RESEARCH ARTICLE

Macroecological diversification and convergence in a clade of keystone symbionts

Matthew P. Nelsen[1,*,†], Steven D. Leavitt[2], Kathleen Heller[1,3], Lucia Muggia[4] and H. Thorsten Lumbsch[1]

[1]The Field Museum, Negaunee Integrative Research Center, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA, [2]Department of Biology and M. L. Bean Life Science Museum, Brigham Young University, 4102 Life Science Building, Provo, UT 84602, USA, [3]Biological Sciences Division, University of Chicago, 5841 S. Maryland Avenue, Chicago, IL 60637, USA and [4]Department of Life Sciences, University of Trieste, via Giorgieri 10, 34127 Trieste, Italy

*Corresponding author: The Field Museum, Negaunee Integrative Research Center, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA. Tel: 312.922.9410; E-mail: mpnelsen@gmail.com

One sentence summary: Lichen algae convergently evolved to occupy shared climates and habitats at a gradual rate, and broadly coincident with the evolution of these biomes and associated taxa.

†Matthew P. Nelsen, http://orcid.org/0000-0002-6866-815X

ABSTRACT

Lichens are classic models of symbiosis, and one of the most frequent nutritional modes among fungi. The ecologically and geographically widespread lichen-forming algal (LFA) genus *Trebouxia* is one of the best-studied groups of LFA and associates with over 7000 fungal species. Despite its importance, little is known about its diversification. We synthesized twenty years of publicly available data by characterizing the ecological preferences of this group and testing for time-variant shifts in climatic regimes over a distribution of trees. We found evidence for limited shifts among regimes, but that disparate lineages convergently evolved similar ecological tolerances. Early *Trebouxia* lineages were largely forest specialists or habitat generalists that occupied a regime whose extant members occur in moderate climates. *Trebouxia* then convergently diversified in non-forested habitats and expanded into regimes whose modern representatives occupy wet-warm and cool-dry climates. We rejected models in which climatic diversification slowed through time, suggesting climatic diversification is inconsistent with that expected under an adaptive radiation. In addition, we found that climatic and vegetative regime shifts broadly coincided with the evolution of biomes and associated or similar taxa. Together, our work illustrates how this keystone symbiont from an iconic symbiosis evolved to occupy diverse habitats across the globe.

Keywords: climate; Cenozoic; lichen; photobiont; symbiosis; *Trebouxia*

INTRODUCTION

Biotic interactions are diverse and pervasive in nature, where they differentially influence organismal fitness and shape the evolution of interacting lineages (Simpson 1953; Ehrlich and Raven 1964; Vermeij 1994; Jablonski 2008; Chomicki *et al.* 2019; Harmon *et al.* 2019; Hembry and Weber 2020). Individual species may be directly influenced by shifts in abiotic variables, such as climate, which may indirectly influence the identity and range of species they interact with (Gilman *et al.* 2010; Walther 2010; Harley 2011; Colwell, Dunn and Harris 2012; Blois *et al.* 2013). Symbioses may be especially vulnerable to shifts in biotic and

abiotic variables, as they may become destabilized through local extirpation of individual symbionts or alteration of cost: benefit ratios (Sachs and Simms 2006; Dunn et al. 2009; Kiers et al. 2010; Colwell, Dunn and Harris 2012; Kikuchi et al. 2016; Sampayo et al. 2016; Baker et al. 2018; Steidinger et al. 2019; Chomicki, Kiers and Renner 2020; Rolshausen et al. 2020). Thus, understanding the macroecological preferences of symbionts in a phylogenetic framework can reveal the processes underlying niche diversification and insight into their potential responses to environmental perturbations.

Lichens, symbiotic associations between heterotrophic fungi and photosynthetic algae or cyanobacteria, are classic examples of symbiosis (Frank 1877; de Bary 1879) and contribute important ecosystem services at local and global scales (Larson 1987; Seaward 2008; Elbert et al. 2012; Porada et al. 2014; Rutherford et al. 2017; Porada, Stan and Kleidon 2018). The facultatively lichenized green algal genus *Trebouxia* (Tschermak-Woess 1978; Mukhtar, Garty and Galun 1994; Sanders and Lücking 2002; Sanders 2005; Hedenäs, Blomberg and Ericson 2007) is one of the most taxonomically diverse and frequent lichen symbionts—supporting over 7000 species of lichen-forming fungi (LFF) (this number is obtained by summing richness estimates (Lücking, Hodkinson and Leavitt 2017) of families and genera exclusively or predominantly associated with *Trebouxia* (Miadlikowska et al. 2014)) from across a wide range of fungal classes. This number exceeds the diversity of extant zooxanthellae-associated scleractinian coral species (~800) (Hoeksema and Cairns 2020) and extant mammal species (~6500) (Burgin et al. 2018), while approaching the maximum estimated number (8500) of ectomycorrhizal plant species (Brundrett and Tedersoo 2018).

The globally distributed genus *Trebouxia* occupies diverse terrestrial biomes, but is most common in temperate, boreal, polar and alpine habitats (Honegger 1998, 2009; Sanders 2001; Pérez-Ortega et al. 2012; Ruprecht, Brunauer and Printzen 2012; Wagner et al. 2020). Climatic variables play important roles in structuring lichen associations (Peksa and Škaloud 2011; Fernández-Mendoza and Printzen 2013; Leavitt et al. 2015; Singh et al. 2017; Dal Grande et al. 2018; Vančurová et al. 2018); however, our understanding of the ecological tolerances of lichen-forming algae (LFA) has long lagged behind those of LFF (Cordeiro et al. 2005). Understanding their tolerances is important as switching algal symbionts may extend the range of the fungus through association with a locally-adapted algal partner or enable the continued persistence in localities undergoing changing environmental conditions (Yahr, Vilgalys and DePriest 2006; Nelsen and Gargas 2008, 2009; Ellis 2012; del Campo et al. 2013; Singh et al. 2017; Dal Grande et al. 2018; Rolshausen et al. 2018, 2020).

Presently, a global synthesis of the phylogenetic *and* ecological diversity of *Trebouxia* is lacking. While several efforts have been made to characterize climatic tolerances of *Trebouxia* algae, they are limited in their geographic scope or to associations with closely related species of lichen-forming fungi (Fernández-Mendoza et al. 2011; Werth and Sork 2014; Lutsak et al. 2016; Singh et al. 2017; Dal Grande et al. 2018; Rolshausen et al. 2018; Mark et al. 2020). Given its high taxonomic diversity, widespread geographic and ecological occurrence, and importance to diverse lineages of LFF (Tschermak-Woess 1988; Voytsekhovich, Mikhailyuk and Darienko 2011; Miadlikowska et al. 2014; Leavitt et al. 2015; Muggia, Leavitt and Barreno 2018; Muggia et al. 2020), it is critical to understand how this macroecological diversity evolved. Ascertaining the evolutionary lability of macroecological preferences of *Trebouxia* algae is important for understanding how deeply entrenched climatic tolerances are in the evolutionary history of these algae. For instance, niche

evolution may have evolved at a constant rate over time (Fig. S1, Supporting Information). Under this scenario, niche divergence between taxa will be linked to the time since these two taxa diverged from one another. Thus, more closely-related taxa are expected to be more similar to one another, with phylogenetic relationships being strong predictors of niche preferences. Trait evolution may instead increase over time (Fig. S1, Supporting Information). For example, ecological diversification may have occurred more recently, such that sub-clades substantially overlap in their occupation of different adaptive or ecological regimes—highlighting their evolutionarily lability (Simpson 1944; Schluter 2000; Harmon et al. 2003).

In contrast, ecological diversification may decrease over time (Fig. S1, Supporting Information). For instance, under an adaptive radiation, lineages rapidly diversify to fill unoccupied adaptive or ecological zones; however, confinement to these zones ultimately limits diversification, which will slow over time (Simpson 1944; Schluter 2000; Harmon et al. 2003). Consequently, niche disparity will be disproportionately concentrated in the early history of a clade, and occupation of distinct adaptive or ecological regimes will be phylogenetically conserved. Such phylogenetic niche conservatism may reflect an inability to disperse to, adapt to, or persist in new adaptive zones; consequently, such lineages may be especially prone to extinction in the face of shrinking habitats or shifting environmental conditions (Crisp and Cook 2012). Several clades of lichen-forming fungi have been suggested to represent adaptive radiations (Lumbsch et al. 2010; Divakar et al. 2013; Gaya et al. 2015; Kraichak et al. 2015; Schneider, Resl and Spribille 2016), including many which associate with *Trebouxia* algae or related lineages, and raises the question of whether *Trebouxia* itself has undergone an adaptive radiation. Moreover, understanding how these algae diversified to occupy unique climatic regimes can also provide insight into the niche differentiation of obligately lichenized fungi associated with *Trebouxia*. Thus, as these algae support diverse fungi, understanding their macroclimatic tolerances and their evolution can shed insight into the evolution of the symbiosis itself.

