Universidad Rey Juan Carlos

TESIS DOCTORAL

Insights in specialization patterns in biotic interactions: integration of metrics and the effect of the scale and ecological drivers in lichen communities

Autora:

Clara Rodríguez Arribas

Directores:

Isabel Martínez Moreno
María Prieto Álvaro
Gregorio Aragón Rubio

Programa de Doctorado en Conservación de Recursos Naturales

Escuela Internacional de Doctorado

2023

Tabla de contenido

Tabla de contenido 1
RESUMEN 7
Antecedentes 7
Objetivos 8
Metodología 9
Resultados 10
Conclusiones 12
SUMMARY 15
Background 15
Objectives 16
Methodology 17
Results 18
Conclusions 19
INTRODUCCIÓN GENERAL 25
1. Especialización 25
2. Especialización en interacciones bióticas 30
3. Líquenes como ejemplo de interacciones bióticas 37
4. Referencias 42
OBJETIVOS GENERALES 55
METODOLOGÍA GENERAL 59
1. Áreas de estudio y diseño experimental 59
1.1. Variables ambientales 60
2. Identificación de líquenes y cianobacterias y análisis de rasgos funcionales 61
2.1. Identificación morfológica de los líquenes 61
2.2. Identificación molecular de las cianobacterias 61
2.3. Rasgos funcionales y medias ponderadas de la comunidad (CWM: Community Weighted Mean). 63
3. Métricas de especialización 64
3.1. Medidas de especialización por especie. 64
3.2. Medidas de la arquitectura de las redes de interacciones 65
4. Diversidad beta 67

5. Análisis estadísticos 68
5.1. Selección de variables ambientales 68
5.2. Curvas de acumulación 69
5.3. Análisis de redundancia (RDA) y partición de la varianza 69
5.4. Modelos Lineales (LMs) 70
5.5. Modelos nulos 70
5.6. Modelos Lineales Mixtos Generalizados (GLMMs). 71
6. Referencias 75
AFILIACIÓN DE LOS COAUTORES 83

CHAPTER 1: Specialization patterns in symbiotic associations: a community perspective over spatial scales.

Abstract 87
Keywords 88
Introduction 88
Methods 93
Sampling 93
Environmental variables 95
Nostoc phylogroup delimitation 95
Availability and composition of *Nostoc* phylogroups along the latitudinal gradient 96
Specialization measures 96
Number of interacting partners and sampling efficiency 97
Indices: Simpson and d′ 97
Generalized linear mixed models 98
Distributional ranges and specialization patterns 99
Spatial scale: Beta diversity and partner replacement 99
Results 100
Nostoc phylogroup delimitation 100
Availability and composition of *Nostoc* phylogroups along the latitudinal gradient 100
Specialization measures 102
Number of interacting partners and sampling efficiency 102
Indices: Simpson and d′ 103
Generalized linear mixed models 104
Distributional ranges and specialization patterns 107

Spatial scale: Beta diversity and partner replacement 107
Discussion 108
References 113
Supporting information 123

CHAPTER 2: Cyanobacterial variability in lichen cephalodia.

Abstract 167
Keywords 167
Introduction 168
Methods 171
Results 172
Discussion 177
References 180

CHAPTER 3: Unravelling network structures and species roles in the lichen symbiosis in a wide latitudinal gradient.

Abstract 189
Keywords 189
Introduction 190
Materials and methods 194
Sampling and data collection 194
Sampling 194
Environmental variables 196
Functional traits: CWM and Rao's index 197
Network architecture measures 198
Statistical analysis 198
Species roles 200
Results 200
Network architecture measures 202
Environmental and functional determinants of network architecture 203
Species' roles 205
Discussion 206
References 212
Supporting information 221

CHAPTER 4: Specialization: a multidimensional and integrative perspective.

Abstract 249
Keywords 249
Introduction 250
Proposal for quantifying specialization 251
Results 255
Discussion 258
References 260
Supporting information 265
DISCUSIÓN GENERAL 273
1. La importancia de la escala 275
1.1. Escala espacial 276
1.2. Escala biológica 278
2. La importancia de los factores ambientales 281
2.1. Influencia de los factores ambientales en la distribución de las cianobacterias 282
2.2. Gradiente latitudinal de especialización 283
3. La importancia de los rasgos funcionales 285
3.1. Módulos en las redes de interacción 288
3.2. Lichen-guilds y especialización a nivel de comunidad 289
4. Métricas para cuantificar la especialización 292
5. Consideraciones finales 296
6. Referencias 298
GENERAL CONCLUSIONS 313

RESUMEN/SUMMARY

RESUMEN

Antecedentes

Comprender los procesos que participan en el ensamblaje de las comunidades y explican la coexistencia de las especies es una de las cuestiones principales en ecología. Por ello, es necesario conocer cuáles son los requerimientos que presentan los distintos organismos, y qué aspectos pueden estar condicionando la composición de las comunidades de las que forman parte. Uno de estos aspectos está relacionado con los patrones de especialización que se dan en las interacciones bióticas que establecen las especies coexistentes y los procesos que condicionan dicha especialización. El análisis de las interacciones bióticas puede enfocarse desde dos aproximaciones: las interacciones que se dan a nivel de especie y las redes de interacción que engloban a toda la comunidad. En cuanto a la primera aproximación, aparecen ciertas limitaciones al cuantificar la especialización, ya que este fenómeno ha sido tradicionalmente abordado desde el número o la riqueza de parejas, sin integrar otras facetas como pueden ser la abundancia de las parejas o la preferencia por ciertas parejas. Además, históricamente, las especies han sido clasificadas como especialistas o generalistas dependiendo de sus requerimientos y su tolerancia ecológica, pero esta perspectiva simplista no describe la enorme complejidad de este fenómeno que representa la especialización. Por ello, una perspectiva integradora que ayude a entender el grado de especialización en el marco de las interacciones bióticas y los factores subyacentes a esta especialización, es una aproximación que puede ayudar a comprender los procesos de ensamblaje de una comunidad.

La especialización ha de ser contextualizada en un eje del nicho de requerimientos de los organismos. En el caso de las interacciones bióticas, la especialización se considera teniendo en cuenta las parejas con las que puede interaccionar un organismo. Además, la escala, tanto biológica como espacial o temporal, afecta en la concepción de la especialización, ya que los patrones que muestra un organismo en escalas pequeñas pueden variar al considerar escalas más amplias. A su vez, la especialización se ve condicionada por factores tanto ecológicos como evolutivos. Por ello, determinar los

factores ambientales y los rasgos funcionales que afectan a las interacciones bióticas puede ser un punto clave para comprender la coexistencia de las especies.

Organismos como los líquenes nos ofrecen un sistema de estudio excepcional para abordar el estudio de las interacciones bióticas dada la simbiosis íntima que representan. Los líquenes se han definido tradicionalmente como organismos compuestos por un hongo principal, el micobionte y uno o más componentes fotosintéticos, los fotobiontes, que pueden ser algas verdes o cianobacterias. El micobionte se encarga principalmente de proteger al fotobionte de la desecación, mientras que el fotobionte es el miembro que aporta nutrientes a través de la fotosíntesis. Además, cuando el fotobionte se trata de una cianobacteria (cianobionte), también se encarga de fijar nitrógeno atmosférico. Esta tesis doctoral se centra en los líquenes, en concreto, en las interacciones que se dan entre el micobionte y el cianobionte. Debido a la intimidad de esta simbiosis, tradicionalmente se ha considerado que los micobiontes muestran un alto grado de especialización por sus fotobiontes, mostrando poca flexibilidad en las asociaciones. No obstante, estudios recientes muestran cómo los líquenes presentan distintos patrones de especialización. Además, la especialización se ha relacionado con distintos factores ambientales y rasgos funcionales como el tipo de reproducción. Sin embargo, estos estudios se han centrado en especies o géneros concretos y poco se sabe cómo funcionan estos procesos a nivel de comunidad.

Objetivos

El objetivo principal de esta tesis doctoral es comprender las interacciones bióticas y los fenómenos de especialización asociados, así como identificar y analizar los principales componentes que intervienen en este proceso. Para ello, se pretende analizar cómo varían los patrones de especialización en función de la escala considerada (tanto biológica como espacial), así como los distintos efectos que tienen sobre la especialización diversos factores, tanto ecológicos, derivados de las condiciones ambientales y geográficas de un amplio gradiente latitudinal, como los distintos rasgos funcionales que presentan las especies. Con todo ello, se quiere proponer un marco conceptual integrador que considere las diferentes facetas que determinan la especialización y que sirva como

herramienta que permita identificar el grado de especialización de una forma más precisa, objetiva, integradora y universal.

Para alcanzar este objetivo general, esta tesis doctoral utiliza los líquenes, un ejemplo clásico de simbiosis, como sistema de estudio en un amplio gradiente latitudinal en el hemisferio sur y desglosa este objetivo general en distintos objetivos específicos desarrollados a lo largo de cuatro capítulos que consisten en: 1) determinar la especialización de las especies de micobionte por sus cianobiontes asociados utilizando 3 índices diferentes a distintas escalas espaciales y analizar los factores ambientales y los rasgos funcionales que influyen en la especialización, 2) estudiar la especialización a distintas escalas biológicas y determinar la variabilidad individual (intratalina), intraespecífica e interespecífica de cianobiontes en distintas especies de cefalolíquenes, 3) analizar las redes micobionte-cianobacteria para comprobar si hay un patrón estructural compartido y determinar cómo influyen las variables ambientales y los rasgos funcionales de las especies en la estructura de las redes y 4) proponer un marco conceptual integrador que considere la especialización desde una perspectiva tridimensional, englobando tres aspectos: *especificidad*, *preferencia* y *selectividad*, aplicable a todos los tipos de interacciones bióticas.

Metodología

Este estudio se centra en comunidades epífitas de líquenes y sus cianobiontes en un extenso gradiente latitudinal en el hemisferio sur, en Chile. Este gradiente se encuentra entre las latitudes 38.63° S y 54.96° S, y engloba la distribución de la lenga o *Nothofagus pumilio*, forófito sobre el que se han muestreado las comunidades liquénicas. Además, este gradiente abarca una amplia variabilidad de condiciones ambientales, que van desde el clima mediterráneo en el norte del gradiente hasta el clima boreal en las localidades situadas más al sur. Se estudiaron 11 bosques pertenecientes a distintas áreas protegidas (Parques o Reservas Nacionales). En los bosques, se recogieron ejemplares de todas las especies epífitas de líquenes y se cuantificaron distintas variables ambientales relacionadas con la estructura del bosque (pendiente, inclinación, orientación, insolación, cobertura del dosel arbóreo y diámetro a la altura del pecho de los árboles). Las especies recogidas fueron identificadas posteriormente con el uso de claves dicotómicas y

monografías. También se identificaron las cianobacterias presentes en estas especies a través de la amplificación del marcador genético *rcbLX* y se desarrollaron posteriores análisis filogenéticos, de redes y de clasificación. Además, se consideraron distintos rasgos funcionales de las especies de líquenes consideradas, que incluyen el tipo de reproducción, la función de la cianobacteria y el biotipo.

Para alcanzar los objetivos propuestos en este estudio, se han utilizado distintos índices para cuantificar las interacciones, que abarcan desde índices que cuantifican la especialización para cada especie, como medidas de la red de interacciones que tiene lugar en la comunidad. Además, se han utilizado distintas aproximaciones estadísticas (ej.: modelos lineares mixtos generalizados y modelos nulos) para analizar cómo influyen en las interacciones tanto los factores ambientales que cubren este amplio gradiente latitudinal, como los rasgos funcionales de las especies estudiadas.

Resultados

En el capítulo 1 encontramos que la composición de cianobiontes que forman parte de las comunidades liquénicas estudiadas se ve condicionada por distintos factores ambientales como la temperatura y el diámetro medio de los árboles. Además, la especialización del micobionte por su cianobacteria varía con la métrica y la escala espacial consideradas. Los factores que influyen en la especialización fueron diferentes dependiendo del índice de especialización utilizado. La función del cianobionte parcialmente explicó la riqueza de cianobiontes y el índice de Simpson, mientras que la identidad del micobionte afectó principalmente al índice d'. Es decir, los cefalolíquenes tienden a ser menos especializados que los cianolíquenes, probablemente debido a la menor dependencia por el cianobionte en el caso de los cefalolíquenes. Por otro lado, hay una tendencia a que disminuya la especialización cuando la escala espacial se amplía, incrementándose el número de cianobiontes con las que puede interactuar una especie de micobionte dada. También se ha identificado que en el caso de los líquenes que presentan amplios rangos de distribución, estos se asocian, o bien con múltiples cianobiontes, o bien con cianobiontes que presentan amplios rangos de distribución. Por otra parte, los resultados indican la dependencia del contexto de la especialización y cómo su consideración cambia con la métrica y la escala espacial considerada. Por tanto, se

sugiere la necesidad de considerar la comunidad entera, así como tener en cuenta la amplitud de la escala espacial considerada, dado que ambos aspectos son cruciales para entender los factores que determinan la especialización.

En el capítulo 2 los resultados indican que existe una alta variabilidad de cianobiontes a diferentes escalas biológicas en el caso de los cefalolíquenes. A nivel individual o intratalino, encontramos distintos cianobiontes en diferentes cefalodios dentro de un mismo talo liquénico. Al ampliar la escala biológica, es decir, desde individuo o talo hasta el nivel de especie, observamos casos en los que disminuye la especialización al aumentar la diversidad de cianobiontes con los que pueden interactuar. En cuanto a la variabilidad interespecífica, observamos que varios cianobiontes se comparten entre las 3 especies de liquen estudiadas, mientras que otros son exclusivos de algunas de las especies. Además, se pueden observar grados variables de especialización por el cianobionte en función del bosque estudiado. Estos resultados, enfatizan la importancia de contextualizar la escala biológica de estudio y determinar la disponibilidad local de cianobiontes.

El capítulo 3 muestra como las redes de líquenes a escala local presentan diferentes patrones, tanto modulares como anidados y aleatorios, con redes poco conectadas, mientras que, al aumentar la escala espacial, se observa un patrón altamente anidado en la metarred. Además, se ha identificado un gradiente latitudinal en la estructura de las redes, con una mayor modularidad hacia el norte del gradiente y una mayor conectancia hacia el sur, principalmente influenciada por la temperatura. Por otro lado, el tipo de reproducción de los líquenes afecta a la conectancia de la red, estando menos conectadas aquellas redes que presentan individuos que carecen de estructuras reproductivas, tanto sexuales como asexuales. Como en otros sistemas mutualistas, la mayoría de las especies fueron periféricas, aunque algunas actuaban como conectores o centros, siendo estos últimos principalmente cefalolíquenes. Por tanto, los cefalolíquenes mostraron un papel estructural más importante que los cianolíquenes, indicando que podrían estar implicados en procesos de facilitación, actuando como fuentes de cianobacterias para otras especies, facilitando así el establecimiento de nuevos talos liquénicos.

En el capítulo 4 se propone un marco integrador combinando tres métricas que proporcionan información complementaria para considerar la especialización de forma más precisa (*especificidad*, *preferencia* y *selectividad*). Este marco ha sido testado en

distintos sistemas de interacciones, tanto antagonistas (parásito hospedador), como mutualistas (micobionte-cianobionte, planta-hormiga, planta-polinizador, planta-dispersor de semillas). Los resultados revelan una tendencia hacia la especialización, pero hacia una especialización laxa, en la que los organismos interactúan con pocas parejas, pero tienden a interactuar de manera equitativa y ser selectivos u oportunistas hacia ellas. Además, se observa una prevalencia de interacciones asimétricas, en las cuales la mayoría de las especies tienen pocas interacciones y unas pocas especies muestran un mayor número de interacciones, y se encuentra que la especialización no está condicionada por la abundancia de los organismos. Este enfoque integrador proporciona una perspectiva más objetiva del fenómeno de la especialización y permite comparaciones entre y dentro de diferentes sistemas de interacción.

Conclusiones

El desarrollo de este trabajo aporta nuevas contribuciones en la comprensión de los patrones de especialización en el contexto de las interacciones bióticas. En concreto, se destaca la dependencia del contexto de la especialización, y cómo ésta varía en función de la escala y de la métrica consideradas. En cuanto a la escala, se observa cómo a escalas espaciales y biológicas más amplias, hay una tendencia a la generalización, al asociarse los micobiontes con mayor número de cianobacterias que a escalas más pequeñas (localidad en lo referido a la escala espacial y talo o individuo en lo referido a la escala biológica). Con las redes observamos cómo la metarred que engloba la totalidad del gradiente tiene una estructura fuertemente anidada, mientras que las redes locales varían entre estructuras anidadas, modulares y aleatorias. En cuanto a las métricas utilizadas, se observa cómo son distintos factores los que explican su variabilidad. Así, la riqueza de cianobiontes y el índice de Simpson se ven afectados por la función de la cianobacteria, mientras que el índice d' se explica en parte por la identidad del micobionte. En base a estos resultados, se propone una nueva aproximación para cuantificar la especialización de una forma más objetiva e integradora, que considera tres facetas fundamentales: la *especificidad*, la *preferencia* y la *selectividad* de las interacciones, considerando estos componentes como complementarios e integrándolos en un espacio tridimensional.

Los resultados demuestran que las interacciones entre los micobiontes y los cianobiontes están condicionadas por distintos factores ambientales y por los rasgos funcionales de las especies. Concretamente, variables relacionadas con la temperatura determinan tanto la composición de las comunidades de cianobiontes en los distintos bosques, lo que determina su disponibilidad, como la modularidad y la conectancia de las redes de interacciones. Por otro lado, tanto la función de la cianobacteria como el tipo de reproducción influyen sobre las interacciones entre los micobiontes y las cianobacterias dependiendo de la escala biológica considerada. En cuanto a la función de la cianobacteria, los cianolíquenes tienden a ser más especializados que los cefalolíquenes al cuantificar la especialización con la riqueza de parejas y el índice de Simpson. Este resultado también se ve reflejado en las redes de interacción, siendo los cefalolíquenes los que predominan en los roles de conectores y de especies centrales (*hub*s) de las redes, ya que, al interactuar con mayor número de cianobacterias, podrían actuar como fuentes de cianobiontes para otros líquenes con mayor grado de especialización. En cuanto a la reproducción, encontramos que las redes menos conectadas son aquellas que cuentan con especies que carecen de estructuras de reproducción, tanto sexuales como asexuales.

Todos los resultados obtenidos han permitido profundizar en la comprensión de las interacciones bióticas, especialmente en las interacciones mutualistas y los fenómenos de especialización asociados. Asimismo, los resultados de esta tesis doctoral han contribuido a aumentar el conocimiento sobre los procesos que influyen en la coexistencia de las especies en comunidades y a entender cómo el establecimiento de unas u otras interacciones interviene en la conformación de dichas comunidades. También queda reflejada la importancia de los factores ambientales y los rasgos funcionales, así como de la escala a considerar en el establecimiento de estas interacciones. Además, el marco teórico propuesto proporciona una perspectiva más objetiva e integradora de la concepción de la especialización.

SUMMARY

Background

One of the main challenges in ecology is to understand the mechanisms behind the assemblage of communities and the coexistence of species. Therefore, it is important to know the requirements of the organisms as well as the factors that might affect the composition of the communities in which they live. One of these aspects is related to the specialization patterns that occur in the biotic interactions established by coexisting species and the processes that influence this specialization. The analysis of biotic interactions can be considered from two perspectives: the interactions that occur at the species level and the interaction networks that encompass the entire community. Regarding the first perspective, certain limitations appear when quantifying specialization, since this phenomenon has traditionally been approached from the number or richness of partners, without integrating other facets such as the abundance of partners and/or the preference for certain partners. In addition, historically, species have been classified as specialists or generalists depending on their requirements and ecological tolerance, but this simplified perspective does not describe the enormous complexity of the phenomenon that specialization represents. Therefore, an integrative perspective that helps to understand the degree of specialization in biotic interactions and the factors underlying this specialization can help to understand community assembly.

Specialization must be contextualized on an axis of the organisms´ requirement niche. In the case of biotic interactions, specialization considers the partners interacting with an organism. In addition, scale, whether biological, spatial or temporal, affects the concept of specialization, since the patterns shown by an organism at small scales may vary when considering larger scales. In turn, specialization is conditioned by both ecological and evolutionary factors. Therefore, determining the environmental factors and functional traits that affect biotic interactions may be a key point in understanding species coexistence.

Organisms such as lichens offer an exceptional study system to understand biotic interactions due to the intimacy of their symbiosis. Lichens have traditionally been

defined as organisms composed by a main fungus, the mycobiont, and one or more photosynthetic components, the photobionts, including green algae, cyanobacteria, or both. The mycobiont is primarily responsible for protecting the photobiont from desiccation, while the photobiont provides nutrients resulting from photosynthesis. In addition, when the photobiont is a cyanobacteria (cyanobiont), it is also responsible of nitrogen fixation. The present doctoral thesis focuses on lichens, specifically on the interactions that occur between the mycobiont and the cyanobiont. The intimacy of this symbiosis has traditionally led to consider mycobionts as having a high degree of specialization for their photobionts, with little flexibility towards its partners. However, recent studies have shown that lichens exhibit distinct patterns of specialization. Furthermore, specialization has been related to different environmental factors and functional traits such as the reproductive mode. However, these studies have focused on specific species or genera, and little is known about how these processes occur at the community level.

Objectives

The main objective of this doctoral thesis is to understand the biotic interactions and the associated specialization phenomenon, as well as to identify and analyse the main factors influencing this process. To this end, the variation of specialization according to the scale considered (both biological and spatial) is analysed, together with the effect of different factors (ecological, derived from environmental and geographical conditions of a wide latitudinal gradient, and the different functional traits of the species) on specialization. With all this, the main aim is to propose an integrative conceptual framework that considers the different facets that determine specialization and that serves as a tool to identify the degree of specialization in a more precise, objective, integrative and universal way.

To achieve this general objective, this PhD thesis uses lichens, a classic example of symbiosis, over a wide latitudinal gradient in the Southern hemisphere as a study system. This main aim is divided into specific objectives developed along the four chapters with the following purposes: 1)to determine the specialization of mycobiont species to their associated cyanobionts with 3 different indices at different spatial scales and analyse the

environmental factors and functional traits that influence specialization, 2) to study specialization at different biological scales and determine individual (intrathaline), intraspecific, and interspecific variability of cyanobionts in different cephalolichen species, 3) to analyse mycobiont-cyanobacterial networks to describe if there is a shared structural pattern and determine how environmental variables and species functional traits influence network structure, and 4) to propose an integrative conceptual framework that considers specialization from a three-dimensional perspective, encompassing three aspects: *specificity*, *preference* and *selectivity*, applicable to all types of biotic interactions.

Methodology

This study focuses on epiphytic lichens communities and their cyanobionts in an extensive latitudinal gradient in the Southern hemisphere, in Chile. This gradient is located between latitudes 38.63° S and 54.96° S, and covers the distribution of *Nothofagus pumilio*, the phorophyte on which lichen communities were sampled. In addition, this gradient includes a wide variability of environmental conditions, ranging from those of the Mediterranean climate in the North of the gradient to those from the boreal climate in the Southernmost forests. Eleven forests located in different protected areas (National Parks or Reserves) were studied. In these forests, organisms of all epiphytic lichen species were collected, and different environmental variables related to forest structure and quality were quantified (slope, inclination, orientation, insolation, canopy cover and diameter at breast height of the trees). The species were subsequently identified with the use of dichotomous keys and monographs. Cyanobacteria present in these species were identified with molecular techniques through the amplification of the *rcbLX* gen region and followed by phylogenetic, network and classification analyses. In addition, different functional traits of the lichen species were considered, including the reproductive mode, the function of the cyanobacterial and the growth form.

To achieve the aims proposed in this study, different indices have been used to quantify the interactions, ranging from indices that quantify the specialization for each species, as well as measures of the network of interactions of the whole community. In addition, different statistical approaches (e.g., generalized linear mixed models and null models) have been used to analyse how interactions are influenced by both, the

environmental factors that cover this wide latitudinal gradient and the functional traits of the species studied.

Results

In Chapter 1, we found that the composition of cyanobionts forming part of the lichen communities is conditioned by different environmental factors such as temperature and the diameter at breast height of the trees. Furthermore, mycobiont specialization for its cyanobacteria varied with the metric and the spatial scale considered. Factors influencing specialization differed depending on the specialization index used. The function of the cyanobacteria partially explained cyanobiont richness and Simpson's index, whereas mycobiont identity mainly affected the d' index. That is, cephalolichens tend to be less specialized than cyanolichens, probably due to a less dependency on the cyanobiont in the case of cephalolichens. On the other hand, there is a tendency to a lower specialization when the spatial scale is widened, increasing the number of cyanobionts interacting with the mycobiont species at larger spatial scales. It has also been identified that lichens exhibiting wide distributional ranges, are associated either with multiple cyanobionts or with cyanobionts with wide distributional ranges. Thus, the results emphasize the context dependency of specialization and how its consideration changes with the metric and the spatial scale considered. Therefore, it is suggested the need to consider the entire community, as well as to define the extent of the spatial scale, since both aspects are crucial to understand the factors determining specialization.

In Chapter 2 the results show a high variability of cyanobionts at different biological scales in cephalolichens. At the individual or intrathalline level, we found different cyanobionts in different cephalodia within the same lichen thallus. When the biological scale was expanded, i.e., from the individual or thallus to the species level, we observe cases in which specialization decreases as they interact with a higher diversity of cyanobionts. In terms of interspecific variability, several cyanobionts were shared among the 3 lichen species studied, while others were exclusive to some of them. Additionally, varying degrees of cyanobiont specialization were observed depending on the forest studied. These results emphasize the importance of contextualizing the biological scale of the study and the determination of the local availability of cyanobionts.

Chapter 3 shows that lichen networks exhibit different structures at the local scale, including modular, nested, and random structures with loosely connected networks, whereas, as the spatial scale increases, a highly nested pattern is observed in the metanetwork. Besides, a latitudinal gradient in the structure of the networks has been identified, with higher modularity towards the North of the gradient and higher connectance towards the South, mainly influenced by temperature. Complementary, the reproductive mode affects network connectance, with networks with individuals lacking reproductive structures, both sexual and/or asexual, being less connected. As in other mutualistic systems, most species were peripheral, although some acted as connectors or hubs, the latter being mainly cephalolichens. Therefore, cephalolichens showed a more relevant structural role than cyanolichens, indicating that they might be involved in facilitation processes. Thus, cephalolichens could be considered sources of cyanobacteria for other species, thus facilitating the establishment of new lichen thalli.

In Chapter 4 an integrative framework combining three metrics that provides complementary information to consider specialization more precisely (specificity, preference and selectivity) is proposed. This framework has been tested in different interaction systems, both antagonistic (host-parasite) and mutualistic (mycobiont-cyanobiont, plant- ant, plant-pollinator, plant-seed disperser). The results reveal a tendency towards specialization, but towards a lax specialization, in which organisms interact with few partners, but with rather even and opportunistic interactions. Furthermore, a prevalence of asymmetric interactions is observed, in which most species have few interactions, and a few species show a higher number of interactions. This integrative approach provides a more objective perspective on the phenomenon of specialization and allows comparisons between and within different interacting systems.

Conclusions

The outcome of this work provides new contributions to the understanding of specialization patterns in the context of biotic interactions. In particular, it highlights the context dependency of specialization, and how it varies depending on the scale and the metric considered. With respect to the scale, we observed how at larger spatial and biological scales, there is a tendency towards generalization, with mycobionts being

associated with a higher number of cyanobacteria than at smaller scales (locality with respect to the spatial scale and thallus or individual with respect to the biological scale). At the community level, the metanetwork of the whole latitudinal gradient showed a highly nested structure, while the local networks varied between nested, modular, and random structures. As for the metrics used, we observe that different factors explain their variability. Thus, cyanobiont richness and Simpson's index are affected by the function of the cyanobacteria, while the d' index is partly explained by the identity of the mycobiont. Based on these results, a new approach is proposed to quantify specialization in a more objective and integrative way, which considers three fundamental facets: *specificity*, *preference*, and *selectivity* of interactions, considering these components as complementary and integrating them in a three-dimensional space.

The results showed that interactions between mycobionts and cyanobionts were conditioned by different environmental factors and by the functional traits of the species. Specifically, temperature-related variables determine both, the composition of cyanobiont communities in different forests, determining their availability, and the modularity and connectance of the networks. On the other hand, both the function of the cyanobacteria and the type of reproduction influence the interactions between mycobionts and cyanobacteria depending on the biological scale considered. Regarding the function of the cyanobacteria, cyanolichens tend to be more specialized than cephalolichens when specialization is quantified with partner richness and Simpson's index. This result is also reflected in the interaction networks, with cephalolichens predominating in the roles of connectors and hub species in the networks. Thus, by interacting with a higher diversity of cyanobacteria, they may act as sources of cyanobionts for other coexisting lichens with a higher degree of specialization. In terms of reproduction, we found that the least connected networks are those with species lacking both, sexual and asexual reproductive structures.

All the results obtained have allowed us to deepen our understanding of biotic interactions, especially in mutualistic interactions and their specialization patterns. Likewise, the results of this doctoral thesis have contributed to increase the knowledge about the processes that influence the coexistence of species in communities and to understand how the establishment of one or other interaction mediates the configuration

of these communities. The importance of environmental factors and functional traits, as well as the scale to be considered in the establishment of these interactions, is also reflected. In addition, the proposed theoretical framework provides a more objective and integrative perspective on the conception of specialization.

INTRODUCCIÓN GENERAL

INTRODUCCIÓN GENERAL

Entender los procesos y las reglas que determinan la coexistencia de las especies y la composición de las comunidades sigue siendo uno de los retos más importantes en ecología de comunidades (Gleason, 1926; Pavoine & Bonsall, 2011). Además, el contexto actual de cambio global hace necesario conocer los efectos que tienen distintas variables ambientales sobre la coexistencia de las especies y la composición de las comunidades para poder predecir su respuesta ante escenarios futuros. Uno de los puntos clave para comprender estos procesos se encuentra en los patrones de especialización que se dan en las interacciones bióticas que establecen las especies coexistentes. Conocer el grado de especialización que presentan las distintas especies que se encuentran conformando una comunidad, así como los procesos que condicionan esa especialización, permite detectar tanto especies más vulnerables a los cambios ambientales, como especies que tengan un papel clave como fuente de recursos para otras especies coexistentes, así como predecir las consecuencias que pueden tener los cambios ambientales en los patrones de especialización. Desentrañar estos procesos puede ayudar, por tanto, a entender cómo se distribuye la biodiversidad y las interacciones bióticas y a desarrollar estrategias de conservación ante los efectos potenciales del cambio global.

1. Especialización

Una de las características de los organismos más reconocidas en ecología es el grado de **especialización** que presentan en relación con las condiciones abióticas y bióticas. En este sentido, cada organismo presenta una serie de requerimientos definidos por distintos factores ambientales y por sus interacciones bióticas, que determinan su supervivencia y reproducción (Forister *et al*., 2012). Por ello, conocer el grado de especialización de un organismo es relevante para entender su biología y sigue siendo un reto en ecología (Forister *et al*., 2012; Futuyma & Moreno, 1988; Thompson, 2005).

El concepto de especialización ha de estar contextualizado dentro del denominado **nicho de requerimientos** (*sensu* Leibold, 1995), entendido como el hipervolumen n-dimensional en el que cada eje hace referencia a distintos factores abióticos o bióticos que determinan la persistencia de las poblaciones y las especies (Carscadden *et al*., 2020; Hutchinson, 1957, 1978). Tradicionalmente, los organismos se han clasificado como

"**especializados**" o "**generalizados**", en función de sus requerimientos y sus límites de tolerancia a lo largo de los distintos ejes del citado nicho (Forister *et al*., 2012; Futuyma & Moreno, 1988). Un organismo se considera especializado cuando sus requerimientos y sus límites de tolerancia son muy estrechos, mientras que un organismo sería considerado generalizado cuando sus requerimientos y sus límites de tolerancia abarcan una mayor amplitud a lo largo del eje del nicho analizado (Figura 1). Un organismo puede considerarse, por tanto, especializado para un eje del nicho, pero generalizado respecto a otro eje. Es importante, por tanto, definir el eje del nicho sobre el que se va a estudiar la especialización. No obstante, mostrar un patrón en un eje puede expandirse a otros ejes del nicho (Peay, 2016). Por ejemplo, según Peay (2016) la habilidad de plantas y hongos micorrizógenos de interactuar con múltiples parejas (tendencia a la generalización) puede contribuir a una ampliación de sus límites de tolerancia o de sus requerimientos abióticos en otros ejes del nicho (generalización) si esas parejas tienen variabilidad funcional, por ejemplo, asimilando distintos nutrientes (Courty *et al*., 2010). Por ello, conocer si la especialización en un eje del nicho conlleva especialización en otros ejes del nicho puede ayudar, entre otras cosas, a predecir qué organismos son más sensibles bajo las condiciones actuales de cambio global (Yang & Rudolf, 2010).

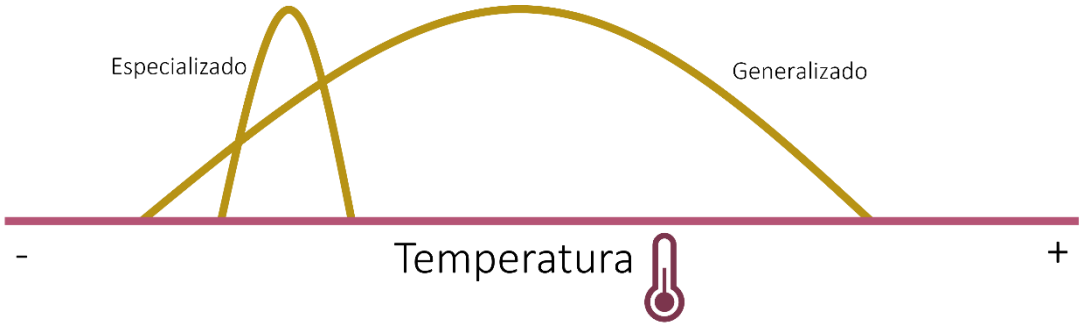


Figure 1. Representación esquemática de los límites de tolerancia del eje del nicho relacionado con la temperatura que presenta un organismo especializado (rango de temperatura reducido) y un organismo generalizado (rango de temperatura amplio).

Por otro lado, la **escala**, ya sea espacial, temporal, biológica o filogenética, también afecta a la especialización, contribuyendo a su **dependencia del contexto** (Figura 2a; Batstone *et al*., 2018; Devictor *et al*., 2010; Fox & Morrow, 1981; Hughes, 2000). En general, se observa una tendencia hacia patrones más generalizados cuando la escala considerada es más amplia (ej.: local vs. regional; especies vs. familias; ver Bolnick *et al*.,

2003; Hughes, 2000). Estos distintos patrones de especialización que pueden presentar los organismos en función de la escala estudiada se deben a cambios en la constancia en el requerimiento de un recurso concreto o un límite de tolerancia entre escalas de baja extensión (Figura 2b; Fox & Morrow, 1981; Herrera *et al*., 2019). Ilustrando este concepto con un ejemplo, una especie (escala filogenética) puede mostrar límites de tolerancia estrechos en distintas localidades (escala espacial), pero diferentes en cada localidad. Este caso se correspondería con un elevado grado de especialización en cada localidad, mientras que la constancia entre los patrones de especialización de la especie en las distintas localidades sería baja. El resultado de esta baja constancia se traduciría en un patrón de generalización en una escala espacial más amplia (escala regional), considerando el conjunto de las distintas localidades.

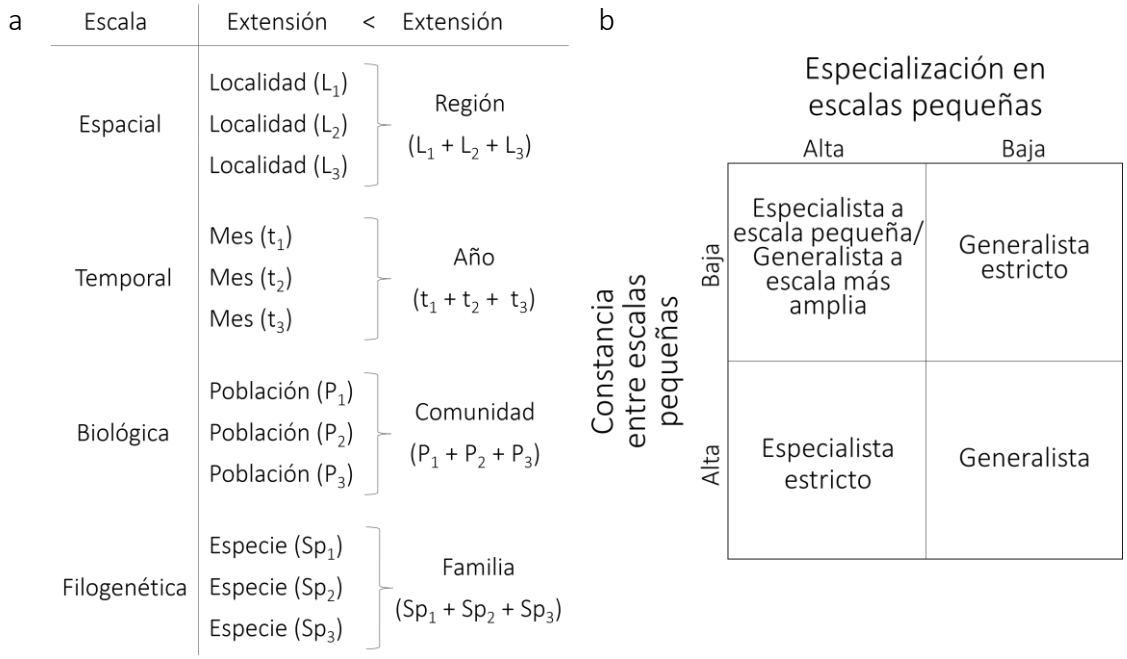


Figure 2. a) Representación de la extensión de distintas escalas (espacial, temporal, biológica y filogenética). A la izquierda se muestra un ejemplo de cada escala con una extensión menor que en el ejemplo de la derecha. b) Adaptación de la figura de Herrera *et al*. (2019) en la que se representa cómo la constancia del grado de especialización de un organismo entre distintas escalas de igual extensión se traduce en la expresión de un grado de especialización igual (alta constancia) o menor (baja constancia) si la extensión de la escala se amplía.

Se han descrito ventajas y desventajas asociadas a los distintos grados de especialización (ej.: Batstone *et al*., 2018; Egan & Funk, 2006). Así, a pesar de que los

organismos más especializados tienden a mostrar un mayor éxito biológico en sus hábitats óptimos, esto les supone un coste cuando se encuentran en otros hábitats (Futuyma & Moreno, 1988). Además, son más sensibles a las perturbaciones dados sus restrictivos requerimientos (MacArthur, 1955). En el caso opuesto, un patrón de generalización podría ser ventajoso para la supervivencia de los organismos bajo un amplio abanico de condiciones abióticas y/o bióticas. Sin embargo, los organismos que muestren este patrón serán peores competidores contra organismos especializados en sus hábitats óptimos (lo que ha sido descrito como "a jack of all trades is a master of none"; MacArthur, 1955). No obstante, estos patrones y sus ventajas y desventajas son muy generales y hay que tener en cuenta que el grado de especialización no es tan simple como una categorización binomial y que los grados de especialización se distribuyen a lo largo de un continuo (Figura 3; Blüthgen *et al*., 2006; Devictor *et al*., 2010; Forister *et al*., 2012; Fox & Morrow, 1981; Novotny *et al*., 2010; Poisot *et al*., 2015).

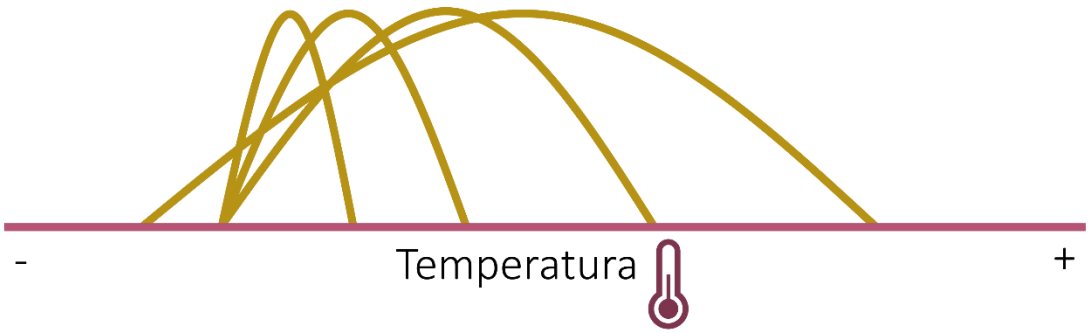


Figure 3. Representación esquemática de la naturaleza continua de los distintos grados de especialización a lo largo del eje del nicho relacionado con la temperatura.

En cuanto a las ventajas evolutivas de la especialización, es importante destacar su importancia en el estudio de los modelos de **radiación adaptativa** (Schluter, 2000). En este contexto, se considera que la especialización limita el solapamiento de nicho al generar nichos discretos, lo que favorece la diversificación de las especies (Funk *et al*., 2002; Gavrilets & Losos, 2009; Schluter, 2000). En otras palabras, la especialización promueve el aislamiento y la división de las poblaciones, lo que restringe el flujo génico y puede facilitar fenómenos de diversificación y especiación (Berlocher & Feder, 2002; Colles *et al*., 2009; De Vienne *et al*., 2013; Funk *et al*., 2006; Futuyma, 2001; Price, 1980; Rundle & Nosil, 2005; Winkler & Mitter, 2008). Aunque simultáneamente y en relación con lo anterior, puede producirse una reducción de la variabilidad genética de las

poblaciones al explotar un número de recursos más limitado de manera más eficiente. Esta reducción de la variabilidad genética junto a los pequeños tamaños poblacionales y los restringidos rangos de distribución, comprometen la habilidad de las especies para adaptarse a situaciones cambiantes y aumentan su riesgo de extinción (Colles *et al*., 2009). Por todo ello, se considera que la especialización tiene costes evolutivos, pudiendo llegar a ser un callejón sin salida que puede llevar a la extinción de especies (Biesmeijer *et al*., 2006; Colles *et al*., 2009; McKinney, 1997; Moran, 1988; Simpson, 1944).

Otro punto importante son los factores que influyen sobre los grados de especialización, pudiendo ser éstos tanto ecológicos como evolutivos (Armbruster, 2006; Herrera *et al*., 2019). Ecológicamente, la especialización se define en base a la estrecha amplitud de los límites de tolerancia o requerimientos de los organismos en un eje del nicho. De igual manera, la especialización se identifica con tener un rango de hábitats restringido y también puede considerarse como una selección por los recursos independientemente de su proporción disponible (Irschick *et al*., 2005). Por tanto, se espera que, hábitats con gran variabilidad de recursos disponibles, favorezcan el aumento en la especialización de los organismos, lo que limitaría el solapamiento de nicho entre dichos organismos (Schemske, 2002; Schemske *et al*., 2009). Asimismo, hábitats climáticamente estables durante largos periodos de tiempo, podrían favorecer la especialización de los organismos, ya que la ausencia de grandes fluctuaciones garantizaría que no se produjesen grandes cambios en la composición de especies de las comunidades, así como cambios en su fenología (ej.: Dalsgaard *et al*., 2013).

Evolutivamente, la especialización hace referencia a las limitaciones genéticas que condicionan la evolución de ciertos rasgos que permiten la explotación de muchos recursos (generalización) versus pocos recursos (especialización) de forma efectiva (Futuyma & Moreno, 1988; Huey & Hertz, 1984; Irschick *et al*., 2005). En este contexto, la especialización se ha relacionado tradicionalmente con el desarrollo de rasgos adaptativos, indicando que un alto grado de adaptación en un determinado rasgo es una fuerte evidencia de especialización (Irschick *et al*., 2005). Además, tradicionalmente se asumía que la adaptación de un determinado rasgo que genera un incremento en la eficacia biológica al explotar un recurso concreto disminuiría la eficacia biológica respecto a la explotación de otro recurso (Aigner, 2001; Wilson & Thomson, 1996). Sin embargo,

hay estudios que postulan que esto no tiene por qué ser así, ya que el desarrollo de un rasgo adaptativo que permita la favorable explotación de un recurso no tiene por qué implicar un detrimento en la explotación de otro recurso (Aigner, 2001). Por tanto, aún es un desafío conocer si la especialización, entendida como los estrechos requerimientos abióticos y/o bióticos y como la expresión de ciertos rasgos dirigidos a la explotación de recursos concretos implica una limitación en la efectividad para explotar un rango de recursos más amplio (Irschick *et al*., 2005).

Todas estas premisas hacen que los grados de especialización sean dependientes del contexto en el que van a ser considerados, tanto en lo relativo al eje del nicho en el que se centra su análisis como a la escala. Esto hace que los términos de especialización y generalización sean relativos y sólo se consideren útiles en el contexto previamente definido (Forister *et al*., 2012). Además, esta contextualización es necesaria para responder a distintas cuestiones tanto macroecológicas como macroevolutivas. Entre estas cuestiones estarían, por ejemplo, entender la distribución de los organismos especializados y generalizados en el espacio y en el tiempo o comprender la evolución de distintos rasgos adaptativos utilizados para satisfacer eficientemente los requerimientos abióticos y/o bióticos de los organismos (Futuyma & Moreno, 1988).

2. Especialización en interacciones bióticas

La especialización dentro del marco de las interacciones bióticas ha sido un tema de gran interés en ecología (Armbruster, 2017; Bascompte & Jordano, 2014; Futuyma & Moreno, 1988) y, en este contexto, los estudios de especialización se han centrado en sistemas antagonistas y mutualistas. Las relaciones antagonistas se definen como interacciones en las que un organismo obtiene beneficio a costa del otro e incluyen, entre otras, las interacciones parásito-hospedador o depredador-presa. Por otro lado, las relaciones mutualistas son aquellas en las que ambos integrantes obtienen beneficio mutuo (Boucher *et al*., 1982) gracias al intercambio recíproco de recursos y/o servicios (Peay, 2016). Algunos ejemplos son los sistemas de polinización, las relaciones planta-micorriza o las relaciones entre el micobionte y el fotobionte en el caso de los líquenes. El número de parejas potenciales presentes en una comunidad definiría el eje del hipervolumen del nicho sobre el que estudiar la especialización en las interacciones

bióticas (Forister *et al*., 2012; Koffel *et al*., 2021). Esta interacción entre distintos organismos puede darse de múltiples formas, que pueden ir desde una especialización recíproca en la que ambos integrantes de la interacción interactúan exclusivamente entre ellos, hasta una generalización total en la que ambos integrantes interactúan con múltiples parejas. También pueden darse casos de especialización intermedia o asimétrica en la que uno de los integrantes se asocia con un amplio número de parejas mientras que el otro se asocia con un número de parejas más limitado, siendo este último el patrón encontrado más frecuentemente en la naturaleza (Bascompte *et al*., 2003; Vázquez & Aizen, 2004).

En el caso de las relaciones bióticas, la interacción con el otro organismo puede llevar a la **expansión o disminución de los límites de tolerancia** o los requerimientos en otros ejes del nicho del organismo de estudio (ej.: Afkhami *et al*., 2014). Por ejemplo, en interacciones de tipo mutualista, esa expansión en otros ejes del nicho puede deberse a que los beneficios de la asociación amplíen los límites de tolerancia de los organismos respecto a los límites de tolerancia de los organismos por separado (ver Peay, 2016 para sistemas planta-micorriza). Por otro lado, también puede ocurrir una disminución en los límites de tolerancia en aquellas interacciones mutualistas obligadas donde los organismos integrantes están condicionados por los límites de tolerancia de sus parejas, lo que genera una restricción en su amplitud de nicho (ej.: Mueller *et al*., 2011; Warren *et al*., 2010).

La **escala** también ha de tenerse en cuenta al estudiar los fenómenos de especialización en interacciones bióticas (Burkle & Alarcón, 2011; CaraDonna *et al*., 2017; Fox & Morrow, 1981). Los cambios en los grados de especialización de un organismo dado en función de si se consideran escalas de menor o de mayor extensión pueden explicarse analizando la **diversidad beta** (Hughes, 2000; Poisot *et al*., 2012). En este sentido, Ventre Lespiaucq *et al*. (2021) definen distintos patrones de recambio de parejas. Estos patrones varían desde el reemplazo total de parejas hasta la ausencia de reemplazo y mantenimiento de las mismas parejas entre dos puntos (ej.: dos localidades en lo referido a la escala espacial). El análisis de la diversidad beta de las parejas interactuantes permite, por tanto, comparar los grados de especialización a distintas escalas (Figura 2a). Adicionalmente, encontramos dos aspectos que influyen en la diversidad beta al

considerar comunidades coexistentes de organismos que interactúan en redes de interacción: el **recambio de especies** y el **recambio de interacciones** (Poisot *et al*., 2012; Poisot *et al*., 2015). Así, un alto grado de generalización al ampliar la extensión de la escala considerada puede darse debido a 1) cambios en la composición de especies de la comunidad (recambio de especies) y la necesidad de asociarse con parejas distintas porque las parejas previamente consideradas dejen de estar disponibles, o a 2) cambios en las interacciones producidos cuando la pareja con la que interactúa un organismo está disponible, pero el organismo interacciona con otras parejas distintas (recambio de interacciones).

Al igual que para la especialización en general, en las interacciones bióticas, factores ecológicos y evolutivos influyen en los patrones de especialización (Armbruster, 2006; Herrera *et al*., 2019). En cuanto a los **factores ecológicos**, la especialización puede verse afectada por la disponibilidad de las parejas interactuantes debido a filtros abióticos y/o bióticos (Iglesias-Prieto *et al*., 2004; Rolshausen *et al*., 2020; Suz *et al*., 2014). Así, se espera que en aquellas regiones con una elevada riqueza de parejas con las que interactuar, los organismos tiendan a ser más especializados, disminuyendo el solapamiento de nicho con otros organismos (Dalsgaard *et al*., 2011; Hillebrand, 2004; Trøjelsgaard & Olesen, 2013). Adicionalmente, como se ha mencionado anteriormente, zonas con elevada estabilidad climática, tanto presente como pasada (ver Dalsgaard *et al*., 2013), tenderán a mostrar organismos con patrones más especializados. Por el contrario, en condiciones ambientales poco estables o con elevadas fluctuaciones, los organismos tenderán a patrones de generalización, asociándose con numerosas parejas para hacer frente a la variabilidad de las condiciones abióticas y/o bióticas (Dalsgaard *et al*., 2011, 2013; Dynesius & Jansson, 2000; Rech *et al*., 2016; Trøjelsgaard & Olesen, 2013; Waser *et al*., 1996). Ambos factores, tanto el mayor número de especies con las que interactuar como la estabilidad de las condiciones ambientales, hacen que se espere un gradiente latitudinal de especialización (Figura 4). Este gradiente reflejaría un mayor grado de especialización en las regiones cercanas al Ecuador, donde se encuentra una elevada riqueza de especies (Teoría del Gradiente Latitudinal de Diversidad; Hillebrand, 2004; Kinlock *et al*., 2018) y elevada estabilidad climática, y una mayor generalización hacia los polos. Sin embargo, esta hipótesis, propuesta en origen por Robert MacArthur (1972), y

que expone que la amplitud de nicho está positivamente asociada con la latitud, no ha sido apoyada completamente por estudios posteriores. Así, se han encontrado resultados dispares al analizar esta teoría del gradiente de especialización en distintos tipos de interacciones bióticas (ver Dalsgaard *et al*., 2017), y se ha sugerido su replanteamiento junto con el de las hipótesis que la sustentan (ver Vázquez & Stevens, 2004). Por ejemplo, para distintos sistemas mutualistas de polinización, mientras que Dalsgaard *et al*. (2011) encuentran un aumento en la especialización cerca del Ecuador, Schleuning *et al*. (2012) encuentran una menor especialización en zonas tropicales respecto a zonas templadas. Por otro lado, en sistemas antagonistas no se encuentran patrones de especialización condicionados por la latitud (Morris *et al*., 2014). Encontrar el patrón opuesto al esperado, en el que se observaría un aumento de especialización hacia los polos, podría ser el resultado de interactuar con parejas mejor adaptadas a condiciones adversas, o de una disponibilidad limitada de parejas con las que interactuar en estas latitudes alejadas del Ecuador, lo que restringiría la posibilidad de presentar un alto grado de generalización (Ollerton & Cranmer, 2002).

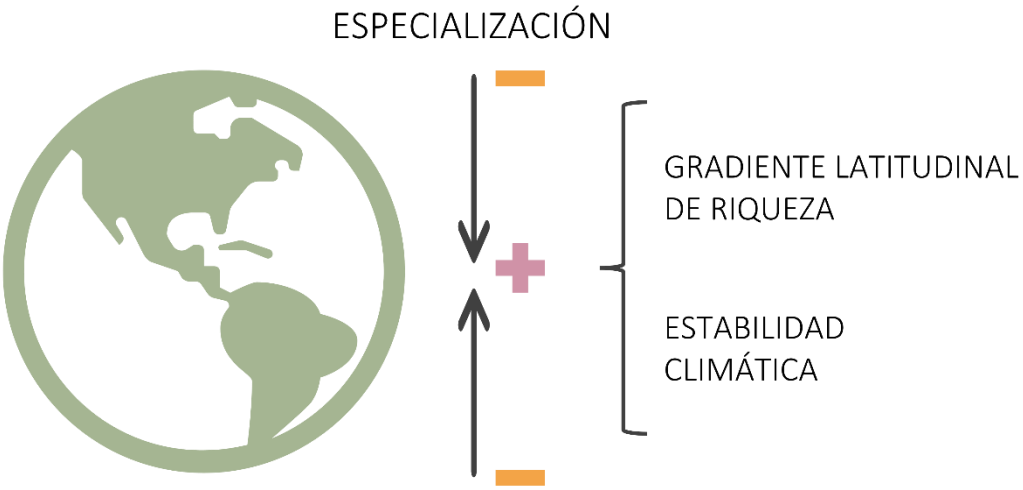


Figure 4. Esquema del gradiente latitudinal de especialización que sugiere una mayor especialización en latitudes menores debido a una mayor estabilidad climática y un elevado número de especies en estas latitudes.

Evolutivamente, la interacción única y repetida entre dos organismos durante largos periodos de tiempo puede promover la **coevolución** y **coespeciación**, haciendo que ambos organismos estén cada vez más especializados entre sí (Armbruster, 2006; De

Vienne *et al*., 2013; Forister *et al*., 2012; Herrera *et al*., 2019; Thompson, 1999, 2005). La coevolución sería el resultado de una selección recíproca a corto plazo que no implica una diversificación (De Vienne *et al*., 2013). Por otro lado, las dinámicas evolutivas a largo plazo podrían deberse a fenómenos de coespeciación de ambos integrantes de la interacción, o de especiación debida al cambio de la pareja interactuante (De Vienne *et al*., 2013). Basadas en estas dos últimas premisas de dinámicas evolutivas a largo plazo, surgen dos hipótesis sobre le evolución de la especialización: la Hipótesis Oscilatoria (Oscillation Hypothesis), que sugiere que la generalización es un estado efímero y transitorio que da lugar a la especiación de taxones especializados, dado que la especialización beneficia la eficacia biológica de las especies (Janz & Nylin, 2008) y la Hipótesis de las sillas musicales (Musical Chairs Hypothesis), que postula que la especiación está dirigida por cambios de una pareja interactuante por otra, lo que indica que los organismos especializados pueden cambiar de pareja sin la necesidad de pasar por un estado intermedio de generalización (Hardy & Otto, 2014; Schluter, 2000). Por ejemplo, en interacciones liquen-hongo liquenícola se ha encontrado este último patrón, sugiriendo que la diversificación de ciertos hongos liquenícolas se ha producido por cambios en el hospedador (Millanes *et al*., 2014).

Es importante destacar que la especialización en las interacciones bióticas tiene ciertas ventajas entre las que destacamos una mejora en la eficacia biológica (ej.: Egan & Funk, 2006). En este sentido, un alto grado de especialización va asociado implícitamente a la idea de que los recursos que utiliza o requiere un organismo especializado, en este caso las parejas interactuantes, son utilizados con una mayor eficacia que con la que lo haría un organismo generalizado (Armbruster, 2017), de nuevo ligado a la frase "a jack of all trades is a master of none" (MacArthur, 1955). En cuanto a la generalización, Batstone *et al*. (2018) señalan que un patrón más generalizado puede ser una ventaja selectiva debido a que la interacción con varias parejas puede suponer: a) un incremento en las posibilidades de interactuar con parejas mejor adaptadas (*sampling effect*), b) una disminución en el impacto que puede conllevar la pérdida de parejas si hay otras parejas que aportan funciones similares a través de distintos mecanismos (*complementarity*), o c) una mejora en el rendimiento de la asociación cuando las parejas tienen dinámicas asincrónicas, interactuando con distintas parejas en distintos escenarios (*portfolio effect*).

Por otra parte, el grado de especialización también influye en los **rangos de distribución** de los organismos (Peay, 2016). La hipótesis principal respecto a los rangos de distribución sugiere que los organismos muy especializados tendrán rangos de distribución más reducidos, al verse condicionados principalmente por la distribución de sus parejas (Magain *et al*., 2017). Sin embargo, los organismos especializados también pueden mostrar amplios rangos de distribución cuando se asocian con una pareja que tiene un amplio rango de distribución (Fedrowitz *et al*., 2011; Zúñiga *et al*., 2017). Por otro lado, se esperaría que los organismos más generalizados tiendan a mostrar rangos de distribución más amplios (ej.: Maher *et al*., 2017; Peay, 2016). Así, la flexibilidad para asociarse con distintas parejas hace que la ausencia de una de ellas no limite el rango de distribución de un organismo, pudiendo éste asociarse con otras parejas. Además, como se ha mencionado anteriormente, la interacción con distintas parejas puede aumentar los límites de tolerancia en distintos ejes del nicho, lo que implicaría un aumento de los grados de tolerancia a otros factores abióticos permitiendo el establecimiento del organismo en un mayor rango de hábitats (Iglesias-Prieto *et al*., 2004; Rolshausen *et al*., 2018; Suz *et al*., 2014).

Finalmente, otro de los temas controvertidos en los estudios de especialización dentro de las interacciones bióticas está relacionado con cómo definir y cómo cuantificar la especialización (ver Armbruster, 2017; Devictor *et al*., 2010; Poisot *et al*., 2012). En este contexto, la especialización se ha medido tradicionalmente considerando exclusivamente el **número de parejas** con el que interactúa un organismo (Blüthgen *et al*., 2006; Sahli & Conner, 2006). Sin embargo, esta aproximación conlleva ciertas restricciones dado que un organismo puede interactuar con varias parejas en distintas proporciones mostrando cierta "preferencia" por algunas de ellas, lo que indicaría cierta especialización, o puede interactuar con las parejas más raras de la comunidad y menos disponibles, mostrando también cierta especialización y un menor oportunismo (ver Blüthgen *et al*., 2006). Por ello, se han propuesto en los últimos años distintas medidas para cuantificar la especialización, utilizando índices de diversidad como el **índice de Simpson** (Magain *et al*., 2018; Sahli & Conner, 2006) o índices de amplitud de nicho como el **índice d'** (Blüthgen *et al*., 2006).

Además, es importante destacar que los sistemas de interacciones son más complejos que la interacción aislada de cada uno de sus integrantes (Thompson, 2005). Esto hace que la cuantificación de la especialización basada en las interacciones por parejas muestre ciertas limitaciones. Por ello, nuevas aproximaciones desde el enfoque de **redes de interacciones** han sido desarrolladas en los últimos 20 años, teniendo en cuenta que los organismos evolucionan y coexisten dentro de redes de interacción (Bascompte & Jordano, 2007). Las redes de interacción pueden ser **unipartitas**, en las que hay un conjunto de nodos que interactúan entre sí (los nodos hacen referencia a los organismos), o **bipartitas**, en las que hay dos clases de nodos y las interacciones se dan entre nodos de ambas clases y no entre nodos de la misma clase. En estas últimas se distinguen dos clases de organismos conformando los nodos: *recursos* (ej.: hospedadores en sistemas de parásito-hospedador o plantas en sistemas de polinización) y *consumidores* (ej.: parásitos en sistemas de parásito-hospedador o polinizadores en sistemas de polinización). Un ejemplo de redes unipartitas serían las redes tróficas, en las que se pueden dar interacciones entre distintos grupos de organismos. Por otro lado, un ejemplo de red bipartita serían los sistemas mutualistas: en sistemas de polinización encontramos dos clases totalmente diferenciadas de organismos que interactúan entre sí y que no interactúan con nodos de su misma clase: los polinizadores (consumidores) y las plantas (recursos).

Distintas medidas informan sobre la estructura de las redes y se han utilizado para encontrar patrones generales que permiten comprender la coexistencia de especies y la estabilidad de las comunidades (Bascompte, 2009; Bascompte *et al*., 2003). Entre estas medidas son comúnmente utilizadas la conectancia, el anidamiento o la modularidad. La **conectancia** hace referencia al número de interacciones observado en relación al número de interacciones posibles (Jordano, 1987) y es una medida de complejidad de las redes (Heleno *et al*., 2012) que presenta un papel estabilizador al proporcionar robustez (ej.: en redes tróficas; Dunne *et al*., 2002). El **anidamiento** indica que los organismos especializados interactúan con subgrupos de organismos con los que también interactúan los organismos generalizados, lo que se traduce en una elevada cohesión en las redes que ofrece respuestas alternativas ante una perturbación y confiere robustez ante la pérdida aleatoria de especies (Bascompte *et al*., 2003; Ramos-Jiliberto *et al*., 2010). Por último, la

modularidad describe una estructura más compartimentada en la que se observan subgrupos más pequeños que incluyen organismos más conectados entre sí que con el resto de la red. La modularidad afecta tanto a la estabilidad de las redes amortiguando la propagación de una perturbación a la red completa, como favoreciendo la coevolución en el caso de las especies que conforman cada módulo (Gilarranz *et al*., 2017; May, 1972; Olesen *et al*., 2007; Stouffer & Bascompte, 2011).

En función del tipo de interacción (antagonista o mutualista) se espera que la estructura de la red confiera distintas propiedades al sistema de interacción. Por ejemplo, Thébault & Fontaine (2010), postulan que, en interacciones antagonistas, como las redes tróficas, una estructura modular aumenta la resiliencia de la red mientras que el anidamiento disminuye su persistencia. Por otro lado, en interacciones mutualistas ocurre al contrario: el anidamiento aumenta la resiliencia y la modularidad reduce la persistencia de la red. Además, una elevada diversidad de especies promueve la persistencia y resiliencia de las redes mutualistas mientras que es la conectancia la que tiene esta función en redes antagonistas (Thébault & Fontaine, 2010). No obstante, la modularidad también se espera en interacciones mutualistas íntimas, ya que los distintos módulos resultantes de la estrecha asociación podrían configurar unidades coevolutivas (Olesen *et al*., 2007; Thompson, 1994). Estas unidades coevolutivas representarían grupos de especies que interactúan preferencialmente entre ellas, desarrollando rasgos complementarios que convergen entre especies filogenéticamente distantes para facilitar esa interacción (Bascompte *et al*., 2006; Dupont & Olesen, 2009; Thompson, 2005).

3. Líquenes como ejemplo de interacciones bióticas

Los líquenes son un ejemplo de relaciones mutualistas. Se trata de una íntima asociación simbiótica en la que interactúan un hongo principal, el micobionte, y una o varias parejas fotosintéticas, fotobiontes, que pueden ser algas verdes, cianobacterias o ambas. Además, formando parte de esta simbiosis se encuentra un número indeterminado de microorganismos como levaduras y bacterias, lo que hace que los líquenes se consideren ecosistemas en miniatura (Allen & Lendemer, 2022; Hawksworth & Grube, 2020). El resultado de este conjunto de interacciones es el talo liquénico, cuya identificación y clasificación se ha basado históricamente en la taxonomía del micobionte

(Nash, 1996). Centrándonos en los componentes principales y en su papel mutualista, encontramos que el micobionte se encarga principalmente de proteger al fotobionte de la desecación, mientras que el fotobionte realiza la fotosíntesis aportando carbohidratos y, en el caso de que se trate de una cianobacteria, también fija nitrógeno atmosférico (Nash, 1996).

Los líquenes que contienen cianobacterias representan cerca del 10 % de las especies descritas de líquenes (Jüriado *et al*., 2019; Rikkinen, 2015), y tienen un importante papel ecológico dada su capacidad de fijar nitrógeno atmosférico (Asplund & Wardle, 2017; Ellis, 2012). Las cianobacterias que forman parte de la simbiosis liquénica pertenecen en su mayoría a los géneros *Nostoc* y *Scytonema* (Rambold *et al*., 1998). Hay dos tipos de líquenes que se asocian con cianobacterias: los cianolíquenes, en los que la cianobacteria es el único fotobionte (líquenes bipartitos), o los cefalolíquenes, en los que el fotobionte principal encargado de la fotosíntesis es un alga verde y las cianobacterias se encuentran en unas estructuras diferenciadas denominadas cefalodios que se encargan principalmente de la fijación de nitrógeno (líquenes tripartitos; Nash, 1996).

La mayoría de los estudios de especialización en la simbiosis liquénica se han realizado desde la perspectiva de las interacciones entre parejas de simbiontes, utilizando la riqueza de parejas de fotobiontes como medida de especialización (ej.: Magain *et al*., 2017; Yahr *et al*., 2004, 2006). Además, la totalidad de estos estudios se centran en la especialización del micobionte por el fotobionte, debido a que los fotobiontes se consideran menos dependientes de la simbiosis al ser encontrados también de vida libre (Magain *et al*., 2017; Zúñiga *et al*., 2017). Estos trabajos analizan distintas escalas biológicas y filogenéticas, incluyendo desde poblaciones, a géneros o familias (ej.: Fernández-Mendoza *et al*., 2011; Leavitt *et al*., 2015; Magain *et al*., 2017; Pino-Bodas & Stenroos, 2021). En ellos se han encontrado representados distintos grados de especialización, desde especialización recíproca hasta generalización por parte de ambos simbiontes (Magain *et al*., 2017; Otálora *et al*., 2010; Pino-Bodas & Stenroos, 2021; Yahr *et al*., 2006).

Otro tema escasamente abordado en estos organismos consiste en analizar la variabilidad de fotobiontes a distintas escalas biológicas (individuo o talo, especie, comunidad). A nivel intratalino (individuo), se destaca que, mientras que en clorolíquenes

la coexistencia de varias especies de algas verdes en el mismo talo es un fenómeno ampliamente reconocido (ej.: Moya *et al*., 2017; Muggia *et al*., 2013; Ohmura *et al*., 2019), en líquenes que contienen cianobacterias hay menos evidencias. En concreto, en cianolíquenes, la mayoría de los estudios encuentran sólo un filogrupo de *Nostoc* formando parte de esta interacción (ej.: Magain *et al*., 2017; O'Brien *et al*., 2013; Ramírez-Fernández *et al*., 2013). Sin embargo, en cefalolíquenes, hay resultados contrastados, donde la mayoría de las especies presentan el mismo filogrupo de *Nostoc* en sus cefalodios (Paulsrud *et al*., 2001), y unas pocas especies presentan distintos cianobiontes en diferentes cefalodios (Paulsrud *et al*., 2000 en el caso de *Peltigera venosa* y Myllys *et al*., 2007 en el caso de *Lobaria pulmonaria*).

Complementariamente, los estudios que trabajan a nivel de comunidad se centran principalmente en fenómenos de facilitación e intercambio de fotobiontes, definiendo distintas agrupaciones o gremios (*guilds*) que comparten un pool de fotobiontes y se diferencian principalmente por sus requerimientos ecológicos (Cardós *et al*., 2019; O'Brien *et al*. , 2013; Rikkinen, 2013; Rikkinen *et al*., 2002). En estos gremios se distinguen entre especies centrales (*core*) que actúan como fuente de fotobiontes para las especies periféricas (*fringe*), que serían las que captan los fotobiontes de las especies centrales (Rikkinen *et al*., 2002). Entre los gremios de los líquenes que contienen cianobacterias se distinguen tradicionalmente el del género *Peltigera*, en el que se encuentran cianolíquenes terrícolas, y el del género *Nephroma*, formado por especies epífitas de cianolíquenes (Rikkinen *et al*., 2002). Los estudios centrados en los gremios sugieren que la especialización por los distintos cianobiontes se da a nivel de comunidad sin encontrar correlación entre las especies de micobionte y los distintos clados de *Nostoc* (Rikkinen *et al*., 2002; Stenroos *et al*., 2006).

En cuanto a la perspectiva de redes de interacciones, los escasos estudios de líquenes realizados hasta la fecha encuentran fuertes patrones modulares en estas redes de interacción, indicando preferencias en las interacciones (Chagnon *et al*., 2018, 2019; Duran-Nebreda & Valverde, 2023; Kaasalainen *et al*., 2021). Chagnon *et al*. (2018, 2019) analizan estas redes en el género *Peltigera*, encontrando que existe una estructura filogenética conservada en la selección de parejas, que se traduce en que especies más cercanas de *Peltigera* tienden a interactuar con las mismas cianobacterias o con

cianobacterias con pequeñas distancias genéticas. De igual manera, las especies de micobionte encontradas en los mismos módulos tienden a estar más emparentadas filogenéticamente que lo esperado por azar. Kaasalainen *et al*. (2021), estudian el intercambio y facilitación de cianobacterias analizando las redes de toda la comunidad de líquenes y encuentran distintos patrones en la estructura de las redes en función de los gremios. En este estudio encuentran mayor anidamiento en el *guild* de *Nephroma* mientras que el *guild* de *Peltigera* es más compartimentado o modular. Además, sugieren que sus resultados reflejan las diferencias ecológicas previamente mencionadas entre los dos gremios (epífitos vs. terrícolas). Por último, Duran-Nebreda & Valverde (2023), encuentran patrones modulares, esta vez analizando las interacciones entre distintas especies de micobiontes y todos los tipos posibles de fotobionte, lo que indica preferencias en las interacciones (Chagnon *et al*., 2018).

La disponibilidad de los fotobiontes ha sido considerada un condicionante de los patrones de especialización en líquenes (Yahr *et al*., 2004). En este sentido, se han encontrado distintos factores ambientales y geográficos condicionando la distribución y la disponibilidad potencial de los fotobiontes (Dal Grande *et al*., 2018; Fernández-Mendoza *et al*., 2011; Magain *et al*., 2017). Fernández-Mendoza *et al*. (2011) encuentran que los fotobiontes de *Cetraria aculeata* muestran una estructura genética determinada por factores ecológicos. Asimismo, factores climáticos relacionados con gradientes altitudinales pueden estructurar la comunidad de los fotobiontes (Dal Grande *et al*., 2014; Vargas Castillo & Beck, 2012). Por tanto, el establecimiento de la simbiosis puede verse limitado por la disponibilidad de fotobiontes adaptados localmente, por lo que pueden darse cambios de un fotobionte por otro con una mejor adaptación local. En condiciones extremas, por ejemplo, se espera que el número de cianobacterias disminuya a causa de los filtros ambientales, lo que se traduce en una disminución de la especialización por parte del micobionte para ampliar el rango de parejas. De esta forma podría aumentar las posibilidades de encontrar parejas mejor adaptadas localmente o que ofrezcan complementariedad que favorezca la supervivencia bajo estas condiciones (Batstone *et al*., 2018; Singh *et al*., 2017; Wirtz *et al*., 2003). Sin embargo, no sólo la disponibilidad del fotobionte condiciona la especialización. Hay estudios que encuentran que, aun estando disponibles los fotobiontes con los que interactúan ciertas especies de micobionte, éstas

pueden asociarse con fotobiontes diferentes, originándose un recambio de interacciones (ej.: Rolshausen *et al*., 2018, 2020). Además, hay especies de micobionte con rangos de distribución más estrechos que los de sus fotobiontes, estando la interacción condicionada por otros factores más allá de la disponibilidad de dichos fotobiontes (ej.: Lu *et al*., 2018).

En cuanto a otros factores que influyen en la especialización en líquenes se han identificado la identidad de los micobiontes y ciertos rasgos funcionales. Hay estudios que muestran que la identidad del micobionte condiciona la especialización (Dal Grande *et al*., 2018; Fedrowitz *et al*., 2011; Jüriado *et al*., 2019; Leavitt *et al*., 2015) y que consideran que es el factor que más influye en las interacciones entre micobiontes y fotobiontes en líquenes (Leavitt *et al*., 2015). Estos resultados sugieren que la especialización podría tener un componente genético. Por otro lado, entre los rasgos funcionales que afectan a la especialización se encuentran la reproducción, la función del fotobionte (es decir, si son micobiontes asociados con algas verdes, con cianobacterias o con ambas) o el biotipo (Beck *et al*., 2002; Berlinches de Gea *et al*., 2023; Blaha *et al*., 2006; Fedrowitz *et al*., 2012; Fernández-Mendoza *et al*., 2011; Kaasalainen *et al*., 2021; Lücking *et al*., 2009; Muggia *et al*., 2008; Otálora *et al*., 2010; Peksa & Škaloud, 2011; Piercey-Normore & Deduke, 2011; Rikkinen, 2003; Yahr *et al*., 2006). Por ejemplo, se ha encontrado una mayor especialización en líquenes con reproducción asexual debido a que el micobionte y el fotobionte se dispersan juntos teniendo lugar una transmisión vertical del fotobionte (Otálora *et al*., 2010; Steinová *et al*., 2019). Esta transmisión vertical puede promover la coevolución y/o la coespeciación de ambos integrantes de la interacción (Thompson, 2005), por lo que hay estudios que sugieren que la reproducción asexual en líquenes puede favorecer la especialización recíproca (Magain *et al*., 2017). En cambio, las estructuras de reproducción sexual en líquenes forman esporas exclusivamente del micobionte, teniendo éste que asociarse con nuevos fotobiontes para reestablecer la simbiosis, por lo que la transmisión del fotobionte es horizontal en este caso. Sin embargo, numerosos estudios han encontrado que se pueden producir cambios de fotobionte en líquenes con reproducción asexual, teniendo lugar también una trasmisión horizontal del fotobionte en esos casos (ej.: Moya *et al*., 2020; Piercey-Normore & DePriest, 2001).

El aumento en la flexibilidad a la hora de interactuar con distintos fotobiontes es considerado una estrategia para mejorar la tolerancia ecológica y ampliar, así, los límites de tolerancia en otros ejes del nicho (Ertz *et al*., 2018). Esto puede permitir a la asociación simbiótica extender su rango de distribución, ocupando diferentes microhábitats y hacer frente a cambios en las condiciones ambientales (Ertz *et al*., 2018; Fernández-Mendoza *et al*., 2011; Yahr *et al*., 2004). Contrariamente, los líquenes especializados se ven limitados por la distribución de su simbionte para expandir su rango de distribución, aunque también se han encontrado amplios rangos de distribución en especies especializadas que se asocian con fotobiontes ampliamente distribuidos (Magain *et al*., 2018). Comprender, por tanto, la flexibilidad que presentan distintas especies de micobiontes para interactuar con sus cianobiontes, podría ayudar a comprender los rangos de distribución que presentan los líquenes y su presencia en hábitats con condiciones ambientales muy adversas (ej.: Green *et al*., 1999; Heber *et al*., 2006; Sancho *et al*., 2007, 2011).

4. Referencias

Afkhami, M. E., McIntyre, P. J., & Strauss, S. Y. (2014). Mutualist-mediated effects on species' range limits across large geographic scales. *Ecology Letters*, *17*(10), 1265–1273. https://doi.org/10.1111/ele.12332

Aigner, P. A. (2001). Optimality modeling and fitness trade offs: when should plants become pollinator specialists? *Oikos*, *95*, 177–184. https://doi.org/10.1034/j.1600-0706.2001.950121.x

Allen, J. L., & Lendemer, J. C. (2022). A call to reconceptualize lichen symbioses. *Trends in Ecology and Evolution*, *37*(7), 582–589. https://doi.org/10.1016/j.tree.2022.03.004

Armbruster, W. S. (2006). Evolutionary and ecological perspectives on specialization: from the arctic to the tropics. In *Plant-Pollinator Interactions: From Specialization to Generalization (eds N. Waser & J. Ollerton). University of Chicago Press, Chicago, IL, USA.* (pp. 260–282).

Armbruster, W. S. (2017). The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology*, *31*(1), 88–100. https://doi.org/10.1111/1365-2435.12783

Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, *92*(3), 1720–1738. https://doi.org/10.1111/brv.12305

Bascompte, J. (2009). Disentangling the web of life. *Science*, *325*(5939), 416–419. https://doi.org/10.1126/science.1170749

Bascompte, J., & Jordano, P. (2007). Plant-animal mutualistic networks: The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *38*(May), 567–593. https://doi.org/10.1146/annurev.ecolsys.38.091206.095818

Bascompte, J., & Jordano, P. (2014). *Mutualistic Networks*. Princeton University Press.

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Bascompte, J., Jordano, P., & Olesen, J. M. (2006). Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science*, *312*(5772), 431–433. https://doi.org/10.1126/science.1123412

Batstone, R. T., Carscadden, K. A., Afkhami, M. E., & Frederickson, M. E. (2018). Using niche breadth theory to explain generalization in mutualisms. *Ecology*, *99*(5), 1039–1050. https://doi.org/10.1002/ecy.2188

Beck, A., Kasalicky, T., & Rambold, G. (2002). Myco-photobiontal selection in a Mediterranean cryptogam community with Fulgensia fulgida. *New Phytologist*, *153*(2), 317–326. https://doi.org/10.1046/j.0028-646X.2001.00315.x

Berlinches de Gea, A., Hautier, Y., & Geisen, S. (2023). Interactive effects of global change drivers as determinants of the link between soil biodiversity and ecosystem functioning. *Global Change Biology*, *29*(2), 296–307. https://doi.org/10.1111/gcb.16471

Berlocher, S. H., & Feder, J. L. (2002). Sympatric speciation in phytophagous insects: moving beyond controversy? *Annual Review of Entomology*, *47*, 773–815. https://doi.org/10.1146/annurev.ento.47.091201.145312

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., … Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, *313*(5785), 351–354. https://doi.org/10.1126/science.1127863

Blaha, J., Baloch, E., & Grube, M. (2006). High photobiont diversity associated with the euryoecious lichen-forming ascomycete Lecanora rupicola (Lecanoraceae, Ascomycota). *Biological Journal of the Linnean Society*, *88*(2), 283–293. https://doi.org/10.1111/j.1095-8312.2006.00640.x

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*. https://doi.org/10.1186/1472-6785-6-9

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist*, *161*(1), 1–28. https://doi.org/10.1086/343878

Boucher, D. H., James, S., & Keeler, K. H. (1982). The ecology of mutualism. *Annual Review of Ecology and Systematics*, *13*, 315–347.

