenum* A in association with *Stereocaulon vesuvianum***
BIO1	$3.9e^{-16}$	0.000108	0.000252	$3.861e^{-06}$
BIO2	$1.221e^{-07}$	0.8077	$6.786e^{-07}$	0.4361
BIO3	0.4156	0.1005	0.7618	0.2455
BIO4	0.0481	0.1083	0.0193	0.108
BIO5	$< 2.2e^{-16}$	0.3672	$1.888e^{-09}$	$5.156e^{-06}$
BIO6	$6.366e^{-07}$	0.0005975	0.01907	0.0003686
BIO7	0.001919	0.1031	0.0001044	0.06981
BIO8	$1.728e^{-14}$	$1.019e^{-05}$	0.009172	$5.103e^{-06}$
BIO9	$< 2.2e^{-16}$	0.3203	$7.36e^{-07}$	$4.267e^{-06}$
BIO10	$< 2.2e^{-16}$	0.1695	$2.584e^{-06}$	$3.523e^{-06}$
BIO11	$1.231e^{-07}$	0.001109	0.003458	0.0003848
BIO12	$5.331e^{-13}$	0.1466	$3.869e^{-10}$	0.02286
BIO13	$7.446e^{-13}$	0.3267	$1.421e^{-06}$	$7.618e^{-05}$
BIO14	$9.298e^{-06}$	0.07341	$4.231e^{-10}$	0.9307
BIO15	$7.683e^{-05}$	0.09019	$5.233e^{-12}$	0.7347
BIO16	$1.527e^{-13}$	0.3982	$1.63e^{-07}$	$6.214e^{-05}$
BIO17	$1.337e^{-08}$	0.065	$9.763e^{-11}$	0.617
BIO18	$9.482e^{-05}$	0.1287	$4.408e^{-11}$	0.9033
BIO19	$2.164e^{-14}$	0.379	$5.673e^{-07}$	$3.624e^{-05}$
altitude	$2.977e^{-08}$	0.05307	0.09813	0.003085

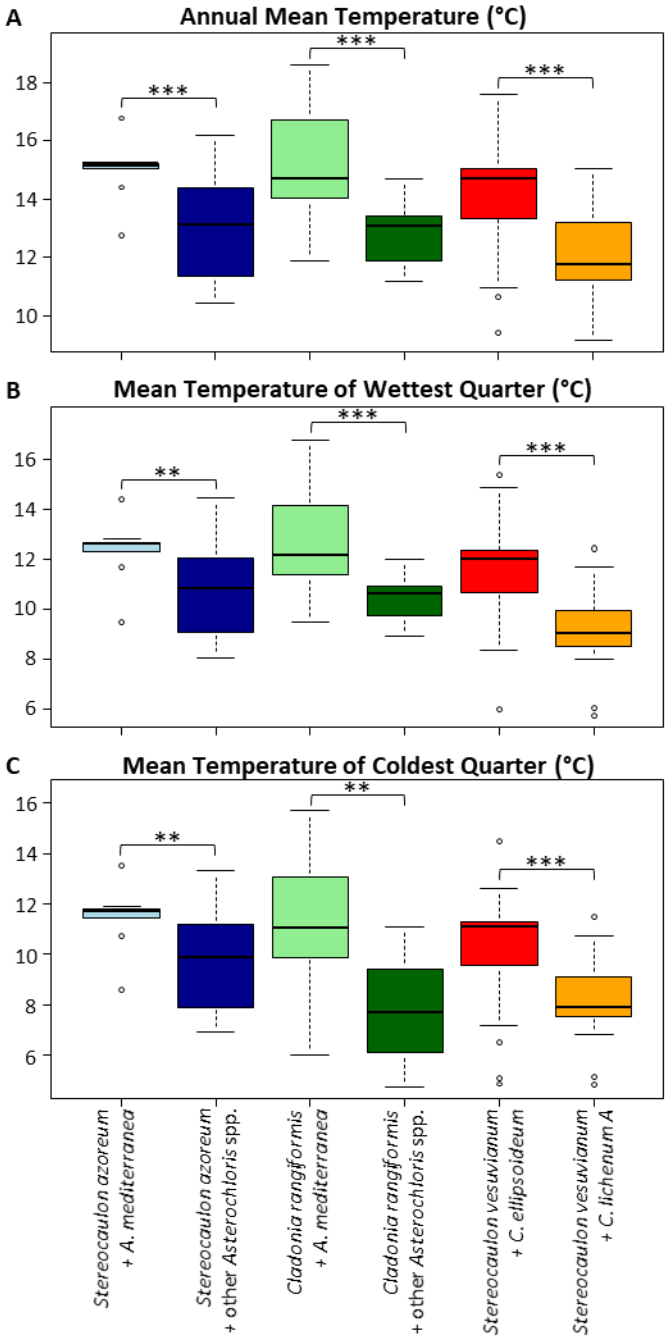


Figure 8. Differences in the distribution of selected phycobiont-mycobiont pairs along the gradients of **(A)** annual mean temperature, **(B)** mean temperature of wettest quarter, and **(C)** mean temperature of coldest quarter. Significance level: two stars (**): $p < 0.01$, three stars (***): $p < 0.001$.

Taxonomy

***Stereocaulon canariense* Malíček & Vančurová sp. nov.**

Mycobank: MB841859

Type: Spain, Canary Islands. El Hierro, Parque rural de Frontera, Ermita de los Reyes, N-facing lava field, 27°43'48.9"N, 18°07'13.6"W, alt. 710 m, on lava rock, leg. L. Vančurová & J. Malíček 16. 5. 2013 (holotype – PRA PRA-00020984; isolate A313). ITS Genbank number OL625487.

Diagnosis: Almost identical with *S. vesuvianum* s. str., but calcium oxalate crystals absent from apothecia and both species differ in molecular characters of nuclear ITS region.

Etymology: Distribution of the new species is restricted to the Canary Islands.

Description: Podetia up to 3.5 cm, (richly) branched, smooth and without tomentum or rarely with rudimental local tomentum on the underside. Cephalodia not observed, but small bunches of *Stigonema* on podetia observed in three collections. In exposed habitats, podetia are usually low and forming more or less compact cushions covered by phyllocladia or even only basal thallus composed of phyllocladia present. Phyllocladia densely cover podetia or form basal thallus in young stages, developing from small white to pale grey granules/squamules without the dark center to typical peltate shape with a distinct white rim and olive center, resembling lecanorine apothecia, up to 1.5 mm in diam (Figure 9A). Margin of phyllocladia elevated, flexuose to rarely crenulate, thick. Hyphal tissue in the olive center of phyllocladia 50–100(–130) µm thick in section, colourless to yellowish/brownish in upper part due to crystals of atranorin (soluble in K), which are very abundant in this layer and upper part of phyllocladia margin, photobiont cells 7–13 µm in diam., medullary hyphae branched, 8–15 µm thick, walls 3–6 µm thick.

Apothecia common in well-developed thalli, lecideine, brown to black, 0.4–0.6 mm in diam., sessile to constricted at base, discs firstly plane, soon convex. Outer exciple pale brown (rarely) to brown, of thick hyphae (c. 10 µm), with abundant fine crystals of atranorin (POL+, soluble in K); hypothecium very pale brown to brown, K+ yellow-brown to dark brown, without crystals; epihymenium brown, POL+ but without any crystalline objects (POL- in K); hymenium 50–75 (–100) µm. Paraphyses 1–2 µm thick, sparsely branched, swollen at tips up to 4(–5) µm, apices often with a dark brown cap. Asci 8-spored, clavate, ca. 35–40 × 8–12(-14) µm. Ascospores 1–3-septate, (18–)23–35 × 2.5–3.5 µm, colourless, straight or slightly curved, with rounded apices.