Here, we synthesize over 20 years of publicly available sequence data to estimate climatic preferences of individual *Trebouxia* algae. We first test whether the rate of climatic niche evolution has decreased through time as expected under an adaptive radiation. We then infer when the invasion of different climatic and vegetative regimes occurred, while placing this in a paleoecological context. Together, this synthesis provides a framework within which the macroecological diversification of the most common clade of lichen symbionts can be better understood.

MATERIALS & METHODS

Data acquisition

In the absence of the ability to evenly sample algae from across the globe, and sequence them using long-read technology, we instead utilized over 20 years of existing barcode data to address these questions. As our goal was to maximize sample size, we concentrated on the internal transcribed spacer (ITS) region of the ribosomal cistron, which is the most commonly sequenced locus for *Trebouxia* algae and has been provisionally regarded as a barcoding marker (Grube and Muggia 2010; Leavitt et al. 2015; Muggia, Leavitt and Barreno 2018). We periodically queried NCBI for *Trebouxia* ITS sequences between March 2013 and June 2018 using the search criteria 'internal transcribed spacer OR 5.8S AND *Trebouxia*[ORGN] AND Chlorophyta[ORGN]'. Accessions

not in our database were downloaded, parsed, and metadata (country, coordinates) recorded. As our final data query was in June 2018, data from more recent studies (Mark *et al.* 2020; Muggia *et al.* 2020; Ruprecht *et al.* 2020; Wagner *et al.* 2020) were not included. We then removed the 18S and 28S using ITSx 1.0.9 (Bengtsson-Palme *et al.* 2013) and a custom hidden Markov model (HMM) that included a greater representation of *Trebouxia* sequences than the default HMM included with ITSx. Sequences were filtered to retain those with a minimum length of 525 bp, maximum length of 700 bp, and no more than three ambiguously coded nucleotides using mothur 1.33.3 (Schloss *et al.* 2009). Following this, sequences were then compared to a database of known sequences using blastn 2.2.29+ (Altschul *et al.* 1990). This database contained ITS sequences of fungi, other lichen-associated algae, and representatives of the four traditional *Trebouxia* clades ('A', 'I', 'C', 'S') *sensu* (Friedl *et al.* 2000; Helms 2003; Leavitt *et al.* 2015). Recent work (Xu *et al.* 2020) has elevated several clade 'S' accessions included in the present study to that of a new clade ('D'). As this was published well after our work was underway, we have retained the traditional four clade system, and emphasize that nearly all analyses were performed over a distribution of topologies (see below) to account for topological uncertainty. Sequences were then assigned to one of the four traditionally-recognized *Trebouxia* clades based on their top match, and sequences matching non-*Trebouxia* accessions were removed. Finally, geographic metadata, such as coordinates and country, were updated or added in consultation with the literature.

Operational Taxonomic Unit (OTU)-selection

Here we adopted an OTU-based approach to group accessions based on sequence similarity. We caution that OTUs are not equivalent to species, and are instead better considered 'species hypotheses' (SH)—reflecting the unknown and imperfect correspondence between the two approaches (Kõljalg *et al.* 2013). Consequently, we regard these OTUs an approximation of species. The OTU-based approach has the benefit of being able to utilize the large number of ITS sequences retrieved, is comparatively fast relative to other methods, and reflects approaches frequently employed in other microbial lineages (Kõljalg *et al.* 2013). The pipeline utilized was similar to that of UNITE (Kõljalg *et al.* 2013), which has been used to delimit SH from fungal ITS sequences. Previous work (Leavitt *et al.* 2015) on *Trebouxia* algae has suggested a 97.5% sequence similarity threshold largely corresponded with groupings designated using the Automatic Barcode Gap Discovery method (ABGD) (Puillandre *et al.* 2012); consequently we adopted this sequence similarity threshold to designate OTUs. First, sequences for each major clade were condensed into OTUs at 97.5% sequence similarity over 70% of the compared sequences using blastclust 2.2.26 (Altschul *et al.* 1997), and a consensus sequence generated for each OTU using megablast 2.2.26 (Zhang *et al.* 2000). For each OTU, the accession that most closely matched this OTU consensus sequence was selected as the representative sequence for that OTU.

Alignment

Representative sequences for each major clade were then separately aligned using MAFFT 7.402 (Katoh and Standley 2013). We then used profile aligning in MAFFT to align the four major clade alignments to one another and add accessions of the related genera *Asterochloris* and *Vulcanochloris* as outgroups. This produced a core alignment containing representatives from across the phylogeny. There is a long history of retaining the most variable portions of the ITS when inferring phylogenetic relationships across *Trebouxia* (Kroken and Taylor 2000; Hauck, Helms and Friedl 2007; Muggia, Grube and Tretiach 2008; Werth 2012; Werth and Sork 2014; Leavitt *et al.* 2015; Mark *et al.* 2020). We adopted this approach to retain distinct OTUs, which may have otherwise been collapsed through the removal of variable portions of the alignment. Arguments may be made that genomic data should instead be utilized, but we lack genomic data from a comparable number of globally sampled accessions. Additionally, the utilization of more conserved markers for individual OTU representatives may increase support along the backbone, as in recent work (Muggia *et al.* 2020). However, multi-locus barcoding efforts and phylogenetic inference will also face challenges, as it will be difficult to link loci in samples containing multiple algal individuals unless long-read sequencing technologies are employed. Future efforts may also benefit from grafting approaches (Beaulieu *et al.* 2012; Uyeda *et al.* 2017) that link resolved clade-level phylogenies to a resolved *Trebouxia* backbone.

Phylogenetic inference

A maximum likelihood (ML) analysis using the GTRGAMMA substitution model was conducted in RAxML (Stamatakis 2014), and 1000 fast bootstrap replicates (Stamatakis, Hoover and Rougemont 2008) performed. A Bayesian analysis was subsequently conducted in BEAST 2.4.4 (Bouckaert *et al.* 2014) using the GTR+G substitution model, a Yule tree prior, and a diffuse gamma prior ($\alpha = 0.001$, $\beta = 1000$) on the ucld.mean. As the individual monophyly of the three genera included (*Asterochloris*, *Vulcanochloris* and *Trebouxia*) and the *Asterochloris+Vulcanochloris* relationship are well-established (Friedl and Zeltner 1994; Friedl and Rokitta 1997; Škaloud and Peksa 2010; Vančurová *et al.* 2015) and received ML bootstrap support values greater than 70 here, we constrained them in our subsequent dating analysis to help root the phylogeny concentrate on inferring relationships among *Trebouxia* OTUs. It is common practice in dating analyses to constrain nodes that have age priors placed on them or to facilitate rooting (Gaya *et al.* 2015; Lutzoni *et al.* 2018). In our dating analysis, we utilized secondary calibrations derived from a previous study (Nelsen *et al.* 2020a) to impose age priors on three nodes. Normal priors encompassing the 95% HPD age estimates (Nelsen *et al.* 2020a) of *Asterochloris+Vulcanochloris* (mean: 68.0, sigma: 27.5), the root (mean: 279.8, sigma: 63.5), and the crown of *Trebouxia* (mean: 121.4, sigma: 34.5) excluding accession AB177830 (OTU $G14_{97.5}$) were employed. This accession was excluded because ML analyses placed this OTU outside of the major *Trebouxia* clades (with low support) and sister to the remainder of *Trebouxia*. Since the age prior was based on the most recent common ancestor (MRCA) of the four major *Trebouxia* clades, this prior was only applied to them. This had the effect of allowing this OTU to group with one of the major clades, in which case the age prior would be applied to the crown of all of *Trebouxia*, or to occupy a position sister to the remainder of *Trebouxia*, in which case the age prior would be applied to the MRCA of the four major *Trebouxia* clades. Additionally, uniform priors (minimum: 1, maximum: 500) were applied to the MRCA's of *Asterochloris* and *Vulcanochloris*. The analysis was run for 150 million generations, sampling every 10,000. A burnin of 25% was applied and ESS values calculated and all found to be over 200. Trees were subsampled every 20,000 generations, and a maximum clade credibility (MCC) tree calculated using median node heights.

Geographic and climatic data

Country and geographic coordinates associated with sequences were retrieved from NCBI when available. Primary publications were consulted to obtain data not located in NCBI accessions. Sequence abundance was assigned to individual administrative units and continents using the ISOA3 and continental designations provided by Natural Earth (http://www.naturalearthdata.com/downloads/10m-cultural-vectors/10m-admin-0-details/). We made minor modifications to continental designations to better reflect geography: Russia was changed to be part of Asia, rather than Europe, and Trinidad and Tobago changed to be part of South America, rather than North America. The geographic distributions of sampled specimens were then plotted to illustrate the number of sequences derived from each country.