Burkle, L. A., & Alarcón, R. (2011). The future of plant-pollinator diversity: Understanding interaction networks acrosss time, space, and global change. *American Journal of Botany*, *98*(3), 528–538. https://doi.org/10.3732/ajb.1000391

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M., & Sanders, N. J. (2017). Interaction rewiring and the rapid turnover of plant–pollinator networks. *Ecology Letters*, *20*(3), 385–394. https://doi.org/10.1111/ele.12740

Cardós, J. L. H., Prieto, M., Jylhä, M., Aragón, G., Molina, M. C., Martínez, I., & Rikkinen, J. (2019). A case study on the re-establishment of the cyanolichen symbiosis: Where do the compatible photobionts come from? *Annals of Botany*, *124*(3), 379–388. https://doi.org/10.1093/aob/mcz052

Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., … Wiens, J. J. (2020). Niche Breadth: Causes and Consequences for Ecology, Evolution, and Conservation. *The Quarterly Review of Biology*, *95*(3), 179–214. https://doi.org/10.1086/710388

Chagnon, P.-L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2018). Strong specificity and network modularity at a very fine phylogenetic scale in the lichen genus Peltigera. *Oecologia*, *187*(3), 767–782. https://doi.org/10.1007/s00442-018-4159-6

Chagnon, P.-L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2019). Species diversification and phylogenetically constrained symbiont switching generated high modularity in the lichen genus Peltigera. *Journal of Ecology*, *107*(4), 1645–1661. https://doi.org/10.1111/1365-2745.13207

Colles, A., Liow, L. H., & Prinzing, A. (2009). Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. *Ecology Letters*, *12*(8), 849–863. https://doi.org/10.1111/j.1461-0248.2009.01336.x

Courty, P.-E., Buée, M., Diedhiou, A. G., Frey-Klett, P., Le Tacon, F., Rineau, F., … Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry*, *42*(5), 679–698. https://doi.org/10.1016/j.soilbio.2009.12.006

Dal Grande, F., Beck, A., Cornejo, C., Singh, G., Cheenacharoen, S., Nelsen, M. P., & Scheidegger, C. (2014). Molecular phylogeny and symbiotic selectivity of the green algal genus Dictyochloropsis s.l. (Trebouxiophyceae): A polyphyletic and widespread group forming photobiont-mediated guilds in the lichen family Lobariaceae. *New Phytologist*, *202*(2), 455–470. https://doi.org/10.1111/nph.12678

Dal Grande, F., Rolshausen, G., Divakar, P. K., Crespo, A., Otte, J., Schleuning, M., & Schmitt, I. (2018). Environment and host identity structure communities of green algal symbionts in lichens. *New Phytologist*, *217*(1), 277–289. https://doi.org/10.1111/nph.14770

Dalsgaard, B., Magård, E., Fjeldså, J., González, A. M. M., Rahbek, C., Olesen, J. M., … Svenning, J. C. (2011). Specialization in plant-hummingbird networks is associated with species richness, contemporary precipitation and quaternary climate-change velocity. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0025891

Dalsgaard, B., Schleuning, M., Maruyama, P. K., Dehling, D. M., Sonne, J., Vizentin-Bugoni, J., … Rahbek, C. (2017). Opposed latitudinal patterns of network-derived and dietary specialization in avian plant–frugivore interaction systems. *Ecography*, *40*(12), 1395–1401. https://doi.org/10.1111/ecog.02604

Dalsgaard, B., Trøjelsgaard, K., Martín González, A. M., Nogués-Bravo, D., Ollerton, J., Petanidou, T., … Olesen, J. M. (2013). Historical climate-change influences modularity and nestedness of pollination networks. *Ecography*, *36*(12), 1331–1340. https://doi.org/10.1111/j.1600-0587.2013.00201.x

De Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E., & Giraud, T. (2013). Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist*, *198*(2), 347–385. https://doi.org/10.1111/nph.12150

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., … Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, *47*(1), 15–25. https://doi.org/10.1111/j.1365-2664.2009.01744.x

Dunne, J. A., Williams, R. J., & Martinez, N. D. (2002). Network structure and biodiversity loss in food webs: Robustness increases with connectance. *Ecology Letters*, *5*(4), 558–567. https://doi.org/10.1046/j.1461-0248.2002.00354.x

Dupont, Y. L., & Olesen, J. M. (2009). Ecological modules and roles of species in heathland plant-insect flower visitor networks. *Journal of Animal Ecology*, *78*(2), 346–353. https://doi.org/10.1111/j.1365-2656.2008.01501.x

Duran-Nebreda, S., & Valverde, S. (2023). Composition, structure and robustness of Lichen guilds. *Scientific Reports*, *13*(1), 1–14. https://doi.org/10.1038/s41598-023-30357-w

Dynesius, M., & Jansson, R. (2000). Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(16), 9115–9120. https://doi.org/10.1073/pnas.97.16.9115

Egan, S. P., & Funk, D. J. (2006). Individual advantages to ecological specialization: Insights on cognitive constraints from three conspecific taxa. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1588), 843–848. https://doi.org/10.1098/rspb.2005.3382

Ellis, C. J. (2012). Lichen epiphyte diversity: A species, community and trait-based review. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*(2), 131–152. https://doi.org/10.1016/j.ppees.2011.10.001

Elvebakk, A., Papaefthimiou, D., Robertsen, E. H., & Liaimer, A. (2008). Phylogenetic patterns among Nostoc cyanobionts within bi- and tripartite lichens of the genus Pannaria. *Journal of Phycology*, *44*(4), 1049–1059. https://doi.org/10.1111/j.1529-8817.2008.00556.x

Ertz, D., Guzow-Krzemińska, B., Thor, G., Łubek, A., & Kukwa, M. (2018). Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the Arthoniomycetes. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-23219-3

Fedrowitz, K., Kaasalainen, U., & Rikkinen, J. (2011). Genotype variability of Nostoc symbionts associated with three epiphytic Nephroma species in a boreal forest landscape. *The Bryologist*, *114*(1), 220. https://doi.org/10.1639/0007-2745-114.1.220

Fedrowitz, K., Kaasalainen, U., & Rikkinen, J. (2012). Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. *Ecology and Evolution*, *2*(9), 2291–2303. https://doi.org/10.1002/ece3.343

Fernández-Mendoza, F., Domaschke, S., García, M. A., Jordan, P., Martín, M. P., & Printzen, C. (2011). Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. *Molecular Ecology*, *20*(6), 1208–1232. https://doi.org/10.1111/j.1365-294X.2010.04993.x

Forister, M. L., Dyer, L. A., Singer, M. S., Stireman III, J. O., & Lill, J. T. (2012). Revisiting the evolution of ecological specialization, with emphasis on insect–plant interactions. *Ecology*, *93*(5), 981–991. https://doi.org/10.1890/11-0650.1

Fox, L. R., & Morrow, P. A. (1981). Specialization: Species property or local phenomenon. *Science*, *211*(4485), 887–893. https://doi.org/10.1126/science.211.4485.887

Funk, D. J., Filchak, K. E., & Feder, J. L. (2002). Herbivorous insects: Model systems for the comparative study of speciation ecology. *Genetica*, *116*(2–3), 251–267. https://doi.org/10.1023/A:1021236510453

Funk, D. J., Nosil, P., & Etges, W. J. (2006). Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(9), 3209–3213. https://doi.org/10.1073/pnas.0508653103

Futuyma, D. J. (2001). Ecological specialization and generalization. In C. W. F. D. A. Roff (Ed.), *Evolutionary Ecology: Concepts and Case Studies*.

Futuyma, D. J., & Moreno, G. (1988). The Evolution of Ecological Specialization. *Annual Review of Ecology and Systematics*, *19*(1988), 207–233. https://doi.org/10.1146/annurev.es.19.110188.001231

Gavrilets, S., & Losos, J. B. (2009). Adaptive Radiation: Contrasting Theory with Data. *Science*, *323*(5915), 732–737. https://doi.org/10.1126/science.1157966

Gilarranz, L. J., Rayfield, B., Liñán-Cembrano, G., Bascompte, J., & Gonzalez, A. (2017). Effects of network modularity on the spread of perturbation impact in experimental metapopulations. *Science*, *357*(6347), 199–201. https://doi.org/10.1126/science.aal4122

Gleason, H. A. (1926). The Individualistic Concept of the Plant Association. *Bulletin of the Torrey Botanical*, *53*(1), 7–26. https://doi.org/10.2307/2479933

Green, T. G. A., Schroeter, B., & Sancho, L. G. (1999). Plant life in Antarctica. In F.I. Pugnaire and F. Valladares (Ed.), *Handbook of Functional Plant Ecology* (pp. 495–543). New York, Basel: Marcel Dekker.

Hardy, N. B., & Otto, S. P. (2014). Specialization and generalization in the diversification of phytophagous insects: Tests of the musical chairs and oscillation hypotheses. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1795). https://doi.org/10.1098/rspb.2013.2960

Hawksworth, D. L., & Grube, M. (2020). Lichens redefined as complex ecosystems. *New Phytologist*, *227*(5), 1281–1283. https://doi.org/10.1111/nph.16630

Heber, U., Lange, O. L., & Shuvalov, V. A. (2006). Conservation and dissipation of light energy as complementary processes: homoiohydric and poikilohydric autotrophs. *Journal of Experimental Botany*, *57*(6), 1211–1223. https://doi.org/10.1093/jxb/erj104

Heleno, R., Devoto, M., & Pocock, M. (2012). Connectance of species interaction networks and conservation value: Is it any good to be well connected? *Ecological Indicators*, *14*(1), 7–10. https://doi.org/10.1016/j.ecolind.2011.06.032

Herrera, P., Suárez, J. P., Sánchez-Rodríguez, A., Molina, M. C., Prieto, M., & Méndez, M. (2019). Many broadly-shared mycobionts characterize mycorrhizal interactions of two coexisting epiphytic orchids in a high elevation tropical forest. *Fungal Ecology*, *39*, 26–36. https://doi.org/10.1016/j.funeco.2018.11.003

Hillebrand, H. (2004). On the generallity of the latutinal diversity gradient. *American Naturalist*, *163*(2), 192–211. https://doi.org/10.1086/381004

Huey, R. B., & Hertz, P. E. (1984). Is a jack-of-all-temperatures a master of none? *Evolution*, *38*(2), 441–444. https://doi.org/10.2307/2408502

Hughes, J. B. (2000). The Scale of Resource Specialization and the Distribution and Abundance of Lycaenid. *Oecologia*, *123*(3), 375–383. https://doi.org/10.1007/s004420051024

Hutchinson, G. E. (1957). Concluding remarks. Cold Spring Harbor Symposium. *Quantitative Biology*, *22*, 415–247. https://doi.org/10.1101/SQB.1957.022.01.039

Hutchinson, G. E. (1978). *An Introduction to Population Biology*. *Yale Univ Press, New Haven, CT*.

Iglesias-Prieto, R., Beltrán, V. H., LaJeunesse, T. C., Reyes-Bonilla, H., & Thomé, P. E. (2004). Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1549), 1757–1763. https://doi.org/10.1098/rspb.2004.2757

Irschick, D., Dyer, L., Sherry, T. W., & Ranta, E. (2005). Phylogenetic Methodologies for Studying Specialization. *Oikos*, *110*(2), 404–408. https://doi.org/10.1111/j.0030-1299.2005.13927.x

Janz, N., & Nylin, S. (2008). The oscillation hypothesis of host-plant range and speciation. *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects*, 203–215. https://doi.org/10.1525/california/9780520251328.003.0015

Jordano, P. (1987). Patterns of Mutualistic Interactions in Pollination and Seed Dispersal: Connectance, Dependence Asymmetries, and Coevolution. *The American Naturalist*, *129*(5), 657–677.

Jüriado, I., Kaasalainen, U., Jylhä, M., & Rikkinen, J. (2019). Relationships between mycobiont identity, photobiont specificity and ecological preferences in the lichen genus Peltigera (Ascomycota) in Estonia (northeastern Europe). *Fungal Ecology*, *39*, 45–54. https://doi.org/10.1016/j.funeco.2018.11.005

Kaasalainen, U., Tuovinen, V., Mwachala, G., Pellikka, P., & Rikkinen, J. (2021). Complex Interaction Networks Among Cyanolichens of a Tropical Biodiversity Hotspot. *Frontiers in Microbiology*, *12*(June), 1–12. https://doi.org/10.3389/fmicb.2021.672333

Kinlock, N. L., Prowant, L., Herstoff, E. M., Foley, C. M., Akin-Fajiye, M., Bender, N., … Gurevitch, J. (2018). Explaining global variation in the latitudinal diversity gradient: Meta-analysis confirms known patterns and uncovers new ones. *Global Ecology and Biogeography*, *27*(1), 125–141. https://doi.org/10.1111/geb.12665

Koffel, T., Daufresne, T., & Klausmeier, C. A. (2021). From competition to facilitation and mutualism: a general theory of the niche. *Ecological Monographs*, *91*(3), 1–31. https://doi.org/10.1002/ecm.1458

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

Leibold, M. A. (1995). The Niche Concept Revisited: Mechanistic Models and Community Context. *Ecology*, *76*(5), 1371–1382. https://doi.org/10.2307/1938141

Lu, J., Magain, N., Miadlikowska, J., Coyle, J. R., Truong, C., & Lutzoni, F. (2018). Bioclimatic factors at an intrabiome scale are more limiting than cyanobiont availability for the lichen-forming genus Peltigera. *American Journal of Botany*, *105*(7), 1198–1211. https://doi.org/10.1002/ajb2.1119

Lücking, R., Lawrey, J. D., Sikaroodi, M., Gillevet, P. M., Chaves, J. L., Sipman, H. J. M., & Bungartz, F. (2009). Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. *American Journal of Botany*, *96*(8), 1409–1418. https://doi.org/10.3732/ajb.0800258

MacArthur, R. H. (1955). Fluctuations of Animal Populations and a Measure of Community Stability. *Ecology*, *36*(3), 533–536.

MacArthur, R. H. (1972). *Geographical Ecology.* Princeton, NJ: Princeton University Press.

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Magain, N., Truong, C., Goward, T., Niu, D., Goffinet, B., Sérusiaux, E., … Miadlikowska, J. (2018). Species delimitation at a global scale reveals high species richness with complex biogeography and patterns of symbiont association in peltigera section peltigera (Lichenized ascomycota: Lecanoromycetes). *Taxon*, *67*(5), 836–870. https://doi.org/10.12705/675.3

Maher, A. M. D., Asaiyah, M. A. M., Brophy, C., Griffin, C. T., & Griffin, C. T. (2017). An Entomopathogenic Nematode Extends Its Niche by Associating with Different Symbionts. *Microbial Ecology*, *73*, 211–223. https://doi.org/10.1007/s00248-016-0829-2

May, R. M. (1972). Will a Large Complex System be Stable? *Nature*, *238*(5364), 413–414. https://doi.org/10.1038/238413a0

McKinney, M. L. (1997). Extinction Vulnerability and Selectivity: Combining Ecological and Paleontological Views. *Annual Review of Ecology and Systematics*, *28*(1), 495–516. https://doi.org/10.1146/annurev.ecolsys.28.1.495

Millanes, A. M., Truong, C., Westberg, M., Diederich, P., & Wedin, M. (2014). Host switching promotes diversity in host-specialized mycoparasitic fungi: uncoupled evolution in the Biatoropsis-Usnea system. *Evolution*, *68*(6), 1576–1593. https://doi.org/10.1111/evo.12374

Moran, N. A. (1988). The Evolution of Host-Plant Alternation in Aphids: Evidence for Specialization as a Dead End. *The American Naturalist*, *132*(5), 681–706. https://doi.org/10.1086/284882

Morris, R. J., Gripenberg, S., Lewis, O. T., & Roslin, T. (2014). Antagonistic interaction networks are structured independently of latitude and host guild. *Ecology Letters*, *17*(3), 340–349. https://doi.org/10.1111/ele.12235

Moya, P., Molins, A., Chiva, S., Bastida, J., & Barreno, E. (2020). Symbiotic microalgal diversity within lichenicolous lichens and crustose hosts on Iberian Peninsula gypsum biocrusts. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-71046-2

Moya, P., Molins, A., Martínez-Alberola, F., Muggia, L., & Barreno, E. (2017). Unexpected associated microalgal diversity in the lichen Ramalina farinacea is uncovered by pyrosequencing analyses. *PLOS ONE*, *12*(4), e0175091. https://doi.org/10.1371/journal.pone.0175091

Mueller, U. G., Mikheyev, A. S., Solomon, S. E., & Cooper, M. (2011). Frontier mutualism: coevolutionary patterns at the northern range limit of the leaf-cutter ant–fungus symbiosis. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1721), 3050–3059. https://doi.org/10.1098/rspb.2011.0125

Muggia, L., Grube, M., & Tretiach, M. (2008). Genetic diversity and photobiont associations in selected taxa of the Tephromela atra group (Lecanorales, lichenised Ascomycota). *Mycological Progress*, *7*(3), 147–160. https://doi.org/10.1007/s11557-008-0560-6

Muggia, L., Vancurova, L., Škaloud, P., Peksa, O., Wedin, M., & Grube, M. (2013). The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS Microbiology Ecology*, *85*(2), 313–323. https://doi.org/10.1111/1574-6941.12120

Myllys, L., Stenroos, S., Thell, A., & Kuusinen, M. (2007). High cyanobiont selectivity of epiphytic

lichens in old growth boreal forest of Finland. *New Phytologist*, *173*(3), 621–629. https://doi.org/10.1111/j.1469-8137.2006.01944.x

Nash, T. H. (1996). *Lichen Biology.* (Cambridge). Cambridge University Press.

Novotny, V., Miller, S. E., Baje, L., Balagawi, S., Basset, Y., Cizek, L., … Weiblen, G. D. (2010). Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *Journal of Animal Ecology*, *79*(6), 1193–1203. https://doi.org/10.1111/j.1365-2656.2010.01728.x

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2005). Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen fungus Peltigera. *European Journal of Phycology*, *40*(4), 363–378. https://doi.org/10.1080/09670260500342647

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2013). Assessing population structure and host specialization in lichenized cyanobacteria. *New Phytologist*, *198*(2), 557–566. https://doi.org/10.1111/nph.12165

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

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104

Ollerton, J., & Cranmer, L. (2002). Latitudinal trends in plant-pollinator interactions: Are tropical plants more specialised? *Oikos*, *98*(2), 340–350. https://doi.org/10.1034/j.1600-0706.2002.980215.x

Otálora, M. A. G., Martínez, I., O'Brien, H., Molina, M. C., Aragón, G., & Lutzoni, F. (2010). Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution*, *56*(3), 1089–1095. https://doi.org/10.1016/j.ympev.2010.05.013

Paulsrud, P., Rikkinen, J., & Lindblad, P. (2000). Spatial patterns of photobiont diversity in some Nostoc-containing lichens. *New Phytologist*, *146*(2), 291–299. https://doi.org/10.1046/j.1469-8137.2000.00647.x

Paulsrud, P., Rikkinen, J., & Lindblad, P. (2001). Field investigations on cyanobacterial specificity in Peltigera aphthosa. *New Phytologist*, *152*(1), 117–123. https://doi.org/10.1046/j.0028-646x.2001.00234.x

Pavoine, S., & Bonsall, M. B. (2011). Measuring biodiversity to explain community assembly: a unified approach. *Biological Reviews*, *86*(4), 792–812. https://doi.org/10.1111/j.1469-185X.2010.00171.x

Peay, K. G. (2016). The Mutualistic Niche: Mycorrhizal Symbiosis and Community Dynamics. *Annual Review of Ecology, Evolution, and Systematics*, *47*, 143–164. https://doi.org/10.1146/annurev-ecolsys-121415-032100

Peksa, O., & Škaloud, P. (2011). Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae). *Molecular Ecology*, *20*(18), 3936–3948. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Piercey-Normore, M. D., & Deduke, C. (2011). Fungal farmers or algal escorts: Lichen adaptation

from the algal perspective. *Molecular Ecology*, *20*(18), 3708–3710. https://doi.org/10.1111/j.1365-294X.2011.05191.x

Piercey-Normore, M. D., & DePriest, P. T. (2001). Algal switching among lichen symbioses. *American Journal of Botany*, *88*(8), 1490–1498. https://doi.org/10.2307/3558457

Pino-Bodas, R., & Stenroos, S. (2021). Global Biodiversity Patterns of the Photobionts Associated with the Genus Cladonia (Lecanorales , Ascomycota). *Microbial Ecology*, *82*, 173–187. https://doi.org/10.1007/s00248-020-01633-3

Poisot, T., Canard, E., Mouillot, D., Mouquet, N., & Gravel, D. (2012). The dissimilarity of species interaction networks. *Ecology Letters*, *15*(12), 1353–1361. https://doi.org/https://doi.org/10.1111/ele.12002

Poisot, T., Canard, E., Mouquet, N., & Hochberg, M. E. (2012). A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, *3*(3), 537–544. https://doi.org/10.1111/j.2041-210X.2011.00174.x

Poisot, T., Kéfi, S., Morand, S., Stanko, M., Marquet, P. A., & Hochberg, M. E. (2015). A continuum of specialists and generalists in empirical communities. *PLoS ONE*, *10*(5), 1–12. https://doi.org/10.1371/journal.pone.0114674

Price, P. W. (1980). *Evolutionary biology of parasites. Monographs in population biology* (Vol. 15). United States: Princeton University Press.

Rambold, G., Friedl, T., & Beck, A. (1998). Photobionts in Lichens: Possible Indicators of Phylogenetic Relationships? *The Bryologist*, *101*(3), 392–397. https://doi.org/10.2307/3244177

Ramírez-Fernández, L., Zúñiga, C., Méndez, M. A., Carú, M., Orlando, J., & Orlando, J. (2013). Genetic diversity of terricolous Peltigera cyanolichen communities in different conservation states of native forest from southern Chile. *International Microbiology*, *16*(4), 243–252. https://doi.org/10.2436/20.1501.01.200

Ramos-Jiliberto, R., Domínguez, D., Espinoza, C., López, G., Valdovinos, F. S., Bustamante, R. O., & Medel, R. (2010). Topological change of Andean plant-pollinator networks along an altitudinal gradient. *Ecological Complexity*, *7*(1), 86–90. https://doi.org/10.1016/j.ecocom.2009.06.001

Rech, A. R., Dalsgaard, B., Sandel, B., Sonne, J., Svenning, J.-C., Holmes, N., & Ollerton, J. (2016). The macroecology of animal versus wind pollination: ecological factors are more important than historical climate stability. *Plant Ecology & Diversity*, *9*(3), 253–262. https://doi.org/10.1080/17550874.2016.1207722

Rikkinen, J. (2003). Ecological and evolutionary role of photobiont-mediated guilds in lichens. *Symbiosis*, *34*(2), 99–110.

Rikkinen, J. (2013). Molecular studies on cyanobacterial diversity in lichen symbioses. *MycoKeys*, *6*, 3–32. https://doi.org/10.3897/mycokeys.6.3869

Rikkinen, J. (2015). Cyanolichens. *Biodiversity and Conservation*, *24*(4), 973–993. https://doi.org/10.1007/s10531-015-0906-8

Rikkinen, J., Oksanen, I., & Lohtander, K. (2002). Lichen guilds share related cyanobacterial symbionts. *Science*, *297*(5580), 357. https://doi.org/10.1126/science.1072961

Rolshausen, G., Dal Grande, F., Sadowska-Deś, A. D., Otte, J., & Schmitt, I. (2018). Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche

expansions. *Ecography*, *41*(8), 1380–1392. https://doi.org/10.1111/ecog.03457

Rolshausen, G., Hallman, U., Grande, F. D., Otte, J., Knudsen, K., & Schmitt, I. (2020). Expanding the mutualistic niche: Parallel symbiont turnover along climatic gradients. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1924). https://doi.org/10.1098/rspb.2019.2311

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Sancho, L. G., De la Torre, R., Horneck, G., Ascaso, C., De Los Rios, A., Pintado, A., … Schuster, M. (2007). Lichens survive in space: Results from the 2005 LICHENS experiment. *Astrobiology*, *7*(3), 443–454. https://doi.org/10.1089/ast.2006.0046

Sancho, L. G., Palacios, D., Green, T. G. A., Vivas, M., & Pintado, A. (2011). Extreme high lichen growth rates detected in recently deglaciated areas in Tierra del Fuego. *Polar Biology*, *34*(6), 813–822. https://doi.org/10.1007/s00300-010-0935-4

Schemske, D. W. (2002). Tropical diversity: patterns and processes. Ecol. In R. C. and T. Whitmore (Ed.), *Ecological and Evolutionary Perspectives on the Origins ofTropical Diversity: Key Papers and Commentaries* (pp. 163–173). Chicago, IL: University of Chicago Press.

Schemske, D. W., Mittelbach, G. G., Cornell, H. V., Sobel, J. M., & Roy, K. (2009). Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics*, *40*, 245–269. https://doi.org/10.1146/annurev.ecolsys.39.110707.173430

Schleuning, M., Fründ, J., Klein, A. M., Abrahamczyk, S., Alarcón, R., Albrecht, M., … Blüthgen, N. (2012). Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Current Biology*, *22*(20), 1925–1931. https://doi.org/10.1016/j.cub.2012.08.015

Schluter, D. (2000). Ecological Character Displacement in Adaptive Radiation. *The American Naturalist*, *156*(S4), 4–16. https://doi.org/10.1086/303412

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

Singh, G., Dal Grande, F., Divakar, P. K., Otte, J., Crespo, A., & Schmitt, I. (2017). Fungal–algal association patterns in lichen symbiosis linked to macroclimate. *New Phytologist*, *214*(1), 317–329. https://doi.org/10.1111/nph.14366

Steinová, J., Škaloud, P., Yahr, R., Bestová, H., & Muggia, L. (2019). Reproductive and dispersal strategies shape the diversity of mycobiont-photobiont association in Cladonia lichens. *Molecular Phylogenetics and Evolution*, *134*(October 2017), 226–237. https://doi.org/10.1016/j.ympev.2019.02.014

Stenroos, S., Ho, F., Myllys, L., Hyvo, J., & Thell, A. (2006). High selectivity in symbiotic associations of lichenized ascomycetes and cyanobacteria. *Cladistics*, *22*, 230–238. https://doi.org/10.1111/j.1096-0031.2006.00101.x

Stouffer, D. B., & Bascompte, J. (2011). Compartmentalization increases food-web persistence. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(9), 3648–3652. https://doi.org/10.1073/pnas.1014353108

Suz, L. M., Barsoum, N., Benham, S., Dietrich, H. P., Fetzer, K. D., Fischer, R., … Bidartondo, M. I. (2014). Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests. *Molecular Ecology*, *23*(22), 5628–5644. https://doi.org/10.1111/mec.12947

Thébault, E., & Fontaine, C. (2010). Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science*, *329*(5993), 853–856. https://doi.org/10.1126/science.1188321

Thompson, J. N. (1994). *The Coevolutionary Process.* Chicago: University of Chicago Press.

Thompson, J. N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, *153*(SUPPL.). https://doi.org/10.1086/303208

Thompson, J. N. (2005). *The geographic mosaic of coevolution.* University of Chicago Press.

Trøjelsgaard, K., & Olesen, J. M. (2013). Macroecology of pollination networks. *Global Ecology and Biogeography*, *22*(2), 149–162. https://doi.org/10.1111/j.1466-8238.2012.00777.x

Vargas Castillo, R., & Beck, A. (2012). Photobiont selectivity and specificity in Caloplaca species in a fog-induced community in the Atacama Desert, northern Chile. *Fungal Biology*, *116*(6), 665–676. https://doi.org/10.1016/j.funbio.2012.04.001

Vázquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology*, *85*(5), 1251–1257. https://doi.org/10.1890/03-3112

Vázquez, D. P., & Stevens, R. D. (2004). The latitudinal gradient in niche breadth: concepts and evidence. *The American Naturalist*, *164*(1), 0–19. https://doi.org/10.1086/421445

Ventre Lespiaucq, A., Jacquemyn, H., Rasmussen, H. N., & Méndez, M. (2021). Temporal turnover in mycorrhizal interactions: a proof of concept with orchids. *New Phytologist*, *230*(5), 1690–1699. https://doi.org/10.1111/nph.17291

Warren, R. J., Giladi, I., & Bradford, M. A. (2010). Ant-mediated seed dispersal does not facilitate niche expansion. *Journal of Ecology*, *98*(5), 1178–1185. https://doi.org/10.1111/j.1365-2745.2010.01694.x

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, *77*(4), 1043–1060. https://doi.org/10.2307/2265575

Wilson, P., & Thomson, J. D. (1996). How do flowers diverge? In *Floral Biology (eds D.G. Lloyd & S.C.H. Barrett). Chapman and Hall, New York, NY, USA.* (pp. 88–111).

Winkler, I. S., & Mitter, C. (2008). The Phylogenetic Dimension of Insect-Plant Interactions: A Review of Recent Evidence. In K. Tilmon (Ed.), *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (pp. 240–263). University of California Press. https://doi.org/10.1525/california/9780520251328.003.0018

Wirtz, N., Lumbsch, H. T., Green, T. G. A., Türk, R., Pintado, A., Sancho, L., & Schroeter, B. (2003). Lichen fungi have low cyanobiont selectivity in maritime Antarctica. *New Phytologist*, *160*(1), 177–183. https://doi.org/10.1046/j.1469-8137.2003.00859.x

Yahr, R., Vilgalys, R., & Depriest, P. T. (2004). Strong fungal specificity and selectivity for algal symbionts in Florida scrub Cladonia lichens. *Molecular Ecology*, *13*(11), 3367–3378. https://doi.org/10.1111/j.1365-294X.2004.02350.x

Yahr, R., Vilgalys, R., & Depriest, P. T. (2006). Geographic variation in algal partners of Cladonia subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytologist*, *171*(4), 847–860. https://doi.org/10.1111/j.1469-8137.2006.01792.x

Yang, L. H., & Rudolf, V. H. W. (2010). Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecology Letters*, *13*(1), 1–10.

https://doi.org/10.1111/j.1461-0248.2009.01402.x

Zúñiga, C., Leiva, D., Carú, M., & Orlando, J. (2017). Substrates of Peltigera Lichens as a Potential Source of Cyanobionts. *Microbial Ecology*, *74*(3), 561–569. https://doi.org/10.1007/s00248-017-0969-z

OBJETIVOS GENERALES

OBJETIVOS GENERALES

Con esta tesis doctoral se pretende comprender los fenómenos de especialización en interacciones bióticas. Más concretamente, se trata de identificar y analizar los principales componentes que intervienen en este fenómeno estudiando diferentes comunidades liquénicas conformadas por líquenes que interaccionan con cianobacterias a lo largo de un amplio gradiente latitudinal en el hemisferio sur y considerando diferentes escalas espaciales y biológicas. A lo largo de cuatro capítulos, se ha abordado la especialización desde distintas aproximaciones, para entender los mecanismos que participan en el establecimiento de estas interacciones con los siguientes objetivos específicos:

Capítulo 1: determinar la especialización de cada especie de liquen asociado con cianobacterias utilizando 3 índices diferentes en bosques de lenga (*Nothofagus pumilio*) a lo largo de un gradiente latitudinal. Además, analizar la influencia de diferentes factores ambientales y rasgos funcionales sobre la especialización y los cambios de dicha especialización en función de la escala espacial.

Capítulo 2: estudiar la especialización a distintas escalas biológicas en cefalolíquenes analizando la variabilidad intratalina, intraespecífica e interespecífica de sus cianobiontes.

Capítulo 3: analizar cómo se estructuran las redes micobionte-cianobacteria y ver si hay un patrón estructural compartido en estas redes en un gradiente latitudinal. Asimismo, determinar el papel de las distintas especies que conforman las redes y la relación con sus rasgos funcionales. Por otra parte, examinar si la estructura de la red se ve afectada por variables ambientales y por los rasgos funcionales de las especies.

Capítulo 4: proponer un marco conceptual integrador que defina la especialización de una forma más objetiva y aplicable a todos los tipos de interacciones bióticas, combinando tres métricas diferentes pero complementarias que integran tres dimensiones diferentes: *especificidad*, *preferencia* y *selectividad*.

METODOLOGÍA GENERAL

METODOLOGÍA GENERAL

En este apartado se realiza una descripción de la metodología general utilizada a lo largo de la tesis. Una mayor concreción de la información particular de cada capítulo se encontrará en el apartado *Materials and Methods* de cada uno de ellos (por ejemplo, el número de muestras utilizadas en cada capítulo, la escala, etc.).

Todos los análisis estadísticos, así como el cálculo de los distintos parámetros que se describen a continuación fueron realizados con el software R v. 4.0.4 (http://www.rproject.org/). Se incluye una figura con la metodología general resumida (Figura 1).

1. Áreas de estudio y diseño experimental

Se seleccionaron 11 bosques de *Nothofagus pumilio* (Poepp. & Endl.) Krasser, a lo largo de un amplio gradiente latitudinal en Chile, situados entre las latitudes 38.63° S y 54.96° S (Figura 1a). Los bosques se localizan en Espacios Naturales Protegidos: Parque Nacional del Conguillío (2 bosques), Parque Nacional de Puyehue (2 bosques), Parque Nacional de Hornopirén (1 bosque), Parque Nacional de Cerro Castillo (2 bosques), Parque Nacional de Torres del Paine (1 bosque), Reserva Nacional de Magallanes (1 bosque) y la Isla de Navarino en la Reserva de la Biosfera de Cabo de Hornos (2 bosques). En cada bosque se seleccionaron cinco parcelas separadas al menos 100 metros del borde del bosque para eliminar el efecto borde de las comunidades muestreadas. Dentro de cada parcela, se muestrearon 10 árboles, en los que se determinó la cobertura en porcentaje de cada especie de liquen utilizando cuadrados de muestreo de 20 x 30 cm (ver Aragón *et al*., 2012). Los cuadrados de muestreo se situaron en dos orientaciones (norte y sur) y en dos alturas (base y altura del pecho). En total, se recogió la información de 2200 cuadrados de muestreo y se calculó la cobertura media de cada especie de liquen a nivel de parcela en un total de 55 parcelas (Figura 1b).

Para el análisis molecular, se recogieron un máximo de ocho muestras de cada especie de cianoliquen y cefaloliquen en cada bosque. Las muestras recolectadas se secaron al aire y se congelaron a-20°C para conservar el material en condiciones óptimas para la amplificación del ADN.

1.1. Variables ambientales

Se han considerado 27 variables ambientales relacionadas con el clima, la geografía, la estructura del bosque y la calidad del hábitat (Appendix S1 del Capítulo 1). De esas 27 variables, 19 son variables climáticas relacionadas con la temperatura y la precipitación obtenidas de la base climática CHELSA (Karger *et al*., 2017). Las ocho variables restantes fueron tomadas in situ en cada una de las parcelas e incluyen latitud, longitud, altitud, orientación, pendiente, insolación, diámetro de los árboles a la altura del pecho (DBH; Diameter at breast height) y cobertura del dosel arbóreo (Figura 1c). Latitud, longitud, altitud, orientación y pendiente fueron medidas con un GPS, un compás y un clinómetro, en cada parcela muestreada. La cobertura del dosel arbóreo y el DBH se midieron a la altura del pecho en cada árbol muestreado (550 árboles totales) usando fotografías hemisféricas y una cinta métrica, respectivamente. Las fotografías hemisféricas se realizaron con una cámara digital nivelada horizontalmente (Canon EOS 5D), dirigida al cenit con un objetivo ojo de pez con un campo de visión de 180° (SIGMA 8 mm F3.5 ex DG Fisheye). Posteriormente fueron analizadas con el programa Gap Light Analyzer v2.0 (http://www.rem.sfu.ca/forestry/index.htm) para estimar el porcentaje de apertura del dosel. Tanto para el DBH como para la cobertura arbórea se calculó la media de estas medidas para cada parcela. La latitud, la orientación y la pendiente se utilizaron para calcular el índice de insolación (Gandullo, 1974) usando las siguientes ecuaciones:

$$Gla = \sin i * \cos p - \cos \alpha * \cos i * \sin p$$

$$Glb = \sin i * \cos p + \cos \alpha * \cos i * \sin p$$

donde Gla es la radiación solar potencial en los sitios orientados cara norte, Glb en los sitios de la cara sur, i es el ángulo de incidencia solar ($i = 90° - latitud$), p es la pendiente, and α es el ángulo formado por la orientación y 0° para Gla y por la orientación y 180° para Glb.

2. Identificación de líquenes y cianobacterias y análisis de rasgos funcionales

2.1. Identificación morfológica de los líquenes

Para la identificación de los líquenes (Figura 1d) se utilizaron diversas claves dicotómicas y monografías en función del género o la familia a identificar: Degelius (1974) para *Collema*, Jørgensen (2000, 2005) para *Fuscopannaria*, Galloway & Jørgensen (1995) para *Leptogium*, White & James (1988) para *Nephroma*, Passo *et al*. (2004), Elvebakk & Bjerke (2005), Passo & Calvelo (2006), Elvebakk (2007), Elvebakk *et al*. (2007), Passo & Calvelo (2011), y Elvebakk (2013) para *Pannaria*, Galloway (1985) para *Parmeliella* y *Psoroma*, Goward *et al*. (1995), Martínez (1999), y Vitikainen (1994) para *Peltigera*, Galloway (1992) y Lücking *et al*. (2017), para *Pseudocyphellaria s. lat.*, Elvebakk *et al*. (2010) para *Psorophorus* y *Xanthopsoroma* y Galloway (1994) para *Sticta*. En total, fueron encontradas 86 especies de líquenes que contienen cianobacterias (53 cianolíquenes y 33 cefalolíquenes).

2.2. Identificación molecular de las cianobacterias

Los filogrupos de *Nostoc* formando parte de la simbiosis liquénica fueron identificados con técnicas moleculares utilizando la región *rbcLX,* previamente utilizada por otros autores para los mismos fines (ej.: Magain *et al*., 2017). Para la extracción del ADN se utilizó la resina quelante Chelex® 100 (Bio-Rad, Hercules, CA, USA). Para la amplificación de la region *rbcLX* por PCR se utilizaron los cebadores *cw* y *cx* (Rudi *et al*., 1998) con los siguientes tiempos y temperaturas: 95 °C 15 min; 35 ciclos de 1 min a 95 °C, 30 s a 54 °C, 30 s a 72 °C; 10 min a 72 °C. Los productos de la PCR se enviaron a Macrogen Spain (www.macrogen.com) donde se realizó la secuenciación utilizando el mismo cebador reverso (*rbcLX cx*) que en la PCR. Se obtuvieron un total de 1120 secuencias de cianobacterias. La edición y el alineamiento de las 1120 secuencias obtenidas se realizó con el software Geneious Prime 2021.0.1 (https://www.geneious.com) en el que se incluyeron secuencias disponibles de Magain *et al*., (2017). Tanto las regiones ambiguas, como los dos espaciadores intergénicos, fueron identificados y eliminados manualmente con el programa Aliview v. 1.26 (Larsson, 2014). El alineamiento está disponible en el repositorio de datos Zenodo (Rodríguez-Arribas *et*

al., 2023). Para el capítulo 2, se seleccionaron seis talos de tres especies de cefalolíquenes (*Nephroma antarcticum*, *Pseudocyphellaria granulata* y *Pannaria farinosa*) en dos de los bosques (Torres del Paine e Isla de Navarino), y se amplificó el ADN de las cianobacterias presentes en 5 cefalodios diferentes para analizar la variabilidad intratalina y comparar la variabilidad entre las especies.

Para delimitar los filogrupos de *Nostoc* se combinaron dos aproximaciones: 1) el método ASAP (Assemble Species by Automatic Partitioning, Puillandre *et al*., 2021) y 2) análisis filogenéticos junto con la información de estudios previos (Magain *et al*., 2017). Los análisis ASAP se realizaron siguiendo un modelo de sustitución Jukes-Cantor (JC69), separando las unidades taxonómicas operacionales (OTUs: Operational Taxonomic Units) por debajo de una probabilidad de 0.01, manteniendo las 10 mejores puntuaciones y con -1 de valor inicial en el servidor https://bioinfo.mnhn.fr/abi/public/asap/. Se seleccionaros las particiones incluidas en un rango de distancia genética entre 0.001-0.01. Los análisis filogenéticos se realizaron mediante análisis de máxima verosimilitud (ML: Maximum likelihood) y análisis Bayesiano. El análisis de máxima verosimilitud se llevó a cabo en el CIPRES Science Gateway Portal (Miller *et al*., 2010) con el programa RAxML v. 8.2.12 (Stamatakis, 2014) y 1000 iteraciones de bootstrap. El análisis bayesiano se realizó en el servidor Agapita (URJC, Área de Biodiversidad y Conservación) utilizando el programa MrBayes 3.2.7a (Huelsenbeck & Ronquist, 2001) con un modelo de sustitución GTR+I+G (Rodríguez *et al*., 1990). El análisis se corrió con 50 millones de generaciones, dos carreras y cuatro cadenas, eliminando el primer 25% de los resultados y obteniendo resultados cada 1000 generaciones. Ambos análisis fueron comparados posteriormente, teniendo en cuenta los nodos soportados (>75% de bootstrap en ML y > 95% de probabilidad posterior en el análisis bayesiano).

Combinando las OTUs obtenidas por ASAP, junto con los clados soportados de los análisis filogenéticos de ML y Bayesianos y la información de estudios previos (Magain *et al*., 2017), se identificaron un total de 64 filogrupos de *Nostoc* formando parte de la simbiosis liquéncia en estas comunidades. En el capítulo 2, se trabajó a escala biológica más pequeña, a nivel de OTU, sin agruparlas en sus correspondientes filogrupos. Además, en el capítulo 2 se realizó una red de haplotipos en PopART v .1.7

(http://popart.otago.ac.nz) con el método TCS (Clement *et al*., 2002) para apoyar los resultados previamente obtenidos (Figura 1e).

2.3. Rasgos funcionales y medias ponderadas de la comunidad (CWM: Community Weighted Mean).

Para los capítulos 1 y 3 se consideraron tres rasgos funcionales cualitativos (Figura 1f). Estos rasgos se determinaron observacionalmente para cada muestra usando una lupa binocular y fueron: el tipo de reproducción (sexual y/o asexual, o ausencia de estructuras reproductoras tanto asexuales como sexuales), el papel de la cianobacteria en el talo (fotobionte principal para cianolíquenes o secundario para cefalolíquenes) y el biotipo o forma de crecimiento (foliáceo de lóbulo ancho, foliáceo de lóbulo estrecho o escuamuloso). Los distintos rasgos considerados tienen papeles fundamentales en distintos aspectos de la biología de los líquenes. El tipo de reproducción está relacionado con la dispersión y el establecimiento (Ellis, 2012; Ellis & Coppins, 2006; Nelson *et al*., 2015; Rapai *et al*., 2012). Los líquenes que tienen estructuras de reproducción asexual dispersan conjuntamente ambos simbiontes, aunque estas estructuras son relativamente grandes, por lo que no se dispersan a grandes distancias. Por otro lado, los líquenes con estructuras reproductivas sexuales dispersan únicamente al micobionte en forma de esporas que pueden recorrer largas distancias, pero que necesitan encontrar un nuevo fotobionte para reestablecer la simbiosis (Martínez *et al*., 2012). La función de la cianobacteria influye en la adquisición de nutrientes, ya que estas se encargan de la fijación de nitrógeno atmosférico y, también, afecta a la actividad fotosintética (Asplund & Wardle, 2017). Por último, el biotipo está relacionado con las estrategias de uso del agua y con la complejidad estructural de los líquenes (Asplund & Wardle, 2017).

Para tener un valor ponderado de la variabilidad de los rasgos en cada parcela, se calculó el CWM (Community Weighted Mean) para cada rasgo considerado en el capítulo 3. El CWM es un índice que aporta información sobre los rasgos dominantes en una comunidad teniendo en cuenta la abundancia relativa de las especies que la conforman (Lavorel *et al*., 2008). Para el cálculo de este índice, se utilizó la función *functcomp()* del paquete FD (v.1.0-12.1, Laliberte & Legendre, 2010) en R, especificando CWM.type = "all" en la función al tratarse de rasgos cualitativos.

3. Métricas de especialización

3.1. Medidas de especialización por especie.

Para conocer el grado de especialización de cada especie de liquen en cada bosque (capítulo 1), así como para objetivar y proponer un marco conceptual integrativo que defina los fenómenos de especialización (capítulo 4) se han utilizado diferentes índices (Figura 1g):

Riqueza de parejas interactuantes ($\boldsymbol{R}$) y riqueza estandarizada ($\boldsymbol{R'}$).

Riqueza (capítulo 1):

$$R_i = \sum_{j=1}^{Nj} N_{ij}$$

donde Nj es el número total de parejas potenciales disponibles en la comunidad y N_{ij} es el número de parejas j que interactúan con la especie i. Este índice se calculó con la función *diversity()* del paquete vegan (v.2.5-7; Oksanen *et al*., 2020) en R. La riqueza de parejas informa, por tanto, del número de fotobiontes con los que interactúa cada micobionte.

Riqueza estandarizada (capítulo 4):

$$R_i{}' = \frac{R_i - 1}{R_{max} - 1}$$

donde R_i es la riqueza de parejas observada para la especie i y R_{max} puede definirse en función de los objetivos del estudio. Para hacer comparaciones dentro de los sistemas de interacciones se sugiere la utilización de la riqueza máxima observada de parejas en un sistema dado como R_{max}. Sin embargo, para comparar entre sistemas de interacciones, se sugiere la utilización de la disponibilidad local de parejas (Nj) como R_{max}.

Índice de Simpson ($\boldsymbol{D}$) y equitatividad de Simpson ($\boldsymbol{E}$).

Índice de Simpson (Simpson, 1949; capítulo 1):

$$D_i = \sum_{j=1}^{R_i} p_{ij}^2$$

donde R_i es el número de parejas que interactúan con la especie i y p_{ij} es la frecuencia de interacciones de la especie i con cada pareja j. Este índice se calculó con la función *diversity()* del paquete vegan (v.2.5-7; Oksanen *et al*., 2020) en R.

Equitatividad de Simpson (Smith & Wilson, 1996; capítulo 4):

$$E_i = \frac{1/D_i}{R_i}$$

donde R_i es el número de parejas que interactúan con la especie i y D_i es el índice de Simpson para la especie *i*.

Índice d' y transformación del índice d'.

Índice d' (Blüthgen *et al*., 2006; capítulo 1):

$$d_i = \sum_{j=1}^{N_j} (p_{ij} ln \frac{p_{ij}}{q_j})$$

donde N_j es el número de parejas potenciales disponibles, p_{ij} es la frecuencia de interacciones de la especie i con cada pareja j y q_j es la disponibilidad relativa de cada pareja *j*. Los autores Blüthgen *et al*. (2006) normalizan esta ecuación dando lugar al índice d'. Este índice se calculó con la función *dfun()* del paquete bipartite (v.2.16; (Dormann, 2011; Dormann *et al*., 2008, 2009) en R.

Transformación del índice d' (capítulo 4):

$$td_i' = 1 - d_i'$$

Este índice se transforma para que los valores oscilen desde 0 (mayor especialización) a 1 (menor especialización), en el mismo sentido que los índices anteriores utilizados en el capítulo 4 (R' y E).

3.2. Medidas de la arquitectura de las redes de interacciones

Para determinar la estructura de las redes bipartitas de interacciones entre micobiontes y fotobiontes (capítulo 3) se han considerado distintos parámetros de la arquitectura de las redes (Figura 1h):

Número de nodos (especies para los micobiontes o filogrupos para los fotobiontes) de cada clase de organismos (N_m, N_p):

$$N_m = Riqueza\ de\ micobiontes$$

$$N_p = Riqueza\ de\ fotobiontes$$

Riqueza de especies de toda la red (S):

$$S = N_m + N_p$$

Tamaño de la red ($Size$):

$$Size = N_m N_p$$

Número total de interacciones presentes en la red (L):

$$L = N\acute{u}mero\ total\ \ de\ interacci\acute{o}nes\ de\ la\ red$$

Conectancia:

$$C = \frac{L}{Size} = \frac{L}{N_m N_p}$$

Anidamiento (N, basado en Fortuna *et al*., 2019):

$$N = \frac{\sum_{i=1,i<j}^{N_m} \frac{c_{ij}}{min\ (k_i, k_j)} + \sum_{i=1,i<j}^{N_p} \frac{c_{ij}}{min\ (k_i, k_j)}}{\frac{N_m(N_m - 1)}{2} + \frac{N_p(N_p - 1)}{2}}$$

donde N_m es el número de micobiontes, N_p el número de fotobiontes, k_i el número de parejas con las que interactúa el nodo i, c_{ij} el número común de parejas interaccionando con los nodos i y j.

El valor que puede tener el anidamiento varía de 0 a 1. Así, $N = 0$ se presentaría cuando no hay ninguna interacción común entre micobiontes y fotobiontes, lo que tendría lugar cuando todas las interacciones son recíprocas ($c_{ij} = 0$). Por otro lado, $N = 1$ sería el resultado de un anidamiento perfecto en el que los micobiontes comparten todos los fotobiontes con los que pueden interactuar y los fotobiontes comparten todos los micobiontes con los que interactúan ($c_{ij} = min\ (k_i, k_j)$).

Esta manera de medir el anidamiento basada en Fortuna *et al*., (2019) es equivalente a la aproximación NODF de (Almeida-Neto *et al*., 2008), pero sin penalizar la contribución de las especies que tienen el mismo número de parejas.

Número de módulos:

El número de módulos se obtuvo con la función *cluster_spinglass()* del paquete igraph (v-1.2.11; Csardi & Nepusz, 2006) que está basada en un modelo "spinglass" junto con hibridación simulada (simulated annealing) (Reichardt & Bornholdt, 2006). Este algoritmo trabaja con redes conectadas sin tener en cuenta los módulos aislados. Los módulos vienen definidos por un conjunto de nodos con un elevado número de interacciones entre sí y pocas interacciones con el resto de la red.

Modularidad (Q, basado en Clauset *et al*., 2004):

$$Q = \frac{1}{2L}\sum_{ij}[A_{ij} - \frac{k_i k_j}{2L}]\delta(c_i, c_j)$$

donde L es el número total de interacciones, A_{ij} es el valor observado de la celda de la matriz de incidencia que representa la interacción entre la especie i y la especie j, k_i es el número de parejas de la especie i, k_j es el número de parejas de la especie j, c_i es el módulo de i y c_j el módulo de j. La suma se realiza sobre la interacción entre cada par de especies de ambos nodos (micobiontes y fotobiontes) y $\delta = 1$ si ambos organismos pertenecen al mismo módulo y $\delta = 0$ si pertenecen a módulos distintos. La modularidad se calculó con la función *modularity()* paquete igraph (v-1.2.11; Csardi & Nepusz, 2006).

4. Diversidad beta

Con el objetivo de estudiar los patrones de especialización a distintas escalas (capítulo 1), se calculó el reemplazo de fotobiontes para cada especie de micobionte entre los distintos bosques del gradiente latitudinal estudiado (Figura 1i). Para ello se utilizaron las medidas de diversidad beta descritas por Carvalho *et al*. (2013):

$$\beta_{total} = \beta_{repl} + \beta_{rich}$$

donde β_{total} es la diversidad beta total, β_{repl} es el componente de la diversidad beta referido al recambio de parejas y β_{rich} hace referencia al componente de la diversidad

beta relacionado con cambios en la riqueza de parejas. Estas dimensiones de la diversidad beta fueron calculadas utilizando la función *beta()* del paquete BAT (v.2.7.1; Cardoso *et al*., 2015). Posteriormente, con los valores obtenidos de cada componente de la diversidad beta se clasificó cada caso siguiendo las categorías propuestas por Ventre Lespiaucq *et al*. (2021):

a) "No turnover" (ausencia de recambio de parejas): el micobionte interactúa con los mismos fotobiontes en todas las localidades.

$$\beta_{total} = 0;\ \beta_{repl} = 0;\ \beta_{rich} = 0$$

b) "Nested loss/gain" (pérdida o ganancia anidada de parejas): el micobionte mantiene la interacción con un grupo constante de fotobiontes, pero pierde o gana otros entre distintas localidades.

$$\beta_{total} = [0.01, 0.99];\ \beta_{repl} = 0;\ \beta_{rich} = 1$$

c) "Partial replacement" (reemplazo parcial): el micobionte pierde unos fotobiontes y gana otros entre distintas localidades, dando lugar a un aumento del número de parejas, una disminución o el mantenimiento del mismo número de parejas.

$$\beta_{total} = [0.01, 0.99];\ \beta_{repl} = [0.01, 0.99];\ \beta_{rich} = [0.01, 0.99]; riqueza\ variable$$

$$\beta_{total} = [0.01, 0.99];\ \beta_{repl} = 1;\ \beta_{rich} = 0; riqueza\ constante$$

d) "Total replacement" (reemplazo total): el micobionte pierde todos los fotobiontes y establece interacciones con fotobiontes diferentes manteniendo o cambiando el número de parejas entre las distintas localidades.

$$\beta_{total} = 1;\ \beta_{repl} = [0.01, 0.99];\ \beta_{rich} = [0.01, 0.99]; riqueza\ variable$$

$$\beta_{total} = 1;\ \beta_{repl} = 1;\ \beta_{rich} = 0; riqueza\ constante$$

5. Análisis estadísticos

5.1. Selección de variables ambientales

Para evitar multicolinearidad en los modelos estadísticos utilizados, se seleccionaron distintas variables en función del capítulo:

a) Capítulo 1: se seleccionaron variables no correlacionadas (r de Pearson < 0.7; p < 0.05). Las variables utilizadas fueron: temperatura media anual (bio01), temperatura mínima del mes más frío (bio06) y precipitación del mes más seco (bio14).
b) Capítulo 3: se realizó un análisis de componentes principales (PCA; Principal Component Analysis) utilizando una rotación "varimax" tanto con las variables climáticas como geográficas (Figura 1j). Posteriormente, se seleccionaron los tres primeros componentes como variables explicativas. Dichos componentes se correspondían con gradientes de temperatura y latitud (PC1), precipitación (PC2) y temperaturas más bajas y precipitaciones más elevadas (PC3).

5.2. Curvas de acumulación

Se realizaron curvas de acumulación para determinar si la eficiencia de muestreo era adecuada y capturaba la mayoría de fotobiontes que forman parte de estas comunidades (capítulo 1; Figura 1k). Estas curvas fueron estimadas por un lado para cada especie de micobionte y, por otro, para cada bosque. Para calcularlas se utilizó la función *specaccum()* del paquete vegan (v.2.5-7; Oksanen *et al*., 2020). El número de fotobiontes estimado para cada especie de micobionte y cada bosque fue calculado en función de la asíntota de cada curva de acumulación utilizando la ecuación de Chao (Chao, 1987) con la función *estimateR()*:

$$S_{Chao} = S_0 + \frac{{f_1}^2}{2f_2}\frac{(N-1)}{N}$$

donde S_{Chao} es la riqueza estimada de fotobiontes, S_0 es la riqueza observada de fotobiontes, f_1 y f_2 son el número de fotobiontes que sólo aparecen en una o dos especies de micobionte (o bosques), respectivamente, y N es el número de talos de cada especie de micobionte (o el número de bosques).

Para detectar diferencias significativas entre el número observado de fotobiontes (S_0) y el estimado (S_{Chao}), se realizó posteriormente una prueba t de Student (t-test).

5.3. Análisis de redundancia (RDA) y partición de la varianza.

Se realizó un análisis de redundancia (RDA; Redundancy Analysis) para determinar si las variables ambientales tenían una influencia sobre la composición de los filogrupos

de fotobiontes en los distintos bosques (capítulo 1; Figura 1l). Posteriormente se realizó una partición de la varianza para estimar la variación explicada por cada variable ambiental y se utilizaron diagramas de Venn para mostrar los resultados. Para ello se utilizaron la función *rda()* y *varpart()* del paquete vegan (v.2.5-7; Oksanen *et al*., 2020), respectivamente.

5.4. Modelos Lineales (LMs)

Se han utilizado modelos lineales, con una distribución gaussiana de los residuos para analizar la relación entre la riqueza de fotobiontes y la latitud y para la relación entre el rango de distribución de cada especie de micobionte (km) y la riqueza de parejas de fotobionte (capítulo 1; Figura 1m). Para realizar estos análisis se utilizó la función *lm()* del paquete stats (R Core Team, 2021) y se verificó que los residuos cumplieran todos los supuestos del modelo.

5.5. Modelos nulos

Con el fin de identificar redes significativamente anidadas, modulares y/o conectadas y para comparar los distintos valores de anidamiento, modularidad y conectancia a lo largo del gradiente latitudinal, se realizaron modelos nulos para obtener los valores estandarizados de estas medidas o "z-scores". Para ello se utilizó el modelo nulo "probabilístico" en el que la probabilidad de ocupar una celda de la matriz de incidencia de las interacciones es proporcional al número de parejas de las especies que constituyen la fila y la columna que la componen. Este modelo se utiliza como "modelo nulo 2" en Bascompte *et al*. (2003) y como modelo "probabilístico" en Fortuna *et al*. (2010). Este modelo tiene una baja proporción de error de tipo I (rechazar la hipótesis nula siendo ésta verdadera) respecto a otros modelos nulos (Rodríguez-Gironés & Santamaría, 2006):

$$\rho_{ij} = \frac{p_i + q_j}{2}; \begin{cases} p_i = \frac{1}{N_m} \sum_{i=1}^{n} A_{ij} \\ q_j = \frac{1}{N_p} \sum_{j=1}^{m} A_{ij} \end{cases}$$

donde p_i es la probabilidad observada de ocupación de la fila i, q_i es la probabilidad observada de ocupación de la columna j, N_m es el número de columnas de la matriz de incidencia (es decir, el número de especies de micobiontes), N_p es el número de filas de la matriz de incidencia (número de filogrupos del fotobionte) y A_{ij} es el valor observado de la celda perteneciente a la fila i y a la columna j.

Posteriormente, los z-scores se calcularon como:

$$z - scores = \frac{x_i - \overline{x}}{\sigma}$$

donde x_i es el valor observado de anidamiento, modularidad o conectancia, $\overline{x}$ es la media de ese valor obtenido de las matrices aleatorias generadas por el modelo nulo y σ la desviación estándar de dichas matrices aleatorias.

5.6. Modelos Lineales Mixtos Generalizados (GLMMs).

Para analizar la relación entre las variables ambientales y los rasgos funcionales y las medidas de especialización (capítulo 1) y entre las variables ambientales, los CWM y los parámetros de las redes (capítulo 3) se utilizaron modelos lineales mixtos generalizados (GLMMs). Para los análisis del capítulo 1 tanto la especie de micobionte como el bosque fueron considerados efectos aleatorios, mientras que para el capítulo 3, sólo el bosque fue tenido en cuenta como efecto aleatorio. En ambos casos se revisó que los residuos cumplieran todas las asunciones de los modelos y se calculó el factor de inflación de la varianza (VIF) para comprobar la ausencia de multicolinealidad entre las variables predictoras.

En el capítulo 1 se consideró el *p*-valor de los modelos y se realizó un análisis de la varianza (ANOVA) tipo III para considerar las variables que afectaban significativamente a las medidas de especialización. Se obtuvieron tanto el R_m^2 (marginal) como el R_c^2 (condicional), que hacen referencia a la proporción de la varianza explicada por los efectos fijos (R_m^2) y por los efectos fijos junto con los aleatorios (R_c^2).

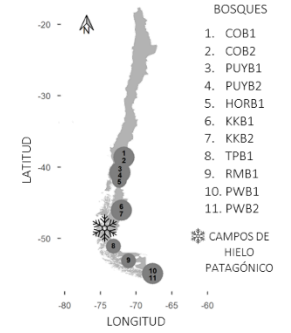
Por otro lado, en el capítulo 3, los modelos se evaluaron utilizando el criterio de información de Akaike corregido (AICc; Figura 1n). Para ello, se realizó una selección de

modelos seleccionando el modelo con un AICc menor y todos los modelos que diferían de él menos de 2 unidades de AICc. Asimismo, se calculó el peso Akaike (w^+) de cada modelo seleccionado y la importancia relativa de cada variable predictora (w_i), considerando sólo aquellas variables con un $w_i > 0.4$ (Burnham, 2015). Para considerar que una variable predictora tuviera un efecto significativo, se tuvo en cuenta si el intervalo de confianza del 95% de los estimadores de los parámetros promediados del modelo excluía el cero (Burnham & Anderson, 2002).

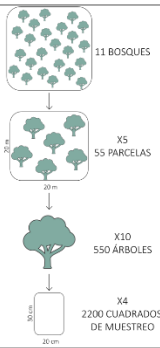
METODOLOGÍA GENERAL

ÁREAS DE ESTUDIO

a) MAPA



b) MUESTREO



c) VARIABLES AMBIENTALES

19 variables climáticas CHELSA
- Temperatura
- Precipitación

8 variables medidas *in situ*
- Latitud
- Longitud
- Altitud
- Pendiente
- Orientación

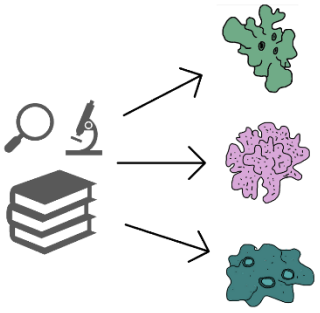
PARCELA

- Inclinación (calculada)
- DBH
- Cobertura del dosel

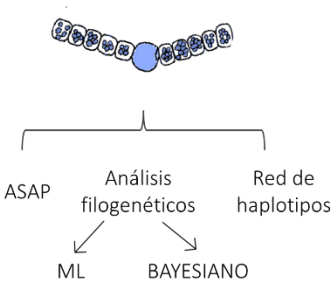
ÁRBOL

IDENTIFICACIÓN Y RASGOS FUNCIONALES

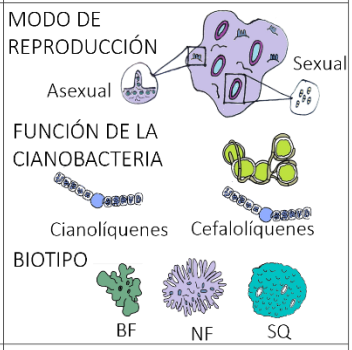
d) MICOBIONTE



e) FOTOBIONTE

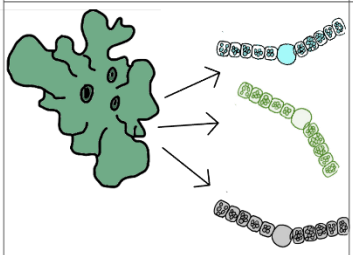


f) RASGOS FUNCIONALES



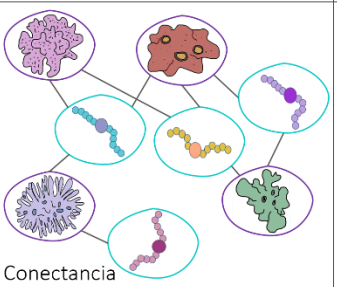
MEDIDAS DE ESPECIALIZACIÓN

g) POR ESPECIE



Número de parejas
Índice y equitatividad de Simpson
Índice d' y 1-d'

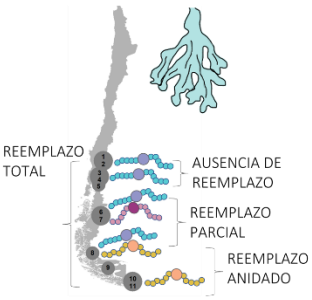
h) REDES



Conectancia
Anidamiento
Modularidad

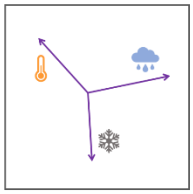
DIVERSIDAD β

i) ESCENARIOS

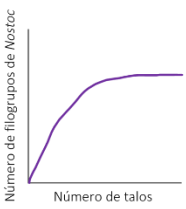


ANÁLISIS ESTADÍSTICOS

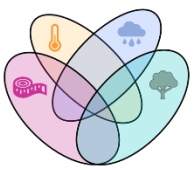
j) PCA



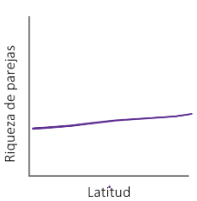
k) CURVAS ACUMULACIÓN



l) PARTICIÓN DE LA VARIANZA



m) LMs



n) GLMMs

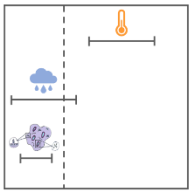


Figure 1. Representación esquemática de la metodología general: a) Mapa de Chile que muestra los bosques muestreados a lo largo del gradiente latitudinal. El tamaño de los círculos refleja se si han muestreado 1 (círculos pequeños) o 2 bosques (círculos grandes). B1 y B2 se corresponde con bosque 1 y bosque 2 respectivamente. El resto de las abreviaturas hacen referencia a Parque Nacional del Conguillío (*CO*), Parque Nacional de Puyehue (*PUY*), Parque Nacional de Hornopirén (*HOR*), Parque Nacional de Cerro Castillo (*KK*), Parque Nacional de Torres del Paine (*TP*), Reserva Nacional de Magallanes (*RM*) y Reserva de la Biosfera de Cabo de Hornos (*PW*). b) Esquema del diseño de muestreo llevado a cada bosque, en los que se seleccionaron 5 parcelas, 10 árboles en cada parcela y se realizaron 4 inventarios por árbol. c) Variables ambientales consideradas en este estudio. d) Técnicas de identificación de los micobiontes bajo la lupa binocular o el microscopio con diversas monografías. e) Técnicas moleculares utilizadas para identificar los cianobiontes: ASAP (Assemble Species by Automatic Partitioning), análisis filogenélicos de máxima verosimilitud (ML) y bayesianos y redes de haplotipos. f) Esquema de los rasgos funcionales considerados en este estudio. Las abreviaturas se corresponden con: Broad-lobed foliose (BF; foliáceo de lóbulo ancho), narrow-lobed foliose (NF; Foliáceo de lóbulo estrecho) y squamulose (SQ; escuamuloso). g) Índices utilizados a nivel de especie. h) Medidas de estructura de redes para cuantificar las interacciones a nivel de comunidad. i) Escenarios de reemplazo de especies que definen la diversidad beta. j) Esquema del análisis de componentes principales (PCA). k) Esquema de las curvas de acumulación. l) Esquema de un diagrama de Venn que ilustra la partición de la varianza. m) Esquema de un modelo lineal (LM). n) Esquema de modelos lineales mixtos generalizados (GLMMs).

6. Referencias

Almeida-Neto, M., Guimarães, P., Guimarães Jr, P. R., Loyola, R. D., & Ulrich, W. (2008). A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos*, *117*(8), 1227–1239. https://doi.org/10.1111/j.0030-1299.2008.16644.x

Aragón, G., Martínez, I., & García, A. (2012). Loss of epiphytic diversity along a latitudinal gradient in southern Europe. *Science of The Total Environment*, *426*, 188–195. https://doi.org/10.1016/j.scitotenv.2012.03.053

Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, *92*(3), 1720–1738. https://doi.org/10.1111/brv.12305

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*. https://doi.org/10.1186/1472-6785-6-9

Burnham, K. P. (2015). Multimodel Inference : Understanding AIC relative variable importance values.

Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Inference: A Practical Information-Theoretic Approach* (2nd Ed). New York: Springer-Verlag. https://doi.org/10.1007/b97636

Cardoso, P., Rigal, F., & Carvalho, J. C. (2015). BAT - Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. *Methods in Ecology and Evolution*, *6*(2), 232–236. https://doi.org/10.1111/2041-210X.12310

Carvalho, J. C., Cardoso, P., Borges, P. A. V., Schmera, D., & Podani, J. (2013). Measuring fractions of beta diversity and their relationships to nestedness: A theoretical and empirical comparison of novel approaches. *Oikos*, *122*(6), 825–834. https://doi.org/10.1111/j.1600-0706.2012.20980.x

Chao, A. (1987). Estimating the Population Size for Capture-Recapture Data with Unequal Catchability. *Biometrics*, *43*(4), 783–791. https://doi.org/10.2307/2531532

Clauset, A., Newman, M. E. J., & Moore, C. (2004). Finding community structure in very large networks. *Physical Review E - Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics*, *70*(6), 6. https://doi.org/10.1103/PhysRevE.70.066111

Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: Estimating gene genealogies. In *Proceedings - International Parallel and Distributed Processing Symposium, IPDPS 2002* (p. 184). https://doi.org/10.1109/IPDPS.2002.1016585

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal Complex Systems*, *Complex Sy*(1695), 1695. Retrieved from http://igraph.sf.net

Degelius, G. (1974). The lichen genus Collema with special reference to the extra-European species. *Symbolae Botanicae Upsalienses*, *20*(2), 1–215.

Dormann, C. F. (2011). How to be a specialist ? Quantifying specialisation in pollination networks, *1*(1), 1–20.

Dormann, C. F., Frund, J., Bluthgen, N., & Gruber, B. (2009). Indices, Graphs and Null Models: Analyzing Bipartite Ecological Networks. *The Open Ecology Journal*, *2*(1), 7–24. https://doi.org/10.2174/1874213000902010007

Dormann, C. F., Gruber, B., & Fründ, J. (2008). Introducing the bipartite Package: Analysing Ecological Networks. *R News*, *8*(2), 8–11. Retrieved from http://cran.r-project.org/doc/Rnews/

Ellis, C. J. (2012). Lichen epiphyte diversity: A species, community and trait-based review. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*(2), 131–152. https://doi.org/10.1016/j.ppees.2011.10.001

Ellis, C. J., & Coppins, B. J. (2006). Contrasting functional traits maintain lichen epiphyte diversity in response to climate and autogenic succession. *Journal of Biogeography*, *33*(9), 1643–1656. https://doi.org/10.1111/j.1365-2699.2006.01522.x

Elvebakk, A. (2007). The panaustral lichen Pannaria sphinctrina (Mont.) Tuck. and the related new species P-lobulifera from New Caledonia. *Cryptogamie Mycologie*, *28*(3), 225–235.

Elvebakk, A. (2013). Pannaria minutiphylla and P. pulverulacea, two new and common, austral species, previously interpreted as Pannaria microphyllizans (Nyl.) P. M. Jørg. *The Lichenologist*, *45*(1), 9–20. https://doi.org/10.1017/S0024282912000679

Elvebakk, A., & Bjerke, J. W. (2005). Pannaria isabellina (Vain.) comb. nov., a remarkable lichen species from Chile. *The Lichenologist*, *37*(1), 47–54. https://doi.org/10.1017/S0024282904014525

Elvebakk, A., Fritt-Rasmussen, J., & Elix, J. A. (2007). The New Zealand lichen Pannaria leproloma (Nyl.) P. M. Jørg. and its panaustral relative P. farinosa nom. nov. *The Lichenologist*, *39*(04), 349–359. https://doi.org/10.1017/S0024282907006913

Elvebakk, A., Robertsen, E. H., Park, C. H., & Hong, S. G. (2010). Psorophorus and Xanthopsoroma, two new genera for yellow-green, corticolous and squamulose lichen species, previously in Psoroma. *The Lichenologist*, *42*(05), 563–585. https://doi.org/10.1017/S0024282910000083

Fortuna, M. A., Barbour, M. A., Zaman, L., Hall, A. R., Buckling, A., & Bascompte, J. (2019). Coevolutionary dynamics shape the structure of bacteria-phage infection networks. *Evolution*, *73*(5), 1001–1011. https://doi.org/10.1111/evo.13731

Fortuna, M. A., Stouffer, D. B., Olesen, J. M., Jordano, P., Mouillot, D., Krasnov, B. R., … Bascompte, J. (2010). Nestedness versus modularity in ecological networks: Two sides of the same coin? *Journal of Animal Ecology*, *79*(4), 811–817. https://doi.org/10.1111/j.1365-2656.2010.01688.x

Galloway, D. J. (1985). *Flora of New Zealand. Lichens.* (Wellington).

Galloway, D. J. (1992). Studies in Pseudocyphellaria (lichens) III. The South American species. *Bibliotheca Lichenologica*, *46*, 1–275.

Galloway, D. J. (1994). Studies on The Lichen Genus Sticta (Schreber) Ach.: I. Southern South American Species. *The Lichenologist*, *26*(03), 223–282. https://doi.org/10.1006/lich.1994.1019

Galloway, D. J., & Jørgensen, P. M. (1995). The lichen genus Leptogium (Collemataceae) in Southern Chile, South America. *Contributions to Lichenology in Honour of Gerhard Follmann. Bot. Inst. Univ. Cologne*, 227–247.

Gandullo, J. M. (1974). *Ensayo de evaluación cuantitativa de la insolación y de la pendiente del terreno*. (An. INIA Ser. Recur. Nat. 1, Ed.).

Goward, T., Goffinet, B., & Vitikainen, O. (1995). Synopsis of the genus Peltigera (lichenized Ascomycetes) in British Columbia, with a key to the North American species. *Canadian Journal of Botany*, *73*(1), 91–111. https://doi.org/10.1139/b95-012

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Jørgensen, P. M. (2000). Survey of the Lichen Family Pannariaceae on the American Continent, North of Mexico. *The Bryologist*, *103*(4), 670–704. https://doi.org/10.1639/0007-2745(2000)103[0670:SOTLFP]2.0.CO;2

Jørgensen, P. M. (2005). A new Atlantic species in Fuscopannaria, with a key to its European species. *Lichenologist*, *37*(3), 221–225. https://doi.org/10.1017/S0024282905014866

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., … Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, *4*, 1–20. https://doi.org/10.1038/sdata.2017.122

Laliberte, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, *91*(1), 299–305. https://doi.org/10.1890/08-2244.1

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N. S. G., Garden, D., Dorrough, J., … Bonis, A. (2008). Assessing functional diversity in the field - Methodology matters! *Functional Ecology*, *22*(1), 134–147. https://doi.org/10.1111/j.1365-2435.2007.01339.x

Lücking, R., Moncada, B., McCune, B., Farkas, E., Goffinet, B., Parker, D., … Yakovchenko, L. S. (2017). Pseudocyphellaria crocata (Ascomycota: Lobariaceae) in the Americas is revealed to be thirteen species, and none of them is P. crocata. *The Bryologist*, *120*(4), 441–500. https://doi.org/10.1639/0007-2745-120.4.14

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Martínez, I. (1999). *Taxonomía del género Peltigera Willd. (Ascomycetes Liquenizados) en la península Ibérica y estudio de sus hongos liquenícolas.* Real Jardín Botánico, C.S.I.C.

Martínez, I., Flores, T., Otálora, M. A. G., Belinchón, R., Prieto, M., Aragón, G., & Escudero, A. (2012). Multiple-scale environmental modulation of lichen reproduction. *Fungal Biology*, *116*(11), 1192–1201. https://doi.org/10.1016/j.funbio.2012.09.005

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *2010 Gateway Computing Environments Workshop (GCE)* (pp. 1–8). https://doi.org/10.1109/GCE.2010.5676129

Nelson, P. R., McCune, B., & Swanson, D. K. (2015). Lichen traits and species as indicators of vegetation and environment. *The Bryologist*, *118*(3), 252. https://doi.org/10.1639/0007-2745-118.3.252

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Stevens, M. H. H. (2020). vegan: Community ecology package. R package version 2.5-6. Retrieved from https://cran.r-project.org/web/packages/vegan/index.html

Passo, A., & Calvelo, S. (2006). New reports and combinations in the family Pannariaceae (Lecanorales, lichenized Ascomycota). *The Lichenologist*, *38*(06), 549–555. https://doi.org/10.1017/S0024282906005688

Passo, A., & Calvelo, S. (2011). Pannaria byssoidea (Pannariaceae), a new squamulose species from southern South America. *The Bryologist*, *114*(4), 756–763. https://doi.org/10.1639/0007-2745-114.4.756

Passo, A., Calvelo, S., & Stocker-Wörgötter, E. (2004). Taxonomic notes on Pannaria pallida from Southern South America and New Zealand. *Mycotaxon (USA)*, *90*, 55–65.

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, *21*(2), 609–620. https://doi.org/10.1111/1755-0998.13281

R Core Team. (2021). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.r-project.org/

Rapai, S. B., McMullin, R. T., & Newmaster, S. G. (2012). The importance of macrolichen traits and phylogeny in forest community assemblage along a high elevation gradient in southwestern British Columbia. *Forest Ecology and Management*, *274*, 231–240. https://doi.org/10.1016/j.foreco.2012.02.018

Reichardt, J., & Bornholdt, S. (2006). Statistical mechanics of community detection. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, *74*(1), 1–16. https://doi.org/10.1103/PhysRevE.74.016110

Rodríguez-Arribas, C., Martínez, I., Aragón, G., Zamorano-Elgueta, C., Cavieres, L., & Prieto, M. (2023). Data from: Specialization patterns in symbiotic associations: a community perspective over spatial scales. In *Zenodo*. https://doi.org/10.5281/zenodo.7674732

Rodríguez-Gironés, M. A., & Santamaría, L. (2006). A new algorithm to calculate the nestedness temperature of presence–absence matrices. *Journal of Biogeography*, *33*(5), 924–935. https://doi.org/10.1111/j.1365-2699.2006.01444.x

Rodríguez, F., Oliver, J. L., Marín, A., & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, *142*(4), 485–501. https://doi.org/10.1016/S0022-5193(05)80104-3

Rudi, K., Skulberg, O. M., & Jakobsen, K. S. (1998). Evolution of cyanobacteria by exchange of genetic material among phyletically related strains. *Journal of Bacteriology*, *180*(13), 3453–3461. https://doi.org/10.1128/jb.180.13.3453-3461.1998

Simpson, E. H. (1949). Measurement of Diversity. *Nature*, *163*(4148), 688. https://doi.org/10.1038/163688a0

Smith, B., & Wilson, J. B. (1996). A Consumer's Guide to Evenness Indices. *Oikos*, *76*(1), 70–82. https://doi.org/10.2307/3545749

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Ventre Lespiaucq, A., Jacquemyn, H., Rasmussen, H. N., & Méndez, M. (2021). Temporal turnover in mycorrhizal interactions: a proof of concept with orchids. *New Phytologist*, *230*(5), 1690–1699. https://doi.org/10.1111/nph.17291

Vitikainen, O. (1994). Notes on some Peltigera of the Neotropics. *Acta Botanica Fennica*, *150*, 217–221.

White, F. J., & James, P. W. (1988). Studies on the Genus Nephroma II. The Southern Temperate Species. *The Lichenologist*, *20*(02), 103–166. https://doi.org/10.1017/S0024282988000167

AFILIACIÓN
COAUTORES

AFILIACIÓN DE LOS COAUTORES

Isabel Martínez (PhD Supervisor): Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid 28933, Spain.

María Prieto (PhD Supervisor): Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid 28933, Spain.

Gregorio Aragón (PhD Supervisor): Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid 28933, Spain.

Lohengrin Cavieres (Chapter 1): Instituto de Ecología y Biodiversidad (IEB), Santiago 8320000, Chile. ECOBIOSIS, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción 4030000, Chile.

Carlos Zamorano-Elgueta (Chapter 1): Universidad de Aysén, Coyhaique, Chile and CR2-Center for Climate and Resilience Research (CR)2, Santiago, Chile

Adrián Escudero (Chapter 2): Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid 28933, Spain.

Jesús López-Angulo (Chapter 2 and 3): Department of Environmental System Science, Swiss Federal Institute of Technology Zurich (ETH), 8092 Zurich, Switzerland and Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, Móstoles, Madrid 28933, Spain.

Jordi Bascompte (Chapter 3): Department of Evolutionary Biology and Environmental Studies, University of Zurich, 8057 Zurich, Switzerland.

CHAPTER 1

Specialization patterns in symbiotic associations: a community perspective over spatial scales.

Clara Rodríguez-Arribas, Isabel Martínez, Gregorio Aragón, Carlos Zamorano-Elgueta, Lohengrin Cavieres and María Prieto

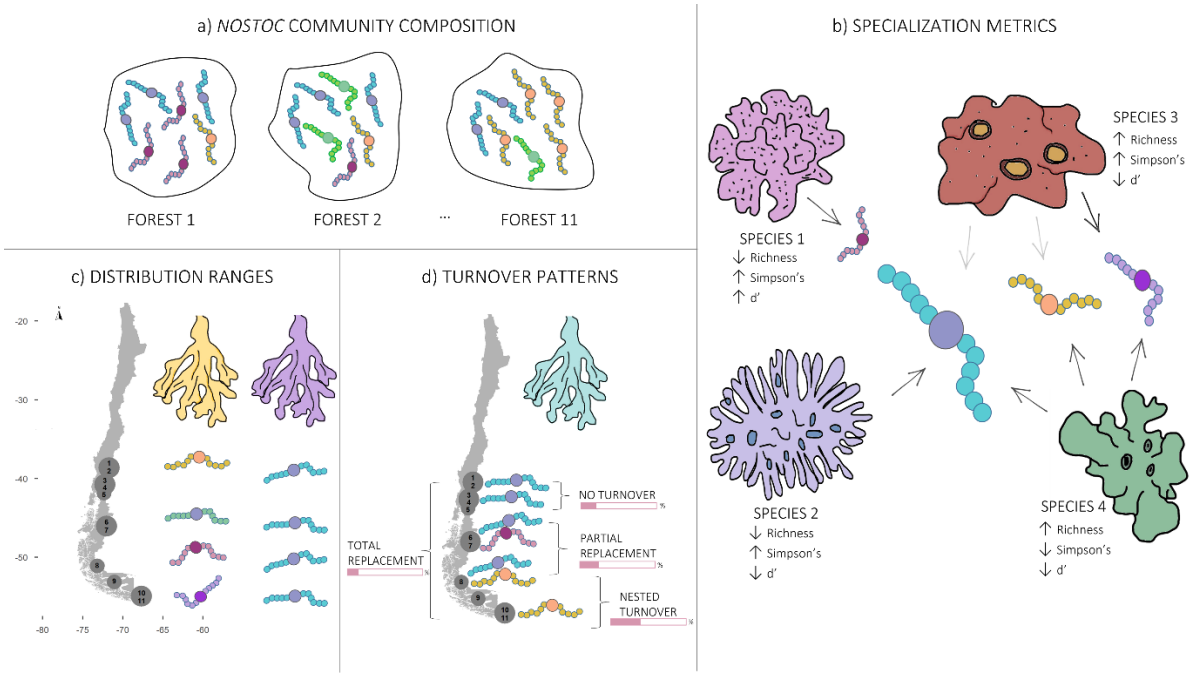
Manuscript published in *Ecology and Evolution* 2023

Abstract

Specialization, contextualized in a resource axis of an organism niche, is a core concept in ecology. In biotic interactions, specialization can be determined by the range of interacting partners. Evolutionary and ecological factors, in combination with the surveyed scale (spatial, temporal, biological, and/or taxonomic), influence the conception of specialization. This study aimed to assess the specialization patterns and drivers in the lichen symbiosis, considering the interaction between the principal fungus (mycobiont) and the associated *Nostoc* (cyanobiont), from a community perspective considering different spatial scales. Thus, we determined *Nostoc* phylogroup richness and composition of lichen communities in 11 *Nothofagus pumilio* forests across a wide latitudinal gradient in Chile. To measure specialization, cyanobiont richness, Simpson's and d' indices were estimated for 37 mycobiont species in these communities. Potential drivers that might shape *Nostoc* composition and specialization measures along the environmental gradient were analysed. Limitations in lichen distributional ranges due to the availability of their cyanobionts were studied. Turnover patterns of cyanobionts were identified at multiple spatial scales. The results showed that environmental factors shaped the *Nostoc* composition of these communities, thus limiting cyanobiont availability to establish the symbiotic association. Besides, specialization changed with the spatial scale and with the metric considered. Cyanolichens were more specialized than cephalolichens when considering partner richness and Simpson's index, whereas the d' index was mostly explained by mycobiont identity. Little evidence of lichen distributional ranges due to the distribution of their cyanobionts was found. Thus, lichens with broad distributional ranges either associated with several cyanobionts or with widely distributed cyanobionts. Comparisons between local and regional scales showed a decreasing degree of specialization at larger scales due to an increase in cyanobiont richness. The results support the context dependency of specialization and how its consideration changes with the metric and the spatial scale considered. Subsequently, we suggest considering the entire community and widening the spatial scale studied as it is crucial to understand factors determining specialization.

Keywords

Lichen, mutualism, niche, specialization, symbiosis.



Graphical abstract. Specialization towards cyanbacteria in lichens is a context-dependent concept, which changes with the metric and the spatial scale considered. As well, environmental factors shape *Nostoc* communities, determining the *Nostoc* phylogroups available in each forest to establish the symbiosis. However, *Nostoc* availability is not the only factor shaping mycobiont distributional ranges along a wide latitudinal gradient in Chile: (a) differences in *Nostoc* phylogroup composition in the different forests; (b) differences in specialization metrics (cyanobacteria richness, Simpson's and d' indices) according to how the interactions are established and the relative abundance of each *Nostoc* phylogroups; (c) wide distributional ranges are either reached by the replacement of partners or by the interaction with wide distributed partners and, (d) percentage found of the different turnover partners of partners within species between forests.