Pycnidia infrequent, in phyllocladia near podetia apices, black, variable in shape, usually 100–200 µm in diam. (in section), wall brown, paraplectenchymatic, conidia straight or one apex slightly curved, 5–7 x 1 µm.

Chemistry: Atranorin (major), stictic acid complex (major), including norstictic acid (minor) detected by TLC (n= 24). Spot reactions: K+ yellow, Pd+ slowly orange, C-, KC-, UV+ dull (yellow-)orange.

Distribution and ecology: The new species is so far known only from the Canary Islands (Gran Canaria, El Hierro, La Palma, Lanzarote, Tenerife). Most of records come from La Palma, El Hierro and Lanzarote. It is common and widely distributed here in lower and middle elevations from 100 m a.s.l. (Gran Canaria) to 960 m a.s.l. (Tenerife). It occurs on lava rocks, where it is among the first colonists. In some areas, it may be a dominant in saxicolous communities. Rarely, it may grow on soil crusts in volcanic rocks or weathered lava ground.

Caloplaca spp. and *Candelariella vitellina* were the most commonly recorded, co-occurring species. *Acarospora* spp., *Buellia* spp., *Cladonia foliacea*, *Cetraria aculeata*, *Lecanora campestris* and *Ramalina* spp. were other associated taxa.

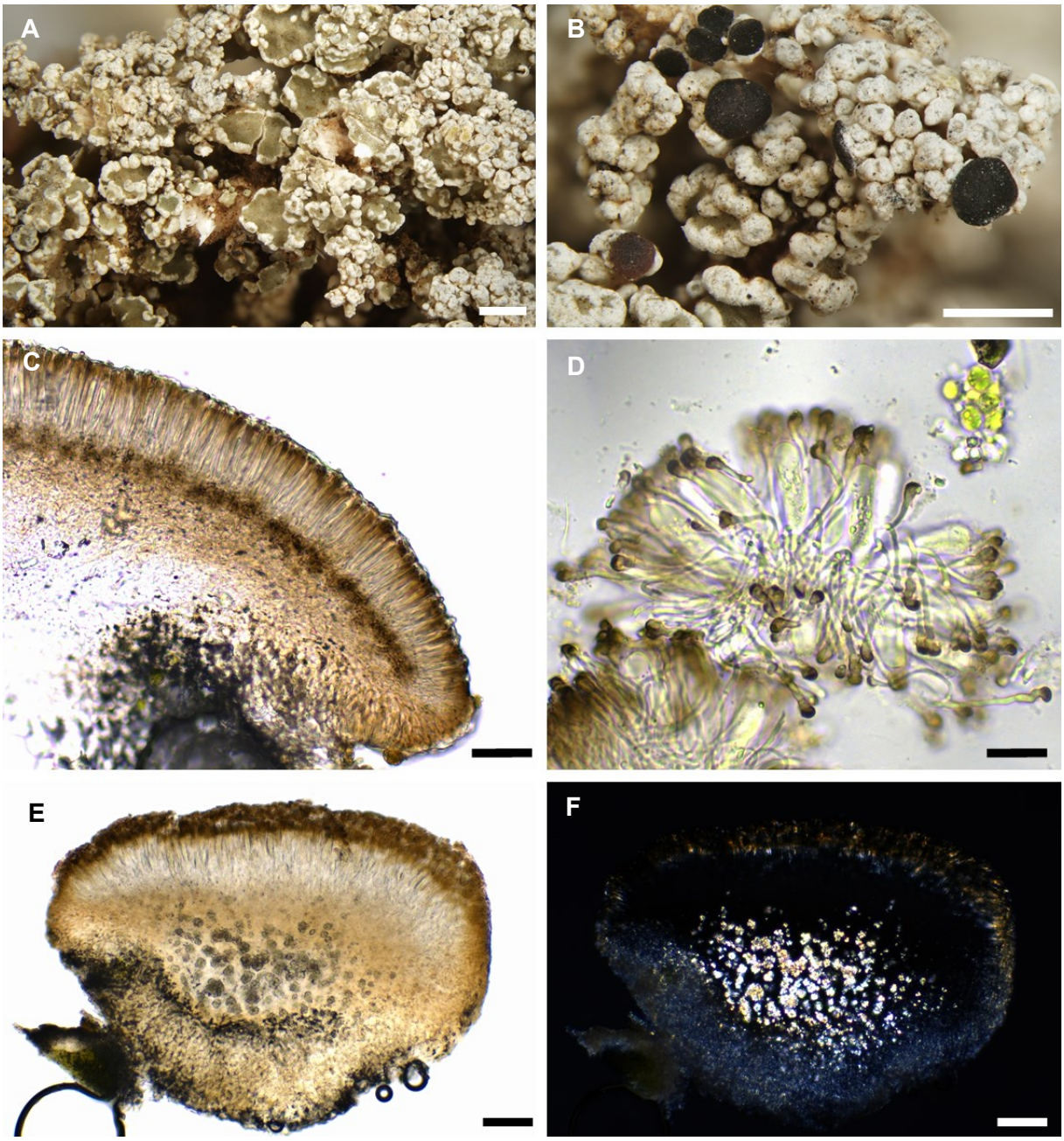


Figure 9. Habitus of *S. canariense* **(A–D)** and *S. vesuvianum* s. str. **(E, F)**. **(A)** Typical peltate phyllocladia, holotype; **(B)** fertile podetia, holotype; **(C)** section of an apothecium in water; **(D)** paraphyses with brown apical cap in KOH; **(E)** section of an apothecium in water; **(F)** section of an apothecium in polarized light. Scales: 1 mm **(A, B)**, 50 µm **(C, E, F)**, 20 µm **(D)**.

Phylogeny: *Stereocaulon canariense* is strongly supported as a distinct clade in the ITS phylogeny; its closest relative is *S. virgatum* (Figure 1). The most similar species, *S. vesuvianum* s. str. (OTU11) and the boreal-montane to arctic-alpine *S. vesuvianum* var. *nodulosum* are (OTU10) not closely related.

Notes: *Stereocaulon canariense* is quite a variable species. Thalli on very dry and exposed sites are often formed by low compact cushions of podetia or even by phyllocladia alone. One sample (L1617) was composed mostly of phyllocladia, forming the basal thallus, with abundant apothecia

and rare podetia up to 3 mm high. In two samples (A315 and A169), local "pseudosoralia" were observed. They contained white blastidia like structures of c. 0.1 mm in diam. with compact hyphae on their surface.

Stereocaulon vesuvianum sensu lato is a very polymorphic and cosmopolitan morphospecies well characterized by its peltate phyllocladia (grey to olive in centers) and the presence of stictic acid (Lamb 1977). However, our molecular studies revealed three distinct lineages in the complex. Here we described only *S. canariense*, but the other taxa should be re-classified in the future. *Stereocaulon vesuvianum* sensu stricto is morphologically identical, but contains calcium oxalate crystals in lower hypothecium. We observed two additional characters, which, however, partially overlap in both species. Epihymenium in *S. vesuvianum* is usually reddish-brown (brown in *S. canariense*) and phyllocladia form generally smaller olive centres, which are often poorly developed or only punctiform comparing to usually large olive centres in *S. canariense*, and its phyllocladia strongly resembling lecanorine apothecia. Both species share similar ecology, but on the Canary Islands, they have not been collected at the same site. Most of localities of *S. vesuvianum* have been recorded above 1000 m a.s.l. (mean = 1426 m, n = 32), whereas *S. canariense* occurs below this elevation (mean = 476 m, n = 25). *Stereocaulon vesuvianum* is not restricted to young stages of lava fields, but grows on various volcanic rocks also in late succession stages, e.g. well-lit forests with rocky substrata.

