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

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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 analysed 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. Based on an analogy, 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 substrates 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
diversity, ecological niches, lichen, phycobiont, specificity, symbiosis

1 | 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 organisms. The exclusive presence of multiple autotrophic and heterotrophic symbionts 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, Friedl, Helms, & Ott, 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, Cannon, Minter, & Stalpers, 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, Friedl, & Beck, 1998; Yahr, Vilgalys, & Depriest, 2004; Yahr, Vilgalys, & DePriest, 2006). Most mycobiont species associate with several lineages of a single algal genus, frequently *Trebouxia* (Casano et al., 2011; Helms, Friedl, Rambold, & Mayrhofer, 2001; Leavitt et al., 2015, 2016; Leavitt, Nelsen, Lumbsch, Johnson, & St. Clair, 2013; Muggia, Perez-Ortega, Kopun, Zellnig, & Grube, 2014; Nyati, Bhattacharya, Werth, & Honegger, 2013; G. 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, Peksa, Škaloud, & Bačkorová, 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, Steinová, Řídká, Vančurová, & Peksa, 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 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 substrates (Medeiros, Fryday, & Rajakaruna, 2014; Purvis & Halls, 1996). Some species of this genus also tolerate submersion (Sadowsky, Hussner, & Ott, 2012), as well as drought (Singh, Ranjan, Nayaka, Pathre, & Shirke, 2013). *Stereocaulon* ranks among the pioneer lichens that grow in harsh conditions on bare substrates, such as lava flows and relatively exposed siliceous blocks, thereby contributing to their weathering (Meunier, Kirman, Strasberg, Grauby, & Dussouillez, 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, Peksa, Němcová, & Škaloud, 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 (Fernández-Mendoza et al., 2011; Grande et al., 2017; Leavitt et al., 2016; Marini, Nascimbene, & Nimis, 2011; Peksa & Škaloud, 2011; G. Singh et al., 2017). As of late, Rolshausen, Dal Grande, Sadowska-Deś, Otte, and Schmitt (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 prefer 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).

As 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: (a) What is the diversity of phycobionts associated with the lichen-forming genus *Stereocaulon* within the entire genus and species-level lineages? (b) Which environmental factors influence the global distribution of phycobionts? (c) Do phycobionts and mycobionts exhibit reciprocal specificity/selectivity, and how does this affect the width of their climatic niches?

2 | MATERIAL AND METHODS

2.1 | 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, Supporting Information Table S1) were analysed. 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 of 17–4,500 m