Introduction

The degree of specialization of the organisms to the abiotic and biotic conditions is a core concept in ecology. Specialization must be defined with reference to a particular axis of the requirement niche of an organism (i.e., resource), understanding the niche as the n-dimensional hypervolume composed by n niche axes which refer to the set of biotic

and abiotic requirements determining species or populations persistence (Carscadden *et al*., 2020; Hutchinson, 1957, 1978). Thus, organisms' requirements along their different niche axes define their specialization patterns (Forister *et al*., 2012; Futuyma & Moreno, 1988), being an organism considered a specialist when its requirements have a narrow amplitude, whereas generalists have a broad requirement breadth for a given resource or niche axis. The ecological relevance of specialization arises from a better performance of specialist organisms in their optimal habitats, at the expense of their performance in other habitats (Futuyma & Moreno, 1988). In addition, specialists could be negatively affected by disturbance, which may increase their probability of extinction (MacArthur, 1955). For this reason, understanding how specialization is driven and determining which species show a specialist pattern can help to find vulnerable species and to develop conservation strategies (Devictor *et al*., 2010).

One of the factors to consider specialization in biotic interactions refers to the interacting partners in both antagonistic and mutualistic systems. The specialization patterns range from reciprocal specialization, where the partners interact exclusively with each other, to generalization in which both partners interact with many others, with intermediate patterns in which both partners show different degrees of specialization (asymmetrical specialization). Remarkably, in biotic interactions, this degree of specialization is often considered from one partner (host) towards the other member of the interaction (e.g., Torres-Martínez *et al*., 2021).

Within this context of biotic interactions, we focus on mutualist interactions. Mutualism is defined as “an interaction between species that is beneficial to both” (Boucher *et al*., 1982). This mutual benefit is due to the reciprocal exchange of resources or services (Peay, 2016) and may be affected by the degree of specialization, as a high specialization has been proposed to influence the selection of certain traits which increase the fitness of the association (Aigner, 2001; Irschick *et al*., 2005; Wilson & Thomson, 1996). Specialization in mutualistic interactions can be influenced by evolutionary and ecological factors (Armbruster, 2006; Herrera *et al*., 2019). From an evolutionary point of view, the unique and repeated interaction with high-quality partners over long periods of time promotes coevolution and cospeciation, resulting in a high specialization (Armbruster, 2006; De Vienne *et al*., 2013; Forister *et al*., 2012; Herrera *et*

al., 2019; Thompson, 1999, 2005). On the contrary, ecological factors might condition specialization by determining local partner availability due to abiotic filtering and/or biotic interactions (Iglesias-Prieto *et al*., 2004; Rolshausen *et al*., 2020; Suz *et al*., 2014). The limitation of interactions due to partner availability is influenced by partner presence/absence, but also by its abundance, which promotes the selection of highly abundant compatible partners (Vázquez *et al*., 2005). In addition, considering the latitudinal diversity gradient (Hillebrand, 2004; Kinlock *et al*., 2018), the number of species is expected to be higher in the tropics, thus promoting an increase in specialization due to niche partitioning of interacting partners (Schluter, 2000). However, the variation of the degree of specialization along environmental gradients is still ambiguous. In this sense, environmental variables are expected to influence specialization patterns, as under extreme environmental conditions, generalist organisms have more chances to find high-quality partners to deal with the extreme environmental conditions (Batstone *et al*., 2018), whereas specialist organisms are expected to be more efficient in habitats where environmental conditions are limited for most of the species (Carboni *et al*., 2016). Moreover, environmental gradients have been found to influence interactions not only in the level of specialization but also in the sign of the interaction, fluctuating between more positive interactions (i.e., mutualisms) under stressful or low-resource conditions and negative (i.e., antagonisms) or neutral interactions under benign conditions (O'Brien *et al*., 2018), emphasizing the importance of the environments in which interactions take place.

The scale, considered as the spatial (i.e., local vs. regional), temporal (i.e., t_0 to t_N), biological (i.e., individuals, populations, species and communities), and/or taxonomic (i.e., species, genera, families) extent, plays an important role determining specialization in mutualist systems. How the scale affects specialization depends on the differences in the constancy of interactions between sites, time points, biological, and/or taxonomic entities (Hughes, 2000). Differences in specialization between different scales (e.g., local vs. regional and species vs. families) can be explained by different turnover scenarios as those proposed by Ventre Lespiaucq *et al*., 2021. These turnover scenarios are based on the analysis of the variation in species composition between different points of a defined scale (beta diversity).

Along with corals, mycorrhizae, legume-rhizobium, pollination and seed-dispersal interactions, lichenized fungi are one of the great examples of terrestrial mutualisms. An individual lichen is a complex ecosystem composed by the interaction of a primary fungus (the mycobiont host), one or more photosynthetic partners (photobionts) and an indeterminate number of other microscopic organisms (i.e., yeasts, bacteria; Hawksworth & Grube, 2020). In the symbiosis, the photobionts (algae or cyanobacteria) provide carbohydrates from photosynthesis, while the mycobiont (host) protects the photobionts from desiccation. Cyanobacterial partners (cyanobionts) also fix atmospheric dinitrogen (Nash, 1996).

Lichens associated with cyanobionts (cyanolichens and cephalolichens) represent nearly 10% of all known lichen-symbiotic fungi (Jüriado *et al*., 2019; Rikkinen, 2015) and are ecologically important due to their nitrogen fixation properties (Asplund & Wardle, 2017; Ellis, 2012). Cyanobacteria is the only photobiont in bipartite cyanolichens, while cephalolichens (tripartite) have a green algae as the principal photobiont while cyanobacteria are held in modified internal or external structures known as cephalodia. The function of the cyanobiont in both cyanolichens and cephalolichens is different as in cyanolichens the cyanobacteria performs photosynthesis and contributes to nitrogen fixation whereas it is mostly in charge of nitrogen fixation in cephalolichens (Nash, 1996). The result of the interaction, the lichen thalli, is treated as an organism itself and is the entity affected by the specialization pattern acquired. The mycobiont is usually more specialized than the photobiont, possibly because the mycobiont is more dependent on the symbiosis, whereas the green algae and cyanobacteria can live independently (Magain *et al*., 2017). Thus, the degree of specialization is often considered from the perspective of the mycobiont (host). The advantage of the high specialization from the mycobiont to the photobiont can be seen as an adaptive process which allows an optimization of the fitness of the lichen thallus (i.e., abundance, growth rate, reproduction allocation and survival) to different local biotic and abiotic environmental variables as a result of the interaction with a well locally adapted photobiont to a set of biotic and abiotic variables (Magain *et al*., 2017; Rolshausen *et al*., 2018). On the contrary, generalization promotes the flexibility to associate with several photobionts which would

translate into an improvement of the ecological tolerance of the lichen thallus (Batstone *et al*., 2018; Ertz *et al*., 2018).

Factors determining specialization patterns between mycobionts and photobionts are still unclear (Mark *et al*., 2020). Photobiont distribution and availability, lichen reproductive mode (linked to a vertical or a horizontal transmission of the photobiont), mycobiont identity, function of the cyanobiont in cyano- and cephalolichens, geography, or environmental variables have been shown to play major roles in shaping these patterns (Beck *et al*., 2002; Blaha *et al*., 2006; Fedrowitz *et al*., 2012; Fernández-Mendoza *et al*., 2011; Kaasalainen *et al*. 2021; Lücking *et al*., 2009; Muggia *et al*. , 2008; Otálora *et al*., 2010; Peksa & Škaloud, 2011; Piercey-Normore & Deduke, 2011; Rikkinen, 2003; Yahr *et al*., 2006). However, most specialization studies of lichens are based on either small biological and/or spatial scales (e.g., Chagnon *et al*., 2018; Jüriado *et al*., 2019; Leavitt *et al*., 2015; Lu ***et al***., 2018), and consequently, there is still a gap in knowledge of specialization patterns in a community context (i.e., considering all the coexisting species of lichens interacting with cyanobionts within forests) and along wide geographical scales.

In this study, we investigated specialization patterns of epiphytic communities of cyano-and cephalolichens growing in *Nothofagus pumilio* (Poepp. & Endl.) Krasser forests across a wide latitudinal gradient in Chile. Specifically, we aimed to: (1) analyse the availability and composition of *Nostoc* cyanobiont phylogroups establishing the lichen symbiosis across a broad latitudinal range, (2) correlate the distribution of these phylogroups with environmental variables that might shape the composition of *Nostoc* communities, (3) detect the specialization patterns of mycobionts towards their cyanobionts and the factors (environmental variables, reproductive mode, function of the cyanobiont and mycobiont identity) driving specialization, (4) determine whether generalist mycobionts result in lichens with wider distribution ranges and if these ranges are conditioned by the distribution ranges of their cyanobionts and (5) compare specialization patterns of mycobionts across spatial scales (local vs. regional). Based on previous knowledge, we propose four hypotheses: First, *Nostoc* availability increases with lower latitudes and *Nostoc* community composition is correlated with environmental factors that change with latitude. Second, environmental variables, reproductive mode, function of the cyanobiont, and/or mycobiont species identity are all correlated with

specialization of the mycobiont. Third, generalized mycobionts are expected to result in lichens with wider distributional ranges, and specialized mycobionts to be conditioned by the distributional ranges of their cyanobionts. And fourth, specialization of the mycobiont decreases with increasing spatial scale, which could encompass several turnover scenarios.

Methods

Sampling

Between 2017 and 2018, 11 forest stands were sampled across a wide latitudinal gradient (from 38.63°S to 54.96°S), nine of them belonging to National Parks or Reserves (Figure 1). Forest stands were mostly formed by *N. pumilio* with over 65% of cover. Within each forest stand, we collected eight thalli of each species of cyanolichen and cephalolichen in different *N. pumilio* trees at a minimum distance to the forest edge of 100 m. Each thallus was considered as an individual. Samples were air-dried and stored at −20°C. Forest structure and habitat quality-related variables (elevation, orientation, slope, diameter at breast high (DBH) and canopy cover) were collected in situ in each forest. Elevation, orientation, and slope (estimated by GPS, compass, and clinometer, respectively) were measured at five locations in each forest, and DBH and canopy cover were quantified in 50 trees per forest. For the canopy cover, hemispherical photographs were taken with a horizontally levelled digital camera (Canon EOS 5D) aimed at the zenith, employing fish-eye lens with a 180 field of view (SIGMA 8 mm F3.5 ex DG Fisheye). The photographs were analysed with Gap Light Analyzer v2.0 (GLA v2) (http://www.rem.sfu.ca/forestry/index.htm), which estimates the canopy openness as a percentage. Subsequently, we calculated the mean of these variables for each forest stand (Appendix S1).

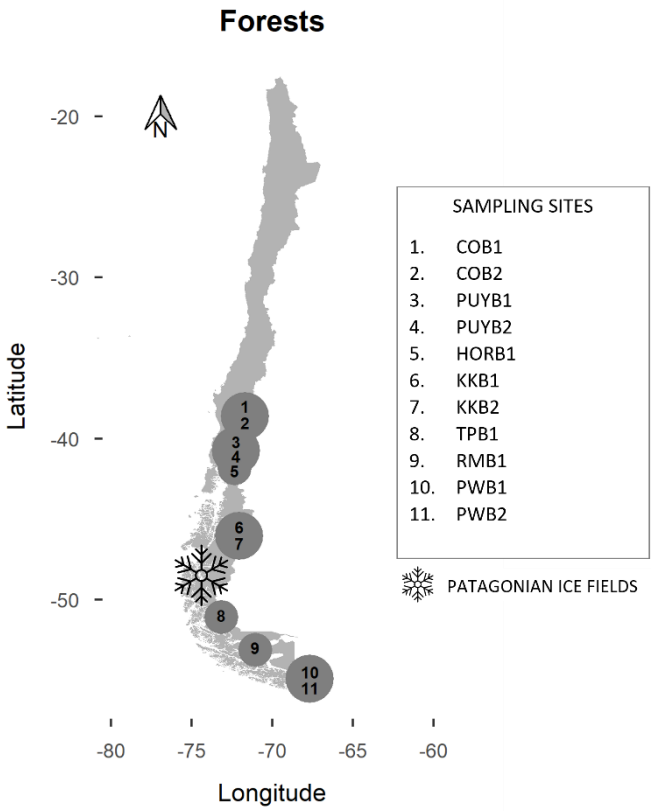


Figure 1. *Nothofagus pumilio* forests sampled along a latitudinal gradient. The size of the circles refers to the number of forests sampled in the same National Park or Reserve (bigger circles contain two forests named B1 and B2, and smaller circles represent one forest). Abbreviations of the areas: CO, Conguillío National Park; HOR, Hornopirén National Park; KK, Cerro Castillo National Park; PUY, Puyehue National Park; PW, Navarino Island; RM, Magallanes National Reserve; and TP, Torres del Paine National Park.

A total of 1492 lichen thalli were collected belonging to 86 mycobiont species (53 cyanolichens and 33 cephalolichens). Lichen identification was based on morphological characters and followed Degelius (1974) for *Collema*, Jørgensen (2000, 2005) for *Fuscopannaria*, Galloway & Jørgensen (1995) for *Leptogium*, White & James (1988) for *Nephroma*, Passo *et al*. (2004), Elvebakk & Bjerke (2005), Passo & Calvelo (2006), Elvebakk (2007), Elvebakk *et al*. (2007), Passo & Calvelo (2011), and Elvebakk (2013) for *Pannaria*, Galloway (1985) for *Parmeliella* and *Psoroma*, Goward *et al*. (1995), Martínez (1999), and Vitikainen (1994) for *Peltigera*, Galloway (1992) and Lücking *et al*. (2017), for *Pseudocyphellaria* s. lat., Elvebakk *et al*. (2010) for *Psorophorus* and *Xanthopsoroma* and Galloway (1994) for *Sticta* (Appendix S2). Data from the reproductive mode (sexual and/or asexual) and the function of the cyanobiont stablishing the symbiosis (principal

photobiont for cyanolichens or secondary photobiont for cephalolichens) were gathered observationally from the samples collected, as the reproductive mode could differ within the same species across the studied gradient.

Environmental variables

We selected 26 environmental variables related to climate, forest structure and habitat quality (Appendix S1). Climatic information was extracted from the CHELSA climate database (Karger *et al*., 2017), including information of 19 temperature and precipitation variables. We also considered the geographic variables of each forest (latitude and longitude). Variables related to forest structure and habitat quality comprise elevation, slope, orientation, canopy cover and tree DBH. To avoid multicollinearity, we selected variables not significantly correlated with each other ($r > 0.7$, $p > 0.05$), calculated with function *rcorr()* from package Hmisc (v.4.4-1; Harrell & Dupont, 2018) and previously shown as being more ecologically relevant to lichen biology (Matos *et al*., 2015). The final variables included in the models were mean annual temperature (bio01), minimum temperature of the coldest month (bio06), precipitation of the driest month (bio14) and DBH.

Nostoc phylogroup delimitation

DNA from the cyanobionts was extracted using Chelex® 100 Chelating Resin (Bio-Rad). Subsequently, rbcLX region was amplified with the primers CW and CX (Rudi *et al*., 1998) using the following program: 95°C 15 min; 35 cycles of 1 min at 95°C, 30 s at 54°C, 30 s at 72°C; and 10 min at 72°C. PCR products were sequenced at Macrogen Spain service (www.macrogen.com) using the same primers employed in the PCR. A total of 1120 cyanobacterial consensus sequences were obtained (Rodríguez-Arribas *et al*., 2023).

The 1120 obtained sequences were edited and aligned using Geneious Prime 2021.0.1 software (https://www.geneious.com). Published sequences from Magain *et al*. (2017) were also added to the alignment. Ambiguous regions (i.e., two intergenic spacers) were delimited manually and excluded for the analysis using Aliview v. 1.26 (Larsson, 2014). Delimitation of *Nostoc* phylogroups was based on the ASAP method (Assemble Species by Automatic Partitioning, Puillandre *et al*., 2021) together with results (i.e., highly

supported clades) obtained from the combined (Maximum likelihood and Bayesian) phylogenetic analysis and previous studies (Magain *et al*., 2017). ASAP analysis was carried out in the webserver (https://bioinfo.mnhn.fr/abi/public/asap/) applying the Jukes-Cantor (JC69) model of substitution (split groups below 0.01 probability, keep 10 best scores and −1 as seed value). Partitions included in the 0.001–0.01 range of genetic distances were selected. Maximum likelihood (ML) analysis was conducted with RAxML v. 8.2.12 (Stamatakis, 2014) in the CIPRES Science Gateway Portal (Miller *et al*., 2010) with 1000 bootstrap iterations. The Bayesian analysis was run in MrBayes 3.2.7a (Huelsenbeck & Ronquist, 2001) in Agapita server (URJC, Biodiversity and Conservation Area) for 50 million generations with two runs and four chains, sampling every 1000 generations with a burning of the 25% and the GTR + I + G substitution model (Rodríguez *et al*., 1990).

Availability and composition of *Nostoc* phylogroups along the latitudinal gradient

We analyse *Nostoc* availability along the whole latitudinal gradient sampled in order to detect varying *Nostoc* diversity (i.e., richness) in the different forests with a linear model, using function *lm()* from package stats (R Core Team, 2021). To predict which of the selected environmental variables might have influenced *Nostoc* composition, a redundancy analysis (RDA) was performed using symbiotic *Nostoc* abundances per forest and the *rda()* function in package vegan (v.2.5-7; Oksanen *et al*., 2020). The variation explained by each variable was estimated with the *varpart()* function from the same package and a Venn diagram.

Specialization measures

We selected those mycobiont species with at least 10 sequences along the latitudinal gradient to ensure a minimum number of specimens for the following analysis. Specialization measures were calculated at the forest level for every mycobiont species from the previously selected with a minimum of four rbcLX sequences in that forest. In total, 37 mycobiont species met these requirements in at least one of the forests.

Number of interacting partners and sampling efficiency

Cyanobiont richness was calculated per mycobiont species per forest as the number of interacting partners with which a mycobiont species establishes in each of the forests for the selected 37 species.

Accumulation curves were estimated per mycobiont species along the latitudinal gradient (for the selected 37 species) and per forest (including all species found in each forest) to determine the sampling efficiency (i.e., if *Nostoc* diversity within species and within forest was well determined) with the function *specaccum()* from the vegan package (v.2.5-7; Oksanen *et al*., 2020). The number of potential *Nostoc* phylogroups was estimated with the Chao 1 equation using *estimateR()*. Differences between the observed cyanobiont richness and the Chao1 estimation within each mycobiont and within each forest were analysed using a t-test with function *t-test()* from the package stats (R Core Team, 2021).

Indices: Simpson and d'

Two different indices were used to quantify specialization at the forest level for each of the previously selected 37 mycobiont species with at least four sequences in the forest.

Simpson's index (Simpson, 1949) was calculated as:

$$D_i = \sum_{j=1}^{N} p_{ij}^2$$

where N is the number of *Nostoc* phylogroups associated with mycobiont species i, and p_{ij} is the frequency of association of species i with each phylogroup j. The Simpson's index considers the richness and the evenness of the partners and accentuates the contribution of common versus rare species (Magurran, 1988; Sahli & Conner, 2006), emphasizing the selectivity or preference towards certain cyanobionts.

d′-index (Blüthgen *et al*., 2006) was calculated using the function *dfun()* from the bipartite package v.2.16 in R (Dormann, 2011; Dormann *et al*., 2008) as:

$$d_i = \sum_{j=1}^{C} (p_{ij} ln \frac{p_{ij}}{q_j})$$

where c is the total number of interacting phylogroup species, p_{ij} is the distribution of the interactions of species i with each partner j, and q_j is the relative availability of each *Nostoc* partner j. This equation is normalized to result in the d′-index (see Blüthgen *et al*., 2006 for further details). The d′ index is a niche breadth index which consists of a standardized form of the Kullback–Leibler distance and considers the proportion of interactions with the different partners in relation to their relative abundance in the community. It quantifies whether a species should be considered as more opportunistic because it uses the resources in the same proportion as they are in the environment (low specialization), in comparison with a species that uses preferentially rare resources (high specialization, e.g., Blüthgen *et al*., 2007).

Both indices (Simpson's and d′-index) range from zero to one. A Simpson's index equal to one means that a mycobiont species will always be associated with the same *Nostoc* phylogroup, while a mycobiont associating with different phylogroups in different proportions will have lower values. On the contrary, a d′-index of one is achieved when reciprocal specialization is reached (i.e., both myco-and cyanobiont only associate between them). However, values close to zero of d′-index are reached when a mycobiont is associated with *Nostoc* phylogroups in the same proportion as their availability in the forest, suggesting that the interaction is opportunistic rather than specialized (Blüthgen *et al*., 2006).

Generalized linear mixed models

Factors associated with the number of interacting partners (cyanobiont richness), and both Simpson's and d′ indices for each mycobiont species per forest were analysed using generalized linear mixed models (GLMMs). The environmental variables selected (DBH, bio01, bio06 and bio14), the reproductive mode observed (sexual, asexual, both, or none) and the function of the cyanobiont (primary photobiont for cyanolichens or secondary photobiont for cephalolichens) were included as fixed effects, while forests and mycobiont species identity were included as random effects. We performed GLMMs with Poisson distribution for cyanobiont richness and Gaussian distribution for Simpson's and

d′ indices with functions *glmer()* or *lmer()*, respectively from the lme4 package (v.1.1-23; Bates *et al.*, 2015). Explanatory variables were scaled using function *scale()* which computes the mean (centre) of a variable, sets the mean to zero and calculates the values from such a mean. The values are then standardized by the standard deviation of each variable. The response variables were transformed to accomplish comparisons and linear mixed model assumptions (ln for Simpson's index and square root for d′-index). We calculated the variance inflation factor (VIF) of each model to verify the absence of multicollinearity (VIF < 4) with the function *vif()* from package psych (v.2.1.3; Revelle, 2021). We tested whether there was spatial autocorrelation using Moran's index with function *testSpatialAutocorrelation()* from package DHARMa (v.0.4.5; Hartig, 2022).

To analyse the significance of the fixed effects, we performed an ANOVA type III from package car (v.3.0-9; Fox & Weisberg, 2019) and a post hoc Tuckey test with function *glht()* from package multcomp (v. 1.4-18; Hothorn *et al*., 2008). Marginal R^2 (R^2_m) and conditional R^2 (R^2_c) were calculated with the *r.squaredGLMM()* function from package MuMIn (v.1.43.17; Bartoń, 2020) to obtain the variance explained by the fixed effects (R^2_m), and by the entire model (with both fixed and random effects, R^2_c), respectively. The residuals met all assumptions of the linear mixed models.

Distributional ranges and specialization patterns

We analysed the distributional range of each mycobiont species and cyanobiont phylogroup considering the distance along the studied gradient in which they were present. Then, we selected those mycobiont species with at least 10 thalli along the entire latitudinal gradient, and we performed linear models with function *lm()* from package stats (R Core Team, 2021) to observe whether generalist mycobiont species associating with many cyanobionts had broader distributional ranges as a lichen. We then determined whether lichen distributional ranges were correlated by the distribution of their *Nostoc* partners.

Spatial scale: Beta diversity and partner replacement

In order to analyse the specialization patterns within mycobiont species among forests and between different spatial scales (local vs. regional), we calculated cyanobiont

turnover (Carvalho *et al*., 2013; Ventre Lespiaucq *et al*., 2021). We used function *beta()* from package BAT (v.2.7.1; Cardoso *et al*., 2015) to obtain β_{total}, β_{repl} and β_{rich} to determine whether the differences in beta diversity within mycobiont species were due to species replacement and/or due to differences in partner richness ($\beta_{total} = \beta_{repl} + \beta_{rich}$). Specifically, several turnover scenarios have been considered (Ventre Lespiaucq *et al*., 2021): (a) No turnover: the mycobiont interacts with the same cyanobiots throughout the gradient, (b) Nested loss/gain: the mycobiont maintains many of the same cyanobionts along the gradient, but either gains or loses phylogroups, (c) Partial replacement: the mycobiont loses some cyanobiont phylogroups and gains others. This process could produce the same, lower or higher cyanobiont richness among sites and (d) Total replacement: a shift in cyanobiont composition from site to site. Richness can remain the same or differ among sites.

All statistical analyses were performed in R v. 4.0.4 (http://www.rproject.org/).

Results

Nostoc phylogroup delimitation

We defined a total of 64 *Nostoc* phylogroups based on the results of the supported clades obtained in the combined phylogenetic analyses (i.e., ML and Bayesian analysis which were congruent), together with the grouping generated by ASAP and previous studies (i.e., Magain *et al*., 2017). Only two phylogroups were shared with phylogroups found by Magain's *et al*. (2017) (Appendix S3). Phylogroups were named from one to 64, where phylogroups XV and VIIIb from Magain *et al*. (2017) corresponded to phylogroups one and two, respectively (Appendix S3).

Availability and composition of *Nostoc* phylogroups along the latitudinal gradient

Nostoc phylogroup richness did not show a latitudinal pattern (Appendix S4). However, *Nostoc* phylogroup composition changed among forests (Figure 2). Each forest stand was dominated by a few abundant *Nostoc* phylogroups, but most phylogroups were

locally rare. Across the latitudinal gradient studied, *Nostoc* phylogroup 38 was the most abundant, representing 37.32% of the sequences obtained, followed by phylogroup 42, which represented 19.29% (Appendix S5). These abundant phylogroups were shared among different and distantly related mycobiont species.

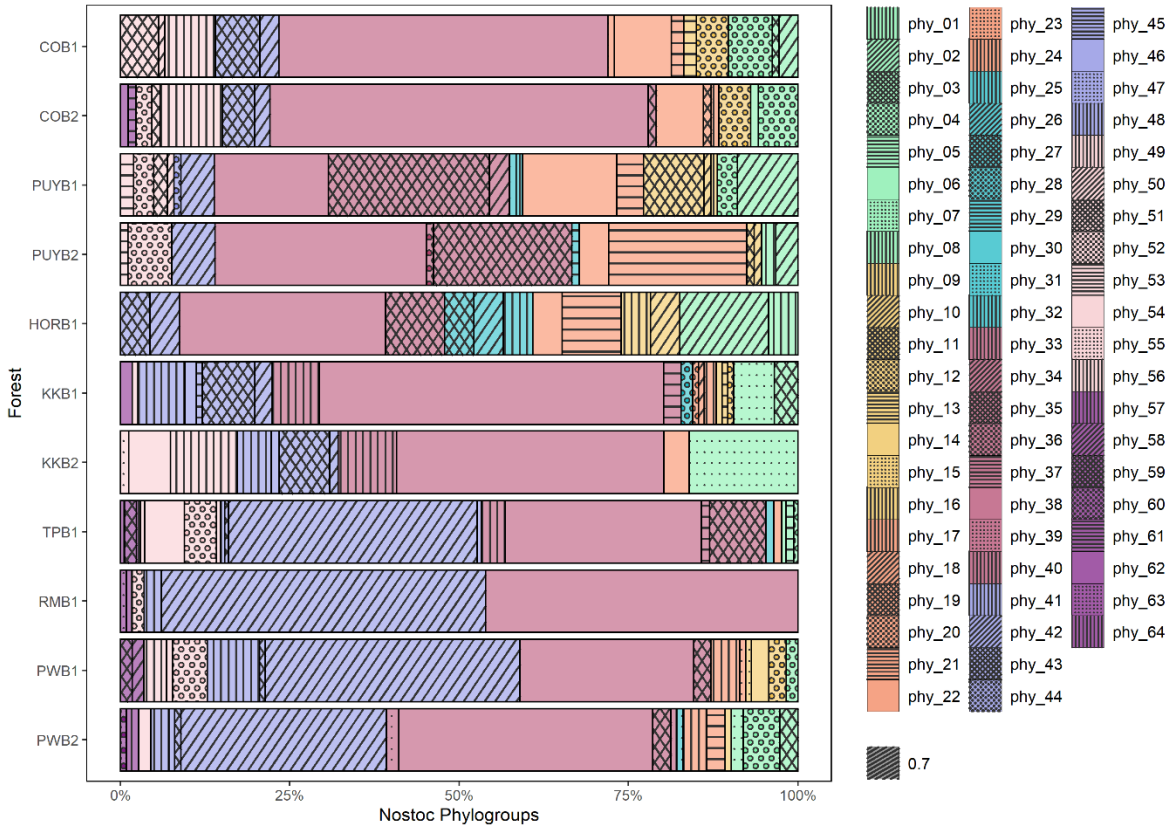


Figure 2. *Nostoc* phylogroups composition on the different forests. Sampling sites: (1) Conguillío National Park (COB1 and COB2), (2) Puyehue National Park (PUYB1 and PUYB2), (3) Hornopirén National Park (HORB1), (4) Cerro Castillo National Park (KKB1 and KKB2), (5) Torres del Paine National Park (TPB1), (6) Magallanes National Reserve (RMB1) and (7) Navarino Island (PWB1 and PWB2).

Mycobiont species differed in their patterns of cyanobiont composition within a forest. Some mycobionts tended to establish with the most abundant *Nostoc* phylogroups while others interacted with rare phylogroups (Appendix S6). Species such as *Nephroma cellulosum, Parmeliella nigrocinta*, *Pseudocyphellaria bartlettii*, *P. dubia*, *P. gilva*, *P.* gr. *argyracea*, *P.* gr. *citrina*, *P. lechlerii*, *P. mallota*, *P. scabrosa*, only associated with the same three *Nostoc* phylogroups (35, 38 and 42), which were the most common phylogroups in the gradient. On the contrary, species such as *Leptogium valdivianum*, *Peltigera collina*, *P. hymenina*, *Sticta fuliginosa* and *S. hypochra* were never found associating with the most

abundant phylogroups. The rest of the species established, at least once, with the most common phylogroups 35, 38, and/or 42.

Cyanobiont phylogroup composition along the gradient was correlated with climate (mean annual temperature—bio01, minimum temperature of the coldest month—bio06 and precipitation of the driest month—bio14) and forest structure (tree diameter at breast height—DBH) as the RDA analyses showed (Appendix S7). The variation explained by these environmental variables was ca. 70%, with bio06 having the biggest contribution (39.2%), followed by bio01 (30.7%), DBH (20.6%) and bio14 (0.91%) (Appendix S8).

Specialization measures

Number of interacting partners and sampling efficiency

Accumulation curves are shown in Appendix S9 for each mycobiont species and Appendix S10 for each forest. The estimated Chao1 revealed different levels of sampling efficiency, with 23 species (62.16%) showing a Chao1 equal to the number of phylogroups found (sampling of 100% of the phylogroups; Appendix S11). The sampling efficiencies of seven of 37 species were lower than 80%. All the species (even those with a lower sampling efficiency) have been included in subsequent analyses (Simpson's and d'-index calculations, GLMMs and beta diversity), as they already associated with a high number of partners, showing a generalist pattern.

The observed cyanobiont richness within each lichen species along the gradient varied from one to 15 (Appendix S11). *Nephroma antarcticum* was the most generalist lichen species. It interacted with 15 *Nostoc* phylogroups and its Chao1 of 30 suggests that 50% of the expected richness was detected. On the contrary, *Peltigera collina*, *Pseudocyphellaria bartletti* and *Sticta fuliginosa* were found to be associated only with one *Nostoc* phylogroup, with an estimated sampling efficiency of 100% for each of them (Chao1 = 1).

Considering each forest, the number of cyanobionts observed did not show big differences from the Chao1 estimate, which indicates that the sample size was adequate for estimating within-forest *Nostoc* richness ($t(18) = -2.08$; $p > .05$) (Appendix S12).

Indices: Simpson and d'

Simpson's index and d'-index varied from 0.22 to 1 and 0 to 1, respectively. In addition, most mycobiont species showed different values of each of the indices per forest indicating that the specialization pattern that a species shows in one forest may change across forests (Figure 3; Appendix S13).

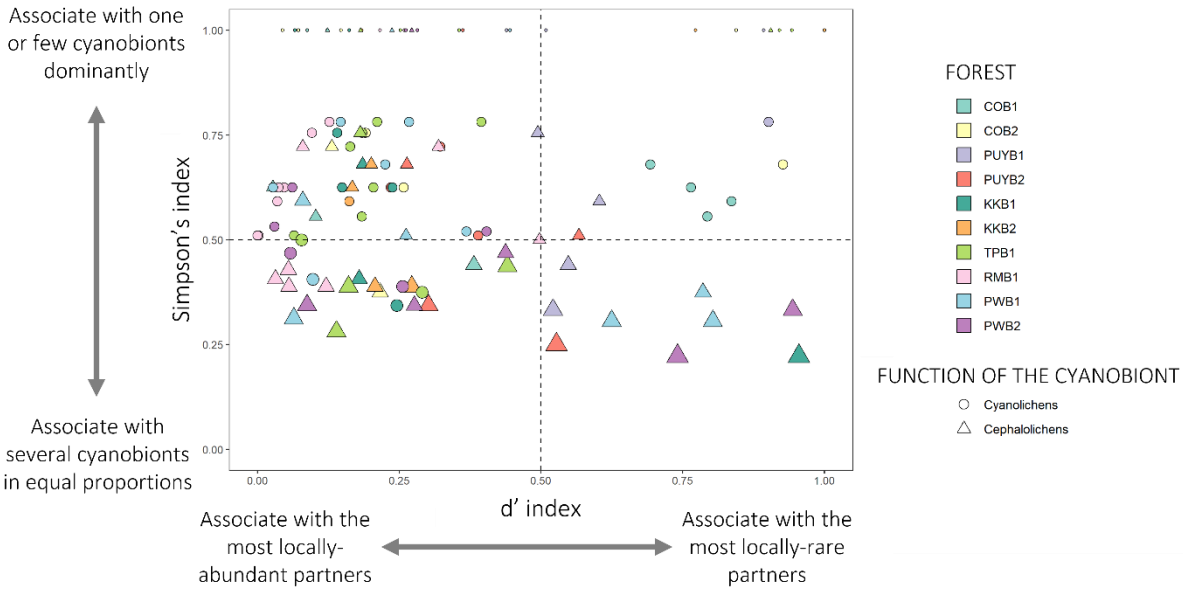


Figure 3. Representation of the values of the three specialization metrics obtained for each lichen species at the forest level (local scale). Circles represent cyanolichens and triangles cephalolichens. The size of circles and triangles refers to the cyanobiont richness of each lichen species, ranging from 1 to 5. The y-axis represents the values obtained by the Simpson's index and the x-axis the values of the d' index. Colours refer to the studied forests.

Only two species, *Nephroma pseudoparile* and *Peltigera collina*, had a value equal to one in both indices in almost all forests (Figure 3; Appendix S13). Nonetheless, in certain forests, several species (e.g., *Pannaria* gr. *sphinctrina* (TPB1), *Peltigera collina* (TPB1), *Peltigera hymenina* (PUYB1), *Sticta fuliginosa* (TPB1) and *Sticta hypochra* (COB2)), showed high values of both indices (S-index = 1; d'-index between 0.94 and 0.84). High values of Simpson's index and low values of d' index were observed in *Nephroma cellulosum* (KKB1), *Parmeliella nigrocinta* (COB2), *Pseudocyphellaria* gr. *citrina* (COB1) and *Pseudocyphellaria hirsuta* (COB2). The opposite pattern (i.e., a low S-index and a high d'-index) was found in fewer species, such as *Pseudocyphellaria freycinettii* (PWB2) and

Psoroma asperellum (KKB1). In addition, examples of varying patterns along the latitudinal gradient were observed in *Pseudocyphellaria* gr. *vaccina* and *Nephroma pseudoparile*. For instance, *Nephroma pseudoparile* showed high values of both indices in the Northernmost forests, above the Patagonian ice fields, but low values (S-index = 0.38; d′-index = 0.29) in its Southern distribution, thus being more specialized towards the North.

Generalized linear mixed models

The results of the GLMMs for the three metrics are presented in Table 1. Among the fixed effects in the model, the function of the cyanobiont was the factor explaining the highest proportion of variation for cyanobiont richness (X^2 (1, N = 120) = 12.10, p > 0.05) and Simpson's index (X^2 (1, N = 120) = 30.53, p > 0.05). Cephalolichens tend to interact with a higher number of cyanobionts than cyanolichens (Figure 4). In addition, cephalolichens show lower values of Simpson's index than cyanolichens, which means they interact with the different partners in more equal proportions, not showing preferential interactions with any of them (Figure 5). Random effects (mycobiont species identity and forest) explained little to no variance in cyanobiont richness and Simpson's index (cyanobiont richness: R^2_m= R^2_c= 0.13; S-index: R^2_m = R^2_c= 0.28). On the contrary, the variation of the d′-index was explained mostly by the random effects (R^2_m = 0.13; R^2_c= 0.75), being the largest part of the variance explained by the mycobiont species identity. The environmental variables (bio01, bio06, bio14 and DBH) and the reproductive mode (sexual vs. asexual) had little effect in all the specialization measures.

Table 1. Results of the GLMMs. Bold values refer to a significant effect of the fixed effects. Random and fixed effects estimates and type III ANOVA for partner richness, Simpson index and d' index of specialization.

	Partner Richness						Simpson index						d' index					
Random Effects	**Variance**			**SD**			**Variance**			**SD**			**Variance**			**SD**		
Mycobiont species	0			0			0			0			0.04			0.2		
Forest	0			0			0			0			0			0.05		
Residual				SD			0.12			0.35			0.02			0.13		
Fixed Effects	**Estimate**	**SD**	**z**	**Chisq**	**Df**	**P**	**Estimate**	**SD**	**t**	**Chisq**	**Df**	**P**	**Estimate**	**SD**	**t**	**Chisq**	**Df**	**P**
Intercept	0.49	0.11	4.43	19.64	1	**9.38e-06 *****	-0.29	0.05	-5.51	30.38	1	**3.58e-08 *****	0.61	0.05	11.2	125.38	1	**<2e-16 *****
Scale(DBH)	0.01	0.12	0.49	0	1	0.96	-0.01	0.06	-0.12	0.01	1	0.91	-0.04	0.04	-1.22	1.48	1	0.22
Scale(bio01)	-0.12	0.12	-1.03	1.05	1	0.31	0.1	0.06	1.82	3.31	1	0.07	0.05	0.04	1.54	2.36	1	0.12
Scale(bio06)	0.07	0.1	0.67	0.45	1	0.5	-0.05	0.05	-1.12	1.269	1	0.26	-0.02	0.03	-0.66	0.44	1	0.51
Scale(bio14)	0.07	0.11	0.68	0.46	1	0.5	-0.05	0.05	-0.89	0.8	1	0.37	0.03	0.03	1.04	1.08	1	0.3
Reproduction				1.02	3	0.8				1.8	3	0.61				3.39	3	0.34
Reproduction: both	0.1	0.18	0.55				-0.04	0.09	-0.42				0.02	0.05	0.41			
Reproduction: none	0.3	0.34	0.89				-0.24	0.21	-1.13				0.25	0.14	1.84			
Reproduction: sexual	0.07	0.14	0.51				-0.07	0.07	-0.89				0.03	0.06	0.44			
Cyanobiont				12.1	1	**5.04e-04 *****				30.53	1	**3.28e-08 *****				2.1	1	0.15
Cyanobiont: cephalolichen	0.45	0.13	3.48				-0.38	0.07	-5.53				-0.11	0.08	-1.45			

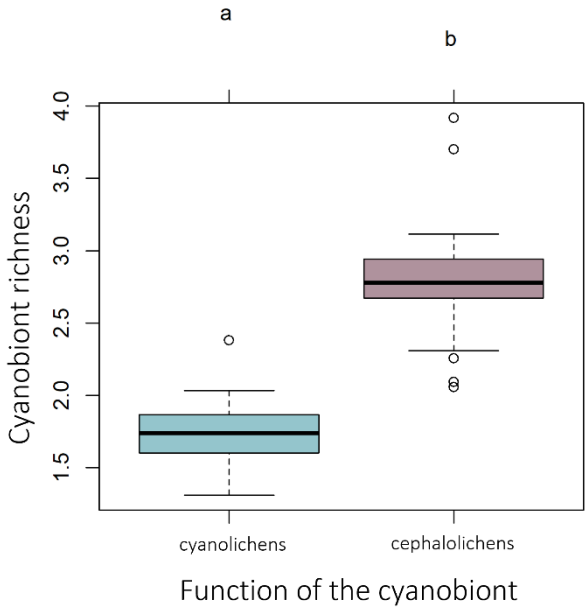


Figure 4. Cyanobiont richness for cyano-and cephalolichens. Letters inform about the significant differences after performing a Tuckey test.

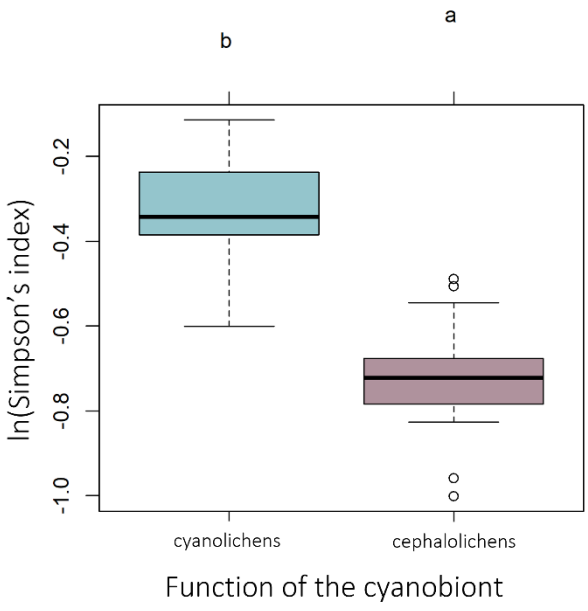


Figure 5. Simpson's index for cyano-and cephalolichens. Letters inform about the significant differences after performing a Tuckey test.

Distributional ranges and specialization patterns

The relationship between the number of partners of each mycobiont species and the geographic distance did not appear to be linear (Appendix S14). Those lichen species with broad distributional ranges are either associated with a high number of interacting partners or (most frequently) associated with a low number of interacting partners with a broad distributional range (e.g., *Pseudocyphellaria* gr. *citrina*). Most mycobiont species established at least once, with widely distributed *Nostoc* phylogroups; thus, cyanobionts were not limiting lichen distributions (Appendices S15 and S16). On the contrary, examples of limitations in the distributional range of a lichen species due to the distributional range of the *Nostoc* partner are rare (besides the cases in which there are few observations). For instance, *Nostoc* phylogroup 02 has a small distributional range (361.24 km). This phylogroup only associates with *Peltigera praetextata* and *Peltigera hymenina*. *Peltigera praetextata* (with five thalli) only associates with phylogroup 02 and shares its distributional range. On the contrary, *Peltigera hymenina*, also sharing the same distributional range, associates likewise with phylogroup 43, which has a distributional range of 1841.91 km. Thus, *Peltigera hymenina* distributional range is not limited by its cyanobionts (Appendices S15 and S16). Only one case with enough number of samples was found in which both partners matched their distributional ranges. Moreover, this case showed reciprocal specialization (*Peltigera collina* with *Nostoc* phylogroup 40).

Spatial scale: Beta diversity and partner replacement

We found that most mycobiont species increased their number of partners when increasing the spatial scale following; mostly, a nested turnover scenario when comparing each forest with the regional gradient (Appendix S17). This increase in generalization in larger spatial scales is due to differences in the frequency and the identity of the interactions between different forests (Appendices S6 and S18). Only four species (*Peltigera collina*, *Pseudocyphellaria bartlettii*, *Pseudocyphellaria mallota* and *Sticta fuliginosa*) were found to show an exclusive pattern of no turnover and, thus, maintaining their specialization pattern across spatial scales. The resting 33 species toggled between no turnover (15 observations) to nested turnover (98 observations) between each forest and the regional scale.

Discussion

Our study highlights the varying levels of specialization in epiphytic lichen communities along a wide latitudinal gradient in Chile. Environmental factors may influence specialization by determining the composition and availability of *Nostoc* communities in the studied forests, therefore conditioning the potential interactions. However, a decrease in *Nostoc* phylogroup richness was not seen towards the south, suggesting that specialization does not seem to be influenced by a lower availability of potential partners in the Southernmost part of the gradient. Moreover, we did not find a relation between the distributional range of lichen species and the distributional range of their cyanobionts. Specialization depends on the analysed index and the spatial scale considered. The functional contribution of the cyanobiont to the lichen association influenced specialization measured as cyanobiont richness and Simpson's index, but with the d′-index, the mycobiont identity was the factor explaining most of the variance. On the contrary, the specification of the spatial scale is essential as specialization patterns changed in most species when considering each forest separately (local scale) or the whole latitudinal gradient (regional scale), showing a tendency towards generalism when the spatial scale increased. Differences in the number and identity of cyanobionts associated with lichen species can be assessed by different turnover scenarios, in which *Nostoc* phylogroups are added or replaced between forests. Therefore, considering the relevance of environmental variables influencing *Nostoc* pool composition, mycobionts are expected to establish with locally adapted cyanobionts.

In contrast with what we expected from the latitudinal diversity gradient hypothesis (Hillebrand, 2004), we did not find a latitudinal diversity pattern for *Nostoc* phylogroup richness along the gradient studied. However, in agreement with other studies, environmental drivers influenced *Nostoc* composition on the different forests (Fernández-Mendoza *et al*., 2011; Muggia *et al*., 2014; Muggia *et al*., 2013; Nadyeina *et al*., 2014; Vargas Castillo & Beck, 2012). Our results showed that temperature-related variables (bio06 and bio01) explained most of the observed composition of *Nostoc* phylogroups along the gradient, followed by the DBH, which informs about the forest structure. These results suggest that differences in cyanobiont pools found along the gradient could be due to environmental filtering leading to the prevalence of better locally

adapted phylogroups to these environmental conditions (Batstone *et al*., 2018; Nelsen *et al*., 2021; Rolshausen *et al*., 2020). In accordance with previous studies, this fact (i.e., establishing an interaction with locally adapted phylogroups) may benefit the fitness of the association (Batstone *et al*., 2018; Magain *et al*., 2017; Thompson, 2005). However, these assumptions should be tested experimentally through physiological or demographical approaches (e.g., photosynthesis performance or growth rate under different environmental conditions of the same mycobiont associating with different partners, among others). Nonetheless, the factors determining the composition of the *Nostoc* pool may be different in other habitats, such as soil or rocks, as in the current study we focused on epiphytic lichen communities growing on *Nothofagus pumilio*.

Mycobiont species showed variable values of the three metrics used to quantify specialization (cyanobiont richness, Simpson's and d′ index) depending on the forest considered. These changes in the specialization metrics across different forests inform about a dynamic pattern of specialization, in which species can change from a specialized to a more generalized pattern in different localities, probably related to the local adaptation of the partners. As a result, the specialization pattern will differ when considering different spatial scales (local vs. regional). Nonetheless, the factors influencing specialization measures were different depending on the specialization index considered. While the function of the cyanobiont partly explained cyanobiont richness and Simpson's index, mycobiont species identity mostly affected the d′-index. Thus, cephalolichens showed a less specialized pattern (higher number of interacting cyanobionts and lower values of Simpson's index) than cyanolichens. Little is known about specialization in cyanolichens and cephalolichens, and in contrast to our results, some studies have found either a high specialization in cephalolichens (Paulsrud *et al*., 2001) or no differences between both (Hoz *et al*., 2018; Wirtz *et al*., 2003). Differences with previous studies may be due to the different sampling designs, as no other study has considered the whole lichen community as we do here or may be due to the lower number of cephalolichens species found in other regions (i.e., Europe). The lower specialization found in cephalolichens may be related to the relative importance of the cyanobiont to fulfil the requirements in the lichen symbiosis in bi-and tripartite lichens (Palmqvist, 2002; Rai, 2002). In cyanolichens, the cyanobionts have the photosynthetic

function, and the interaction is obliged to result in the lichen symbiosis; meanwhile, in cephalolichens, the main photosynthetic partner is a green alga and the cyanobiont's main function is nitrogen fixation (Paulsrud *et al*., 2001; Rai *et al*., 2000). This is translated in a smaller dependency for the cyanobiont in cephalolichens, explaining their lower specialization. On the contrary, the d'-index is influenced by mycobiont species identity, being the main factor conditioning the interactions. Previous studies (Dal Grande *et al*., 2018; Fedrowitz *et al*., 2011; Jüriado *et al*., 2019; Leavitt *et al*., 2015) also found that the mycobiont identity determined specialization with little influence of the environment or other factors. Thus, considering the whole *Nostoc* pool, the association with more abundant (opportunism) or rare phylogroups is determined by the fungus identity, which may be genetically constrained. Nonetheless, we observed a tendency in which those cephalolichens establishing with a larger number of partners, interacted with rather rare cyanobionts, showing higher values of the d'-index. Thus, increasing the flexibility to associate with higher number of partners could allow interacting with low abundant partners in the community. Environmental drivers did not explain any of the specialization metrics directly, but as previously described, may influence specialization through their effect in the composition and potential availability of the *Nostoc* community within a forest. This may be related to the small variation in the temperature-related variables along the gradient.

Surprisingly and contrary to previous studies (Cao *et al*., 2015; Dal Grande *et al*., 2014, 2018; Fedrowitz *et al*., 2011, 2012; Otálora *et al*., 2010), lichens' reproductive mode did not drive specialization in the studied communities. Cyanobint switching or turnover in asexually reproducing lichens could be the explanation of the lack of relationship between the reproductive mode and specialization. This cyanobiont switching would lead to a shift from a vertical transmission of the cyanobiont (in which both symbionts are dispersed together) to a horizontal transmission by replacement of the previous cyanobiont (Ertz *et al*., 2018; Ohmura *et al*., 2019; Piercey-Normore & DePriest, 2001; Rolshausen *et al*., 2018; Vidal-Russell & Messuti, 2017). As a result, in both reproductive modes (sexual and asexual), the cyanobiont can be newly acquired (horizontally transmitted), explaining the absence of a differential specialization pattern between sexual and asexually reproducing lichens. These findings are in agreement with the

observed changing pools of *Nostoc* cyanobionts along the gradient as mycobionts can switch to a locally adapted cyanobiont. This might contribute to different geographic mosaics of symbiotic interactions (Fedrowitz *et al*., 2012; Magain *et al*., 2017; Thompson, 2005).

When considering the distribution of the lichen, we found that lichens with wide distributional ranges, could either belong to mycobiont species that associate with a high number of *Nostoc* phylogroups in the entire gradient by replacing their partners along their distributional range, or with a low number of widely distributed *Nostoc* phylogroups, as phylogroup 38 (Magain *et al*., 2017; Rolshausen *et al*., 2020; Ventre Lespiaucq *et al*., 2021). Thus, cyanobiont switches to locally adapted partners could be a mechanism to increase the geographic and ecological niche of a given lichen species (Fernández-Mendoza *et al*., 2011; Muggia *et al*., 2014; Peksa & Škaloud, 2011). However, partner availability is not necessarily the unique limiting factor for lichens' distributions and other variables should be taken into account, as several species have narrow distributional ranges, but associate with wide-distributed cyanobionts (e.g., *Pseudocyphellaria mallota*; Lu *et al*., 2018).

Our results highlight the importance of defining the spatial scale in the study of specialization patterns in symbiotic organisms, as other studies have previously shown, in order to understand the relationship between cyanobiont pools and environmental filtering (Rolshausen *et al*., 2018, 2020). As expected, the studied lichen species showed a tendency to increase the number of interacting partners, and a decline of specialization when the spatial scale was increased, instead of maintaining the same number of partners along their distribution range. The decrease in specialization at larger scales is in agreement with studies proposing that partner replacement could occur in order to establish with better adapted partners (e.g., Fernández-Mendoza *et al*., 2011). A transplantation experiment could be useful to test this turnover of partners under different environments (see Williams *et al*., 2017).

Not considering the complete community may limit the consideration of specialization. Most studies are focused on low biological ranks, thus missing the photobiont pool of the local community (e.g., Dal Grande *et al*., 2018; Leavitt *et al*., 2015; Magain *et al*., 2017; Pino-Bodas & Stenroos, 2021; Werth & Sork, 2010). The

consideration of the total community allows to determine the pool of photobionts and their proportions in the community, which are paramount to consider specialization (Blüthgen *et al*., 2006; Vázquez *et al*., 2007). With this information, specialization can be measured not only in terms of the number and frequency of associations (partner richness and Simpson's index, respectively), but also considering the opportunism of those interactions (d' index). The combination of the three metrics offers an integrative approach of the consideration of specialization within its continuum nature. The community context, together with the wide latitudinal gradient studied, provided results that could be extrapolated to other lichen communities from other habitat types, helping and improving our knowledge about specialization in this intimate symbiotic system.

In summary, our study shows how specialization varies within the axis of partner availability in the hypervolume of the niche in lichenized symbiotic associations. Mycobiont species showed a variable number of cyanobionts and different values of Simpson and d' indices. Therefore, the metric used to quantify specialization influences the understanding of specialization in mutualistic interactions. Cyanolichens were more specialized when considering the cyanobiont richness and Simpson index than cephalolichens, probably due to the relative obliged interaction with the cyanobiont to stablish the symbiosis. However, when considering the whole pool of *Nostoc* symbionts when measuring the d'-index for each mycobiont species, is the mycobiont identity what mostly affects the specialization pattern acquired. As well, we found that specialization can be considered differently depending on the spatial scale, with a general pattern of changes in the constancy of associations by adding or replacing cyanobionts between different forests. Because of the relevance of environmental factors shaping the composition of *Nostoc* cyanobiont communities, mycobionts may interact with different cyanobionts across their distributional range, which could enhance the chances of finding better locally adapted phylogroups. Thus, mycobionts distributional ranges are barely affected by cyanobiont availability. As a result, we found a tendency to increase partner richness when increasing the spatial scale, with a dominant nested turnover scenario produced by the addition or loss of some cyanobionts and the maintenance of certain cyanobionts among forests. As a whole, our results encourage to consider the whole community and the relative abundance of available partners, as well as the proportion of

interactions with each partner to be able to quantify specialization more informatively with complementary metrics. Nonetheless, we also promote the importance of considering specialization as a scale-dependent concept.

References

Aigner, P. A. (2001). Optimality modeling and fitness trade offs: when should plants become pollinator specialists? *Oikos*, *95*, 177–184. https://doi.org/10.1034/j.1600-0706.2001.950121.x

Armbruster, W. S. (2006). Evolutionary and ecological perspectives on specialization: from the arctic to the tropics. In *Plant-Pollinator Interactions: From Specialization to Generalization (eds N. Waser & J. Ollerton). University of Chicago Press, Chicago, IL, USA.* (pp. 260–282).

Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, *92*(3), 1720–1738. https://doi.org/10.1111/brv.12305

Bartoń, K. (2020). MuMIn. R package version 1.43.17. Retrieved from https://cran.r-project.org/web/packages/MuMIn/index.html

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Batstone, R. T., Carscadden, K. A., Afkhami, M. E., & Frederickson, M. E. (2018). Using niche breadth theory to explain generalization in mutualisms. *Ecology*, *99*(5), 1039–1050. https://doi.org/10.1002/ecy.2188

Beck, A., Kasalicky, T., & Rambold, G. (2002). Myco-photobiontal selection in a Mediterranean cryptogam community with Fulgensia fulgida. *New Phytologist*, *153*(2), 317–326. https://doi.org/10.1046/j.0028-646X.2001.00315.x

Blaha, J., Baloch, E., & Grube, M. (2006). High photobiont diversity associated with the euryoecious lichen-forming ascomycete Lecanora rupicola (Lecanoraceae, Ascomycota). *Biological Journal of the Linnean Society*, *88*(2), 283–293. https://doi.org/10.1111/j.1095-8312.2006.00640.x

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*. https://doi.org/10.1186/1472-6785-6-9

Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., & Blüthgen, N. (2007). Specialization, Constraints, and Conflicting Interests in Mutualistic Networks. *Current Biology*, *17*(4), 341–346. https://doi.org/10.1016/j.cub.2006.12.039

Boucher, D. H., James, S., & Keeler, K. H. (1982). The ecology of mutualism. *Annual Review of Ecology and Systematics*, *13*, 315–347.

Cao, S., Zhang, F., Liu, C., Hao, Z., Tian, Y., Zhu, L., & Zhou, Q. (2015). Distribution patterns of haplotypes for symbionts from Umbilicaria esculenta and U. muehlenbergii reflect the

importance of reproductive strategy in shaping population genetic structure. *BMC Microbiology*, *15*(1). https://doi.org/10.1186/s12866-015-0527-0

Carboni, M., Zelený, D., & Acosta, A. T. R. (2016). Measuring ecological specialization along a natural stress gradient using a set of complementary niche breadth indices. *Journal of Vegetation Science*, *27*(5), 892–903. https://doi.org/10.1111/jvs.12413

Cardoso, P., Rigal, F., & Carvalho, J. C. (2015). BAT - Biodiversity Assessment Tools, an R package for the measurement and estimation of alpha and beta taxon, phylogenetic and functional diversity. *Methods in Ecology and Evolution*, *6*(2), 232–236. https://doi.org/10.1111/2041-210X.12310

Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., … Wiens, J. J. (2020). Niche Breadth: Causes and Consequences for Ecology, Evolution, and Conservation. *The Quarterly Review of Biology*, *95*(3), 179–214. https://doi.org/10.1086/710388

Carvalho, J. C., Cardoso, P., Borges, P. A. V., Schmera, D., & Podani, J. (2013). Measuring fractions of beta diversity and their relationships to nestedness: A theoretical and empirical comparison of novel approaches. *Oikos*, *122*(6), 825–834. https://doi.org/10.1111/j.1600-0706.2012.20980.x

Chagnon, P.-L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2018). Strong specificity and network modularity at a very fine phylogenetic scale in the lichen genus Peltigera. *Oecologia*, *187*(3), 767–782. https://doi.org/10.1007/s00442-018-4159-6

Dal Grande, F., Beck, A., Cornejo, C., Singh, G., Cheenacharoen, S., Nelsen, M. P., & Scheidegger, C. (2014). Molecular phylogeny and symbiotic selectivity of the green algal genus Dictyochloropsis s.l. (Trebouxiophyceae): A polyphyletic and widespread group forming photobiont-mediated guilds in the lichen family Lobariaceae. *New Phytologist*, *202*(2), 455–470. https://doi.org/10.1111/nph.12678

Dal Grande, F., Rolshausen, G., Divakar, P. K., Crespo, A., Otte, J., Schleuning, M., & Schmitt, I. (2018). Environment and host identity structure communities of green algal symbionts in lichens. *New Phytologist*, *217*(1), 277–289. https://doi.org/10.1111/nph.14770

De Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E., & Giraud, T. (2013). Cospeciation vs host-shift speciation: Methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist*, *198*(2), 347–385. https://doi.org/10.1111/nph.12150

Degelius, G. (1974). The lichen genus Collema with special reference to the extra-European species. *Symbolae Botanicae Upsalienses*, *20*(2), 1–215.

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., … Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, *47*(1), 15–25. https://doi.org/10.1111/j.1365-2664.2009.01744.x

Dormann, C. F. (2011). How to be a specialist ? Quantifying specialisation in pollination networks, *1*(1), 1–20.

Dormann, C. F., Gruber, B., & Fründ, J. (2008). Introducing the bipartite Package: Analysing Ecological Networks. *R News*, *8*(2), 8–11. Retrieved from http://cran.r-project.org/doc/Rnews/

Ellis, C. J. (2012). Lichen epiphyte diversity: A species, community and trait-based review. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*(2), 131–152. https://doi.org/10.1016/j.ppees.2011.10.001

Elvebakk, A. (2007). The panaustral lichen Pannaria sphinctrina (Mont.) Tuck. and the related new species P-lobulifera from New Caledonia. *Cryptogamie Mycologie*, *28*(3), 225–235.

Elvebakk, A. (2013). Pannaria minutiphylla and P. pulverulacea, two new and common, austral species, previously interpreted as Pannaria microphyllizans (Nyl.) P. M. Jørg. *The Lichenologist*, *45*(1), 9–20. https://doi.org/10.1017/S0024282912000679

Elvebakk, A., & Bjerke, J. W. (2005). Pannaria isabellina (Vain.) comb. nov., a remarkable lichen species from Chile. *The Lichenologist*, *37*(1), 47–54. https://doi.org/10.1017/S0024282904014525

Elvebakk, A., Fritt-Rasmussen, J., & Elix, J. A. (2007). The New Zealand lichen Pannaria leproloma (Nyl.) P. M. Jørg. and its panaustral relative P. farinosa nom. nov. *The Lichenologist*, *39*(04), 349–359. https://doi.org/10.1017/S0024282907006913

Elvebakk, A., Robertsen, E. H., Park, C. H., & Hong, S. G. (2010). Psorophorus and Xanthopsoroma, two new genera for yellow-green, corticolous and squamulose lichen species, previously in Psoroma. *The Lichenologist*, *42*(05), 563–585. https://doi.org/10.1017/S0024282910000083

Ertz, D., Guzow-Krzemińska, B., Thor, G., Łubek, A., & Kukwa, M. (2018). Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the Arthoniomycetes. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-23219-3

Fedrowitz, K., Kaasalainen, U., & Rikkinen, J. (2011). Genotype variability of Nostoc symbionts associated with three epiphytic Nephroma species in a boreal forest landscape. *The Bryologist*, *114*(1), 220. https://doi.org/10.1639/0007-2745-114.1.220

Fedrowitz, K., Kaasalainen, U., & Rikkinen, J. (2012). Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. *Ecology and Evolution*, *2*(9), 2291–2303. https://doi.org/10.1002/ece3.343

Fernández-Mendoza, F., Domaschke, S., García, M. A., Jordan, P., Martín, M. P., & Printzen, C. (2011). Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. *Molecular Ecology*, *20*(6), 1208–1232. https://doi.org/10.1111/j.1365-294X.2010.04993.x

Forister, M. L., Dyer, L. A., Singer, M. S., Stireman III, J. O., & Lill, J. T. (2012). Revisiting the evolution of ecological specialization, with emphasis on insect–plant interactions. *Ecology*, *93*(5), 981–991. https://doi.org/10.1890/11-0650.1

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third edit). Thousand Oaks

CA: SAGE Publications Ltd.

Futuyma, D. J., & Moreno, G. (1988). The Evolution of Ecological Specialization. *Annual Review of Ecology and Systematics*, *19*(1988), 207–233. https://doi.org/10.1146/annurev.es.19.110188.001231

Galloway, D. J. (1985). *Flora of New Zealand. Lichens.* (Wellington).

Galloway, D. J. (1992). Studies in Pseudocyphellaria (lichens) III. The South American species. *Bibliotheca Lichenologica*, *46*, 1–275.

Galloway, D. J. (1994). Studies on The Lichen Genus Sticta (Schreber) Ach.: I. Southern South American Species. *The Lichenologist*, *26*(03), 223–282. https://doi.org/10.1006/lich.1994.1019

Galloway, D. J., & Jørgensen, P. M. (1995). The lichen genus Leptogium (Collemataceae) in Southern Chile, South America. *Contributions to Lichenology in Honour of Gerhard Follmann. Bot. Inst. Univ. Cologne*, 227–247.

Goward, T., Goffinet, B., & Vitikainen, O. (1995). Synopsis of the genus Peltigera (lichenized Ascomycetes) in British Columbia, with a key to the North American species. *Canadian Journal of Botany*, *73*(1), 91–111. https://doi.org/10.1139/b95-012

Harrell, F. E., & Dupont, C. (2018). Hmisc: harrell miscellaneous. R package version 4.1-1. Retrieved from https://cran.r-project.org/package=Hmisc

Hartig, F. (2022). DHARMa: Residual diagnostics for hierarchical (multi-level/ mixed) regression models. R package version 0.4.5. Retrieved from https://cran.r-project.org/package=DHARMa

Hawksworth, D. L., & Grube, M. (2020). Lichens redefined as complex ecosystems. *New Phytologist*, *227*(5), 1281–1283. https://doi.org/10.1111/nph.16630

Herrera, P., Suárez, J. P., Sánchez-Rodríguez, A., Molina, M. C., Prieto, M., & Méndez, M. (2019). Many broadly-shared mycobionts characterize mycorrhizal interactions of two coexisting epiphytic orchids in a high elevation tropical forest. *Fungal Ecology*, *39*, 26–36. https://doi.org/10.1016/j.funeco.2018.11.003

Hillebrand, H. (2004). On the generallity of the latutinal diversity gradient. *American Naturalist*, *163*(2), 192–211. https://doi.org/10.1086/381004

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal. Biometrische Zeitschrift*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Hoz, C. J. P. D. La, Magain, N., Lutzoni, F., Goward, T., Restrepo, S., & Miadlikowska, J. (2018). Contrasting Symbiotic Patterns in Two Closely Related Lineages of Trimembered Lichens of the Genus Peltigera. *Frontiers in Microbiology*, *9*, 1–14. https://doi.org/10.3389/fmicb.2018.02770

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hughes, J. B. (2000). The Scale of Resource Specialization and the Distribution and Abundance of Lycaenid. *Oecologia*, *123*(3), 375–383. https://doi.org/10.1007/s004420051024

Hutchinson, G. E. (1957). Concluding remarks. Cold Spring Harbor Symposium. *Quantitative Biology*, *22*, 415–247. https://doi.org/10.1101/SQB.1957.022.01.039

Hutchinson, G. E. (1978). *An Introduction to Population Biology*. *Yale Univ Press, New Haven, CT*.

Iglesias-Prieto, R., Beltrán, V. H., LaJeunesse, T. C., Reyes-Bonilla, H., & Thomé, P. E. (2004). Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1549), 1757–1763. https://doi.org/10.1098/rspb.2004.2757

Irschick, D., Dyer, L., Sherry, T. W., & Ranta, E. (2005). Phylogenetic Methodologies for Studying Specialization. *Oikos*, *110*(2), 404–408. https://doi.org/10.1111/j.0030-1299.2005.13927.x

Jørgensen, P. M. (2000). Survey of the Lichen Family Pannariaceae on the American Continent, North of Mexico. *The Bryologist*, *103*(4), 670–704. https://doi.org/10.1639/0007-2745(2000)103[0670:SOTLFP]2.0.CO;2

Jørgensen, P. M. (2005). A new Atlantic species in Fuscopannaria, with a key to its European species. *Lichenologist*, *37*(3), 221–225. https://doi.org/10.1017/S0024282905014866

Jüriado, I., Kaasalainen, U., Jylhä, M., & Rikkinen, J. (2019). Relationships between mycobiont identity, photobiont specificity and ecological preferences in the lichen genus Peltigera (Ascomycota) in Estonia (northeastern Europe). *Fungal Ecology*, *39*, 45–54. https://doi.org/10.1016/j.funeco.2018.11.005

Kaasalainen, U., Tuovinen, V., Mwachala, G., Pellikka, P., & Rikkinen, J. (2021). Complex Interaction Networks Among Cyanolichens of a Tropical Biodiversity Hotspot. *Frontiers in Microbiology*, *12*(June), 1–12. https://doi.org/10.3389/fmicb.2021.672333

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., … Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, *4*, 1–20. https://doi.org/10.1038/sdata.2017.122

Kinlock, N. L., Prowant, L., Herstoff, E. M., Foley, C. M., Akin-Fajiye, M., Bender, N., … Gurevitch, J. (2018). Explaining global variation in the latitudinal diversity gradient: Meta-analysis confirms known patterns and uncovers new ones. *Global Ecology and Biogeography*, *27*(1), 125–141. https://doi.org/10.1111/geb.12665

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

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

Lu, J., Magain, N., Miadlikowska, J., Coyle, J. R., Truong, C., & Lutzoni, F. (2018). Bioclimatic factors at an intrabiome scale are more limiting than cyanobiont availability for the lichen-forming

genus Peltigera. *American Journal of Botany*, *105*(7), 1198–1211. https://doi.org/10.1002/ajb2.1119

Lücking, R., Lawrey, J. D., Sikaroodi, M., Gillevet, P. M., Chaves, J. L., Sipman, H. J. M., & Bungartz, F. (2009). Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. *American Journal of Botany*, *96*(8), 1409–1418. https://doi.org/10.3732/ajb.0800258

Lücking, R., Moncada, B., McCune, B., Farkas, E., Goffinet, B., Parker, D., … Yakovchenko, L. S. (2017). Pseudocyphellaria crocata (Ascomycota: Lobariaceae) in the Americas is revealed to be thirteen species, and none of them is P. crocata. *The Bryologist*, *120*(4), 441–500. https://doi.org/10.1639/0007-2745-120.4.14

MacArthur, R. H. (1955). Fluctuations of Animal Populations and a Measure of Community Stability. *Ecology*, *36*(3), 533. https://doi.org/10.2307/1929601

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Magurran, A. E. (1988). *Ecological Diversity and Its Measurements*. Princeton, NJ: Princeton University Press. https://doi.org/10.1007/978-94-015-7358-0

Mark, K., Laanisto, L., Bueno, C. G., Niinemets, Ü., Keller, C., & Scheidegger, C. (2020). Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *New Phytologist*, *227*(5), 1362–1375. https://doi.org/10.1111/nph.16475

Martínez, I. (1999). *Taxonomía del género Peltigera Willd. (Ascomycetes Liquenizados) en la península Ibérica y estudio de sus hongos liquenícolas.* Real Jardín Botánico, C.S.I.C.

Matos, P., Pinho, P., Aragón, G., Martínez, I., Nunes, A., Soares, A. M. V. M., & Branquinho, C. (2015). Lichen traits responding to aridity. *Journal of Ecology*, *103*(2), 451–458. https://doi.org/10.1111/1365-2745.12364

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *2010 Gateway Computing Environments Workshop (GCE)* (pp. 1–8). https://doi.org/10.1109/GCE.2010.5676129

Muggia, L., Grube, M., & Tretiach, M. (2008). Genetic diversity and photobiont associations in selected taxa of the Tephromela atra group (Lecanorales, lichenised Ascomycota). *Mycological Progress*, *7*(3), 147–160. https://doi.org/10.1007/s11557-008-0560-6

Muggia, L., Pérez-Ortega, S., Kopun, T., Zellnig, G., & Grube, M. (2014). Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Annals of Botany*, *114*(3), 463–475. https://doi.org/10.1093/aob/mcu146

Muggia, L., Vancurova, L., Škaloud, P., Peksa, O., Wedin, M., & Grube, M. (2013). The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS Microbiology Ecology*, *85*(2), 313–323. https://doi.org/10.1111/1574-6941.12120

Nadyeina, O., Dymytrova, L., Naumovych, A., Postoyalkin, S., Werth, S., Cheenacharoen, S., & Scheidegger, C. (2014). Microclimatic differentiation of gene pools in the Lobaria pulmonaria symbiosis in a primeval forest landscape. *Molecular Ecology*, *23*(21), 5164–5178. https://doi.org/10.1111/mec.12928

Nash, T. H. (1996). *Lichen Biology.* (Cambridge). Cambridge University Press.

Nelsen, M. P., Leavitt, S. D., Heller, K., Muggia, L., & Lumbsch, H. T. (2021). Macroecological diversification and convergence in a clade of keystone symbionts. *FEMS Microbiology Ecology*, *97*(6). https://doi.org/10.1093/femsec/fiab072

O'Brien, A. M., Sawers, R. J. H., Ross-Ibarra, J., & Strauss, S. Y. (2018). Evolutionary responses to conditionality in species interactions across environmental gradients. *American Naturalist*, *192*(6), 715–730. https://doi.org/10.1086/700118

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

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Stevens, M. H. H. (2020). vegan: Community ecology package. R package version 2.5-6. Retrieved from https://cran.r-project.org/web/packages/vegan/index.html

Otálora, M. A. G., Martínez, I., O'Brien, H., Molina, M. C., Aragón, G., & Lutzoni, F. (2010). Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution*, *56*(3), 1089–1095. https://doi.org/10.1016/j.ympev.2010.05.013

Palmqvist, K. (2002). Cyanolichens: Carbon Metabolism. In Amar N Rai, B. Bergman, & U. Rasmussen (Eds.), *Cyanobacteria in Symbiosis* (pp. 73–96). Dordrecht: Springer Netherlands. https://doi.org/10.1007/0-306-48005-0_5

Passo, A., & Calvelo, S. (2006). New reports and combinations in the family Pannariaceae (Lecanorales, lichenized Ascomycota). *The Lichenologist*, *38*(06), 549–555. https://doi.org/10.1017/S0024282906005688

Passo, A., & Calvelo, S. (2011). Pannaria byssoidea (Pannariaceae), a new squamulose species from southern South America. *The Bryologist*, *114*(4), 756–763. https://doi.org/10.1639/0007-2745-114.4.756

Passo, A., Calvelo, S., & Stocker-Wörgötter, E. (2004). Taxonomic notes on Pannaria pallida from Southern South America and New Zealand. *Mycotaxon (USA)*, *90*, 55–65.

Paulsrud, P., Rikkinen, J., & Lindblad, P. (2001). Field investigations on cyanobacterial specificity in Peltigera aphthosa. *New Phytologist*, *152*(1), 117–123. https://doi.org/10.1046/j.0028-646x.2001.00234.x

Peay, K. G. (2016). The Mutualistic Niche: Mycorrhizal Symbiosis and Community Dynamics. *Annual Review of Ecology, Evolution, and Systematics*, *47*, 143–164. https://doi.org/10.1146/annurev-ecolsys-121415-032100

Peksa, O., & Škaloud, P. (2011). Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae). *Molecular Ecology*, *20*(18), 3936–3948. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Piercey-Normore, M. D., & Deduke, C. (2011). Fungal farmers or algal escorts: Lichen adaptation from the algal perspective. *Molecular Ecology*, *20*(18), 3708–3710. https://doi.org/10.1111/j.1365-294X.2011.05191.x

Piercey-Normore, M. D., & DePriest, P. T. (2001). Algal switching among lichen symbioses. *American Journal of Botany*, *88*(8), 1490–1498. https://doi.org/10.2307/3558457

Pino-Bodas, R., & Stenroos, S. (2021). Global Biodiversity Patterns of the Photobionts Associated with the Genus Cladonia (Lecanorales , Ascomycota). *Microbial Ecology*, *82*, 173–187. https://doi.org/10.1007/s00248-020-01633-3

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, *21*(2), 609–620. https://doi.org/10.1111/1755-0998.13281

R Core Team. (2021). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.r-project.org/

Rai, A. N. (2002). Cyanolichens: Nitrogen Metabolism. In Amar N Rai, B. Bergman, & U. Rasmussen (Eds.), *Cyanobacteria in Symbiosis* (pp. 97–115). Dordrecht: Springer Netherlands. https://doi.org/10.1007/0-306-48005-0_6

Rai, A. N., Söderbäck, E., & Bergman, B. (2000). Tansley Review No. 116. Cyanobacterium–plant symbioses. *New Phytologist*, *147*(3), 449–481. https://doi.org/10.1046/j.1469-8137.2000.00720.x

Revelle, W. (2021). psych: Procedures for psychological, psychometric, and personality research. R package version 2.1.9. Northwestern University. Retrieved from https://cran.r-project.org/package=psych

Rikkinen, J. (2003). Ecological and evolutionary role of photobiont-mediated guilds in lichens. *Symbiosis*, *34*(2), 99–110.

Rikkinen, J. (2015). Cyanolichens. *Biodiversity and Conservation*, *24*(4), 973–993. https://doi.org/10.1007/s10531-015-0906-8

Rodríguez-Arribas, C., Martínez, I., Aragón, G., Zamorano-Elgueta, C., Cavieres, L., & Prieto, M. (2023). Data from: Specialization patterns in symbiotic associations: a community perspective over spatial scales. https://doi.org/10.5281/zenodo.7674732

Rodríguez, F., Oliver, J. L., Marín, A., & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, *142*(4), 485–501. https://doi.org/10.1016/S0022-5193(05)80104-3

Rolshausen, G., Dal Grande, F., Sadowska-Deś, A. D., Otte, J., & Schmitt, I. (2018). Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions. *Ecography*, *41*(8), 1380–1392. https://doi.org/10.1111/ecog.03457

Rolshausen, G., Hallman, U., Grande, F. D., Otte, J., Knudsen, K., & Schmitt, I. (2020). Expanding

the mutualistic niche: Parallel symbiont turnover along climatic gradients. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1924). https://doi.org/10.1098/rspb.2019.2311

Rudi, K., Skulberg, O. M., & Jakobsen, K. S. (1998). Evolution of cyanobacteria by exchange of genetic material among phyletically related strains. *Journal of Bacteriology*, *180*(13), 3453–3461. https://doi.org/10.1128/jb.180.13.3453-3461.1998

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Schluter, D. (2000). Ecological Character Displacement in Adaptive Radiation. *The American Naturalist*, *156*(S4), 4–16. https://doi.org/10.1086/303412

Simpson, E. H. (1949). Measurement of Diversity. *Nature*, *163*(4148), 688. https://doi.org/10.1038/163688a0

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Suz, L. M., Barsoum, N., Benham, S., Dietrich, H. P., Fetzer, K. D., Fischer, R., … Bidartondo, M. I. (2014). Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests. *Molecular Ecology*, *23*(22), 5628–5644. https://doi.org/10.1111/mec.12947

Thompson, J. N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, *153*(SUPPL.). https://doi.org/10.1086/303208

Thompson, J. N. (2005). *The geographic mosaic of coevolution.* University of Chicago Press.

Torres-Martínez, L., Porter, S. S., Wendlandt, C., Purcell, J., Ortiz-Barbosa, G., Rothschild, J., … Sachs, J. L. (2021). Evolution of specialization in a plant-microbial mutualism is explained by the oscillation theory of speciation. *Evolution*, *75*(5), 1070–1086. https://doi.org/10.1111/evo.14222

Vargas Castillo, R., & Beck, A. (2012). Photobiont selectivity and specificity in Caloplaca species in a fog-induced community in the Atacama Desert, northern Chile. *Fungal Biology*, *116*(6), 665–676. https://doi.org/10.1016/j.funbio.2012.04.001

Vázquez, D. P., J. Melián, C., M. Williams, N., Blüthgen, N., R. Krasnov, B., & Poulin, R. (2007). Species abundance and asymmetric interaction strength in ecological networks. *Oikos*, *116*(7), 1120–1127. https://doi.org/10.1111/j.2007.0030-1299.15828.x

Vázquez, D. P., Poulin, R., Krasnov, B. R., & Shenbrot, G. I. (2005). Species abundance and the distribution of specialization in host-parasite interaction networks. *Journal of Animal Ecology*, *74*(5), 946–955. https://doi.org/10.1111/j.1365-2656.2005.00992.x

Ventre Lespiaucq, A., Jacquemyn, H., Rasmussen, H. N., & Méndez, M. (2021). Temporal turnover in mycorrhizal interactions: a proof of concept with orchids. *New Phytologist*, *230*(5), 1690–1699. https://doi.org/10.1111/nph.17291

Vidal-Russell, R., & Messuti, M. I. (2017). Phylogenetic signal of photobiont switches in the lichen genus Pseudocyphellaria s. l. follows a Brownian motion model. *Symbiosis*, *72*(3), 215–223.

https://doi.org/10.1007/s13199-016-0458-z

Vitikainen, O. (1994). Notes on some Peltigera of the Neotropics. *Acta Botanica Fennica*, *150*, 217–221.

Werth, S., & Sork, V. L. (2010). Identity and genetic structure of the photobiont of the epiphytic lichen Ramalina menziesii on three oak species in southern California. *American Journal of Botany*, *97*(5), 821–830. https://doi.org/10.3732/ajb.0900276

White, F. J., & James, P. W. (1988). Studies on the Genus Nephroma II. The Southern Temperate Species. *The Lichenologist*, *20*(02), 103–166. https://doi.org/10.1017/S0024282988000167

Williams, L., Colesie, C., Ullmann, A., Westberg, M., Wedin, M., & Büdel, B. (2017). Lichen acclimation to changing environments: Photobiont switching vs. climate-specific uniqueness in Psora decipiens. *Ecology and Evolution*, *7*(8), 2560–2574. https://doi.org/10.1002/ece3.2809

Wilson, P., & Thomson, J. D. (1996). How do flowers diverge? In *Floral Biology (eds D.G. Lloyd & S.C.H. Barrett). Chapman and Hall, New York, NY, USA.* (pp. 88–111).

Wirtz, N., Lumbsch, H. T., Green, T. G. A., Türk, R., Pintado, A., Sancho, L., & Schroeter, B. (2003). Lichen fungi have low cyanobiont selectivity in maritime Antarctica. *New Phytologist*, *160*(1), 177–183. https://doi.org/10.1046/j.1469-8137.2003.00859.x

Yahr, R., Vilgalys, R., & Depriest, P. T. (2006). Geographic variation in algal partners of Cladonia subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytologist*, *171*(4), 847–860. https://doi.org/10.1111/j.1469-8137.2006.01792.x

Supporting information

CHAPTER 1

Specialization patterns in symbiotic associations: a community perspective over spatial scales.

Clara Rodríguez-Arribas, Isabel Martínez, Gregorio Aragón, Carlos Zamorano-Elgueta, Lohengrin Cavieres and María Prieto

Manuscript published in *Ecology and Evolution* 2023

Appendix S1. Environmental variables: 1) Geographic variables: Latitude and Longitude (º), 2) Forest structure and habitat quality related variables: Altitude (mlsa), Orientation (º), Inclination (º), DBH (m) and canopy cover (%), 3)Climate related variables extracted from CHELSA climate database (Karger *et al*. 2017): Annual Mean Temperature (bio1), Mean Diurnal Range (BIO2), Isothermality (BIO3), Temperature Seasonality (BIO4), Max Temperature of Warmest Month (BIO5), Min Temperature of Coldest Month (BIO6), Temperature Annual Range (BIO7), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Mean Temperature of Warmest Quarter (bio10), Mean Temperature of Coldest Quarter (bio11), Annual Precipitation (bio12), Precipitation of Wettest Month (bio13), Precipitation of Driest Month (bio14), Precipitation Seasonality (bio15), Precipitation of Wettest Quarter (bio16), Precipitation of Driest Quarter (bio17), Precipitation of Warmest Quarter (bio18), and Precipitation of Coldest Quarter (bio19). Temperature in ºC, precipitation in mm.

Sampling sites: 1) Conguillío National Park (COB1 and COB2), 2) Puyehue National Park (PUYB1 and PUYB2), 3) Hornopirén National Park (HORB1), 4) Cerro Castillo National Park (KKB1 and KKB2), 5) Torres del Paine National Park (TPB1), 6) Magallanes National Reserve (RMB1) and 7) Navarino Island (PWB1 and PWB2).