The most widely distributed lineage of "*S. vesuvianum*" (OTU10) well corresponds to var. *nodulosum* (Wallr.) Lamb, described also as *S. denudatum* Flörke (Lamb 1977), which is characterized by rare apothecia, frequent globose soralia at tips (usually present at well-developed samples) and the absence of calcium oxalate crystals in apothecia. Its podetia are simple or sparsely branched, usually in upper half. This taxon distinctly differs from Mediterranean *S. vesuvianum* s. str. in ecology: it is a boreal-montane to arctic-alpine species, occurring usually on acidic siliceous stones and rocks.

Additional specimens examined: SPAIN, Canary Islands. ***El Hierro:*** 1.9 km NNE of Faro de Orchilla, lava field, 27°43'24.6"N, 18°8'43.4"W, alt. 280 m, leg. L. Vančurová & J. Malíček 16. 5. 2013 (PRA; A315); 3 km NNW of La Restinga, lava field, 27°39'57.5"N, 17°59'38.6"W, alt. 330 m, leg. L. Vančurová & J. Malíček 15. 5. 2013 (PRA; A290, A291); ***El Hierro***, El Sabinar, heap of stones, soil and lava stones, 27.7454517N, 18.1229275W, alt. 665 m, leg. J. Vančurová 5. 3. 2020 (PRA; A690)**.** ***Gran Canaria***: Agaete, ancient necropolis, lava field, 28°05'49.2"N, 15°41'30.9"W, alt. 100 m, leg. L. Vančurová & J. Malíček 2. 6. 2013 (PRA; A201, A202). ***La Palma***: 2.5 km NE from Puerto de Naos, lava field, 28°36'17.0"N, 17°53'43.4"W, alt. 440 m, leg. L. Vančurová & J. Malíček 20. 5. 2013 (PRA; A104); Ibid.: 28°35'55.7"N, 17°53'36.2"W, alt. 480 m, leg. L. Vančurová & J. Malíček 20. 5. 2013 (PRA; A105); 3 km E of El Paso, lava rock on the border of the N-facing lava field, 28°39'11.4"N, 17°51'4.3"W, alt. 860 m, leg. L. Vančurová & J. Malíček 19. 5. 2013 (PRA; A97); Ibid.: 28°39'10.1"N, 17°51'4.3"W, alt. 865 m, leg. L. Vančurová & J. Malíček 19. 5. 2013 (PRA; A98); at base of volcano San Antonio, lava stone, 28°28'39.7"N, 17°51'1.3"W, alt. 390 m, leg. L.

Vančurová & J. Malíček 17. 5. 2013 (PRA; A77); at base of volcano Teneguía, little lava stones around the path, 28°27'50.9"N, 17°50'43.2"W, alt. 175 m, leg. L. Vančurová & J. Malíček 17. 5. 2013 (PRA; A80); Santa Cruz de La Palma, San Isidro, E-slope of mountain ridge Cumre Nueva just above the village, weathered volcanic rock, 28°37'49"N, 17°48'1"W, alt. 675 m, leg. J. Vondrák 16. 3. 2014 (PRA-Vondrák 12151; O56); Volcan Teneguía, lava stone by the path, 28°28'23"N, 17°50'50"W, alt. 340 m, leg. L. Vančurová 16. 10. 2011 (PRA; L1620); volcano San Antonio, stone on the top of a volcano, 28°29'7.8"N, 17°50'59.7"W, alt. 610 m, leg. L. Vančurová & J. Malíček 17. 5. 2013 (PRA; A72, A73); Ibid.: little lava stones around the path, 28°29'13.8"N, 17°50'56.9"W, alt. 630 m, leg. L. Vančurová & J. Malíček 17. 5. 2013 (PRA; A74); Ibid.: 28°29'11.4"N, 17°50'59.2"W, alt. 615 m, leg. L. Vančurová & J. Malíček 17. 5. 2013 (PRA; A75); Ibid.: 28°28'55"N, 17°50'58"W, alt. 595 m, leg. L. Vančurová 16. 10. 2011 (PRA; L1616); Ibid.: 28°28'53"N, 17°50'44"W, alt. 480 m, leg. L. Vančurová 16. 10. 2011 (PRA; L1617, L1618); volcano Teneguía, lava rock, 28°28'29.0"N, 17°51'3.7"W, alt. 380 m, leg. L. Vančurová & J. Malíček 17. 5. 2013 (PRA; A78). ***Lanzarote***: Paisaje Protegido de La Geria, lava field, 28°59'1.4"N, 13°40'53.5"W, alt. 330 m, leg. L. Vančurová & J. Malíček 25. 5. 2013 (PRA; A169); Parque Natural de Los Volcanes, lava field, 29°2'39.6"N, 13°42'32.4"W, alt. 240 m, leg. L. Vančurová & J. Malíček 24. 5. 2013 (PRA; A162); Ibid.: 29°2'27.9"N, 13°43'19.6"W, alt. 255 m, leg. L. Vančurová & J. Malíček 24. 5. 2013 (PRA; A163); Ibid.: 29°0'50.6"N, 13°43'51.5"W, alt. 300 m, leg. L. Vančurová & J. Malíček 24. 5. 2013 (PRA; A164, A165); Ibid.: 29°2'38.5"N, 13°43'22.5"W, alt. 205 m, leg. L. Vančurová & J. Malíček 24. 5. 2013 (PRA; A167). ***Tenerife***: Arguayo, near soccer field, N-facing lava field, 28°16'20.4"N, 16°48'18.8"W, alt. 960 m, leg. L. Vančurová & J. Malíček 7. 5. 2013 (PRA; A254).

Discussion

Phycobiont diversity in the study area

We found 15 species-level lineages of phycobionts in 338 lichen thalli belonging to the genera *Stereocaulon*, *Cladonia*, and *Lepraria*. Out of these 15 lineages, only eight belonged to the genus *Asterochloris*. This is a relatively surprising finding considering the results of previous studies focusing on photobiont diversity of corresponding mycobiont genera. For example, Kosecka (2021) found 21 *Asterochloris* and one *Vulcanochloris* species-level lineages in Bolivia with a considerably smaller sampling of the same lichen genera. Vančurová (2020) sampled only two *Stereocaulon* species-level lineageas in the Swiss Alps and found 14 *Asterochloris* and three other trebouxiophycean species-level lineages. Peksa and Škaloud (2011) recovered 10 *Asterochloris* lineages associated with *Lepraria* in the Czech Republic. In the study area, globally abundant *Asterochloris* species (*Asterochloris irregularis*, *A. pseudirregularis*, *A. lobophora*, *A. phycobiontica*, *A. gaertneri*, *A. friedlii*, and others) were not detected. One of the most common *Asterochloris* species, *A. glomerata* (Pino-Bodas & Stenroos 2020), was present in three samples and belonged to the single genotype that is known to be exclusively from the study area.

We hypothesized that the isolation of oceanic islands may explain the limited phycobiont species richness. However, the islands in Macaronesia showed higher species richness than the less isolated

Sicily and Aeolian Islands. It is more likely that phycobiont distribution can be restricted by a limited number of substrate/habitat types. The main reason for the absence of many *Asterochloris* lineages might be their preference for cold and wet climates (Pino-Bodas & Stenroos 2020; Vančurová *et al.* 2018). Nevertheless, there were a few cases in which phycobionts that are typical of cold climates were recorded; for example, one sample included *A. stereocaulonicola,* which was originally described in Antarctica (Kim *et al.* 2020). In contrast, *A. mediterranea*, the most abundant phycobiont in the study area, copes well with warm and dry climates (Moya *et al.*, 2015; Pino-Bodas and Stenroos, 2020). Madeira, the wettest and coldest island in our study, showed the highest phycobiont species richness. Interestingly, we found all three *Vulcanochloris* species with a high haplotype diversity on the warmest and driest island in the study area, Lanzarote. This island seemed to be the hot-spot of *Vulcanochloris* diversity. In contrast, lichens associated with *Asterochloris* (including *A. mediterranea*, which performs well in warmer habitats) were extremely rare in that island. Lindgren *et al.* (2020) proposed a hypothesis explaining the association *between Haveochlorella* and several *Sticta* spp. on Madagascar and Reunion. A long-distance dispersal of mycobionts may have been followed by an association with locally adapted phycobionts and the subsequent diversification of mycobionts. This pattern can be true also for the relationship between *Stereocaulon canariense* and *Vulcanochloris* spp.