We then verified coordinate data using the nearestCountry function in rangeBuilder (Title 2017). Our dataset included a high number of samples from Antarctica; however, the World-Clim (Hijmans et al. 2005) database lacks models for this region, and only a limited number of variables have been extended to include this region (Wagner et al. 2018). We instead utilized 19 bioclimatic variables derived from the MERRAclim (Vega, Pertierra and Olalla-Tárraga 2017) dataset, which included estimates for Antarctica, and yields comparable values to those in WorldClim. For each variable (estimated under the mean relative humidity value), we created a layer that averaged data for the three available decades (1980s, 1990s and 2000s) at a resolution of 2.5 arc sine minutes. Values were then extracted for individual coordinate sets. Temperature variables were divided by 10 to place values on the degrees Celsius scale, while specific humidity values were retained at the scale provided. Mean estimates for each bioclimatic variable were then calculated for each OTU and used in downstream analyses (Smith and Beaulieu 2009). As in other studies that have characterized the ecological or climatic tolerances of algal OTUs, we did not rarefy or weight samples to reduce spatial sample clustering (Singh et al. 2017; Rolshausen et al. 2018).

Ordination of climatic data

We visualized the distribution of OTUs in multidimensional climate space using a phylogenetic principal components analysis (pPCA) (Revell 2009). This analysis was conducted in phytools (Revell 2012) using the pruned MCC tree (only including OTUs with climatic data) and a correlation matrix with the correlation structure obtained under Brownian motion (BM). Results were plotted using a modified version of the ggbiplot function in ggbiplot (Vu 2011).

Multivariate climatic niche evolution

We employed a two-step approach to identify multivariate climatic regimes and subsequently determine whether transitions among these adaptive regimes were best characterized by a time-invariant or time-variant model of evolution. Since PCA and pPCA axes may erroneously appear to have evolved under an early burst model of evolution (Uyeda, Caetano and Pennell 2015)—a model we explicitly test below—we instead relied on a restricted set of variables to characterize the macroclimatic niche of lichen algae. The microclimate in which lichens are active may differ substantially from the macroclimate (Kershaw 1985); however, in the absence of detailed microhabitat data, we relied on macroclimatic estimators of mean annual temperature

(BIO1) and specific humidity (BIO12) to characterize the niche of *Trebouxia* algae. Temperature and moisture are important factors regulating photosynthetic performance in lichen algae (Green, Nash and Lange 2008) and setting range limits in diverse lineages (Liu, Ye and Wiens 2020).

We then utilized l1ou (Khabbazian et al. 2016) to model evolutionary shifts in climatic regimes. The l1ou package identifies the optimal number and location of shifts in phenotypic regimes using the phylogenetic Least Absolute Shrinkage and Selection Operator (LASSO) (Khabbazian et al. 2016). For each trait, l1ou constrains the selection and rate parameters across adaptive regimes, while allowing the optima to vary across regimes (Khabbazian et al. 2016). A maximum of 42 rate shifts (approximately half the number of taxa) were permitted, with the 'OUfixedRoot' model applied to the root state, the best shift configuration selected using the phylogenetic Bayesian Information Criterion (pBIC) (Khabbazian et al. 2016), and default values used for remaining arguments. Convergent regimes were subsequently identified using backward steps and again evaluated using the pBIC.

We then tested whether shifts among these multivariate adaptive regimes were best-characterized as being time-invariant, or exponentially increasing or decreasing through time. To achieve this, we adopted an approach in which discrete Markov models of trait evolution (ER: equal transition rates among all states; SYM: transition rates between individual pairs of states were symmetric; ARD: all transition rates may be unique) were fit with and without branch length transformations (Slater and Friscia 2019). We first fit the three models in geiger (Pennell et al. 2014) without branch length transformations, and then conducted two additional sets of analyses using the EB transformation with each model: one set (EREBD, SYMEBD, ARDEBD) in which rates decreased (D) through time (alpha constrained to be negative), and another (EREBI, SYMEBI, ARDEBI) in which rates increased (I) through time (alpha constrained to be positive). Support among the nine models (three time-constant, six time-variant) was then evaluated using the relative weights of the Akaike Information Criterion corrected for small samples ($AICc_w$). Analyses were conducted using the MCC tree and a random sample of trees derived from the posterior, thereby integrating over topological and temporal uncertainty. We randomly sampled 1000 trees from the posterior, but due to the computationally-intense nature of these analyses, we subsequently restricted analyses to 100 trees randomly derived from this subset. All trees were pruned to only include taxa with climatic data.

Diversification in non-forested habitats

Finally, we assessed when OTUs became highly dependent on and diversified in non-forested habitats. We divided their occurrence into two coarse character states that reflected a full or partial-dependence on forested habitats (closed-canopy) versus a strict occupation of non-forested, open canopy habitats. To achieve this, we first extracted vegetative biome information (Olson et al. 2001) for unique sets of coordinates from each OTU, and determined the OTU occurrence frequency for each biome. A small number of records (76) and OTUs (5) that possessed bioclimatic information lacked biome designations. The proportion of accessions recorded from biomes with a more or less continuous canopy of trees (forested) versus those occupying open canopy (non-forested) biomes (Table S1, Supporting Information) was then calculated. OTUs that occupied forested biomes

at a frequency of 100%–33% were then treated as relying exclusively or partially on forested biomes (1). In contrast, those that primarily occupied non-forested habitats (<33% of occurrences in forested biomes) were considered to rely exclusively on non-forested habitats (2). We fit the all rates different (ARD) model of trait evolution to the MCC tree and 1000 trees derived from the posterior (described above) using the raydisc function in corHMM (Beaulieu and O'Meara 2017). Marginal probabilities for node states were estimated, and probabilities at the root were weighted using the 'maddfitz' approach (FitzJohn, Maddison and Otto 2009).

Analyses were performed at the Grainger Bioinformatics Center (Field Museum) and the CIPRES portal (Miller, Pfeiffer and Schwartz 2010), and data files and code are available on GitHub (https://github.com/mpnelsen/Trebouxia_Macroecology).

RESULTS

General

Our initial data set consisted of 6180 accessions, 5329 ('A': 2178; 'I': 1050; 'C': 314; 'S': 1787) of which passed quality control filters. At a sequence similarity of 97.5%, we recovered 137 OTUs ('A': 72; 'I': 20; 'C': 23; 'S': 22), 91 of which ('A': 49; 'I': 11; 'C': 16; 'S': 15) that were represented by more than one accession, while 46 were singletons ('A': 23; 'I': 9; 'C': 7; 'S': 7). RAxML and BEAST analyses revealed that several clade 'A' OTUs with representative sequences differed solely in the presence or absence of large indels. Due to the high homology and the short branches formed discerning these pairs, we merged $A27_{97.5}$ (KR912550) into $A2_{97.5}$, $A24_{97.5}$ (KR913092) into $A7_{97.5}$, $A60_{97.5}$ (AJ007385) into $A5_{97.5}$, and $A56_{97.5}$ (HQ026172) into $A9_{97.5}$, resulting in a total of 133 OTUs with clade 'A' comprised of 47 multi-sequence OTUs, and 21 singletons (Table S2, Supporting Information).

The 133-tip ML and Bayesian topologies and support values are included in Figs S2 and S3 (Supporting Information). With the exception of clade 'C' in the ML analysis only, all four traditionally recognized clades were strongly supported as monophyletic (bootstrap proportion ≥ 70 and Bayesian posterior probability ≥ 0.95). Several clade 'S' OTUs ($S7_{97.5}$, $S10_{97.5}$, $S12_{97.5}$, $S13_{97.5}$, $S21_{97.5}$, $S22_{97.5}$) that formed a strongly-supported clade in both ML and Bayesian analyses have recently been elevated to a fifth major clade not designated here (see Materials & Methods for further discussion). As monophyly of clade 'C' was strongly supported in the Bayesian analysis, and the isolated clade 'C' OTU was not strongly supported in a contradictory location, we regarded clade 'C' as a monophyletic entity, and all subsequent analyses were performed on the 133 OTU data set. However, relationships among the four traditional clades remained uncertain, and we again emphasize that nearly all subsequent analyses were performed on a distribution of trees to account for topological and temporal uncertainty.

We then acquired country-level data from NCBI for 5097 (95.6%) of the sequences that passed quality control filters, and manually added or corrected country-level data for 495 accessions (Table S3 and Fig. S4, Supporting Information). Coordinates were acquired for 2359 (44.3%) accessions (660 from NCBI, while 1699 were manually added or corrected) that passed quality control filters (Fig. S5, Supporting Information). Together with the country-level data added, approximately 2200 geographic metadata improvements were made here.