	GEOGRAPHIC VARIABLES		FOREST STRUCTURE AND HABITAT QUALITY VARIABLES					CLIMATE																		
								TEMPERATURE RELATED VARIABLES											PRECIPITATION RELATED VARIABLES								
BOSQUE	Longitude	Latitude	Altitude	Orientation	Inclination	DBH	Canopy	bio01	bio02	bio03	bio04	bio05	bio06	bio07	bio08	bio09	bio10	bio11	bio12	bio13	bio14	bio15	bio16	bio17	bio18	bio19
COB1	-71.7	-38.6	1373.2	117.2	11	1.92	77.60	6.1	9.1	40	438.9	19.2	-3.5	22.7	1.6	12.5	12.5	0.4	1611	271	36	60	788	114	114	767
COB2	-71.6	-38.6	1487.2	185.6	18.3	1.49	84.00	6	9.1	39.9	443.2	19.1	-3.7	22.8	1.4	12.4	12.4	0.2	1459	247	34	60	718	107	107	685
PUYB1	-72.2	-40.8	978.4	75.6	9	1.21	69.50	7	7.2	37.9	380.4	17.8	-1.2	19.1	3.1	12.5	12.5	2	2370	323	96	43	965	292	292	931
PUYB2	-72.2	-40.8	1237.6	288	28.7	1.25	73.40	5.9	7.2	38	381	16.8	-2.3	19.1	2	11.4	11.4	0.9	2388	320	103	41	958	312	312	923
HORB1	-72.34	-41.84	913	64.8	12.8	1.15	71.72	6.7	6.1	36.7	336.4	16	-0.5	16.5	3.4	10.2	11.6	2.3	1680	226	86	35	664	270	288	638
KKB1	-72.0	-46.1	1092.6	196.4	16	1.41	86.00	2.9	5.8	34.5	353.9	11.9	-5	16.9	-2.1	7.8	7.8	-2.1	778	117	31	47	321	100	100	321
KKB2	-72.2	-46.1	857.2	119.2	17.1	1.43	85.60	5.1	5.8	34.5	349.3	14	-2.8	16.8	0.2	10	10	0.2	722	104	32	42	282	103	103	282
TPB1	-73.2	-51.1	340.4	142.6	14.8	1.45	84.80	5.1	4.7	33.9	315.6	12	-1.8	13.8	3.8	6.4	9.2	0.5	853	98	51	20	284	163	164	232
RMB1	-71.0	-53.1	387	189.6	8.9	1.35	86.50	4.2	4.3	34	297.6	10.7	-2	12.7	3	3.7	8.2	0	444	47	27	17	140	86	112	96
PWB1	-67.7	-55.0	294	288.8	17.4	1.29	85.00	4.9	4.1	35.2	268.9	11	-0.7	11.7	8.5	5.9	8.6	1.2	528	53	34	14	156	104	139	119
PWB2	-67.6	-55.0	407	328.4	25.6	1.20	81.50	4.4	4.1	35.2	269.5	10.4	-1.2	11.7	7.9	5.4	8	0.6	648	68	42	15	196	129	178	138

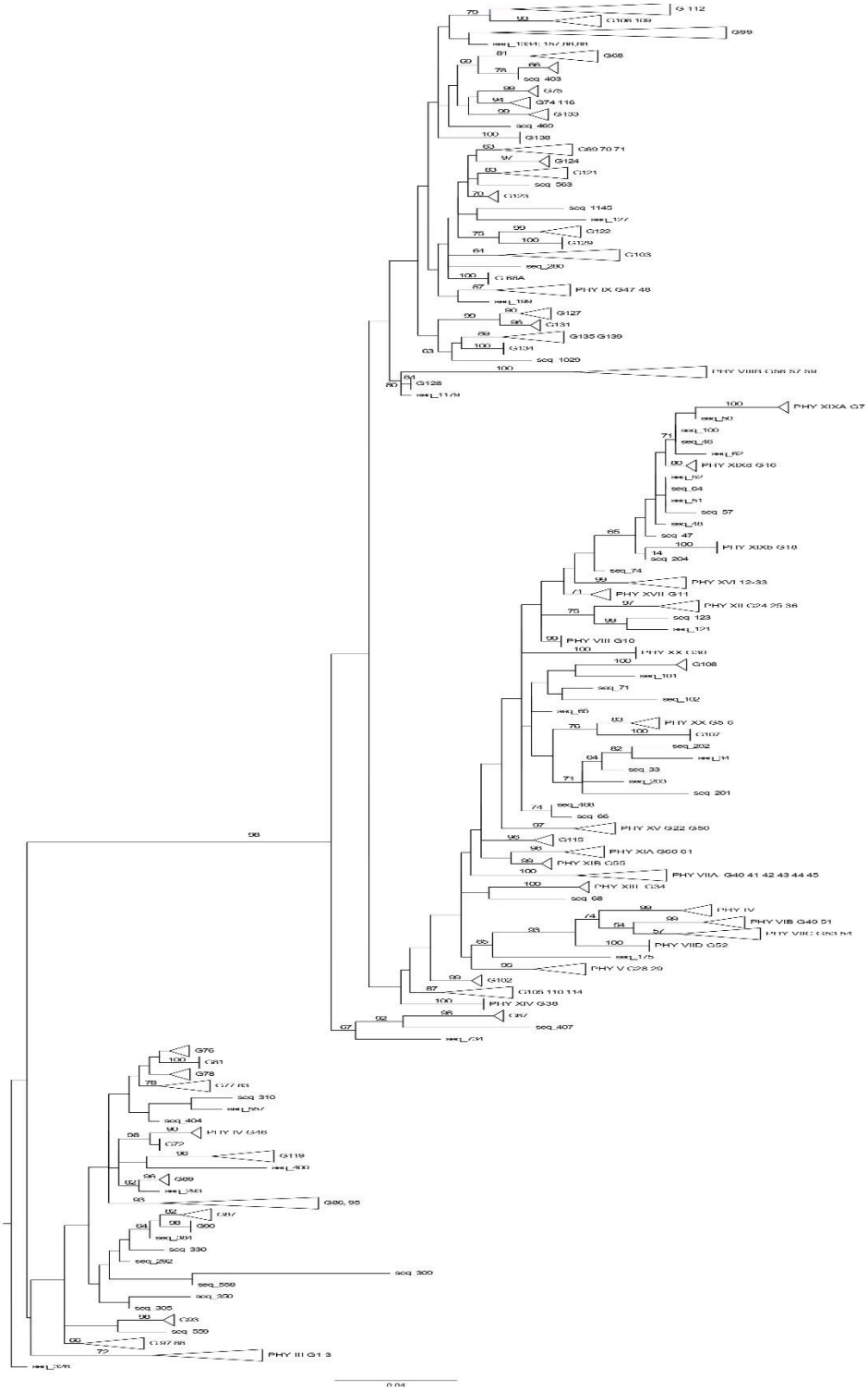
Appendix S2. Lichen species found along the gradient with the number of cyanobiont sequences per species (N sequences), the number of forest (N forests) in which the species is present and the number of interacting phylogroups (partner richness).

Specie	N sequences	N forests	Partner richness
Collema flaccidum (Ach.) Ach.	18	7	7
Collema glaucophthalmum Nyl.	4	1	3
Crocodia guilleminii (Mont.) Nyl.	26	6	4
Cyanisticta obvoluta (Sw.) C.W. Dodge	37	8	6
Fuscopannaria mediterranea (Tav.) P.M. Jørg.	4	1	1
Fuscopannaria minor (Darb.) P.M. Jørg.	13	5	3
Fuscopannaria sp. *1*	3	2	2
Leciophysma sp. *1*	1	1	1
Leptogium aff. *tenuissimum*	7	1	6
Leptogium azureum (Sw.) Mont.	3	2	2
Leptogium cochleatum (Dicks.) P.M. Jørg. & P. James	1	1	1
Leptogium decipiens P.M. Jørg.	15	5	5
Leptogium laceroides B. de Lesd.	9	3	3
Leptogium menziesii (Sm.) Mont.	6	3	2
Leptogium patagonicum Zahlbr.	5	1	2
Leptogium sp. *1*	1	1	1
Leptogium valdivianum M. Lindstr.	12	4	4
Nephroma analogicum Nyl. *(+ N. chubutense* I.M. Lamb*)*	11	3	6
Nephroma antarcticum (Wulfen) Nyl.	61	11	15
Nephroma cellulosum (Ach.) Ach.	49	10	3
Nephroma kuehnemannii I.M. Lamb *(+ N. microphyllum* Henssen*)*	2	2	2
Nephroma parile (Ach.) Ach.	28	4	3
Nephroma plumbeum (Mont.) Mont.	2	2	2
Nephroma pseudoparile (Räsänen) Zahlbr.	4	2	1
Nephroma skottsbergii F.J. White & P. James *(+ N. papillosum* F.J. White & P. James*)*	20	4	7
Pannaria aff. *implexa*	5	1	2
Pannaria aff. *patagonica*	2	1	1
Pannaria arthroophylla (Stirt.) Elvebakk & D.J.Galloway	3	1	2
Pannaria byssoidea Passo & Calvelo	7	1	2
Pannaria contorta (Müll. Arg.) Passo & Calvelo	1	1	1
Pannaria farinose Elvebakk & Fritt-Rasm	23	5	4
Pannaria gr. *sphinctrina*	20	5	6
Pannaria pallida (Nyl.) Hue	24	8	3
Pannaria pulverulacea Elvebakk	20	7	8
Pannaria sp. *1*	2	1	1
Parmeliella nigrata (Müll. Arg.) P.M. Jørg. & D.J. Galloway	4	3	2
Parmeliella nigrocinta (Mont.) Müll. Arg.	18	6	3

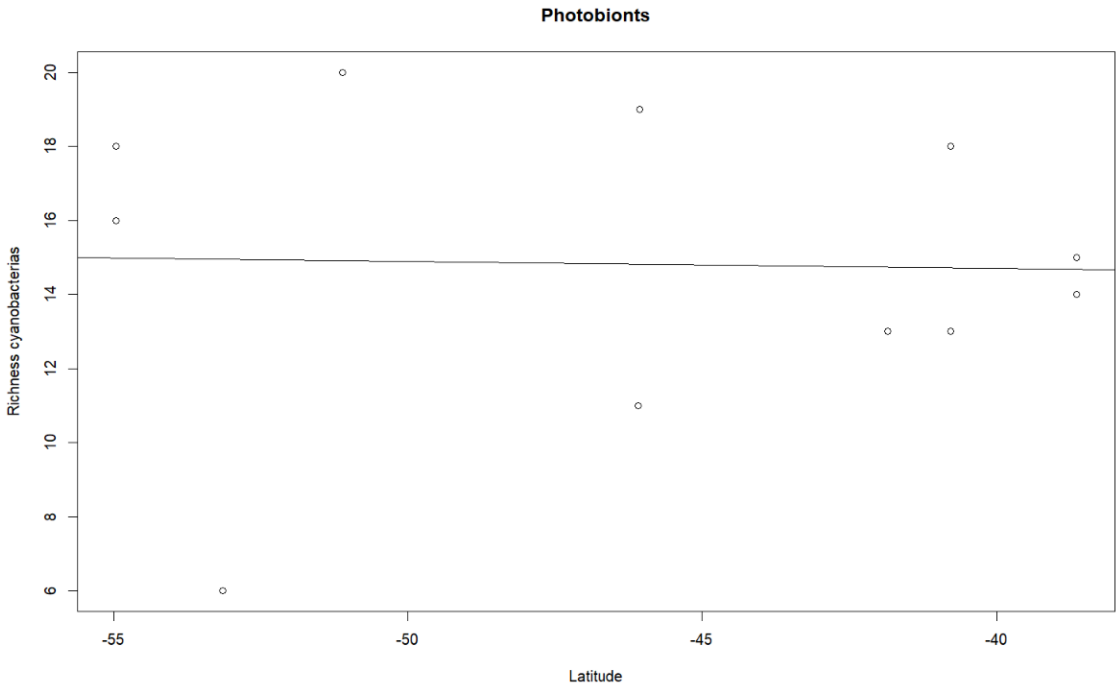
Parmeliella sp. *1*	1	1	1
Phormopsora isabellina (Vain.) Elvebakk, S.G. Hong & C.H. Park	1	1	1
Peltigera canina (L.) Willd.	6	2	2
Peltigera collina (Ach.) Schrad.	20	3	1
Peltigera degenii Gyeln.	2	1	1
Peltigera hymenina (Ach.) Delise	20	5	2
Peltigera membranacea (Ach.) Nyl.	8	3	1
Peltigera polydactylon (Neck.) Hoffm.	5	3	1
Peltigera praetextata (Flörke ex Sommerf.) Zopf	4	4	1
Peltigera rufescens (Weiss) Humb.	4	1	1
Peltigera sp. *1*	2	1	1
Podostictina berberina (G. Forst.) B. Moncada & Lücking	3	3	1
Podostictina encoensis (R. Sant.) D.J. Galloway & de Lange	1	1	1
Podostictina flavicans (Hook. f. & Taylor) B. Moncada & Lücking	8	3	3
Pseudocyphellaria bartlettii D.J. Galloway	10	3	1
Pseudocyphellaria coppinsii D.J. Galloway	7	2	3
Pseudocyphellaria divulsa (Taylor) Imshaug	1	1	1
Pseudocyphellaria dubia Du Rietz	23	4	2
Pseudocyphellaria faveolate (Delise) Malme	17	5	3
Pseudocyphellaria freycinetti (Delise) Malme	15	3	8
Pseudocyphellaria gilva (Ach.) Malme	34	6	3
Pseudocyphellaria glabra (Hook. f. & Taylor) C.W. Dodge	21	5	4
Pseudocyphellaria gr. *argyracea*	58	10	2
Pseudocyphellaria gr. *citrina*	67	11	3
Pseudocyphellaria gr. *vaccina*	36	7	13
Pseudocyphellaria granulate (C. Bab.) Malme	45	8	7
Pseudocyphellaria hirsuta (Mont.) Malme	40	9	5
Pseudocyphellaria intricata (Delise) Vain.	22	3	3
Pseudocyphellaria lechleri (Müll. Arg.) Du Rietz	32	6	2
Pseudocyphellaria mallota (Tuck.) H. Magn.	12	4	2
Pseudocyphellaria norvegica (Gyeln.) P. James	13	5	4
Pseudocyphellaria nudata (Zahlbr.) D.J. Galloway	2	1	1
Pseudocyphellaria piloselloides (Räsänen) H. Magn.	6	6	1
Pseudocyphellaria scabrosa (R. Sant.) D.J. Galloway & de Lange	34	7	3
Pseudocyphellaria sp. *1*	1	1	1
Pseudocyphellaria valdiviana (Nyl.) Follmann	7	2	4
Pseudocyphellaria wandae D.J. Galloway	1	1	1
Psoroma asperellum Nyl.	11	3	7
Psoroma hirsutulum Nyl.	2	2	2
Psoroma hypnorum (Vahl) Gray	3	1	2
Psoroma polychidioides (Zahlbr.) P.M. Jørg.	3	2	1
Psorophorus pholidotus (Mont.) Elvebakk & S.G. Hong	1	1	1
Sticta ainoae D.J. Galloway & J. Pickering	1	1	1
Sticta caulescens De Not.	5	1	1
Sticta fuliginosa (With.) Ach.	12	4	1
Sticta gr. *sublimbata*	5	3	3

Sticta hypochra Vain.	15	5	2
Xanthopsoroma contextum (Stirt.) Elvebakk & S.G. Hong	1	1	1
Xanthopsoroma soccatum (R. Br. ex Cromb.) Elvebakk	1	1	1

Appendix S3. Best tree obtained from Maximum likelihood analysis (with RAxML) with the definition of phylogroups. Bootstrap values are shown in the nodes. PHY: phylogroups defined by Magain *et al*. (2017), G: groups defined by ASAP, seq: name of sequences belonging to different phylogroups.



Appendix S4. Photobiont richness along the latitudinal gradient studied (R^2 =-0.11; P = 0.93).

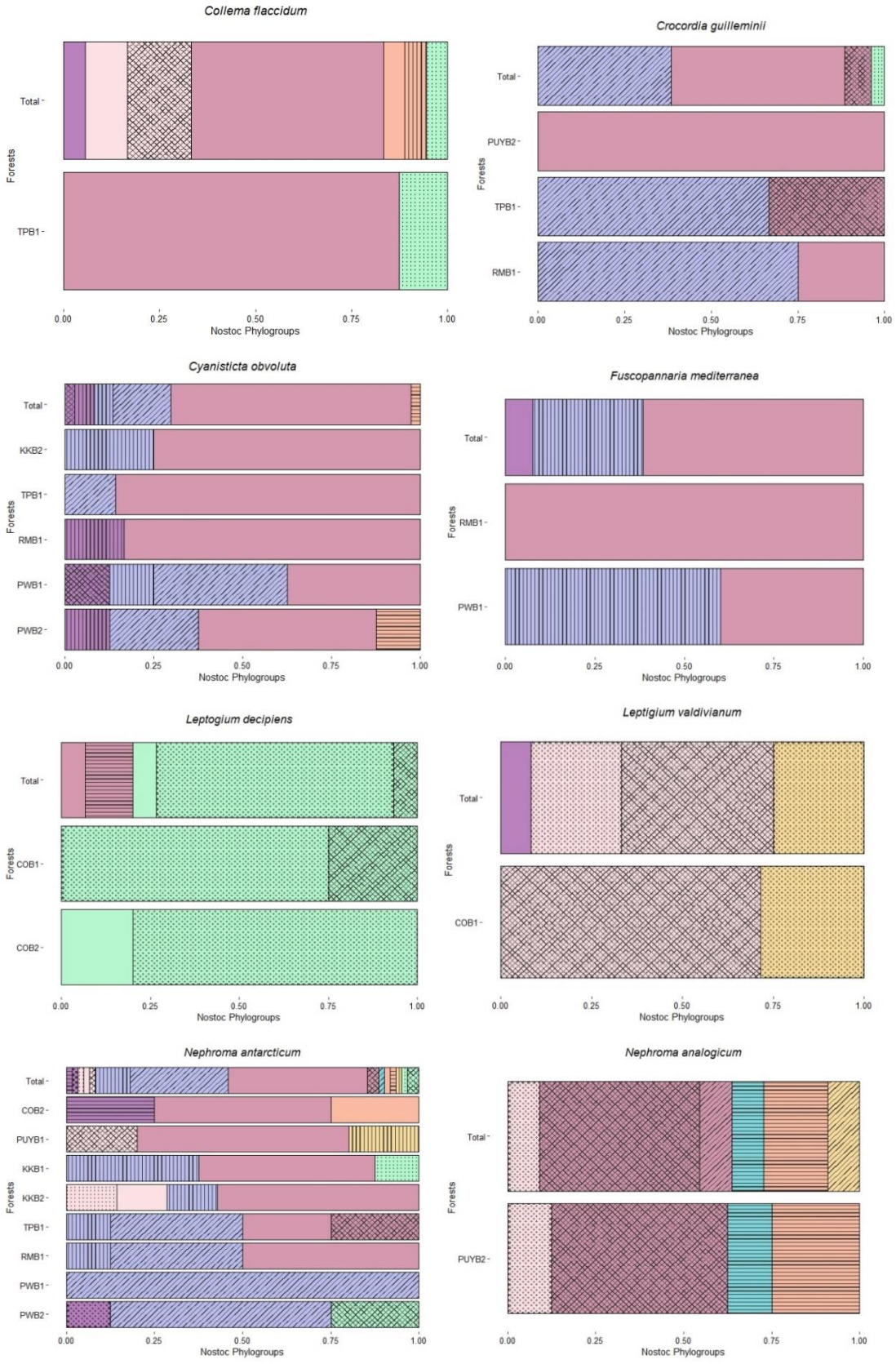


Appendix S5 *Nostoc* phylogroups, number of sequences of each phylogroups and percentage of the different phylogroups in the whole latitudinal gradient.

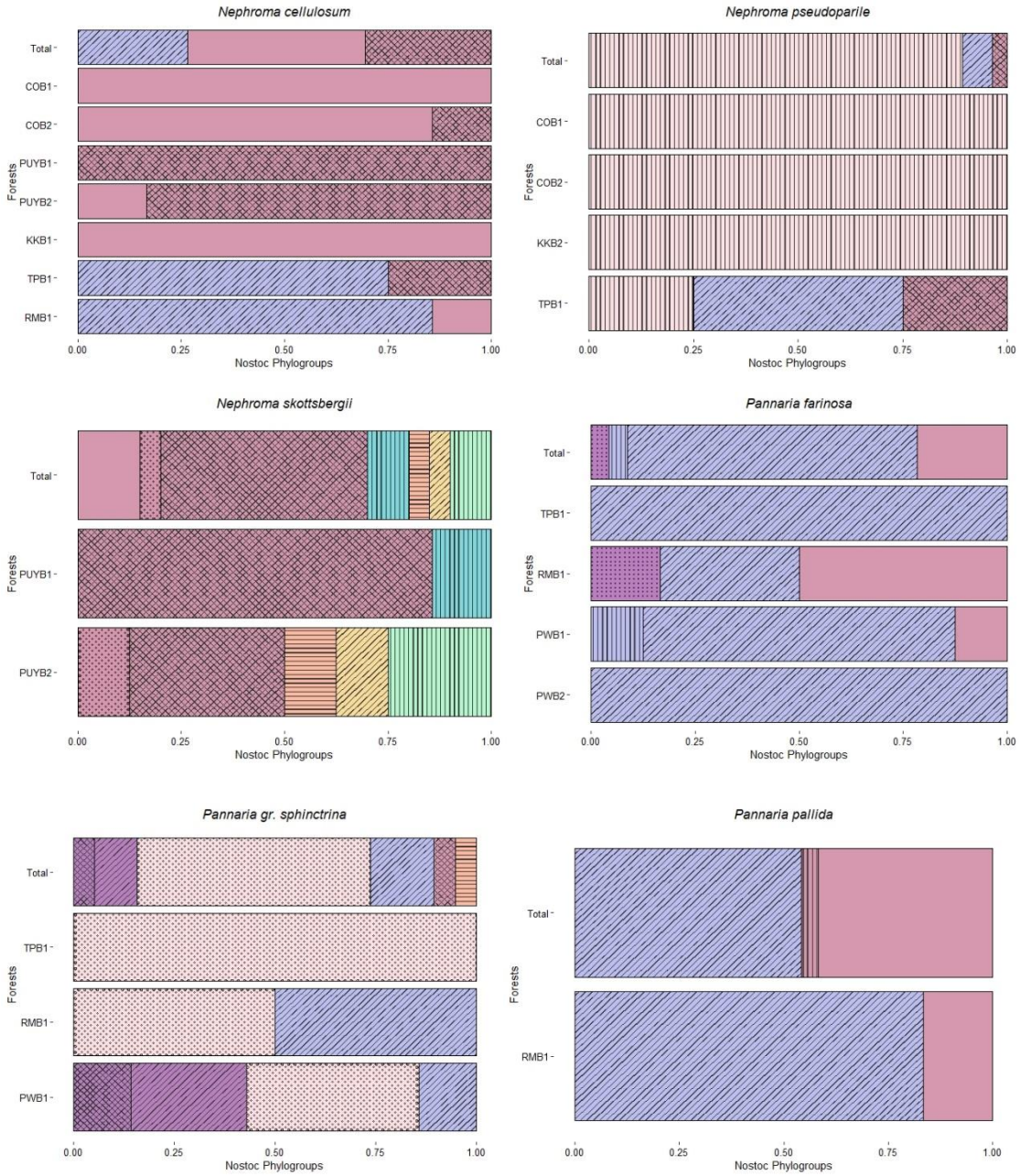
PHYLOGROUP IDENTITY	NUMBER OF SEQUENCES	PERCENTAGE
1	1	0.09
2	18	1.61
3	8	0.71
4	24	2.14
5	2	0.18
6	1	0.09
7	23	2.05
8	2	0.18
9	1	0.09
10	3	0.27
11	10	0.89
12	13	1.16
13	3	0.27
14	5	0.45
15	1	0.09
16	1	0.09
17	3	0.27
18	1	0.09
19	1	0.09
20	1	0.09
21	30	2.68
22	39	3.48
23	2	0.18
24	9	0.80
25	2	0.18
26	1	0.09
27	1	0.09
28	2	0.18
29	1	0.09
30	1	0.09
31	1	0.09
32	2	0.18
33	1	0.09
34	3	0.27
35	66	5.89
36	1	0.09
37	5	0.45
38	418	37.32
39	2	0.18
40	21	1.88
41	1	0.09
42	216	19.29
43	30	2.68

44	1	0.09
45	1	0.09
46	3	0.27
47	1	0.09
48	28	2.50
49	25	2.23
50	2	0.18
51	9	0.80
52	27	2.41
53	3	0.27
54	19	1.70
55	1	0.09
56	5	0.45
57	4	0.36
58	2	0.18
59	5	0.45
60	1	0.09
61	1	0.09
62	3	0.27
63	1	0.09
64	1	0.09

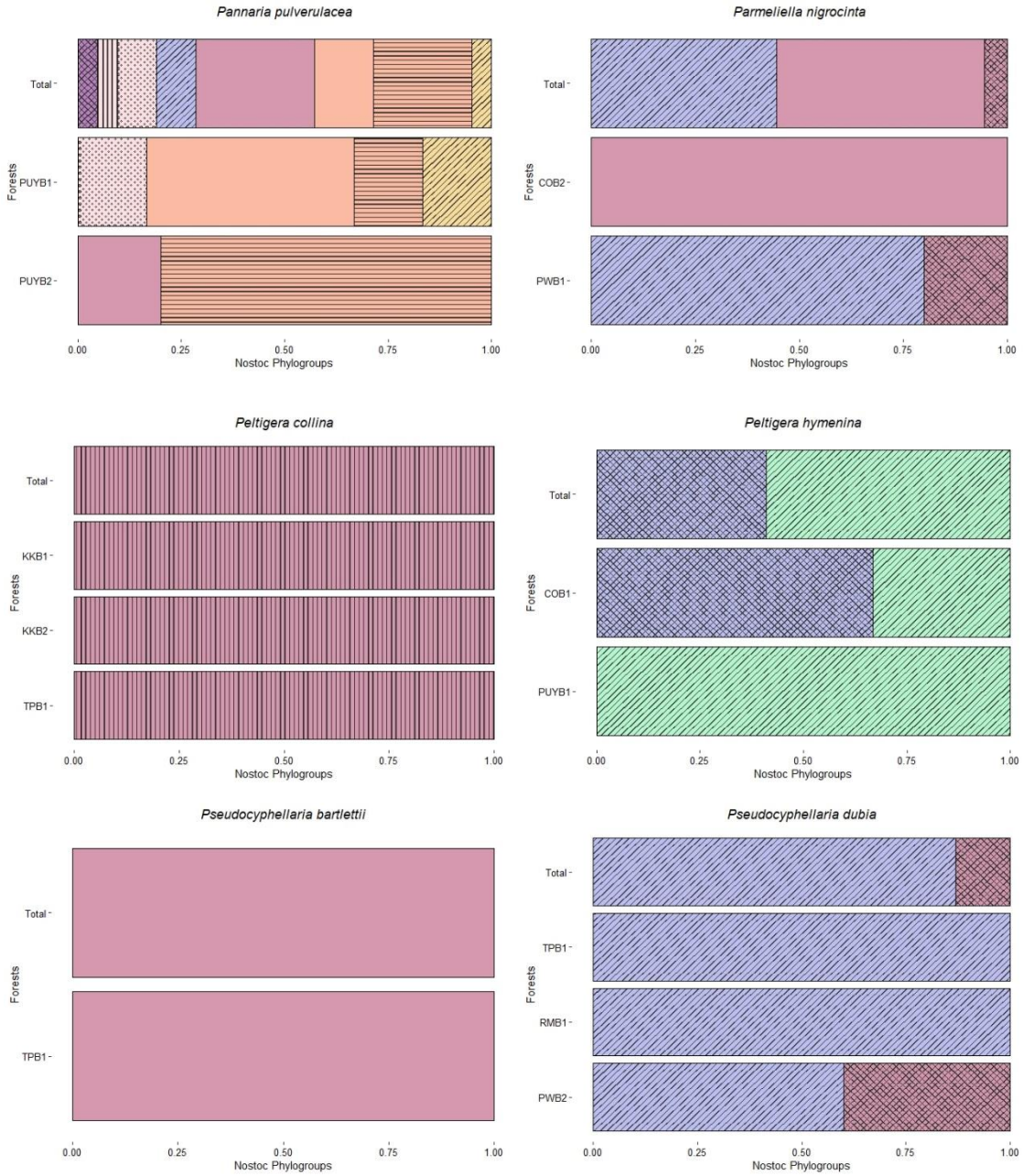
Appendix S6. *Nostoc* phylogroups composition for each mycobiont species in the studied forests and the total latitudinal gradient, from North (top of the graphs) to South (bottom of the graphs).



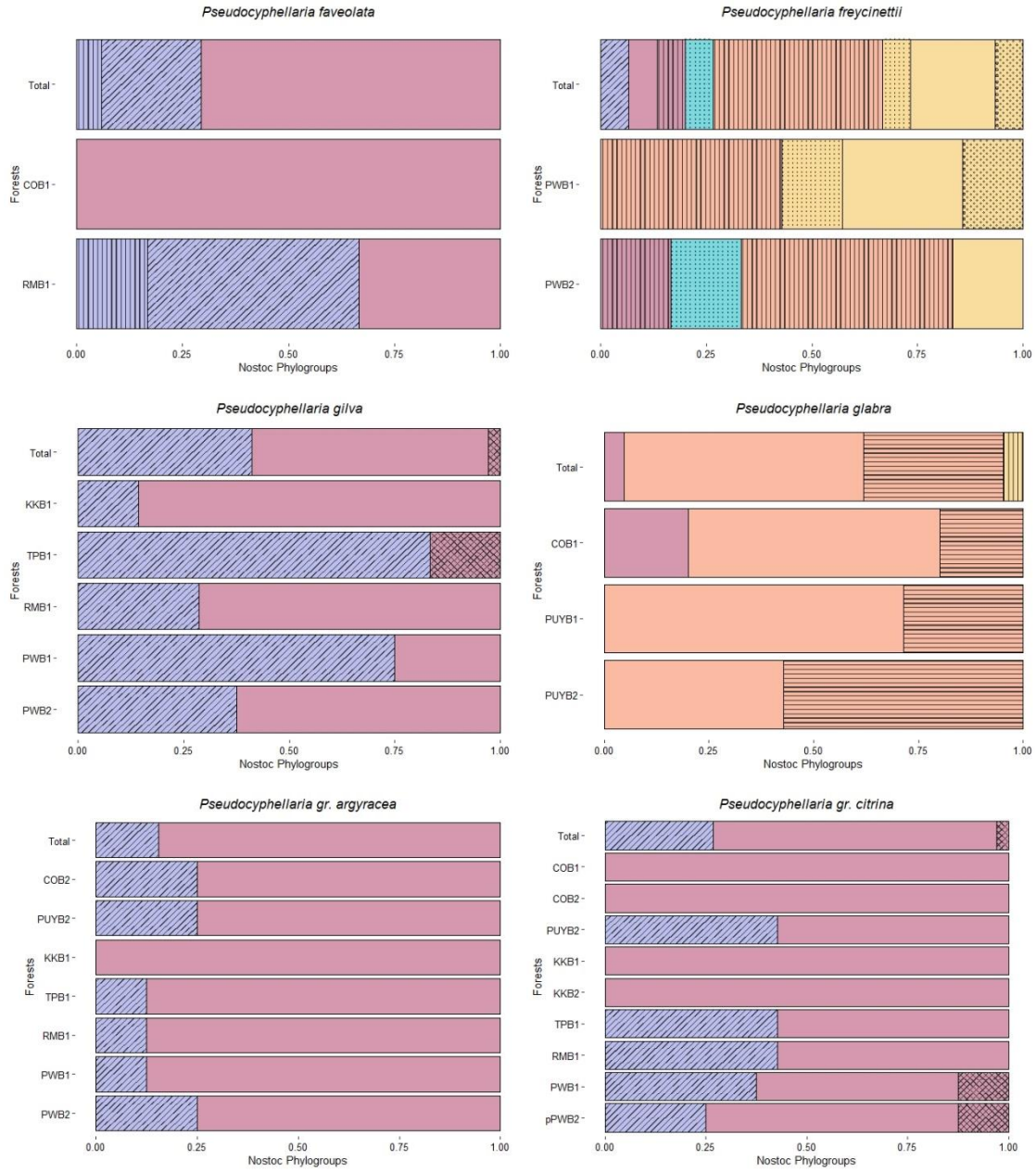
Appendix S6 *Nostoc* phylogroups composition for each mycobiont species in the studied forests and the total latitudinal gradient, from North (top of the graphs) to South (bottom of the graphs) (cont.).



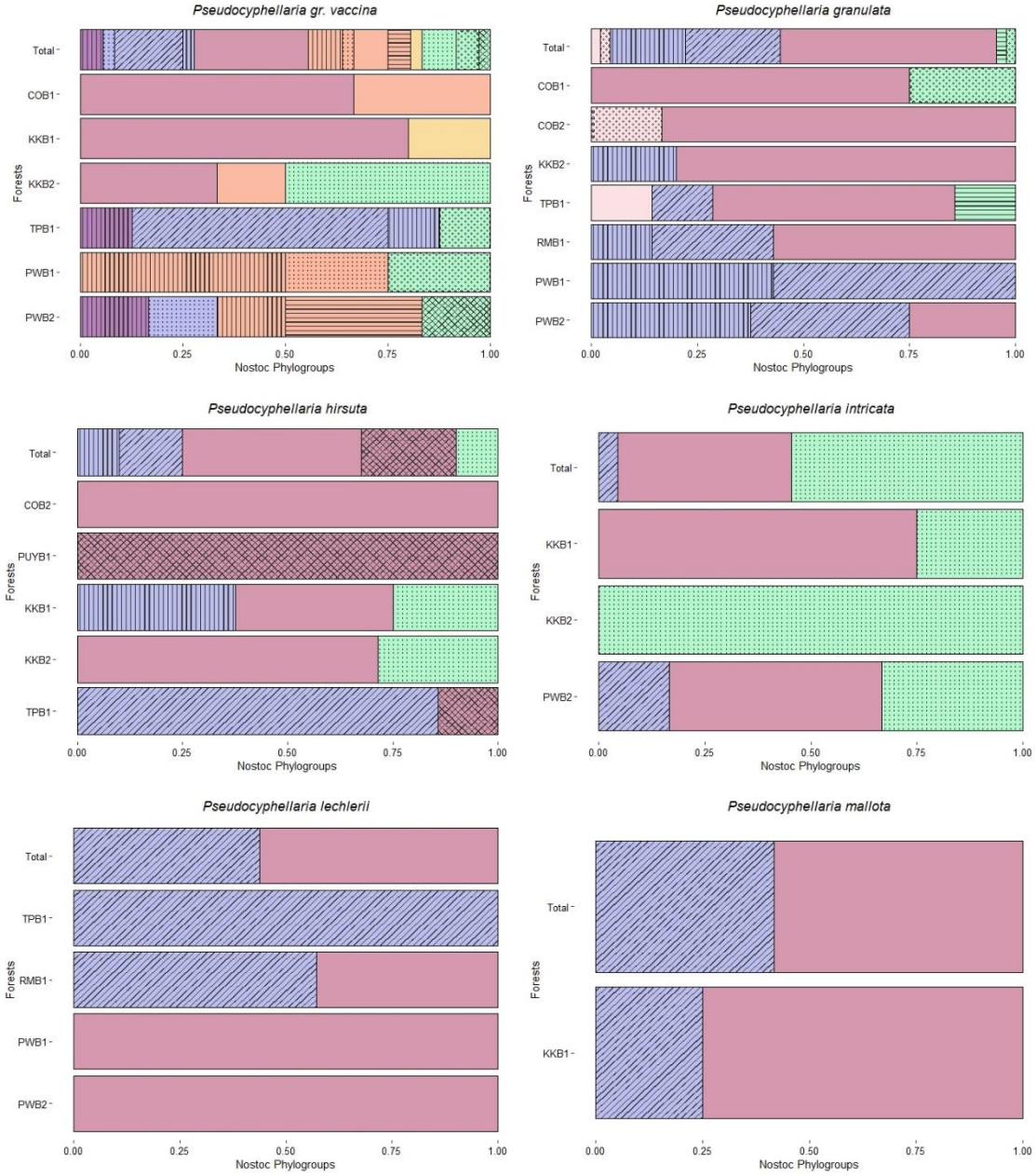
Appendix S6. *Nostoc* phylogroups composition for each mycobiont species in the studied forests and the total latitudinal gradient, from North (top of the graphs) to South (bottom of the graphs) (cont.).



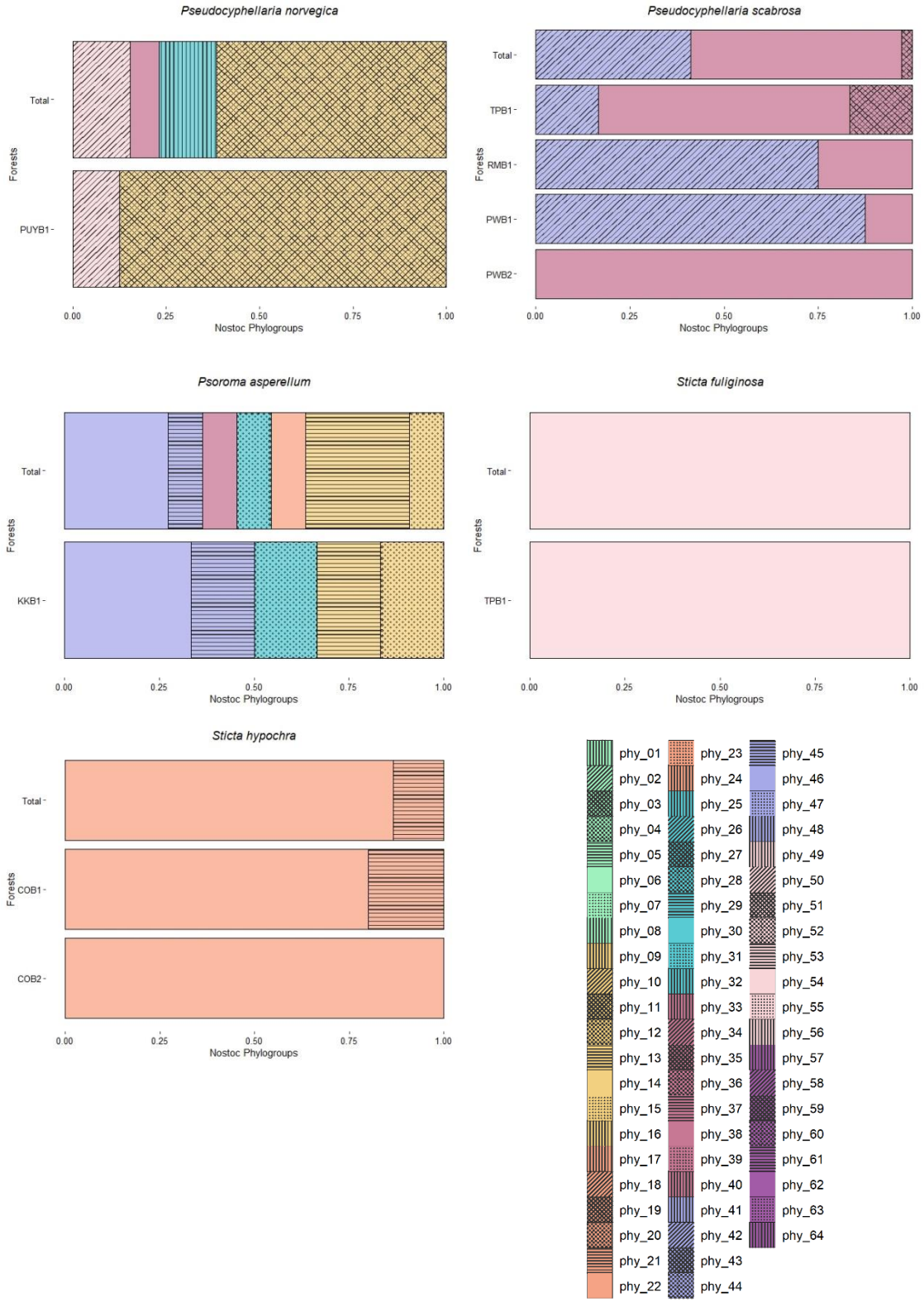
Appendix S6. *Nostoc* phylogroups composition for each mycobiont species in the studied forests and the total latitudinal gradient, from North (top of the graphs) to South (bottom of the graphs) (cont.).



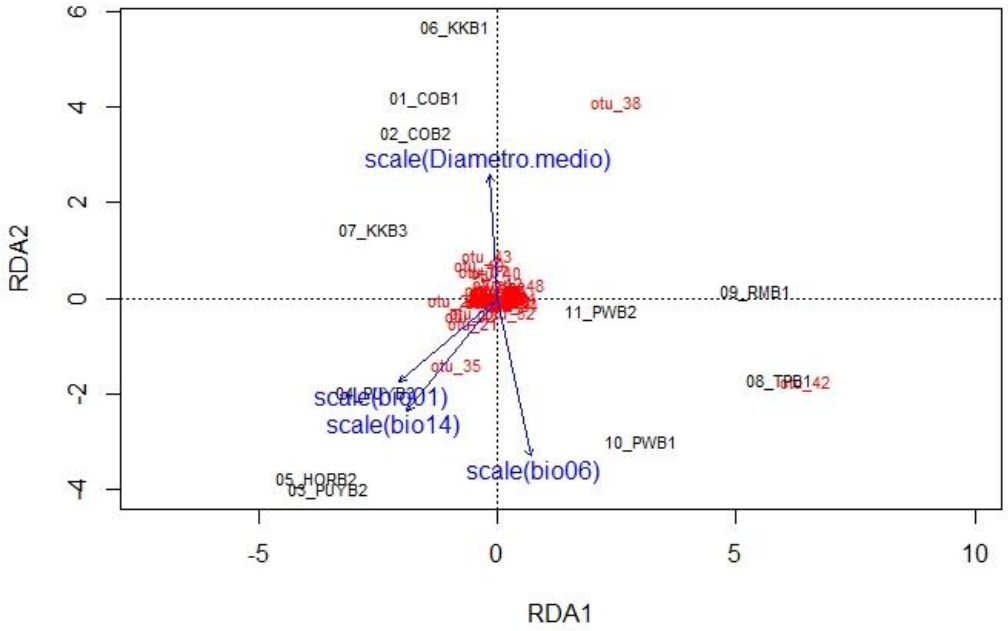
Appendix S6. *Nostoc* phylogroups composition for each mycobiont species in the studied forests and the total latitudinal gradient, from North (top of the graphs) to South (bottom of the graphs) (cont.).



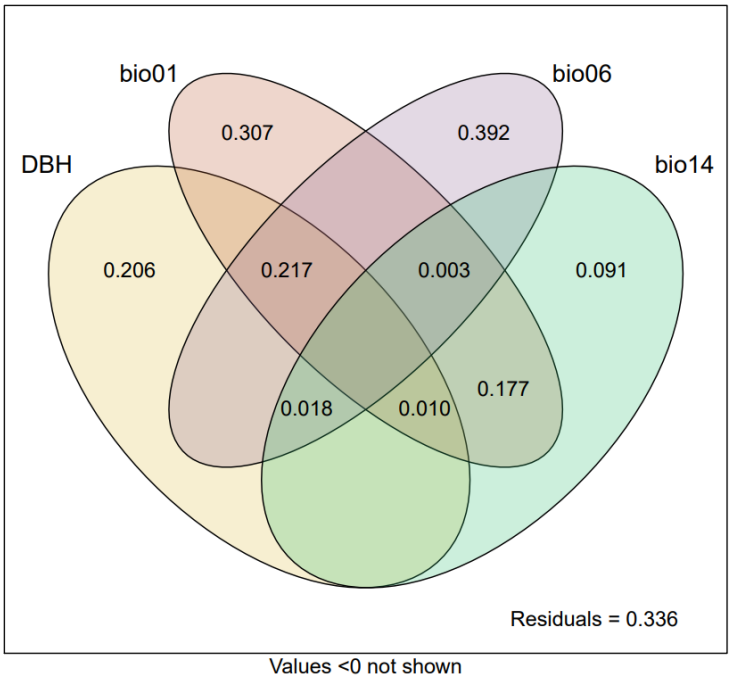
Appendix S6. *Nostoc* phylogroups composition for each mycobiont species in the studied forests and the total latitudinal gradient, from North (top of the graphs) to South (bottom of the graphs) (cont.).



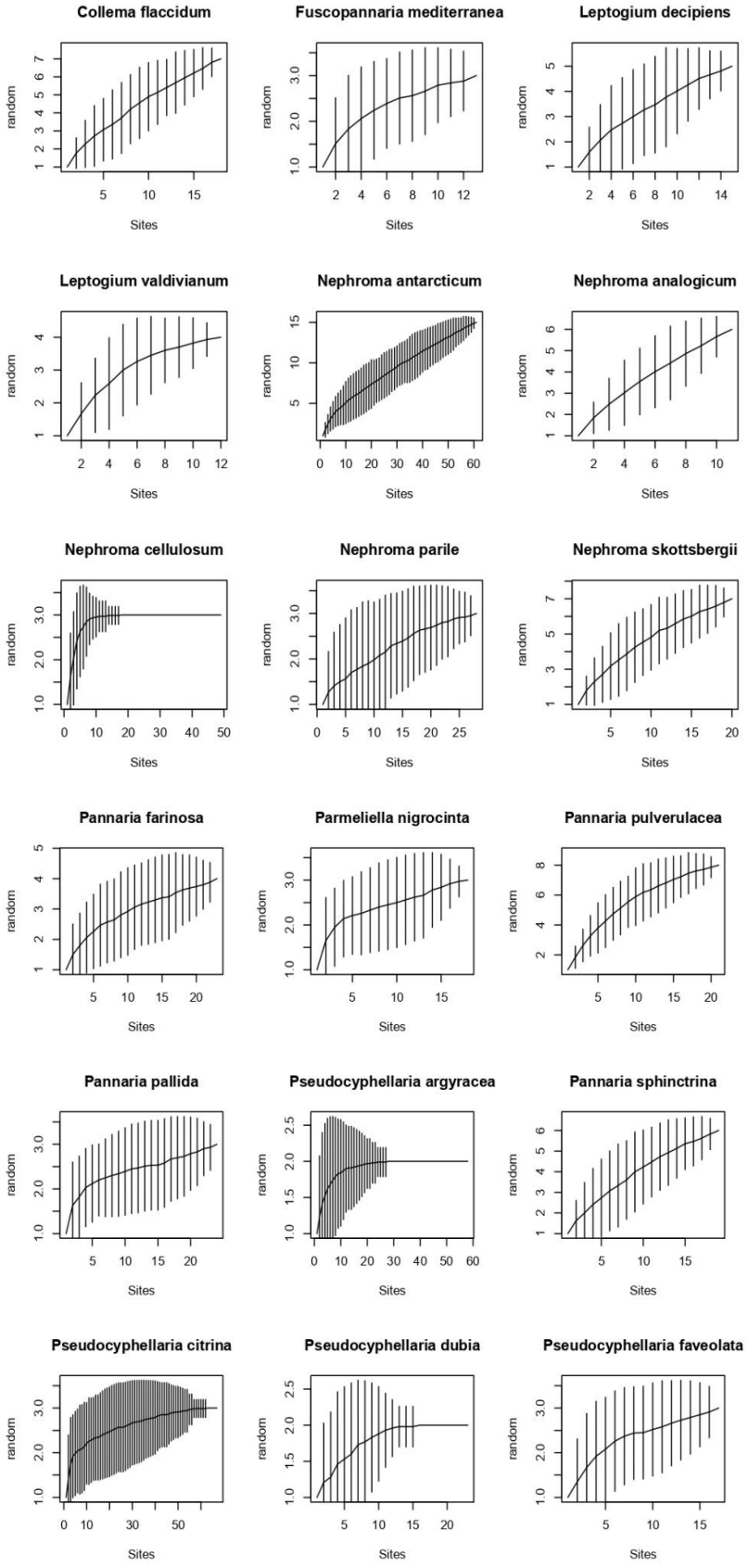
Appendix S7. Redundancy Analysis (RDA) of *Nostoc* phylogroups composition and environmental variables ((DBH, Mean Annual Temperature - bio01, Minimum Temperature of the Coldest Month- bio06, and Precipitation of the Driest Month- bio14).



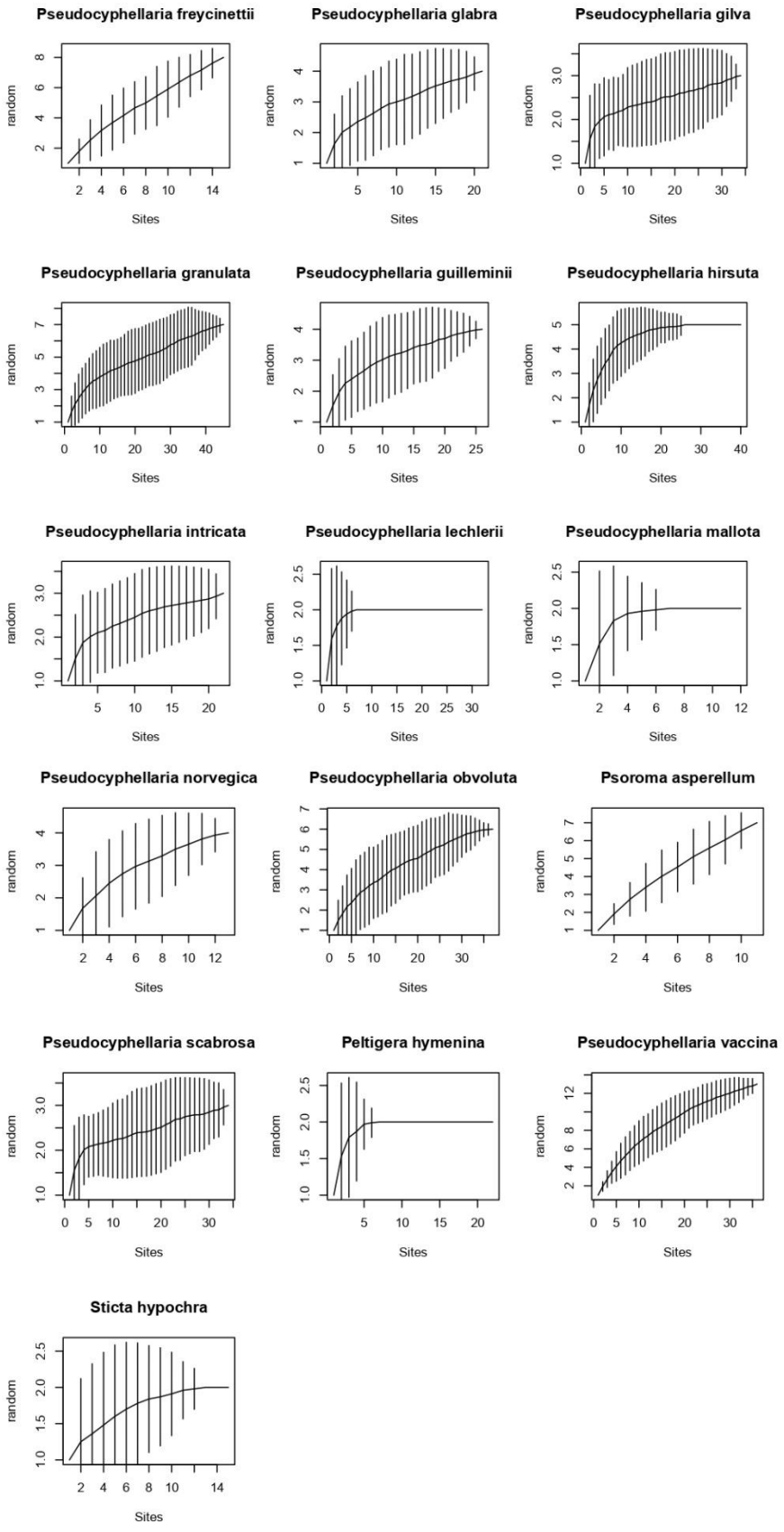
Appendix S8. Venn diagrams showing the variation partitioning of the photobiont composition explained by each group of explanatory variables (DBH, Mean Annual Temperature - bio01, Minimum Temperature of the Coldest Month - bio06, and Precipitation of the Driest Month- bio14).



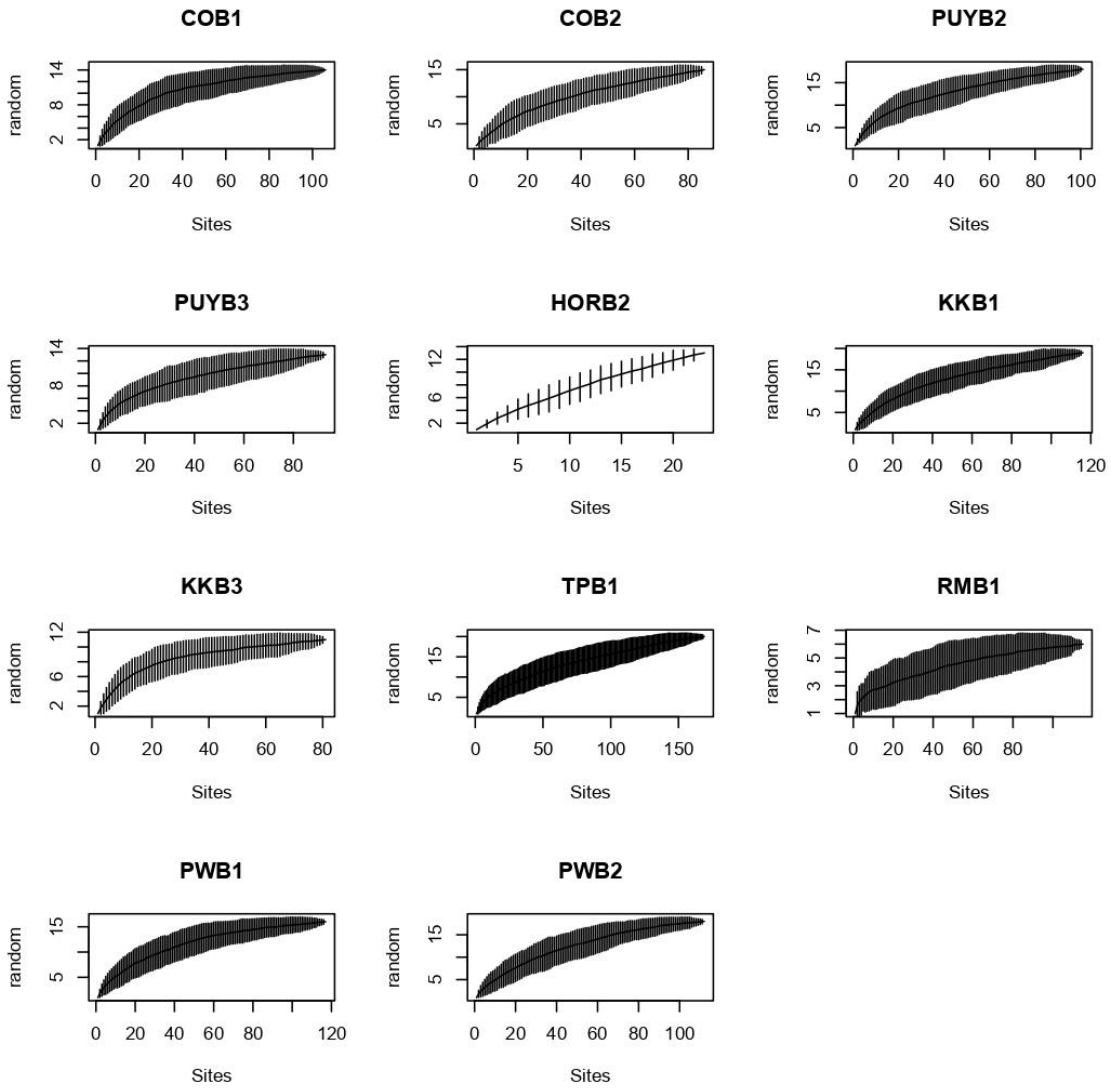
Appendix S9. Mycobiont species accumulation curves for the number of interacting cyanobionts (partner richness).



Appendix S9. Mycobiont species accumulation curves for the number of interacting cyanobionts (partner richness) (cont.).



Appendix S10. Forest accumulation curves for the *Nostoc* phylogroups.



Appendix S11. Number of *Nostoc* phylogroups observed for those mycobiont species with at least 10 sequenced thalli (PARTNER RICHNESS), richness estimated with Chao1 (S.CHAO1), standard deviation of Chao1 (SE.CHAO1), estimated percentage sampled comparing S.OBS and S. CHAO1 (% SAMPLED).

SPECIE	PARTNER RICHNESS	S.CHAO1	SE.CHAO1	% SAMPLED
Collema flaccidum	7	10	4.11	70
Crocodia guilleminii	4	4	0.41	100
Cyanisticta obvoluta	6	6.33	0.41	94.74
Fuscopannaria mediterranea	3	3	0.41	100
Leptogium decipiens	5	6.5	2.53	76.92
Leptogium valdivianum	4	4	0.43	100
Nephroma analogicum	6	9	12.80	66.67
Nephroma antarcticum	15	30	4.10	50
Nephroma cellulosum	3	3	0.00	100
Nephroma pseudoparile	3	3	0.20	100
Nephroma skottsbergii	7	8	1.79	87.50
Pannaria farinosa	4	5	2.17	80
Pannaria gr. sphinctrina	6	7.5	0.41	80
Pannaria pallida	3	3	0.41	100
Pannaria pulverulacea	8	9	1.80	88.89
Parmeliella nigrocinta	3	3	0.00	100
Peltigera collina	1	1	2.54	100
Peltigera hymenina	2	2	0.00	100
Pseudocyphellaria bartlettii	1	1	0.00	100
Pseudocyphellaria dubia	2	2	13.51	100
Pseudocyphellaria faveolata	3	3	2.17	100
Pseudocyphellaria freycinettii	8	23	0.41	34.78
Pseudocyphellaria gilva	3	3	7.08	100
Pseudocyphellaria glabra	4	5	0.22	80
Pseudocyphellaria gr. *argyracea*	2	2	0.00	100
Pseudocyphellaria gr. *citrina*	3	3	0.41	100
Pseudocyphellaria gr. *vaccina*	13	15.5	0.00	83.87
Pseudocyphellaria granulata	7	13	0.00	53.85
Pseudocyphellaria hirsuta	5	5	0.00	100
Pseudocyphellaria intricata	3	3	0.00	100
Pseudocyphellaria lechlerii	2	2	0.14	100
Pseudocyphellaria mallota	2	2	0.91	100
Pseudocyphellaria norvegica	4	4	10.10	100
Pseudocyphellaria scabrosa	3	3	0.00	100
Psoroma asperellum	7	17	3.15	41.18
Sticta fuliginosa	1	1	0.00	100
Sticta hypochra	2	2	0	100

Appendix S12. Mycobiont species richness per forest (MYCOBIONT SPS.), *Nostoc* phylogroup richness observed per forest (S.OBS), richness estimated with Chao1 per forest (S.CHAO1), standard deviation of Chao1 (SE.CHAO1).

BOSQUE	MYCOBIONT SPS	S.OBS	S.CHAO1	SE.CHAO1
COB1	37	14	15	1.81
COB2	24	15	22	7.08
PUYB1	35	18	23	5.51
PUYB2	25	13	18	5.98
HORB1	16	13	25	10.73
KKB1	30	19	23.2	4.33
KKB2	22	11	14	4.51
TPB1	36	20	27.2	6.43
RMB1	24	6	6.5	1.27
PWB1	25	16	17.5	2.22
PWB2	23	18	21	3.41

Appendix S13. Mycobiont specialization measures, reproductive mode and function of cyanobiont per forest. Specialization metrics were estimated for those mycobiont species with at least ten thalli along the latitudinal gradient and in those forests in which they had a minimum of four thalli. N thalli: Number of thalli sequenced per species and forest, Partner richness: number of *Nostoc* phylogroups found per species and forest, Simpson index, d' index, reproductive mode, and function of the cyanobiont. Abbreviations of the forests: CO, Conguillío National Park; PUY, Puyehue National Park; HOR, Hornopirén National Park; KK, Cerro Castillo National Park; TP, Torres del Paine National Park; RM, Magallanes National Reserve; and PW, Navarino Island. B1 and B2 refers to forest 1 or 2, respectively.

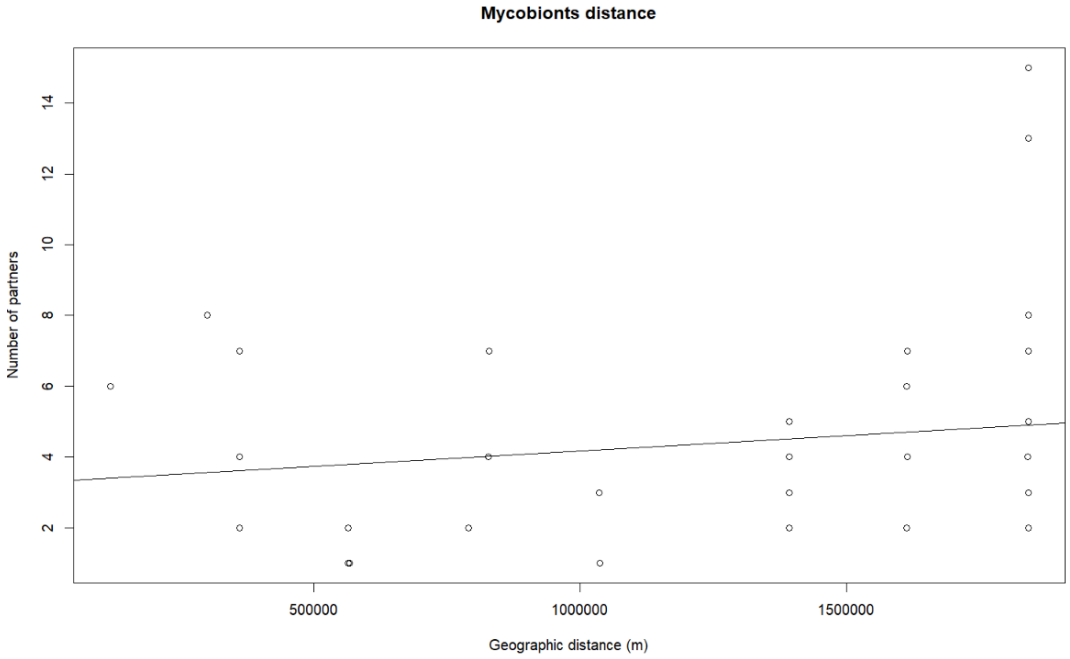
SPECIES	FOREST	N THALLI	PARTNER RICHNESS	SIMPSON INDEX	d' INDEX	REPRODUCTIVE MODE	FUNCTION OF THE CYANOBIONT
Collema							
C. flaccidum	TPB1	8	2	0.78	0.39	asexual	cyanolichen
Crocordia							
C. guilleminii	PUYB2	6	1	1.00	0.36	Sexual	cyanolichen
C. guilleminii	TPB1	6	2	0.56	0.18	Sexual	cyanolichen
C. guilleminii	RMB1	8	2	0.63	0.05	Sexual	cyanolichen
Cyanisticta							
C. obvoluta	KKB2	4	2	0.63	0.17	Sexual	cephalolichen
C. obvoluta	TPB1	7	2	0.76	0.18	Sexual	cephalolichen
C. obvoluta	RMB1	6	2	0.72	0.32	Sexual	cephalolichen
C. obvoluta	PWB1	8	4	0.31	0.06	Sexual	cephalolichen
C. obvoluta	PWB2	8	4	0.34	0.09	Sexual	cephalolichen
Fuscopannaria							
F. mediterranea	RMB1	4	1	1.00	0.22	sexual	cyanolichen
F. mediterranea	PWB1	5	2	0.52	0.37	sexual	cyanolichen
Leptogium							
L. decipiens	COB1	4	2	0.63	0.76	asexual	cyanolichen
L. decipiens	COB2	5	2	0.68	0.93	asexual	cyanolichen
L. valdivianum	COB1	7	2	0.59	0.84	sexual	cyanolichen
Nephroma							
N. analogicum	PUYB2	8	4	0.34	0.30	asexual	cephalolichen
N. antarcticum	COB2	4	3	0.38	0.22	asexual	cephalolichen
N. antarcticum	PUYB1	5	3	0.44	0.55	sexual	cephalolichen
N. antarcticum	KKB1	8	3	0.41	0.18	both	cephalolichen
N. antarcticum	KKB2	7	4	0.39	0.27	both	cephalolichen
N. antarcticum	TPB1	8	4	0.28	0.14	both	cephalolichen
N. antarcticum	RMB1	8	3	0.41	0.03	sexual	cephalolichen
N. antarcticum	PWB1	6	1	1.00	0.24	sexual	cephalolichen
N. antarcticum	PWB2	8	3	0.47	0.44	sexual	cephalolichen
N. cellulosum	COB1	6	1	1.00	0.12	sexual	cyanolichen

N. cellulosum	COB2	7	2	0.76	0.19	both	cyanolichen
N. cellulosum	PUYB1	6	1	1.00	0.44	both	cyanolichen
N. cellulosum	PUYB2	6	2	0.72	0.32	both	cyanolichen
N. cellulosum	KKB1	5	1	1.00	0.07	both	cyanolichen
N. cellulosum	TPB1	8	2	0.63	0.20	sexual	cyanolichen
N. cellulosum	RMB1	7	2	0.76	0.10	sexual	cyanolichen
N. pseudoparile	COB1	8	1	1.00	1.00	both	cyanolichen
N. pseudoparile	COB2	8	1	1.00	1.00	asexual	cyanolichen
N. pseudoparile	KKB2	8	1	1.00	1.00	asexual	cyanolichen
N. pseudoparile	TPB1	4	3	0.38	0.29	asexual	cyanolichen
N. skottsbergii	PUYB1	7	2	0.76	0.49	asexual	cephalolichen
N. skottsbergii	PUYB2	8	5	0.25	0.53	both	cephalolichen
Pannaria							
P. farinosa	TPB1	4	1	1.00	0.18	asexual	cephalolichen
P. farinosa	RMB1	6	3	0.39	0.12	asexual	cephalolichen
P. farinosa	PWB1	8	3	0.59	0.08	asexual	cephalolichen
P. farinosa	PWB2	4	1	1.00	0.27	asexual	cephalolichen
P. gr. *sphinctrina*	TPB1	6	1	1.00	0.91	sexual	cephalolichen
P. gr. *sphinctrina*	RMB1	4	2	0.50	0.50	sexual	cephalolichen
P. gr. *sphinctrina*	PWB1	7	4	0.31	0.62	sexual	cephalolichen
P. pallida	RMB1	6	2	0.72	0.08	sexual	cephalolichen
P. pulverulacea	PUYB1	6	4	0.33	0.52	both	cephalolichen
P. pulverulacea	PUYB2	5	2	0.68	0.26	asexual	cephalolichen
Parmeliella							
P. nigrocinta	COB2	4	1	1.00	0.04	sexual	cyanolichen
P. nigrocinta	PWB1	5	2	0.68	0.23	sexual	cyanolichen
Peltigera							
P. collina	KKB1	8	1	1.00	1.00	asexual	cyanolichen
P. collina	KKB2	7	1	1.00	1.00	asexual	cyanolichen
P. collina	TPB1	5	1	1.00	0.94	Asexual	cyanolichen
P. hymenina	COB1	6	2	0.56	0.79	Sexual	cyanolichen
P. hymenina	PUYB1	7	1	1.00	0.89	Sexual	cyanolichen
Pseudocyphellaria							
P. bartlettii	TPB1	8	1	1.00	0.36	Asexual	cyanolichen
P. dubia	TPB1	7	1	1.00	0.25	Both	cyanolichen
P. dubia	RMB1	8	1	1.00	0.26	Asexual	cyanolichen
P. dubia	PWB2	5	2	0.52	0.40	Asexual	cyanolichen
P. faveolata	COB1	6	1	1.00	0.12	Both	cephalolichen
P. faveolata	RMB1	6	3	0.39	0.06	Asexual	cephalolichen
P. freycinettii	PWB1	7	4	0.31	0.80	None	cephalolichen
P. freycinettii	PWB2	6	4	0.33	0.94	None	cephalolichen
P. gilva	KKB1	7	2	0.76	0.14	None	cyanolichen
P. gilva	TPB1	6	2	0.72	0.16	Sexual	cyanolichen
P. gilva	RMB1	7	2	0.59	0.03	Both	cyanolichen
P. gilva	PWB1	4	2	0.63	0.03	Sexual	cyanolichen
P. gilva	PWB2	8	2	0.53	0.03	Sexual	cyanolichen
P. glabra	COB1	5	3	0.44	0.38	Asexual	cephalolichen
P. glabra	PUYB1	7	2	0.59	0.60	Both	cephalolichen

P. glabra	PUYB2	7	2	0.51	0.57	Asexual	cephalolichen
P. gr. *argyracea*	COB2	8	2	0.63	0.26	Asexual	cyanolichen
P. gr. *argyracea*	PUYB2	4	2	0.63	0.23	Asexual	cyanolichen
P. gr. *argyracea*	KKB1	8	1	1.00	0.16	Both	cyanolichen
P. gr. *argyracea*	TPB1	8	2	0.78	0.21	Both	cyanolichen
P. gr. *argyracea*	RMB1	8	2	0.78	0.13	Asexual	cyanolichen
P. gr. *argyracea*	PWB1	8	2	0.78	0.27	Asexual	cyanolichen
P. gr. *argyracea*	PWB2	8	2	0.63	0.06	Both	cyanolichen
P. gr. *citrina*	COB1	5	1	1.00	0.09	Asexual	cyanolichen
P. gr. *citrina*	COB2	8	1	1.00	0.15	Asexual	cyanolichen
P. gr. *citrina*	PUYB2	7	2	0.51	0.39	Asexual	cyanolichen
P. gr. *citrina*	KKB1	8	1	1.00	0.16	Asexual	cyanolichen
P. gr. *citrina*	KKB2	4	1	1.00	0.18	Asexual	cyanolichen
P. gr. *citrina*	TPB1	7	2	0.51	0.06	Asexual	cyanolichen
P. gr. *citrina*	RMB1	7	2	0.51	0.00	Asexual	cyanolichen
P. gr. *citrina*	PWB1	8	3	0.41	0.10	Asexual	cyanolichen
P. gr. *citrina*	PWB2	8	3	0.47	0.06	Asexual	cyanolichen
P. gr. *vaccina*	COB1	6	2	0.56	0.10	Sexual	cephalolichen
P. gr. *vaccina*	KKB1	5	2	0.68	0.19	Sexual	cephalolichen
P. gr. *vaccina*	KKB2	6	3	0.39	0.21	Sexual	cephalolichen
P. gr. *vaccina*	TPB1	8	4	0.44	0.44	Sexual	cephalolichen
P. gr. *vaccina*	PWB1	4	3	0.38	0.79	Sexual	cephalolichen
P. gr. *vaccina*	PWB2	6	5	0.22	0.74	Sexual	cephalolichen
P. granulata	COB1	4	2	0.63	0.03	Asexual	cephalolichen
P. granulata	COB2	6	2	0.72	0.13	Asexual	cephalolichen
P. granulata	KKB2	5	2	0.68	0.20	Asexual	cephalolichen
P. granulata	TPB1	7	4	0.39	0.16	Asexual	cephalolichen
P. granulata	RMB1	7	3	0.43	0.05	Asexual	cephalolichen
P. granulata	PWB1	7	2	0.51	0.26	Asexual	cephalolichen
P. granulata	PWB2	8	3	0.34	0.28	Asexual	cephalolichen
P. hirsuta	COB2	5	1	1.00	0.07	Sexual	cyanolichen
P. hirsuta	PUYB1	8	1	1.00	0.51	Sexual	cyanolichen
P. hirsuta	KKB1	8	3	0.34	0.25	Sexual	cyanolichen
P. hirsuta	KKB2	7	2	0.59	0.16	Sexual	cyanolichen
P. hirsuta	TPB1	7	2	0.76	0.18	Sexual	cyanolichen
P. intricata	KKB1	8	2	0.63	0.15	Asexual	cyanolichen
P. intricata	KKB2	8	1	1.00	0.77	Both	cyanolichen
P. intricata	PWB2	6	3	0.39	0.26	Both	cyanolichen
P. lechlerii	TPB1	8	1	1.00	0.27	Asexual	cyanolichen
P. lechlerii	RMB1	7	2	0.51	0.00	Asexual	cyanolichen
P. lechlerii	PWB1	8	1	1.00	0.45	Asexual	cyanolichen
P. lechlerii	PWB2	7	1	1.00	0.26	Both	cyanolichen
P. mallota	KKB1	8	2	0.63	0.24	Asexual	cyanolichen
P. norvegica	PUYB1	8	2	0.78	0.90	Asexual	cyanolichen
P. scabrosa	TPB1	6	3	0.50	0.08	Asexual	cyanolichen
P. scabrosa	RMB1	4	2	0.63	0.04	Asexual	cyanolichen
P. scabrosa	PWB1	8	2	0.78	0.15	Asexual	cyanolichen
P. scabrosa	PWB2	8	1	1.00	0.28	Asexual	cyanolichen

Psoroma							
P. asperellum	KKB1	6	5	0.22	0.95	Sexual	cephalolichen
Sticta							
S. fuliginosa	TPB1	8	1	1.00	0.92	Asexual	cyanolichen
S. hypochra	COB1	5	2	0.68	0.69	Both	cyanolichen
S. hypochra	COB2	4	1	1.00	0.84	Asexual	cyanolichen

Appendix S14. Number of partners per species of mycobiont with at least 10 thalli along the latitudinal gradient with the geographic distance (m) (R^2 =-0.0008, P = 0.33).



Appendix S15. *Nostoc* phylogroups and geographic distance (ordered by distance in km, followed by number of forest).

Nostoc	Thalli	Richness of partners	Distance (km)	Number of forests
phylogroup_01	1	1	0	1
phylogroup_06	1	1	0	1
phylogroup_09	1	1	0	1
phylogroup_15	1	1	0	1
phylogroup_16	1	1	0	1
phylogroup_18	1	1	0	1
phylogroup_19	1	1	0	1
phylogroup_20	1	1	0	1
phylogroup_26	1	1	0	1
phylogroup_27	1	1	0	1
phylogroup_29	1	1	0	1
phylogroup_30	1	1	0	1
phylogroup_31	1	1	0	1
phylogroup_33	1	1	0	1
phylogroup_36	1	1	0	1
phylogroup_41	1	1	0	1
phylogroup_44	1	1	0	1
phylogroup_45	1	1	0	1
phylogroup_47	1	1	0	1
phylogroup_55	1	1	0	1
phylogroup_60	1	1	0	1
phylogroup_61	1	1	0	1
phylogroup_63	1	1	0	1
phylogroup_64	1	1	0	1
phylogroup_08	2	1	0	1
phylogroup_32	2	1	0	1
phylogroup_39	2	1	0	1
phylogroup_58	2	1	0	1
phylogroup_05	2	2	0	1
phylogroup_28	2	2	0	1
phylogroup_34	3	3	0	1
phylogroup_24	9	2	1.65	2
phylogroup_53	3	1	2.21	2
phylogroup_11	10	3	2.21	2
phylogroup_46	3	1	10.87	2
phylogroup_25	2	1	118.49	2
phylogroup_10	3	3	118.49	3
phylogroup_50	2	1	243.20	2
phylogroup_51	9	3	24320	3
phylogroup_02	18	2	361.24	4

phylogroup_56	5	3	565.13	2
phylogroup_59	5	4	565.13	2
phylogroup_57	4	2	565.61	3
phylogroup_37	5	3	568.19	2
phylogroup_40	21	1	568.19	3
phylogroup_13	3	1	827.51	2
phylogroup_17	3	2	827.78	2
phylogroup_62	3	3	827.78	2
phylogroup_14	5	3	1036.64	3
phylogroup_07	23	7	1036.64	4
phylogroup_54	19	5	1036.64	5
phylogroup_48	28	8	1036.64	6
phylogroup_49	25	1	1393.77	4
phylogroup_22	39	9	1393.77	7
phylogroup_35	66	17	1840.97	7
phylogroup_52	27	10	1841.41	6
phylogroup_03	8	4	1841.91	3
phylogroup_21	30	12	1841.91	5
phylogroup_04	24	8	1841.91	6
phylogroup_43	30	7	1841.91	8
phylogroup_42	216	27	1841.91	11
phylogroup_38	418	47	1841.91	11
phylogroup_23	2	2	1842.35	2
phylogroup_12	13	5	1842.35	4

Appendix S16. Mycobiont species and geographic distance (ordered by distance in km, followed by number of forest).

Mycobionts	Thalli	Richness of partners	*Nostoc* phylogroup identity	Distance (km)	Number of forests
Leciophysma sp. 1	1	1	19	0	1
Leptogium cochleatum	1	1	44	0	1
Leptogium sp. 1	1	1	4	0	1
Pannaria contorta	1	1	42	0	1
Pannaria isabellina	1	1	35	0	1
Parmeliella sp. 1	1	1	38	0	1
Pseudocyphellaria encoensis	1	1	42	0	1
Pseudocyphellaria sp1	1	1	11	0	1
Pseudocyphellaria divulsa	1	1	34	0	1
Psorophorus pholidotus	1	1	38	0	1
Pseudocyphellaria wandae	1	1	38	0	1
Sticta ainoae	1	1	30	0	1
Xanthopsoroma contextum	1	1	42	0	1
Xanthopsoroma soccatum	1	1	38	0	1
Pannaria sp1	2	1	52	0	1
Pannaria aff patagonica	2	1	52	0	1
Pseudocyphellaria nudata	2	1	38	0	1
Peltigera degenii	2	1	43	0	1
Pannaria arthroophylla	3	2	59, 64	0	1
Psoroma hypnorum	3	2	12, 14	0	1
Fuscopannaria mediterranea	4	1	35	0	1
Peltigera rufescens	4	1	43	0	1
Collema glaucophthalmum	4	3	37, 52, 56	0	1
Sticta caulescens	5	1	21	0	1
Leptogium patagonicum	5	2	04, 39	0	1
Pannaria aff implexa	5	2	52, 56	0	1
Pannaria byssoidea	7	2	35, 38	0	1
Leptogium aff. tenuissimum	7	6	07, 18, 20, 28, 37, 38	0	1
Psoroma polychinoides	3	1	53	2.21	2
Pseudocyphellaria coppinsii	7	3	35, 38, 52	2.21	2
Fuscopannaria sp. 1	3	2	38, 48	10.87	2
Nephroma kuehenmanii (+ N. microphyllum)	2	2	22, 38	118.49	2
Nephroma plumbeum	2	2	01, 38	118.49	2
Pseudocyphellaria valdiviana	7	4	21, 22, 27, 34	118.49	2
Nephroma analogicum (+ N. chubutense)	11	6	10, 21, 29, 34, 35, 52	118.49	3
Pseudocyphellaria flavicans	8	3	04, 21, 38	242.50	3
Leptogium azureum	3	2	23, 38	243.20	2
Pseudocyphellaria freycinetti	15	8	12, 14, 15, 24, 31, 33, 38, 42	300.33	3
Pseudocyphellaria berberina (+ P. coerulescens)	3	1	38	361.24	3

Peltigera polydactylon	5	1	2	361.24	3
Nephroma skottsbergii (+ N. papillosum)	20	7	08, 10, 21, 25, 35, 36, 38	361.24	4
Peltigera hymenina	20	2	02, 43	361.24	5
Pseudocyphellaria glabra	21	4	16, 21, 22, 38	361.24	5
Psoroma hirsutulum	2	2	05, 38	565.13	2
Pseudocyphellaria bartlettii	10	1	38	565.13	3
Pseudocyphellaria dubia	23	2	35, 42	565.61	4
Peltigera collina	21	1	40	568.19	3
Pseudocyphellaria mallota	12	2	38, 42	790.86	4
Leptogium laceroides	9	3	04, 12, 17	827.51	3
Leptogium valdivianum	12	4	12, 51, 52, 62	827.51	4
Nephroma pseudoparile	4	1	38	830.08	2
Peltigera membranacea	8	1	43	830.08	3
Psoroma asperellum	11	7	12, 13, 22, 28, 38, 45, 46	830.08	3
Peltigera canina	6	2	03, 43	1036.64	2
Pseudocyphellaria intricata	22	3	07, 38, 42	1036.64	3
Pseudocyphellaria gilva	34	3	35, 38, 42	1036.64	6
Sticta fuliginosa	12	1	54	1037.54	4
Sticta gr. *weigelii*	5	3	11, 38, 54	1152.41	3
Pseudocyphellaria piloselloides	6	1	38	1378.82	6
Parmeliella nigrata	4	2	38, 42	1393.77	3
Nephroma parile	28	3	35, 42, 49	1393.77	4
Pseudocyphellaria norvegica	13	4	11, 32, 38, 50	1393.77	5
Sticta hypochra	15	2	21, 22	1393.77	5
Leptogium decipiens	15	5	03, 04, 06, 37, 38	1393.77	5
Peltigera praetextata	4	1	43	1394.34	4
Pseudocyphellaria lechleri	32	2	38, 42	1613.61	6
Pseudocyphellaria obvoluta	37	6	21, 38, 42, 48, 57, 59	1613.73	8
Pannaria gr. *Sphinctrina*	20	6	21, 35, 42, 52, 58, 59	1614.08	5
Pseudocyphellaria guillemini	26	4	07, 35, 38, 42	1615.51	6
Collema flaccidum	18	7	07, 17, 22, 38, 51, 54, 62	1615.51	7
Pannaria farinosa	23	4	38, 42, 48, 63	1840.97	5
Leptogium meziensii	6	2	04, 43	1841.91	3
Pseudocyphellaria faveolata	17	3	38, 42, 48	1841.91	5
Parmeliella nigrocinta	18	3	35, 38, 42	1841.91	6
Pannaria pulverulacea	21	8	10, 21, 22, 38, 42, 52, 56, 59	1841.91	7
Pseudocyphellaria scabrosa	34	3	35, 38, 42	1841.91	7
Pseudocyphellaria gr. *vaccina*	36	13	03, 04, 07, 14, 21, 22, 23, 24, 38, 41, 42, 47, 57	1841.91	7
Pannaria pallida	23	2	38, 42	1841.91	8
Pseudocyphellaria granulata	45	7	04, 05, 38, 42, 48, 52, 54	1841.91	8
Pseudocyphellaria hirsuta	40	5	07, 35, 38, 42, 48	1841.91	9
Nephroma cellulosum	49	3	35, 38, 42	1841.91	10

Pseudocyphellaria gr. *argyracea*	57	2	38, 42	1841.91	10
Nephroma antarcticum	61	15	03, 07, 09, 21, 22, 26, 35, 38, 42, 48, 51, 54, 55, 60, 61	1841.91	11
Pseudocyphellaria gr. *citrina*	67	3	35, 38, 42	1841.91	11
Fuscopannaria minor	13	3	38, 48, 62	1842.35	5

Appendix S17. Beta diversity between forests and the regional scale. Comparisons are made within species for each the forest the species is present with the regional scale. β_{tot}: total beta diversity, reflecting both species replacement and loss/gain ($\beta_{total} = \beta_{repl} + \beta_{rich}$). β_{repl}: beta diversity explained by replacement of species alone. β_{rich}: beta diversity explained by species loss/gain (richness differences) alone. Description: turnover strategy following Ventre Lespiaucq *et al*., 2021.