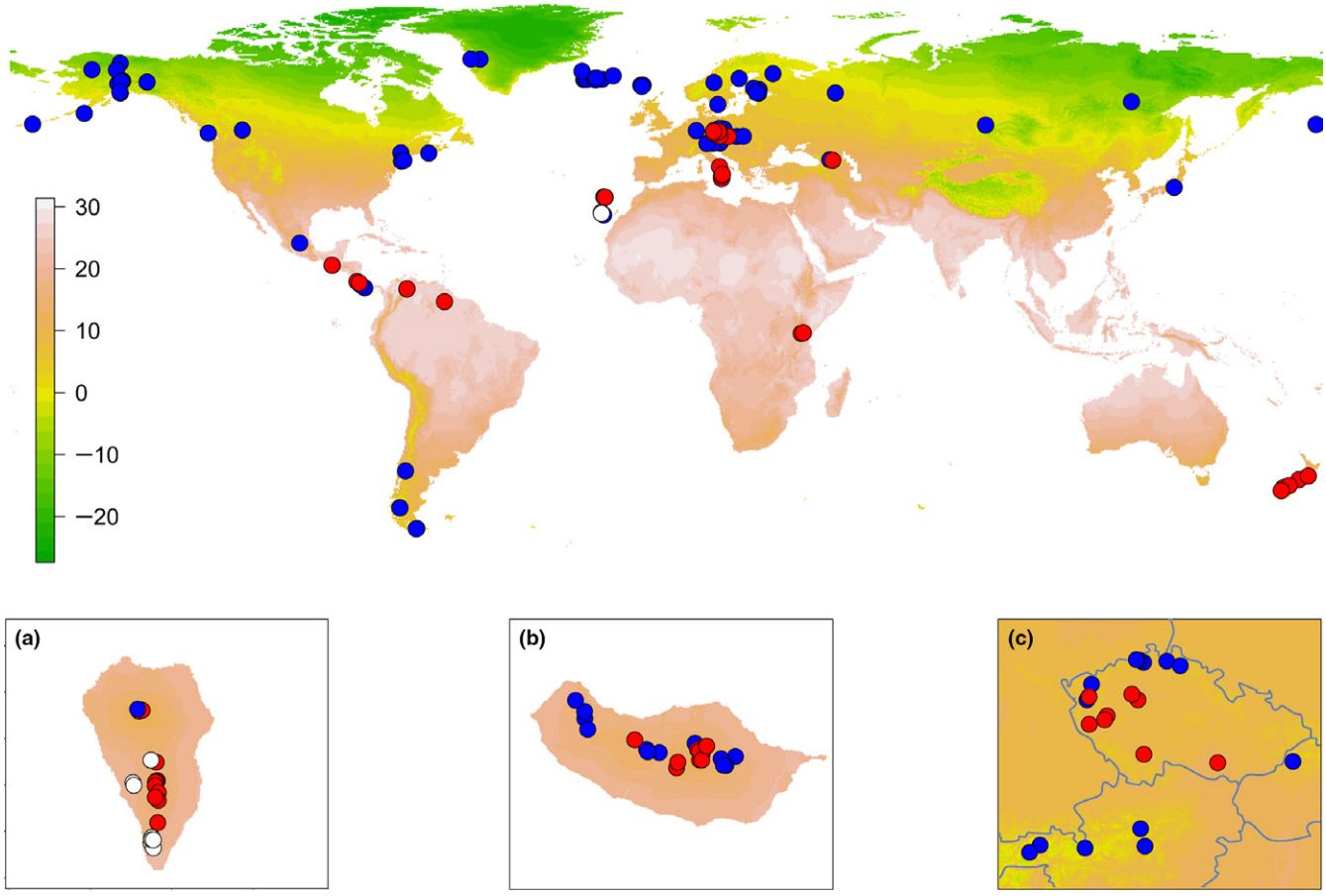


FIGURE 1 Distribution of phycobionts associating with the lichen-forming fungal genus *Stereocaulon*. Blue dots—*Asterochloris*, red dots—*Chloroidium*, white dots—*Vulcanochloris*. Legend—annual mean temperature gradient. Magnified cut-outs—(a) La Palma (Canary Islands), (b) Madeira, (c) Czech Republic

(Supporting Information 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 precoated glass plates in solvent systems A, B and C according to Orange, James, and White (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.

2.2 | 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, Crespo, Fatehi, & Bridge, 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 Supporting Information 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 (Supporting Information 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 (Supporting Information Table S1).

2.3 | 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 analysed as a single locus data set for the ITS rDNA (data not shown) and as a concatenated data set 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 data set 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 data set 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, Stecher, Peterson, Filipski, & Kumar, 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 (Supporting Information Appendix S1) and 66 actin type I sequences (Supporting Information Appendix S2); missing actin data were replaced with question marks.

The *Chloroidium* ITS rDNA data set comprised 111 sequences: 80 newly obtained sequences from *Stereocaulon* specimens and 31 representative sequences from all known free-living *Chloroidium* species (Supporting Information 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, Lorenz, Bernhart, Neuböck, & Hofacker, 2008) with default settings. After removing identical sequences, the resulting alignment comprised 45 sequences (Supporting Information Appendix S3).

The *Stereocaulon* mycobiont ITS rDNA data set 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 data set to differentiate among ITS1, 5.8 S and ITS2 rDNA, actin intron 206, actin intron 248 and actin exon regions. Substitution models (Supporting Information Table S4) were selected using the Bayesian information criterion (BIC) as implemented in JModelTest2 (Darriba, Taboada, Doallo, & Posada, 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 1,000 random sequence addition replicates and random addition of sequences (the number was limited to 10,000 per replicate). ML and MP bootstrap support values were obtained from 100 and 1,000 bootstrap replicates, respectively. Only one search replicate was applied for ML bootstrapping.

2.4 | 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) data sets. As the presence of identical sequences may result in artefactual species trees (Hoef-Emden, 2012), we merged all identical sequences in our data set. First, we performed the Bayesian analyses with BEAST 1.8.2 (Drummond, Suchard, Xie, & Rambaut, 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 (Supporting Information 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, Drummond, Xie, Baele, & Suchard, 2018), and the five tree files were merged using the burn-in set to 3 million generations (all ESS values of the merged data set 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. At last, 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 P_{min} 0.001, P_{max} 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 (a) the original name given to the lineage when it was first published (e.g., A9); (b) the name of a known species that had been formally described in previous phylogenetic studies; (c) the names *A. aff. irregularis*, *A. aff. italiana* and *C. aff. ellipsoideum* indicating affinity to that species; or (d) 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 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, and therefore, the species names (*S. vesuvianum*, *S. azoreum* and *S. nanodes*) were assigned to the lineages only when their identity was obvious.

2.5 | Variation partitioning

From the entire data set 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 data set 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 analysed 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 5 km 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). Therefore, PC1–PC4 were selected. Based on an analogy, the presence/absence matrix of 12 substrate/habitat variables (Supporting Information Table S5) was 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 neighbour matrices (PCNM) vectors representing the geographical distances at various spatial scales (Borcard, Legendre, Avois-Jacquet, & Tuomisto, 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 for the analysis. All analyses were performed in R (R Core Team, 2017).