In one case, we noticed *Myrmecia* sp. as the phycobiont of *Cladonia*. The genus *Asterochloris* has generally been considered the exclusive phycobiont of this broadly distributed mycobiont genus (Piercey-Normore & DePriest 2001; Pino-Bodas & Stenroos 2020; Steinová *et al.* 2019; Yahr *et al.* 2006). However, its ability to associate with *Trebouxia* and *Chloroidium* under stressful conditions has recently been demonstrated (Osyczka *et al.* 2020; Park *et al.* 2015). Moreover, Elshobary *et al.* (2015) discovered unknown trebouxiophycean algae as phycobionts of *Cladonia macrophylla*.

Phycobiont pool sharing

Kaasalainen *et al.* (2021) demonstrated that cyanolichens share photobionts and revealed the presence of photobiont-mediated lichen guilds. Most of the mycobionts shared photobionts with other non-related fungal species but other photobiont-mycobiont pairs remained isolated. In our study area, the vast majority of *Cladonia* and *Lepraria* mycobionts associated with the *Asterochloris mediterranea* phycobiont. On the other hand, phycobionts of *Stereocaulon* were highly diverse. After downsampling to a sample size of 22 (the number of *Lepraria* samples) *Stereocaulon* mycobionts associated with almost two times more phycobiont species than *Cladonia* or *Lepraria* (Supplementary Figure 5). The *Stereocaulon* species-level lineages showed a high level of specificity towards their phycobionts (on the level of algal genera, but not on the level of algal species). *Stereocaulon azoreum* was associated with the same phycobionts as *Cladonia* and *Lepraria* on the Canary Islands, whereas in Madeira, it associated with another algal species. *Stereocaulon vesuvianum* and *S. canariense* were associated with *Chloroidium* spp. and *Vulcanochloris* spp., respectively. Although *Cladonia* and *Lepraria* mycobionts often grow

together with *Stereocaulon* mycobionts, *Stereocaulon vesuvianum* and *S. canariense* never shared phycobionts with them in our samples. Moreover, at localities where more than one thallus was sampled with *Asterochloris*, the number of species-level lineages varied. In some cases, all samples shared one species, but at at other localities, each sample contained different species of *Asterochloris*.

Stereocaulon is not the only mycobiont genus able to associate with various phycobiont genera. The mycobiont genus *Sticta* has been shown to associate (besides cyanobacteria) with multiple trebouxiophycean algae (*Haveochlorella*, *Chloroidium*, *Symbiochloris*, and *Elliptochloris*); some *Sticta* species are specific towards particular phycobionts, but others can associate with up to three trebouxiophycean genera (Lindgren *et al.* 2020). Previous studies hypothesized that *Chloroidium* phycobionts are well-adapted to volcanic substrates (Vančurová *et al.*, 2018). However, *Cladonia* mycobionts in association with *Asterochloris* can also cope with this substrate. This fact points to the role of specificity or other mycobiont features on phycobiont distribution. On the other hand, *Stereocaulon canariense* was able to grow at arid localities in association with *Vulcanochloris* spp. Nevertheless, we could not find any lichen usually associated with *Asterochloris* (*Cladonia* and *Lepraria*) in the most arid parts of the study area (Fuerteventura and most of the localities on Lanzarote), even though these have previously been reported (van den Boom & Etayo 2006). In contrast, the driest areas were dominated by lichens known to be associated with *Trebouxia* (for example *Ramalina* spp.). The ability of some *Cladonia* species to associate with *Trebouxia* phycobionts (Osyczka *et al.* 2020; Shishido *et al.* 2021) might be helpful under such conditions (Candotto-Carniel *et al.* 2016; Leavitt *et al.* 2016; Romeike *et al.* 2002; Sadowsky & Ott 2012; de Vera 2012); however we did not find any sample containing *Trebouxia*.

In a few cases, we found *Asterochloris* phycobionts in thalli of *S. vesuvianum* and *S. canariense*, even though these have high specificity towards other phycobiont genera. In some of these cases, we also found common phycobionts in the same thallus. In the case of *S. vesuvianum*, we repeatedly detected more than one *Chloroidium* species in a single thallus. Furthermore, we were almost unable to obtain a non-mixed sequence of phycobionts of *Stereocaulon vesuvianum* at the localities on the slopes of Mt. Etna. In that area, Darienko *et al.* (2018) reported multiple free-living *Chloroidium* species that can coexist in *Stereocaulon* thalli. Paul *et al.* (2018) mentioned difficulties with Sanger sequencing as a possible indicator of algal plurality (i.e., the co-occurrence of multiple phycobionts in individual lichen thalli). Several authors found phycobionts with different ecological optima in a single thallus (Casano *et al.* 2011; Gasulla *et al.* 2020; Molins *et al.* 2020), which was also the case of multiple *Chloroidium* spp. in *Stereocaulon vesuvianum* thalli. Vančurová *et al.* (2020) found additional algal phycobionts in *Stereocaulon* thalli, which were otherwise detected as the predominant phycobionts in other mycobiont species at the same locality. Therefore, we believe that phycobiont sharing is possible even in cases when the lichens differ in a predominant phycobiont. Since different lichen genera and species differ in the incidence of this

phenomenon (Dal Grande *et al.* 2017; Smith *et al.* 2020), we cannot reliably estimate whether other lichen genera in the study area also show such algal plurality.

Climatic factors driving phycobiont distribution

Even though almost all phycobionts in the study area are known to be well adapted to warm areas, they spread out along the climatic gradient. We examined the differences between those phycobionts distributed in the relatively colder and more humid areas and those taking up the widest space in the middle of the climatic niche (Figure 7B). Since both mycobionts and phycobionts contribute to the ecology of the holobiont, we tested various combinations of partners. *Asterochloris mediterranea* and other *Asterochloris* phycobionts in *Stereocaulon azoreum* differed significantly in 17 out of 19 tested climatic variables. However, this contrasting pair of phycobionts in thalli of *Cladonia rangiformis* showed significant differences along four variables related to temperature. We considered the possibility that different thallus structure of lichen genera affected their water regime as suggested by Sadowsky *et al.* (2012); however, the pair of *Chloroidium* species in thalli of *Stereocaulon vesuvianum* also differed somewhat in temperature-related variables. *Stereocaulon azoreum* was distributed only in Macaronesia, where temperature and precipitation are correlated. Hence, it is impossible to estimate what factor is crucial for driving the distribution of their phycobionts in this area. On the other hand, *Stereocaulon vesuvianum* and *Cladonia rangiformis* both grew on Sicily and the Aeolian Islands, where the climate characteristics are different. The three factors that were significant in all combinations were related to temperature, emphasizing the importance of this factor in the selection of the appropriate phycobiont. Several previous studies also found temperature to be the critical factor determining the distribution of phycobionts (Molins *et al.* 2020; Rolshausen *et al.* 2020; Vančurová *et al.* 2018). Rolshausen *et al.* (2020) recently proposed parallel symbiont turnover zones as demarcated regions where symbiont replacement is most likely to occur. In all gradients, this symbiont turnover zone is characterized by approximately 12°C average annual temperature. We found this pattern in all combinations, but the average annual temperature of the turnover zone was slightly higher and not uniform among the mycobiont species/genera (Figure 10). We attribute this difference to the additional influence of mycobiont selectivity. Previous studies did not determine specific turnover zones, but described the gradual change of phycobionts along the altitudinal gradient (Gasulla *et al.* 2020; Vargas Castillo & Beck 2012). Moreover, Molins *et al.* (2020) studied *Trebouxia* phycobionts of *Buellia zoharyi* on Lanzarote, Fuerteventura, and Tenerife. They found three species that differed on their distribution ranges and the level of their tolerance to high temperatures (even under laboratory conditions). Interestingly, in corals, which is another symbiotic association similar to lichens in many aspects, the ability to cooperate with symbionts characterized by various temperature optima has been documented as an advantageous adaptive strategy (Silverstein *et al.* 2015). Similarly, under a scenario of global change, it may also be a critical factor for lichens (Ellis 2019), favoring those with the ability to associate with several