Regional	Local	β_{tot}	Description
Collema flaccidum			
	TPB1	0.71	Nested turnover, variable richness
Crocodia guilleminii			
	PUYB2	0.75	Nested turnover, variable richness
	TPB1	0.50	Nested turnover, variable richness
	RMB1	0.50	Nested turnover, variable richness
Cyanisticta obvoluta			
	KKB2	0.67	Nested turnover, variable richness
	TPB1	0.67	Nested turnover, variable richness
	RMB1	0.67	Nested turnover, variable richness
	PWB1	0.33	Nested turnover, variable richness
	PWB2	0.33	Nested turnover, variable richness
Fuscopannaria mediterranea			
	RMB1	0.67	Nested turnover, variable richness
	PWB1	0.33	Nested turnover, variable richness
Leptogium decipiens			
	COB1	0.60	Nested turnover, variable richness
	COB2	0.60	Nested turnover, variable richness
Leptogium valdivianum			
	COB1	0.50	Nested turnover, variable richness
Nephroma analogicum			
	PUYB2	0.33	Nested turnover, variable richness
Nephroma antarcticum			
	COB2	0.80	Nested turnover, variable richness
	PUYB1	0.80	Nested turnover, variable richness
	KKB1	0.80	Nested turnover, variable richness
	KKB2	0.73	Nested turnover, variable richness
	TPB1	0.73	Nested turnover, variable richness
	RMB1	0.80	Nested turnover, variable richness
	PWB1	0.93	Nested turnover, variable richness
	PWB2	0.80	Nested turnover, variable richness
Nephroma cellulosum			
	COB1	0.67	Nested turnover, variable richness
	COB2	0.33	Nested turnover, variable richness
	PUYB1	0.67	Nested turnover, variable richness

	PUYB2	0.33	Nested turnover, variable richness
	KKB1	0.67	Nested turnover, variable richness
	TPB1	0.33	Nested turnover, variable richness
	RMB1	0.33	Nested turnover, variable richness
Nephroma pseudoparile			
	COB1	0.67	Nested turnover, variable richness
	COB2	0.67	Nested turnover, variable richness
	KKB2	0.67	Nested turnover, variable richness
	TPB1	0.00	No turnover, constant richness
Nephroma skottsbergii			
	PUYB1	0.71	Nested turnover, variable richness
	PUYB2	0.29	Nested turnover, variable richness
Pannaria farinosa			
	TPB1	0.75	Nested turnover, variable richness
	RMB1	0.25	Nested turnover, variable richness
	PWB1	0.25	Nested turnover, variable richness
	PWB2	0.75	Nested turnover, variable richness
Pannaria gr. *sphinctrina*			
	TPB1	0.83	Nested turnover, variable richness
	RMB1	0.67	Nested turnover, variable richness
	PWB1	0.33	Nested turnover, variable richness
Pannaria pallida			
	RMB1	0.33	Nested turnover, variable richness
Pannaria pulverulacea			
	PUYB1	0.50	Nested turnover, variable richness
	PUYB2	0.75	Nested turnover, variable richness
Parmeliella nigrocinta			
	COB2	0.67	Nested turnover, variable richness
	PWB1	0.33	Nested turnover, variable richness
Peltigera collina			
	KKB1	0.00	No turnover, constant richness
	KKB2	0.00	No turnover, constant richness
	TPB1	0.00	No turnover, constant richness
Peltigera hymenina			
	COB1	0.00	No turnover, constant richness
	PUYB1	0.50	Nested turnover, variable richness
Pseudocyphellaria bartlettii			
	TPB1	0.00	No turnover, constant richness
Pseudocyphellaria dubia			
	TPB1	0.50	Nested turnover, variable richness
	RMB1	0.50	Nested turnover, variable richness
	PWB2	0.00	No turnover, constant richness
Pseudocyphellaria faveolata			
	COB1	0.67	Nested turnover, variable richness
	RMB1	0.00	No turnover, constant richness

Pseudocyphellaria freycinettii			
	PWB1	0.50	Nested turnover, variable richness
	PWB2	0.50	Nested turnover, variable richness
Pseudocyphellaria gilva			
	KKB1	0.33	Nested turnover, variable richness
	TPB1	0.33	Nested turnover, variable richness
	RMB1	0.33	Nested turnover, variable richness
	PWB1	0.33	Nested turnover, variable richness
	PWB2	0.33	Nested turnover, variable richness
Pseudocyphellaria glabra			
	COB1	0.25	Nested turnover, variable richness
	PUYB1	0.50	Nested turnover, variable richness
	PUYB2	0.50	Nested turnover, variable richness
Pseudocyphellaria gr. *argyracea*			
	COB2	0.00	No turnover, constant richness
	PUYB2	0.00	No turnover, constant richness
	KKB1	0.50	Nested turnover, variable richness
	TPB1	0.00	No turnover, constant richness
	RMB1	0.00	No turnover, constant richness
	PWB1	0.00	No turnover, constant richness
	PWB2	0.00	No turnover, constant richness
Pseudocyphellaria gr. *citrina*			
	COB1	0.67	Nested turnover, variable richness
	COB2	0.67	Nested turnover, variable richness
	PUYB2	0.33	Nested turnover, variable richness
	KKB1	0.67	Nested turnover, variable richness
	KKB2	0.67	Nested turnover, variable richness
	TPB1	0.33	Nested turnover, variable richness
	RMB1	0.33	Nested turnover, variable richness
	PWB1	0.00	No turnover, constant richness
	PWB2	0.00	No turnover, constant richness
Pseudocyphellaria gr. *vaccina*			
	COB1	0.85	Nested turnover, variable richness
	KKB1	0.85	Nested turnover, variable richness
	KKB2	0.77	Nested turnover, variable richness
	TPB1	0.69	Nested turnover, variable richness
	PWB1	0.77	Nested turnover, variable richness
	PWB2	0.62	Nested turnover, variable richness
Pseudocyphellaria granulata			
	COB1	0.71	Nested turnover, variable richness
	COB2	0.71	Nested turnover, variable richness
	KKB2	0.71	Nested turnover, variable richness
	TPB1	0.43	Nested turnover, variable richness
	RMB1	0.57	Nested turnover, variable richness
	PWB1	0.71	Nested turnover, variable richness
	PWB2	0.57	Nested turnover, variable richness

Pseudocyphellaria hirsuta			
	COB2	0.80	Nested turnover, variable richness
	PUYB1	0.80	Nested turnover, variable richness
	KKB1	0.40	Nested turnover, variable richness
	KKB2	0.60	Nested turnover, variable richness
	TPB1	0.60	Nested turnover, variable richness
Pseudocyphellaria intricata			
	KKB1	0.33	Nested turnover, variable richness
	KKB2	0.67	Nested turnover, variable richness
	PWB2	0.00	No turnover, constant richness
Pseudocyphellaria lechlerii			
	TPB1	0.50	Nested turnover, variable richness
	RMB1	0.00	No turnover, constant richness
	PWB1	0.50	Nested turnover, variable richness
	PWB2	0.50	Nested turnover, variable richness
Pseudocyphellaria mallota			
	KKB1	0.00	No turnover, constant richness
Pseudocyphellaria norvegica			
	PUYB1	0.50	Nested turnover, variable richness
Pseudocyphellaria scabrosa			
	TPB1	0.00	No turnover, constant richness
	RMB1	0.33	Nested turnover, variable richness
	PWB1	0.33	Nested turnover, variable richness
	PWB2	0.67	Nested turnover, variable richness
Psoroma asperellum			
	KKB1	0.29	Nested turnover, variable richness
Sticta fuliginosa			
	TPB1	0.00	No turnover, constant richness
Sticta hypochra			
	COB1	0.00	No turnover, constant richness
	COB2	0.50	Nested turnover, variable richness

Appendix S18. Beta diversity between forests. Comparisons are made within species forest by forests in each the forest the species is present. βtot: total beta diversity, reflecting both species replacement and loss/gain (βtotal = βrepl + βrich). βrepl: beta diversity explained by replacement of species alone. βrich: beta diversity explained by species loss/gain (richness differences) alone. Description: turnover strategy following Ventre Lespiaucq *et al*., 2021.

Specie	Forest 1	Forest 2	β_{tot}	β_{repl}	β_{rich}	Description
Crocodia guilleminii						
	PUYB2	TPB1	1.00	0.67	0.33	Total replacement, variable richness
	PUYB2	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	TPB1	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
Cyanisticta obvoluta						
	KKB2	TPB1	0.67	0.67	0.00	Partial replacement, constant richness
	KKB2	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
	KKB2	PWB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB2	PWB2	0.80	0.40	0.40	Partial replacement, variable richness
	TPB1	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
	TPB1	PWB1	0.50	0.00	0.50	Nested turnover, variable richness
	TPB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
	RMB1	PWB1	0.80	0.40	0.40	Partial replacement, variable richness
	RMB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
	PWB1	PWB2	0.67	0.67	0.00	Partial replacement, constant richness
Fuscopannaria mediterranea						
	COB1	PWB1	0.50	0.00	0.50	Nested turnover, variable richness
Leptogium decipiens						
	COB2	COB2	0.67	0.67	0.00	Partial replacement, constant richness
Nephroma antarcticum						
	COB2	PUYB1	0.80	0.80	0.00	Partial replacement, constant richness
	COB2	KKB1	0.80	0.80	0.00	Partial replacement, constant richness
	COB2	KKB2	0.83	0.67	0.17	Partial replacement, variable richness
	COB2	TPB1	0.83	0.67	0.17	Partial replacement, variable richness
	COB2	RMB1	0.80	0.80	0.00	Partial replacement, constant richness
	COB2	PWB1	1.00	0.50	0.50	Total replacement, variable richness
	COB2	PWB2	1.00	1.00	0.00	Total replacement, constant richness
	PUYB1	KKB1	0.80	0.80	0.00	Partial replacement, constant richness
	PUYB1	KKB2	0.83	0.67	0.17	Partial replacement, variable richness
	PUYB1	TPB1	0.83	0.67	0.17	Partial replacement, variable richness
	PUYB1	RMB1	0.80	0.80	0.00	Partial replacement, constant richness
	PUYB1	PWB1	1.00	0.50	0.50	Total replacement, variable richness
	PUYB1	PWB2	1.00	1.00	0.00	Total replacement, constant richness
	KKB1	KKB2	0.60	0.40	0.20	Partial replacement, variable richness
	KKB1	TPB1	0.60	0.40	0.20	Partial replacement, variable richness

	KKB1	RMB1	0.50	0.50	0.00	Partial replacement, constant richness
	KKB1	PWB1	1.00	0.50	0.50	Total replacement, variable richness
	KKB1	PWB2	1.00	1.00	0.00	Total replacement, constant richness
	KKB2	TPB1	0.67	0.67	0.00	Partial replacement, constant richness
	KKB2	RMB1	0.60	0.40	0.20	Partial replacement, variable richness
	KKB2	PWB1	1.00	0.40	0.60	Total replacement, variable richness
	KKB2	PWB2	1.00	0.86	0.14	Total replacement, variable richness
	TPB1	RMB1	0.25	0.00	0.25	Nested turnover, variable richness
	TPB1	PWB1	0.75	0.00	0.75	Nested turnover, variable richness
	TPB1	PWB2	0.83	0.67	0.17	Partial replacement, variable richness
	RMB1	PWB1	0.67	0.00	0.67	Nested turnover, variable richness
	RMB1	PWB2	0.80	0.80	0.00	Partial replacement, constant richness
	PWB1	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
Nephroma cellulosum						
	COB1	COB2	0.50	0.00	0.50	Nested turnover, variable richness
	COB1	PUYB1	1.00	1.00	0.00	Total replacement, constant richness
	COB1	PUYB2	0.50	0.00	0.50	Nested turnover, variable richness
	COB1	KKB1	0.00	0.00	0.00	No turnover, constant richness
	COB1	TPB1	1.00	0.67	0.33	Total replacement, variable richness
	COB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	PUYB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	PUYB2	0.00	0.00	0.00	No turnover, constant richness
	COB2	KKB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	TPB1	0.67	0.67	0.00	Partial replacement, constant richness
	COB2	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
	PUYB1	PUYB2	0.50	0.00	0.50	Nested turnover, variable richness
	PUYB1	KKB1	1.00	1.00	0.00	Total replacement, constant richness
	PUYB1	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	PUYB1	RMB1	1.00	0.67	0.33	Total replacement, variable richness
	PUYB2	KKB1	0.50	0.00	0.50	Nested turnover, variable richness
	PUYB2	TPB1	0.67	0.67	0.00	Partial replacement, constant richness
	PUYB2	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
	KKB1	TPB1	1.00	0.67	0.33	Total replacement, variable richness
	KKB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	TPB1	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
Nephroma pseudoparile						
	COB1	COB2	0.00	0.00	0.00	No turnover, constant richness
	COB1	KKB2	0.00	0.00	0.00	No turnover, constant richness
	COB1	TPB1	0.67	0.00	0.67	Nested turnover, variable richness
	COB2	KKB2	0.00	0.00	0.00	No turnover, constant richness
	COB2	TPB1	0.67	0.00	0.67	Nested turnover, variable richness
	KKB2	TPB1	0.67	0.00	0.67	Nested turnover, variable richness
Nephroma skottsbergii						
	PUYB1	PUYB2	0.83	0.33	0.50	Partial replacement, variable richness
Pannaria farinosa						
	TPB1	RMB1	0.67	0.00	0.67	Nested turnover, variable richness

	TPB1	PWB1	0.67	0.00	0.67	Nested turnover, variable richness
	TPB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
	RMB1	PWB1	0.50	0.50	0.00	Partial replacement, constant richness
	RMB1	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
	PWB1	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
Pannaria gr. *sphinctrina*						
	TPB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	TPB1	PWB1	0.75	0.00	0.75	Nested turnover, variable richness
	RMB1	PWB1	0.50	0.00	0.50	Nested turnover, variable richness
Pannaria pulverulacea						
	PUYB1	PUYB2	0.80	0.40	0.40	Partial replacement, variable richness
Parmeliella nigrocinta						
	COB2	PWB1	1.00	0.67	0.33	Total replacement, variable richness
Peltigera collina						
	KKB1	KKB2	0.00	0.00	0.00	No turnover, constant richness
	KKB1	TPB1	0.00	0.00	0.00	No turnover, constant richness
	KKB2	TPB1	0.00	0.00	0.00	No turnover, constant richness
Peltigera hymenina						
	COB1	PUYB1	0.50	0.00	0.50	Nested turnover, variable richness
Pseudocyphellaria dubia						
	TPB1	RMB1	0.00	0.00	0.00	No turnover, constant richness
	TPB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
	RMB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
Pseudocyphellaria faveolata						
	COB1	RMB1	0.67	0.00	0.67	Nested turnover, variable richness
Pseudocyphellaria freycinettii						
	PWB1	PWB2	0.67	0.67	0.00	Partial replacement, constant richness
Pseudocyphellaria glabra						
	COB1	PUYB1	0.33	0.00	0.33	Nested turnover, variable richness
	COB1	PUYB2	0.33	0.00	0.33	Nested turnover, variable richness
	PUYB1	PUYB2	0.00	0.00	0.00	No turnover, constant richness
Pseudocyphellaria gilva						
	KKB1	TPB1	0.67	0.67	0.00	Partial replacement, constant richness
	KKB1	RMB1	0.00	0.00	0.00	No turnover, constant richness
	KKB1	PWB1	0.00	0.00	0.00	No turnover, constant richness
	KKB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
	TPB1	RMB1	0.67	0.67	0.00	Partial replacement, constant richness
	TPB1	PWB1	0.67	0.67	0.00	Partial replacement, constant richness
	TPB1	PWB2	0.67	0.67	0.00	Partial replacement, constant richness
	RMB1	PWB1	0.00	0.00	0.00	No turnover, constant richness
	RMB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
	PWB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
Pseudocyphellaria gr. *argyracea*						
	COB2	PUYB2	0.00	0.00	0.00	No turnover, constant richness
	COB2	KKB1	0.50	0.00	0.50	Nested turnover, variable richness

	COB2	TPB1	0.00	0.00	0.00	No turnover, constant richness
	COB2	RMB1	0.00	0.00	0.00	No turnover, constant richness
	COB2	PWB1	0.00	0.00	0.00	No turnover, constant richness
	COB2	PWB2	0.00	0.00	0.00	No turnover, constant richness
	PUYB2	KKB1	0.50	0.00	0.50	Nested turnover, variable richness
	PUYB2	TPB1	0.00	0.00	0.00	No turnover, constant richness
	PUYB2	RMB1	0.00	0.00	0.00	No turnover, constant richness
	PUYB2	PWB1	0.00	0.00	0.00	No turnover, constant richness
	PUYB2	PWB2	0.00	0.00	0.00	No turnover, constant richness
	KKB1	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	PWB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
	TPB1	RMB1	0.00	0.00	0.00	No turnover, constant richness
	TPB1	PWB1	0.00	0.00	0.00	No turnover, constant richness
	TPB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
	RMB1	PWB1	0.00	0.00	0.00	No turnover, constant richness
	RMB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
	PWB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
Pseudocyphellaria gr. *citrina*						
	COB1	COB2	0.00	0.00	0.00	No turnover, constant richness
	COB1	PUYB2	0.50	0.00	0.50	Nested turnover, variable richness
	COB1	KKB1	0.00	0.00	0.00	No turnover, constant richness
	COB1	KKB2	0.00	0.00	0.00	No turnover, constant richness
	COB1	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB1	PWB1	0.67	0.00	0.67	Nested turnover, variable richness
	COB1	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
	COB2	PUYB2	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	KKB1	0.00	0.00	0.00	No turnover, constant richness
	COB2	KKB2	0.00	0.00	0.00	No turnover, constant richness
	COB2	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	PWB1	0.67	0.00	0.67	Nested turnover, variable richness
	COB2	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
	PUYB2	KKB1	0.50	0.00	0.50	Nested turnover, variable richness
	PUYB2	KKB2	0.50	0.00	0.50	Nested turnover, variable richness
	PUYB2	TPB1	0.00	0.00	0.00	No turnover, constant richness
	PUYB2	RMB1	0.00	0.00	0.00	No turnover, constant richness
	PUYB2	PWB1	0.33	0.00	0.33	Nested turnover, variable richness
	PUYB2	PWB2	0.33	0.00	0.33	Nested turnover, variable richness
	KKB1	KKB2	0.00	0.00	0.00	No turnover, constant richness
	KKB1	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	PWB1	0.67	0.00	0.67	Nested turnover, variable richness
	KKB1	PWB2	0.67	0.00	0.67	Nested turnover, variable richness

	KKB2	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB2	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB2	PWB1	0.67	0.00	0.67	Nested turnover, variable richness
	KKB2	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
	TPB1	RMB1	0.00	0.00	0.00	No turnover, constant richness
	TPB1	PWB1	0.33	0.00	0.33	Nested turnover, variable richness
	TPB1	PWB2	0.33	0.00	0.33	Nested turnover, variable richness
	RMB1	PWB1	0.33	0.00	0.33	Nested turnover, variable richness
	RMB1	PWB2	0.33	0.00	0.33	Nested turnover, variable richness
	PWB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
Pseudocyphellaria gr. *vaccina*						
	COB1	KKB1	0.67	0.67	0.00	Partial replacement, constant richness
	COB1	KKB2	0.33	0.00	0.33	Nested turnover, variable richness
	COB1	TPB1	1.00	0.67	0.33	Total replacement, variable richness
	COB1	PWB1	1.00	0.80	0.20	Total replacement, variable richness
	COB1	PWB2	1.00	0.57	0.43	Total replacement, variable richness
	KKB1	KKB2	0.75	0.50	0.25	Partial replacement, variable richness
	KKB1	TPB1	1.00	0.67	0.33	Total replacement, variable richness
	KKB1	PWB1	1.00	0.80	0.20	Total replacement, variable richness
	KKB1	PWB2	1.00	0.57	0.43	Total replacement, variable richness
	KKB2	TPB1	1.00	0.86	0.14	Total replacement, variable richness
	KKB2	PWB1	1.00	1.00	0.00	Total replacement, constant richness
	KKB2	PWB2	1.00	0.75	0.25	Total replacement, variable richness
	TPB1	PWB1	0.83	0.67	0.17	Partial replacement, variable richness
	TPB1	PWB2	0.88	0.75	0.13	Partial replacement, variable richness
	PWB1	PWB2	0.86	0.57	0.29	Partial replacement, variable richness
Pseudocyphellaria granulata						
	COB1	COB2	0.67	0.67	0.00	Partial replacement, constant richness
	COB1	KKB2	0.67	0.67	0.00	Partial replacement, constant richness
	COB1	TPB1	0.80	0.40	0.40	Partial replacement, variable richness
	COB1	RMB1	0.75	0.50	0.25	Partial replacement, variable richness
	COB1	PWB1	1.00	1.00	0.00	Total replacement, constant richness
	COB1	PWB2	0.75	0.50	0.25	Partial replacement, variable richness
	COB2	KKB2	0.67	0.67	0.00	Partial replacement, constant richness
	COB2	TPB1	0.80	0.40	0.40	Partial replacement, variable richness
	COB2	RMB1	0.75	0.50	0.25	Partial replacement, variable richness
	COB2	PWB1	1.00	1.00	0.00	Total replacement, constant richness
	COB2	PWB2	0.75	0.50	0.25	Partial replacement, variable richness
	KKB2	TPB1	0.80	0.40	0.40	Partial replacement, variable richness
	KKB2	RMB1	0.33	0.00	0.33	Nested turnover, variable richness
	KKB2	PWB1	0.67	0.67	0.00	Partial replacement, constant richness
	KKB2	PWB2	0.33	0.00	0.33	Nested turnover, variable richness
	TPB1	RMB1	0.60	0.40	0.20	Partial replacement, variable richness
	TPB1	PWB1	0.80	0.40	0.40	Partial replacement, variable richness
	TPB1	PWB2	0.60	0.40	0.20	Partial replacement, variable richness
	RMB1	PWB1	0.33	0.00	0.33	Nested turnover, variable richness

	RMB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
	PWB1	PWB2	0.33	0.00	0.33	Nested turnover, variable richness
Pseudocyphellaria hirsuta						
	COB2	PUYB1	1.00	1.00	0.00	Total replacement, constant richness
	COB2	KKB1	0.67	0.00	0.67	Nested turnover, variable richness
	COB2	KKB2	0.50	0.00	0.50	Nested turnover, variable richness
	COB2	TPB1	1.00	0.67	0.33	Total replacement, variable richness
	PUYB1	KKB1	1.00	0.50	0.50	Total replacement, variable richness
	PUYB1	KKB2	1.00	0.67	0.33	Total replacement, variable richness
	PUYB1	TPB1	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	KKB2	0.33	0.00	0.33	Nested turnover, variable richness
	KKB1	TPB1	1.00	0.80	0.20	Total replacement, variable richness
	KKB2	TPB1	1.00	1.00	0.00	Total replacement, constant richness
Pseudocyphellaria intricata						
	KKB1	KKB2	0.50	0.00	0.50	Nested turnover, variable richness
	KKB1	PWB2	0.33	0.00	0.33	Nested turnover, variable richness
	KKB2	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
Pseudocyphellaria lechlerii						
	TPB1	RMB1	0.50	0.00	0.50	Nested turnover, variable richness
	TPB1	PWB1	1.00	1.00	0.00	Total replacement, constant richness
	TPB1	PWB2	1.00	1.00	0.00	Total replacement, constant richness
	RMB1	PWB1	0.50	0.00	0.50	Nested turnover, variable richness
	RMB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
	PWB1	PWB2	0.00	0.00	0.00	No turnover, constant richness
Pseudocyphellaria scabrosa						
	TPB1	RMB1	0.33	0.00	0.33	Nested turnover, variable richness
	TPB1	PWB1	0.33	0.00	0.33	Nested turnover, variable richness
	TPB1	PWB2	0.67	0.00	0.67	Nested turnover, variable richness
	RMB1	PWB1	0.00	0.00	0.00	No turnover, constant richness
	RMB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
	PWB1	PWB2	0.50	0.00	0.50	Nested turnover, variable richness
Sticta hypochra						
	COB1	COB2	0.50	0.00	0.50	Nested turnover, variable richness

CHAPTER 2

Cyanobacterial variability in lichen cephalodia.

María Prieto, Natalia Montané, Gregorio Aragón, Isabel Martínez and Clara Rodríguez-Arribas

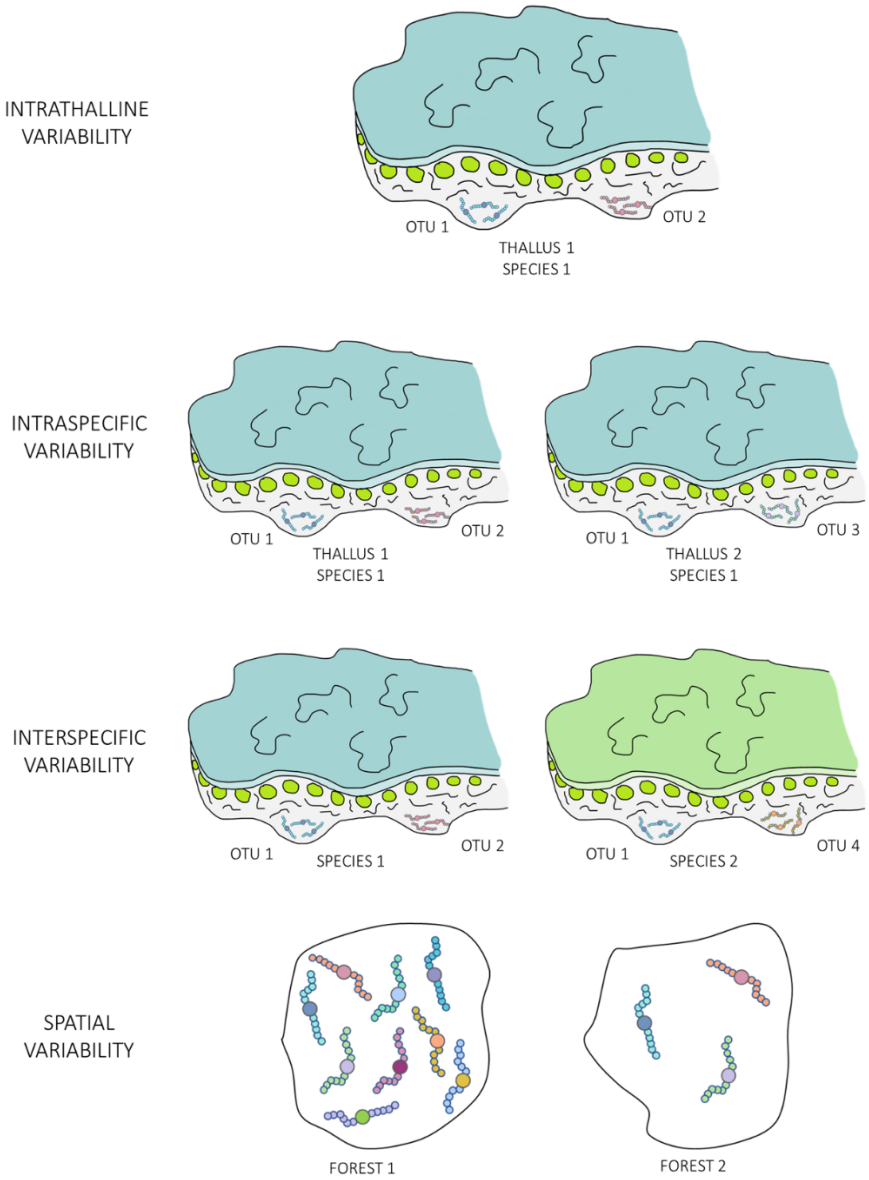
Manuscript published in *Journal of Fungi* 2023

Abstract

The ecological success of lichens is related to both myco- and photobionts which condition the physiological limits of the lichen symbioses and thus affect their ecological niches and geographic ranges. A particular type of lichen, called cephalolichen, is characterized by housing both green algal and cyanobacterial symbionts—the latter is restricted to special structures called cephalodia. In this type of lichen, questions related to specialization within species or within individuals are still unsolved as different patterns have previously been observed. In order to study the variability at the intrathalline, intraspecific, and interspecific level, cyanobionts from different cephalodia within the same thalli and from different thalli were genetically analysed in three cephalolichen species at two different forests (18 thalli, 90 cephalodia). The results showed variability in the cephalodial *Nostoc* OTUs in all the studied species, both at the intrathalline and intraspecific levels. The variability of *Nostoc* OTUs found in different cephalodia of the same thallus suggests low specialization in this relationship. Additionally, differences in OTU diversity in the three studied species and in the two forests were found. The variability observed may confer an increased ecological plasticity and an advantage to colonize or persist under additional or novel habitats or conditions.

Keywords

Cyanolichens, intrathalline, rbcLX, *Nephroma antarcticum*, *Nostoc*, *Pannaria farinosa*, *Pseudocyphellaria granulata*, specialization, symbiosis.



Graphical abstract. Intrathalline, intraspecific, interspecific and spatial variability.

Introduction

Lichenization is a successful nutritional strategy, with almost 20% of all fungal species being lichenized (Kirk *et al*., 2008) and dominating about 8% of the land surface of the world (Ahmadjian, 1995). According to photobiont association, lichens are divided into two groups. Bipartite lichens are characterized by a mycobiont establishing with green algae (chlorolichen) or cyanobacteria (cyanolichen), in which the photosynthetic partner (phycobiont) provides carbon products to the mycobiont. In tripartite lichens (cephalolichens), the fungus is simultaneously associated with both green algae

(photobiont) and cyanobacteria (cyanobiont). In these lichens, the photobiont is distributed through the thallus, providing fixed carbon, and the cyanobiont is often confined to special structures, called cephalodia, supplying the fixed nitrogen. In this case, cyanobionts are specialized for nitrogen fixation; they are predominantly heterotrophic and show an increase in heterocyst proportions over 30% of non-symbiotic cyanobacterial cells and lichenized cyanobionts of bipartite cyanolichens (Kershaw, 1985; Rai, 1998).

Although lichens are compound organisms, and the physiological limits of the lichen symbiosis are driven by the association as an integrated whole, some aspects are specific to the photosynthetic symbiont (Stanton *et al*., 2023). Thus, photobionts are involved in photosynthesis, secondary compound production, nitrogen fixation, etc., and their preferences regarding abiotic conditions may limit the ecological niches and geographic ranges of lichens (Fernández-Mendoza *et al*., 2011; Peksa & Škaloud, 2011; Rolshausen *et al*., 2018, 2020). In this respect, the specialization of the fungal photobiont association is related to the potential acclimation to local environmental conditions and colonization of new niches and geographic regions. Thus, low specialization between myco- and photobionts may preclude limitations if mycobionts have the ability to lichenize with different photobionts (Muggia *et al*., 2014) as they can expand their ecological niches and geographic distributions outside the physiological limits imposed by a single photobiont species. On the other hand, specialized species may have narrower geographical distributions and ecological niches (Leavitt *et al*., 2015; Magain *et al*., 2017). While there are different attributes to characterize specialization in biotic interactions, the classification of organisms as specialists or generalist has been mostly based on the number of interacting partners (Blüthgen *et al*., 2006; Sahli & Conner, 2006); however see Rodríguez-Arribas *et al*., *under review*. The variability in the photobiont partner is, thus, related to the specialization of the mycobiont for the photobiont and may be observed at different levels (biological, geographical, or ecological; Fedrowitz *et al*., 2012; Fox & Morrow, 1981; Herrera *et al*., 2019; Lu *et al*., 2018; Muggia *et al*., 2014; Rodríguez-Arribas *et al*., 2023). At the biological level (individual, species, or genus levels), numerous studies have shown contrasting patterns: from a high specialization of mycobiont towards the photobiont, (i.e., Leavitt *et al*., 2015; Myllys *et al*., 2007; O'Brien *et al*., 2013; Paulsrud *et al*., 1998; Piercey-Normore & DePriest, 2001), to a common pattern of generalization (i.e.,

a high number of interacting photobionts; Muggia *et al*., 2013; Sadowska-Deś *et al*., 2014; Wirtz *et al*., 2003). When comparing specialization between bi-membered (cyanolichens and chlorolichens) and tri-membered (cephalolichens) lichen species, several patterns have been found. A high specialization hypothesized for cephalolichens (Paulsrud *et al*., 2001) differed from the results of (Rodríguez-Arribas *et al*., 2023), showing that cephalolichens are more generalized than cyanolichens, and from other studies, which found no differences between both (Hoz *et al*., 2018; Wirtz *et al*., 2003). At the intrathalline (individual) level, and concerning cephalolichens specifically, it has been shown that most tripartite lichens contain the same *Nostoc* strain in all cephalodia of individual thalli (Paulsrud *et al*., 2001), with the exception of *Peltigera venosa*, *Lobaria pulmonaria*and three species of *Pannaria* (*P. farinosa*, *P. sphinctrina* and *P. lobulifera*), which housed different cyanobionts in different cephalodia (Elvebakk *et al*., 2008; Myllys *et al*., 2007; Paulsrud *et al*., 2000). However, this conclusion is based on very few studies analysing specifically the intrathalline variation in cephalodia. Additionally, concerning species with green algae, it has been shown that the co-occurrence of several photobionts in individual lichen thalli is relatively common (Bačkor *et al*. , 2010; Moya *et al*., 2017; Muggia *et al*., 2014; Onuţ-Brännström *et al*., 2018; Vančurová *et al*., 2018). At the community scale, it has been revealed that lichens do not show a one-to-one relationship as different species of cyanobacteria are shared between different lichen species (Onuţ-Brännström *et al*., 2018; Paulsrud *et al*., 1998, 2000; Peksa *et al*., 2022), like the Lichen Guilds theory proposes (Rikkinen, 2003; Rikkinen *et al*., 2002).

On the other hand, factors driving photobiont selection are diverse, and related to phylogenetic relationships, reproductive strategy, the availability of photobionts, or ecological factors (Hoz *et al*., 2018; Lu *et al*., 2018; Magain *et al*., 2017; Mark *et al*., 2020; O'Brien *et al*., 2013; Otálora *et al*., 2010; Peksa *et al*., 2022; Singh *et al*., 2017; Yahr *et al*., 2006). In a recent study, the authors showed that photobiont availability, the function of the cyanobiont (principal photobiont for cyanolichens or secondary photobiont for cephalolichens) and the mycobiont species were factors related with specialization in cyanobacterial lichens (Rodríguez-Arribas *et al*., 2023).

Based on this previous study, of which shows a high variability of *Nostoc* phylogroups in different lichen species along a latitudinal gradient in Chile, a question

arose on whether this variability was also present at the intrathalline (individual) level, and if this variation may be related with the geographic gradient studied. As stated before, this variability had been observed in several tripartite lichen species previously (Elvebakk *et al*., 2008; Myllys *et al*., 2007; Paulsrud *et al*., 2000). Thus, the aims of this study were to investigate if the individual thalli of tripartite lichens host different *Nostoc* genotypes depending on the species and on the studied forest. For this purpose, three cephalolichens species were selected at two different forests at different latitudes in Chile, and different cephalodia per thallus were genetically characterized.

Methods

Two forests from a previous study carried out in Chile and sampled between 2017 and 2018, were used (Parque Nacional Torres del Paine and Isla Navarino; Rodríguez-Arribas *et al*., 2023). Forest stands were mostly formed by *Nothofagus pumilio* with over 65% of cover. Within these forests, three species of Peltigerales with cephalodia were collected: *Pannaria farinosa*, *Nephroma antarcticum* and *Pseudocyphellaria granulata*. From each species, 6 thalli were selected (3 at each forest), and from each thallus, 5 different cephalodia were analysed. Samples were air-dried and stored at-20ºC. DNA from different cephalodia in each thallus was extracted using Chelex® 100 Chelating Resin (Bio-Rad, Hercules, CA, USA). Region rbcLX was amplified using primers CW and CX (Rudi *et al*., 1998) and the following program: 95 ºC 15 min; 35 cycles of 1 min at 95 ºC, 30 s at 54 ºC, 30 s at 72 ºC; and 10 min at 72 ºC. The PCR products were sequenced at Macrogen Spain service (www.macrogen.com (accessed on 21st September 2020)) using the same primers employed in the PCR.

The obtained sequences were edited and aligned using Geneious Prime v. 2021.0.1 software (https://www.geneious.com (accessed on 11th January 2021)). Some samples failed in the PCR or were too short and were discarded. Ambiguous regions and introns were delimited manually and excluded for the analysis using AliView v. 1.26, Uppsala, Sweeden (Larsson, 2014). *Nostoc* sequences were grouped into operational taxonomic units (OTUs). The delimitation of *Nostoc* OTUs was based on the ASAP method (Assemble Species by Automatic Partitioning; Puillandre *et al*., 2021), which proposes

partitions of species hypotheses using genetic distances calculated between DNA sequences. Additionally, we performed a phylogenetic and network analysis. The ASAP analysis was carried out in the webserver (https://bioinfo.mnhn.fr/abi/public/asap/ (accessed on 9th March 2021)) applying the Jukes-Cantor (JC69) model of substitution (groups below 0.01 probability were split, the 10 best scores were kept, and-1 was the seed value). Partitions included in the 0.001-0.01 range of genetic distances were selected. A maximum likelihood phylogenetic analysis (ML) was conducted with RAxML v. 8.2.12, Karlsruhe, Germany (Stamatakis, 2014), assuming the GTRGAMMA model. The node support was estimated with the rapid bootstrap algorithm, using 1000 pseudoreplicates. A haplotype network was constructed using the TCS method (Clement *et al*., 2002), as implemented in PopART v .1.7, Dunedin, New Zeland (http://popart.otago.ac.nz (accessed on 17th July 2023)).

Results

A total of 61 cyanobacterial consensus sequences were obtained (Table 1). The best partition obtained in ASAP was selected based on the lower score. Thus, a total of nine OTUs were found consorting with the three species (Table 1). This result is congruent with the ML phylogenetic analyses and the haplotype network, as shown in Figures 1 and 2. *Nostoc* OTUs were shared between the species. Thus, OTUs 2 (present in 25 cephalodia out of 58), 1 (11), 3 (7) and 6 (6) were the most abundant, while the rest of OTUs (except 4) were only found in 1 cephalodia (Table 1; Figure 3).

Table 2. Voucher and GenBank numbers of the species studied with thalli, cephalodia, and forest information and OTU classification based on the ASAP analysis, ML phylogenetic analysis, and TCS haplotype network. TP: Torres del Paine, IN: Navarino Island.

Species	Thallus	Cephalodia	OTU ASAP	Forest	Voucher	GenBank
Nephroma antarcticum	NM490	A3	1	TP	ARAN20283	OR344442
Nephroma antarcticum	NM490	A6	2	TP		OR344444
Nephroma antarcticum	NM490	A7	2	TP		OR344445
Nephroma antarcticum	NM490	A8	2	TP		OR344446
Nephroma antarcticum	NM491	A3	3	TP	ARAN20284	OR344447

Nephroma antarcticum	NM491	A1	3	TP		OR344449
Nephroma antarcticum	NM491	A2	3	TP		OR344450
Nephroma antarcticum	NM491	A4	3	TP		OR344451
Nephroma antarcticum	NM491	A5	4	TP		OR344448
Nephroma antarcticum	NM493	A5	5	TP	ARAN20285	OR344452
Nephroma antarcticum	NM493	A2	6	TP		OR344454
Nephroma antarcticum	NM493	A3	6	TP		OR344455
Nephroma antarcticum	NM493	A4	6	TP		OR344456
Nephroma antarcticum	NM722	A3	2	IN	ARAN20292	OR344474
Nephroma antarcticum	NM722	A6	2	IN		OR344476
Nephroma antarcticum	NM722	A5	2	IN		OR344475
Nephroma antarcticum	NM722	A7	2	IN		OR344477
Nephroma antarcticum	NM724	A6	1	IN	ARAN20293	OR344478
Nephroma antarcticum	NM724	A7	2	IN		OR344479
Nephroma antarcticum	NM727	A3	3	IN	ARAN20294	OR344481
Nephroma antarcticum	NM727	A2	2	IN		OR344480
Nephroma antarcticum	NM727	A4	2	IN		OR344482
Nephroma antarcticum	NM727	A6	2	IN		OR344484
Nephroma antarcticum	NM727	A8	2	IN		OR344486
Nephroma antarcticum	NM727	A5	2	IN		OR344483
Nephroma antarcticum	NM727	A7	2	IN		OR344485
Pannaria farinosa	NM473	A4	1	TP		OR344431
Pannaria farinosa	NM473	A8	1	TP	ARAN20286	OR344432
Pannaria farinosa	NM477	A1	1	TP	ARAN20287	OR344433
Pannaria farinosa	NM477	A2	2	TP		OR344434
Pannaria farinosa	NM478	A5	1	TP	ARAN20288	OR344441
Pannaria farinosa	NM741	A5	2	IN	ARAN20295	OR344487
Pannaria farinosa	NM743	A6	1	IN	ARAN20296	OR344488
Pannaria farinosa	NM746	A1	2	IN	ARAN20297	OR344489
Pseudocyphellaria granulata	NM547	A4	6	TP	ARAN20289	OR344460
Pseudocyphellaria granulata	NM547	A5	6	TP		OR344461

Pseudocyphellaria granulata	NM547	A2	6	TP		OR344458
Pseudocyphellaria granulata	NM547	A3	3	TP		OR344459
Pseudocyphellaria granulata	NM547	A1	4	TP		OR344457
Pseudocyphellaria granulata	NM548	A5	3	TP	ARAN20290	OR344465
Pseudocyphellaria granulata	NM548	A2	7	TP		OR344462
Pseudocyphellaria granulata	NM548	A4	7	TP		OR344463
Pseudocyphellaria granulata	NM548	A1	2	TP		OR344464
Pseudocyphellaria granulata	NM549	A4B	7	TP	ARAN20291	OR344469
Pseudocyphellaria granulata	NM549	A5	7	TP		OR344470
Pseudocyphellaria granulata	NM549	A3	8	TP		OR344471
Pseudocyphellaria granulata	NM549	A4	9	TP		OR344472
Pseudocyphellaria granulata	NM549	A2B	1	TP		OR344467
Pseudocyphellaria granulata	NM549	A3B	1	TP		OR344468
Pseudocyphellaria granulata	NM549	A1	1	TP		OR344466
Pseudocyphellaria granulata	NM783	A3	2	IN	ARAN20298	OR344491
Pseudocyphellaria granulata	NM783	A6	2	IN		OR344492
Pseudocyphellaria granulata	NM784	A5	2	IN	ARAN20299	OR344496
Pseudocyphellaria granulata	NM784	A2	2	IN		OR344494
Pseudocyphellaria granulata	NM784	A1	2	IN		OR344493
Pseudocyphellaria granulata	NM784	A3	2	IN		OR344495
Pseudocyphellaria granulata	NM788	A7	1	IN	ARAN20300	OR344500
Pseudocyphellaria granulata	NM788	A6	2	IN		OR344499
Nephroma antarcticum	NM490	A3	1	TP	ARAN20283	OR344442
Nephroma antarcticum	NM490	A6	2	TP		OR344444
Nephroma antarcticum	NM490	A7	2	TP		OR344445
Nephroma antarcticum	NM490	A8	2	TP		OR344446

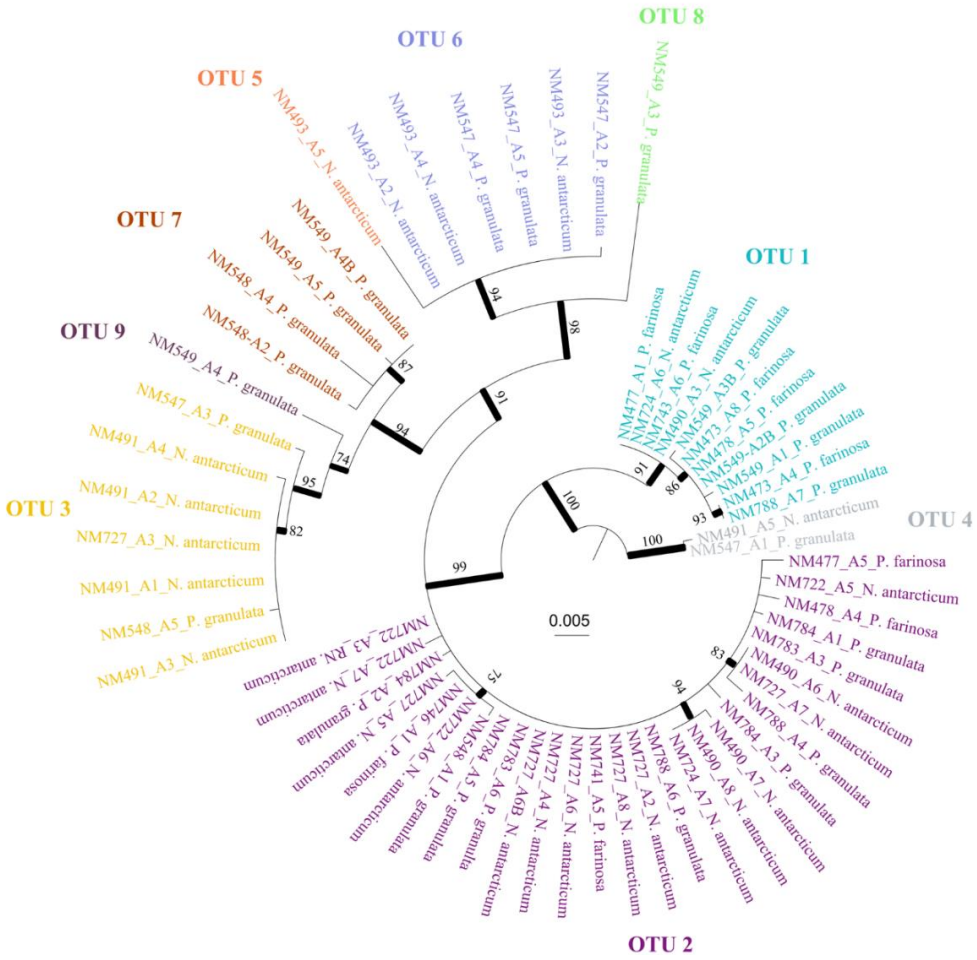


Figure 1. Best tree from the ML analysis of the rbcLX region. Bootstrap values ≥ 70% are indicated on or below the branches and with thicker lines. OTUs delimited based on the ASAP results are depicted in the tree and represented with different colours.

The obtained results were different in the different species and in both forests (Table 1 and Figure 3). Thus, *Pseudocyphellaria granulata* had the highest variability in OTUs followed by *Nephroma antarcticum*, which also showed high variability. The results in *Pannaria farinosa* showed lower variability in its cyanobionts. In addition, the two OTUs found in *Pannaria farinosa* (OTU 1 and 2) were shared with the other two lichen species. Also, OTUs 3, 4 and 6 were found in both *Pseudocyphellaria granulata* and *Nephroma antarcticum*. On the other hand, OTU 5 was exclusive from *Nephroma antarcticum*, while OTUs 7, 8, and 9 were only found in *Pseudocyphellaria granulata*.

When comparing forests, a significant difference was observed between Torres del Paine and Navarino Island, as the latter showed lower diversity in the cyanobionts found, and there were also differences in their abundances. Nine OTUs were found in Torres del

Paine with a dominance of OTU 1; meanwhile, three OTUs were found in Navarino, with OTU 2 being dominant. Nonetheless, the OTUs from Navarino Island were a subset of those from Torres del Paine, showing a nested pattern.

The three species also showed differences in their cyanobionts depending on the forest. *Nephroma antarcticum* had a similar proportion of OTUs 2 and 3 in Torres del Paine, while *Pseudocyphellaria granulata* had a higher proportion of OTU 7. All three species showed a dominance of OTU 2 in Navarino.

All the species showed variability in their cephalodial *Nostoc* at the intrathaline (individual) and intraspecific levels. All thalli except one of *N. antarcticum* (five in total) had two different OTUs per thallus. In *Pseudocyphellaria granulata*, three thalli presented between three and four *Nostoc* OTUs. In this species, two thalli from Navarino only had one OTU and other sample had two OTUs. Despite the poor results obtained in *Pannaria farinosa*, as only nine cephalodia were successfully sequenced, the studied thalli had two OTUs.

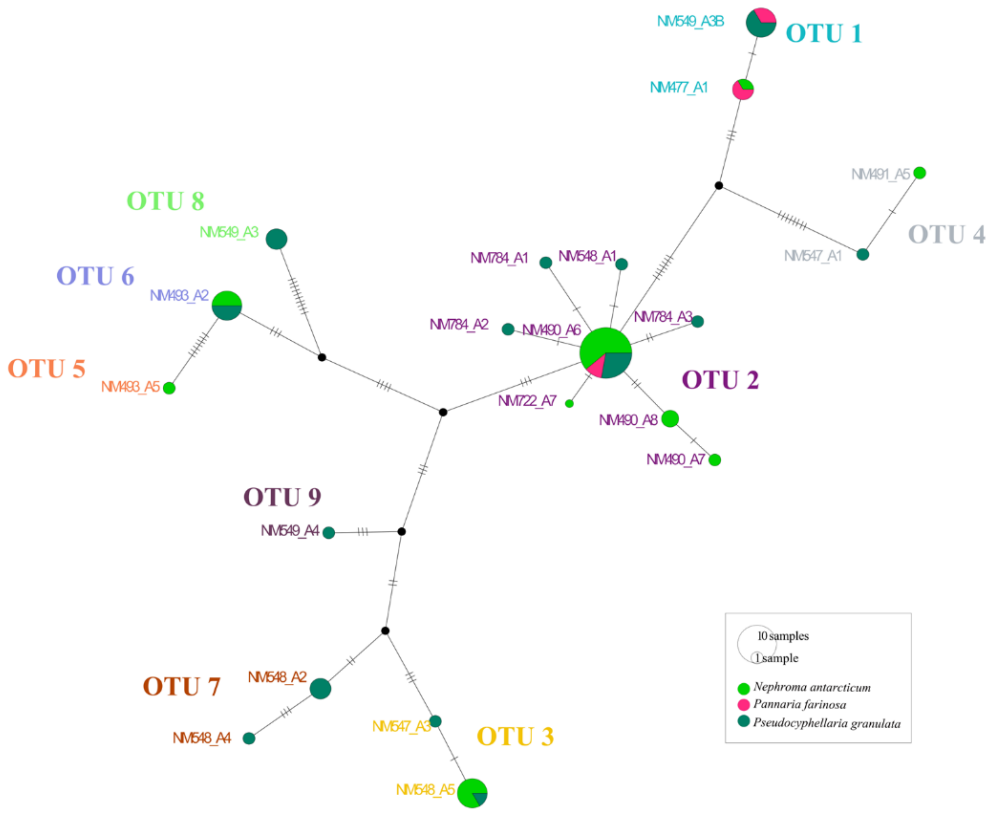


Figure 2. The TCS haplotype network for *Nostoc* rbcLX sequences. The size of the pie chart is proportional to the number of thalli belonging to the haplotype, and the colour is according to the 3

studied species. The dash on the line represents one mutational step of the haplotype sequence. Black-filled circles indicate missing haplotypes. Haplotypes are grouped in OTUs obtained by the ASAP analysis.

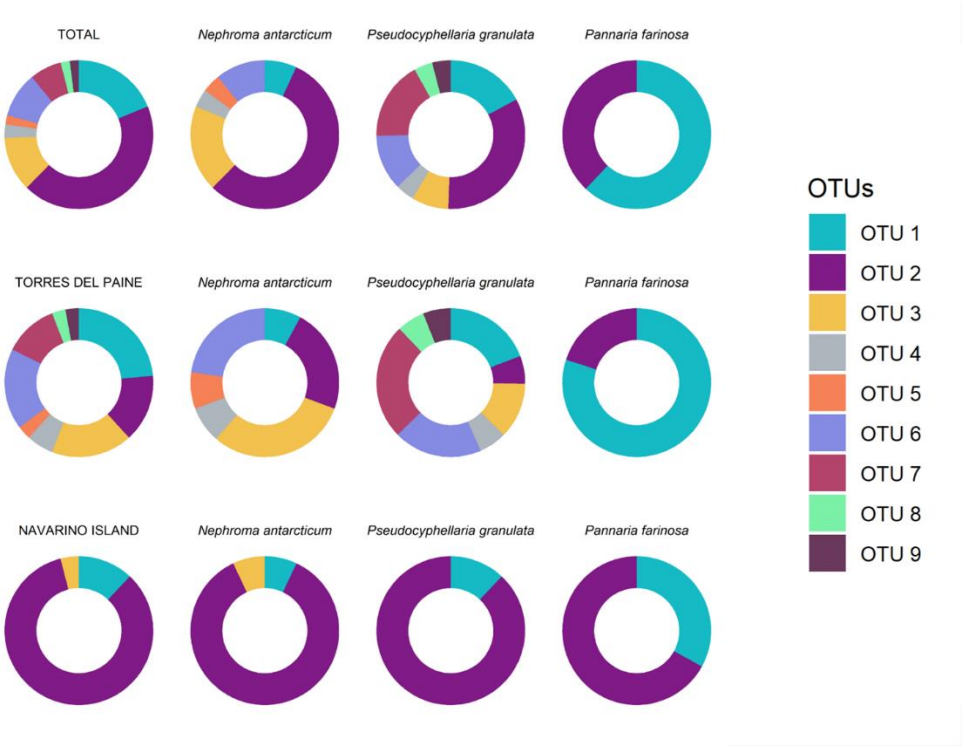


Figure 3. The abundance (richness) of *Nostoc* OTUs found in the studied species at the different levels.

Discussion

In relation to the variability of the photobiont partners in lichenized fungi, many basic questions remain unknown. An important step is to learn about this variability of photobionts in lichens at different levels, from the individual to the ecological communities. The results from this study, based on the richness of cyanobionts in tripartite lichens (cephalolichens), showed differences in the *Nostoc* OTUs from cephalodia at different scales: thallus, species, and forests.

At the lowest level, the thallus (individual), a high variability of cyanobionts was found in different cephalodia of the same thallus. Although there was a difference in the number of OTUs per thalli between the three studied species, all three showed the ability

to harbour more than one and different *Nostoc* OTUs within a thallus. The coexistence inside a single lichen thallus of different *Trebouxia* species has been previously demonstrated (Blázquez *et al*., 2022; Del-Campo *et al*., 2010; Muggia *et al*., 2013; Piercey-Normore *et al*., 2006), but it has rarely been studied in tripartite lichens (e.g., in *Lobaria pulmonaria*, *Peltigera venosa* and *Pannaria*; Elvebakk *et al*., 2008; Myllys *et al*., 2007; Paulsrud *et al*., 2000). In the case of *Lobaria pulmonaria* (Myllys *et al*., 2007), only one of the studied thalli showed different *Nostoc* genotypes in different cephalodia. The results obtained in *Pannaria*, showed non identical *Nostoc* 16S sequences from different cephalodia in the same thallus in three *Pannaria* individuals belonging to *P. farinosa*, *P. sphinctrina* and *P. lobulifera* (Elvebakk *et al*., 2008). However, the genetic distance between these sequences is not enough to consider them as different haplotypes, at least in *P. sphinctrina* and *P. lobulifera*. In addition, previous morphological studies not using molecular data observed different cyanobacterial morphotypes within single thalli and occasionally even in the same cephalodium (Jordan & Rickson, 1971; Tschermak-Woess, 1989).

The ecological significance of photobiont coexistence in the same thallus is not clear. Additionally, it is not clear if this occurrence is a widespread phenomenon. On the one hand, the same mycobiont with different photobionts haa shown differences in various aspects of its physiology (Casano *et al*., 2011). On the other hand, (Blázquez *et al*., 2022) demonstrated that the contribution of the secondary photobionts was marginal in most thalli. Additionally, (Paulsrud *et al*., 2000) suggested the possibility of the existence of different degrees of lichenization with different *Nostoc* strains, ranging from loosely associated colonies to well-corticated cephalodia in *Peltigera venosa*. It is unlikely that a loose association between the myco- and cyanobiont occurs in cephalodia- it has been shown that there is a high specific biorecognition process involved in the acquisition of the cephalodial *Nostoc* (Lehr *et al*., 2000), performed by specific lectins produced and secreted by the mycobiont. Thus, lectins produced by a vegetative fungal component in *Peltigera aphthosa* were shown to have a similar function in selecting the compatible cephalodial cyanobacterium as lectins produced by germinating spores. The observed photobiont diversity was already predicted to operate on the level of single cyanolichen thalli, especially in the case of cephalodiate species (Rikkinen, 2013).

At the species level (comparing different thalli from the same species), numerous studies have shown contrasting patterns: from a high specialization of mycobiont towards the photobiont (Leavitt *et al*., 2015; Myllys *et al*., 2007; O'Brien *et al*., 2013; Paulsrud *et al*., 1998; Piercey-Normore & DePriest, 2001), to a common pattern of generalization (i.e., a high number of interacting photobionts) (Elvebakk *et al*., 2008; Muggia *et al*., 2013; Sadowska-Deś *et al*., 2014; Wirtz *et al*., 2003). Previous results (Paulsrud *et al*., 1998) have shown that the identity of cyanobionts was related with the species identity of the lichen-forming fungus rather than the geographical area where the lichen was growing. In this previous example, the same lichen species collected in Sweden and Finland (i.e., *Peltigera aphthosa*, *P. canina* and *Nephroma arcticum*) had the same identical intron sequences in different samples of the same lichen species. Conversely, in the present study, the location (forests) determined the cyanobiont variability. Thus, in Navarino Island, there was a lower diversity of cyanobionts for the three studied species, suggesting that environmental variables may determine the cyanobiont pool found in ecological communities (Rodríguez-Arribas *et al*., 2023).

In many cases (O'Brien *et al*., 2005; Piercey-Normore & DePriest, 2001), one fungal species can associate with more than one symbiont, and these are often also shared by several taxonomically unrelated cyanolichen species. This is also in line with the Lichen Guild theory (Peksa *et al*., 2022), where different species shared the same *Nostoc* genotype. As in our results, the most common *Nostoc* OTUs were shared between the three species, pointing out the existence of facilitation in a community context where some species may act as core species (source of cyanobionts), whereas others may act as fringe species, capturing their photobionts from the former ones (Cardós *et al*., 2019). Species with the ability to associate with many OTUs (generalized), may host compatible OTUs for other more specialized species, with the former acting as a core species, as could be *Pseudocyphellaria granulata* in the current study.

Several mechanisms have been proposed to explain the differences in specialization in lichens. Previous studies suggest that geographic and ecological factors including macroclimatic variables can drive the differences in the specialization of the association in both cyano- and chlorolichens (Leavitt *et al*., 2015; Mark *et al*., 2020). In general, a low specialization towards phycobionts allows for the host to associate with

ecologically diversified or locally adapted algae, thereby broadening the lichen ecological amplitude (Peksa & Škaloud, 2011; Vančurová *et al*., 2018). For instance, a previous study analyzed *Nostoc* cyanobionts from five lichen species in maritime Antarctica and determined that lichens from those regions were more generalized than lichens from temperate and boreal regions; this was regarded as lichens adapting to extreme environmental stress (Wirtz *et al*., 2003). However, this finding opposed the results obtained here. In addition, environmental factors have been found to affect the *Nostoc* pool at the community scale (Rodríguez-Arribas *et al*., 2023). In Navarino Island, the diversity of *Nostoc* genotypes found was lower than in Torres del Paine, harbouring a subset of the genotypes found at northern locations (i.e., Torres del Paine). The ability of mycobiont species to interact with different cyanobionts in different localities, even though they are present, may allow for some fungal hosts to associate with the cyanobacterial genotypes that are optimally adapted to the conditions (Rikkinen, 2013).

Our study also notices the high variability of *Nostoc* OTUs (low specialization) at different biological and spatial scales in cephalolichens. This high variability of partners, even at the intrathalline level, could be due to a low dependence on the cyanobiont in those tri-membered lichens, as the main photosynthetic partner is green algae, which conducts the photosynthetic activity, whereas the cyanobiont's main function is nitrogen fixation (Paulsrud *et al*., 2001; Rai *et al*., 2000). In addition, this study emphasizes the importance of the contextualization of the scale, as results based on one site could change when widening the spatial scale, and thus limit the understanding of specialization. Also, it highlights the importance of determining the local availability of cyanobionts, as differences in specialization between locations may be due to a different pool of the cyanobionts available to interact.

References

Ahmadjian, V. (1995). Lichens are more important than you think. *BioScience*, *45*(3), 124. https://doi.org/10.1093/bioscience/45.3.124

Bačkor, M., Peksa, O., Škaloud, P., & Bačkorová, M. (2010). Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences. *Ecotoxicology and Environmental Safety*, *73*(4), 603–612. https://doi.org/10.1016/j.ecoenv.2009.11.002

Blázquez, M., Hernández-Moreno, L. S., Gasulla, F., Pérez-Vargas, I., & Pérez-Ortega, S. (2022). The Role of Photobionts as Drivers of Diversification in an Island Radiation of Lichen-Forming Fungi. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.784182

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*. https://doi.org/10.1186/1472-6785-6-9

Cardós, J. L. H., Prieto, M., Jylhä, M., Aragón, G., Molina, M. C., Martínez, I., & Rikkinen, J. (2019). A case study on the re-establishment of the cyanolichen symbiosis: Where do the compatible photobionts come from? *Annals of Botany*, *124*(3), 379–388. https://doi.org/10.1093/aob/mcz052

Casano, L. M., Del Campo, E. M., García-Breijo, F. J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., … Barreno, E. (2011). Two Trebouxia algae with different physiological performances are ever-present in lichen thalli of Ramalina farinacea. Coexistence versus Competition? *Environmental Microbiology*, *13*(3), 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x

Clement, M., Snell, Q., Walke, P., Posada, D., & Crandall, K. (2002). TCS: Estimating gene genealogies. In *Proceedings - International Parallel and Distributed Processing Symposium, IPDPS 2002* (p. 184). https://doi.org/10.1109/IPDPS.2002.1016585

Del-Campo, E. M., Gimeno, J., De-Nova, J. P. G., Casano, L. M., Gasulla, F., García-Breijo, F. J., … Barreno, E. (2010). South European populations of Ramalina farinacea (L.) Ach. share different Trebouxia algae. *Biology of Lichens – Symbiosis, Ecology, Environm. Monitoring, Systematics, Cyber Applications*, *105*(23), 247–256. https://doi.org/10.13140/RG.2.1.1107.1449

Elvebakk, A., Papaefthimiou, D., Robertsen, E. H., & Liaimer, A. (2008). Phylogenetic patterns among Nostoc cyanobionts within bi- and tripartite lichens of the genus Pannaria. *Journal of Phycology*, *44*(4), 1049–1059. https://doi.org/10.1111/j.1529-8817.2008.00556.x

Fedrowitz, K., Kaasalainen, U., & Rikkinen, J. (2012). Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. *Ecology and Evolution*, *2*(9), 2291–2303. https://doi.org/10.1002/ece3.343

Fernández-Mendoza, F., Domaschke, S., García, M. A., Jordan, P., Martín, M. P., & Printzen, C. (2011). Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. *Molecular Ecology*, *20*(6), 1208–1232. https://doi.org/10.1111/j.1365-294X.2010.04993.x

Fox, L. R., & Morrow, P. A. (1981). Specialization: Species property or local phenomenon. *Science*, *211*(4485), 887–893. https://doi.org/10.1126/science.211.4485.887

Herrera, P., Suárez, J. P., Sánchez-Rodríguez, A., Molina, M. C., Prieto, M., & Méndez, M. (2019). Many broadly-shared mycobionts characterize mycorrhizal interactions of two coexisting epiphytic orchids in a high elevation tropical forest. *Fungal Ecology*, *39*, 26–36.

https://doi.org/10.1016/j.funeco.2018.11.003

Hoz, C. J. P. D. La, Magain, N., Lutzoni, F., Goward, T., Restrepo, S., & Miadlikowska, J. (2018). Contrasting Symbiotic Patterns in Two Closely Related Lineages of Trimembered Lichens of the Genus Peltigera. *Frontiers in Microbiology*, *9*, 1–14. https://doi.org/10.3389/fmicb.2018.02770

Jordan, W. P., & Rickson, F. R. (1971). Cyanophyte cephalodia in the lichen genus Nephroma. *American Journal of Botany*, *58*(6), 562–568. https://doi.org/10.1002/j.1537-2197.1971.tb10005.x

Kershaw, K. A. (1985). *Physiological ecology of lichens*. Cambridge, UK: Cambridge University Press.

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). *Dictionary of the Fungi* (10th Ed). Wallingford, UK,.

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

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

Lehr, H., Galun, M., Ott, S., Jahns, H. M., & Fleminger, G. (2000). Cephalodia of the lichen Peltigera aphthosa (L.) Willd. Specific recognition of the compatible photobiont. *Symbiosis*, *29*(4), 357–365.

Lu, J., Magain, N., Miadlikowska, J., Coyle, J. R., Truong, C., & Lutzoni, F. (2018). Bioclimatic factors at an intrabiome scale are more limiting than cyanobiont availability for the lichen-forming genus Peltigera. *American Journal of Botany*, *105*(7), 1198–1211. https://doi.org/10.1002/ajb2.1119

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Mark, K., Laanisto, L., Bueno, C. G., Niinemets, Ü., Keller, C., & Scheidegger, C. (2020). Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *New Phytologist*, *227*(5), 1362–1375. https://doi.org/10.1111/nph.16475

Moya, P., Molins, A., Martínez-Alberola, F., Muggia, L., & Barreno, E. (2017). Unexpected associated microalgal diversity in the lichen Ramalina farinacea is uncovered by pyrosequencing analyses. *PLOS ONE*, *12*(4), e0175091. https://doi.org/10.1371/journal.pone.0175091

Muggia, L., Pérez-Ortega, S., Kopun, T., Zellnig, G., & Grube, M. (2014). Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Annals of Botany*, *114*(3), 463–475. https://doi.org/10.1093/aob/mcu146

Muggia, L., Vancurova, L., Škaloud, P., Peksa, O., Wedin, M., & Grube, M. (2013). The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS Microbiology Ecology*, *85*(2), 313–323. https://doi.org/10.1111/1574-6941.12120

Myllys, L., Stenroos, S., Thell, A., & Kuusinen, M. (2007). High cyanobiont selectivity of epiphytic lichens in old growth boreal forest of Finland. *New Phytologist*, *173*(3), 621–629. https://doi.org/10.1111/j.1469-8137.2006.01944.x

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2005). Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen fungus Peltigera. *European Journal of Phycology*, *40*(4), 363–378. https://doi.org/10.1080/09670260500342647

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2013). Assessing population structure and host specialization in lichenized cyanobacteria. *New Phytologist*, *198*(2), 557–566. https://doi.org/10.1111/nph.12165

Onuţ-Brännström, I., Benjamin, M., Scofield, D. G., Heiŋmarsson, S., Andersson, M. G. I., Lindström, E. S., & Johannesson, H. (2018). Sharing of photobionts in sympatric populations of Thamnolia and Cetraria lichens: Evidence from high-throughput sequencing. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-22470-y

Otálora, M. A. G., Martínez, I., O'Brien, H., Molina, M. C., Aragón, G., & Lutzoni, F. (2010). Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution*, *56*(3), 1089–1095. https://doi.org/10.1016/j.ympev.2010.05.013

Paulsrud, P., Rikkinen, J., & Lindblad, P. (1998). Cyanobiont Specificity in Some Nostoc-Containing Lichens and in a Peltigera aphthosa Photosymbiodeme. *New Phytologist*, *139*(3), 517–524. https://doi.org/10.1046/j.1469-8137.1998.00220.x

Paulsrud, P., Rikkinen, J., & Lindblad, P. (2000). Spatial patterns of photobiont diversity in some Nostoc-containing lichens. *New Phytologist*, *146*(2), 291–299. https://doi.org/10.1046/j.1469-8137.2000.00647.x

Paulsrud, P., Rikkinen, J., & Lindblad, P. (2001). Field investigations on cyanobacterial specificity in Peltigera aphthosa. *New Phytologist*, *152*(1), 117–123. https://doi.org/10.1046/j.0028-646x.2001.00234.x

Peksa, O., Gebouská, T., Škvorová, Z., Vančurová, L., & Škaloud, P. (2022). The guilds in green algal lichens-an insight into the life of terrestrial symbiotic communities. *FEMS Microbiology Ecology*, *98*(2). https://doi.org/10.1093/femsec/fiac008

Peksa, O., & Škaloud, P. (2011). Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae). *Molecular Ecology*, *20*(18), 3936–3948. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Piercey-Normore, M. D., Coxson, D., Goward, T., & Goffinet, B. (2006). Phylogenetic position of a Pacific Northwest North American endemic cyanolichen, Nephroma occultum (Ascomycota, Peltigerales). *The Lichenologist*, *38*(5), 441–456. https://doi.org/DOI: 10.1017/S0024282906005950

Piercey-Normore, M. D., & DePriest, P. T. (2001). Algal switching among lichen symbioses. *American Journal of Botany*, *88*(8), 1490–1498. https://doi.org/10.2307/3558457

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, *21*(2), 609–620. https://doi.org/10.1111/1755-0998.13281

Rai, A. N. (1998). Nitrogen metabolism. In E. Galun, M. (Ed.), *Handbook of Lichenology* (1st ed, pp. 201–237). Boca Raton, FL, USA: Press Inc.

Rai, A. N., Söderbäck, E., & Bergman, B. (2000). Tansley Review No. 116. Cyanobacterium–plant symbioses. *New Phytologist*, *147*(3), 449–481. https://doi.org/10.1046/j.1469-8137.2000.00720.x

Rikkinen, J. (2003). Ecological and evolutionary role of photobiont-mediated guilds in lichens. *Symbiosis*, *34*(2), 99–110.

Rikkinen, J. (2013). Molecular studies on cyanobacterial diversity in lichen symbioses. *MycoKeys*, *6*(April 2013), 3–32. https://doi.org/10.3897/mycokeys.6.3869

Rikkinen, J., Oksanen, I., & Lohtander, K. (2002). Lichen guilds share related cyanobacterial symbionts. *Science*, *297*(5580), 357. https://doi.org/10.1126/science.1072961

Rodríguez-Arribas, C., Martínez, I., Aragón, G., Zamorano-Elgueta, C., Cavieres, L., & Prieto, M. (2023). Specialization patterns in symbiotic associations: A community perspective over spatial scales. *Ecology and Evolution*, *13*(7), e10296. https://doi.org/10.1002/ece3.10296

Rodríguez-Arribas, C., Prieto, M., Aragón, G., López-Angulo, J., Escudero, A., & Martínez, I. Specialization: a new multidimensional and integrative perspective. *Ecology*, *under review*.

Rolshausen, G., Dal Grande, F., Sadowska-Deś, A. D., Otte, J., & Schmitt, I. (2018). Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions. *Ecography*, *41*(8), 1380–1392. https://doi.org/10.1111/ecog.03457

Rolshausen, G., Hallman, U., Grande, F. D., Otte, J., Knudsen, K., & Schmitt, I. (2020). Expanding the mutualistic niche: Parallel symbiont turnover along climatic gradients. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1924). https://doi.org/10.1098/rspb.2019.2311

Rudi, K., Skulberg, O. M., & Jakobsen, K. S. (1998). Evolution of cyanobacteria by exchange of genetic material among phyletically related strains. *Journal of Bacteriology*, *180*(13), 3453–

3461. https://doi.org/10.1128/jb.180.13.3453-3461.1998

Sadowska-Deś, A. D., Dal Grande, F., Lumbsch, H. T., Beck, A., Otte, J., Hur, J.-S., … Schmitt, I. (2014). Integrating coalescent and phylogenetic approaches to delimit species in the lichen photobiont Trebouxia. *Molecular Phylogenetics and Evolution*, *76*, 202–210. https://doi.org/10.1016/j.ympev.2014.03.020

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Singh, G., Dal Grande, F., Divakar, P. K., Otte, J., Crespo, A., & Schmitt, I. (2017). Fungal–algal association patterns in lichen symbiosis linked to macroclimate. *New Phytologist*, *214*(1), 317–329. https://doi.org/10.1111/nph.14366

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stanton, D. E., Ormond, A., Koch, N. M., & Colesie, C. (2023). Lichen ecophysiology in a changing climate. *American Journal of Botany*, *110*(2), e16131. https://doi.org/10.1002/ajb2.16131

Tschermak-Woess, E. 1989. (1989). The algal partner. *Hnadbook of Lichenology*, *1*, 39–92.

Vančurová, L., Muggia, L., Peksa, O., Řídká, T., & Škaloud, P. (2018). The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in Stereocaulon (lichenized Ascomycota). *Molecular Ecology*, *27*(14), 3016–3033. https://doi.org/10.1111/mec.14764

Wirtz, N., Lumbsch, H. T., Green, T. G. A., Türk, R., Pintado, A., Sancho, L., & Schroeter, B. (2003). Lichen fungi have low cyanobiont selectivity in maritime Antarctica. *New Phytologist*, *160*(1), 177–183. https://doi.org/10.1046/j.1469-8137.2003.00859.x

Yahr, R., Vilgalys, R., & Depriest, P. T. (2006). Geographic variation in algal partners of Cladonia subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytologist*, *171*(4), 847–860. https://doi.org/10.1111/j.1469-8137.2006.01792.x

CHAPTER 3

Unravelling network structures and species roles in the lichen symbiosis in a wide latitudinal gradient.

Clara Rodríguez-Arribas, Jordi Bascompte, María Prieto, Jesús López-Angulo, Gregorio Aragón and Isabel Martínez

Abstract

The study of biotic interactions through the structural organization of networks has been proved to be a useful tool to understand species coexistence and community stability. Lichens are one of the main examples of intimate symbiosis, but results obtained from network approaches are scarce. In order to understand the factors shaping lichen community assemblage, we aim to unravel how interacting networks are built, and which drivers influence network structures. For this purpose, we analysed 41 lichen local networks and the metanetwork along a wide latitudinal gradient in the Southern Hemisphere with a broad variability of environmental conditions. The results showed that there is not a commonly shared structure in these local networks characterizing the lichen system itself. Yet, the metanetwork showed a nested structure, in which specialists interact with subsets of the species interacting with the generalists. Nonetheless, local networks were affected by environmental factors and functional traits. Thus, modularity increases at higher latitudes while connectance decreases, mostly driven by temperature variability, denoting a more generalized pattern towards the South. In relation with functional traits, the absence of reproductive structures in lichens decreased connectance probably limiting processes of photobiont sharing and facilitation through these structures. Few species were structurally important in the local networks and the metanetwork, being cephalolichens (i.e., lichens with cyanobateria in cephalodia), the mycobiont species found with more structural roles besides peripherals, being considered as a source of cyanobacteria for coexisting species. The few cases of reciprocal interactions found and the lack of a pattern of convergent trait organization on the different modules limit the consideration of modules as coevolutionary units. Our results indicate that the symbiosis of lichens associated with cyanobacteria does not have a defined network structure per se and that environmental processes as well as functional traits shape the structure of these networks in different ways.

Keywords

Lichen, networks, latitudinal specialization gradient, species' roles.

Introduction

Biotic interactions have been studied from many perspectives through the history of biology, with a greater emphasis from the perspective of pairwise interactions. However, as ecological systems are more complex than the isolated interaction of their components (Thompson, 2005), the application of network approaches has been described as a useful tool to understand the structure and dynamics of communities of interacting species (Bascompte & Jordano, 2007, 2014; Levine *et al*., 2017). Network analytical techniques contribute to provide new perspectives to the knowledge of the factors that influence community assembly and species coevolution (e.g., Jordano *et al*., 2003; Rezende *et al*., 2007; Rohr *et al*., 2014) as they provide a powerful representation of the ecological interactions among species and their global interdependence.

Most studies based on networks of interacting species have focused on finding common structural patterns considering different network properties such as nestedness (i.e., specialists are found interacting with a subset of the species that generalist interact too; Bascompte, 2009; Bascompte *et al*., 2003) or modularity (i.e., the networks consist of smaller modules of highly connected species; Olesen *et al*. , 2007). Networks properties have been assessed to play different roles in ecosystems and in the maintenance of biodiversity. Thus, nested and heterogeneous networks have been proposed to generate a more stable association in interacting systems providing alternative responses to perturbations (Bascompte *et al*., 2003) and conferring robustness against the random loss of species (Ramos-Jiliberto *et al*., 2010). Besides, modularity affects not only the stability of the networks by buffering the spread of a perturbation on the entire network and slowing down the rate of biodiversity loss (Gilarranz *et al*., 2017; May, 1972; Stouffer & Bascompte, 2011; Wilmers, 2007), but also may favour the potential coevolution of their components (e.g., floral syndromes of plant-animal interactions and the selection of matching suites of traits; Olesen *et al*., 2007). Additionally, within a network, species play different topological roles (hubs, connectors, and peripherals), which may also have implications for network functioning and conservation. Thus, it is important to identify attributes of hub species, which act as network keystones (Dupont & Olesen, 2009), as if these species go extinct, their modules may fragment causing cascading loss of other

species. By comparison, if connectors are lost, modules become decoupled, and the modularity of the network becomes more pronounced.

Biotic interactions range from antagonism, in which one partner benefits from another that it harms, to mutualism, in which both partners take advantages of the interaction. In this context, mutualisms have been considered a key factor in ecosystem functioning since the early history of life (Bascompte & Jordano, 2014; Gomes *et al*., 2022; Thompson, 2005). Focusing on mutualistic interactions, they are represented by bipartite networks in which there are two classes of nodes (i.e., organisms) interacting between them but with no interaction within each class. The studies in mutualistic networks such as pollination and seed-dispersal systems have revealed different structural patterns including nestedness, modularity or both (Bascompte, 2009; Bascompte *et al*., 2003; Olesen *et al*., 2007). Besides the studies unravelling network general patterns in interacting systems, little is known about the drivers influencing the observed network structure. By one hand, networks have been related with latitude. In accordance with the latitudinal diversity gradient, most hypothesis postulate that specialization increases towards the equator, expecting networks to show higher modularity, due to an increase in species diversity and climatic stability (Dalsgaard *et al*., 2011; Hillebrand, 2004; Trøjelsgaard & Olesen, 2013)(Dalsgaard *et al*., 2011; Hillebrand, 2004; Trøjelsgaard & Olesen, 2013). Additionally, environmental factors have been shown to shape network structure, providing contrasting results (see Dalsgaard *et al*., 2017). For instance, past climate stable conditions have been found to increase modularity for pollination networks towards the tropics (Dalsgaard *et al*., 2013; Trøjelsgaard & Olesen, 2013), which is in line with the proposed hypothesis, while seed-dispersal networks have been found to decrease modularity towards the tropics due to current lower temperature seasonality (Schleuning *et al*., 2014). As well, other studies have shown how climatic variables such as temperature, influence the nestedness of the networks (e.g., Rico-Gray *et al*., 2012 for plant-ant interactions). The different patterns found to date could be due to differences between systems, or by the use of different methods and scales to compare networks (Pellissier *et al*., 2018).

Other drivers influencing network structure are related with the functional traits of the organisms conforming the network (Thompson, 2005). Body-size for food webs or

floral syndromes in pollination networks are good examples of how functional traits influence network structure (Fenster *et al*., 2004; Woodward *et al*., 2005). For instance, floral syndromes have been assessed to contribute to the delimitation of different modules within a network (Olesen *et al*., 2007). The high functional similarity of traits concurring within a module found, which could be conserved phylogenetically or not (Fenster *et al*., 2004), allows the interaction between organisms showing matching traits, and might represent co-evolutionary units (Thompson, 2005). However, functional traits of the organisms of a network have been barely considered as drivers of network structure or poorly associated with the role of the organisms within networks (Schleuning *et al*., 2014), but they should be taken into account as traits are ecologically relevant due to differences in the functional importance of the individual species composing the networks (Stouffer *et al*., 2012).