Ordination of climatic data

The first three pPCA axes accounted for 94.2% of the variation (Fig. 1, Table 1, Figs 6 and 7, Supporting Information). The first PC was primarily associated with climatic variables that reflected a temperate (positive) to tropical (negative) gradient. PC1 (55.0% of variation) was associated with cooler temperatures (BIO1, BIO6, BIO8, BIO9, BIO10, BIO11) and lower specific humidity (BIO12, BIO13, BIO14, BIO16, BIO17, BIO18, BIO19), while PC2 (27.3% of variation) was linked to decreased seasonality (BIO4, BIO7, BIO15) and diurnal temperature range (BIO2). In contrast, PC3 (11.9% of variation) was largely negatively associated with diurnal temperature fluctuations and isothermality (BIO2, BIO3). At coarse scales, major clades often occupied somewhat distinct portions of the pPCA; however, substantial overlap existed such that OTUs from different clades exhibited similar climatic tolerances. Major clades varied substantially in their position along PC1, such that members of clade 'C' were largely restricted to warm and wet habitats, while members of clade 'S' occupied drier and cooler habitats. Members of clade 'I' occupied positions along PC1 that were somewhat intermediate between those of clades 'C' and 'S'. In contrast, members of clade 'A' were widely distributed across PC1. With the exception of clades 'C' and 'S', all clades overlapped in their position PC1. This trend was increased along the seasonality gradient (PC2), where all clades overlapped in their positions along this axis. Members of clades 'A', 'I' and 'S' were widely distributed along this axis, while members of clade 'C' occupied a more limited subset of the ranges occupied by other clades. We emphasize that exceptions to these broader trends certainly exist; for instance, many clade 'C' accessions lacking coordinate data were recovered from New Zealand and Russia, indicating this clade is not strictly confined to wet, tropical-warm temperate climates (Table S3, Supporting Information). Further sampling is required to verify the general patterns revealed here through this synthesis of 20 years of publicly available data.

Climatic niche evolution

We then accounted for topological and temporal uncertainty by performing subsequent analyses on both the MCC tree and 100 trees derived from the posterior. Our multivariate l1ou analyses recovered strong evidence for the diversification and convergent evolution of multiple unique climatic regimes. Six regime shifts among five unique climatic regimes were identified on the MCC tree (Fig. 2), while analyses of the 100 trees derived from the posterior recovered an average of 5.96 regimes (min = 4, max = 9) with an average of 7.98 regime shifts (min = 4, max = 14). Examination of the MCC tree revealed an ancestral regime characterized by taxa occupying moderate habitats loosely comparable to the average among extant taxa (Fig. 2). Regimes characterized by taxa occupying warm and wet habitats first evolved in clade 'C' during the Cretaceous (regime 2) and was followed by the mid-late Cenozoic evolution of regime (5) in clade 'A'. In contrast, regimes comprised of taxa occupying comparatively cool and dry habitats repeatedly evolved through the Cenozoic across clades 'A', 'I' and 'S' (regimes 3 & 4). Climatic regimes evolved repeatedly, typically among distantly related OTUs (regimes 3 & 4). Our subsequent analyses revealed that the time-invariant model (ER) best characterized the evolution of these climatic regimes on the MCC tree, while the time-invariant model best characterized most trees derived from the posterior (ER = 99 trees, EREBI = 1 tree). Thus, despite uncertainties in age estimates and topology, shifts among climatic regimes did
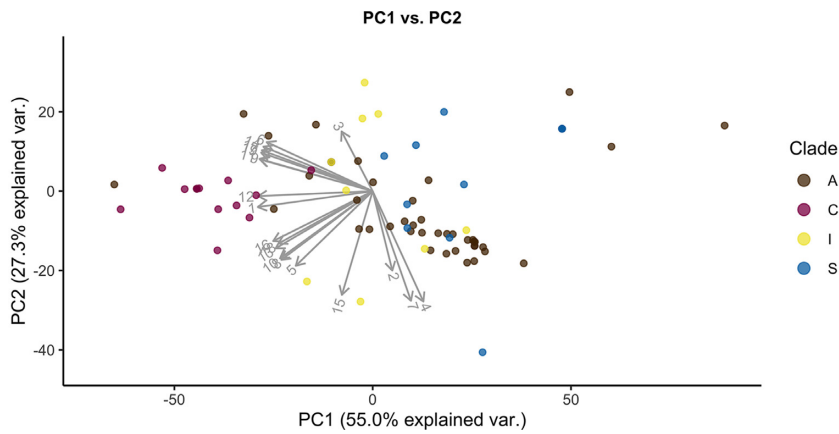
Figure 1. Phylogenetic principal component analysis (pPCA) of 19 bioclimatic variables with OTUs shaded by clade (PC1 vs. PC2). The proportion of variance explained is shown along each axis, and correlations with bioclimatic variables are indicated in grey lines labeled by bioclimatic variable number (1–19). See Table 1 for explanation of variables and correlation with PC axes.

Table 1. Correlation coefficients (loadings) between individual variables and first three axes in the pPCA. Correlations ≥0.65 are in bold and italics, and indicate the variables most strongly correlated with individual axes.

Abbreviation	Variable	PC1	PC2	PC3
BIO1	Annual Mean Temperature	−0.94	−0.13	−0.27
BIO2	Mean Diurnal Range Temperature	0.16	−0.65	−0.65
BIO3	Isothermality (BIO2/BIO7) (∗100)	−0.25	0.49	−0.69
BIO4	Temperature Seasonality (Standard Deviation ∗100)	0.41	−0.90	−0.01
BIO5	Max Temperature of Warmest Month	−0.63	−0.61	−0.47
BIO6	Min Temperature of Coldest Month	−0.86	0.40	−0.14
BIO7	Temperature Annual Range (BIO5-BIO6)	0.31	−0.89	−0.25
BIO8	Mean Temperature of Most Humid Quarter	−0.74	−0.56	−0.28
BIO9	Mean Temperature of Least Humid Quarter	−0.92	0.26	−0.25
BIO10	Mean Temperature of Warmest Quarter	−0.76	−0.55	−0.28
BIO11	Mean Temperature of Coldest Quarter	−0.92	0.26	−0.22
BIO12	Annual Mean Specific Humidity	−0.93	−0.04	0.28
BIO13	Specific Humidity of Most Humid Month	−0.79	−0.47	0.37
BIO14	Specific Humidity of Least Humid Month	−0.89	0.36	0.08
BIO15	Specific Humidity Seasonality (Coefficient of Variation)	−0.25	−0.85	0.41
BIO16	Specific Humidity Mean of Most Humid Quarter	−0.81	−0.41	0.39
BIO17	Specific Humidity Mean of Least Humid Quarter	−0.90	0.33	0.10
BIO18	Specific Humidity Mean of Warmest Quarter	−0.77	-0.43	0.39
BIO19	Specific Humidity Mean of Coldest Quarter	−0.91	0.31	0.10
	Proportion of Variance	0.550	0.273	0.119
	Cumulative Proportion of Variance	0.550	0.823	0.942

not exponentially decrease through time, and limited evidence existed for an exponential increase in shifts.

Diversification in non-forested biomes

Finally, it is unclear whether early *Trebouxia* lineages diversified exclusively in non-forested habitats or instead relied fully or partially on forested habitats. However, our analyses suggest that Late Cretaceous-early Cenozoic *Trebouxia* lineages likely diversified in forested habitats or were habitat generalists that occupied both forested and non-forested habitats (Fig. 2; Table S4, Supporting Information). Lineages specializing on non-forested habitats were instead recovered with confidence relatively recently (Eocene-present). These open habitat specialists occupied varied climatic regimes; for instance, clades 'S', 'I' and 'A' have convergently evolved to occupy non-forested habitats in cool-dry climates, while clades 'C' and 'A' have independently

evolved to regularly occupy open habitats in warmer and wetter climates (Fig. 2).