2.6 | 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 this study, these environmental dimensions were described based on 19 Bioclim variables (Karger et al., 2017). The climatic hypervolumes were constructed by multivariate kernel density estimation (Blonder, Lamanna, Violle, & Enquist, 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 5,000 random background points, using the alpha-hull 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 was inspected as correlation between the number of accepted partners and size of climatic hypervolume. As 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 (Supporting Information 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. As 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 μ_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 burn-in. We fit the regression model in program JAGS 4.2.0 (Plummer, 2003) through the R2JAGS package in R.

3 | RESULTS

3.1 | 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 (Supporting Information Table S1), and the alignments have been deposited as Supporting Information Appendices S1–S3.

Based on 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 Supporting Information 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* data set 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 Supporting Information Figure S3. The phylogenetic relationships are congruent with those identified by Darienko et al. (2010). According to the three DNA species delimitation analyses (GMYC, bPTP and ABGD), putative species boundaries in *Chloroidium* data set were estimated. The species were delimited based on the consensus of these analyses, leading to the delimitation of 12 species clusters. The *Chloroidium* phycobionts analysed 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* data set was previously analysed 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

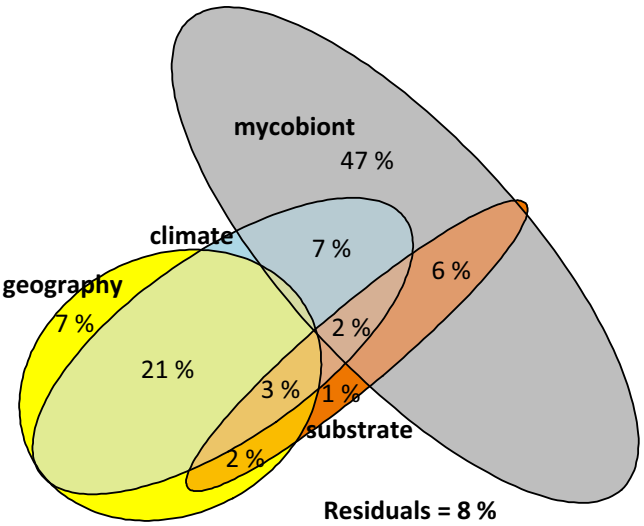


FIGURE 2 Venn's diagram showing the variation in distribution of phycobionts associated with the lichen-forming fungal genus *Stereocaulon* explained by effects of climate, substrate/habitat, geographical distance and the mycobiont [Colour figure can be viewed at wileyonlinelibrary.com]

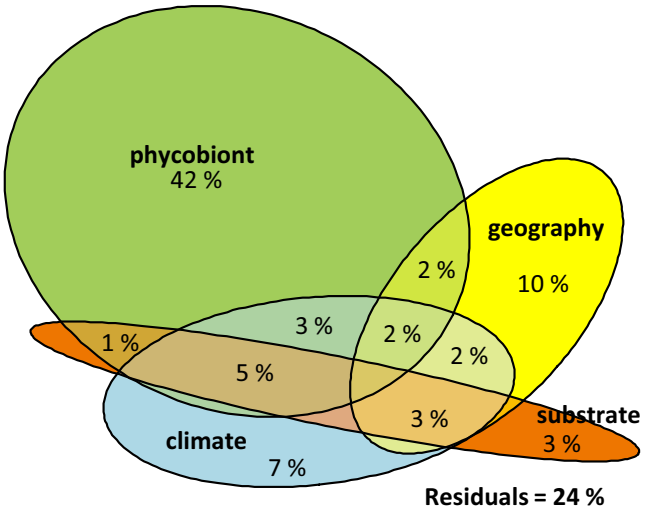


FIGURE 3 Venn's diagram showing the variation in distribution of *Stereocaulon* mycobionts explained by effects of climate, substrate/habitat, geographical distance and the phycobiont [Colour figure can be viewed at wileyonlinelibrary.com]