On top of all these uncertainties, conclusions coming from network studies traditionally are based on plant-animal interactions (Bascompte *et al*., 2003; Burns, 2013; Guimarães *et al*., 2006) and plant-fungal symbioses (e.g., mycorrhizae; Chagnon *et al*., 2015; Martos *et al*., 2012; Montesinos-Navarro *et al*., 2012, and fungal endophytes; Chagnon *et al*., 2016) but neglect lichens, a well-known example of intimate symbiosis. This mutualism consists of a principal fungus (mycobiont) which interacts with several organisms (being considered as miniature ecosystems by Hawksworth & Grube, 2020), but remarkably, with a photosynthetic partner (photobiont), which can be a green alga, a cyanobacteria or both. The mycobiont protects the photobiont from desiccation, whereas the photobiont performs photosynthesis and nitrogen fixation (the latter only when it is a cyanobacteria; Nash, 1996). When the mycobiont associates with a cyanobacteria, the cyanobacteria can be either the principal photobiont, mostly in charge of photosynthesis and nitrogen fixation (cyanolichens), or a secondary photobiont, with the main function of fixing nitrogen and located in differentiated structures called cephalodia (cephalolichens; Nash, 2008). The studies of mycobiont-photobiont interactions forming part of lichens have been assessed mainly from the perspective of pairwise interactions (e.g., Magain *et al*., 2017, 2018; Yahr *et al*., 2004, 2006), and the few network-based approaches have been focused on concrete families (e.g., Chagnon *et al*., 2018, 2019), without considering the complete coexisting community. At the community scale, several

studies have focused on determining photobiont sharing and facilitation, finding different photobiont-sharing guilds distinguished by their ecological requirements (Cardós *et al*., 2019; O'Brien *et al*., 2013; Rikkinen, 2013; Rikkinen *et al*., 2002).

Unlike mycorrhizae and fungal endophytes, the lichen symbiosis converges in the lichen thallus, which is the resulting symbiotic unit and needs both components to be developed (Nash, 2008). The mycobiont is not able to live without its photosynthetic partner unless a loss of lichenization occurs in a specific fungal clade (Lutzoni *et al*., 2001; Wedin *et al*., 2004). This high dependence of the mycobiont limits reciprocal specialization as the interaction with any compatible photobiont is better than none. Nonetheless, if the association with different photobionts is translated in varying levels of fitness for the mycobiont, natural selection could imply specialization towards few photobionts. If these few optimal photobionts are shared with many fungal species, the result could be asymmetrical specialization (Lu *et al*., 2018; Magain *et al*., 2017) which is typical in nested networks (Joppa *et al*., 2010; Vázquez & Aizen, 2004). However, even though a nested architecture of the lichen networks is in line with previous studies finding the prevalence of nestedness in natural mutualistic networks (e.g., Bascompte *et al*., 2003; Fontaine *et al*., 2011), the high level of intimacy and dependence on these interactions to result in the lichen thallus leads us to expect high modularity in these networks (Olesen *et al*., 2007; Thompson, 2005). All these supports the potential of network-based analysis to understand the underlying mechanisms influencing the lichen symbiosis.

Based on the cited premises we aim to contribute to general conclusions emerging from network analysis, by studying network structure and its drivers in lichen communities in order to explain the establishment and maintenance of lichenic interactions at the community level. To do so, we characterized lichen interacting networks at two spatial scales (local and regional) across a wide latitudinal gradient in Chile in different well-preserved *Nothofagus pumilio* forests. This latitudinal gradient covers a broad range of environmental conditions to use as drivers in order to understand how environmental variability affects lichen network structure. Additionally, these forests harbour impressive diverse lichen communities, especially characterized by a huge number of cyanolichens and a remarkable number of cephalolichens. Thus, specific aims

are: 1) to assess if there is a common network structure in the lichen symbiosis, 2) to determine if network structure changes depending on the spatial scale considered, 3) to analyse the environmental and/or functional drivers that shape the variability of network structure, and 4) to identify which topological roles (i.e., peripherals, connectors and hubs) play the different species and the functional traits prevailing in each of these roles. We expect to find 1) a common modular structure of the lichen networks as this interaction consists of a long-term intimate mutualism, 2) the maintenance of a modular structure at different spatial scales, 3) environmental drivers and mycobionts' functional traits to explain part of the variation of the network structure, and 4) cephalolichens to stand out as hubs and/or connectors acting as a source of cyanobacterias, as cephalolichens have the cyanobacteria in specialized structures (cephalodia), which could make the cyanobacteria more reachable for other coexisting species.

Materials and methods

Sampling and data collection

Sampling

Eleven *Nothofagus pumilio* (Poepp. & Endl.) Krasser forests were sampled in a wide latitudinal gradient covering most of *N. pumilio* distributional range (from 38.63º S to 54.96º S) between 2017 and 2018, nine of them belonging to National Parks or Reserves (Figure 1a). Within each forest stand, five plots were established at least 100 m away from the forest edge. Within each plot, ten trees were selected, and four 20 × 30 cm grids were placed in each of the ten trees at two orientations (north and south) and heights (breast height and base). Cover (%) of each lichen species found in each grid was visually determined. In total, information of 2200 grids was collected and used to calculate the mean lichen cover (%) at plot level in 55 plots (Figure 1b).

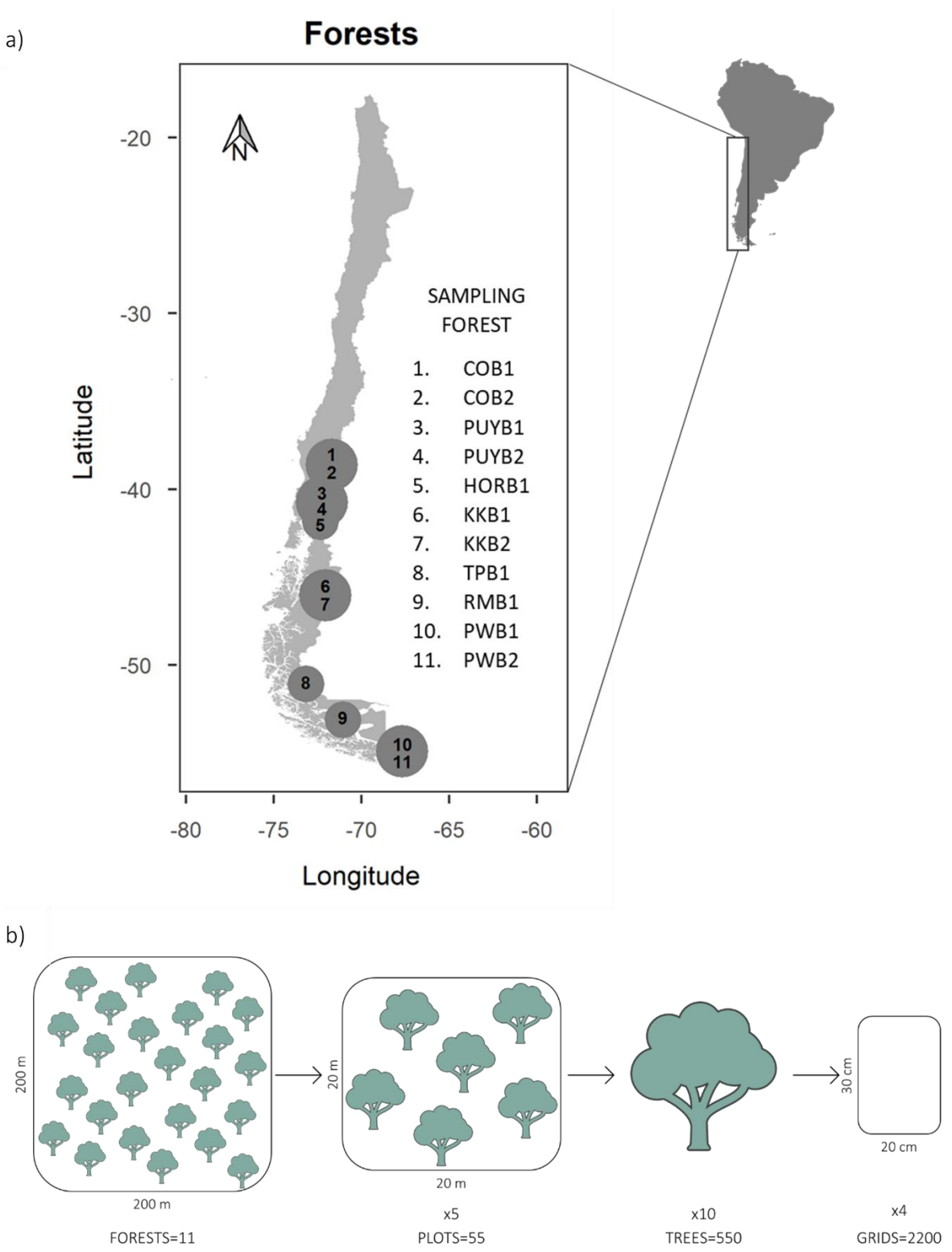


Figure 1. a) Sampling sites: Conguillío National Park (COB1 and COB2), Puyehue National Park (PUYB1 and PUYB2), Hornopirén National Park (HORB1), Cerro Castillo National Park (KKB1 and KKB2), Torres del Paine National Park (TPB1), Magallanes National Reserve (RMB1) and Navarino Island (PWB1 and PWB2), b) Sampling design in each site.

Lichen species were identified based on morphological characters following several monographs depending on the genera (Degelius (1974) for *Collema*, Jørgensen (2000, 2005) for *Fuscopannaria*, Galloway & Jørgensen (1995) for *Leptogium*, White & James (1988) for *Nephroma*, Passo *et al*. (2004), Elvebakk & Bjerke (2005), Passo & Calvelo (2006), Elvebakk (2007), Elvebakk *et al*. (2007), Passo & Calvelo (2011), and Elvebakk (2013) for *Pannaria*, Galloway (1985) for *Parmeliella* and *Psoroma*, Goward *et al*. (1995), Martínez (1999), and Vitikainen (1994) for *Peltigera*, Galloway (1992) and Lücking *et al*. (2017), for *Pseudocyphellaria* s. lat., Elvebakk *et al*. (2010) for *Psorophorus* and *Xanthopsoroma* and Galloway (1994) for *Sticta*). A total of 68 lichen species were identified (46 cyanolichens and 22 cephalolichens), from families *Collemataceae* (i.e., *Collema* and *Leptogium*), *Nephromataceae* (i.e., *Nephroma*), *Pannariaceae* (i.e., *Fuscopannaria*, *Pannaria*, *Psoroma*, *Psorophorus*, *Xanthopsoroma*), *Peltigeraceae* (i.e., *Peltigera*), *Lobariaceae* (i.e., *Lobariaceae* and *Sticta*).

In addition, a maximum of eight thalli were collected per forest for each species of cyanolichen and cephalolichen in different *N. pumilio* trees. Samples were air-dried and stored at -20ºC for DNA amplification and sequencing. *Nostoc* phylogroup delimitation was based on the *rbcLX* gene region (see Suppl. 1) obtaining a total of 58 phylogroups.

Environmental variables

Twenty-five environmental variables related to climate, geography, forest structure and habitat quality were selected (Suppl. 2). Climatic information included 19 temperature and precipitation variables was extracted from the CHELSA climate database (Karger *et al*., 2017). Geographic and environmental variables related with forest structure and habitat quality were collected *in situ*. Latitude, longitude, elevation, orientation, and slope (measured with a GPS, a compass, and a clinometer respectively), were collected in each plot, and diameter at breast height (DBH) and canopy cover (hemispherical photographs with fish-eyed lens) were measured in each of the 550 trees. Subsequently, the average of the DBH and the canopy cover was calculated for each plot. Orientation and slope were used to calculate potential solar radiation following López-Angulo *et al*. (2018).

In order to avoid multicollinearity, climatic and geographic variables were standardized and summarized in a principal components analysis (PCA) with *varimax* rotation with function *principal()* from package psych (v- .2.3; Revelle, 2021) in R (R Core Team, 2021). We selected the three first components of the PCA, which explained 95% of the variance, to be used as explanatory variables in the subsequent analysis. The axes represented variation in temperature and latitude (49% of explained variance), precipitation (27%) and temperature of coldest and wettest quarters (19%; Suppl. 3).

Functional traits: CWM and Rao's index

Data belonging to three categorical functional traits were gathered observationally from the 1064 thalli collected. These traits included the reproductive mode (sexual and/or asexual), the function of the cyanobacteria establishing the symbiosis (principal photobiont for cyanolichens or secondary photobiont for cephalolichens), and the growth form (foliose broad-lobed, FB; foliose narrow-lobed, FN; and squamulose, SQ). These traits are related to different aspects of lichen biology. For instance, the reproductive mode influences dispersion and establishment (Ellis, 2012; Ellis & Coppins, 2006; Nelson *et al*., 2015; Rapai *et al*., 2012). Also, lichens with sexual reproduction obtain their photobiont horizontally as only fungal spores are dispersed and they need to find a new photobiont to establish, whereas in asexual structures the mycobiont and the photobiont are dispersed jointly, and thus it is considered a vertical transmission. The function of the cyanobacteria is related with nutrient uptake (e.g., nitrogen fixation) and photosynthetic activity (Asplund & Wardle, 2017) and the growth form influences water use and structural complexity of the lichen thallus (Asplund & Wardle, 2017).

Community weighted means (CWM) and Rao's index were calculated at the plot level for each of the functional traits considered. The CWM reflects the dominant traits in a community and is calculated weighting the mean trait value of each species in a community by the relative abundance of that species (Lavorel *et al*., 2008). As all the traits considered were categorical, the CWM calculated the proportion of the total abundance for each category of the trait (Götzenberger *et al*., 2020). Rao's quadratic entropy index (hereafter 'Rao'; Rao, 1982) is an indicator of functional dissimilarity and, also, considers the relative abundance of the species within a community. We used the function *functcomp()* to calculate the CWM (with the argument *CWM.type = "all"* for categorical

traits) and function *dbFD()* to calculate Rao's index from package FD (v-1.0.12.1; Laliberte & Legendre, 2010) in R (R Core Team, 2021; Suppl. 4).

Network architecture measures

Binary incidence matrices of the bipartite networks between the mycobiont and the *Nostoc* partners were built for each of the 55 studied plots. Networks with at least five nodes in each class (mycobionts and cyanobacteria, as in Gaiarsa *et al*., 2021 and Simmons *et al*., 2019) and a minimum of 12 total number of species were selected, resulting in a total of 41 networks. The metanetwork including the complete latitudinal gradient studied was also built. Isolated modules were not considered as a part of the network to calculate the network parameters. For the isolated modules, we determined the taxonomic family and the functional traits of the mycobiont species in order to determine if there is a common pattern in the composition of these isolated modules (i.e., if they are dominated by a certain mycobiont family or by a certain functional trait).

For each local network and the metanetwork the number of nodes of each class, network richness (total number of nodes of both classes of organisms), network size (potential number of interactions), number of links, connectance, nestedness (following Fortuna *et al*., 2019), number of modules (based in a spin-glass model and simulated annealing by Reichardt & Bornholdt, 2006) and modularity (by Clauset *et al*., 2004), were calculated with package igraph (v-1.2.11; Csardi & Nepusz, 2006). Equations used to calculate all these metrics are detailed in Suppl. 5.

Statistical analysis

Null models were performed to quantify whether the structure properties of the networks (nestedness, modularity and connectance) deviated from randomness. The observed values of these properties of the local networks and the metanetwork were compared to expected values generated from the "probabilistic null model" (null model 2 in Bascompte *et al*., 2003). In this null model, the probability to occupy a given cell is defined by the average of the probabilities of its row and column observed occupation. The biological implication of this null model is that the probability of interaction of two species is proportional to the observed level of generalization of them. For each of the 41

local networks and the metanetwork, 999 random matrices were generated, in which we assured there were no isolated modules. Afterwards, z-scores were calculated to estimate whether these networks were significantly nested, modular and/or connected generating a normal distribution function to obtain the p-value with function *pnorm()* from package stats (v-4.1.3; R Core Team, 2021), considering one-tailed tests for nestedness and modularity and using two-tailed tests for connectance.. For those significantly modular networks, contingency tables were built in order to identify which mycobiont families, genera and/or functional traits were dominant in each module.

We conducted linear mixed models (Pinheiro & Bates, 2000) to test whether the z-score values obtained for nestedness, modularity and connectance were different from a null expectation of zero and to analyse the effect of environmental and functional predictors on the z-score values. Function *lmer()* from the package lme4 (v-1.1.28; Bates *et al*., 2015) was used for this purpose. We employed the environmental variables summarized in the PCA, together with potential solar radiation, DBH and canopy cover, and the CWM and Rao index as fixed effects. Forest was considered as a random effect. We examined the correlations between predictors and tested the absence of multicollinearity with the variance inflation factor (VIF), ensuring that VIF values were lower than 4 (Zuur *et al*., 2010), to fulfil model assumptions. A maximum of four predictors was set in the models in order to avoid overparameterization. The models for each of the z-scores were evaluated using the corrected Akaike information criterion (AICc), selecting the model with the lowest AICc and all models differing from the former in less than 2 AICc units using function *dredge()* from package MuMIn (v-1.46.0; Bartoń, 2020). Akaike weights (w^+) of each selected model and the relative importance of each predictor (w_i) were calculated. Only those predictors with $w_i > 0.4$ (Burnham, 2015) were included and estimated the 95% confidence intervals (CI) of model-averaged parameter estimates with function *model.avg()* from package MuMIn to consider a parameter as significant if the 95% CI excluded zero (Burnham & Anderson, 2002). The CI of the Intercept of the models was also used to determine if there were nestedness, modularity and connectance patterns on the local networks.

Species roles

To determine the role of each node (peripheral, connector, module hub and network hub) within each network and in the metanetwork, the position of each node in the parameter space determined by the within module versus between modules degree was estimated following the cut-offs of Guimerà & Nunes Amaral, 2005 and Olesen *et al*., 2007 (see Suppl. 6 for further details).

Results

Considering the 41 networks with at least 5 nodes from each class and 12 nodes of total richness, a total of 42 *Nostoc* phylogroups and 54 mycobiont species were found conforming them. From the 42 *Nostoc* phylogroups of the cyanobacteria class, 26 were always found within the networks, and 16 were part of both isolated modules and networks. Twelve additional *Nostoc* phylogroups were always found in isolated modules. From those 54 species belonging to the mycobionts class, 36 were always found as a part of the network, and 18 species were found either in isolated modules or inside the network depending on the plot. Additionally, 12 mycobiont species were only found establishing interactions outside of the main network in isolated modules (Suppl. 7). The mycobiont species found exclusively in isolated modules belonged to the genera *Leptogium* (3 species), *Pannaria* (1), *Peltigera* (5), *Pseudocyphellaria* (1), and *Psoroma* (2). All the species of *Peltigera* were isolated from the rest of the network. Isolated modules were composed by *Collemataceae* (27%), followed by *Peltigeraceae* (25%), *Lobariaceae* (24%), *Pannariaceae* (12%), and *Nephromataceae* (11%). In addition, isolated modules were constituted mostly by cyanolichens (91%) (Figure 2).

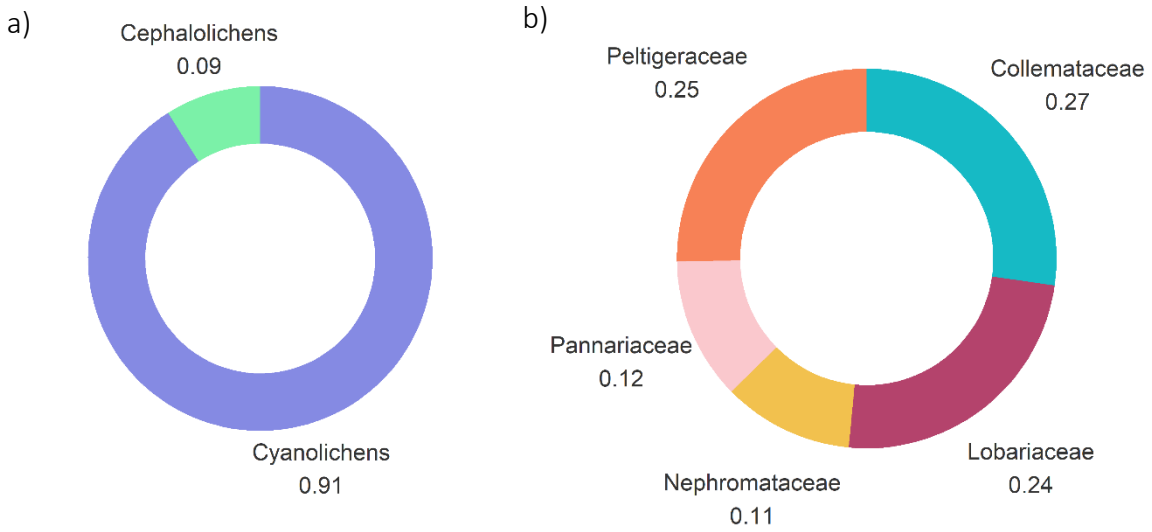


Figure 2. a) Percentage of nodes in isolated modules of each type of lichen associated with cyanobacteria (cyanolichens in blue; cephelolichens in green) and b) Percentage of nodes in isolated modules of each type mycobiont family (*Collemataceae* in blue, *Lobariaceae* in dark pink, *Nephromaraceae* in yellow, *Pannariaceae* in light pink and *Peltigeraceae* in orange).

Taking into account the isolated modules of the local networks, we found only three cases of exclusively one-to-one interactions: *Leptogium cyanescens* with phylogroup 44, *Peltigera collina* with phylogroup 40 and *Psoroma polychidioides* with phylogroup 53. The rest of the isolated modules were composed by more than two nodes, thus being those interactions non-exclusive for at least one of the partners (e.g., in cob1 plot 2 phylogroup 02 associates exclusively with *Peltigera hymenina*, but *Peltigera hymenina* also interacts with phylogroup 43).

When considering the whole latitudinal gradient (regional scale) to build the metanetwork, five isolated modules were found belonging to reciprocal interactions. These isolated modules were established by: *Sticta ainoae* and phylogroup 30, *Leptogium* sp. 1 and phylogroup 19, *Leptogium cyanescens* and phylogroup 44, *Peltigera collina* and phylogroup 40, and *Psoroma polychidioides* and phylogroup 53. Thus, the three cases of exclusive one-to-one interactions found at the networks built at the plot level (local scale), were consistent when considering the metanetwork, being these associations highly

specialized. The other two cases found in the metanetwork were from plots discarded for not reaching the requirements of 5 nodes of each class and 12 of total richness.

Network architecture measures

From the 41 networks selected, we found networks ranging from 6 to 23 mycobiont species and 5 to 13 *Nostoc* phylogroups. The number of links varied between 12 and 45, and network richness and size ranged from 13 to 36 and 42 to 299, respectively. The metanetwork was composed by 80 mycobiont species and 59 *Nostoc* phylogroups, with 238 links, network richness of 139, and size of 4720 (Suppl. 8).

Eleven significantly nested networks were found (27%) (Suppl. 9), 10 significantly modular (24%) (Suppl. 10) with 20 of them following a random structure (49%) (Figure 3). The metanetwork was significantly nested (Figure 4). None of the studied networks was both, nested and modular (Suppl. 11). In addition, 19 networks were less connected than expected by chance (46%). Nonetheless, the intercepts estimated in the linear mixed models were significant for the three metrics, indicating deviations from the null model. For nestedness and modularity the intercepts were positive, indicating a tendency towards nested and modular architectures. For connectance the intercept was negative, revelling lower connectance than expected from the null model.

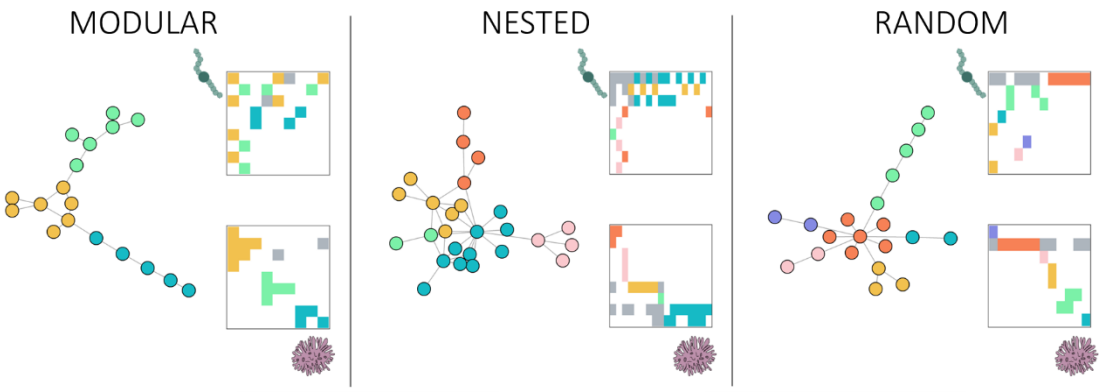


Figure 3. Examples of each of the structural patterns found in lichen networks (modular, ¼; nested, ¼; random, ½) with a representation of the network, the nested matrix on the top and the modular matrix on the bottom. The y axis of the matrices represents the cyanobacteria phylogroups and the x axis the mycobiont species. The colours represent the different modules.

Regarding mycobiont families, genera and/or functional traits no patterns were found composing each of the modules (Suppl. 12), except one module consistently formed exclusively by the same mycobiont species in *Psoroma asperellum*.

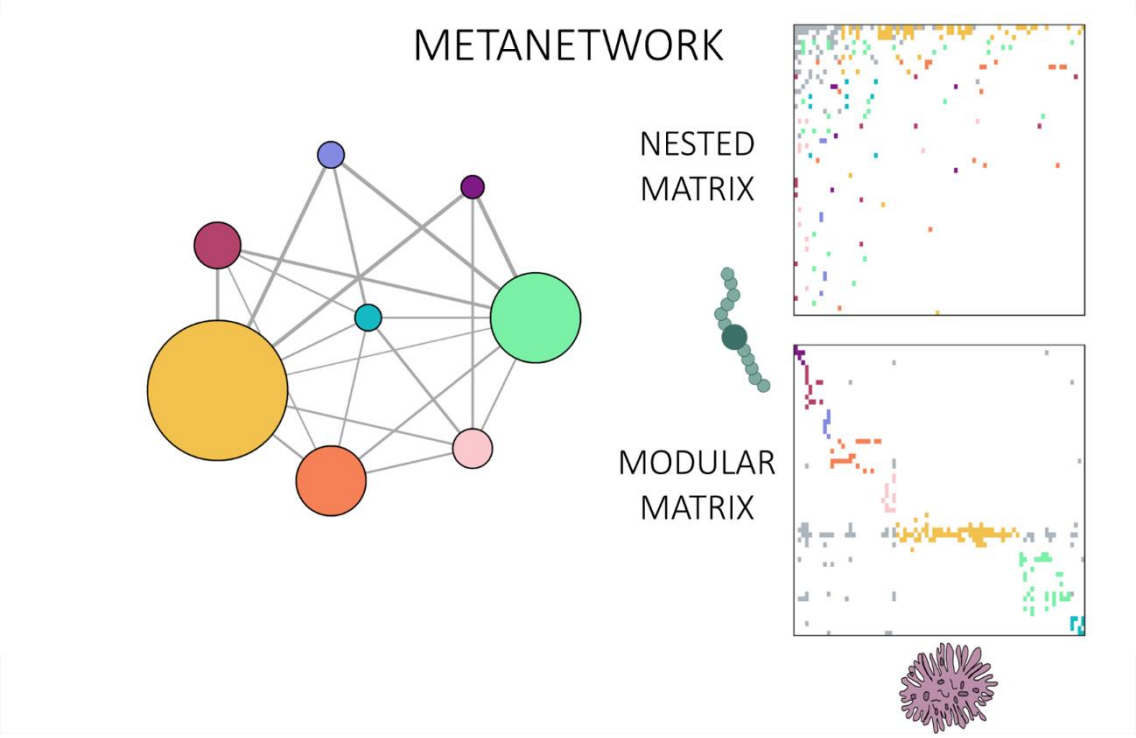


Figure 4. Representation of the modules of the metanetwork with the size of the circles representing the number of nodes within each module, and the width of the edges the number of links between modules, the nested matrix on the top and the modular matrix on the bottom. The y axis of the matrices represents the cyanobacteria phylogroups and the x axis the mycobiont species. The colours represent the different modules.

Environmental and functional determinants of network architecture

Modularity (z-score values) significantly increased with increasing temperature and latitude (average-estimate = 0.58) (Suppl. 13) and diminished with lower temperatures of coldest and wettest annual quarters (average-estimate =-0.45). Both, temperature and latitude related variables and temperatures of coldest and wettest annual quarters related variables had a relative importance of w_i= 1. On the other hand, connectance was significantly and negatively affected by temperature and latitude and by the absence of reproductive structures (sexual and/or asexual) (average-estimate =-0.33 and-0.17, respectively), both predictors having a relative importance of one. Nonetheless,

we found no effect in the variation of the nestedness z-scores of neither environmental drivers nor the functional traits (Table 1; Figure 5).

Table 1. Results of the Generalized Mixed Model for the z-scores of nestedness, modularity and connectance. CI= Confidence interval, VIF: Variance Inflaction Factor.

Predictor	Estimate	2.5% CI	97.5% CI	VIF
z-score Nestedness				
(Intercept)	**0.71**	**0.18**	**1.25**	
Temp cold-wet	0.29	-0.14	0.72	1.93
z-score Modularity				
(Intercept)	**0.86**	**0.57**	**1.16**	
Temp and Latitude	**0.58**	**0.25**	**0.91**	2.02
Temp cold-wet	**-0.45**	**-0.72**	**-0.18**	1.65
z-score Connectance				
(Intercept)	**-1.57**	**-1.74**	**-1.40**	
Rep none	**-0.17**	**-0.26**	**-0.07**	1.83
Temp and Latitude	**-0.33**	**-0.48**	**-0.18**	1.85

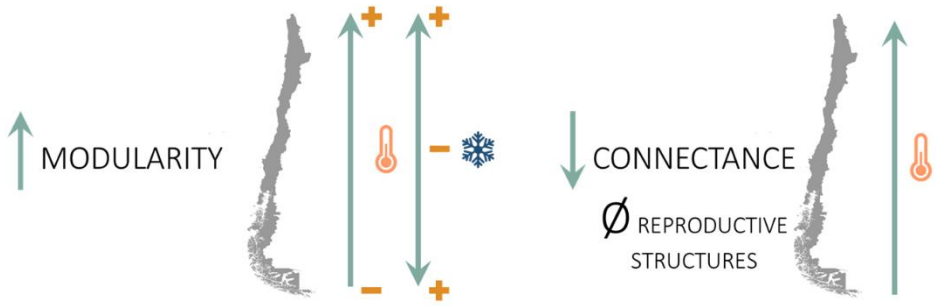


Figure 5. Environmental and functional drivers influencing z-scores of modularity and connectance. Modularity is positively affected by temperature related variables and minimum temperature of colder and wettest quarters. Connectance is negatively affected by temperature related variables and the absence of reproductive structures.

Species' roles

The number of modules varied between three and six. In the local network, most mycobiont species and *Nostoc* phylogroups were peripheral species, which could be considered highly specialized (Suppl. 14). Most of the connector mycobiont species consisted of cephalolichens (79%), while the only module-hub found was a cyanolichen from the *Collemataceae* family. Network hubs were present in nested or non-structured networks, but not in modular networks and were always cyanobacteria (Suppl. 15). When focusing on the metanetwork, most of the species and phylogroups were also peripheral (86%), with 7% being connectors, 5% module hubs and the remaining 2% belonged to network hubs or supergeneralist species. Species and phylogroups belonging to the metanetwork hubs were *Nephroma antarcticum*, *Pseudocyphellaria vaccina* and *Nostoc* phylogroup 38 (Figure 6). Mycobiont connectors were mainly cephalolichens (75%) and module or network hubs were always cephalolichens (Suppl. 15; Figure 7).

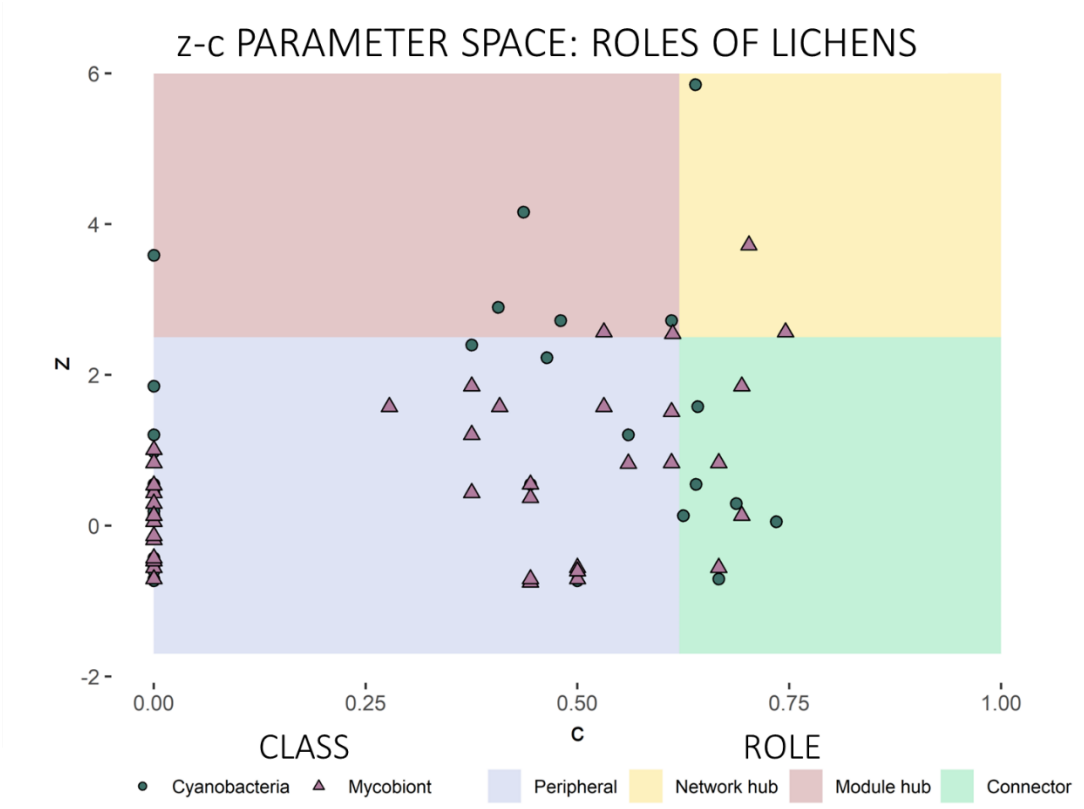


Figure 6. Role of the mycobiont species and *Nostoc* phylogroups found in the metanetwork.

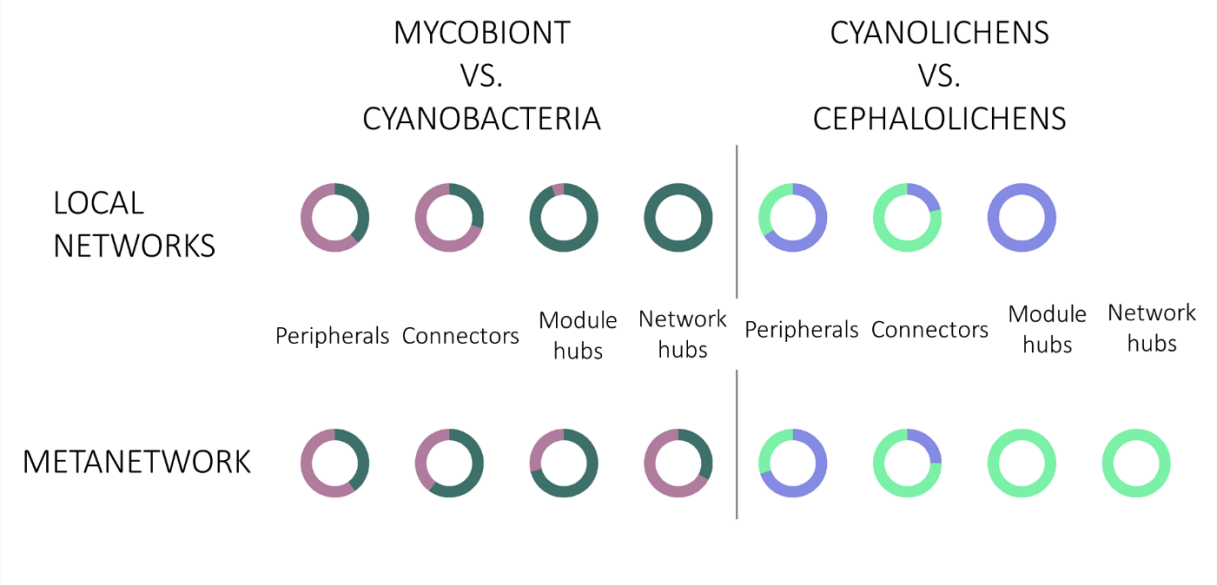


Figure 4. On the left: proportions of mycobionts (pink) and cyanobacteria (dark green) for each of the structural roles of the networks. On the right proportions of cyanolichens (blue) and cephalolichens (green) for each of the structural roles of the networks. On the top the proportions refer to the local networks and on the bottom to the metanetwork.

Discussion

Our study shows modular or nested structures in half of the local networks studied across the latitudinal gradient, suggesting the effect of ecological factors and/or functional traits shaping lichen network structure rather than a common network structure of the lichen system *per se*. Nonetheless, a tendency towards both, nested and modular patterns seems to define lichen networks in overall terms. Furthermore, our study reveals a prevalence of low connected lichen networks. When increasing the spatial scale, the metanetwork considering the whole latitudinal gradient showed a highly nested pattern. In addition, we found a latitudinal gradient of network structure, with modularity increasing towards the North of the gradient and connectance to the South, mostly influenced by temperature. In addition, our results provide evidence on how the reproductive mode of lichens may act as a driver of network connectance. Considering the topological roles of the species in the networks, most mycobiont species and *Nostoc* phylogroups were found to be peripherals, but a few acted as connectors or hubs, being network hubs exclusively cyanobacteria in the local networks and only present in nested

or random structured networks. As well, the topological roles of many of the species varied between local networks and the metanetwork. Nonetheless, non-peripheral mycobiont species were mainly cephalolichens for both, local networks and the metanetwork. Only three cases of one-to-one reciprocal specialization were found in local networks, that were consistent when considering the metanetwork.

Mutualistic networks tend to show a prevalent nested structure (Bascompte *et al*., 2003; Suweis *et al*., 2013). Nonetheless, long-term intimate mutualisms are expected to strengthen modularity (Olesen *et al*., 2007). We hypothesized that mycobiont-cyanobacteria networks would show a significant modular pattern as a result of the intimate interaction of both partners (Duran-Nebreda & Valverde, 2023; Kaasalainen *et al*., 2021; Olesen *et al*., 2007; Thompson, 2005)(Duran-Nebreda & Valverde, 2023; Kaasalainen *et al*., 2021; Olesen *et al*., 2007). However, we found lichen network structure to be highly represented by random networks not differing than what is expected by chance (half of the local networks). The variation of network structures could be influenced by other factors rather than the nature of the lichen-symbiosis system itself, which did not show a commonly shared network architecture besides its low connectance. The intimacy of the lichen symbiosis could be the reason of the low connected pattern, as mycobionts are highly dependent on the photobionts and may interact with few optimal cyanobacteria in order to maximize the fitness of the association (Magain *et al*., 2017). Furthermore, modular networks are expected to be highly robust because modularity enhances stability and may slow down the rate of biodiversity loss (Gilarranz *et al*., 2017; May, 1972; Stouffer & Bascompte, 2011; Wilmers, 2007). On the other hand, nested networks also stabilize interacting mutualistic systems and provide robustness to the network which may prevent coextinction cascades (Bascompte *et al*., 2003; Ramos-Jiliberto *et al*., 2010). Even though studies revealed that randomly interacting networks show a more resilient pattern than those with a nested structure (see Suweis *et al*., 2013), the mainly found random structure in the mycobiont-cyanobacteria networks together with the low connectance, could indicate certain instability of this symbiotic system. This result encourages to consider the vulnerability of these networks and their lower protection from secondary extinctions, and the need to develop further studies. However, we found an overall nested and modular tendency in

the local networks, which could confer different properties such as robustness and stability to lichen networks, even though in lower degree, allowing these communities to persist.

Interestingly, the resulting metanetwork from the complete latitudinal gradient showed a nested instead of a modular structure, in accordance with other mutualist systems (Bascompte *et al*., 2003). This means that specialized species interact with subsets of the species that generalized species interact too, being a rather asymmetrical specialization. However, this result is in contrast with previous studies of networks of interacting species in lichens, which reveal a highly modular structure, in which the species show higher number of interactions with species from the same module than with species from other modules (Chagnon *et al*., 2019; Duran-Nebreda & Valverde, 2023). Though, these studies are focused on different biological or taxonomic entities, not considering the whole community of coexisting species at a given locality (Chagnon *et al*., 2019; Duran-Nebreda & Valverde, 2023). Thus, the results obtained in previous studies limit the understanding of lichens network strutures as they do not consider the actual interactions that take place in a given locality of all coexisting species and only infer them at wider biological scales (i.e., genus). The observed nested structure in the metanetwork suggests that at larger spatial scales, many species may have the hablity to replace their partners for others which could be better adapted to the local environmental conditions, aquiring a more generalized pattern at regional scale (Rodríguez-Arribas *et al*., 2023). In addition, several species may act as supergeneralist (see below) facilitating the cyanobacteria to other species. Besides, the nested structure contributes to stabilize the metanetwork and confer robustness, indicating a lower vulnerability of lichen communities at regional scales and the importance of focusing conservation strategies at the local scale.

The variability of the structure of the local networks is related to ecological factors and functional traits. Overall, modularity increases at higher latitudes, with an opposite pattern for connectance. This latitudinal pattern is in accordance with previous studies of pollination networks (Dalsgaard *et al*., 2013; Trøjelsgaard & Olesen, 2013), and is mainly shaped by a temperature gradient, with lower temperatures towards the South. Thus, lichen networks become more connected under colder temperatures, suggesting an

increase in generalization in order to cope with more adverse environmental conditions (Batstone *et al*., 2018; Dalsgaard *et al*., 2013). The effect of environmental conditions in species richness, diversity and composition of lichen communities has been previously observed in wide latitudinal gradients (e.g., Hurtado *et al*., 2020) and with this study, is also observed in their interacting networks. The absence of reproductive structures, both sexual and asexual, negatively influenced connectance. Thus, networks with lower number of links tended to have higher proportion of lichens lacking reproductive structures (both, sexual and asexual). Lichens conforming these low connected networks would reproduce rather by thallus fragments diminishing their need to find new cyanobacteria. Therefore processes of photobiont sharing and facilitation may be limited between the coexisting mycobionts, reducing the connectance values of the network (Duran-Nebreda & Valverde, 2022; Otálora *et al*., 2010; Rikkinen, 2003; Rikkinen *et al*., 2002).

Most species showed a peripheral role in the modular networks, establishing most of their links with species from the same module as in other mutualistic systems (Olesen *et al*., 2007). However, a small proportion of mycobiont species and *Nostoc* phylogroups were acting as connectors or hubs. Interestingly, network hubs were not present in modular networks, suggesting that an increment in these latter supergeneralist species gluing the network together and blurring the borders of the modules, might be the reason of the nested or random structures (Dalsgaard *et al*., 2013). These network hubs are exclusively cyanobacteria in the local networks (phylogroups 38 and 42), and cyanobacteria and mycobiont species (phylogroup 38, *Nephroma antarcticum* and *Pseudocyphellaria vaccina*) in the metanetwork. This result of solely cyanobacteria being networks hubs at a local scale agrees with our expectation for nested lichen networks, in which few optimal cyanobacteria selected by their better input in the fitness of the interaction are shared with many fungal species (Lu *et al*., 2018; Magain *et al*., 2017). The dominance of cephalolichens with roles different from peripheral species may be related with their location of the cyanobacteria in the lichen thallus. Cephalolichens develop specialized structures, known as cephalodia, to storage the cyanobacteria (Nash, 2008). Thus, cyanobacteria could be more reachable for other coexisting mycobiont species to be captured and re-establish the symbiosis. These cephalolichens could be therefore

consider as a source of cyanobacteria for coexisting species, facilitating the establishment of new lichen thalli. In accordance, previous studies found cephalolichens showing high levels of generalization interacting with many cyanobacteria (Kaasalainen *et al*., 2021; Rodríguez-Arribas *et al*., 2023), which could favour the cyanobacteria requirements of many different mycobiont. Moreover, cephalodia emancipation from the lichen thallus followed by divergence has been described as a potential evolutionary driver in certain lichen families (Magain & Sérusiaux, 2014).

Interestingly, we found that reciprocal one-to-one interactions were rather rare, as it has been showed in other examples of intimate symbiotic mutualisms (Otálora *et al*., 2010; Thompson, 1994, 2005). Only three mycobiont species showed an exclusive interaction with their *Nostoc* partners: *Leptogium cyanescens*-phylogroup 44, *Peltigera collina*- phylogroup 40 and *Psoroma polychidioides*- phylogroup 56, which were consistent when considering the metanetwork. Thus, the sharing of partners in lichen networks is commonly found as in many other mutualisms (Bascompte, 2009; Bascompte *et al*., 2003; Batstone *et al*., 2018). Nonetheless, several mycobiont species and *Nostoc* phylogroups were never found as a part of the local networks, interacting exclusively in isolated modules. This was the case of the species belonging to the genus *Peltigera* which interacted with three *Nostoc* phylogroups always found in isolated modules (phylogroup 02, phylogroup 40 and phylogroup 43) and two phylogroups which appeared sometimes as a part of the main network (phylogroup 03 and phylogroup 04). This strong specialization and the modular pattern found was previously observed in the genus *Peltigera* (Chagnon *et al*., 2018, 2019). In agreement with previous studies, *Peltigera* species have a different pool of photobionts, described as the "*Peltigera-guild*" (Rikkinen, 2003; Rikkinen *et al*., 2002). This "*Peltigera-guild*" has been associated to their ecological requirements, as most of the species are terricolous, growing on soil and mixed with bryophytes. However, as they also interact with two phylogroups that sometimes enter in the network, only *Peltigera collina* remains isolated when considering the metanetwork. Thus, in contrast with the findings of previous studies, *Peltigera* species may be considered as "facultative" epiphytes and the interaction with phylogroups found in the main network may suggest some sharing and facilitation coming from other epiphytic species (Kaasalainen *et al*., 2021; Rikkinen, 2003; Rikkinen *et al*., 2002).

There were no patterns in the configuration of modules in the modular networks, with most modules formed by combinations of several traits and mycobiont families. This result contrasts with pollination networks, in which floral syndromes are well compartmentalized (Olesen *et al*., 2007). However, in this study, matching traits could not be analysed, as the functional traits considered are associated to the mycobiont and not with suits of traits which should match between both components of the symbiosis. Thus, we have little evidence of selected co-evolved traits and of modules being treated as co-evolutionary units (Olesen *et al*., 2007; Thompson, 2005).

To sum up, local mycobiont-cyanobacteria networks from lichens are variable in their structural patterns considering a wide latitudinal gradient, finding modular, nested, and mostly random networks. This result reveals the vulnerability of these interacting system at local scales, due to the lack of a structure in most of the networks which could provide robustness to the loss of species or buffer its effects. However, the metanetwork obtained of the whole gradient showed a highly nested structure, showing the prevalence of asymmetric interactions, which could contribute to stabilize the mutualistic networks preventing coextinction cascades and which reveals an increase in generalization when the spatial scale increases. Nonetheless, ecological factors and functional traits influence the structure of these networks. We found a tendency towards generalization at lower temperatures and latitudes with the increase of network connectance and a decrease in the modularity. As well, a decrease in connectance is observed with the absence of reproductive structures. As in other mutualistic systems, most species were peripherals, but cephalolichens showed a more structural role than cyanolichens, indicating they may be involved in facilitation processes. The absence of a pattern in the composition of the modules and the few cases on one-to-one reciprocal specialization limit the unveil about co-evolution between partners of mycobiont-cyanobacteria.

References

Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, *92*(3), 1720–1738. https://doi.org/10.1111/brv.12305

Bartoń, K. (2020). MuMIn. R package version 1.43.17. Retrieved from https://cran.r-project.org/web/packages/MuMIn/index.html

Bascompte, J. (2009). Disentangling the web of life. *Science*, *325*(5939), 416–419. https://doi.org/10.1126/science.1170749

Bascompte, J., & Jordano, P. (2007). Plant-animal mutualistic networks: The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *38*(May), 567–593. https://doi.org/10.1146/annurev.ecolsys.38.091206.095818

Bascompte, J., & Jordano, P. (2014). *Mutualistic Networks*. Princeton University Press.

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Batstone, R. T., Carscadden, K. A., Afkhami, M. E., & Frederickson, M. E. (2018). Using niche breadth theory to explain generalization in mutualisms. *Ecology*, *99*(5), 1039–1050. https://doi.org/10.1002/ecy.2188

Burnham, K. P. (2015). Multimodel Inference : Understanding AIC relative variable importance values.

Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Inference: A Practical Information-Theoretic Approach* (2nd Ed). New York: Springer-Verlag. https://doi.org/10.1007/b97636

Burns, K. C. (2013). What causes size coupling in fruit–frugivore interaction webs? *Ecology*, *94*(2), 295–300. https://doi.org/10.1890/12-1161.1

Cardós, J. L. H., Prieto, M., Jylhä, M., Aragón, G., Molina, M. C., Martínez, I., & Rikkinen, J. (2019). A case study on the re-establishment of the cyanolichen symbiosis: Where do the compatible photobionts come from? *Annals of Botany*, *124*(3), 379–388. https://doi.org/10.1093/aob/mcz052

Chagnon, P.-L., Bradley, R. L., & Klironomos, J. N. (2015). Trait-based partner selection drives mycorrhizal network assembly. *Oikos*, *124*(12), 1609–1616. https://doi.org/10.1111/oik.01987

Chagnon, P.-L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2018). Strong specificity and network modularity at a very fine phylogenetic scale in the lichen genus Peltigera. *Oecologia*, *187*(3), 767–782. https://doi.org/10.1007/s00442-018-4159-6

Chagnon, P.-L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2019). Species diversification and phylogenetically constrained symbiont switching generated high modularity in the lichen genus Peltigera. *Journal of Ecology*, *107*(4), 1645–1661. https://doi.org/10.1111/1365-

2745.13207

Chagnon, P.-L., U'Ren, J. M., Miadlikowska, J., Lutzoni, F., & Elizabeth Arnold, A. (2016). Interaction type influences ecological network structure more than local abiotic conditions: evidence from endophytic and endolichenic fungi at a continental scale. *Oecologia*, *180*(1), 181–191. https://doi.org/10.1007/s00442-015-3457-5

Clauset, A., Newman, M. E. J., & Moore, C. (2004). Finding community structure in very large networks. *Physical Review E - Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics*, *70*(6), 6. https://doi.org/10.1103/PhysRevE.70.066111

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal Complex Systems*, *Complex Sy*(1695), 1695. Retrieved from http://igraph.sf.net

Dalsgaard, B., Magård, E., Fjeldså, J., González, A. M. M., Rahbek, C., Olesen, J. M., … Svenning, J. C. (2011). Specialization in plant-hummingbird networks is associated with species richness, contemporary precipitation and quaternary climate-change velocity. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0025891

Dalsgaard, B., Schleuning, M., Maruyama, P. K., Dehling, D. M., Sonne, J., Vizentin-Bugoni, J., … Rahbek, C. (2017). Opposed latitudinal patterns of network-derived and dietary specialization in avian plant–frugivore interaction systems. *Ecography*, *40*(12), 1395–1401. https://doi.org/10.1111/ecog.02604

Dalsgaard, B., Trøjelsgaard, K., Martín González, A. M., Nogués-Bravo, D., Ollerton, J., Petanidou, T., … Olesen, J. M. (2013). Historical climate-change influences modularity and nestedness of pollination networks. *Ecography*, *36*(12), 1331–1340. https://doi.org/10.1111/j.1600-0587.2013.00201.x

Degelius, G. (1974). The lichen genus Collema with special reference to the extra-European species. *Symbolae Botanicae Upsalienses*, *20*(2), 1–215.

Dupont, Y. L., & Olesen, J. M. (2009). Ecological modules and roles of species in heathland plant-insect flower visitor networks. *Journal of Animal Ecology*, *78*(2), 346–353. https://doi.org/10.1111/j.1365-2656.2008.01501.x

Duran-Nebreda, S., & Valverde, S. (2023). Composition, structure and robustness of Lichen guilds. *Scientific Reports*, *13*(1), 1–14. https://doi.org/10.1038/s41598-023-30357-w

Ellis, C. J. (2012). Lichen epiphyte diversity: A species, community and trait-based review. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*(2), 131–152. https://doi.org/10.1016/j.ppees.2011.10.001

Ellis, C. J., & Coppins, B. J. (2006). Contrasting functional traits maintain lichen epiphyte diversity in response to climate and autogenic succession. *Journal of Biogeography*, *33*(9), 1643–1656. https://doi.org/10.1111/j.1365-2699.2006.01522.x

Elvebakk, A. (2007). The panaustral lichen Pannaria sphinctrina (Mont.) Tuck. and the related new species P-lobulifera from New Caledonia. *Cryptogamie Mycologie*, *28*(3), 225–235.

Elvebakk, A. (2013). Pannaria minutiphylla and P. pulverulacea, two new and common, austral species, previously interpreted as Pannaria microphyllizans (Nyl.) P. M. Jørg. *The Lichenologist*, *45*(1), 9–20. https://doi.org/10.1017/S0024282912000679

Elvebakk, A., & Bjerke, J. W. (2005). Pannaria isabellina (Vain.) comb. nov., a remarkable lichen species from Chile. *The Lichenologist*, *37*(1), 47–54. https://doi.org/10.1017/S0024282904014525

Elvebakk, A., Fritt-Rasmussen, J., & Elix, J. A. (2007). The New Zealand lichen Pannaria leproloma (Nyl.) P. M. Jørg. and its panaustral relative P. farinosa nom. nov. *The Lichenologist*, *39*(04), 349–359. https://doi.org/10.1017/S0024282907006913

Elvebakk, A., Robertsen, E. H., Park, C. H., & Hong, S. G. (2010). Psorophorus and Xanthopsoroma, two new genera for yellow-green, corticolous and squamulose lichen species, previously in Psoroma. *The Lichenologist*, *42*(05), 563–585. https://doi.org/10.1017/S0024282910000083

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 375–403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Fontaine, C., Guimarães, P. R., Kéfi, S., Loeuille, N., Memmott, J., van der Putten, W. H., … Thébault, E. (2011). The ecological and evolutionary implications of merging different types of networks. *Ecology Letters*, *14*(11), 1170–1181. https://doi.org/10.1111/j.1461-0248.2011.01688.x

Fortuna, M. A., Barbour, M. A., Zaman, L., Hall, A. R., Buckling, A., & Bascompte, J. (2019). Coevolutionary dynamics shape the structure of bacteria-phage infection networks. *Evolution*, *73*(5), 1001–1011. https://doi.org/10.1111/evo.13731

Gaiarsa, M. P., Kremen, C., & Ponisio, L. C. (2021). Pollinator interaction flexibility across scales affects patch colonization and occupancy. *Nature Ecology and Evolution*, *5*(6), 787–793. https://doi.org/10.1038/s41559-021-01434-y

Galloway, D. J. (1985). *Flora of New Zealand. Lichens.* (Wellington).

Galloway, D. J. (1992). Studies in Pseudocyphellaria (lichens) III. The South American species. *Bibliotheca Lichenologica*, *46*, 1–275.

Galloway, D. J. (1994). Studies on The Lichen Genus Sticta (Schreber) Ach.: I. Southern South American Species. *The Lichenologist*, *26*(03), 223–282. https://doi.org/10.1006/lich.1994.1019

Galloway, D. J., & Jørgensen, P. M. (1995). The lichen genus Leptogium (Collemataceae) in Southern Chile, South America. *Contributions to Lichenology in Honour of Gerhard Follmann. Bot. Inst. Univ. Cologne*, 227–247.

Gilarranz, L. J., Rayfield, B., Liñán-Cembrano, G., Bascompte, J., & Gonzalez, A. (2017). Effects of network modularity on the spread of perturbation impact in experimental metapopulations. *Science*, *357*(6347), 199–201. https://doi.org/10.1126/science.aal4122

Gomes, S. I. F., Fortuna, M. A., Bascompte, J., & Merckx, V. S. F. T. (2022). Mycoheterotrophic plants preferentially target arbuscular mycorrhizal fungi that are highly connected to autotrophic plants. *New Phytologist*, *235*(5), 2034–2045. https://doi.org/10.1111/nph.18310

Götzenberger, L., de Bello, F., Dias, A. T. C., Moretti, M., Berg, M. P., & Carmona, C. P. (2020).

Trait-based ecology tools in R. https://doi.org/10.20350/digitalCSIC/12663

Goward, T., Goffinet, B., & Vitikainen, O. (1995). Synopsis of the genus Peltigera (lichenized Ascomycetes) in British Columbia, with a key to the North American species. *Canadian Journal of Botany*, *73*(1), 91–111. https://doi.org/10.1139/b95-012

Guimarães, P. R., Rico-Gray, V., Furtado Dos Reis, S., & Thompson, J. N. (2006). Asymmetries in specialization in ant-plant mutualistic networks. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1597), 2041–2047. https://doi.org/10.1098/rspb.2006.3548

Guimerà, R., & Nunes Amaral, L. A. (2005). Functional cartography of complex metabolic networks. *Nature*, *433*(7028), 895–900. https://doi.org/10.1038/nature03288

Hawksworth, D. L., & Grube, M. (2020). Lichens redefined as complex ecosystems. *New Phytologist*, *227*(5), 1281–1283. https://doi.org/10.1111/nph.16630

Hillebrand, H. (2004). On the generallity of the latutinal diversity gradient. *American Naturalist*, *163*(2), 192–211. https://doi.org/10.1086/381004

Hurtado, P., Prieto, M., Aragón, G., de Bello, F., & Martínez, I. (2020). Intraspecific variability drives functional changes in lichen epiphytic communities across Europe. *Ecology*, *101*(6), e03017. https://doi.org/10.1002/ecy.3017

Joppa, L. N., Montoya, J. M., Solé, R., Sanderson, J., & Pimm, S. L. (2010). On nestedness in ecological networks. *Evolutionary Ecology Research*, *12*(1), 35–46.

Jordano, P., Bascompte, J., & Olesen, J. M. (2003). Invariant properties in coevolutionary networks of plant-animal interactions. *Ecology Letters*, *6*(1), 69–81. https://doi.org/10.1046/j.1461-0248.2003.00403.x

Jørgensen, P. M. (2000). Survey of the Lichen Family Pannariaceae on the American Continent, North of Mexico. *The Bryologist*, *103*(4), 670–704. https://doi.org/10.1639/0007-2745(2000)103[0670:SOTLFP]2.0.CO;2

Jørgensen, P. M. (2005). A new Atlantic species in Fuscopannaria, with a key to its European species. *Lichenologist*, *37*(3), 221–225. https://doi.org/10.1017/S0024282905014866

Kaasalainen, U., Tuovinen, V., Mwachala, G., Pellikka, P., & Rikkinen, J. (2021). Complex Interaction Networks Among Cyanolichens of a Tropical Biodiversity Hotspot. *Frontiers in Microbiology*, *12*(June), 1–12. https://doi.org/10.3389/fmicb.2021.672333

Karger, D. N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R. W., … Kessler, M. (2017). Climatologies at high resolution for the earth's land surface areas. *Scientific Data*, *4*, 1–20. https://doi.org/10.1038/sdata.2017.122

Laliberte, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, *91*(1), 299–305. https://doi.org/10.1890/08-2244.1

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N. S. G., Garden, D., Dorrough, J., … Bonis, A. (2008). Assessing functional diversity in the field - Methodology matters! *Functional Ecology*, *22*(1), 134–147. https://doi.org/10.1111/j.1365-2435.2007.01339.x

Levine, J. M., Bascompte, J., Adler, P. B., & Allesina, S. (2017). Beyond pairwise mechanisms of

species coexistence in complex communities. *Nature*, *546*(7656), 56–64. https://doi.org/10.1038/nature22898

López-Angulo, J., Swenson, N. G., Cavieres, L. A., & Escudero, A. (2018). Interactions between abiotic gradients determine functional and phylogenetic diversity patterns in Mediterranean-type climate mountains in the Andes. *Journal of Vegetation Science*, *29*(2), 245–254. https://doi.org/10.1111/jvs.12607

Lu, J., Magain, N., Miadlikowska, J., Coyle, J. R., Truong, C., & Lutzoni, F. (2018). Bioclimatic factors at an intrabiome scale are more limiting than cyanobiont availability for the lichen-forming genus Peltigera. *American Journal of Botany*, *105*(7), 1198–1211. https://doi.org/10.1002/ajb2.1119

Lücking, R., Moncada, B., McCune, B., Farkas, E., Goffinet, B., Parker, D., … Yakovchenko, L. S. (2017). Pseudocyphellaria crocata (Ascomycota: Lobariaceae) in the Americas is revealed to be thirteen species, and none of them is P. crocata. *The Bryologist*, *120*(4), 441–500. https://doi.org/10.1639/0007-2745-120.4.14

Lutzoni, F., Pagel, M., & Reeb, V. (2001). Major fungal lineages are derived from lichen symbiotic ancestors. *Nature*, *411*(6840), 937–940. https://doi.org/10.1038/35082053

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Magain, N., & Sérusiaux, E. (2014). Do photobiont switch and cephalodia emancipation act as evolutionary drivers in the lichen symbiosis? A case study in the Pannariaceae (Peltigerales). *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0089876

Magain, N., Truong, C., Goward, T., Niu, D., Goffinet, B., Sérusiaux, E., … Miadlikowska, J. (2018). Species delimitation at a global scale reveals high species richness with complex biogeography and patterns of symbiont association in peltigera section peltigera (Lichenized ascomycota: Lecanoromycetes). *Taxon*, *67*(5), 836–870. https://doi.org/10.12705/675.3

Martínez, I. (1999). *Taxonomía del género Peltigera Willd. (Ascomycetes Liquenizados) en la península Ibérica y estudio de sus hongos liquenícolas.* Real Jardín Botánico, C.S.I.C.

Martos, F., Munoz, F., Pailler, T., Kottke, I., Gonneau, C., & Selosse, M.-A. (2012). The role of epiphytism in architecture and evolutionary constraint within mycorrhizal networks of tropical orchids. *Molecular Ecology*, *21*(20), 5098–5109. https://doi.org/10.1111/j.1365-294X.2012.05692.x

May, R. M. (1972). Will a Large Complex System be Stable? *Nature*, *238*(5364), 413–414. https://doi.org/10.1038/238413a0

Montesinos-Navarro, A., Segarra-Moragues, J. G., Valiente-Banuet, A., & Verdú, M. (2012). The network structure of plant–arbuscular mycorrhizal fungi. *New Phytologist*, *194*(2), 536–547. https://doi.org/10.1111/j.1469-8137.2011.04045.x

Nash, III, T. (2008). *Lichen Biology* (2nd ed.). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511790478

Nash, T. H. (1996). *Lichen Biology.* (Cambridge). Cambridge University Press.

Nelson, P. R., McCune, B., & Swanson, D. K. (2015). Lichen traits and species as indicators of vegetation and environment. *The Bryologist*, *118*(3), 252. https://doi.org/10.1639/0007-2745-118.3.252

O'Brien, H. E., Miadlikowska, J., & Lutzoni, F. (2013). Assessing population structure and host specialization in lichenized cyanobacteria. *New Phytologist*, *198*(2), 557–566. https://doi.org/10.1111/nph.12165

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104

Otálora, M. A. G., Martínez, I., O'Brien, H., Molina, M. C., Aragón, G., & Lutzoni, F. (2010). Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution*, *56*(3), 1089–1095. https://doi.org/10.1016/j.ympev.2010.05.013

Passo, A., & Calvelo, S. (2006). New reports and combinations in the family Pannariaceae (Lecanorales, lichenized Ascomycota). *The Lichenologist*, *38*(06), 549–555. https://doi.org/10.1017/S0024282906005688

Passo, A., & Calvelo, S. (2011). Pannaria byssoidea (Pannariaceae), a new squamulose species from southern South America. *The Bryologist*, *114*(4), 756–763. https://doi.org/10.1639/0007-2745-114.4.756

Passo, A., Calvelo, S., & Stocker-Wörgötter, E. (2004). Taxonomic notes on Pannaria pallida from Southern South America and New Zealand. *Mycotaxon (USA)*, *90*, 55–65.

Pellissier, L., Albouy, C., Bascompte, J., Farwig, N., Graham, C., Loreau, M., … Gravel, D. (2018). Comparing species interaction networks along environmental gradients. *Biological Reviews*, *93*(2), 785–800. https://doi.org/10.1111/brv.12366

Pinheiro, J. C., & Bates, D. M. (2000). *Mixed-effects models in S and Splus.* Princeton, NJ: Springer Verlag, New York. Purves.

R Core Team. (2021). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.r-project.org/

Ramos-Jiliberto, R., Domínguez, D., Espinoza, C., López, G., Valdovinos, F. S., Bustamante, R. O., & Medel, R. (2010). Topological change of Andean plant-pollinator networks along an altitudinal gradient. *Ecological Complexity*, *7*(1), 86–90. https://doi.org/10.1016/j.ecocom.2009.06.001

Rao, C. R. (1982). Diversity and dissimilarity coefficients: A unified approach. *Theoretical Population Biology*, *21*(1), 24–43. https://doi.org/10.1016/0040-5809(82)90004-1

Rapai, S. B., McMullin, R. T., & Newmaster, S. G. (2012). The importance of macrolichen traits and phylogeny in forest community assemblage along a high elevation gradient in southwestern British Columbia. *Forest Ecology and Management*, *274*, 231–240. https://doi.org/10.1016/j.foreco.2012.02.018

Reichardt, J., & Bornholdt, S. (2006). Statistical mechanics of community detection. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, *74*(1), 1–16.

https://doi.org/10.1103/PhysRevE.74.016110

Revelle, W. (2021). psych: Procedures for psychological, psychometric, and personality research. R package version 2.1.9. Northwestern University. Retrieved from https://cran.r-project.org/package=psych

Rezende, E. L., Lavabre, J. E., Guimarães, P. R., Jordano, P., & Bascompte, J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature*, *448*(7156), 925–928. https://doi.org/10.1038/nature05956

Rico-Gray, V., Díaz-Castelazo, C., Ramírez-Hernández, A., Guimarães, P. R., & Nathaniel Holland, J. (2012). Abiotic factors shape temporal variation in the structure of an ant–plant network. *Arthropod-Plant Interactions*, *6*(2), 289–295. https://doi.org/10.1007/s11829-011-9170-3

Rikkinen, J. (2003). Ecological and evolutionary role of photobiont-mediated guilds in lichens. *Symbiosis*, *34*(2), 99–110.

Rikkinen, J. (2013). Molecular studies on cyanobacterial diversity in lichen symbioses. *MycoKeys*, *6*(April 2013), 3–32. https://doi.org/10.3897/mycokeys.6.3869

Rikkinen, J., Oksanen, I., & Lohtander, K. (2002). Lichen guilds share related cyanobacterial symbionts. *Science*, *297*(5580), 357. https://doi.org/10.1126/science.1072961

Rodríguez-Arribas, C., Martínez, I., Aragón, G., Zamorano-Elgueta, C., Cavieres, L., & Prieto, M. (2023). Specialization patterns in symbiotic associations: A community perspective over spatial scales. *Ecology and Evolution*, *13*(7), e10296. https://doi.org/10.1002/ece3.10296

Rohr, R. P., Saavedra, S., & Bascompte, J. (2014). On the structural stability of mutualistic systems. *Science*, *345*(6195). https://doi.org/10.1126/science.1253497

Schleuning, M., Ingmann, L., Strauß, R., Fritz, S. A., Dalsgaard, B., Matthias Dehling, D., … Dormann, C. F. (2014). Ecological, historical and evolutionary determinants of modularity in weighted seed-dispersal networks. *Ecology Letters*, *17*(4), 454–463. https://doi.org/10.1111/ele.12245

Simmons, B. I., Cirtwill, A. R., Baker, N. J., Wauchope, H. S., Dicks, L. V., Stouffer, D. B., & Sutherland, W. J. (2019). Motifs in bipartite ecological networks: uncovering indirect interactions. *Oikos*, *128*(2), 154–170. https://doi.org/10.1111/oik.05670

Stouffer, D. B., & Bascompte, J. (2011). Compartmentalization increases food-web persistence. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(9), 3648–3652. https://doi.org/10.1073/pnas.1014353108

Stouffer, D. B., Sales-Pardo, M., Sirer, M. I., & Bascompte, J. (2012). Evolutionary conservation of species' roles in food webs. *Science*, *335*(6075), 1489–1492. https://doi.org/10.1126/science.1216556

Suweis, S., Simini, F., Banavar, J. R., & Maritan, A. (2013). Emergence of structural and dynamical properties of ecological mutualistic networks. *Nature*, *500*(7463), 449–452. https://doi.org/10.1038/nature12438

Thompson, J. N. (2005). *The geographic mosaic of coevolution.* University of Chicago Press.

Trøjelsgaard, K., & Olesen, J. M. (2013). Macroecology of pollination networks. *Global Ecology and Biogeography*, *22*(2), 149–162. https://doi.org/10.1111/j.1466-8238.2012.00777.x

Vázquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology*, *85*(5), 1251–1257. https://doi.org/10.1890/03-3112

Vitikainen, O. (1994). Notes on some Peltigera of the Neotropics. *Acta Botanica Fennica*, *150*, 217–221.

Wedin, M., Döring, H., & Gilenstam, G. (2004). Saprotrophy and lichenization as options for the same fungal species on different substrata: environmental plasticity and fungal lifestyles in the Stictis–Conotrema complex. *New Phytologist*, *164*(3), 459–465. https://doi.org/10.1111/j.1469-8137.2004.01198.x

White, F. J., & James, P. W. (1988). Studies on the Genus Nephroma II. The Southern Temperate Species. *The Lichenologist*, *20*(02), 103–166. https://doi.org/10.1017/S0024282988000167

Wilmers, C. C. (2007). Understanding ecosystem robustness. *Trends in Ecology & Evolution*, *22*(10), 504–506. https://doi.org/10.1016/j.tree.2007.08.008

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J. M., Olesen, J. M., Valido, A., & Warren, P. H. (2005). Body size in ecological networks. *Trends in Ecology and Evolution*, *20*(7), 402–409. https://doi.org/10.1016/j.tree.2005.04.005

Yahr, R., Vilgalys, R., & Depriest, P. T. (2004). Strong fungal specificity and selectivity for algal symbionts in Florida scrub Cladonia lichens. *Molecular Ecology*, *13*(11), 3367–3378. https://doi.org/10.1111/j.1365-294X.2004.02350.x

Yahr, R., Vilgalys, R., & Depriest, P. T. (2006). Geographic variation in algal partners of Cladonia subtenuis (Cladoniaceae) highlights the dynamic nature of a lichen symbiosis. *New Phytologist*, *171*(4), 847–860. https://doi.org/10.1111/j.1469-8137.2006.01792.x

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

Supporting information

CHAPTER 3

Unravelling network structures and species roles in the lichen symbiosis in a wide latitudinal gradient.

Clara Rodríguez-Arribas, Jordi Bascompte, María Prieto, Jesús López-Angulo, Gregorio Aragón and Isabel Martínez

Appendix S1. *Nostoc* phylogroup delimitation.

DNA extraction of the cyanobacteria was performed with Chelex® 100 Chelating Resin (Bio-Rad, Hercules, CA, USA). For the amplification of the *rbcLX* genetic region, primers *cw* and *cx* (Rudi *et al*., 1998) were used in a Polymerase Chain Reaction (PCR). The PCR conditions were as follows: 95 ºC 15 min; 35 cycles of 1 min at 95 ºC, 30 s at 54 ºC, 30 s at 72 ºC; 10 min at 72 ºC. PCR products were sequenced by Macrogen Spain service (www.macrogen.com) with the same primers employed in the PCR. The sequences obtained were edited and aligned using Geneious Prime 2021.0.1 software (https://www.geneious.com). In the alignment, published sequences from Magain *et al*. (2017) were also included in order to have referenced phylogroups. Ambiguous regions (i.e., two intergenic spacers) were delimited manually and excluded for the analysis using Aliview v. 1.26 (Larsson, 2014).

To delimit *Nostoc* phylogroups two techniques were combined. First, we delimit the operational taxonomic units (OTUs) using ASAP (Assemble Species by Automatic Partitioning, Puillandre *et al*., 2021) in the webserver https://bioinfo.mnhn.fr/abi/public/asap/. A Jukes-Cantor (JC69) model of substitution was applied, which split groups below 0.01 probability, kept 10 best scores, and set -1 as seed value. OTUs were selected in the 0.001-0.01 range of genetic distances. Second, the highly supported clades obtained from the phylogenetic analysis (Maximum likelihood and Bayesian), were used to merge OTUs from the same clade and obtained *Nostoc* phylogroups, following previous criteria used by Magain *et al*. (2017). The maximum likelihood (ML) analysis was conducted in the CIPRES Science Gateway Portal (Miller *et al*., 2010) with RAxML v. 8.2.12 (Stamatakis, 2014) and 1000 bootstrap iterations. The Bayesian analysis was run in MrBayes 3.2.7a (Huelsenbeck & Ronquist, 2001) using Agapita server (URJC, Biodiversity and Conservation Area). This latter analysis was performed for 50 million generations with two runs and four chains, with a burning of the 25%, sampling every 1000 generations and a substitution model GTR+I+G (Rodríguez *et al*., 1990).

References:

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *2010 Gateway Computing Environments Workshop (GCE)* (pp. 1–8). https://doi.org/10.1109/GCE.2010.5676129

Puillandre, N., Brouillet, S., & Achaz, G. (2021). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, *21*(2), 609–620. https://doi.org/10.1111/1755-0998.13281

Rodríguez, F., Oliver, J. L., Marín, A., & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, *142*(4), 485–501. https://doi.org/10.1016/S0022-5193(05)80104-3

Rudi, K., Skulberg, O. M., & Jakobsen, K. S. (1998). Evolution of cyanobacteria by exchange of genetic material among phyletically related strains. *Journal of Bacteriology*, *180*(13), 3453–3461. https://doi.org/10.1128/jb.180.13.3453-3461.1998

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Appendix S2. Environmental variables: 1) Geographic variables: Latitude and Longitude (º), 2) Forest structure and habitat quality related variables: Altitude (mlsa), Orientation (º), Inclination (º), DBH (m) and canopy cover (%), 3)Climate related variables extracted from CHELSA climate database (Karger *et al*. 2017): Annual Mean Temperature (bio1), Mean Diurnal Range (BIO2), Isothermality (BIO3), Temperature Seasonality (BIO4), Max Temperature of Warmest Month (BIO5), Min Temperature of Coldest Month (BIO6), Temperature Annual Range (BIO7), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Mean Temperature of Warmest Quarter (bio10), Mean Temperature of Coldest Quarter (bio11), Annual Precipitation (bio12), Precipitation of Wettest Month (bio13), Precipitation of Driest Month (bio14), Precipitation Seasonality (bio15), Precipitation of Wettest Quarter (bio16), Precipitation of Driest Quarter (bio17), Precipitation of Warmest Quarter (bio18), and Precipitation of Coldest Quarter (bio19). Temperature in ºC, precipitation in mm. Sampling sites: 1) Conguillío National Park (COB1 and COB2), 2) Puyehue National Park (PUYB1 and PUYB2), 3) Hornopirén National Park (HORB1), 4) Cerro Castillo National Park (KKB1 and KKB2), 5) Torres del Paine National Park (TPB1), 6) Magallanes National Reserve (RMB1) and 7) Navarino Island (PWB1 and PWB2).

	GEOGRAPHIC VARIABLES		FOREST STRUCTURE AND HABITAT QUALITY VARIABLES						CLIMATE																	
									TEMPERATURE RELATED VARIABLES											PRECIPITATION RELATED VARIABLES							
Plot	Longitude	Latitude	Altitude	Orientation	Inclination	DBH	Canopy	Isolation	bio01	bio02	bio03	bio04	bio05	bio06	bio07	bio08	bio09	bio10	bio11	bio12	bio13	bio14	bio15	bio16	bio17	bio18	bio19
cob1_1	-71.68	-38.64	1400	172	6.6	190.3	79.25	0.70	6.1	9.1	40	438.9	19.2	-3.5	22.7	1.6	12.5	12.5	0.4	1611	271	36	60	788	114	114	767
cob1_2	-71.68	-38.64	1383	16	7.4	191.4	83.54	0.85	6.1	9.1	40	438.9	19.2	-3.5	22.7	1.6	12.5	12.5	0.4	1611	271	36	60	788	114	114	767
cob1_3	-71.68	-38.64	1372	18	5.6	195.9	69.38	0.84	6.1	9.1	40	438.9	19.2	-3.5	22.7	1.6	12.5	12.5	0.4	1611	271	36	60	788	114	114	767
cob1_4	-71.69	-38.64	1373	210	22	207.9	72.79	0.52	6.1	9.1	40	438.9	19.2	-3.5	22.7	1.6	12.5	12.5	0.4	1611	271	36	60	788	114	114	767
cob1_5	-71.69	-38.64	1338	170	13.2	167.2	79.07	0.62	6.1	9.1	40	438.9	19.2	-3.5	22.7	1.6	12.5	12.5	0.4	1611	271	36	60	788	114	114	767
cob2_1	-71.60	-38.63	1585	226	24	145.7	78.47	0.54	6	9.1	39.9	443.2	19.1	-3.7	22.8	1.4	12.4	12.4	0.2	1459	247	34	60	718	107	107	685
cob2_2	-71.60	-38.64	1538	206	10.4	148.9	78.41	0.67	6	9.1	39.9	443.2	19.1	-3.7	22.8	1.4	12.4	12.4	0.2	1459	247	34	60	718	107	107	685
puyb1_1	-72.22	-40.78	970	180	4.8	112.3	77.13	0.70	7	7.2	37.9	380.4	17.8	-1.2	19.1	3.1	12.5	12.5	2	2370	323	96	43	965	292	292	931
puyb1_2	-72.22	-40.78	984	12	3.8	108.4	72.83	0.80	7	7.2	37.9	380.4	17.8	-1.2	19.1	3.1	12.5	12.5	2	2370	323	96	43	965	292	292	931
puyb1_3	-72.22	-40.78	986	28	3.6	125.7	76.09	0.79	7	7.2	37.9	380.4	17.8	-1.2	19.1	3.1	12.5	12.5	2	2370	323	96	43	965	292	292	931
puyb1_4	-72.22	-40.78	978	140	14.2	108.6	70.15	0.61	7	7.2	37.9	380.4	17.8	-1.2	19.1	3.1	12.5	12.5	2	2370	323	96	43	965	292	292	931
puyb1_5	-72.23	-40.78	974	18	18.8	120.5	63.82	0.92	7	7.2	37.9	380.4	17.8	-1.2	19.1	3.1	12.5	12.5	2	2370	323	96	43	965	292	292	931
puyb2_1	-72.19	-40.78	1281	304	30.6	119.3	71.11	0.84	5.9	7.2	38	381	16.8	-2.3	19.1	2	11.4	11.4	0.9	2388	320	103	41	958	312	312	923

puyb2_2	-72.19	-40.78	1302	276	28.8	117.8	68.69	0.70	5.9	7.2	38	381	16.8	-2.3	19.1	2	11.4	11.4	0.9	2388	320	103	41	958	312	312	923
puyb2_3	-72.19	-40.79	1339	324	37.4	117.3	65.09	0.92	5.9	7.2	38	381	16.8	-2.3	19.1	2	11.4	11.4	0.9	2388	320	103	41	958	312	312	923
puyb2_4	-72.20	-40.78	1147	260	20.4	131.2	66.60	0.67	5.9	7.2	38	381	16.8	-2.3	19.1	2	11.4	11.4	0.9	2388	320	103	41	958	312	312	923
puyb2_5	-72.20	-40.78	1119	276	26.4	138.2	77.82	0.71	5.9	7.2	38	381	16.8	-2.3	19.1	2	11.4	11.4	0.9	2388	320	103	41	958	312	312	923
kkb1_1	-72.03	-46.07	1140	0	30.2	156.1	87.18	0.96	2.9	5.8	34.5	353.9	11.9	-5	16.9	-2.1	7.8	7.8	-2.1	778	117	31	47	321	100	100	321
kkb1_2	-72.02	-46.07	1083	320	5.8	158.5	84.44	0.75	2.9	5.8	34.5	353.9	11.9	-5	16.9	-2.1	7.8	7.8	-2.1	778	117	31	47	321	100	100	321
kkb1_3	-72.02	-46.07	1092	20	10.4	139.9	84.60	0.80	2.9	5.8	34.5	353.9	11.9	-5	16.9	-2.1	7.8	7.8	-2.1	778	117	31	47	321	100	100	321
kkb1_4	-72.01	-46.07	1088	330	21.4	142.7	85.18	0.87	2.9	5.8	34.5	353.9	11.9	-5	16.9	-2.1	7.8	7.8	-2.1	778	117	31	47	321	100	100	321
kkb1_5	-72.01	-46.06	1060	312	12	137.2	85.99	0.78	2.9	5.8	34.5	353.9	11.9	-5	16.9	-2.1	7.8	7.8	-2.1	778	117	31	47	321	100	100	321
kkb2_1	-72.16	-46.08	910	122	17.8	129.9	83.32	0.54	5.1	5.8	34.5	349.3	14	-2.8	16.8	0.2	10	10	0.2	722	104	32	42	282	103	103	282
kkb2_2	-72.16	-46.09	867	110	14.8	122.4	84.64	0.61	5.1	5.8	34.5	349.3	14	-2.8	16.8	0.2	10	10	0.2	722	104	32	42	282	103	103	282
kkb2_3	-72.15	-46.09	804	148	12.8	144.9	88.75	0.54	5.1	5.8	34.5	349.3	14	-2.8	16.8	0.2	10	10	0.2	722	104	32	42	282	103	103	282
kkb2_4	-72.16	-46.09	898	66	24.2	148.6	87.03	0.75	5.1	5.8	34.5	349.3	14	-2.8	16.8	0.2	10	10	0.2	722	104	32	42	282	103	103	282
kkb2_5	-72.15	-46.09	807	150	16	147.5	85.97	0.49	5.1	5.8	34.5	349.3	14	-2.8	16.8	0.2	10	10	0.2	722	104	32	42	282	103	103	282
tpb1_1	-73.15	-51.13	657	124	11.4	155.2	83.44	0.53	5.1	4.7	33.9	315.6	12	-1.8	13.8	3.8	6.4	9.2	0.5	853	98	51	20	284	163	164	232
tpb1_2	-73.15	-51.13	597	117	7.2	152.5	85.43	0.58	5.1	4.7	33.9	315.6	12	-1.8	13.8	3.8	6.4	9.2	0.5	853	98	51	20	284	163	164	232
tpb1_3	-73.17	-51.11	86	68	4.8	148	83.95	0.65	5.1	4.7	33.9	315.6	12	-1.8	13.8	3.8	6.4	9.2	0.5	853	98	51	20	284	163	164	232
tpb1_4	-73.18	-51.10	161	334	25.2	210.1	84.81	0.87	5.1	4.7	33.9	315.6	12	-1.8	13.8	3.8	6.4	9.2	0.5	853	98	51	20	284	163	164	232
tpb1_5	-73.18	-51.10	201	70	25.6	212.1	83.12	0.68	5.1	4.7	33.9	315.6	12	-1.8	13.8	3.8	6.4	9.2	0.5	853	98	51	20	284	163	164	232
rmb1_1	-71.03	-53.14	311	118	7	109.1	84.49	0.55	4.2	4.3	34	297.6	10.7	-2	12.7	3	3.7	8.2	0	444	47	27	17	140	86	112	96
rmb1_2	-71.04	-53.14	415	114	8.8	107.1	83.72	0.54	4.2	4.3	34	297.6	10.7	-2	12.7	3	3.7	8.2	0	444	47	27	17	140	86	112	96
pwb1_1	-67.66	-54.97	232	292	15.4	152.3	88.64	0.63	4.9	4.1	35.2	268.9	11	-0.7	11.7	8.5	5.9	8.6	1.2	528	53	34	14	156	104	139	119
pwb1_2	-67.66	-54.97	168	250	5.8	122.5	84.70	0.54	4.9	4.1	35.2	268.9	11	-0.7	11.7	8.5	5.9	8.6	1.2	528	53	34	14	156	104	139	119
pwb1_3	-67.65	-54.96	140	310	15.8	125.1	84.81	0.70	4.9	4.1	35.2	268.9	11	-0.7	11.7	8.5	5.9	8.6	1.2	528	53	34	14	156	104	139	119
pwb2_1	-67.63	-54.96	466	328	15.4	131	82.33	0.74	4.4	4.1	35.2	269.5	10.4	-1.2	11.7	7.9	5.4	8	0.6	648	68	42	15	196	129	178	138
pwb2_2	-67.63	-54.96	404	328	31	118.6	78.56	0.85	4.4	4.1	35.2	269.5	10.4	-1.2	11.7	7.9	5.4	8	0.6	648	68	42	15	196	129	178	138
pwb2_3	-67.63	-54.96	338	322	27	120.2	78.11	0.80	4.4	4.1	35.2	269.5	10.4	-1.2	11.7	7.9	5.4	8	0.6	648	68	42	15	196	129	178	138
pwb2_4	-71.68	-38.64	1400	172	6.6	190.3	79.25	0.70	4.4	4.1	35.2	269.5	10.4	-1.2	11.7	7.9	5.4	8	0.6	648	68	42	15	196	129	178	138

Appendix S3. Correlations between each PCA axis and the climatic and geographic variables.