DISCUSSION

By synthesizing publicly available sequence data from the past twenty years, we provide a provisional geographic and climatic overview of macroecological diversification for *Trebouxia*. Our work demonstrates the convergent evolution of shared climatic tolerances. While previous work has demonstrated the co-occurrence of distantly related *Trebouxia* isolates (Doering and Piercey-Normore 2009; Werth 2012; Leavitt *et al.* 2013, 2015), these lacked the comparative analyses necessary to disentangle whether these represent shared ancestral preferences or the independent evolution of shared climatic tolerances. The ancestral regime occupied by *Trebouxia* is comprised of taxa whose modern representatives are characterized by the occupation
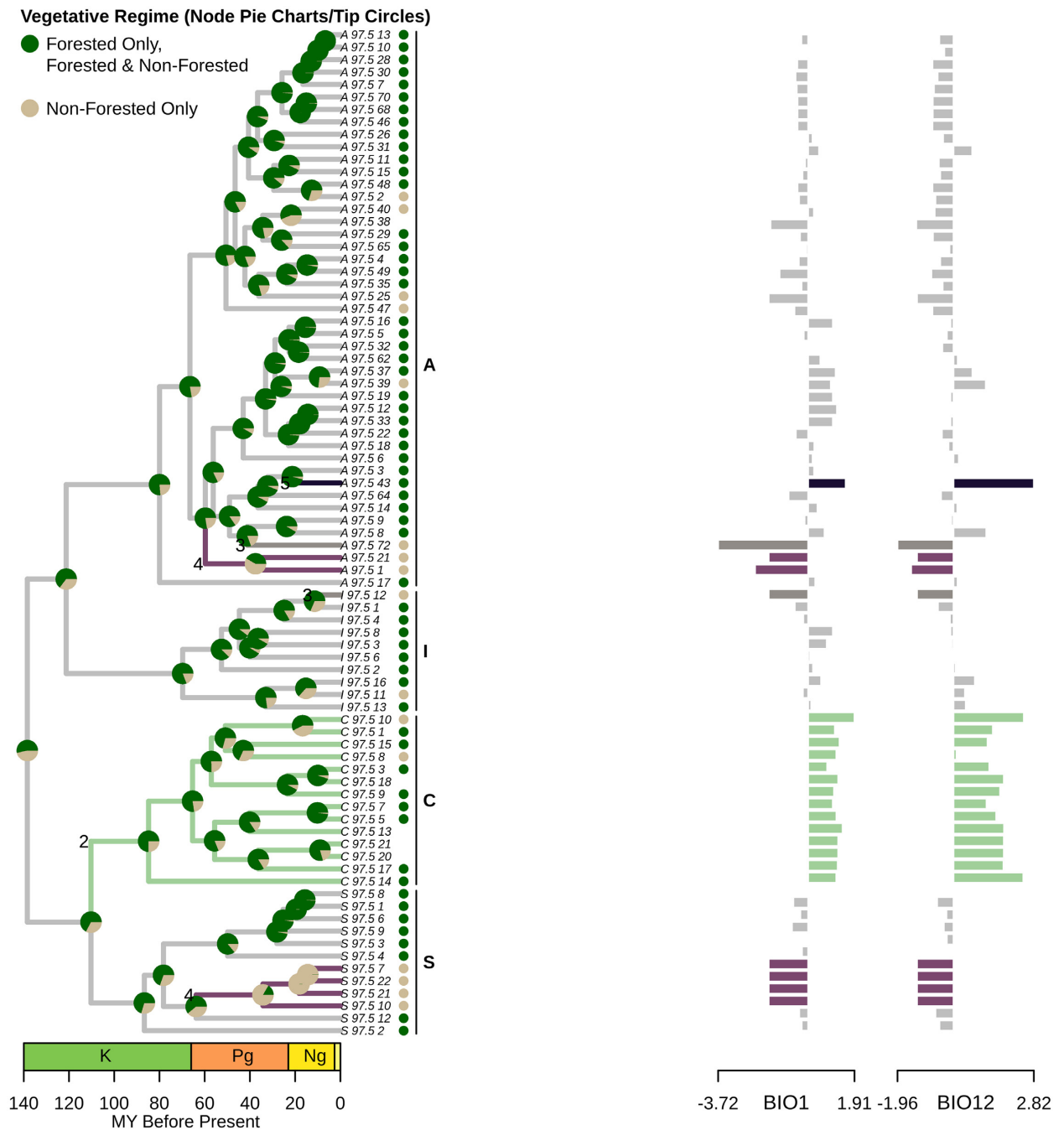
Figure 2. The MCC tree illustrating evolutionary shifts among vegetative and climatic regimes. The x-axis indicates millions of years before present and geological periods (K = Cretaceous, Pg = Paleogene; Ng = Neogene). Vegetative regimes are shown at the tips and pie charts over nodes reflect marginal probabilities for the occupation of each vegetative regime. Branches on the phylogeny are shaded by ancestral climatic regimes derived from the l1ou analysis. The ancestral climatic regime is shaded pale grey, and all other unique climatic regimes are indicated by numbers with branches shaded in color. Barplots to the right are shaded according to climatic regime and illustrate z-scores (number of standard deviations each OTU value is away from the mean) for each of the two variables. Mean and standard deviation (sd) values for each variable are: BIO1 (mean = 11.1°C, sd = 9.4), BIO12 (mean = 714.4 cg water/kg air, sd = 328).

of climates with moderate temperature and moisture. This is consistent with the presence of warm-cool temperate habitats during the Middle Jurassic-Early Cretaceous (Rees, Ziegler and Valdes 1999; Beerling and Woodward 2001; Ziegler *et al.* 2003). While it is unclear whether the most recent common ancestor (MRCA) of *Trebouxia* occupied forested habitats or was instead restricted to non-forested habitats, ancestral state estimates

suggest multiple Cretaceous *Trebouxia* lineages occurred partially or exclusively in forested biomes and were not restricted to non-forested habitats.

Our analyses subsequently revealed the diverse evolutionary pathways major clades traversed to occupy varied climatic and vegetative regimes. For instance, clade 'C' was the first lineage to expand into a regime whose extant members occupy

hot and wet climates in partially or exclusively forested habitats. This Late Cretaceous timing is consistent with both the expansion of angiosperm-dominated rainforests and paleoclimatic modelling, as well as the increased speciation observed in lineages of LFF occupying these habitats (Parrish, Ziegler and Scotese 1982; Morley 2000; Beerling and Woodward 2001; Ziegler *et al.* 2003; Boyce and Lee 2010, 2016; Boyce *et al.* 2010; Kraichak *et al.* 2015). In contrast, clade 'S' likely occupied forested habitats (partially or exclusively), and expanded during the Early Cenozoic into a regime comprised of taxa occupying cooler and drier climates—coinciding with the continued development of cool, temperate habitats in the Late Cretaceous-Early Cenozoic (Saward 1992; Deconto *et al.* 1999; Beerling and Woodward 2001). This clade subsequently diversified in non-forested habitats during the Paleocene-Miocene, coinciding with the diversification of several *Trebouxia*-associated lineages of LFF (such as the cetrarioid core, *Xanthoparmelia* and *Rhizoplaca*) that are common in these habitats (Nelsen *et al.* 2020b; Divakar *et al.* 2015; Kraichak *et al.* 2015; Leavitt *et al.* 2016; Huang *et al.* 2019). Clade 'I' is inferred to have partially or extensively relied on forested habitats, and isolated lineages underwent a Cenozoic transition into regimes whose modern members occupy warmer, as well as cooler and drier habitats. Finally, clade 'A' also exclusively or partially occupied forested habitats, and lineages subsequently extended during the Cenozoic to occupy regimes characterized by cooler and drier habitats—loosely coincident with or following the invasion of similar climatic regimes by clade 'S'. While our data may at first appear to conflict with the Cretaceous occupation of exposed habitats by *Trebouxia*-associated Teloschistaceae fungi (Gaya *et al.* 2015), we emphasize that our forest specialist/generalist category includes the partial occupation of non-forested habitats as well as exposed gaps in larger forested biomes.

Our findings may also be placed in the context of the fossil record, as numerous lichen associations have been recovered from Bitterfeld, Dominican and Baltic amber deposits. Dominican amber formed 15–20 Ma in wet tropical forests of the western Dominican Republic, (Grimaldi 1996; Poinar and Poinar 1999), while Bitterfeld and Baltic ambers formed 24–47 Ma in humid temperate forests of Europe (Kaasalainen, Schmidt and Rikkinen 2017). While *Trebouxia* itself has not been identified in these fossils, modern representatives from clearly recognizable fungal lineages preserved in amber (such as *Anzia*, *Calicium*, Parmeliaceae, Physciaceae (Poinar, Peterson and Platt 2000; Rikkinen and Poinar 2002; Rikkinen 2003; Kaasalainen *et al.* 2015; Kaasalainen, Schmidt and Rikkinen 2017)) obligately associate with *Trebouxia* algae (Tschermak-Woess 1988; Ihda *et al.* 1993; Voytsekhovich, Mikhailyuk and Darienko 2011; Miadlikowska *et al.* 2014). Thus, the presence of these lichenized fungi may indirectly suggest the presence of *Trebouxia* in these habitats and is consistent with our inferences of *Trebouxia* having occupied wet tropical forests and humid temperate forests during the Cenozoic or earlier.