phycobionts covering a greater temperature gradient. However, it is possible that *Cladonia*, *Lepraria*, and *Stereocaulon* mycobionts or their phycobionts reached their maximum temperature limits, thus restricting their distribution ranges, because we did not find them on the warmest sites of the study area (Chazarra *et al.* 2011).

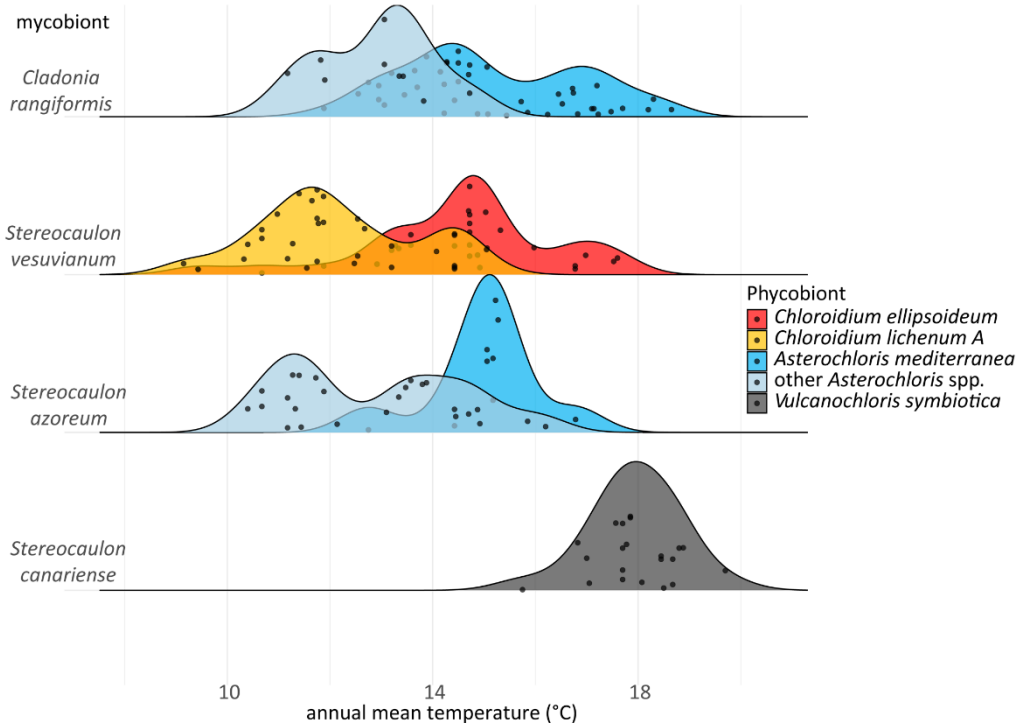


Figure 10. Ridgeplot depicting replacing of phycobionts along the gradient of annual mean temperature.

Stereocaulon canariense as endemic species

Macaronesia is known for high level of endemism (Fernández-Palacios & Whittaker 2008). As an oceanic island system, it is an attractive object for evolution studies (Emerson 2002). Besides well-known examples of beetles and angiosperms, examples of lichen speciation have been documented within this area (Sérusiaux *et al.* 2011). *Stereocaulon canariense* was recorded only in the Canary Islands so far. However, its phycobiont, *Vulcanochloris*, has been rarely reported from other parts of the world as phycobiont of *Stereocaulon* (Kosecka *et al.* 2021) or other lichens (Vaiglová 2017). The communication between Macaronesian and Mediterranean biota has been repeatedly documented (Carine *et al.* 2004; Vondrák *et al.* 2020). Consequently, other localities of this species can be found in the future. Moreover, the origin of this possibly endemic lichen can be a topic of future research.

Data Availability Statement

The data presented in the study are deposited in GenBank, accession numbers OL622077–OL622095 and OL625120–OL625607, and Mendeley Data: http://dx.doi.org/10.17632/428v52svtp.1.

Author Contributions

LV, JM, and PŠ designed the study. LV and JM conducted the sampling and the laboratory work. JM and JS determined the samples. LV, JS, and PŠ conducted the phylogenetic and the statistical analyses. LV and JM wrote the manuscript and produced the figures with contributions from JS and PŠ. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Charles University Science Foundation project GAUK (grant no. 570313), Primus Research Programme of Charles University (grant no. SCI/13), and by a long-term research development grant RVO 67985939.

Acknowledgments

We are grateful to J. Vančurová, J. Vondrák, L. Vančura, and K. Vančura for collecting material and/or help with field work, Z. Sejfová for help with TLC and photos, B. McCune for valuable discussions about the taxonomy of *Stereocaulon*, and the reviewers for their useful comments on the original version of the manuscript.

References

Ahmadjian, V. & Jacobs, J.B. (1981) Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck. *Nature* 289: 169–172. https://doi.org/10.1038/289169a0

del Arco Aguilar, M.J. & Rodríguez Delgado, O. (2018) Vegetation of the Canary Islands in *Vegetation of the Canary Islands*. pp. 83–319.

Bačkor, M., Peksa, O., Škaloud, P. & Bačkorová, M. (2010) Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. *Ecotoxicology and environmental safety* 73: 603–12. https://doi.org/10.1016/j.ecoenv.2009.11.002

Baruffo, L., Zedda, L., Elix, J.A. & Tretiach, M. (2006) A revision of the lichen genus *Lepraria* s.lat. in Italy. *Nova Hedwigia* 83: 387–430. https://doi.org/10.1127/0029-5035/2006/0083-0387

Beck, A. (2002) Selektivität der Symbionten schwermetalltoleranter Flechten. Ludwig-Maxmilians-Universität München

Beiggi, S. & Piercey-Normore, M.D. (2007) Evolution of ITS Ribosomal RNA Secondary Structures in Fungal and Algal Symbionts of Selected Species of *Cladonia* sect. *Cladonia*. *Journal of Molecular Evolution* 64: 528–542. https://doi.org/10.1007/s00239-006-0115-x

Blonder, B., Lamanna, C., Violle, C. & Enquist, B.J. (2014) The n-dimensional hypervolume. *Global Ecology and Biogeography* 23: 595–609. https://doi.org/10.1111/geb.12146

Boluda, C.G., Rico, V.J., Divakar, P.K., Nadyeina, O., Myllys, L., McMullin, R.T., Zamora, J.C.,

Scheidegger, C. & Hawksworth, D.L. (2019) Evaluating methodologies for species delimitation: The mismatch between phenotypes and genotypes in lichenized fungi (*Bryoria* sect. *implexae*, Parmeliaceae). *Persoonia: Molecular Phylogeny and Evolution of Fungi* 42: 75–100. https://doi.org/10.3767/persoonia.2019.42.04

van den Boom, P.P.G. & Clerc, P. (2015) New Records of Lichens and Lichenicolous Fungi from La Gomera (Canary Islands, Spain), including the new species: *Usnea boomiana* P. Clerc. *Candollea* 70: 165–177. https://doi.org/http://www.bioone.org/doi/pdf/10.15553/c2015v702a1

van den Boom, P.P.G. & Ertz, D. (2013) Further New or Interesting Lichens and Lichenicolous Fungi of Tenerife (Canary Islands , Spain). *Stapfia reports* 99: 52–60.

van den Boom, P.P.G. & Etayo, J. (2006) New records of lichens and lichenicolous fungi from Fuerteventura (Canary Islands), with descriptions of some new species. *Cryptogamie, Mycologie* 27: 341–374.

van den Boom, P.P.G. & Magain, N. (2020) Three new lichen species from Macaronesia belonging in Ramalinaceae, with the description of a new genus. *Plant and Fungal Systematics* 65: 167–175. https://doi.org/10.35535/pfsyst-2020-0011

Burgaz, A.R. & Ahti, T. (2009) *Flora Liquenológica Ibérica. Vol 4. Cladoniaceae*. Sociedad Española de Liquenología, Madrid. 111 pp.