Climatic and Geographic variables	RC1	RC2	RC3
Longitude	-0.36	-0.23	0.51
Latitude	**0.87**	0.33	-0.35
Altitude	**0.73**	0.23	-0.59
Annual Mean Temperature (bio1)	**0.73**	0.31	0.56
Mean Diurnal Range (bio02)	**0.95**	0.15	-0.25
Isothermality (bio3)	**0.88**	0.26	0.08
Temperature Seasonality (bio4)	**0.91**	0.12	-0.38
Maximum Temperature of Warmest Month (bio5)	**0.96**	0.26	-0.03
Minimum Temperature of Coldest Month (bio6)	-0.24	0.21	**0.93**
Temperature Annual Range (bio7)	**0.92**	0.16	-0.35
Mean Temperature of Wettest Quarter (bio8)	-0.32	-0.01	**0.89**
Mean Temperature of Driest Quarter (bio9)	**0.89**	0.29	-0.13
Mean Temperature of Warmest Quarter (bio10)	**0.93**	0.28	0.17
Mean Temperature of Coldest Quarter (bio11)	0.3	0.33	**0.87**
Annual Precipitation (bio12)	0.57	**0.82**	-0.03
Precipitation of Wettest Month (bio13)	0.7	0.69	-0.1
Precipitation of Driest Month (bio14)	0.17	**0.98**	0.1
Precipitation Seasonality (bio15)	**0.83**	0.03	-0.52
Precipitation of Wettest Quarter (bio16)	0.69	**0.71**	-0.08
Precipitation of Driest Quarter (bio17)	0.17	**0.98**	0.08
Precipitation of Warmest Quarter (bio18)	0.06	**0.97**	0.19
Precipitation of Coldest Quarter (bio19)	0.7	0.69	-0.11
Proportion of variance	0.49	0.27	0.19
Cumulative proportion of variance	0.49	0.76	0.95

Appendix S4. Community Weighted Mean of each categorical functional trait and Rao's dissimilarity index. BL = Broad-lobed foliose, NL = Narrow-lobed foliose, SQ = Squamulose.

	Reproduction				Function of the Photobiont		Growth form			Rao
	Sexual	Asexual	Both	None	Cyanolichens	Cephalolichens	BL	NL	SQ	
cob1_1	0.25	0.40	0.35	0.00	0.77	0.23	0.13	0.67	0.20	0.17
cob1_2	0.46	0.19	0.36	0.00	0.71	0.29	0.28	0.38	0.35	0.20
cob1_3	0.64	0.25	0.12	0.00	0.85	0.15	0.12	0.61	0.27	0.13
cob1_4	0.29	0.19	0.46	0.06	0.92	0.08	0.24	0.64	0.12	0.11
cob1_5	0.59	0.08	0.33	0.00	0.85	0.15	0.17	0.43	0.40	0.13
cob2_4	0.57	0.38	0.05	0.00	0.81	0.19	0.22	0.26	0.52	0.15
cob2_5	0.61	0.26	0.12	0.00	0.80	0.20	0.12	0.30	0.58	0.14
puyb1_1	0.04	0.77	0.19	0.00	0.54	0.46	0.82	0.16	0.02	0.14
puyb1_2	0.15	0.75	0.11	0.00	0.74	0.26	0.90	0.10	0.00	0.11
puyb1_3	0.12	0.65	0.23	0.00	0.44	0.56	0.88	0.12	0.00	0.14
puyb1_4	0.10	0.50	0.40	0.00	0.19	0.81	0.94	0.05	0.01	0.10
puyb1_5	0.08	0.71	0.21	0.00	0.18	0.82	0.93	0.07	0.00	0.08
puyb2_1	0.25	0.54	0.16	0.05	0.11	0.89	0.75	0.25	0.00	0.11
puyb2_2	0.44	0.41	0.02	0.12	0.65	0.35	0.60	0.40	0.00	0.19
puyb2_3	0.47	0.37	0.02	0.14	0.59	0.41	0.53	0.47	0.00	0.21
puyb2_4	0.40	0.39	0.21	0.00	0.47	0.53	0.62	0.37	0.00	0.21
puyb2_5	0.14	0.74	0.12	0.00	0.32	0.68	0.89	0.11	0.00	0.12
kkb1_1	0.32	0.14	0.46	0.09	0.50	0.50	0.64	0.09	0.27	0.21
kkb1_2	0.53	0.32	0.15	0.00	0.77	0.23	0.55	0.04	0.41	0.17
kkb1_3	0.26	0.17	0.44	0.13	0.59	0.41	0.65	0.16	0.19	0.21
kkb1_4	0.21	0.29	0.46	0.04	0.57	0.43	0.77	0.10	0.13	0.19
kkb1_5	0.34	0.28	0.27	0.11	0.74	0.26	0.48	0.19	0.33	0.21
kkb2_1	0.10	0.09	0.76	0.05	0.37	0.63	0.96	0.02	0.03	0.12
kkb2_2	0.15	0.26	0.60	0.00	0.63	0.37	0.82	0.04	0.13	0.15
kkb2_3	0.14	0.26	0.56	0.03	0.64	0.36	0.82	0.11	0.07	0.15
kkb2_4	0.13	0.09	0.77	0.00	0.32	0.68	0.96	0.01	0.04	0.12
kkb2_5	0.09	0.04	0.87	0.00	0.20	0.80	0.96	0.02	0.03	0.09
tpb1_1	0.33	0.03	0.64	0.00	0.37	0.63	0.70	0.03	0.27	0.20
tpb1_2	0.50	0.05	0.45	0.00	0.44	0.56	0.87	0.10	0.03	0.16
tpb1_3	0.15	0.48	0.37	0.00	0.74	0.26	0.77	0.16	0.06	0.15
tpb1_4	0.02	0.53	0.46	0.00	0.97	0.03	0.92	0.04	0.04	0.04
tpb1_5	0.20	0.08	0.72	0.00	0.30	0.70	0.90	0.05	0.05	0.12
rmb1_1	0.16	0.77	0.07	0.00	0.72	0.28	0.90	0.10	0.00	0.14
rmb1_5	0.44	0.53	0.01	0.01	0.58	0.42	0.73	0.13	0.14	0.18
pwb1_1	0.15	0.31	0.54	0.00	0.73	0.27	0.91	0.06	0.03	0.13
pwb1_2	0.89	0.04	0.07	0.00	0.22	0.78	0.83	0.11	0.06	0.11
pwb1_3	0.66	0.16	0.18	0.00	0.32	0.68	0.69	0.22	0.08	0.16
pwb2_2	0.45	0.32	0.22	0.00	0.56	0.44	0.86	0.05	0.09	0.17
pwb2_3	0.62	0.18	0.20	0.00	0.40	0.60	0.95	0.05	0.01	0.14
pwb2_4	0.45	0.22	0.33	0.00	0.55	0.45	0.95	0.05	0.00	0.15
pwb2_5	0.78	0.09	0.13	0.00	0.36	0.64	0.91	0.03	0.06	0.12

Appendix S5. Equations used to estimate each parameter of network structure.

Name	Equation	Explanation
Network architecture metrics used		
Number of nodes of each class	$N_m = Number\ of\ mycobionts$ $N_p = Number\ of\ photobionts$	
Richness	$S = N_m + N_p$	
Size	$Size = N_m N_p$	
Links	$L = Number\ of\ interactions$	
Connectance	$C = \frac{L}{Size} = \frac{L}{N_m N_p}$	
Nestedness (Fortuna *et al*., 2019)	$N = \frac{\sum_{i=1,i<j}^{N_m} \frac{c_{ij}}{min\ (k_i, k_j)} + \sum_{i=1,i<j}^{N_p} \frac{c_{ij}}{min\ (k_i, k_j)}}{\frac{N_m(N_m-1)}{2} + \frac{N_p(N_p-1)}{2}}$	where N_m is the number of mycobionts, N_p the number of cyanobacteria, k_i the number of partners interacting with of node i, k_j the number of partners interacting with node j, c_{ij} the common number of partners interacting with nodes i and j.
Modularity (Clauset *et al*., 2004)	$Q = \frac{1}{2L}\sum_{ij}[A_{ij} - \frac{k_i k_j}{2L}]\delta(c_i, c_j)$	where L is the number of links, A_{ij} is the presence or absence of interaction between node i and node j, k_i the number of partners interacting with of node i, k_j the number of partners interacting with of node j, c_i is the module of node i y c_j is the module of node j. If nodes i and j belong to the same module $\delta = 1$ and $\delta = 0$ otherwise.
Null model		
Probabilistic null model (Bascompte *et al*., 2003)	$\rho_{ij} = \frac{p_i + q_j}{2}; \begin{cases} p_i = \frac{1}{N_m}\sum_{i=1}^{n} A_{ij} \\ q_j = \frac{1}{N_p}\sum_{j=1}^{m} A_{ij} \end{cases}$	where p_i is the observed probability of occupancy of row i, q_j is the observed probability of occupancy of column j, N_m is the number of mycobionts (number of rows of the incidence matrix), N_p the number of cyanobacteria (number of columns of the incidence matrix), A_{ij} is the presence or absence of interaction between row i and column j.

z-scores	$z - scores = \frac{x_i - \overline{x}}{\sigma}$	Where $x_i$is the observed value of nestedness, modularity or connectance, $\overline{x}$ is the average of that value in the random matrices generated by the null model and σ is the standard deviation of those matrices.

References:

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. Proceedings of the National Academy of Sciences of the United States of America, 100(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Clauset, A., Newman, M. E. J., & Moore, C. (2004). Finding community structure in very large networks. Physical Review E- Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics, 70(6), 6. https://doi.org/10.1103/PhysRevE.70.066111

Fortuna, M. A., Barbour, M. A., Zaman, L., Hall, A. R., Buckling, A., & Bascompte, J. (2019). Coevolutionary dynamics shape the structure of bacteria-phage infection networks. Evolution, 73(5), 1001–1011. https://doi.org/10.1111/evo.13731

Appendix S6. zc-parameter space.

Species roles are defined by their number of interacting partners within their own module (z), and by their interactions with species from different modules (c):

$$z = \frac{k_{is} - \bar{k}_s}{SD_{ks}}$$

$$c = 1 - \sum_{t=1}^{N_M} \left(\frac{k_{it}}{k_i}\right)^2$$

where k_{is} is the number of links of node i to other nodes from its own module s, $\bar{k}_s$ is the average and SD_{ks} the standard deviation of the number of interacting partners of each species within module s, k_i is the total number of partners interacting with node i, and k_{it} is the number of partners interacting with node i in module t (t considers all the modules in the network, including $i's$ own module).

The z and c values, define the position of the species in the zc-parameter space. Olesen *et al*., 2007 simplified the roles proposed by Guimerà and Amaral (2005) in peripherals, connectors, module hubs and network hubs. In both studies, the cutoffs in the zc-parameter space to characterize the role of the species were $z = 2.5$ and $c = 0.62$ which have a specific topological meaning (Olesen *et al*., 2007). Thus, species with low z and c ($z \leq 2.5$ and $c \leq 0.62$) would be peripheral species (i.e., species with few links and most of them with species from the same module), species with low z and high c ($z \leq 2.5$ and $c > 0.62$) would be connectors (i.e., ''glue'' modules together), species with high z and low c ($z > 2.5$ and $c \leq 0.62$) would be module hubs (i.e., highly connected species within their own module), and species with high z and c ($z > 2.5$ and $c > 0.62$) would be network hubs (i.e., species acting as connector and module hubs). Taking into account this information, peripherals would be considered as specialized, connectors and module hubs as generalized, and network hubs would be super generalized.

References:

Guimerà, R. & Amaral, L. A. N. (2005). Functional cartography of complex metabolic networks. Nature, 433, 895–900. https://doi.org/10.1038/nature03288

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination

networks. Proceedings of the National Academy of Sciences of the United States of America, 104(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104

Appendix S7. Exclusively isolated species, exclusively network species, species either belonging to the network or outside the network depending on the plot.

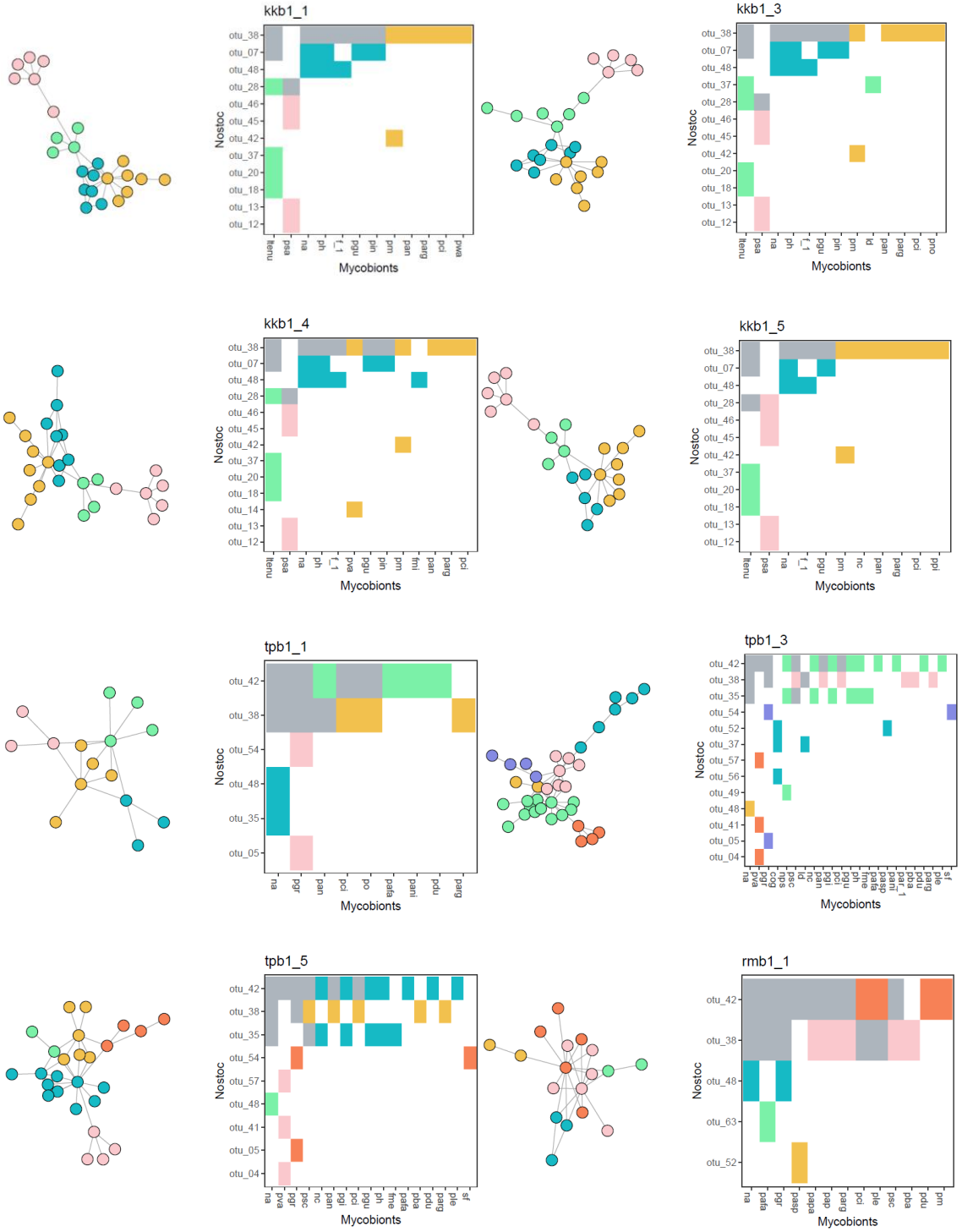
Mycobionts	
Exclusively isolated	*Leptogium cyanescens, Leptogium menziesii, Leptogium patagonicum, Pannaria arthroophylla, Peltigera canina, Peltigera collina, Peltigera hymenina, Peltigera membranacea, Peltigera rufescens, Pesudocyphellaria encoensis, Psoroma hypnorum, Psoroma polychidioides*
Exclusively within the network	*Fuscopannaria sp. 1, Fuscopannaria mediterranea, Fuscopannaria minor, Leptogium azureum, Leptigum aff. tenuissimum, Nephroma antarcticum, Nephroma analogicum, Nephroma parile, Nehroma skottsbergii, Pannaria farinosa, Pannaria pallida, Parmeliella nigrata, Parmeliella nigrocinta, Parmeliella sp. 1, Pseudocyphellaria* gr. *argyracea, Pseudocyphellaria bartlettii., Pseudocyphellaria coppinsii, Pseudocyphellaria dubia, Pseudocyphellaria flavicans, Pseudocyphellaria freycinettii, Pseudocyphellaria gilva, Pseudocyphellaria granulata, Pseudocyphellaria guilleminii, Pseudocyphellaria intricate, Pseudocyphellaria mallota, Pseudocyphellaria obvolute, Pseudocyphellaria piloselloidesm Pseudocyphellaria scabrosa, Pseudocyphellaria valdiviana, Pseudocyphellaria* gr. *vaccina, Pseudocyphellaria wandae, Psoroma asperellum, Psorophorus pholidotus, Sticta caulescens, Xanthopsoroma contextum, Xhanthopsoroma soccatum*
Isolated and within the network	*Collema flaccidum, Collema glaucophthalmum, Leptigum decipiens, Leptogium laceroides, Leptogium valdivianum, Nephroma celullosum, Nephroma pseudoparile, Pannaria pulverulacea, Pannaria sphinctrina, Pseudocyphellaria* gr. *citrina, Pseudocyphellaria glabra, Pseudocyphellaria hirsuta, Pseudocyphellaria lechlerii, Pseudocyphellaria norvegica, Psoroma hirsutulum, Sticta fuliginosa, Sticta hypochra, Sticta* gr. *weigelii*
Cyanobacteria	
Exclusively isolated	Phylogroups: 02, 06, 11, 17, 32, 39, 40, 43, 44, 50, 53, 64
Exclusively within the network	Phylogroups: 07, 08, 09, 10, 13, 18, 20, 21, 24, 25, 28, 29, 34, 36, 38, 41, 45, 46, 47, 48, 55, 57, 58, 60, 61, 63
Isolated and within the network	Phylogroups: 03, 04, 05, 12, 14, 22, 35, 37, 42, 49, 51, 52, 54, 56, 59, 62

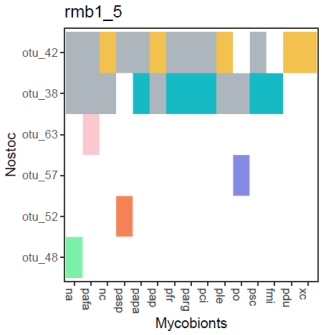
Appendix S8. Number of mycobionts, cyanobacteria, links, total richnes, connected modules in the netwotk, isolaated modules, mycobionts of isolated modules and cyanobacteria of isolated modules of each of the local network studied.

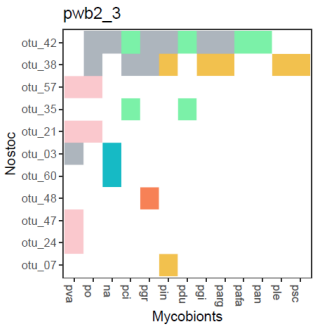
Network	Mycobionts	Cyanobacteria	Links	Richness	Modules network	Isolated modules	Isolated Mycobionts	Isolated Cyanobacteria
cob1_1	13	8	20	21	5	1	1	1
cob1_2	15	8	23	23	5	2	2	3
cob1_3	13	8	22	21	4	2	2	3
cob1_4	13	8	21	21	5	3	3	4
cob1_5	11	6	17	17	4	2	2	3
cob2_4	12	8	19	20	6	3	3	5
cob2_5	11	6	16	17	5	4	5	6
puyb1_1	6	7	12	13	4	4	4	4
puyb1_2	6	7	12	13	4	6	10	7
puyb1_3	13	10	23	23	4	4	6	5
puyb1_4	9	9	18	18	3	3	4	3
puyb1_5	9	9	18	18	4	1	1	1
puyb2_1	10	7	21	17	4	0	0	0
puyb2_2	10	9	24	19	4	1	1	1
puyb2_3	10	7	20	17	3	0	0	0
puyb2_4	10	7	22	17	4	2	2	2
puyb2_5	10	10	26	20	5	1	1	1
kkb1_1	12	12	29	24	4	4	4	5
kkb1_2	10	12	25	22	5	4	4	6
kkb1_3	13	12	30	25	4	3	3	4
kkb1_4	13	13	31	26	4	3	3	5
kkb1_5	11	12	25	23	4	3	4	4
kkb2_1	10	7	18	17	5	3	3	3
kkb2_2	12	7	20	19	5	2	2	2
kkb2_3	11	7	17	18	5	3	3	3
kkb2_4	8	6	15	14	5	2	2	2
kkb2_5	9	6	17	15	3	2	2	2
tpb1_1	9	6	18	15	4	0	0	0
tpb1_2	11	5	20	16	4	3	3	5
tpb1_3	23	13	45	36	6	2	2	3
tpb1_4	18	7	30	25	5	3	3	5
tpb1_5	17	9	34	26	5	3	3	4
rmb1_1	13	5	26	18	5	0	0	0
rmb1_5	15	6	29	21	6	0	0	0
pwb1_1	11	5	23	16	3	3	3	5
pwb1_2	9	7	21	16	4	1	1	2
pwb1_3	14	8	30	22	5	2	2	4
pwb2_2	9	6	19	15	5	1	1	2
pwb2_3	13	11	31	24	4	1	1	2
pwb2_4	12	9	26	21	5	1	1	2

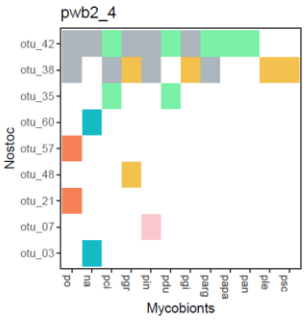
pwb2_5	10	8	22	18	5	1	2	3
Metanetwork	80	59	238	139	12	5	5	5

Appendix S9. Nested networks.

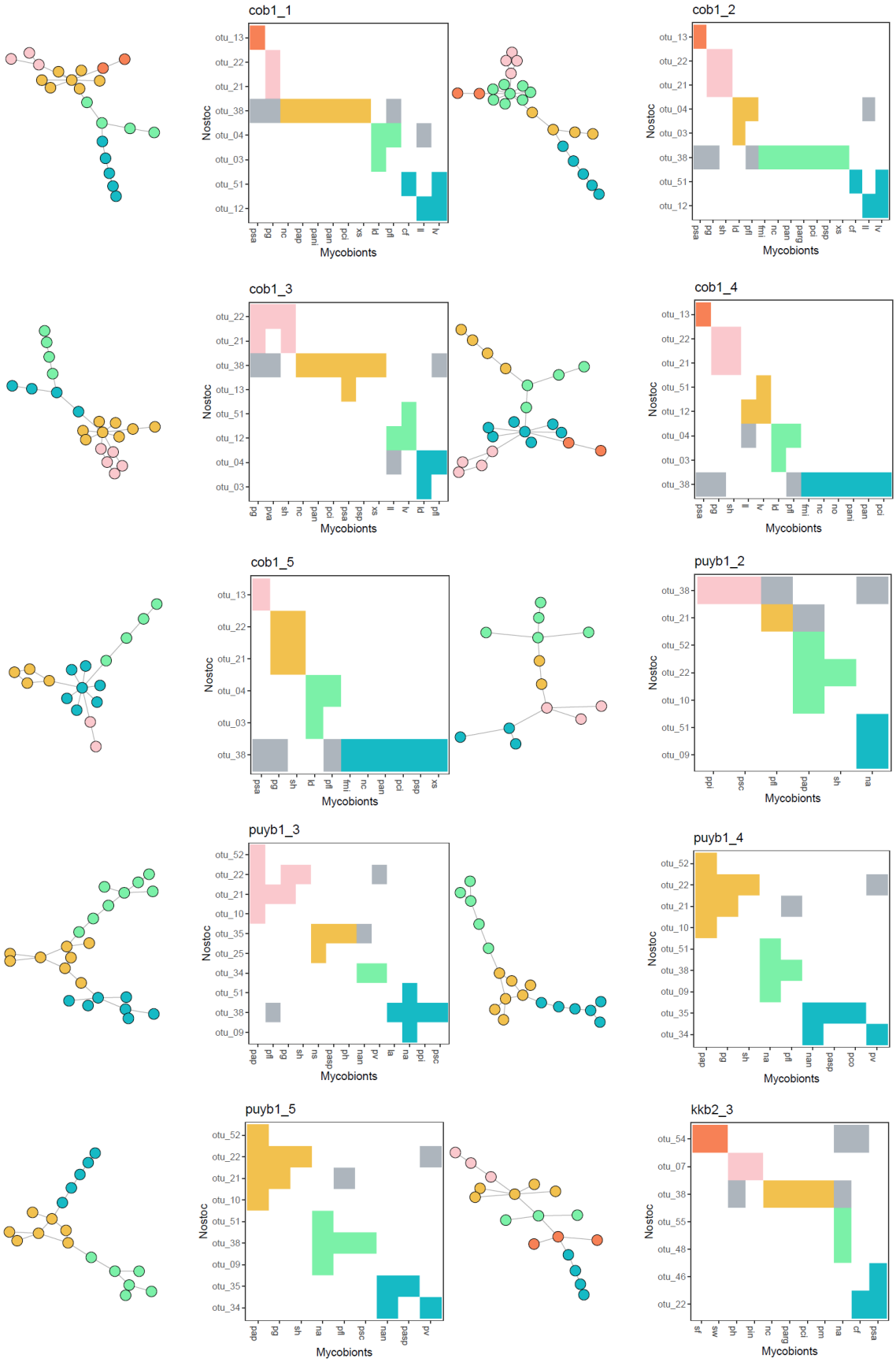



rmb1_5
otu_42
otu_38
otu_63
otu_57
otu_52
otu_48
Nostoc
na
pda
nc
pdp
pdi
ple
po
psc
xc
Mycobionts


otu_42
otu_38
otu_57
otu_35
otu_21
otu_03
otu_60
otu_48
otu_47
otu_24
otu_07
Nostoc
pva
po
na
pci
pgr
pti
pdu
pgi
pag
pap
pan
ple
psc
Mycobionts


pwb2_4
otu_42
otu_38
otu_35
otu_60
otu_57
otu_48
otu_21
otu_07
otu_03
Nostoc
po
na
pci
pgr
pin
pdu
pgi
pag
papa
pan
ple
psc
Mycobionts

Appendix S10. Modular networks.



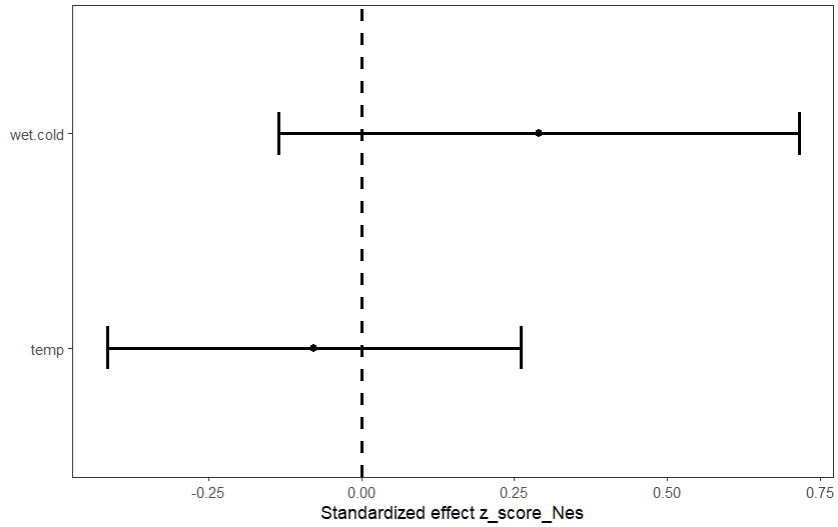
Appendix S11. z-scores and p-value of nestedness, modularity and connectance of each of the local networks studied.

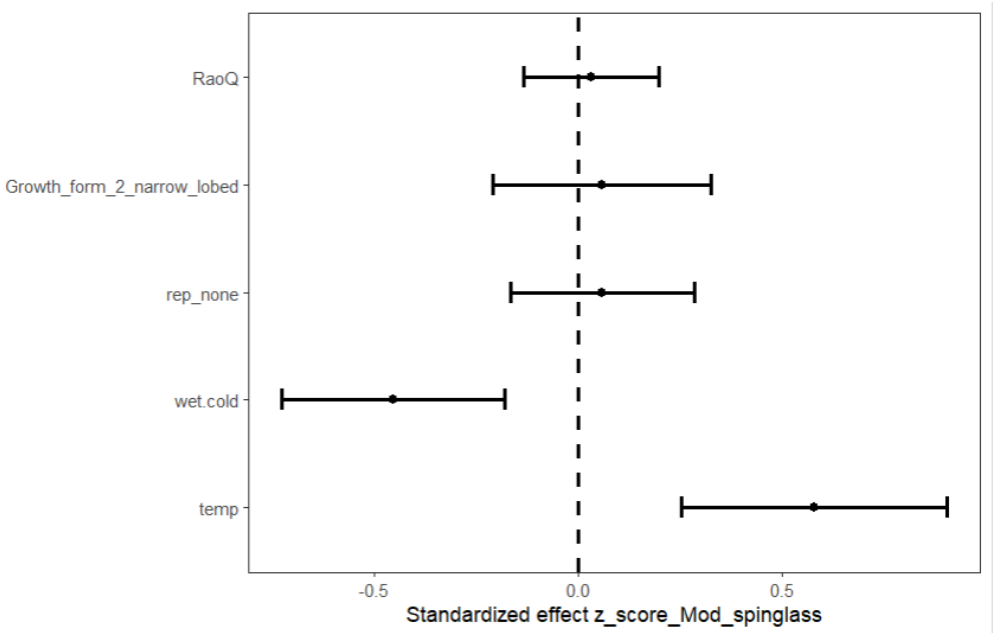
Network	zscore Nestedness	pvalue Nestedness	zscore Modularity	pvalue Modularity	zscore Connectance	pvalue Connectance
cob1_1	0.17	0.43	2.11	**0.02**	-2.42	**0.01**
cob1_2	0.17	0.43	2.59	**0.00**	-2.22	**0.01**
cob1_3	0.15	0.44	1.93	**0.03**	-1.85	**0.03**
cob1_4	0.18	0.43	2.50	**0.01**	-2.15	**0.02**
cob1_5	0.55	0.29	2.20	**0.01**	-1.89	**0.03**
cob2_4	0.68	0.25	1.37	0.09	-2.35	**0.01**
cob2_5	0.33	0.37	1.14	0.13	-2.01	**0.02**
puyb1_1	-1.44	0.93	1.60	0.05	-1.86	**0.03**
puyb1_2	-0.59	0.72	1.95	**0.03**	-1.88	**0.03**
puyb1_3	-0.79	0.79	2.12	**0.02**	-2.22	**0.01**
puyb1_4	-1.21	0.89	2.16	**0.02**	-2.06	**0.02**
puyb1_5	-1.13	0.87	2.08	**0.02**	-1.90	**0.03**
puyb2_1	0.21	0.42	0.51	0.30	-1.22	0.11
puyb2_2	0.59	0.28	1.40	0.08	-1.21	0.11
puyb2_3	0.44	0.33	0.81	0.21	-1.29	0.10
puyb2_4	-0.06	0.52	0.71	0.24	-0.95	0.17
puyb2_5	0.11	0.46	0.98	0.16	-1.15	0.12
kkb1_1	2.33	**0.01**	0.75	0.23	-1.64	0.05
kkb1_2	1.56	0.06	1.10	0.14	-1.79	**0.04**
kkb1_3	2.07	**0.02**	0.84	0.20	-1.77	**0.04**
kkb1_4	1.94	**0.03**	0.69	0.25	-1.82	**0.03**
kkb1_5	2.06	**0.02**	1.28	0.10	-2.03	**0.02**
kkb2_1	-0.86	0.80	0.95	0.17	-1.61	0.05
kkb2_2	-0.33	0.63	1.41	0.08	-1.87	**0.03**
kkb2_3	-0.41	0.66	2.39	**0.01**	-2.16	**0.02**
kkb2_4	-0.17	0.57	0.90	0.18	-1.40	0.08
kkb2_5	-0.15	0.56	0.72	0.23	-1.28	0.10
tpb1_1	2.11	**0.02**	0.29	0.38	-1.18	0.12
tpb1_2	0.37	0.35	0.29	0.38	-1.08	0.14
tpb1_3	2.14	**0.02**	0.65	0.26	-1.93	**0.03**
tpb1_4	1.00	0.16	0.50	0.31	-1.61	0.05
tpb1_5	2.21	**0.01**	0.23	0.41	-1.42	0.08
rmb1_1	2.19	**0.01**	-1.24	0.89	-0.76	0.22
rmb1_5	2.20	**0.01**	-2.03	0.98	-0.95	0.17
pwb1_1	0.94	0.17	0.21	0.42	-0.61	0.27
pwb1_2	0.86	0.19	0.23	0.41	-0.92	0.18
pwb1_3	1.25	0.11	0.27	0.39	-1.06	0.15
pwb2_2	0.95	0.17	-0.19	0.57	-1.03	0.15
pwb2_3	1.84	**0.03**	0.60	0.28	-1.37	0.09
pwb2_4	1.91	**0.03**	0.06	0.48	-1.46	0.07
pwb2_5	1.46	0.07	0.24	0.40	-1.30	0.10
Metanetwork	9.44	**1.88e-21**	0.08	0.47	-2.26	**0.01**

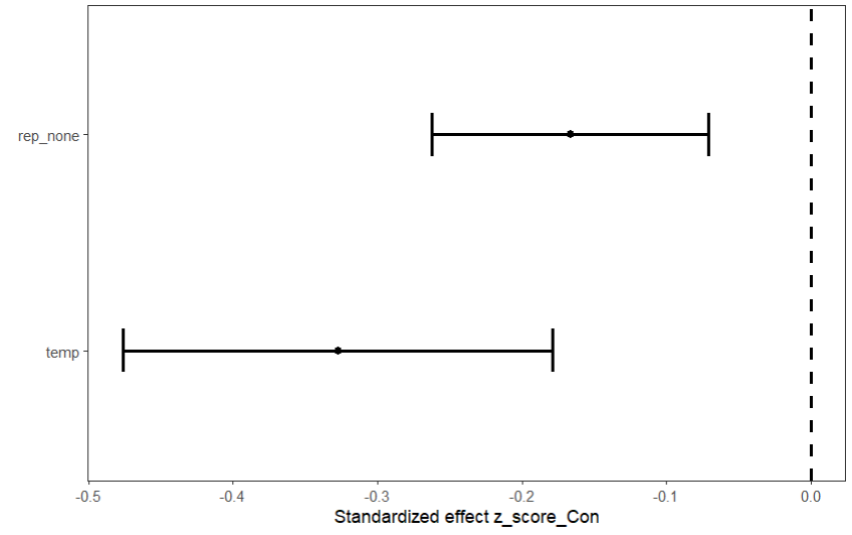
Appendix S12. Number and proportion of modules from the total of 41 modules found in the modular networks composed exclusively from the different categories of the studied functional traits, mycobiont families and genera. Abbreviations for Growth form refer to: BF = broad-lobed foliose, NF = narrow-lobed foliose, and SQ = squamulose.

	Number of modules	Proportion of modules
Reproductive mode		
Asexual	3	0.075
Both	2	0.05
Sexual	4	0.1
Function of cyanobacteria		
Cyanolichens	9	0.225
Cephalolichens	11	0.275
Growth form		
BF	13	0.325
NF	4	0.1
SQ	4	0.1
Family		
Collemataceae	4	0.1
Lobariaceae	9	0.225
Nephromataceae	2	0.05
Pannariaceae	4	0.1
Genera		
Collema	0	0
Fuscopannaria	0	0
Leptogium	2	0.05
Nephroma	2	0.05
Pannaria	0	0
Parmeliella	0	0
Pseudocyphellaria	4	0.1
Psoroma	4	0.1
Psorophorus	0	0
Sticta	1	0.025
Xanthopsoroma	0	0

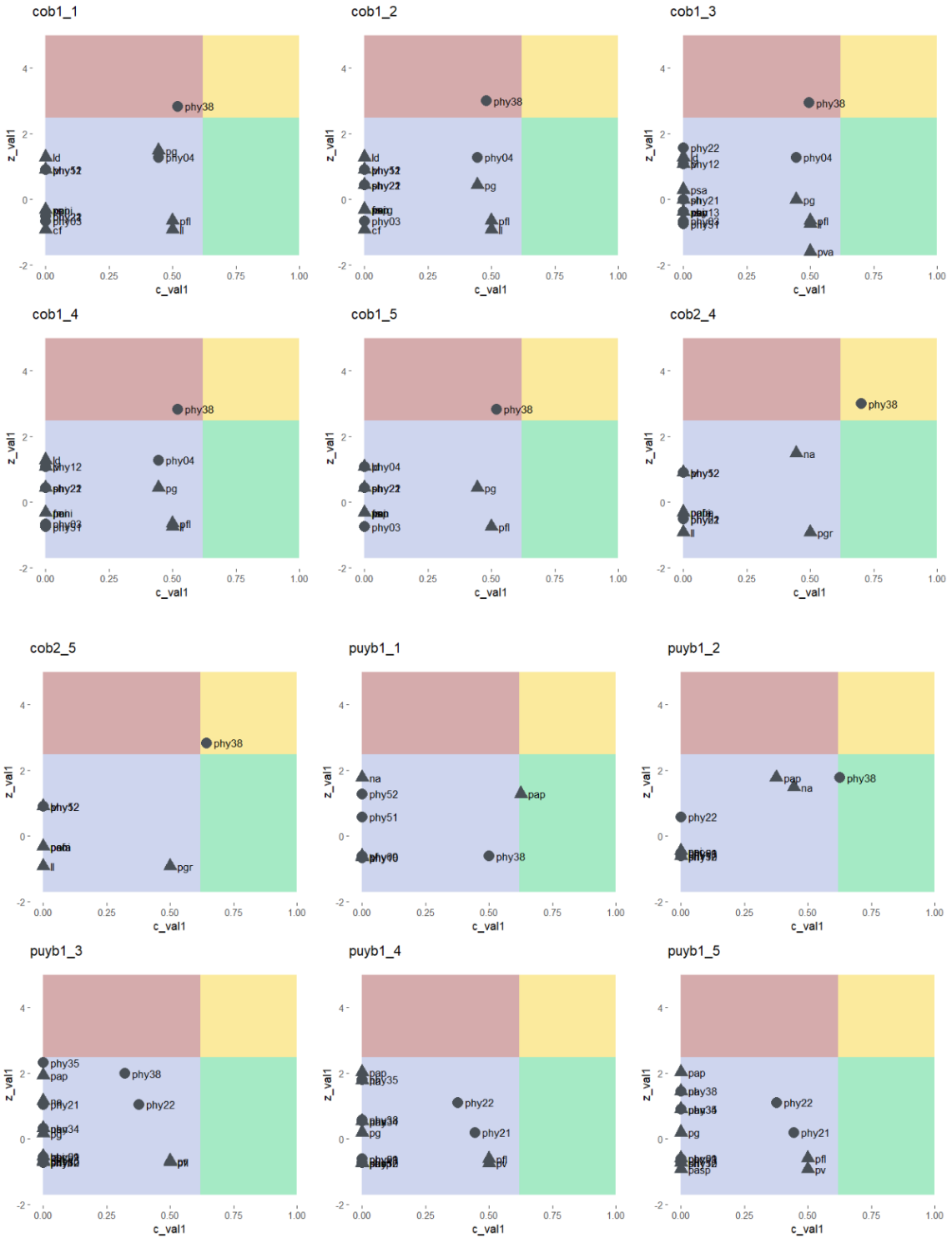
Appendix S13. Results of the GLMMs

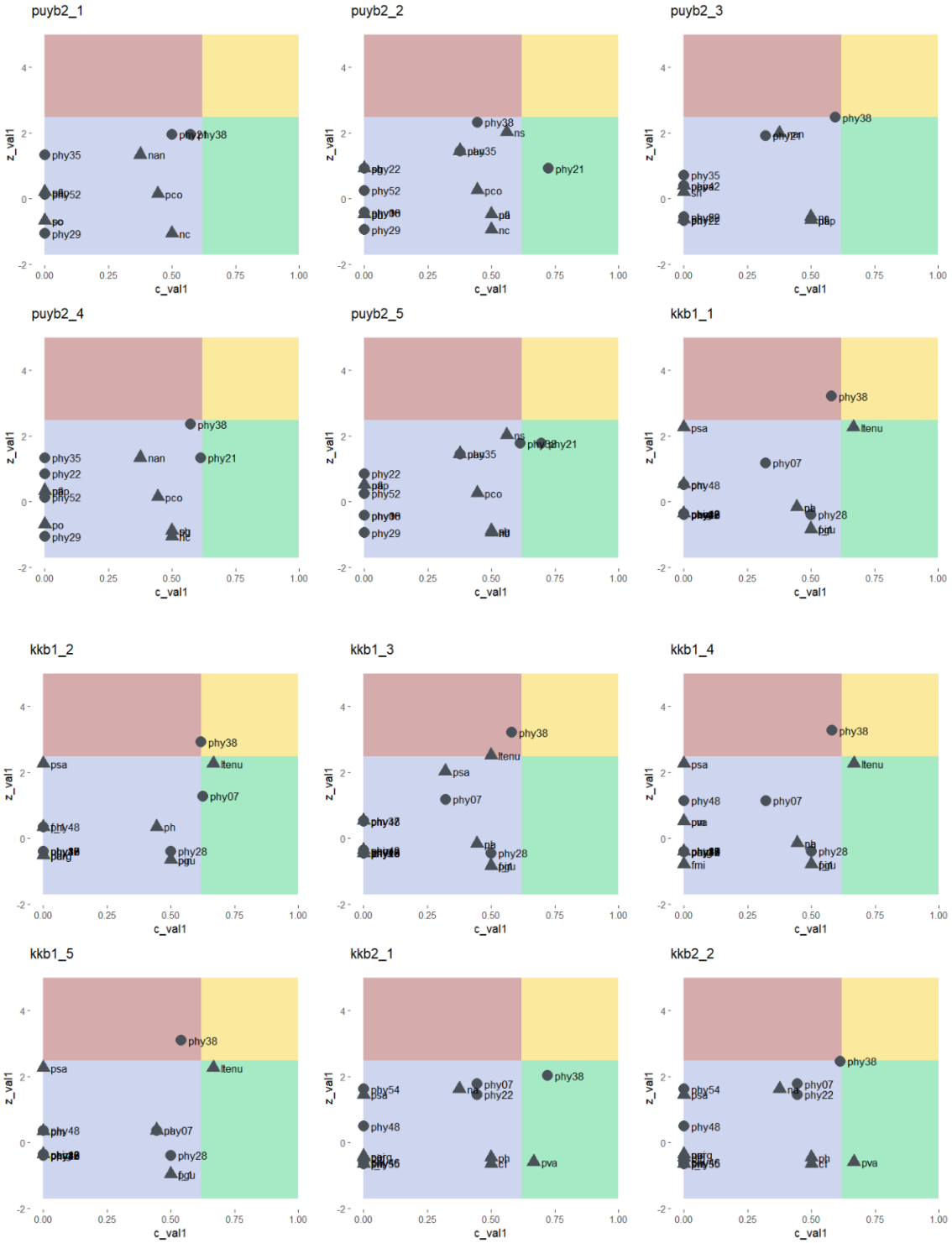


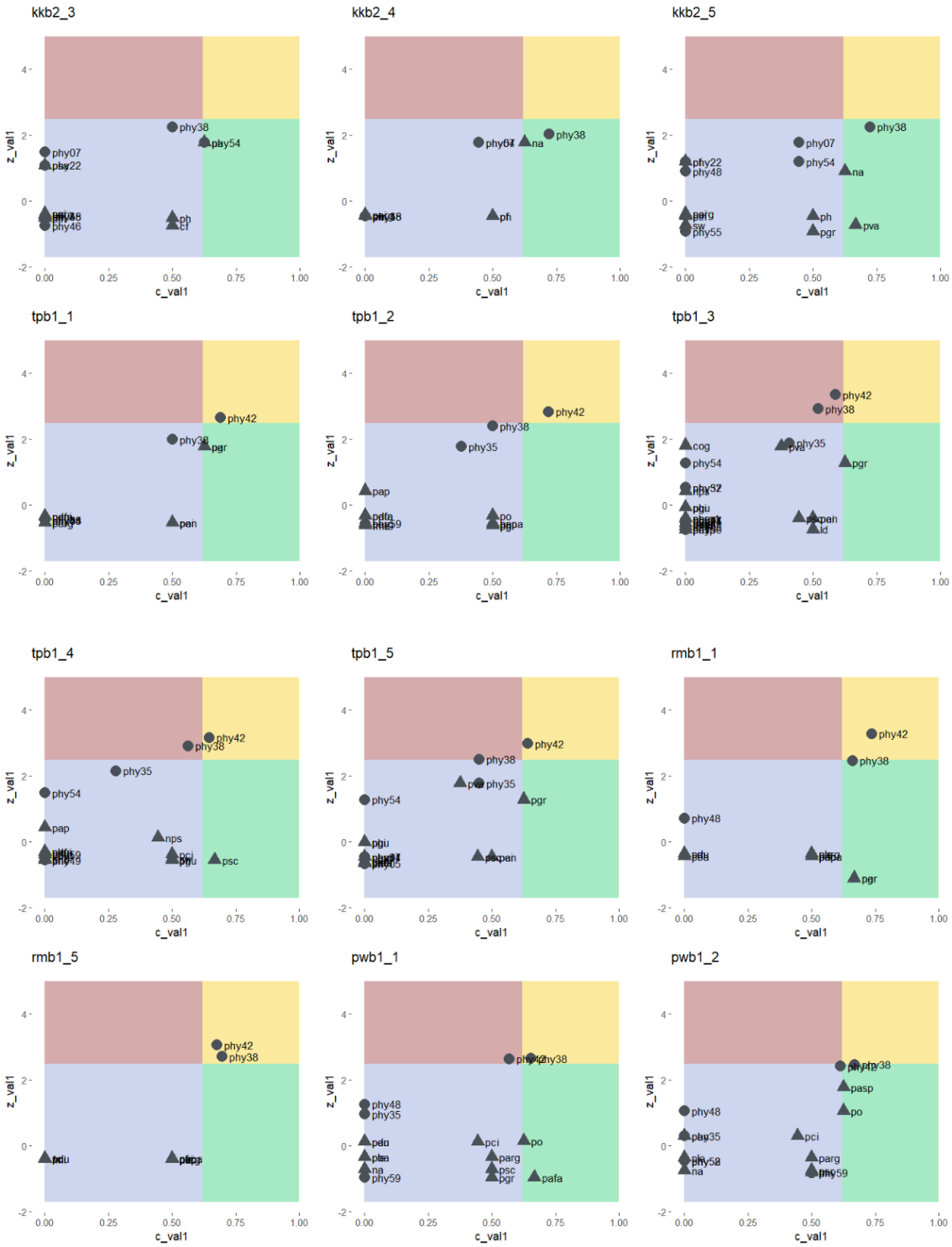


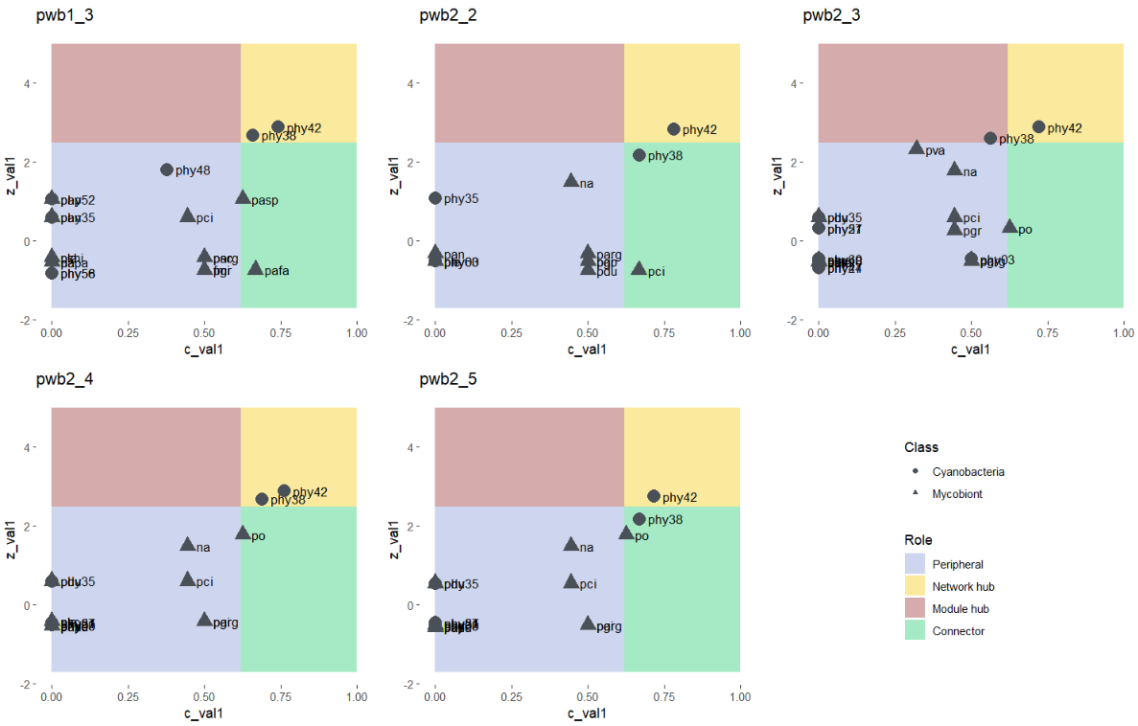


Appendix S14. zc-parameter space and role of each mycobiont species and cyanobacteria phylogroup for each of the local networks.




puyb2_1
puyb2_2
puyb2_3
puyb2_4
puyb2_5
kkb1_1
kkb1_2
kkb1_3
kkb1_4
kkb1_5
kkb2_1
kkb2_2
z_val1
c_val1


kkb2_3
kkb2_4
kkb2_5
tpb1_1
tpb1_2
tpb1_3
tpb1_4
tpb1_5
rmb1_1
rmb1_5
pwb1_1
pwb1_2
z_val1
c_val1


pwb1_3
pwb2_2
pwb2_3
pwb2_4
pwb2_5
z_val1
c_val1
phy42
phy38
phy48
phy52
phy35
pasp
pci
pafa
pgr
na
pva
po
pgrg
Class
Cyanobacteria
Mycobiont
Role
Peripheral
Network hub
Module hub
Connector

Appendix S15. Proportion of observations of the different roles in the local networks and the metanetwork.

	Peripherals	Connectors	Module hubs	Network hubs
LOCAL NETWORKS				
Network structure				
Modular	0.95	0.02	0.03	0
Nested	0.88	0.05	0.04	0.03
Random	0.88	0.08	0.1	0.03
Proportion Cyanobacteria	0.38	0.3	0.94	1
Proportion Mycobiont	0.62	0.7	0.06	0
Cyanobacteria	0.85	0.04	0.05	0.06
Mycobiont	0.62	0.05	0.025	0.025
Function of cyanobacteria				
Cyanolichens	0.66	0.21	1	0
Cephalolichens	0.34	0.79	0	0
Growth Form				
BF	0.63	0.68	0	0
NF	0.20	0.32	1	0
SQ	0.16	0	0	0
Family				
Collemataceae	0.07	0.14	1	0
Lobariaceae	0.55	0.5	0	0
Nephromataceae	0.13	0.18	0	0
Pannariaceae	0.25	0.18	0	0
Peltigeraceae	0	0	0	0
METANETWORK				
Proportion Cyanobacteria	0.39	0.6	0.71	0.33
Proportion Mycobiont	0.61	0.4	0.29	0.67
Cyanobacteria	0.8	0.1	0.08	0.02
Mycobiont	0.9	0.05	0.025	0.025
Function of cyanobacteria				
Cyanolichens	0.7	0.25	0	0
Cephalolichens	0.3	0.75	1	1
Growth Form				
BF	0.53	0.5	0.5	1
NF	0.32	0.5	0	0
SQ	0.15	0	0.5	0
Family				
Collemataceae	0.1	0.25	0	0
Lobariaceae	0.4	0.5	0.5	0.5
Nephromataceae	0.1	0	0	0.5
Pannariaceae	0.3	0.25	0.5	0
Peltigeraceae	0.1	0	0	0

CHAPTER 4

Specialization: a multidimensional and integrative perspective.

Clara Rodríguez Arribas, María Prieto, Gregorio Aragón, Jesús López-Angulo, Adrián Escudero and Isabel Martínez

Manuscript submitted to *Ecology* 2023

Abstract

Specialization remains as a controversial and ambiguous term in ecology. Although it has been usually measured using a dichotomic and simplified classification of *specialists* and *generalists*, its nature is by far more complex. In the case of biotic interactions, the assignation of these two labels, is usually based on the number of interacting partners (one or few versus many). Here we provide a more precise, quantitative, and objective interpretation of the specialization phenomenon combining three different dimensions and metrics (partner richness, Simpson's evenness, and d'-index) that offer complementary information to quantify specialization. Hence, partner richness is a metric associated with *specificity*, Simpson's evenness is related with *preference* and d' index with *selectivity* of the biotic interactions. Consequently, we propose a 3D specialization space combining these three metrics which allows to identify the degree of biotic specialization fleeing from its simplified historical interpretation. The proposed space was subsequently evaluated in five natural interacting systems (host-parasite, plant-ant, plant-pollinator, plant-disperser, and mycobiont-cyanobacteria) using available data comprising 110 networks with quantitative observations. The results indicate the prevalence of a lax specialization, where most organisms tended to show low values in at least one of the metrics. Predominantly, observations showed high values of specificity and low values of preference and selectivity. This relaxed specialization may provide advantages of being specialized, without sentencing specialization to its limitations when being too tight. In order to interpret this specialization space, we propose a categorization based on a symmetrical partition of each axis, obtaining eight specialization categories to be applied by other users. The implementation of this framework provides a useful tool that allows to identify the degree of specialization in a more objective, integrative, and universal way for future specialization studies.

Keywords

Specialization, Generalization, Specialization continuum, Biotic interactions, Specialization categories.

Introduction

Defining the degree of specialization of an organism remains a major challenge in ecology (Armbruster, 2017; Fox & Morrow, 1981; Futuyma & Moreno, 1988). Historically, organisms have been classified using a binomial approach in which they were considered *generalists* or *specialists* depending on their requirements and ecological tolerance. However, this simplified perspective can hardly describe the enormous diversity and complexity of this phenomenon, which is currently assumed to be arranged along a gradient of specialization (Poisot *et al*., 2015). In parallel, the specialization concept has been contextualized within the so-called requirement niche (Hutchinson, 1957) and assimilated to an axis of the niche hypervolume (i.e., one particular biotic or abiotic factor or resource which is more or less critical for the survival and growth of the organism) instead of considering it as an intrinsic and dichotomic property of an organism (Forister *et al*., 2012). Thus, the terms *specialist* and *generalist* should be considered relative (Forister *et al*., 2012) and the assigned category, somewhat arbitrary.

Focusing on biological interactions, both antagonistic and mutualistic, the available potential partners has been used as an axis of the requirement niche to define specialization (Forister *et al*., 2012; Koffel, *et al*., 2021). These partners belong to the different classes of organisms constituting the biological interaction: *resources* or *consumers*. When the level of specialization is high in both, resources and consumers, the degree of niche overlap is low among coexisting species, resulting in a pattern that could trigger critical evolutionary processes including character displacement, but also more complex coevolutionary dynamics (Schluter, 2000). For instance, it may lead to resistance in the case of parasitism (shifting from host specialization to generalization; see Brown, 2014) or tighter dependences in the case of mutualisms.

The number of interacting partners (partner richness) is by far the most common metric used to characterize this specialization niche dimension (Blüthgen, *et al*., 2006; Sahli & Conner, 2006). However, a complete array of metrics has also been proposed, including several diversity or niche breath indices, such as Simpson's which informs about the "preferences" of the interaction between partners (Magain *et al*., 2018; Sahli & Conner, 2006) or the d'-index which informs about the selectivity towards the partners

from all the partners available (Blüthgen *et al*., 2006). Although some comparisons between different specialization indices have been conducted (Poisot, *et al*., 2012), these metrics which inform about different aspects of specialization, should be combined to better explain the specialization phenomenon. For instance, should an organism interacting with a broad number of partners-high partner richness- but preferentially with only one of them be considered a generalist? Or should an organism interacting mostly with one partner which is the most abundant be considered a specialist?

To define and measure specialization in a more objective way, we have developed an integrative framework. For this purpose, we combined three metrics which provide complementary and non-overlapping information about specialization, obtaining a 3D continuous volume which integrates different dimensions: *specificity*, *preference* and *selectivity*. Finally, we evaluated the strengths of the proposed framework by applying it to different interacting systems, both antagonists (host-parasite) and mutualists (lichens, plant-ant, plant-pollinator, and plant-seed-disperser) and visualized organisms´ specialization using this 3D space.

Proposal for quantifying specialization

To reduce the simplified and arbitrary assignation of current classifications, we propose a framework combining three widely used metrics which inform about different but complementary dimensions of specialization. Partner richness (Equation 1) informs about the number of partners with which an organism interacts (hereafter *specificity*, see glossary). Simpson's Evenness (Equations 2 and 3) constitutes the second orthogonal dimension and indicates the frequency distribution of the interactions. In other words, Simpson's Evenness informs about whether an organism shows "preferences" towards some partners or if it interacts with the different partners in the same proportions (from now on it is called *preference*). The last orthogonal dimension is based on the d'-index (Equations 4, 5 and 6) and considers the proportion of interactions of each partner with the overall community, showing the *selectivity* of the interactions (i.e., if these interactions are established with commonly shared partners within the community, being the organism opportunistic, or with rare partners, then being selective). It is worth to note

that these metrics need weighted data of species interactions as well as the information of the complete coexisting community (e.g., all the pollinators in the case of plant-pollinator interactions).

We propose to define specialization by building a 3D space in which each specialization dimension is evaluated independently (i.e., the standardized number of interacting partners, the interaction strength with each partner and the interaction based on the proportion of each partner's interactions). Consequently, specialization will be the integrative vector combining the three dimensions (Figure 1).

Dimension 1: Specificity.

Equation 1. Partner Richness:

$$R_i = \sum_{j=1}^{Nj} N_{ij}$$

where Nj is the total number of potential partners available in the community, N_{ij} is the number of partners j interacting with the target species i.

Partner richness is then min-max standardized to range from 0 (high specialization) to 1 (interacting with all available species; see Equation 1.1) as the number of partners is a quantitative discrete variable, and its standardization allows to treat it as a continuous variable.

Standardization of Partner Richness:

The minimum value used in this standardization is 1 (interacting only with one partner). The maximum value to standardize this metric could be different depending on the question asked. Here, we used the maximum number of local interacting partners found for each interacting system ($\max(R_i)_s$). We consider this value the *interacting carrying capacity* as it is the maximum observed value for each of the systems of interaction (Equation 1.1). Nonetheless, the local availability of partners (Nj) could also be used as the maximum in partner richness' standardization if the aim is to determine the richness of partners depending on the availability of them in order to compare different systems of interaction.

Equation 1.1. min-max standardization:

$$R_i{}' = \frac{R_i - 1}{\max{(R_i)_s} - 1}$$

where R_i is the observed partner richness of species i and $\max{(R_i)_s}$is the maximum richness found in each interacting system in a certain locality.

In this context, values of $R' = 0$ represent the highest specificity, with organisms interacting just with one partner. On the other hand, values of $R' = 1$ belong to the lowest specificity, with organisms reaching their carrying capacity of interactions depending on the system.

Dimension 2: Preference.

This second dimension is based on Simpson's diversity index (Equation 2), which is then used to calculate Simpson's evenness of interactions (Equation 3).

Equation 2 Simpson's index (Simpson, 1949):

$$D_i = \sum_{j=1}^{R_i} p_{ij}^2$$

where R_i is the number of partners interacting with species i, and p_{ij} is the frequency of association of species i with each partner j.

Equation 3 Simpson's Evenness (Smith & Wilson, 1996):

$$E_i = \frac{1/D_i}{R_i}$$

where R_i is the number of partners interacting with species *i*, and D_i is Simpson's index for species *i*.

Dimension 3: Selectivity.

Equation 4 d'-index (Blüthgen *et al*., 2006):

$$d_i = \sum_{j=1}^{N_j} (p_{ij} ln \frac{p_{ij}}{q_j})$$

where N_j is the number of potential partners available, p_{ij} is the distribution of the interactions of species *i* with each partner *j*, and q_j is the relative availability of each partner *j*.

Equation 5 Normalization of d'-index (see Blüthgen *et al*., 2006 for further details):

$$d_i' = \frac{d_i - \min(d)}{\max(d) - \min(d)}$$

In addition, as R' and E range from 0 to 1, with 0 being the highest specialization and 1 the lowest specialization. Thus, we suggest using Equation 6 to interpreted d' index in the same way (0 more selective and 1 more opportunistic).

Equation 6 Transformation of d'-index:

$$td_i' = 1 - d_i'$$

Thus, values of $td' = 1$ are related with opportunistic interactions, whereas values of $td' = 0$ refer to selection against the preferred interacting partner within the community.

This framework permits to locate each interaction accurately in the specialization volume considering its different components: *specificity*, *preference*, and *selectivity*. As well, with the information provided by the three metrics, comparisons between and within different interacting systems can be conducted more precisely.

To evaluate this specialization space, we calculated the three dimensions of specialization in different biotic interaction systems. For this purpose, we used our own data set consisting of lichenized fungi associated with cyanobacteria (in which we have information of the interactions from the mycobionts) and available data from different biological networks from the Web of Life repository (https://www.web-of-life.es/; Fortuna *et al*., 2014). We selected all available bipartite networks with weighted data, and a minimum of 10 nodes of each class (i.e., 10 organisms belonging to the class *resources* and 10 organisms belonging to the class *consumers*), resulting in a total of 110 networks of different interaction systems. Thus, we retained 28 antagonistic networks (28 host-parasite networks) and 82 mutualistic networks (3 plant-ant networks, 62 plant-pollinator networks, 8 plant-seed-disperser networks, and 9 mycobiont-cyanobacteria networks;

Rodríguez-Arribas *et al*., 2022). We aim to determine and compare the distribution pattern of specialization measured with the combination of the three dimensions in the different interacting systems analysed.

The three different metrics of specialization (R', E, and td') were calculated in R v. 4.0.4 (http://www.rproject.org/) for those species with at least four interactions within a network, called from now on as observations. Each network was treated as a locality. Within each network all species belonging to both interacting classes were considered (e.g., plants and pollinators for plant-pollinator systems). Partner richness and Simpson's index were calculated with functions *specnumber()* and *diversity()*, respectively using *vegan* package (v.2.5-7; Oksanen *et al*., 2020) to quantify *selectivity* and *preference*. To calculate the d' index (i.e., *selectivity*), function *dfun()* from package *bipartite* (v.2.16; Dormann *et al*., 2008, 2009) was used. We also calculated the maximum partner richness of each interacting system ($\max(R_i)_s$) to standardize R'. We further defined eight specialization subspaces or categories to help in the interpretability of this specialization volume (see Appendix S1).

Results

In natural systems of interactions, each dimensions showed a different pattern (Figure 1), with *specificity* showing more representation of low values, whereas *preference* and *selectivity* have most values over 0.5.

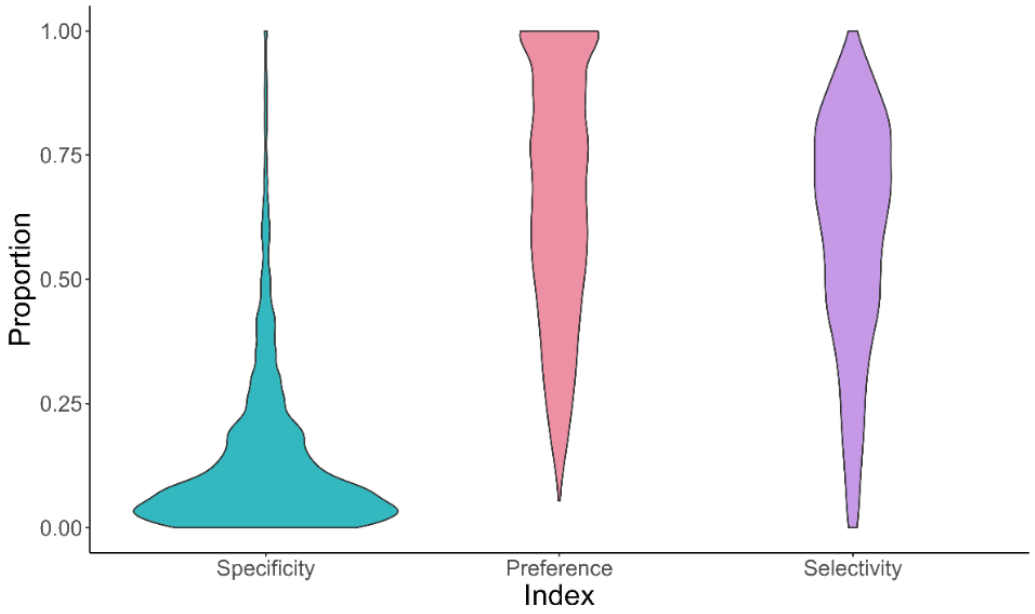


Figure 1. Distribution of the proportion of each facet of specialization (*specificity* measured with R', *preference* quantified with E, and *selectivity* measured with td').

Correlations between metrics and results for each metric in the different systems are shown in Figure 2. Organisms with low *specificity* tend to show a relatively high *preference* (Figure 2B and 2D) and low levels of *selectivity* (Figure 2C and 2G). Thus, organisms associating with a high number of partners, tend to establish preferential interactions with few of them and associate with the most shared ones in the community (*opportunism*). Plant-pollinators systems have the highest values of *specificity* (low R'; Table 1). In addition, all the studied systems show a positive skewed distribution, with a dominance of low values of R'. In relation with *preference*, lichens symbiotic systems show the highest values of E, meaning that mycobionts establish interactions with their cyanobacteria without preferences towards them. Moreover, most E values are higher than 0.5 in lichens, making them the less *preference-driven* system. In relation with the observed td' values, all systems seem to have a negative skewed distribution except in plant-ant systems, which showed a bimodal distribution. Plant-seed-dispersers showed the highest density at high values of td', being the less selective system.

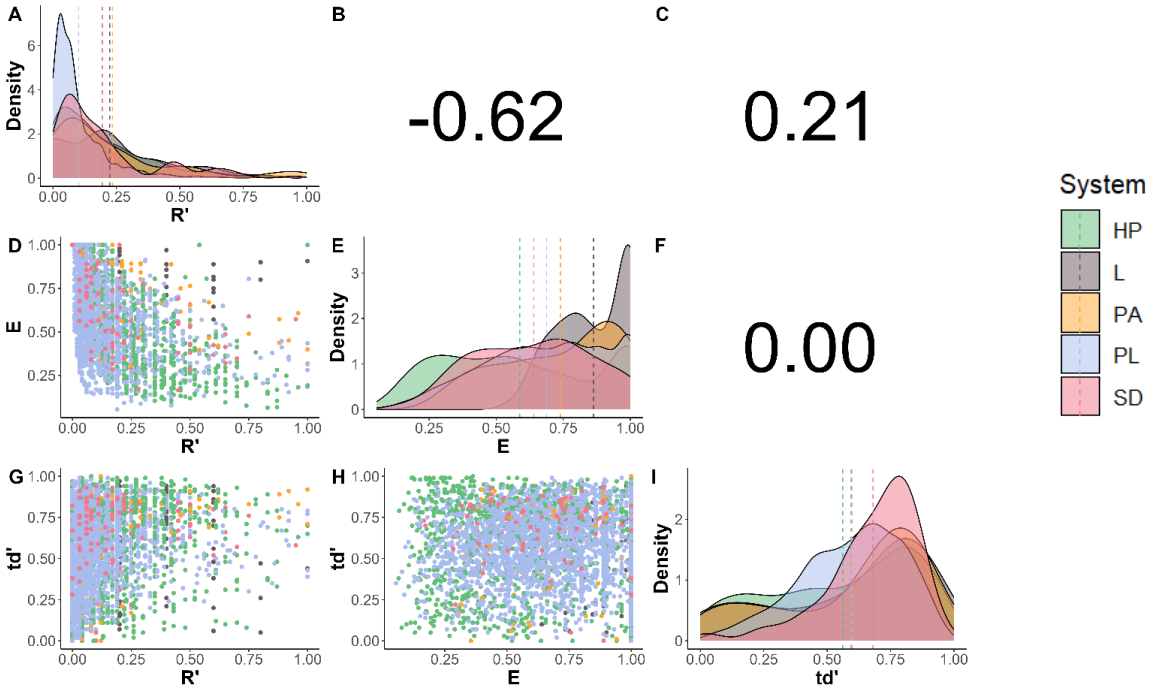


Figure 2. Representation of the three indices in each interacting system and correlations between indices. A), E), and I) are the density plots of each index (R', E, and td', respectively) for each system. D), G), and H) represent the relationship between each index by pairs (R' and E,

R' and td' and E and td', respectively). B), C), and F) show the Spearman correlation coefficient between each index (R' and E, R' and td' and E and td', respectively). Colours refer to each of the systems (HP = Host-Parasite, L = Mycobiont-Cyanobiont, PA = Plant-Ant, PL = Plant-Pollinator, and SD = Plant-Seed Disperser).

Table 1. Mean ± SD of the three different metrics for each of the interacting systems.

System	R'	E	td'
	Mean ± SD	Mean ± SD	Mean ± SD
Host-Parasite	0.28 ± 0.24	0.55 ± 0.25	0.58 ± 0.29
Lichens	0.21 ± 0.22	0.80 ± 0.11	0.63 ± 0.30
Plant-Ant	0.21 ± 0.22	0.72 ± 0.21	0.53 ± 0.31
Plant-Pollinator	0.16 ± 0.19	0.66 ± 0.21	0.61 ± 0.21
Plant-Seed-disperser	0.28 ± 0.24	0.60 ± 0.22	0.72 ± 0.16

Focusing on each system separately and on each class of organisms (consumers vs. resources), we observed that consumers and resources showed similar patterns, with higher differences between systems (Figure 3).

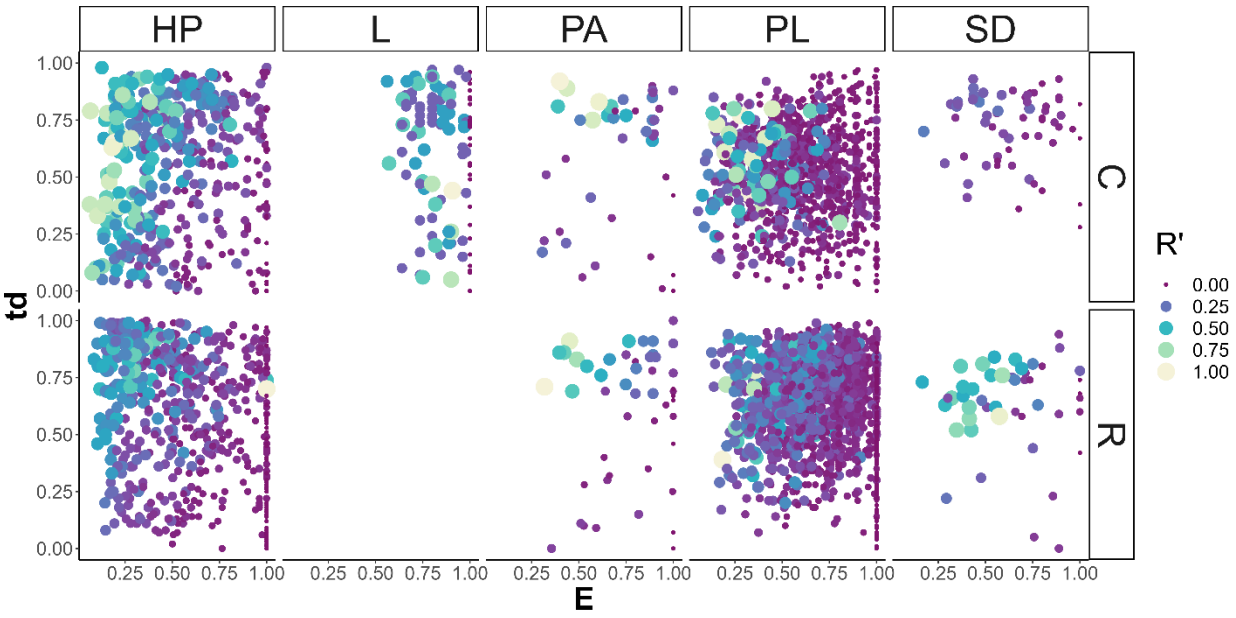


Figure 3. Values of the three indices for each system analysed (HP = Host-Parasite, L = Mycobiont-Cyanobiont, PA = Plant-Ant, PL = Plant-Pollinator, and SD = Plant-Seed Disperser) and each class of

organisms (C = Consumers, and R = Resources). E in the x-axis, td' in the y-axis, and R' represented with circles (size and colour show the different values).

Reciprocal specialization ($R' = 0,\ E = 1,\ td' = 0$) was rare in all interacting systems analysed, not reaching the 5% of the observations in any of them. Remarkably, plant-seed-disperser systems had zero observations of reciprocal specialization.

Discussion

The integrative framework proposed to consider the specialization phenomenon combining three metrics which provide complementary information about the *specificity*, *preference*, and *selectivity* of the interactions lead us to identify specialization patterns very precisely. This framework permits to define specialization, avoiding the simplistic characterization in *specialist* and *generalist* traditionally used and mostly based solely on partner richness. Additionally, the use of the proposed specialization 3D space allows the thorough comparison between and within interacting systems. This framework was evaluated in 110 networks belonging to five bipartite interacting systems, both antagonist and mutualist, providing clear evidence of the potential for objectivization and conceptual unification of the specialization phenomenon.

A tendency towards specialization was found in natural interacting systems as the number of interacting partners observed was relatively low as previously shown (e.g., Egan & Funk, 2006; Poisot *et al*., 2012; Waser, *et al*., 1996). However, the results obtained here reveal a very lax specialization, in which organisms interact with few partners but tend to interact evenly and be either selective or opportunistic towards them when this interactions are highly *specialist*. This pattern seems advantageous and blurs the problems associated to specialization's "dark side" (e.g., evolutionary dead end of specialization; Batstone *et al*., 2018; Futuyma & Moreno, 1988; Thompson, 2005). In addition, we did not observe the pattern mentioned in previous studies in which they find that specialization is conditioned by organisms' abundance, with specialized organisms interacting with the most abundant ones (Vázquez *et al*., 2005, 2007). As in previous studies, a prevalence of asymmetric interactions was found, in which most species have few interactions, but few species show high number of interactions (Bascompte &

Jordano, 2014; Bascompte *et al*., 2003; Vázquez & Aizen, 2004; Vázquez *et al*., 2007, 2005). Even in these cases a lax specialization is maintained as, organisms tend to show preferences towards their partners when their specificity is low, even if opportunistic interactions prevail. Thus, when combining the three different metrics, we found that at least one of them maintains low values, keeping a certain degree of specialization of the organisms, without finding high values of all of them (i.e., supergeneralist organisms).

Several studies postulate that host-parasite systems and intimate mutualisms are more specialized than free-living interacting organisms such as plants and their pollinators or seed dispersers (Blüthgen *et al*., 2007; Olesen *et al*., 2007; Thompson, 2005). However, few studies directly compare different systems simultaneously (see Blüthgen *et al*., 2007), a comparison that becomes very easy with our 3D framework. The application of our framework to find differences between systems of interactions helps to position specialization in a coevolutionary context and to determine if antagonist and mutualistic interactions are translated in different specialization patterns (Thompson, 2005).

The proposed framework also permits to analyse specialization within interacting systems in order to obtain information about which organisms are the most and the less specialized within each system. In addition, comparisons could be made to observe differences between both classes of organisms (*consumers* and *resources*) within a given interacting system. Contrasting results have been published about this topic. Some studies found that organisms belonging to *resources* (i.e., host and plants) showed higher specialization than the *consumers* (i.e., parasites and animals; e.g., Blüthgen *et al*., 2006, 2007; Egan & Funk, 2006), whereas others, suggested that *consumers* are more *preference-driven* than *resources* (Traveset *et al*., 2016), being the latter more selective. This application within interacting systems would be especially useful to develop conservation strategies targeted to the most specialized organisms which could be in higher risk of extinction or, even more, to generalized organisms for which its extinction could affect the entire network. On the one hand, highly specialized organisms may be extremely vulnerable due to their tighter dependence on exclusive partners (Clavel *et al*., 2011). However, these organisms would not generate major changes in the total network. On the other hand, the loss of highly generalized organisms, which add cohesivity to the network acting as network hubs, could cause extinction cascades affecting the rest of the

network (Bascompte, 2009; Bascompte & Jordano, 2007). A categorical classification of specialization considering the three metrics is proposed in the Supplemental information as a useful tool to apply for the mentioned purposes.

Worth the note that we are not considering the phylogeny, neither the functional component of specialization. We are aware that is not the same to interact with species belonging to distant clades than with close relatives (e.g., Novotny & Basset, 2005; Waser *et al*., 1996). In addition, groups of convergent traits developed to specifically interact with some matching partners, as could be the well describe "floral syndromes", also influence the understanding of specialization (Johnson & Steiner, 2000; Weinstein & Graham, 2017). Following the review of Armbruster (2017), and menging the terms currently used in community ecology with the specialization context, we suggest distinguishing between taxonomic, functional, and phylogenetic specialization in order to compile all the information within local networks. From this perspective, our proposal would be a kind of "taxonomical specialization", and a first step, to lately introduce functional and phylogenetic specializations.

In summary, the integration of three different metrics to consider specialization provides a more objective perspective of this biological complex phenomenon. These metrics inform about the *specificity*, the *preference*, and the *selectivity* of the interactions, three crucial aspects to understand the specialization of the organisms. We additionally provided a delimitation of specialization in eight different categories based on these facets of specialization which could be useful to define and precisely categorize organisms in the specialization continuum, leading to a more objective and comparable classification. As a general rule we found a tendency to a lax specialization in which most of the observations show certain degree of specialization in the metrics considered. This lax specialization captures both, advantages of specialization and generalization. Comparisons between systems of interactions and organisms within systems can be done more accurately with the application of our framework.