While global sampling is non-random and the grid cell size employed is inadequate to detect variation in microclimate or vegetation structure, our synthesis of available data and subsequent analyses provide a provisional framework within which the evolution of ecological preferences may be viewed and expanded upon. However, we anticipate increased sampling will continue to reinforce our findings here, which demonstrate overlap among major clades both in terms of convergent regimes occupied and similar niche space in the pPCA. Our work also demonstrates that climatic regimes are not deeply entrenched, and we again suggest increased geographic sampling is likely

to reveal further evidence of convergence in climatic tolerances. While the inclusion of more taxa and the refinement of niches through the inclusion of additional climatic variables or smaller grid cell sizes may reveal differences in the number of climatic regimes and degree of convergence, we argue that diversification among regimes characterized by mean thermal and annual moisture is not disproportionately concentrated in the early evolutionary history of *Trebouxia*.

Together, our analyses provide a first estimate of how *Trebouxia* evolved to occupy diverse habitats globally and become one of the most common algal symbionts in lichen associations. Our work indicates that *Trebouxia* lineages diversified to occupy novel macroecological regimes at a relatively constant or increasing rate through time. Thus, the macroecological breadth observed among extant *Trebouxia* lineages was not achieved early in the history of this clade, as would be expected under an adaptive radiation. This implies that lineages of LFF obligately associated with early *Trebouxia* lineages may have also been restricted to a limited range of macroclimatic regimes, and subsequently expanded through the Cenozoic as *Trebouxia* increased the breadth of habitats occupied.

DATA ACCESSIBILITY

No new sequences were generated in this study. Data and code are available on GitHub (https://github.com/mpnelsen/Trebouxia_Macroecology).

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

REFERENCES

Altschul SF, Gish W, Miller W *et al.* Basic local alignment search tool. *J Mol Biol* 1990;**215**:403–10.

Altschul SF, Madden TL, Schäffer AA *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;**25**:3389–402.

Baker DM, Freeman CJ, Wong JCY *et al.* Climate change promotes parasitism in a coral symbiosis. *ISME J* 2018;**12**:921–30.

Beaulieu JM, O'Meara BC. *corHMM: analysis of binary character evolution*, https://CRAN.R-project.org/package=corHMM, 2017.

Beaulieu JM, Ree RH, Cavender-Bares J *et al.* Synthesizing phylogenetic knowledge for ecological research. *Ecology* 2012;**93**:S4–13.

Beerling D, Woodward FI. *Vegetation and the Terrestrial Carbon Cycle: The First 400 Million Years*. Cambridge, UK: Cambridge University Press, 2001.

Bengtsson-Palme J, Ryberg M, Hartmann M *et al*. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Meth Ecol Evol* 2013;**4**: 914–9.

Blois JL, Zarnetske PL, Fitzpatrick MC *et al*. Climate change and the past, present, and future of biotic interactions. *Science* 2013;**341**:499–504.

Bouckaert R, Heled J, Kühnert D *et al*. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 2014;**10**:e1003537.

Boyce CK, Lee J-E, Feild TS *et al*. Angiosperms helped put the rain in the rainforests: the impact of plant physiological evolution on tropical biodiversity. *Ann Missouri Botan Garden* 2010;**97**:527–40.

Boyce CK, Lee J-E. An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity. *Proceed Royal Soc London B: Biolog Sci* 2010;**277**: 3437–43.

Boyce CK, Lee J-E. Plant evolution and climate over geological timescales. *Annu Rev Earth Planet Sci* 2016;**45**:61–87.

Brundrett MC, Tedersoo L. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol* 2018;**220**:1108–15.

Burgin CJ, Colella JP, Kahn PL *et al*. How many species of mammals are there? *J Mammal* 2018;**99**:1–14.

Chomicki G, Kiers ET, Renner SS. The evolution of mutualistic dependence. *Annu Rev Ecol Evol Syst* 2020;**51**, 409–32.

Chomicki G, Weber M, Antonelli A *et al*. The impact of mutualisms on species richness. *Trends Ecol Evol* 2019;**34**:698–711.

Colwell RK, Dunn RR, Harris NC. Coextinction and persistence of dependent species in a changing world. *Annu Rev Ecol Evol Syst* 2012;**43**:183–203.

Cordeiro LMC, Reis RA, Cruz LM *et al*. Molecular studies of photobionts of selected lichens from the coastal vegetation of Brazil. *FEMS Microbiol Ecol* 2005;**54**:381–90.

Crisp MD, Cook LG. Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? *New Phytol* 2012;**196**:681–94.

Dal Grande F, Rolshausen G, Divakar PK *et al*. Environment and host identity structure communities of green algal symbionts in lichens. *New Phytol* 2018;**217**:277–89.

de Bary A. *Die Erscheinung der Symbiose*. Strassburg, Germany: Verlag von Karl J. Trübner, 1879.

Deconto RM, Brady EC, Bergengren J *et al*. Late Cretaceous climate, vegetation, and ocean interactions. In: Huber BT, Macleod KG, Wing SL (eds). *Warm Climates in Earth History*. Cambridge, U.K.: Cambridge University Press, 1999, 275–96.

del Campo EM, Catalá S, Gimeno J *et al*. The genetic structure of the cosmopolitan three-partner lichen *Ramalina farinacea* evidences the concerted diversification of symbionts. *FEMS Microbiol Ecol* 2013;**83**:310–23.

Divakar PK, Crespo A, Wedin M *et al*. Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytol* 2015;**208**:1217–26.

Divakar PK, Kauff F, Crespo A *et al*. Understanding phenotypical character evolution in parmelioid lichenized fungi (Parmeliaceae, Ascomycota). *PLoS One* 2013;**8**:e83115.

Doering M, Piercey-Normore MD. Genetically divergent algae shape an epiphytic lichen community on Jack Pine in Manitoba. *The Lichenologist* 2009;**41**:69–80.

Dunn RR, Harris NC, Colwell RK *et al*. The sixth mass coextinction: are most endangered species parasites and mutualists? *Proc Royal Soc B: Biological Sciences* 2009;**276**:3037–45.

Ehrlich PR, Raven PH. Butterflies and plants: a study in coevolution. *Evolution* 1964;**18**:586–608.

Elbert W, Weber B, Burrows S *et al*. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nat Geosci* 2012;**5**:459–62.

Ellis CJ. Lichen epiphyte diversity: a species, community and trait-based review. *Perspectives in Plant Ecol Evol Systematics* 2012;**14**:131–52.

Fernández-Mendoza F, Domaschke S, García MA *et al*. Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. *Mol Ecol* 2011;**20**:1208–32.

Fernández-Mendoza F, Printzen C. Pleistocene expansion of the bipolar lichen *Cetraria aculeata* into the Southern hemisphere. *Mol Ecol* 2013;**22**:1961–83.

FitzJohn RG, Maddison WP, Otto SP. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. *Syst Biol* 2009;**58**:595–611.

Frank AB. Über die geologischen Verhältnisse des Thallus einiger Krustenflechten (Cohn's). *Beiträge zur Biologie der Pflanzen* 1877;**2**:123–200.

Friedl T, Besendahl A, Pfeiffer P *et al*. The distribution of group I introns in lichen algae suggests that lichenization facilitates intron lateral transfer. *Mol Phylogenet Evol* 2000;**14**:342–52.

Friedl T, Rokitta C. Species relationships in the lichen alga *Trebouxia* (Chlorophyta, Trebouxiophyceae): molecular analyses of nuclear-encoded large subunit rRNA gene sequences. *Symbiosis* 1997;**23**:125–48.

Friedl T, Zeltner C. Assessing the relationships of some coccoid green lichen algae and the Microthamniales (Chlorophyta) with 18s ribosomal RNA gene sequence comparisons. *J Phycol* 1994;**30**:500–6.

Gaya E, Fernández-Brime S, Vargas R *et al*. The adaptive radiation of lichen-forming Teloschistaceae is associated with sunscreening pigments and a bark-to-rock substrate shift. *Proc Natl Acad Sci* 2015;**112**:11600–5.

Gilman SE, Urban MC, Tewksbury J *et al*. A framework for community interactions under climate change. *Trends Ecol Evol* 2010;**25**:325–31.

Green TGA, Nash TH III, Lange OL. Physiological ecology of carbon dioxide exchange. In: Nash TH III (ed). *Lichen Biology*. 2nd ed. Cambridge, UK: Cambridge University Press, 2008, 152–81.

Grimaldi DA. *Amber: Window to the Past*. New York, USA: Harry N. Abrahams Inc. & American Museum of Natural History, 1996.

Grube M, Muggia L. Identifying algal symbionts in lichen symbioses. In: Nimis PL, Vignes Lebbe R (eds). *Tools for Identifying Biodiversity: Progress and Problems*. Trieste, Italy: Edizioni Universitàdi Trieste, 2010, 295–9.

Harley CDG. Climate change, keystone predation, and biodiversity loss. *Science* 2011;**334**:1124–7.

Harmon LJ, Andreazzi CS, Débarre F *et al*. Detecting the macroevolutionary signal of species interactions. *J Evol Biol* 2019;**32**:769–82.