Candotto-Carniel, F., Gerdol, M., Montagner, A., Banchi, E., De Moro, G., Manfrin, C., Muggia, L., Pallavicini, A. & Tretiach, M. (2016) New features of desiccation tolerance in the lichen photobiont *Trebouxia gelatinosa* are revealed by a transcriptomic approach. *Plant Molecular Biology* 91: 319–339. https://doi.org/10.1007/s11103-016-0468-5

Carine, M.A., Russell, S.J., Santos-Guerra, A. & Francisco-Ortega, J. (2004) Relationships of the Macaronesian and Mediterranean floras: Molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). *American Journal of Botany* 91: 1070–1085. https://doi.org/10.3732/ajb.91.7.1070

Casano, L.M., del Campo, E.M., García-Breijo, F.J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., Guéra, A. & Barreno-Rodríguez, E. (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? *Environmental microbiology* 13: 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Chazarra, A., Baceló, A.M., Pires, V., Cunha, S., Silva, A., Marques, J., Carvalho, F., Mendes, M., Neto, J. & Mendes, L.C. (2011) *Atlas climático de los archipiélagos de canarias, madeira y azores atlas climático dos arquipélagos das canárias, da madeira e dos açores*. Lisbon. 79 pp.

Crespo, A., Arguello, A., Lumbsch, H.T., Llimona, X. & Tønsberg, T. (2006) A new species of *Lepraria* (Lecanorales: Stereocaulaceae) from the Canary Islands and the typification of *Lepraria isidiata*. *Lichenologist* 38: 213–221. https://doi.org/10.1017/S0024282906005846

Cubero, O.F., Crespo, A., Fatehi, J. & Bridge, P.D. (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution* 216: 243–249. https://doi.org/10.1007/BF01084401

Dal Grande, F., Rolshausen, G., Divakar, P.K., Crespo, A., Grande, F.D., Otte, J., Schleuning, M. & Schmitt, I. (2017) Environment and host identity structure communities of green algal symbionts in lichens. *New Phytologist* 217: 277–289. https://doi.org/10.1111/nph.14770

Darienko, T., Lukešová, A. & Pröschold, T. (2018) The polyphasic approach revealed new species of *Chloroidium* (Trebouxiophyceae, Chlorophyta). *Phytotaxa* 372: 51–66. https://doi.org/10.11646/phytotaxa.372.1.4

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Dormann, C.F., Gruber, B. & Fruend, J. (2008) Introducing the bipartite Package: Analysing Ecological Networks. *R News* 8: 8–11.

Ellis, C. (2019) Climate Change, Bioclimatic Models and the Risk to Lichen Diversity. *Diversity* 11: 54.

https://doi.org/10.3390/d11040054

Elshobary, M.E., Osman, M.E.H., Abushady, A.M. & Piercey-Normore, M.D. (2015) Comparison of Lichen-Forming Cyanobacterial and Green Algal Photobionts with Free-Living Algae. *Cryptogamie, Algologie* 36: 81–100. https://doi.org/10.7872/crya.v36.iss1.2015.81

Emerson, B.C. (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology* 11: 951–966.

Engelen, A., Convey, P. & Ott, S. (2010) Life history strategy of *Lepraria borealis* at an Antarctic inland site, Coal Nunatak. *The Lichenologist* 42: 339–346. https://doi.org/10.1017/S0024282909990600

Fernández-Palacios, J.M. & Whittaker, R.J. (2008) The Canaries: An important biogeographical meeting place. *Journal of Biogeography* 35: 379–387. https://doi.org/10.1111/j.1365-2699.2008.01890.x

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.

Gasulla, F., Barrasa, J.M., Casano, L.M. & del Campo, E.M. (2020) Symbiont composition of the basidiolichen *Lichenomphalia meridionalis* varies with altitude in the Iberian Peninsula. *The Lichenologist* 52: 17–26. https://doi.org/10.1017/S002428291900046X

Giovino, A., Marino, P., Domina, G., Scialabba, A., Schicchi, R., Diliberto, G., Rizza, C. & Scibetta, S. (2016) Evaluation of the DNA barcoding approach to develop a reference data-set for the threatened flora of Sicily. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* 150: 631–640. https://doi.org/10.1080/11263504.2014.989285

Di Gristina, E., Gottschlich, G. & Raimondo, F.M. (2016) Rediscovery of *Hieracium nebrodense* (Asteraceae), a little-known endemic of Sicily (Italy). *Phytotaxa* 265: 59–66. https://doi.org/10.11646/phytotaxa.265.1.5

Guindon, S. & Gascuel, O. (2003) A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. *Systematic Biology* 52: 696–704. https://doi.org/10.1080/10635150390235520

Hafellner, J. (2008) Additions and Corrections to the Checklist and Bibliography of Lichens and Lichenicolous Fungi of Insular Laurimacaronesia. IV. *Fritschiana* 64: 1–28.

Hernández Padrón, C.E. & Pérez-Vargas, I. (2010) Lichenes, lichenicolous fungi. *In*: M. Arechavaleta Hernández, S. Rodríguez Núñez, N. Zurita Pérez, & A. García Ramírez (eds.) *Lista de especies silvestres de Canarias. Hongos, plantas y animales terrestres. 2009*. Gobierno de Canarias, Santa Cruz de Tenerife, pp. 71–105.

Högnabba, F. (2006) Molecular phylogeny of the genus *Stereocaulon* (Stereocaulaceae, lichenized ascomycetes). *Mycological research* 110: 1080–1092. https://doi.org/10.1016/j.mycres.2006.04.013

Hutchinson, G.E. (1957) Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology* 22: 415–427. https://doi.org/10.1101/SQB.1957.022.01.039

Kaasalainen, U., Tuovinen, V., Mwachala, G., Pellikka, P. & Rikkinen, J. (2021) Complex Interaction Networks Among Cyanolichens of a Tropical Biodiversity Hotspot. *Frontiers in Microbiology* 12: 1–12. https://doi.org/10.3389/fmicb.2021.672333

Kanz, B., Brackel, W. von, Cezanne, R., Eichler, M., Hohmann, M.-L., Teuber, D. & Printzen, C. (2015) DNA Barcodes for the Distinction of Reindeer Lichens: A Case Study Using *Cladonia rangiferina* and *C. stygia*. *Herzogia* 28: 445–464. https://doi.org/10.13158/heia.28.2.2015.445

Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P. & Kessler, M. (2017) Climatologies at high resolution for the earth's land surface areas. *Scientific Data* 4: 170122. https://doi.org/10.1038/sdata.2017.122

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772–80. https://doi.org/10.1093/molbev/mst010

Kelly, L.J., Hollingsworth, P.M., Coppins, B.J., Ellis, C.J., Harrold, P., Tosh, J. & Yahr, R. (2011) DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. *New*