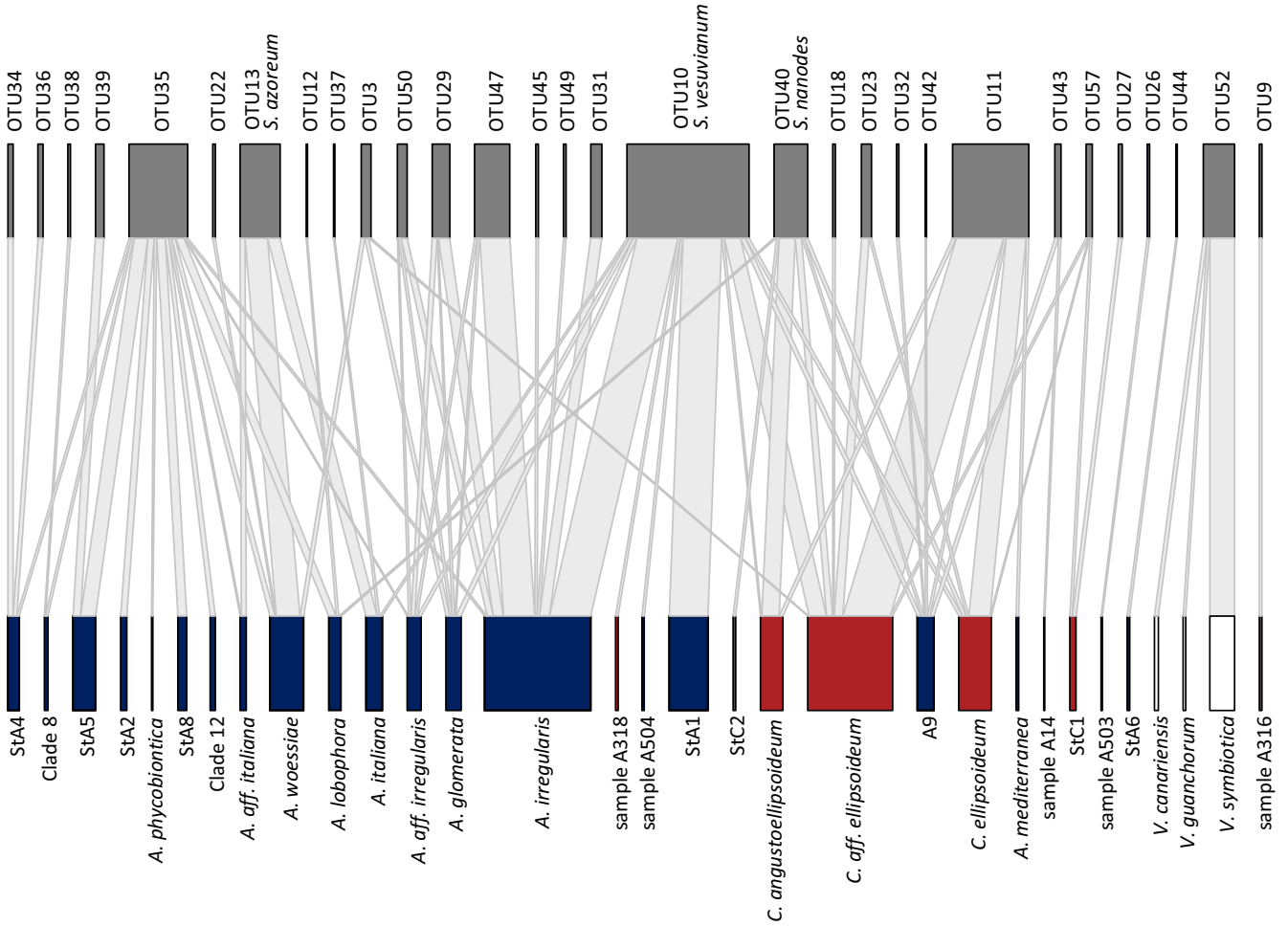


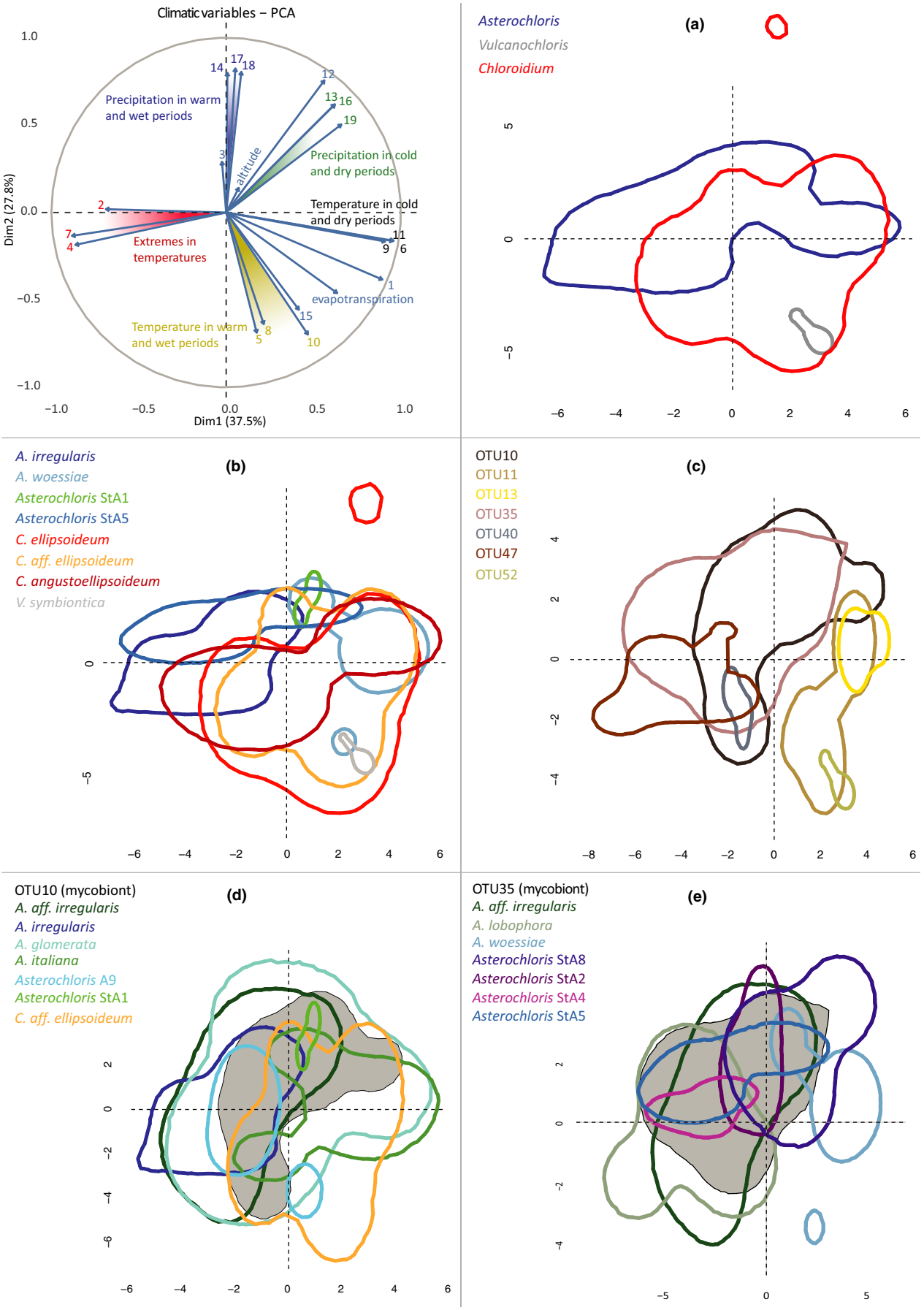
FIGURE 4 Interaction network structure between lichen mycobiont species-level lineages in the genus *Stereocaulon* and phycobiont species-level lineages. The width of the links is proportional to the number of specimens forming the association [Colour figure can be viewed at wileyonlinelibrary.com]

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 data set 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.

FIGURE 5 Climatic niche hypervolumes for (a) algal genera *Asterochloris*, *Vulcanochloris* and *Chloroidium*, (b) eight most abundant algal species-level lineages (phycobionts), (c) seven most abundant fungal species-level lineages (mycobionts), (d) fungal OTU10 (grey filled) with its seven most abundant (of total 11) associating phycobionts, (e) fungal OTU35 (grey filled) with its seven 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)


Climatic variables – PCA
Dim1 (37.5%)
Dim2 (27.8%)
Precipitation in warm and wet periods
altitude
Precipitation in cold and dry periods
Temperature in cold and dry periods
Extremes in temperatures
evapotranspiration
Temperature in warm and wet periods
Asterochloris
Vulcanochloris
Chloroidium
(a)
A. irregularis
A. woessiae
Asterochloris StA1
Asterochloris StA5
C. ellipsoideum
C. aff. ellipsoideum
C. angustoellipsoideum
V. symbiontica
(b)
OTU10
OTU11
OTU13
OTU35
OTU40
OTU47
OTU52
(c)
OTU10 (mycobiont)
A. aff. irregularis
A. irregularis
A. glomerata
A. italiana
Asterochloris A9
Asterochloris StA1
C. aff. ellipsoideum
(d)
OTU35 (mycobiont)
A. aff. irregularis
A. lobophora
A. woessiae
Asterochloris StA8
Asterochloris StA2
Asterochloris StA4
Asterochloris StA5
(e)

3.2 | 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 (Figures 2 and 3). We analysed the relative contributions of climate, habitat/substrate, geographical distance and symbiotic partner to phycobiont and mycobiont distribution.

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, although 33% was shared with other variables. The variables associated with substrate and habitat independently explained 1% of the variability, although 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, although 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, although 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).

3.3 | 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. In conclusion, *Chloroidium* phycobionts were found at an annual mean temperature above 0°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.

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). It is an interesting fact that *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; Supporting Information Figure S4).

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 data set 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; Supporting Information Figures S5 and 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).

4 | DISCUSSION

4.1 | 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 (Supporting Information Figure S2), although the second and the third most prevalent genera are *Chloroidium* and *Vulcanochloris*, respectively.