Harmon LJ, Schulte JA, Larson A *et al*. Tempo and mode of evolutionary radiation in iguanian lizards. *Science* 2003;**301**:961–4.

Hauck M, Helms G, Friedl T. Photobiont selectivity in the epiphytic lichens *Hypogymnia physodes* and *Lecanora conizaeoides*. *The Lichenologist* 2007;**39**:195–204.

Hedenås H, Blomberg P, Ericson L. Significance of old aspen (*Populus tremula*) trees for the occurrence of lichen photobionts. *Biol Conserv* 2007;**135**:380–7.

Helms G. *Taxonomy and Symbiosis in Associations of Physciaceae and Trebouxia*, Göttingen, Germany: Doctoral Dissertation from Georg-August Universität, 2003.

Hembry DH, Weber MG. Ecological interactions and macroevolution: a new field with old roots. *Annu Rev Ecol Evol Syst* 2020;**51**:215–43.

Hijmans RJ, Cameron SE, Parra JL *et al*. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 2005;**25**:1965–78.

Hoeksema BW, Cairns S. *World List of Scleractinia*, http://www.marinespecies.org/scleractinia/, 2020.

Honegger R. Lichen-forming fungi and their photobionts. In: Deising HB (ed). *The Mycota - Plant Relationships*, Vol. 5. 2nd ed., Berlin, Heidelberg: Springer Berlin Heidelberg, 2009, 307–33.

Honegger R. The lichen symbiosis—what is so spectacular about it? *The Lichenologist* 1998;**30**:193–212.

Huang J-P, Kraichak E, Leavitt SD *et al*. Accelerated diversifications in three diverse families of morphologically complex lichen-forming fungi link to major historical events. *Sci Rep* 2019;**9**:1–10.

Ihda T-A, Nakano T, Yoshimura I *et al*. Phycobionts isolated from Japanese species of *Anzia* (Lichenes). *Archiv für Protistenkunde* 1993;**143**:163–72.

Jablonski D. Biotic interactions and macroevolution: extensions and mismatches across scales and levels. *Evolution* 2008;**62**:715–39.

Kaasalainen U, Heinrichs J, Krings M *et al*. Alectorioid morphologies in Paleogene lichens: new evidence and re-evaluation of the fossil *Alectoria succini* Mägdefrau. *PLoS One* 2015;**10**:e0129526.

Kaasalainen U, Schmidt AR, Rikkinen J. Diversity and ecological adaptations in Palaeogene lichens. *Nature Plants* 2017;**3**:17049.

Katoh K, Standley DM. MAFFT Multiple sequence alignment software Version 7: improvements in performance and usability. *Mol Biol Evol* 2013;**30**:772–80.

Kershaw KA. *Physiological Ecology of Lichens*. Cambridge, UK: Cambridge University Press, 1985.

Khabbazian M, Kriebel R, Rohe K *et al*. Fast and accurate detection of evolutionary shifts in Ornstein–Uhlenbeck models. *Methods in Ecology and Evolution* 2016;**7**:811–24.

Kiers ET, Palmer TM, Ives AR *et al*. Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters* 2010;**13**:1459–74.

Kikuchi Y, Tada A, Musolin DL *et al*. Collapse of insect gut symbiosis under simulated climate change. *mBio* 2016;**7**:e01578–16.

Kõljalg U, Nilsson RH, Abarenkov K *et al*. Towards a unified paradigm for sequence-based identification of fungi. *Mol Ecol* 2013;**22**:5271–7.

Kraichak E, Divakar PK, Crespo A *et al*. A Tale of Two Hyperdiversities: diversification dynamics of the two largest families of lichenized fungi. *Sci Rep* 2015;**5**:10028.

Kroken S, Taylor JW. Phylogenetic species, reproductive mode, and specificity of the green alga *Trebouxia* forming lichens with the fungal genus *Letharia*. *The Bryologist* 2000;**103**:645–60.

Larson DW. The absorption and release of water by lichens. *Bibliotheca Lichenologica* 1987;**25**:351–60.

Leavitt SD, Kraichak E, Nelsen MP *et al*. 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). *Mol Ecol* 2015;**24**:3779–97.

Leavitt SD, Kraichak E, Vondrak J *et al*. Cryptic diversity and symbiont interactions in rock-posy lichens. *Mol Phylogenet Evol* 2016;**99**:261–74.

Leavitt SD, Nelsen MP, Lumbsch HT *et al*. Symbiont flexibility in subalpine rock shield lichen communities in the Southwestern USA. *The Bryologist* 2013;**116**:149–61.

Liu H, Ye Q, Wiens JJ. Climatic-niche evolution follows similar rules in plants and animals. *Nat Ecol Evol* 2020;**4**:753–63.

Lücking R, Hodkinson BP, Leavitt SD. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* 2017;**119**:361–416.

Lumbsch HT, Parnmen S, Rangsiruji A *et al*. Phenotypic disparity and adaptive radiation in the genus *Cladia* (Lecanorales, Ascomycota). *Australian Systematic Botany* 2010;**23**:239–47.

Lutsak T, Fernández-Mendoza F, Kirika P *et al*. Mycobiont-photobiont interactions of the lichen *Cetraria aculeata* in high alpine regions of East Africa and South America. *Symbiosis* 2016;**68**:25–37.

Lutzoni F, Nowak MD, Alfaro ME *et al*. Contemporaneous radiations of fungi and plants linked to symbiosis. *Nat Commun* 2018;**9**:5451.

Mark K, Laanisto L, Bueno CG *et al*. Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *New Phytol* 2020;**227**:1362–75.

Miadlikowska J, Kauff F, Högnabba F *et al*. A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Mol Phylogenet Evol* 2014;**79**:132–68.

Miller MA, Pfeiffer W, Schwartz T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010*. New Orleans, LA, USA, 2010, 1–8.

Morley RJ. *Origin and Evolution of Tropical Rain Forests*. West Sussex, UK: John Wiley & Sons, Ltd, 2000.

Muggia L, Grube M, Tretiach M. Genetic diversity and photobiont associations in selected taxa of the *Tephromela atra* group (Lecanorales, lichenised Ascomycota). *Mycological Progress* 2008;**7**:147.

Muggia L, Leavitt S, Barreno E. The hidden diversity of lichenised Trebouxiophyceae (Chlorophyta). *Phycologia* 2018;**57**:503–24.

Muggia L, Nelsen MP, Kirika PM *et al*. Formally described species woefully underrepresent phylogenetic diversity in the common lichen photobiont genus *Trebouxia* (Trebouxiophyceae, Chlorophyta): an impetus for developing an integrated taxonomy. *Mol Phylogenet Evol* 2020;**149**:106821.

Mukhtar A, Garty J, Galun M. Does the lichen alga *Trebouxia* occur free-living in nature: further immunological evidence. *Symbiosis* 1994;**17**:247–53.

Nelsen MP, Gargas A. Dissociation and horizontal transmission of codispersing lichen symbionts in the genus *Lepraria* (Lecanorales: Stereocaulaceae). *New Phytol* 2008;**177**:264–75.

Nelsen MP, Gargas A. Symbiont flexibility in *Thamnolia vermicularis* (Pertusariales: Icmadophilaceae). *The Bryologist* 2009;**112**:404–17.

Nelsen MP, Lücking R, Boyce CK *et al*. No support for the emergence of lichens prior to the evolution of vascular plants. *Geobiology* 2020a;**18**:3–13.

Nelsen MP, Lücking R, Boyce CK *et al*. The macroevolutionary dynamics of symbiotic and phenotypic diversification in lichens. *Proc Natl Acad Sci* 2020b;**117**:21495–503.

Olson DM, Dinerstein E, Wikramanayake ED *et al*. Terrestrial Ecoregions of the World: a new map of life on Earth: a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. *Bioscience* 2001;**51**:933–8.

Parrish JT, Ziegler AM, Scotese CR. Rainfall patterns and the distribution of coals and evaporites in the Mesozoic and Cenozoic. *Palaeogeogr Palaeoclimatol Palaeoecol* 1982;**40**:67–101.

Peksa O, Škaloud P. Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Mol Ecol* 2011;**20**:3936–48.

Pennell MW, Eastman JM, Slater GJ *et al*. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* 2014;**30**:2216–8.

Pérez-Ortega S, Ortiz-Álvarez R, Green TGA *et al*. Lichen myco- and photobiont diversity and their relationships at the edge of life (McMurdo Dry Valleys, Antarctica). *FEMS Microbiol Ecol* 2012;**82**:429–48.

Poinar GO, Peterson EB, Platt JL. Fossil *Parmelia* in New World amber. *The Lichenologist* 2000;**32**:263–9.

Poinar GO, Poinar R. *The Amber Forest: A Reconstruction of the Vanished World*. Princeton, New Jersey, USA: Princeton University Press, 1999.