Phytologist 191: 288–300. https://doi.org/10.1111/j.1469-8137.2011.03677.x

Kim, J.I., Kim, Y.J., Nam, S.W., So, J.E., Hong, S.G., Choi, H.G. & Shin, W. (2020) Taxonomic study of three new Antarctic *Asterochloris* (Trebouxiophyceae) based on morphological and molecular data. *Algae* 35: 17–32. https://doi.org/10.4490/algae.2020.35.2.23

Korchikov, E.S., Bolgov, E.V., Ilyina, E.S. & Pankratov, T.A. (2018) Species diversity and photobiont localization specificities in epigene lichens (on the example of the genus *Cladonia*). *Samara Journal of Science* 7: 59–64. https://doi.org/10.17816/snv201873111

Kosecka, M., Guzow-Krzemińska, B., Černajová, I., Škaloud, P., Jabłońska, A. & Kukwa, M. (2021) New lineages of photobionts in Bolivian lichens expand our knowledge on habitat preferences and distribution of *Asterochloris* algae. *Scientific Reports* 11: 8701. https://doi.org/10.1038/s41598-021-88110-0

Lamb, I.M. (1977) A Conspectus of the Lichen Genus *Stereocaulon*(Schreb.)Hoffm. *Journal of the Hattori Botanical Laboratory* 43: 191–355.

Lamb, I.M. (1978) Keys to the species of the lichen genus *Stereocaulon* (Schreb.) Hoffm. *The Journal of the Hattori Botanical Laboratory* 22: 209–250.

Leavitt, S.D., Kraichak, E., Nelsen, M.P., Altermann, S., Divakar, P.K. & Alors, D. (2015) Fungal specificity and selectivity for algae play a major role in determining lichen partnerships across diverse ecogeographic regions in the lichen-forming family Parmeliaceae (Ascomycota). *Molecular Ecology* 24: 3779–3797. https://doi.org/10.1111/mec.13271

Leavitt, S.D., Kraichak, E., Vondrák, J., Nelsen, M.P., Sohrabi, M., Perez-Ortega, S., St Clair, L.L. & Thorsten Lumbsch, H. (2016) Cryptic diversity and symbiont interactions in rock-posy lichens. *Molecular Phylogenetics and Evolution* 99: 261–274. https://doi.org/10.1016/j.ympev.2016.03.030

Lindgren, H., Moncada, B., Lücking, R., Magain, N., Simon, A., Goffinet, B., Sérusiaux, E., Nelsen, M.P., Mercado-Díaz, J.A., Widhelm, T.J. & Lumbsch, H.T. (2020) Cophylogenetic patterns in algal symbionts correlate with repeated symbiont switches during diversification and geographic expansion of lichen-forming fungi in the genus *Sticta* (Ascomycota, Peltigeraceae). *Molecular Phylogenetics and Evolution* 150: 106860. https://doi.org/10.1016/j.ympev.2020.106860

Lücking, R. (2019) Stop the Abuse of Time! Strict Temporal Banding is not the Future of Rank-Based Classifications in Fungi (Including Lichens) and Other Organisms. *Critical Reviews in Plant Sciences* 38: 199–253. https://doi.org/10.1080/07352689.2019.1650517

Molins, A., Chiva, S., Calatayud, Á., Marco, F., García-Breijo, F., Reig-Armiñana, J., Carrasco, P. & Moya, P. (2020) Multidisciplinary approach to describe *Trebouxia* diversity within lichenized fungi *Buellia zoharyi* from the Canary Islands. *Symbiosis* 82: 19–34. https://doi.org/10.1007/s13199-020-00722-8

Moya, P., Chiva, S., Molins, A., Jadrná, I., Škaloud, P., Peksa, O. & Barreno, E. (2018) *Myrmecia israeliensis* as the primary symbiotic microalga in squamulose lichens growing in European and Canary Island terricolous communities. *Fottea* 18: 72–85. https://doi.org/10.5507/fot.2017.022

Moya, P., Škaloud, P., Chiva, S., García-Breijo, F.J., Reig-Armiñana, J., Vančurová, L. & Barreno, E. (2015) Molecular phylogeny and ultrastructure of the lichen microalga *Asterochloris mediterranea* sp. nov. from Mediterranean and Canary Islands ecosystems. *International journal of systematic and evolutionary microbiology* 65: 1838–54. https://doi.org/10.1099/ijs.0.000185

Muggia, L., Perez-Ortega, S., Kopun, T., Zellnig, G. & Grube, M. (2014) Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Annals of Botany* 114: 463–475. https://doi.org/10.1093/aob/mcu146

Nelsen, M.P. & Gargas, A. (2006) Actin type I introns offer potential for increasing phylogenetic resolution in *Asterochloris* (Chlorophyta: Trebouxiophyceae). *The Lichenologist* 38: 435–440. https://doi.org/10.1017/S0024282906005779

Nelsen, M.P. & Gargas, A. (2008) Dissociation and horizontal transmission of codispersing lichen symbionts in the genus *Lepraria* (Lecanorales: Stereocaulaceae). *The New phytologist* 177: 264–275. https://doi.org/10.1111/j.1469-8137.2007.02241.x

Orange, A., James, P.W. & White, F.J. (2001) *Microchemical Methods for the Identification of Lichens*. British Lichen Society

Osyczka, P., Lenart-Boroń, A., Boroń, P. & Rola, K. (2020) Lichen-forming fungi in postindustrial habitats involve alternative photobionts. *Mycologia* 00: 1–13. https://doi.org/10.1080/00275514.2020.1813486

Park, C.H., Kim, K.M., Elvebakk, A., Kim, O.-S., Jeong, G. & Hong, S.G. (2015) Algal and Fungal Diversity in Antarctic Lichens. *Journal of Eukaryotic Microbiology* 62: 196–205. https://doi.org/10.1111/jeu.12159

Pateiro-Lopez, B. & Rodriguez-Casal, A. (2016) alphahull: Generalization of the Convex Hull of a Sample of Points in the Plane.

Paul, F., Otte, J., Schmitt, I. & Dal Grande, F. (2018) Comparing Sanger sequencing and high-throughput metabarcoding for inferring photobiont diversity in lichens. *Scientific Reports* 8: 8624. https://doi.org/10.1038/s41598-018-26947-8

Peksa, O., Gebouská, T., Škvorová, Z., Vančurová, L. & Škaloud, P. Photobiont-mediated guilds in green algal lichens – an insight into the life of terrestrial symbiotic communities. in press.

Peksa, O. & Škaloud, P. (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae). *Molecular Ecology* 20: 3936–48. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Piercey-Normore, M.D. & DePriest, P.T. (2001) Algal Switching among Lichen Symbioses. *American Journal of Botany* 88: 1490–1498. https://doi.org/10.2307/3558457

Pino-Bodas, R., Martín, M.P., Burgaz, A.R. & Lumbsch, H.T. (2013) Species delimitation in *Cladonia* (Ascomycota): a challenge to the DNA barcoding philosophy. *Molecular Ecology Resources* 13: 1058–1068. https://doi.org/10.1111/1755-0998.12086

Pino-Bodas, R. & Stenroos, S. (2020) Global Biodiversity Patterns of the Photobionts Associated with the Genus *Cladonia* (Lecanorales, Ascomycota). *Microbial Ecology*. https://doi.org/10.1007/s00248-020-01633-3

Rambold, G., Friedl, T. & Beck, A. (1998) Photobionts in Lichens: Possible Indicators of Phylogenetic Relationships? *Bryologist, The* 101: 392–397.