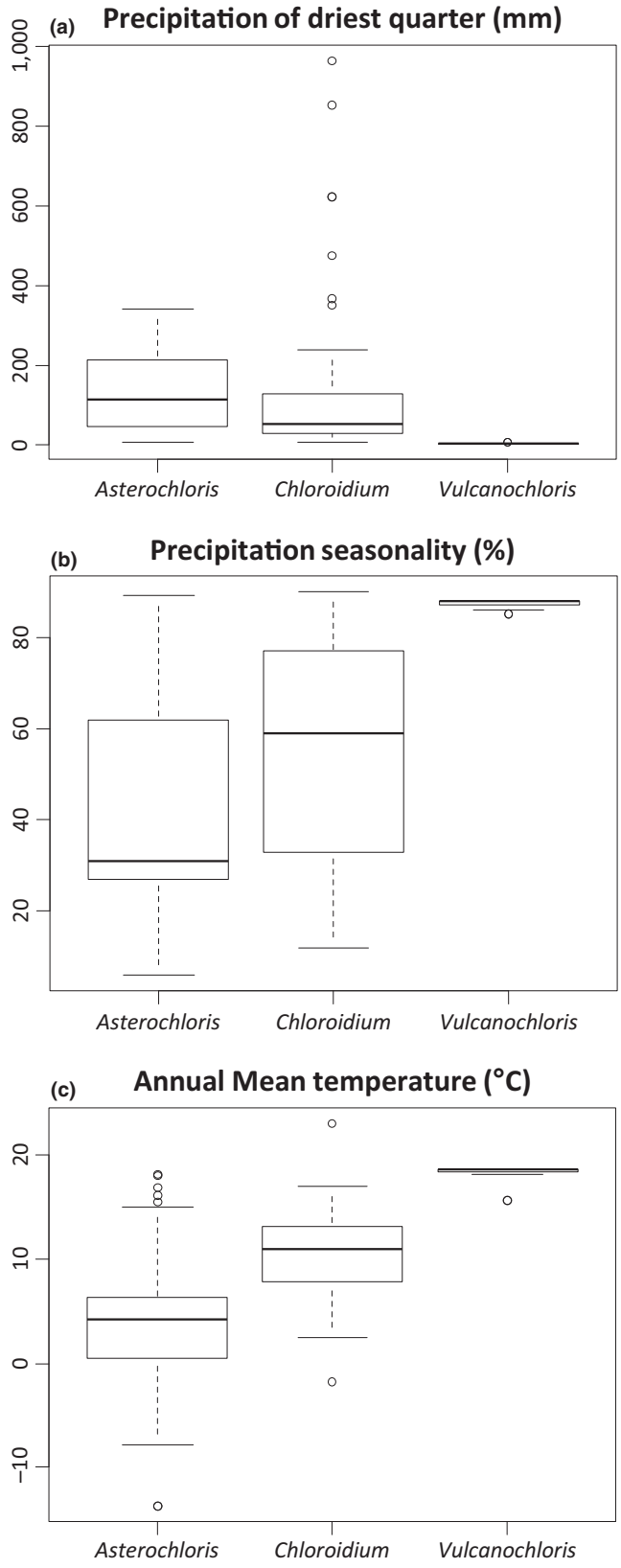


FIGURE 6 Differences in the distribution of three phycobiont genera associated with the lichen-forming fungal genus *Stereocaulon* along the gradient of (a) precipitation of driest quarter; (b) precipitation seasonality; (c) annual mean temperature

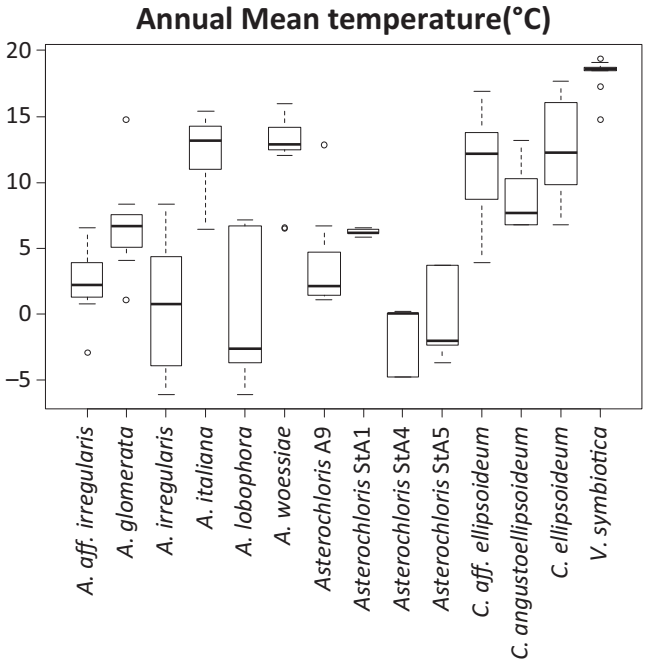


FIGURE 7 Differences in the distribution of 14 most abundant (≥5 specimens) phycobiont species-level lineages associated with the lichen-forming fungal genus *Stereocaulon* along the gradient of annual mean temperature