Porada P, Stan JTV, Kleidon A. Significant contribution of non-vascular vegetation to global rainfall interception. *Nat Geosci* 2018;**11**:563–7.

Porada P, Weber B, Elbert W *et al*. Estimating impacts of lichens and bryophytes on global biogeochemical cycles. *Global Biogeochem Cycles* 2014;**28**:71–85.

Puillandre N, Lambert A, Brouillet S *et al*. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol* 2012;**21**:1864–77.

Rees PM, Ziegler AM, Valdes PJ. Jurassic phytogeography and climates: new data and model comparisons. In: Huber BT, Macleod KG, Wing SL (eds). *Warm Climates in Earth History*. Cambridge, U.K.: Cambridge University Press, 1999, 297–318.

Revell LJ. phytools: an R package for phylogenetic comparative biology (and other things). *Meth Ecol Evol* 2012;**3**:217–23.

Revell LJ. Size-correction and principal components for interspecific comparative studies. *Evolution* 2009;**63**:3258–68.

Rikkinen J, Poinar GO. Fossilised *Anzia* (Lecanorales, lichen-forming Ascomycota) from European Tertiary amber. *Mycol Res* 2002;**106**:984–90.

Rikkinen J. Calicioid lichens from European Tertiary amber. *Mycologia* 2003;**95**:1032–6.

Rolshausen G, Grande FD, Sadowska-Deś AD *et al*. Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions. *Ecography* 2018;**41**:1380–92.

Rolshausen G, Hallman U, Grande FD *et al*. Expanding the mutualistic niche: parallel symbiont turnover along climatic gradients. *Proc Royal Soc B: Biolog Sci* 2020;**287**:20192311.

Ruprecht U, Brunauer G, Printzen C. Genetic diversity of photobionts in Antarctic lecideoid lichens from an ecological view point. *The Lichenologist* 2012;**44**:661–78.

Ruprecht U, Fernández-Mendoza F, Türk R *et al*. High levels of endemism and local differentiation in the fungal and algal symbionts of saxicolous lecideoid lichens along a latitudinal gradient in southern South America. *The Lichenologist* 2020;**52**:287–303.

Rutherford WA, Painter TH, Ferrenberg S *et al*. Albedo feedbacks to future climate via climate change impacts on dryland biocrusts. *Sci Rep* 2017;**7**:44188.

Sachs JL, Simms EL. Pathways to mutualism breakdown. *Trends Ecol Evol* 2006;**21**:585–92.

Sampayo EM, Ridgway T, Franceschinis L *et al*. Coral symbioses under prolonged environmental change: living near tolerance range limits. *Sci Rep* 2016;**6**:36271.

Sanders WB, Lücking R. Reproductive strategies, relichenization and thallus development observed *in situ* in leaf-dwelling lichen communities. *New Phytol* 2002;**155**:425–35.

Sanders WB. Lichens: the interface between mycology and plant morphology. *Bioscience* 2001;**51**:1025–35.

Sanders WB. Observing microscopic phases of lichen life cycles on transparent substrata placed *in situ*. *The Lichenologist* 2005;**37**:373–82.

Saward SA. A global view of Cretaceous vegetation patterns. *Geological Society of America Special Papers* 1992;**267**:17–36.

Schloss PD, Westcott SL, Ryabin T *et al*. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009;**75**:7537–41.

Schluter D. *The Ecology of Adaptive Radiation*. Oxford, New York: Oxford University Press, 2000.

Schneider K, Resl P, Spribille T. Escape from the cryptic species trap: lichen evolution on both sides of a cyanobacterial acquisition event. *Mol Ecol* 2016;**25**:3453–68.

Seaward MRD. Environmental role of lichens. In: Nash TH III (ed). *Lichen Biology*. 2nd ed. Cambridge, UK: Cambridge University Press, 2008, 274–98.

Simpson GG. *Tempo and Mode in Evolution*. New York: Columbia University Press, 1944.

Simpson GG. *The Major Features of Evolution*. New York, USA: Columbia University Press, 1953.

Singh G, Grande FD, Divakar PK *et al*. Fungal–algal association patterns in lichen symbiosis linked to macroclimate. *New Phytol* 2017;**214**:317–29.

Škaloud P, Peksa O. Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga *Asterochloris* (Trebouxiophyceae, Chlorophyta). *Mol Phylogenet Evol* 2010;**54**:36–46.

Slater GJ, Friscia AR. Hierarchy in adaptive radiation: a case study using the Carnivora (Mammalia). *Evolution* 2019;**73**:524–39.

Smith SA, Beaulieu JM. Life history influences rates of climatic niche evolution in flowering plants. *Proceedings of the Royal Society B: Biological Sciences* 2009;**276**:4345–52.

Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 2008;**57**:758–71.

Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;**30**:1312–3.

Steidinger BS, Crowther TW, Liang J *et al*. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* 2019;**569**:404–8.

Title P. *rangeBuilder: occurrence filtering, geographic and taxonomic standardization and generation of species range polygons*, https://CRAN.R-project.org/package=rangeBuilder, 2017.

Tschermak-Woess E. *Myrmecia reticulata* as a phycobiont and free-living—free-living *Trebouxia*— the problem of *Stenocybe septata*. *The Lichenologist* 1978;**10**:69–79.

Tschermak-Woess E. The algal partner. In: Galun M (ed). *CRC Handbook of Lichenology*, **Vol. 1**. Boca Raton, FL, USA: CRC Press, 1988, 39–92.

Uyeda JC, Caetano DS, Pennell MW. Comparative analysis of principal components can be misleading. *Syst Biol* 2015;**64**:677–89.

Uyeda JC, Pennell MW, Miller ET *et al*. The evolution of energetic scaling across the vertebrate Tree of Life. *Am Nat* 2017;**190**:185–99.

Vančurová L, Muggia L, Peksa O *et al*. The complexity of symbiotic interactions influences the ecological amplitude of the host: a case study in *Stereocaulon* (lichenized Ascomycota). *Mol Ecol* 2018;**27**:3016–33.

Vančurová L, Peksa O, Němcová Y *et al*. *Vulcanochloris* (Trebouxiales, Trebouxiophyceae), a new genus of lichen photobiont from La Palma, Canary Islands, Spain. *Phytotaxa* 2015;**219**:118–32.

Vega GC, Pertierra LR, Olalla-Tárraga MÁ. MERRAclim, a high-resolution global dataset of remotely sensed bioclimatic variables for ecological modelling. *Scientific Data* 2017;**4**:170078.

Vermeij GJ. The evolutionary interaction among species: selection, escalation, and coevolution. *Ann Rev Ecol Syst* 1994;**25**:219–36.

Voytsekhovich AA, Mikhailyuk TI, Darienko TM. Lichen photobionts. 2: origin and correlation with mycobiont. *Algologia* 2011;**21**:151–77.

Vu VQ. *ggbiplot: a ggplot2 based biplot*, http://github.com/vqv/ggbiplot, 2011.

Wagner M, Bathke AC, Cary SC *et al*. Myco- and photobiont associations in crustose lichens in the McMurdo Dry Valleys (Antarctica) reveal high differentiation along an elevational gradient. *Polar Biology* 2020;**43**:1967–83.

Wagner M, Trutschnig W, Bathke AC *et al*. A first approach to calculate BIOCLIM variables and climate zones for Antarctica. *Theor Appl Climatol* 2018;**131**:1397–415.

Walther G-R. Community and ecosystem responses to recent climate change. *Philos Transac Royal Soc B: Biological Sci* 2010;**365**:2019–24.

Werth S, Sork VL. Ecological specialization in *Trebouxia* (Trebouxiophyceae) photobionts of *Ramalina menziesii* (Ramalinaceae) across six range-covering ecoregions of western North America. *Am J Bot* 2014;**101**:1127–40.

Werth S. Fungal-algal interactions in *Ramalina menziesii* and its associated epiphytic lichen community. *The Lichenologist* 2012;**44**:543–60.

Xu M, De Boer H, Olafsdottir ES *et al*. Phylogenetic diversity of the lichenized algal genus *Trebouxia* (Trebouxiophyceae, Chlorophyta): a new lineage and novel insights from fungal-algal association patterns of Icelandic cetrarioid lichens (Parmeliaceae, Ascomycota). *Botanical J Linnean Soc* 2020;**194**:460–8.

Yahr R, Vilgalys R, DePriest PT. Geographic variation in algal partners of *Cladonia subtenuis* (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytol* 2006;**171**:847–60.

Zhang Z, Schwartz S, Wagner L *et al*. A greedy algorithm for aligning DNA sequences. *J Comput Biol* 2000;**7**:203–14.

Ziegler A, Eshel G, Rees PM *et al*. Tracing the tropics across land and sea: permian to present. *Lethaia* 2003;**36**:227–54.