Rolshausen, G., Hallman, U., Grande, F.D., Otte, J., Knudsen, K. & Schmitt, I. (2020) Expanding the mutualistic niche: parallel symbiont turnover along climatic gradients. *Proceedings of the Royal Society B: Biological Sciences* 287: 20192311. https://doi.org/10.1098/rspb.2019.2311

Romeike, J., Friedl, T., Helms, G. & Ott, S. (2002) Genetic Diversity of Algal and Fungal Partners in Four Species of *Umbilicaria* (Lichenized Ascomycetes) Along a Transect of the Antarctic Peninsula. *Molecular Biology* 19: 1209–1217.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Sadowsky, A., Hussner, A. & Ott, S. (2012) Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane, Norway. *Nova Hedwigia* 94: 323–334. https://doi.org/10.1127/0029-5035/2012/0014

Sadowsky, A. & Ott, S. (2012) Photosynthetic symbionts in Antarctic terrestrial ecosystems: the physiological response of lichen photobionts to drought and cold. *Symbiosis* 58: 81–90. https://doi.org/10.1007/s13199-012-0198-7

Sérusiaux, E., Villarreal A., J.C., Wheeler, T. & Goffinet, B. (2011) Recent origin, active speciation and dispersal for the lichen genus *Nephroma* (Peltigerales) in Macaronesia. *Journal of Biogeography* 38: 1138–1151. https://doi.org/10.1111/j.1365-2699.2010.02469.x

Shishido, T.K., Wahlsten, M., Laine, P., Rikkinen, J., Lundell, T. & Auvinen, P. (2021) Microbial Communities of *Cladonia* Lichens and Their Biosynthetic Gene Clusters Potentially Encoding Natural Products. *Microorganisms* 9: 1347. https://doi.org/10.3390/microorganisms9071347

Silverstein, R.N., Cunning, R. & Baker, A.C. (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*

21: 236–249. https://doi.org/10.1111/gcb.12706

Škaloud, P. & Peksa, O. (2010) Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris*. *Molecular Phylogenetics and Evolution* 54: 36–46. https://doi.org/10.1016/j.ympev.2009.09.035

Škaloud, P., Steinová, J., Řídká, T., Vančurová, L. & Peksa, O. (2015) Assembling the challenging puzzle of algal biodiversity: species delimitation within the genus *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 51: 507–527. https://doi.org/10.1111/jpy.12295

Smith, H.B., Dal Grande, F., Muggia, L., Keuler, R., Divakar, P.K., Grewe, F., Schmitt, I., Lumbsch, H.T. & Leavitt, S.D. (2020) Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis. *Symbiosis* 82: 133–147. https://doi.org/10.1007/s13199-020-00699-4

Sparrius, L.B., Aptroot, A., Sipman, H.J.M., Pérez-Vargas, I., Matos, P., Gerlach, A. & Vervoort, M. (2017) Estimating the population size of the endemic lichens *Anzia centrifuga* (Parmeliaceae) and *Ramalina* species (Ramalinaceae) on Porto Santo (Madeira archipelago). *Bryologist* 120: 293–301. https://doi.org/10.1639/0007-2745-120.3.293

Steinová, J., Škaloud, P., Yahr, R., Bestová, H. & Muggia, L. (2019) Reproductive and dispersal strategies shape the diversity of mycobiont-photobiont association in *Cladonia* lichens. *Molecular Phylogenetics and Evolution* 134: 226–237. https://doi.org/10.1016/j.ympev.2019.02.014

Swofford, D.L. (2003) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).

Tretiach, M., Muggia, L. & Baruffo, L. (2009) Species delimitation in the *Lepraria isidiata-L. santosii* group: A population study in the Mediterranean-Macaronesian region. *Lichenologist* 41: 1–15. https://doi.org/10.1017/S0024282909008093

Troia, A. (2012) Insular endemism in the Mediterranean vascular flora: The case of the Aeolian Islands (Sicily , Italy). *Biodiversity Journal* 3: 369–374.

Vaiglová, Z. (2017) Dynamics of a lichen symbiosis. Charles University

Vančurová, L., Kalníková, V., Peksa, O., Škvorová, Z., Malíček, J., Moya, P., Chytrý, K., Černajová, I. & Škaloud, P. (2020) Symbiosis between river and dry lands: Phycobiont dynamics on river gravel bars. *Algal Research* 51: 102062. https://doi.org/10.1016/j.algal.2020.102062

Vančurová, L., Muggia, L., Peksa, O., Řídká, T. & Škaloud, P. (2018) The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in *Stereocaulon* (lichenized Ascomycota). *Molecular Ecology* 27: 3016–3033. https://doi.org/10.1111/mec.14764

Vančurová, L., Peksa, O., Němcová, Y. & Škaloud, P. (2015) Vulcanochloris (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain. *Phytotaxa* 219: 118–132. https://doi.org/10.11646/phytotaxa.219.2.2

Vargas Castillo, R. & Beck, A. (2012) Photobiont selectivity and specificity in *Caloplaca* species in a fog-induced community in the Atacama Desert, northern Chile. *Fungal Biology* 116: 665–676. https://doi.org/10.1016/j.funbio.2012.04.001

de Vera, J.-P. (2012) Lichens as survivors in space and on Mars. *Fungal Ecology* 5: 472–479. https://doi.org/10.1016/j.funeco.2012.01.008

Vondrák, J., Frolov, I., Košnar, J., Arup, U., Veselská, T., Halıcı, G., Malíček, J. & Søchting, U. (2020) Substrate switches, phenotypic innovations and allopatric speciation formed taxonomic diversity within the lichen genus *Blastenia*. *Journal of Systematics and Evolution* 58: 295–330. https://doi.org/10.1111/jse.12503

Weber, K. & Kabsch, W. (1994) Intron Positions in Actin Genes Seem Unrelated To the Secondary Structure of the Protein. *Embo Journal* 13: 1280–1286.

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

Yahr, R., Vilgalys, R. & Depriest, P.T. (2004) Strong fungal specificity and selectivity for algal symbionts in Florida scrub *Cladonia* lichens. *Molecular Ecology* 13: 3367–3378. https://doi.org/10.1111/j.1365-294X.2004.02350.x

Yahr, R., Vilgalys, R. & DePriest, P.T. (2006) Geographic variation in algal partners of *Cladonia* subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *The New phytologist* 171: 847–60. https://doi.org/10.1111/j.1469-8137.2006.01792.x

Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

Supplementary Table 1. GenBank accession numbers, phycobiont species-level lineage, mycobiont genus, GPS coordinates and locality of samples.

Supplementary Table 2. Total number of sequences included in particular alignments, newly obtained sequences, previously published sequences originated from the study area, reference sequences retrieved from GenBank, and unique sequences after deleting identical ones.

Supplementary Table 3. Substitution models selected using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Darriba *et al.* 2012; Guindon & Gascuel 2003).

Supplementary Table 4. *Asterochloris* reference sequences retrieved from GenBank with accession numbers.

Supplementary Figure 1. Location of the sampling sites. The annual mean temperature at the locality is indicated by the color of the dot.

Supplementary Figure 2. Phylogenetic hypothesis (unrooted tree) of *Cladonia* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold.

Supplementary Figure 3. Phylogenetic hypothesis (unrooted tree) of *Lepraria* resulting from Bayesian analysis of ITS rDNA. Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. Newly obtained sequences are marked in bold.

Supplementary Figure 4. Part of phylogenetic tree of *Asterochloris* (Figure 2) containing solely reference sequences (accession numbers are listed in Supplementary Table 4). Values at the nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap

(middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site.

Supplementary Figure 5. Rarefaction curves for three mycobiont genera. Vertical line is drawn at smallest sample size in the data set with horizontal lines for the rarefied number of species-level lineages of associated phycobionts.

The Supplementary Material for this article can be found online at:
https://www.frontiersin.org/articles/10.3389/fmicb.2021.769304/full#supplementary-material