The 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, for example, *Protoparmelia*, *Rhizoplaca*, *Tephromela*, *Xanthoparmelia*, *Xanthoria* and *Xanthomendoza* (Leavitt et al., 2013, 2016; Muggia et al., 2014; Muggia, Leavitt, & Barreno, in press; 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). It is an interesting fact that 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., 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 (Supporting Information Figure S3) and four samples from Central America probably represent still undescribed species within *Chloroidium*. Also, Sanders, Pérez-Ortega, Nelsen, Lücking, and de los Ríos (2016)

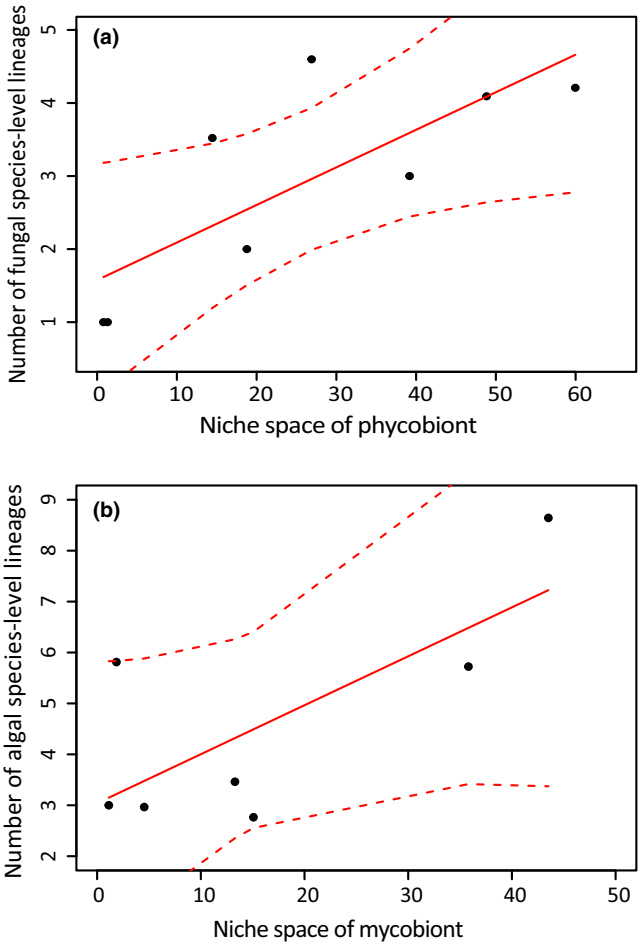


FIGURE 8 (a) Bayesian linear regression of algal niche space (hypervolume) as a predictor of the number of accepted species-level fungal lineages. (b) Bayesian linear regression of fungal niche space (hypervolume) as a predictor of the number of accepted species-level algal lineages. Dashed lines show the 95% CRI around the regression line [Colour figure can be viewed at wileyonlinelibrary.com]

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). By bad luck, 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 analyse 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, Molins, Martinez-Alberola, Muggia, & Barreno, 2017; Muggia, Baloch, Stabentheiner, Grube, & Wedin, 2011; Muggia et al., 2014; Onuț-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, Devlin-Durante, & Lajeunesse, 2014). Particular lineages of *Symbiodinium* show distinct ecological preferences (Baker, 2003; Pettay, Wham, Smith, Iglesias-Prieto, & LaJeunesse, 2015; Rowan, 2004), and some are well adapted to high temperatures and irradiance (Iglesias-Prieto, Beltrán, LaJeunesse, Reyes-Bonilla, & Thomé, 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, Carmi-Veal, Fine, & Goulet, 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).

4.2 | 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*

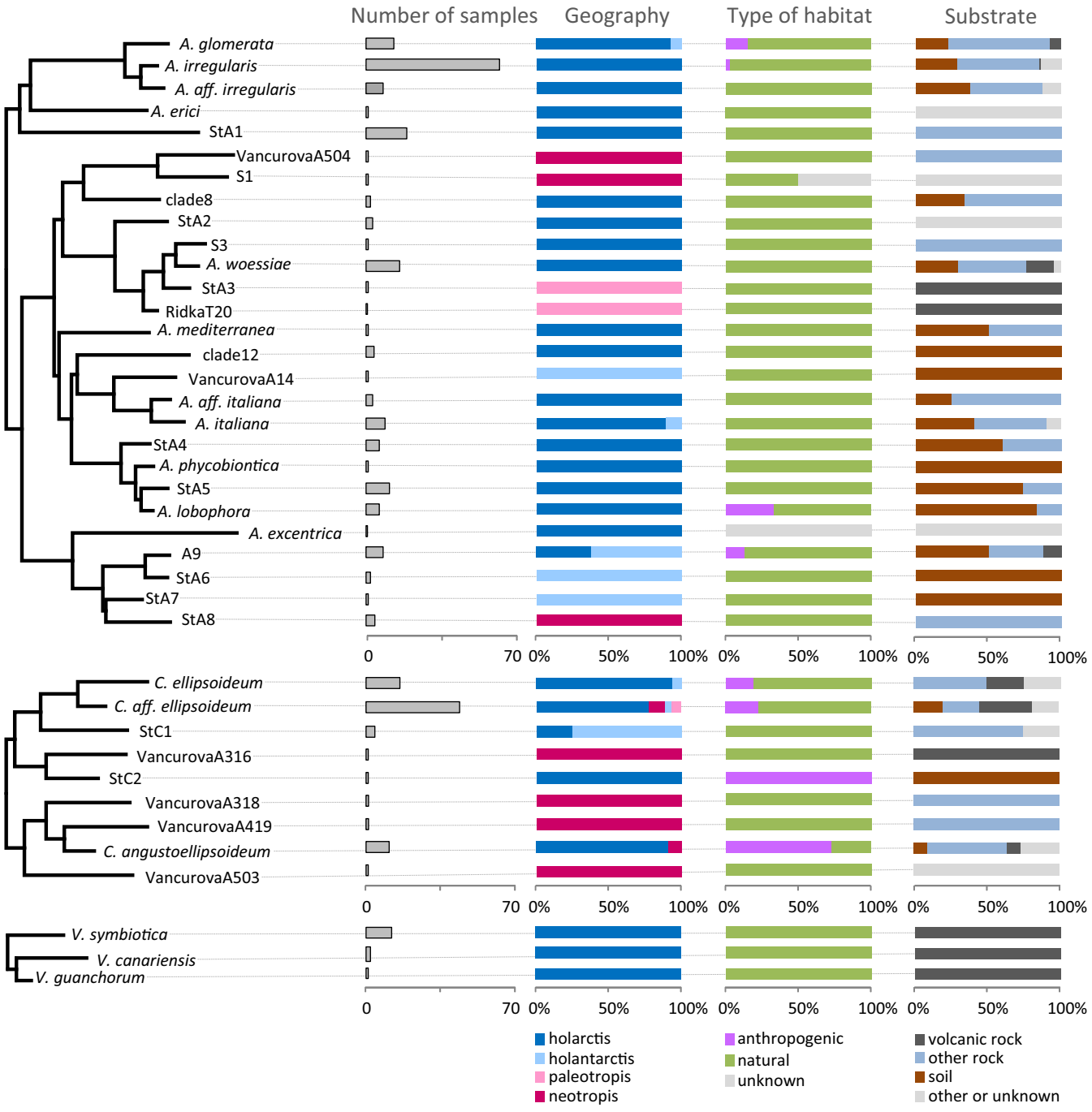


FIGURE 9 Visualized abundance, geographic and habitat, and substrate distribution of phycobionts associating with the lichen-forming genus *Stereocaulon*. From left to right: phylogenetic hypotheses based on ITS rDNA + actin type I gene (*Asterochloris*) or sole ITS rDNA sequences (other two genera);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

phycobionts are distributed in areas with an annual mean temperature above 0°C. Although 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* harbours *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 data set. 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/year 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, Hell, Braga, del Campo, & Casano, 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" harbouring distinct flora, including lichens (Medeiros et al., 2014). Many of the analysed samples came from metal-rich substrates, which in our data set 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, Hoppert, Mudimu, & Friedl, 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 metal (Cu, Zn, Pb, Cd and Ni) levels vary according to the growth morphology of the lichens (Bačkor et al., 2010; Rola, Osyczka, & Kafel, 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, Bianchi, & Pieri, 1996; Cutler, Belyea, & Dugmore, 2008; Stretch & Viles, 2002) principally associate with *Chloroidium* and *Vulcanochloris* phycobionts (Figure 9).

In contrast, *Asterochloris* species rarely associated with *Stereocaulon* species colonizing metal-rich substrates. On volcanic rocks, mainly *Asterochloris woessiae* was recovered. At an earlier time, 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* are 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, Elster, Kováčik, & Škaloud, 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, Hrčková, & Škaloud, 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, Brunauer, & Türk, 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.

4.3 | 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, although 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.

As mutualistic symbiosis can affect niche width (Duffy & Johnson, 2017; Gerz, Guillermo Bueno, Ozinga, Zobel, & Moora, 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.

It is an interesting fact that 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: (a) Most of our samples came from anthropogenic habitats (as indicated by substrate and habitat variables including plastic, metal, mine and sludgebed). A similar behaviour 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., 2013); and (b) 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. Without much doubt, 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, Büdel, Hurry, & Green, 2017; Matos et al., 2015).

5 | 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.

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DATA ACCESSIBILITY

DNA sequences are available in GenBank under Accession nos MH382116–MH382150 and MH414969–MH415451. DNA alignments are available as Supporting Information Appendices 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. analysed the data. L.V., O.P., L.M. and P.Š. wrote the manuscript.

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