References

Armbruster, W. S. (2017). The specialization continuum in pollination systems: diversity of

concepts and implications for ecology, evolution and conservation. *Functional Ecology*, *31*(1), 88–100. https://doi.org/10.1111/1365-2435.12783

Bascompte, J. (2009). Disentangling the web of life. *Science*, *325*(5939), 416–419. https://doi.org/10.1126/science.1170749

Bascompte, J., & Jordano, P. (2007). Plant-animal mutualistic networks: The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *38*(May), 567–593. https://doi.org/10.1146/annurev.ecolsys.38.091206.095818

Bascompte, J., & Jordano, P. (2014). *Mutualistic Networks*. Princeton University Press.

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Batstone, R. T., Carscadden, K. A., Afkhami, M. E., & Frederickson, M. E. (2018). Using niche breadth theory to explain generalization in mutualisms. *Ecology*, *99*(5), 1039–1050. https://doi.org/10.1002/ecy.2188

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*. https://doi.org/10.1186/1472-6785-6-9

Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., & Blüthgen, N. (2007). Specialization, Constraints, and Conflicting Interests in Mutualistic Networks. *Current Biology*, *17*(4), 341–346. https://doi.org/10.1016/j.cub.2006.12.039

Brown, J. H. (2014). Why are there so many species in the tropics? *Journal of Biogeography*, *41*(1), 8–22. https://doi.org/10.1111/jbi.12228

Clavel, J., Julliard, R., & Devictor, V. (2011). Worldwide decline of specialist species: Toward a global functional homogenization? *Frontiers in Ecology and the Environment*, *9*(4), 222–228. https://doi.org/10.1890/080216

Dormann, C. F., Frund, J., Bluthgen, N., & Gruber, B. (2009). Indices, Graphs and Null Models: Analyzing Bipartite Ecological Networks. *The Open Ecology Journal*, *2*(1), 7–24. https://doi.org/10.2174/1874213000902010007

Dormann, C. F., Gruber, B., & Fründ, J. (2008). Introducing the bipartite Package: Analysing Ecological Networks. *R News*, *8*(2), 8–11. Retrieved from http://cran.r-project.org/doc/Rnews/

Egan, S. P., & Funk, D. J. (2006). Individual advantages to ecological specialization: Insights on cognitive constraints from three conspecific taxa. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1588), 843–848. https://doi.org/10.1098/rspb.2005.3382

Forister, M. L., Dyer, L. A., Singer, M. S., Stireman III, J. O., & Lill, J. T. (2012). Revisiting the evolution of ecological specialization, with emphasis on insect–plant interactions. *Ecology*, *93*(5), 981–991. https://doi.org/10.1890/11-0650.1

Fortuna, M. A., Ortega, R., & Bascompte, J. (2014). Web of life. Retrieved from www.web-of-life.es

Fox, L. R., & Morrow, P. A. (1981). Specialization: Species property or local phenomenon. *Science*, *211*(4485), 887–893. https://doi.org/10.1126/science.211.4485.887

Futuyma, D. J., & Moreno, G. (1988). The Evolution of Ecological Specialization. *Annual Review of Ecology and Systematics*, *19*(1988), 207–233. https://doi.org/10.1146/annurev.es.19.110188.001231

Hutchinson, G. E. (1957). Concluding remarks. Cold Spring Harbor Symposium. *Quantitative Biology*, *22*, 415–247. https://doi.org/10.1101/SQB.1957.022.01.039

Johnson, S. D., & Steiner, K. E. (2000). Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution*, *15*(4), 140–143. https://doi.org/10.1016/S0169-5347(99)01811-X

Koffel, T., Daufresne, T., & Klausmeier, C. A. (2021). From competition to facilitation and mutualism: a general theory of the niche. *Ecological Monographs*, *91*(3), 1–31. https://doi.org/10.1002/ecm.1458

Magain, N., Truong, C., Goward, T., Niu, D., Goffinet, B., Sérusiaux, E., … Miadlikowska, J. (2018). Species delimitation at a global scale reveals high species richness with complex biogeography and patterns of symbiont association in peltigera section peltigera (Lichenized ascomycota: Lecanoromycetes). *Taxon*, *67*(5), 836–870. https://doi.org/10.12705/675.3

Novotny, V., & Basset, Y. (2005). Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1568), 1083–1090. https://doi.org/10.1098/rspb.2004.3023

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Stevens, M. H. H. (2020). vegan: Community ecology package. R package version 2.5-6. Retrieved from https://cran.r-project.org/web/packages/vegan/index.html

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104

Poisot, T., Canard, E., Mouquet, N., & Hochberg, M. E. (2012). A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, *3*(3), 537–544. https://doi.org/10.1111/j.2041-210X.2011.00174.x

Poisot, T., Kéfi, S., Morand, S., Stanko, M., Marquet, P. A., & Hochberg, M. E. (2015). A continuum of specialists and generalists in empirical communities. *PLoS ONE*, *10*(5), 1–12. https://doi.org/10.1371/journal.pone.0114674

Rodríguez-Arribas, C., Prieto, M., Aragón, G., López-Angulo, J., Escudero, A., & Martínez, I. (2022). Data from: Specialization: a new multidimensional and integrative perspective. https://doi.org/10.5281/zenodo.7483511

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Schluter, D. (2000). Ecological Character Displacement in Adaptive Radiation. *The American Naturalist*, *156*(S4), 4–16. https://doi.org/10.1086/303412

Simpson, E. H. (1949). Measurement of Diversity. *Nature*, *163*(4148), 688. https://doi.org/10.1038/163688a0

Smith, B., & Wilson, J. B. (1996). A Consumer's Guide to Evenness Indices. *Oikos*, *76*(1), 70–82. https://doi.org/10.2307/3545749

Thompson, J. N. (2005). *The geographic mosaic of coevolution.* University of Chicago Press.

Traveset, A., Tur, C., Trøjelsgaard, K., Heleno, R., Castro-Urgal, R., & Olesen, J. M. (2016). Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*, *25*(7), 880–890. https://doi.org/10.1111/geb.12362

Vázquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology*, *85*(5), 1251–1257. https://doi.org/10.1890/03-3112

Vázquez, D. P., J. Melián, C., M. Williams, N., Blüthgen, N., R. Krasnov, B., & Poulin, R. (2007). Species abundance and asymmetric interaction strength in ecological networks. *Oikos*, *116*(7), 1120–1127. https://doi.org/10.1111/j.2007.0030-1299.15828.x

Vázquez, D. P., Poulin, R., Krasnov, B. R., & Shenbrot, G. I. (2005). Species abundance and the distribution of specialization in host-parasite interaction networks. *Journal of Animal Ecology*, *74*(5), 946–955. https://doi.org/10.1111/j.1365-2656.2005.00992.x

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, *77*(4), 1043–1060. https://doi.org/10.2307/2265575

Weinstein, B. G., & Graham, C. H. (2017). On comparing traits and abundance for predicting species interactions with imperfect detection. *Food Webs*. https://doi.org/10.1016/j.fooweb.2017.05.002

Supporting information

CHAPTER 4

Specialization: a multidimensional and integrative perspective.

Clara Rodríguez Arribas, María Prieto, Gregorio Aragón, Jesús López-Angulo, Adrián Escudero and Isabel Martínez

Manuscript submitted to *Ecology* 2023

Appendix S1. A categorization of specialization.

A categorial classification of specialization considering the three metrics suggested (R', E and td') is proposed. For such categorization we divided each aspect of specialization (*specificity*, *preference*, and *selectivity*) in two halves, obtaining eight specialization categories (Table 1, Figure 1).

Specificity.

We used standardized partner richness (R'), which refers to the number of interacting partners of a given organism, being $R' = 0$ when the organism interacts with one partner, and $R' = 1$ when the organism interacts with the maximum number of partners, to divided organisms as specialist or generalist along the specialization continuum.

$$R'_i < 0.5; specialist$$
$$R'_i \geq 0.5; generalist$$

Preference.

We also integrate the concept of *preference* using Simpson's Evenness (E). For this index, values of $E = 0$ refer to the highest preference, found when an organism has a $R' = 0$, and E values close to 1 are related with an even distribution of the interactions with the different partners, not showing preferences towards any of them. We divided organisms in considering E in two types as follows:

$$E_i < 0.5; preference - driven$$
$$E_i \geq 0.5; preference - less$$

Selectivity.

Referring to the selectivity, organisms are classified as *selective* or *opportunistic* with td'. A value of $td' = 0$ indicates a reciprocal specialization in the interaction, with both organisms interacting exclusively between them, while values close to one are reached when an organism interacts with the most shared partner between the organisms in the community, which is the most abundantly registered.

$$td'_i < 0.5; selective$$
$$td'_i \geq 0.5; opportunist$$

A maximum specialization will be reached when an organism interacts only with one partner and this interaction is reciprocal:

$$R'_i = 0; E'_i = 0; td'_i = 0$$

On the other extreme of the continuum, a maximum generalization will be reached when having high values of R' , E', and td', being thus considered generalist, preference-less and opportunistic:

$$R'_i = 1 ; E'_i = 1 ; td'_i = 1$$

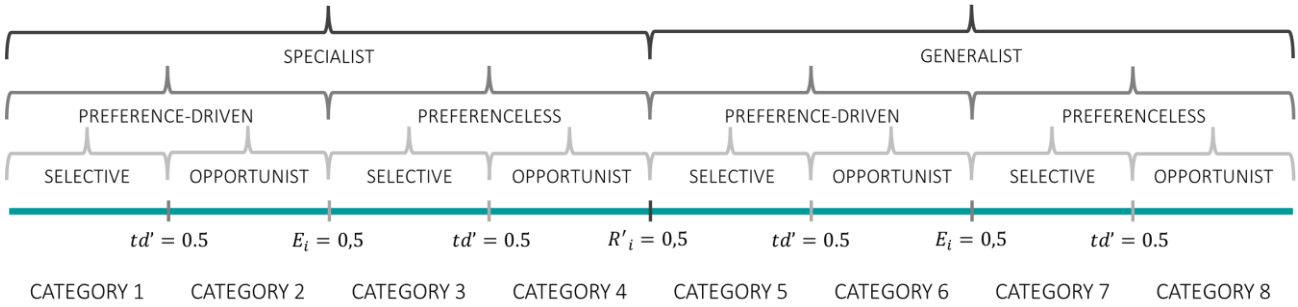


Figure S1. Scheme of the division and integration of the three metrics to describe the eight specialization categories.

Table S1. Specialization categories integrating the three different metrics proposed.

Category	R'	E	td	R' axis	E axis	td axis
1	Specialist	Preference-driven	Selective	[0, 0.5)	[0, 0.5)	[0, 0.5)
2	Specialist	Preference-driven	Opportunist	[0, 0.5)	[0, 0.5)	[0.5, 1]
3	Specialist	Preference-less	Selective	[0, 0.5)	[0.5, 1]	[0, 0.5)
4	Specialist	Preference-less	Opportunist	[0, 0.5)	[0.5, 1]	[0.5, 1]
4	Generalist	Preference-driven	Selective	[0.5, 1]	[0, 0.5)	[0, 0.5)
6	Generalist	Preference-driven	Opportunist	[0.5, 1]	[0, 0.5)	[0.5, 1]
7	Generalist	Preference-less	Selective	[0.5, 1]	[0.5, 1]	[0, 0.5)
8	Generalist	Preference-less	Opportunist	[0.5, 1]	[0.5, 1]	[0.5, 1]

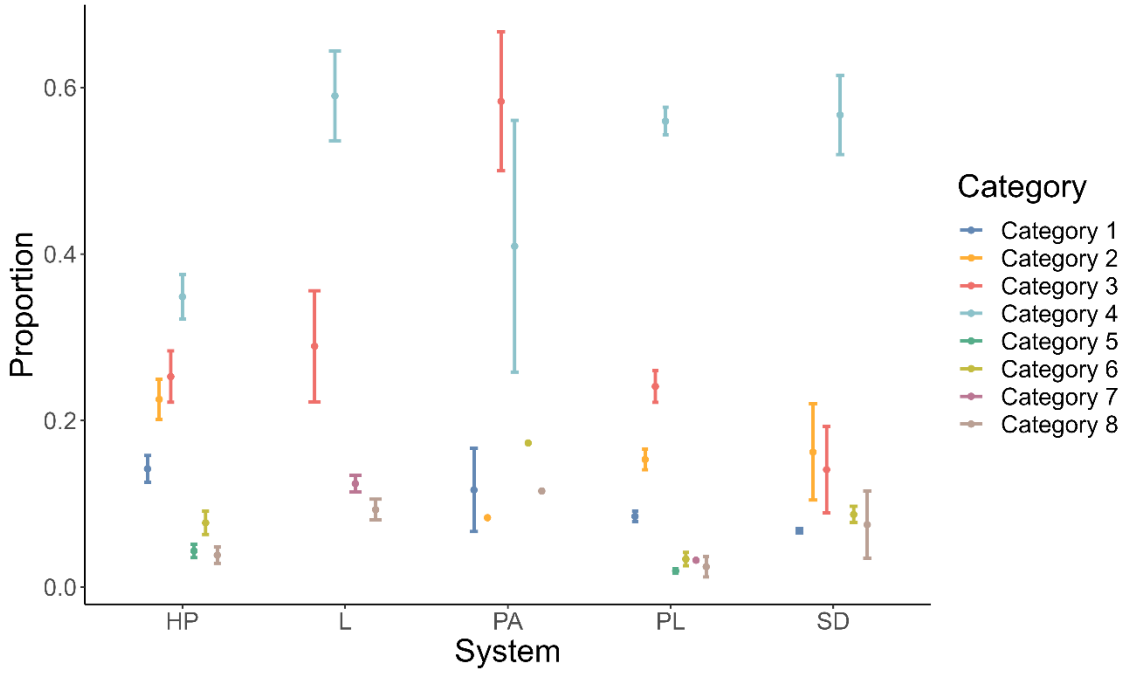


Figure S2. Proportion of observations and standard error of each category within each interacting system (HP = Host-Parasite, L = Mycobiont-Cyanobiont, PA = Plant-Ant, PL = Plant-Pollinator, and SD = Plant-Seed Disperser).

DISCUSIÓN GENERAL

DISCUSIÓN GENERAL

El estudio de las interacciones bióticas es clave para comprender la coexistencia de las especies en las comunidades. Estas interacciones se constituyen con distintos patrones de especialización (Futuyma & Moreno, 1988). El estudio de estas interacciones se ha basado, tradicionalmente, en interacciones antagónicas, como relaciones de competencia o de depredador-presa (ej.: Ehrlich & Raven, 1964). Sin embargo, a partir de estudios como los de Boucher *et al*. (1982) y May (1982), se ha ido introduciendo el análisis de estos patrones en sistemas mutualistas (ej.: Bascompte *et al*., 2003; Johnson & Steiner, 1997; Johnson & Steiner, 2000; Jordano, 1987). Por otro lado, la mayoría de los estudios sobre interacciones bióticas y el análisis del grado de especialización se centraban en evaluar el número de parejas con las que un organismo era capaz de interactuar (Blüthgen *et al*., 2006). No obstante, en los últimos años, han surgido nuevas propuestas para definir y cuantificar la especialización (Armbruster, 2017; Blüthgen *et al*., 2006; Devictor *et al*., 2010; Poisot *et al*., 2012), así como nuevas aproximaciones desde el estudio de las redes de interacción (Bascompte *et al*., 2003; Jordano, 1987). Además, cada vez hay más estudios que tratan de desentrañar la variabilidad biogeográfica de la especialización (Dalsgaard *et al*., 2017; Olesen & Jordano, 2002; Ollerton & Cranmer, 2002), así como la influencia de los factores ambientales, los rasgos funcionales o las relaciones filogenéticas sobre las interacciones que presentan los distintos organismos (Devoto *et al*., 2005; Lara-Romero *et al*., 2019; Pinheiro *et al*., 2016; Rezende *et al*., 2007).

A pesar del aumento de estudios que han evaluado el grado de especialización en distintos tipos de interacciones mutualistas (ej.: Iglesias-Prieto *et al*., 2004 en corales; Peay *et al*., 2010 en micorrizas; Johnson & Steiner, 2000 en polinización), en los líquenes, que son un ejemplo clásico de simbiosis entre distintos organismos (Grube & Wedin, 2016) el conocimiento de los patrones de especialización es limitado. Es por ello, que el objetivo principal de esta tesis doctoral es evaluar los patrones de especialización en la simbiosis liquénica y, más concretamente, en aquellas especies coexistentes en las que participan cianobacterias en la simbiosis. La íntima asociación que se da en estos organismos entre el micobionte (hongo principal) y el cianobionte (cianobacteria fotosintética y fijadora de nitrógeno) hace que se esperen elevados niveles de especialización en este tipo de interacciones (Blüthgen *et al*., 2007; Guimarães *et al*.,

2007; Pires & Guimarães, 2013). Esto se traduciría en un número reducido de cianobacterias asociadas con cada especie de micobionte. Adicionalmente, al haber una mayor dependencia del micobionte por el fotobionte que viceversa (Magain *et al*., 2017; Zúñiga *et al*., 2017), se espera que esas cianobacterias sean compartidas entre varias especies de micobionte y que aparezcan patrones de especialización a nivel de comunidad (Rikkinen, 2013).

Sin embargo, este estudio ha revelado la ausencia de un patrón generalizable en el establecimiento de este tipo de interacciones, tanto a nivel de especie como a nivel de comunidad (redes de interacción). En el primer caso, a nivel de especie, encontramos que la especialización varía desde la especialización recíproca, donde los dos organismos interactúan exclusivamente entre sí, hasta elevados niveles de generalización, donde ambos organismos interactúan a su vez con varias parejas. Por otro lado, la misma especie puede mostrar niveles variables de especialización en función de la localización geográfica. Esto refleja un patrón dinámico, en el que las especies tienen cierta flexibilidad para cambiar de altos a bajos niveles de especialización en las distintas localidades, que pueden estar condicionados por la disponibilidad y adaptación local de las parejas con las que interactúan. En el segundo caso, a nivel de comunidad, analizando las redes de interacción locales, se observa de nuevo la ausencia de un patrón generalizable. Mientras unas redes son significativamente modulares, otras son significativamente anidadas, con la mitad de ellas mostrando una estructura aleatoria en la conformación de las interacciones

Los resultados encontrados en los distintos capítulos de la presente tesis doctoral recalcan la dependencia del contexto que presentan los fenómenos de especialización y la necesidad de especificar las condiciones de su estudio (capítulos 1, 2 y 3). Las diferencias encontradas en los patrones de especialización se hacen evidentes al analizar distintas escalas biológicas (individuos *vs*. especies *vs*. comunidades) y espaciales (local *vs*. regional). Además, la importancia de definir el eje del nicho sobre el que estudiar la especialización (Forister *et al*., 2012; Futuyma & Moreno, 1988; Peay, 2016) también aparece reflejada en este estudio. En este sentido, se han encontrado líquenes que se asocian con pocas cianobacterias (lo que revela una elevada especialización en ese eje del nicho), pero con amplios límites de tolerancia (es decir, generalización) en otros ejes

de su nicho de requerimientos (ej.: temperatura), lo que se traduce en amplios rangos de distribución.

Por otro lado, se han encontrado dificultades para definir de forma objetiva y concreta qué es la especialización y decidir qué métrica es más informativa a la hora de cuantificarla a nivel de especie. De igual manera, se ha identificado que la variación de las métricas utilizadas responde a distintos factores, como pueden ser distintos rasgos funcionales o la identidad del micobionte (capítulo 1). Por todo ello, se ha propuesto un marco conceptual en el que se integran distintas facetas relacionadas con la especialización (*especificidad*, *preferencia* y *selectividad*), que permite concretar y detectar con más precisión y objetividad los grados de especialización que presentan las distintas especies y poder comparar entre diferentes sistemas de interacción (capítulo 4).

1. La importancia de la escala

La importancia de definir la escala es ampliamente reconocida en ecología (Bunnell & Huggard, 1999; Chave, 2013; Estes *et al*., 2018; Levin, 1992; Sandel & Smith, 2009). En las interacciones bióticas, la escala presenta un papel importante determinando la especialización de los organismos (Devictor *et al*., 2010; Hagen *et al*., 2012; Hughes, 2000). El papel de la escala en los fenómenos de especialización aparece representado en el mantenimiento o la constancia de las interacciones en escalas de pequeña extensión (ej.: localidades, individuos; Herrera *et al*., 2019; Hughes, 2000). Cuando se pretenden evaluar los cambios en las interacciones entre distintos puntos, ya sean espaciales, temporales o entre distintas entidades biológicas, la diversidad beta se presenta como la mejor herramienta (CaraDonna *et al*., 2017; Novotny, 2009; Poisot *et al*., 2012; Trøjelsgaard *et al*., 2015; Ventre Lespiaucq *et al*., 2021). Así, la diversidad beta se desglosa en dos componentes que explican los distintos patrones de interacción encontrados en las distintas escalas: el recambio de especies y el recambio de interacciones (CaraDonna *et al*., 2017; Poisot *et al*., 2012).

En el presente estudio se han analizado las interacciones entre los micobiontes y cianobacterias que conforman el talo liquénico, a distintas escalas tanto biológicas como espaciales. A escala biológica, se ha analizado la variabilidad de las interacciones a nivel de individuo (intratalo), intraespecífico e interespecífico (capítulos 1 y 2) y a nivel de

comunidad (capítulo 3). De igual manera, se han tratado distintas escalas espaciales. En el capítulo 1 se analiza la especialización de distintas especies micobionte tanto a escala local como a escala regional. En el capítulo 3 se evalúa la estructura de las redes y el papel de las especies en las redes de interacciones tanto a escala local como a escala regional con la meta red de todo el gradiente latitudinal estudiado.

1.1. Escala espacial

La escala espacial hace referencia a la extensión espacial considerada, que puede abarcar desde localidades concretas a regiones constituidas por la suma de esas localidades (Wiens, 1989). Los organismos pueden presentar un grado invariable de especialización entre escalas espaciales o pueden variar el grado de especialización entre escalas locales y regionales (Krasnov *et al*., 2008). En esta tesis doctoral se ha encontrado que, la mayoría de las especies cambian su patrón de especialización al analizar cada localidad por separado o al analizar el gradiente latitudinal completo. Estas diferencias se deben a cambios en el número y/o la identidad de las cianobacterias asociadas con cada especie de micobionte en cada localidad y se explican con distintos escenarios de recambio de especies, que pueden ir desde la adición de nuevas cianobacterias, al reemplazo total o parcial de las cianobacterias entre localidades (Ventre Lespiaucq *et al*., 2021). Por tanto, las especies de micobionte tienden a aumentar el número de filogrupos de cianobacterias con las que se asocian al considerar extensiones espaciales más amplias, mostrando una menor especialización al aumentar la escala espacial (Figura 1).

En concreto, esta variabilidad de interacciones que muestra la mayoría de las especies entre distintas localidades puede deberse a cambios en la composición de las comunidades de los cianobiontes (recambio de especies) o a la interacción con cianobiontes que estén mejor adaptados a las condiciones locales de cada localidad, lo que mejoraría el fitness de la interacción (recambio de interacciones; Poisot *et al*., 2012; Trøjelsgaard *et al*., 2015). Esta flexibilidad de interactuar con distintas parejas en función de la localidad puede influir, a su vez, en el rango de distribución de las especies (Batstone *et al*., 2018; Iglesias-Prieto *et al*., 2004; Magain *et al*., 2017; Peay, 2016).

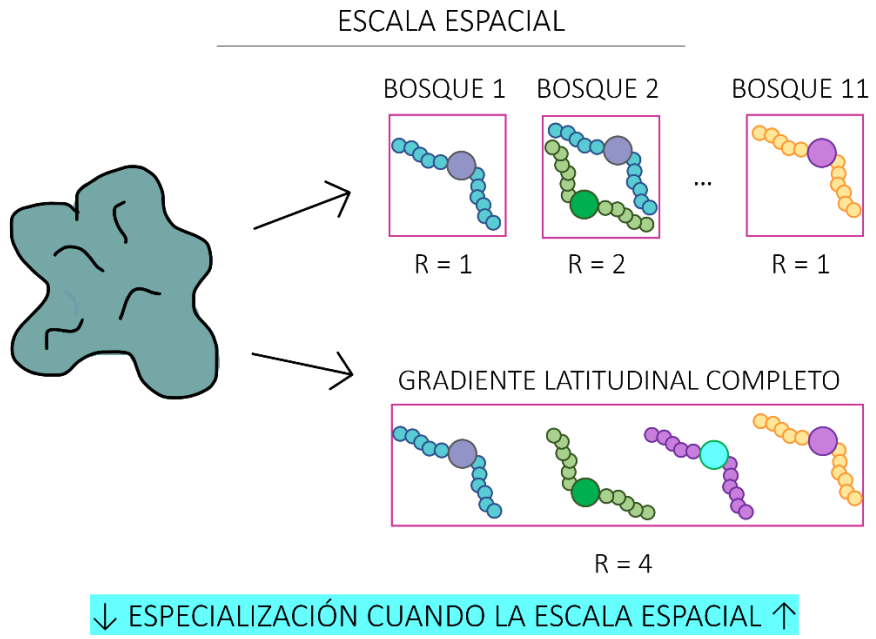


Figura 1. Representación esquemática de la variabilidad de la especialización en función de la escala espacial. En escalas locales (bosques), una especie puede mostrar una elevada especialización al interactuar con un número limitado de cianobacterias mientras que, en escalas regionales, la especialización disminuye cuando la constancia de las interacciones es baja entre las distintas localidades (bosques).

En este estudio, no hemos encontrado relación entre el grado de especialización de las distintas especies de micobionte y su rango de distribución, al contrario de lo que postulan otros estudios (ej.: Swarts *et al.*, 2010 para orquídeas). Encontramos que algunas especies interactúan con distintas cianobacterias en las distintas localidades, mostrando como un patrón más generalizado puede considerarse un mecanismo para ampliar el rango geográfico y el nicho ecológico, tal como indican otros autores (Fernández-Mendoza *et al.*, 2011; Magain *et al.*, 2017; Muggia *et al.*, 2013, 2014; Peksa & Škaloud, 2011). Sin embargo, encontramos especies que se asocian con un pequeño número de cianobacterias, mostrando elevada especialización, pero que tienen amplios rangos de distribución debido a la amplia distribución de las cianobacterias con las que interactúan, de acuerdo con lo que encuentran otros estudios (Davis *et al.*, 2015; Magain *et al.*, 2017). Además, los resultados muestran que la disponibilidad de las cianobacterias no tiene por qué ser un condicionante de la distribución de estos líquenes como se describe en estudios previos (Davis *et al.*, 2015; Lu *et al.*, 2018). Hemos encontrado líquenes que tienen rangos de distribución reducidos, pero que se asocian con cianobacterias con

rangos de distribución más amplios. En estos casos, otras variables estarían limitando el rango de distribución de las especies (Davis *et al*., 2015; Lu *et al*., 2018).

A nivel de comunidad, estudios previos encuentran que las redes suelen mantener su estructura estable al analizar distintas localidades, aunque varíen la composición de especies y las interacciones (ej.: Dáttilo *et al*., 2013; Trøjelsgaard *et al*., 2015). Sin embargo, la comparación entre distintas escalas es escasa, aunque se recalca su importancia (Hagen *et al*., 2012; Tylianakis & Morris, 2017). En nuestro estudio, encontramos cambios en la estructura de las redes de interacciones en función de la escala espacial. En las distintas localidades, las redes pueden presentar una estructura anidada, modular o aleatoria, siendo esta última la más dominante. Sin embargo, al analizar la metarred resultante de las interacciones en todo el gradiente latitudinal estudiado, encontramos que presenta una estructura fuertemente anidada, que refleja que los líquenes especializados interactúan con cianobacterias con las que también interactúan los líquenes más generalizados en contraste con estudios previos en líquenes (Chagnon *et al*., 2018; Duran-Nebreda & Valverde, 2023).

Este resultado contrasta con lo esperado dado que, al tratarse de una simbiosis tan íntima, se esperaba encontrar una estructura altamente modular (Guimarães *et al*., 2007; Pires & Guimarães, 2013; Thompson, 2005). Esta estructura modular resultaría en subgrupos de especies (módulos), que mostrarían cierto grado de especialización entre sí (Dalsgaard *et al*., 2013; Olesen *et al*., 2007). El patrón anidado encontrado en la metarred puede ser debido al aumento de organismos supergeneralistas que actúan como especies centrales (*hubs*) o conectoras que ensamblan toda la red y difuminan los límites entre los módulos (Dalsgaard *et al*., 2013). Por tanto, el patrón anidado encontrado al aumentar la escala espacial puede reflejar un aumento en el número de parejas de algunas de las especies, lo que concuerda con lo detectado al analizar la variabilidad de interacciones a nivel de especie en el capítulo 1.

1.2. Escala biológica

Al igual que sucede con la escala espacial, esperamos que, al ampliar el nivel biológico, disminuya la especialización de las interacciones (Brosi, 2016; Waser *et al*., 1996). Centrándonos en niveles biológicos finos, es decir, a nivel de individuo (intratalino),

estudios previos en líquenes encuentran resultados contrastados dependiendo de la pareja fotosintética. En clorolíquenes (con algas verdes como principal fotobionte), se han descrito distintos taxones de algas verdes dentro de un mismo talo (ej.: Casano *et al.*, 2011; Moya *et al.*, 2017; Muggia *et al.*, 2013). Sin embargo, en cianolíquenes es más frecuente que solo aparezca un único tipo de cianobacteria (ej.: Elvebakk *et al.*, 2008; Paulsrud & Lindblad, 1998). Y en el caso de los cefalolíquenes, se requiere más atención dado el limitado número de estudios, aunque suelen encontrarse distintos filogrupos de cianobiontes en distintos cefalodios (Elvebakk *et al.*, 2008; Myllys *et al.*, 2007). En el capítulo 2 de esta tesis doctoral se han identificado distintas cianobacterias en distintos cefalodios de un mismo individuo (talo) en las tres especies de cefalolíquenes estudiadas (Figura 2).

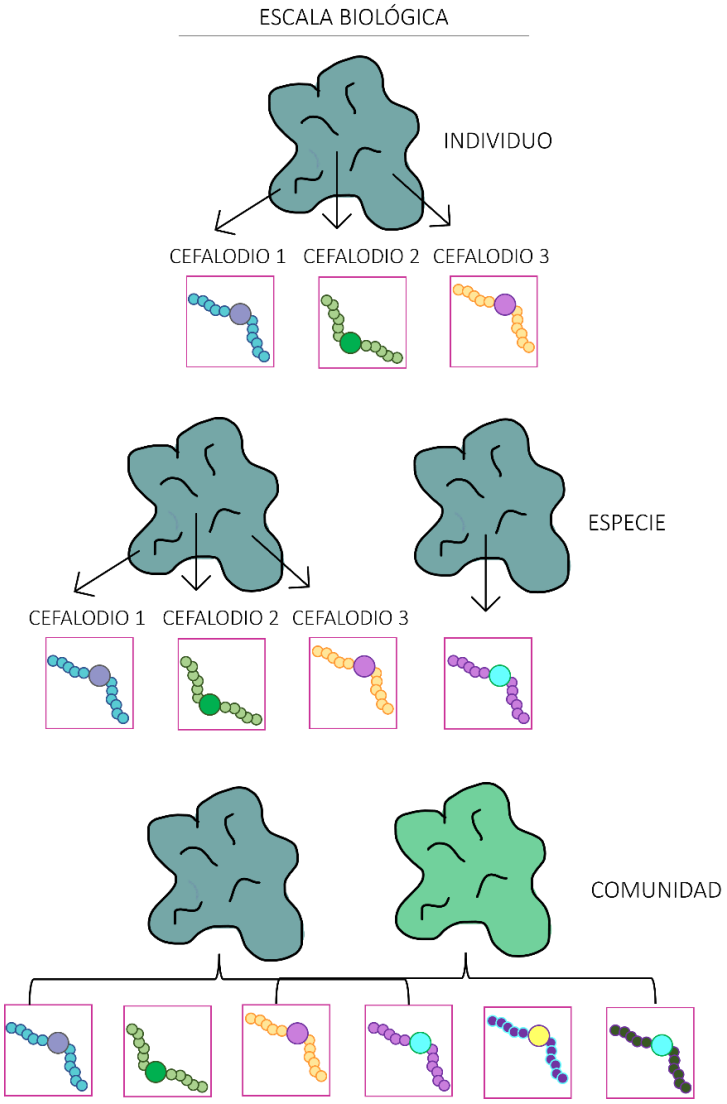


Figura 2. Representación esquemática de la variación de la especialización en función de la escala biológica. Al aumentar la escala biológica (de individuos a especies), se observa una disminución de la especialización al aumentar la riqueza de cianobacterias con las que interactúan. La variabilidad interespecífica de interacciones muestra cómo algunas cianobacterias son compartidas mientras otras interactúan exclusivamente con algunas especies.

No está claro cuál es el significado ecológico de la coexistencia de diferentes fotobiontes en el mismo talo. La diversidad de parejas a nivel intratalino se ha relacionado con respuestas ecofisiológicas complementarias por parte de los fotobiontes que favorecen la adaptación del liquen a distintas condiciones ambientales (Casano *et al*., 2011). Sin embargo, parece que la contribución de los fotobiontes secundarios a la simbiosis liquénica es marginal (Blázquez *et al*., 2022). Algunos estudios señalan que la presencia de varios fotobiontes en el mismo talo se debe a una primera interacción con un fotobionte disponible y un posterior cambio de fotobionte por otro mejor adaptado localmente, por ejemplo en el caso de líquenes liquenícolas (Friedl, 1987; Moya *et al*., 2020). En el caso de los cefalolíquenes, los cianobiontes tienen una función principal como fijadores de nitrógeno, mientras que el organismo fotosintetizador principal es un alga verde (Nash, 1996). Esto podría explicar la flexibilidad para interactuar con varios cianobiontes a nivel intratalino, al no depender de ellos para las necesidades básicas de la simbiosis.

En niveles biológicos superiores, es decir a nivel de especie (capítulo 1 y 2) y a nivel de comunidad (capítulo 3), se observa cómo las especies muestran niveles de especialización variables (capítulo 1 y 2) y las cianobacterias son compartidas entre las especies coexistentes (capítulo 1, 2 y 3). Esto concuerda con estudios previos que postulan que los fenómenos de especialización en líquenes tienen lugar a nivel de comunidad (Rikkinen, 2013; Rikkinen *et al*., 2002). Este resultado recalca que los casos de especialización recíproca son escasos en este sistema de estudio (Magain *et al*., 2017; Otálora *et al*., 2010), dominando los casos de especialización asimétrica en los que las especies especializadas interactúan con parejas generalizadas, como ocurre en otros sistemas de estudio (Bascompte *et al*., 2003; Joppa *et al*., 2009; Vázquez & Aizen, 2004) .

Además, las interacciones a nivel de especie o a nivel de comunidad responden a distintos factores. Por un lado, la especialización a nivel de especie (capítulo 1), podría ser

en parte explicada con la función de la cianobacteria cuando se cuantifica con la riqueza de parejas y el índice de Simpson. Este resultado indica que los cianolíquenes son más especializados que los cefalolíquenes. Adicionalmente, la identidad del micobionte es el factor que mayor variación explica del índice d', lo que indica que la asociación con cianobiontes ampliamente compartidos (bajos valores de d') o raros (altos valores de d') en la comunidad tiene un fuerte componente genético. Por otro lado, las interacciones cuantificadas desde una perspectiva de redes de interacciones (capítulo 3), se ven influenciadas por la variabilidad ambiental y el modo reproductivo. Así, la modularidad de las redes tiende a aumentar con la latitud y la temperatura, mientras que la conectancia sigue un patrón opuesto. Además, la ausencia de estructuras de reproducción sexuales y asexuales también afectan a que las redes estén poco conectadas. Estos resultados se discuten en mayor profundidad en el siguiente apartado.

2. La importancia de los factores ambientales

La variabilidad climática y ambiental engloba un conjunto de factores clave que determinan el ensamblaje de las comunidades y la coexistencia de las especies (Gleason, 1926; Götzenberger *et al*., 2012; Keddy, 1992). Además, el cambio climático es uno de los principales motores del presente cambio global y está relacionado con la actual pérdida de diversidad (Bellard *et al*., 2012). El cambio climático puede afectar a la biodiversidad mediante la modificación de las interacciones entre las especies (Harley, 2011). Esto puede originar cambios en la composición y dinámica de las comunidades incidiendo en el funcionamiento de los ecosistemas (Bellard *et al*., 2012). Los estudios basados en amplios gradientes latitudinales nos permiten evaluar el efecto de una gran variabilidad de condiciones climáticas sobre la distribución de la biodiversidad y sus interacciones (McGill *et al*., 2006).

En el marco de las interacciones bióticas, los factores ambientales tienen gran relevancia al definir el gradiente latitudinal de especialización (Dalsgaard *et al*., 2011, 2013; Jocque *et al*., 2010; MacArthur, 1972; Schleuning *et al*., 2012, 2014). Este gradiente latitudinal de especialización viene definido por una mayor especialización en las zonas tropicales que en las zonas templadas, a causa de una mayor diversidad de especies en estas regiones y de la presencia de condiciones climáticas más estables (Dyer *et al*., 2007;

Hillebrand, 2004; Kinlock *et al*., 2018; MacArthur, 1972; Olesen & Jordano, 2002). La gran diversidad de especies encontrada a menores latitudes promueve la especialización con el fin de evitar el solapamiento de nicho de especies coexistentes (Connell, 1978; Dyer *et al*., 2007; Macarthur & Levins, 1967; Olesen & Jordano, 2002). Además, la estabilidad climática, tanto presente como pasada tiene un papel relevante en las interacciones bióticas establecidas (Dalsgaard *et al*., 2013; Schemske *et al*., 2009; Schleuning *et al*., 2012). No obstante, este gradiente latitudinal de especialización no parece ser generalizable a todos los sistemas de estudio (Dalsgaard *et al*., 2017; Morris *et al*., 2014; Ollerton & Cranmer, 2002; Ollerton *et al*., 2006; Vázquez & Stevens, 2004).

En la presente tesis doctoral, se observa el efecto que los factores ambientales tienen sobre la composición de las comunidades de cianobacterias en cada una de las localidades (capítulo 1). Además, aunque estos factores no contribuyen a explicar la variabilidad de los índices utilizados para cuantificar la especialización a nivel de especie (capítulo 1), si tienen un papel relevante en las redes de interacciones (capítulo 3).

2.1. Influencia de los factores ambientales en la distribución de las cianobacterias

Los resultados del capítulo 1 muestran que la riqueza de cianobacterias no sigue un gradiente latitudinal de mayor riqueza de especies en menores latitudes, en contraste a lo esperado al considerar la teoría del gradiente latitudinal de diversidad (Hillebrand, 2004). Sin embargo, a pesar de la aceptación general de esta teoría y los numerosos estudios posteriores que la confirman (ej.: Kinlock *et al*., 2017), hay diversos grupos de organismos que no siguen esté patrón (ej.: Laenen *et al*., 2018; Wang *et al*., 2017). Al no encontrar un patrón latitudinal en la riqueza de cianobacterias, los distintos niveles de especialización de las especies de micobionte no parecen estar determinados por la finalidad de generar nichos discretos para disminuir la competencia entre especies coexistentes (contrario a Dyer *et al*., 2007; Olesen & Jordano, 2002).

No obstante, los resultados de esta tesis doctoral muestran que los factores ambientales influyen en la composición de las comunidades de cianobiontes y, por tanto, en su disponibilidad. Los cambios en la composición de las comunidades de cianobiontes pueden condicionar los niveles de especialización que presentan las distintas especies de micobiontes, en la misma línea que en estudios previos (Fernández-Mendoza *et al*., 2011;

Muggia *et al*., 2013, 2014; Nadyeina *et al*., 2014; Vargas Castillo & Beck, 2012). La mayor parte de la variabilidad en la composición de las comunidades de cianobiontes a lo largo del gradiente latitudinal la explican variables ambientales relacionadas con la temperatura (temperatura media anual y temperatura mínima del mes más frío), seguidas del DBH. Esto indica que, en función de si los bosques son más o menos viejos (DBH) y de la variabilidad de la temperatura, encontramos comunidades de cianobiontes distintas. Por tanto, hay un filtrado ambiental que influye en la disponibilidad de los cianobiontes (Batstone *et al*., 2018; Nelsen *et al*., 2021; Rolshausen *et al.*, 2018, 2020).

Por otro lado, distintos factores geográficos pueden estar influyendo en la composición de estas comunidades. En el gradiente de estudio, encontramos los campos de hielo patagónicos, que pueden actuar como barrera geográfica limitando la dispersión de las cianobacterias, como ocurre con otros organismos (Zúñiga-Reinoso *et al*., 2013). Por ejemplo, la abundancia del filogrupo 42 se hace notable al sur de los campos de hielo patagónicos, sugiriendo que este filogrupo, o bien está mejor adaptado a las condiciones ambientales del sur del gradiente, o bien encuentra limitaciones en la dispersión al encontrarse con esta barrera geográfica.

2.2. Gradiente latitudinal de especialización

En el capítulo 1, en el que se analizan las interacciones bióticas a nivel de especie, no se ha encontrado un efecto de los factores ambientales sobre la especialización, lo que revela la ausencia de un gradiente latitudinal de especialización para los líquenes a nivel de especie. Este resultado podría explicarse por la ausencia de un gradiente latitudinal de diversidad de los cianobiontes, ya mencionado previamente (Figura 3a). La ausencia de un patrón latitudinal de especialización ha sido encontrada en sistemas antagonistas (Morris *et al*., 2014).

En el capítulo 3, en cambio, al analizar las comunidades considerando toda la red de interacciones, se encuentra que tanto la modularidad como la conectancia varían a lo largo del gradiente latitudinal. Esta variabilidad latitudinal está influenciada mayormente por variables ambientales relacionadas con la temperatura. Mientras que la modularidad aumenta hacia zonas de menor latitud (mayor temperatura), la conectancia presenta el patrón inverso (Figura 3b). Este patrón latitudinal concuerda con los patrones

encontrados en estudios de redes de polinización y de dispersores de semillas (Dalsgaard *et al*., 2011, 2013; Olesen & Jordano, 2002; Trøjelsgaard & Olesen, 2013). Este gradiente latitudinal de especialización sugiere un aumento en la generalización de la red hacia el sur del gradiente, lo que puede permitir al sistema hacer frente a condiciones ambientales adversas de muy bajas temperaturas, aportando estabilidad (Batstone, *et al*., 2018; Dalsgaard *et al*., 2013).

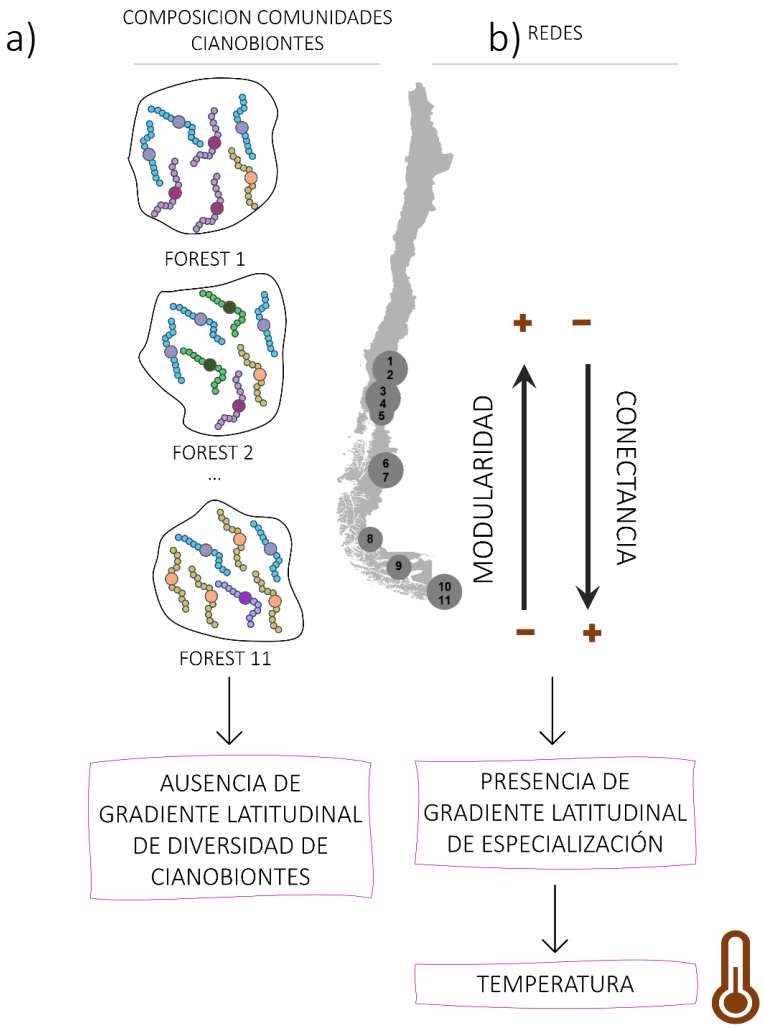


Figura 3. Representación esquemática de la influencia de los factores ambientales en el gradiente latitudinal estudiado: a) las variables ambientales afectan a la composición de las comunidades de cianobiontes, aunque no se observa un patrón latitudinal de diversidad con una disminución de filogrupos hacia latitudes menores y b) gradiente latitudinal de especialización influenciado principalmente por la temperatura, con patrones de mayor modularidad hacia latitudes menores y mayor conectancia hacia latitudes mayores.

3. La importancia de los rasgos funcionales

Los rasgos funcionales son una pieza clave en la ecología de comunidades. Esto es debido a que se asume en parte que las especies coexisten en comunidades en función de sus rasgos funcionales (Götzenberger *et al*., 2012; Shipley *et al*., 2016). Por ello, se espera que, distintos factores, tanto bióticos como abióticos, filtren aquellas especies con un determinado conjunto de rasgos que permiten la supervivencia ante determinadas condiciones para conformar comunidades (Le Bagousse-Pinguet *et al*., 2017). En el contexto de las interacciones bióticas, también se ha destacado la importancia de los rasgos funcionales desde los primeros estudios de Darwin (Darwin, 1862). En concreto, se han identificado rasgos compatibles (*matching traits*) entre las especies que forman parte de una interacción, como puede ser la longitud de la probóscide de un insecto y la longitud de la corola de la flor que poliniza (Dalsgaard *et al*., 2010; Danieli-Silva *et al*., 2012; Fenster *et al*., 2004; Olesen *et al*., 2007; Ollerton *et al*., 2009; Pedraza & Bascompte, 2021). En interacciones de planta-polinizadores, esto ha sido tradicionalmente descrito como *síndromes de polinización* (*pollination syndroms*; ej.: Fenster *et al*., 2004; Martín González *et al*., 2012; Ollerton *et al*., 2009).

En el caso de los líquenes, recientes estudios han recalcado la importancia de los rasgos funcionales en el ensamblaje de las comunidades de líquenes (Ellis *et al*., 2021). Sin embargo, son escasos los relacionados con el papel que tienen los rasgos funcionales en las interacciones bióticas. En estos estudios destaca la importancia de las estructuras de reproducción, donde los líquenes con reproducción asexual tienden a ser más especializados que los líquenes que se reproducen sexualmente (ej.: Hoz *et al*., 2018; Otálora *et al*., 2010; Steinová *et al*., 2019).

En esta tesis doctoral se obtienen resultados consistentes en los distintos capítulos. A nivel de especie (capítulo 1), se observa que parte de la variación tanto de la riqueza de parejas como del índice de Simpson se ve explicada por la función del cianobionte (Figura 4). Es decir, los cianolíquenes presentan mayores niveles de especialización, al considerar estas dos facetas, que los cefalolíquenes. Del mismo modo, en el capítulo 3, se encuentra una mayor especialización en los cianolíquenes que en los cefalolíquenes. Esto se ve reflejado tanto en la conformación de los módulos aislados

como en los papeles que desempeñan las distintas especies dentro de la red. Por un lado, se observa que los módulos aislados de especies que no forman parte de la red se encuentran mayoritariamente formados por especies de cianolíquenes, apoyando el mayor nivel de especialización de éstas. Por otro lado, al analizar los distintos papeles de las especies dentro de las redes, se puede observar que los cefalolíquenes integran a la mayoría de las especies que actúan como conectores o como especies centrales (*hubs*), tanto dentro de los módulos como de toda la red. Por tanto, estas especies actúan como fuente de cianobacterias para otros micobiontes coexistentes dada su capacidad de asociación con múltiples cianobiontes. Además, en el capítulo 2, encontramos cómo los cefalolíquenes presentan variabilidad en sus cianobiontes incluso a nivel intratalino, recalcando su baja especialización incluso a nivel individual.

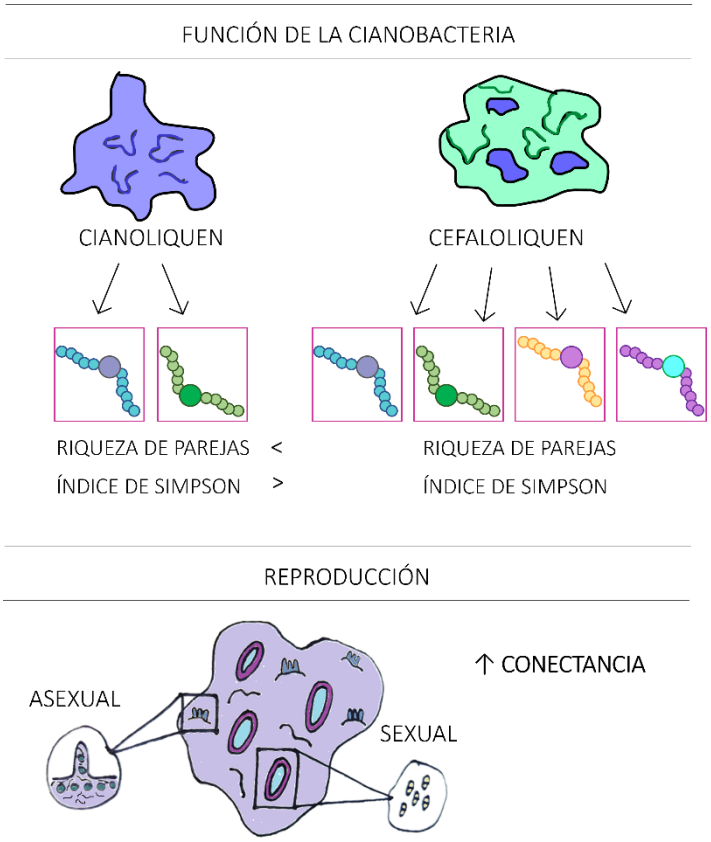


Figura 4. Representación esquemática de los rasgos funcionales considerados y su efecto sobre las interacciones entre micobiontes-cianobiontes. En la parte superior de la imagen se representa la influencia de la función de la cianobacteria sobre la especialización analizada con índices de diversidad (los cianolíquenes son más especializados que los cefalolíquenes). En la parte interior de la imagen se representa el papel que tienen las estructuras de reproducción sexual y/o asexual sobre la conectancia (la presencia de estas estructuras aumenta la conectancia).

Todos estos resultados coinciden en que los cefalolíquenes están menos especializados que los cianolíquenes en términos generales. Los escasos trabajos desarrollados hasta el momento que han analizado las diferencias entre ambos tipos de líquenes no han encontrado diferencias entre ellos (Pardo de la Hoz *et al*., 2018; Wirtz *et al*., 2003). El mayor grado de especialización detectado en los cianolíquenes respecto a los cefalolíquenes puede estar relacionado con la importancia relativa que presenta la cianobacteria para cumplir con los requerimientos de la simbiosis liquénica (Palmqvist, 2002; Rai, 2002). En los primeros, la cianobacteria es el fotobionte principal encargado de realizar la fotosíntesis, resultando en una interacción obligada para dar lugar al talo liquénico. En los segundos, el fotobionte principal es un alga verde mientras que la cianobacteria tiene una función principal como fijadora de nitrógeno atmosférico (Nash, 1996; Paulsrud *et al*., 2001; Rai *et al*., 2000). Como la función fotosintética en los cefalolíquenes está asegurada por el alga verde, esto podría conllevar una menor dependencia del micobionte por la cianobacteria, explicando su menor grado de especialización (Blüthgen *et al*., 2007; Guimarães *et al*., 2007).

Otro rasgo funcional destacable es el modo reproductivo de las especies. En este sentido, se espera que las especies que presentan estructuras de reproducción asexual muestren una mayor especialización. Esto es debido a que estas estructuras asexuales dispersan conjuntamente a ambos simbiontes (micobionte y cianobionte), dando lugar a una transferencia vertical del cianobionte. Sin embargo, cuando los líquenes se reproducen sexualmente, sólo se reproduce el hongo que libera las esporas, que al germinar necesita un nuevo cianobionte para reestablecer la simbiosis (transferencia horizontal del cianobionte; Dal Grande *et al*., 2012; Grube & Spribille, 2012; Hoz *et al*., 2018; Otálora *et al*., 2010, 2013).

El efecto que tiene el modo reproductivo sobre la especialización se observa en el capítulo 3 (Figura 4). En las redes de interacciones, la ausencia de estructuras reproductivas, tanto sexuales como asexuales, tiene un efecto negativo sobre la conectancia. Las redes menos conectadas son aquellas que presentan una mayor proporción de talos que carecen de estas estructuras, probablemente debido a limitaciones para que haya intercambio de cianobiontes y tengan lugar procesos de

facilitación (Chagnon *et al*., 2016; Duran-Nebreda & Valverde, 2023; Otálora *et al*., 2010; Rikkinen, 2003; Rikkinen *et al*., 2002).

Sin embargo, en el capítulo 1, no se observa que la especialización esté relacionada con el modo reproductivo. Hay varios estudios que han encontrado que los líquenes, incluso en los casos en los que se reproducen de forma asexual con ambos simbiontes juntos (transmisión vertical de la cianobacteria), pueden cambiar el fotobionte por otro mejor adaptado a las condiciones locales (*photobiont switching*; DePriest, 2004; Ertz *et al*., 2018; Ohmura *et al*., 2019; Piercey-Normore & DePriest, 2001; Rolshausen *et al*., 2018; Williams *et al*., 2017). De esta manera, el fotobionte original ayuda al talo liquénico a establecerse y a empezar a desarrollarse, y cuando éste puede interactuar con un fotobionte mejor adaptado a las condiciones locales, la cambia por la primera (Moya *et al*., 2020; Rolshausen *et al*., 2018, 2020; Wedin *et al*., 2016). Estos cambios de cianobacterias podrían ser la razón por la que el modo de reproducción no explique significativamente parte de la variación de las distintas medidas de especialización en los resultados del capítulo 1.

3.1. Módulos en las redes de interacción

Los módulos dentro de las redes de interacciones son compartimentos de especies que interactúan entre sí con mucha más frecuencia que con especies pertenecientes al resto de la red (Olesen *et al*., 2007). Los módulos tienden a considerarse como unidades coevolutivas en las que se dan presiones selectivas recíprocas entre las parejas de la interacción, que resultan en la coevolución de rasgos complementarios entre ellas (Darwin, 1862; Dupont & Olesen, 2009; Olesen *et al*., 2007; Thompson, 2005). Incluso grupos alejados filogenéticamente, pueden converger en los rasgos funcionales implicados en la interacción, como ocurre con los anteriormente descritos síndromes de polinización (ej.: Fenster *et al*., 2004). Por ejemplo, en redes tróficas, Rezende *et al*. (2007), encuentran que la filogenia, el tamaño corporal y la preferencia del hábitat están correlacionados con la pertenencia a los distintos módulos.

Contrariamente a lo esperado, no se ha detectado que los rasgos funcionales analizados ni las distintas familias de micobiontes determinen la conformación de los distintos módulos de las redes (Figura 5). Sin embargo, es importante destacar que este

análisis presenta sus limitaciones dado que sólo se ha contado con información de rasgos funcionales de uno de los dos organismos: el micobionte. Esto condiciona poder determinar si existe un conjunto de rasgos compatibles que encajen entre ambas clases de organismos (*matching traits*), para que pueda darse la interacción (Fenster *et al*., 2004; Olesen *et al*., 2007; Thompson, 2005). Al tratarse de una simbiosis tan íntima, es posible que los rasgos compatibles que influyen en este aspecto aparezcan a niveles más fisiológicos como por ejemplo en el reconocimiento de unas parejas u otras (Lehr *et al*., 2000; Sacristán *et al*., 2007). Por tanto, los rasgos funcionales analizados limitan la consideración de los módulos encontrados en las redes modulares como unidades coevolutivas en nuestro sistema de estudio (Olesen *et al*., 2007; Thompson, 2005).

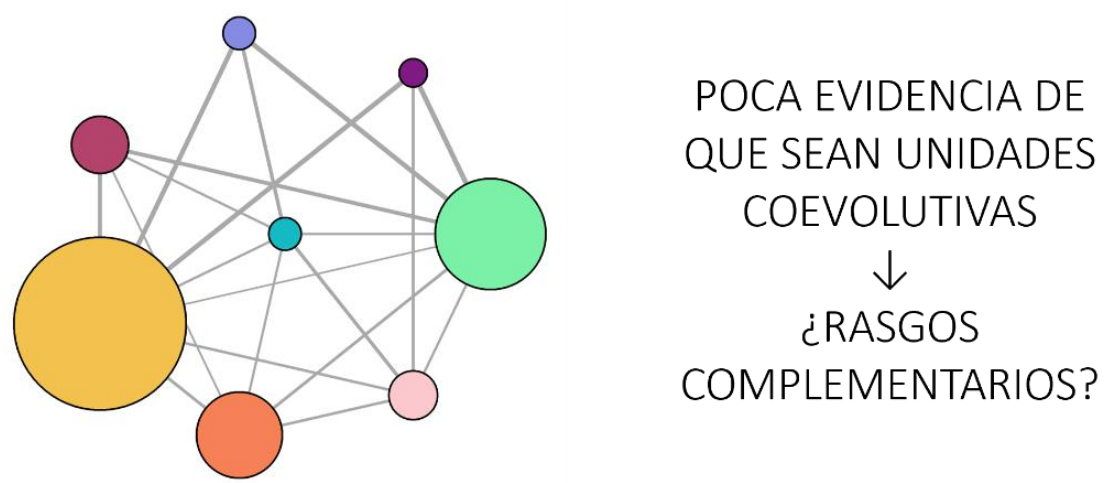


Figura 5. Representación esquemática de los módulos de una red modular y de las limitaciones para considerar estos módulos como unidades coevolutivas.

Además, en el capítulo 1 también se ha encontrado que la variación del índice d' es explicada principalmente por la identidad del micobionte. Esto puede hacer que no tanto los rasgos, si no la especie de micobionte en sí misma y sus demás requerimientos en los distintos ejes del nicho condicione la interacción con unas cianobacterias u otras en función de la abundancia de las últimas (Francesco Dal Grande *et al*., 2018; Jüriado *et al*., 2019; Leavitt *et al*., 2015; Vančurová *et al*., 2018; Vázquez *et al*., 2005).

3.2. Lichen-guilds y especialización a nivel de comunidad

En líquenes asociados con cianobacterias se postula que los fenómenos de especialización se dan a nivel de comunidad (Rikkinen *et al*., 2002). Esta afirmación surge por el hecho de que líquenes coexistentes comparten los cianobiontes (Fedrowitz *et al*.,

2012; Kaasalainen *et al*., 2021; Paulsrud *et al*., 2001; Rikkinen *et al*., 2002). Tradicionalmente se han distinguido distintos gremios (*guilds*) de líquenes en función de sus requerimientos ecológicos (Rikkinen, 2003, 2013). Dentro de estos grupos se describe un sistema de especies centrales o donadoras (*core*), que actúan como fuente de cianobiontes al liberar propágulos que contienen cianobacterias que pueden capturar otras especies periféricas coexistentes (Rikkinen, 2003). De esta forma, los procesos de facilitación en las comunidades de líquenes tienen como resultado una especialización a nivel de comunidad, con líquenes coexistentes compartiendo sus cianobacterias (Rikkinen *et al*., 2002).

A lo largo de esta tesis doctoral, se muestra como numerosos cianobiontes se comparten entre distintas especies de líquenes, incluso aunque no sean cercanos filogenéticamente, de acuerdo con esa especialización mencionada a nivel de comunidad (Rikkinen *et al*., 2002). En las redes locales del capítulo 3, se identifican cianobiontes supergeneralistas que se comparten entre múltiples micobiontes. Este resultado indica la existencia de fenómenos de facilitación, en los que algunas especies pueden actuar como fuente de cianobiontes, mientras que otras interactúan con cianobiontes procedentes de las primeras (Rikkinen, 2003). Entre estas especies supergeneralistas podrían encontrarse *Nephroma antarcticum* o *Pseudocyphellaria vaccina,* que aparecen como especies centrales (*hubs*) cuando se observa su papel en la metarred. Ambas especies son cefalolíquenes y son capaces de interactuar con múltiples cianobiontes. Este resultado consistente de baja especialización en cefalolíquenes (capítulos 1, 2 y 3), sugiere que este grupo de líquenes pueden albergar cianobacterias compatibles para otras especies más especializadas, actuando como donadoras de cianobiontes, al ser capaces de interactuar con mayor número de cianobiontes (Figura 6; Cardós *et al*., 2019).

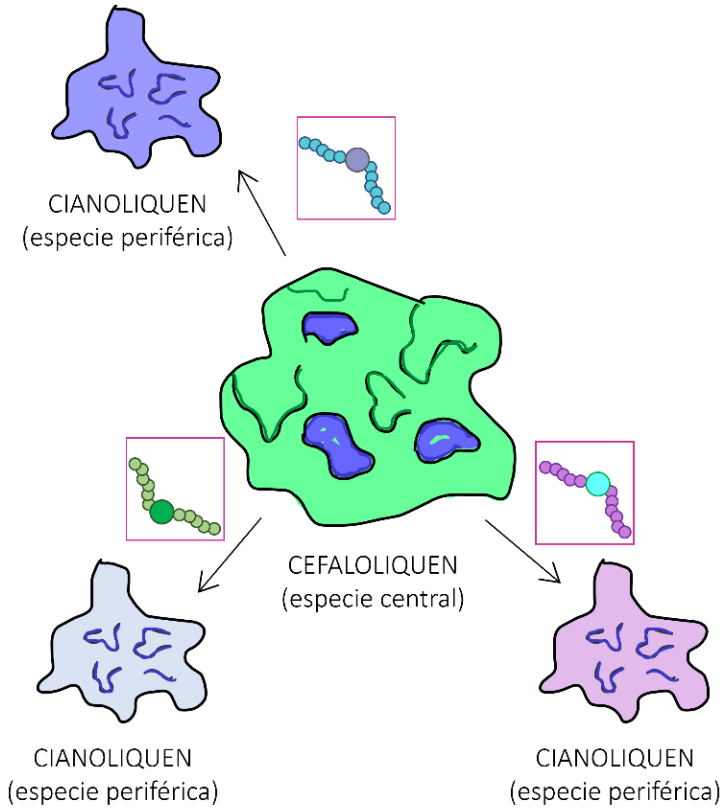


Figura 6. Representación esquemática de los gremios de los líquenes en los que, principalmente los cefalolíquenes albergan más variabilidad de cianobacterias y podrían actuar como especies donadoras de cianobacterias para especies coexistente más especializadas.

Otro resultado del capítulo 3 que concuerda con los estudios previos de los gremios es la distinción entre dos grupos de cianobacterias: las que pertenecen al grupo *Peltigera (Peltigera-guild)* y las que pertenecen al grupo *Nephroma* (*Nephroma-guild*) (Figura 7; Kaasalainen *et al*., 2021; Rikkinen, 2003, 2013). La separación de estos dos grupos de cianobacterias se relaciona con las diferencias que presentan estas especies en cuanto a sus requerimientos ecológicos (Rikkinen *et al*., 2002). De este modo, el grupo *Peltigera* estaría conformado por especies terrícolas que crecen en el suelo mezcladas con distintos briófitos (Rikkinen, 2003; Rikkinen *et al*., 2002). En cambio, el grupo *Nephroma* consiste en especies mayoritariamente epífitas que crecen sobre distintos forófitos (Rikkinen, 2003; Rikkinen *et al*., 2002). En este capítulo se ha encontrado que todas las especies del género *Peltigera* aparecen en módulos aislados en las redes locales. Sin embargo, al analizar la metarred, la mayoría de las especies de *Peltigera* (con la excepción de *Peltigera collina*) entran a formar parte de la red. La presencia de estas *Peltigeras* en los troncos de *Nothofagus pumilio* hace que estas especies puedan

considerarse “epífitas facultativas” y que puedan interaccionar con cianobiontes del grupo de *Nephroma* gracias a fenómenos de facilitación (Kaasalainen *et al.*, 2021; Rikkinen, 2003; Rikkinen *et al.*, 2002).

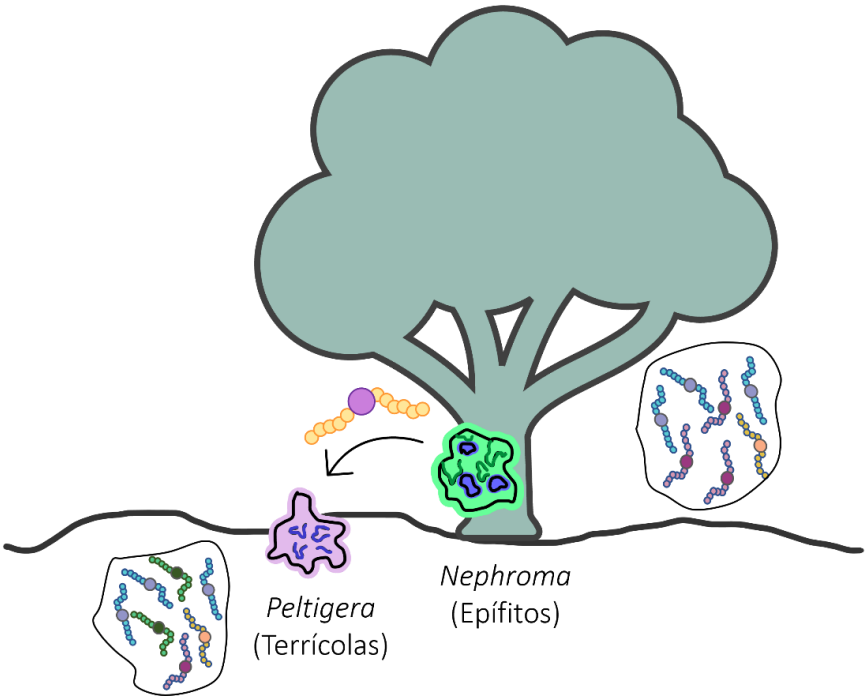


Figura 7. Representación esquemática de los gremios reconocidos dentro de líquenes: *Peltigera* y *Nephroma*, distinguidos principalmente por sus requerimientos ecológicos referidos al sustrato en el que se desarrollan. Asimismo, se ilustra la capacidad de especies pertenecientes al gremio *Peltigera* de captar cianobiotes del gremio *Nephroma* cuando tienen un crecimiento epífito.

4. Métricas para cuantificar la especialización

La especialización dentro de las interacciones bióticas ha sido tradicionalmente considerada desde la perspectiva del número de parejas con las que un organismo dado es capaz de interactuar (Blüthgen *et al.*, 2006). Desde esta perspectiva a nivel de organismo, en los últimos años, distintos índices, tanto de diversidad (ej.: índice de Simpson, índice de Shannon), como de amplitud de nicho (índice d’) han sido considerados (ej.: Blüthgen *et al.*, 2006; Poisot *et al.*, 2012; Sahli & Conner, 2006). No sólo eso, si no que han surgido diferentes propuestas a la hora de definir y cuantificar la especialización (Armbruster, 2017; Devictor *et al.*, 2010; Forister *et al.*, 2012; Poisot *et al.*, 2012).

En el capítulo 1 se ha identificado que las medidas utilizadas para cuantificar el grado de especialización de los micobiontes muestran resultados inconsistentes: mientras que para una métrica unas especies se considerarían especializadas, para otra podrían considerarse especies con alto grado de generalización. Además, la variación de estas métricas es explicada por distintos factores. Los cianolíquenes son más especializados que los cefalolíquenes si se considera la riqueza de parejas y el índice de Simpson, probablemente debido a la interacción obligada de los primeros con la cianobacteria para establecer la simbiosis. Sin embargo, cuando se consideran las interacciones con el índice d', es la identidad del micobionte la que explica la mayor parte de la variabilidad de la especialización. Este resultado respecto al índice d' coincide con estudios previos (Dal Grande *et al*., 2018; Fedrowitz *et al*., 2012; Jüriado *et al*., 2019) que encuentran que la identidad del micobionte determina el grado de especialización con poca influencia del ambiente (Leavitt *et al*., 2015) o de otros factores. Con este resultado, sugerimos en este primer capítulo que la métrica influye en la comprensión de la especialización (Figura 8).

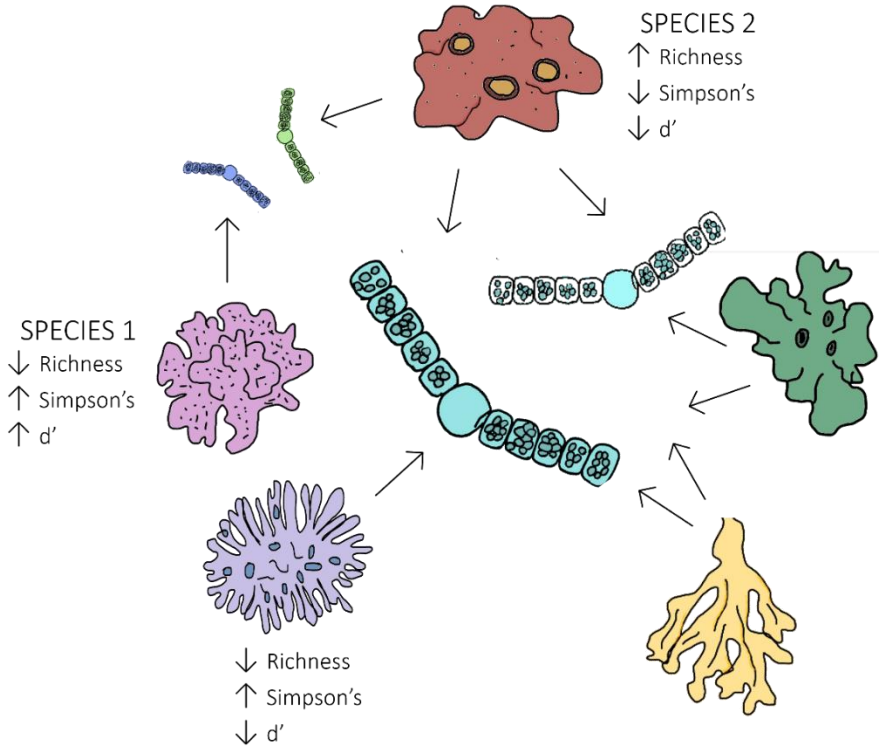


Figura 8. Representación esquemática de cómo los distintos índices utilizados en el Capítulo 1 de la presente tesis doctoral influyen sobre la consideración de la especialización en una comunidad esquemática.

Por tanto, ¿qué métrica considerar para analizar si un organismo es especializado? En el capítulo 4 se propone un marco conceptual en el que se integran tres métricas que ofrecen información complementaria para identificar la especialización. Cada métrica se asocia con una faceta de la especialización: la riqueza de parejas estandarizada hace referencia a la especificidad, la equitatividad de Simpson a la preferencia y el índice d' a la selectividad de las interacciones. Combinando estas tres métricas, se ha generado un espacio 3D en el que cada eje hace referencia a una faceta de la especialización (Figura 9).

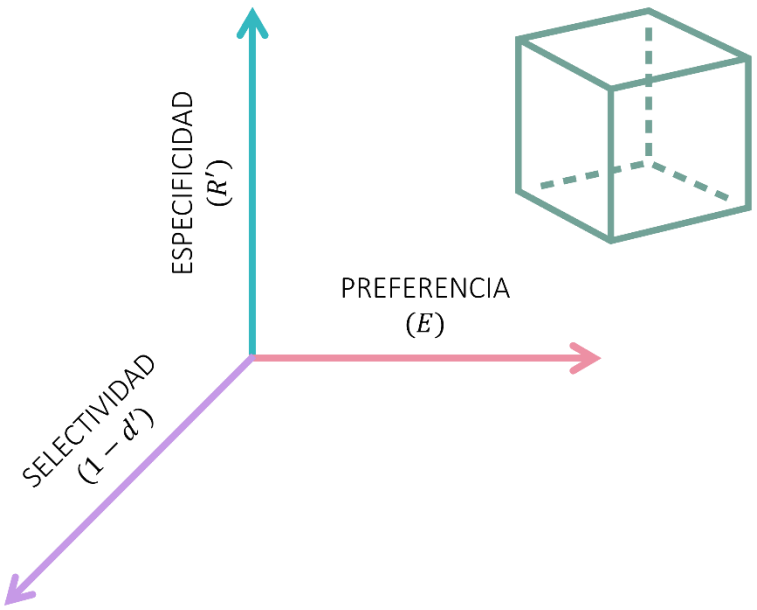


Figura 9. Representación esquemática del volumen 3D propuesto en el Capítulo 4 para considerar la especialización desde una perspectiva integradora que engloba sus distintas facetas: *especificidad*, *preferencia* y *selectividad*.

Cuando aplicamos la nueva propuesta en diferentes sistemas naturales de interacciones, se observa en la mayoría de los organismos analizados una tendencia a la especialización debida a una baja especificidad como mencionan estudios previos (ej.: Egan & Funk, 2006; Poisot *et al*., 2012; Waser *et al*., 1996). No obstante, los resultados revelan una especialización muy laxa, en la que, aunque los organismos tienden a interactuar con pocas parejas, estas interacciones son más equitativas y pueden ser selectivas u oportunistas. Con este patrón se potencian las ventajas y se difuminan las desventajas de la especialización, que puede constituir un callejón sin salida evolutivo (Batstone *et al*., 2018; Futuyma & Moreno, 1988; Thompson, 2005). Además, los

resultados muestran una prevalencia de interacciones asimétricas, en las que la mayoría de las especies interactúan con pocas parejas y pocas especies interactúan con numerosas parejas (Bascompte & Jordano, 2014; Bascompte *et al*., 2003; Vázquez & Aizen, 2004; Vázquez *et al*., 2007, 2005).

Varios estudios postulan que los sistemas huésped-parásito y los mutualismos íntimos están más especializados que los organismos que interactúan en libertad, como las plantas y sus polinizadores o dispersores de semillas (Blüthgen *et al*., 2007; Olesen *et al*., 2007; Thompson, 2005). Sin embargo, pocos estudios comparan directamente diferentes sistemas de forma simultánea (véase Blüthgen *et al*., 2007), una comparación que resulta muy sencilla con el marco 3D que hemos propuesto. La aplicación del citado marco para encontrar diferencias entre sistemas de interacciones ayuda a determinar si las interacciones antagonistas y mutualistas muestran diferentes patrones de especialización (Thompson, 2005).

Nuestra propuesta también permite desentrañar las distintas facetas de la especialización para los organismos dentro de cada sistema. De este modo, se pueden comparar ambas clases de organismos (consumidores y recursos) dentro de un determinado sistema de interacción. Respecto a este tema se han publicado resultados contradictorios. Unos estudios encuentran que los organismos considerados recursos (es decir, hospedadores y plantas) muestran una mayor especialización que los consumidores (es decir, parásitos y animales; ej.: Blüthgen *et al*., 2006, 2007; Egan & Funk, 2006), mientras que otros, sugieren que los consumidores interactúan más preferencialmente que los recursos (Traveset *et al*., 2016), siendo estos últimos más selectivos.

En cualquier caso, hay que tener en cuenta que en la propuesta que se plantea no se ha considerado ni la filogenia ni los rasgos funcionales que participan en las interacciones. Hay que tener en cuenta que no es lo mismo interactuar con especies que estén cercanas filogenéticamente que con especies que pertenecen a clados alejados (ej.: Novotny & Basset, 2005; Waser *et al*., 1996). Asimismo, los rasgos compatibles entre parejas que interactúan también participan en los fenómenos de especialización. Por ello, se sugiere que esta nueva propuesta se englobe dentro de la *especialización taxonómica*, siendo un primer paso para introducir posteriormente la especialización funcional y filogenética.

5. Consideraciones finales

Una de las fortalezas de la presente tesis doctoral es que se analizan los fenómenos de especialización entre micobiontes y cianobiontes considerando todas las especies coexistentes en un amplio gradiente latitudinal en el hemisferio sur. Este análisis ha sido realizado, a su vez, teniendo en cuenta distintas escalas espaciales (localidades *vs*. región) y biológicas (individuos *vs*. especies *vs*. comunidad). Nuestros resultados muestran cuáles son los principales patrones de especialización que se dan en estos organismos y cómo es la estructura de las redes de interacciones que conforman, así como cuáles son los factores que influyen en esta variabilidad. Además, una aportación importante de este trabajo es que propone herramientas que permiten identificar especies clave sobre las que enfocar distintas medidas de conservación, bien porque actúen como donadoras o fuentes de cianobacterias para otras especies en estas comunidades o porque estén altamente especializadas y sean más vulnerables a las condiciones ambientales en un contexto de cambio global.

Los resultados muestran como los micobiontes estudiados son capaces de interactuar con diversos cianobiontes, lo que puede ser ventajoso para lograr el objetivo de la simbiosis liquénica ya que una elevada especialización puede suponer que ambos simbiontes no lleguen a encontrarse y que no llegue a establecerse la asociación simbiótica (Magain *et al*., 2017). Asimismo, la capacidad observada de asociarse con distintas parejas que se encuentren mejor adaptadas a las condiciones locales puede considerarse una ventaja adaptativa para mejorar la eficacia biológica (Batstone *et al*., 2018; Magain *et al*., 2017; Thompson, 2005). De hecho, se ha demostrado que hay micobiontes que presentan diferencias en varios aspectos de su fisiología en función del fotobionte con el que interaccionen (Casano *et al*., 2011). Presentar, por tanto, cierta generalización puede ser ventajoso debido a que 1) puede aumentar las probabilidades de asociarse con parejas mejor adaptadas para hacer frente a condiciones ambientales cambiantes ("*sampling effect*" de Batstone *et al*., 2018) y 2) permite la interacción con otros cianobiontes si se da una pérdida de diversidad de parejas potenciales, lo que aporta robustez y estabilidad en las comunidades.

La flexibilidad para reemplazar unas parejas por otras en distintas localidades, además, puede ampliar los rangos de distribución de las especies. Esto último se traduce en la tendencia hacia la generalización cuando la escala espacial es más amplia que encontramos en los capítulos 1 y 3. No obstante, también podemos encontrar amplios rangos de distribución en líquenes cuando la interacción, aunque sea muy especializada, se dé con parejas ampliamente distribuidas (ej.: Magain *et al*., 2017).

Por otro lado, entre las ventajas de tener un alto grado de especialización se encuentra el aumento en la eficacia y eficiencia de la interacción (Egan & Funk, 2006; Fox & Morrow, 1981; Futuyma & Moreno, 1988). Esto podría darse cuando ha habido coevolución o coespeciación de ambos simbiontes, que puede conllevar la existencia rasgos compatibles en ambos que permitirían optimizar los beneficios de la interacción (Fenster *et al*., 2004; Irschick *et al*., 2005; Olesen *et al*., 2007; Thompson, 2005; Tripp & Manos, 2008). Este resultado lo encontramos principalmente en la especialización que presentan los organismos con distintos requerimientos ecológicos como se han mencionado previamente en las diferencias entre los grupos *Peltigera* y *Nephroma*. Las cianobacterias asociadas a cada uno de estos grupos podrían tener distintas adaptaciones a causa de las diferencias de sustrato, beneficiando la asociación con unas u otras en función de si son líquenes terrícolas o epífitos (Rikkinen, 2003). Sin embargo, entre las desventajas de la elevada especialización encontramos que las especies que presentan este tipo de interacciones pueden ser más vulnerables a los cambios ambientales y a las perturbaciones (Futuyma & Moreno, 1988; Swarts & Dixon, 2009; Swarts *et al*., 2010).

Todos estos resultados contribuyen y proponen herramientas para comprender el ensamblaje y la coexistencia de las especies en las comunidades. Observamos como la red conformada por todas las especies a lo largo del gradiente, muestra una estructura altamente anidada, lo que contribuye a su estabilidad y previene las cascadas de extinción (Bascompte *et al*., 2003; Ramos-Jiliberto *et al*., 2010). Sin embargo, al considerar las comunidades locales, predominan patrones aleatorios y poco conectados en la conformación de estas redes lo que podría indicar que, a nivel local, las comunidades son inestables (Bascompte, 2010; Krause *et al*., 2003; May, 1972; Melián & Bascompte, 2002). Este resultado recalca la vulnerabilidad de estas redes y la necesidad de desarrollar

nuevos estudios para prevenir el impacto negativo del cambio global en estas comunidades.

Adicionalmente, una aportación especialmente relevante de esta tesis doctoral es el marco conceptual propuesto en el capítulo 4, en el que se considera la especialización como un volumen que incluye distintos componentes, lo que permite comparar distintos sistemas de interacciones. Incluso, dentro del mismo sistema, se puede identificar de manera más precisa y objetiva si existen diferencias en la especialización de las distintas clases de organismos que conforman un sistema, así como la especialización de especies concretas de una forma más acertada, teniendo en cuenta la *especificidad*, la *preferencia* y la *selectividad*. Esta aplicación es especialmente útil para desarrollar estrategias de conservación. Por un lado, los organismos altamente especializados pueden ser extremadamente vulnerables debido a su mayor dependencia por la pareja con la que interactúan (Clavel *et al*., 2011), aunque no generarían grandes cambios en el conjunto de la comunidad. Por otro lado, la pérdida de organismos altamente generalizados, que añaden cohesión a la red, podría causar cascadas de extinción que afectarían al conjunto de las especies de la comunidad (Bascompte, 2009; Bascompte & Jordano, 2007).

6. Referencias

Armbruster, W. S. (2017). The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology*, *31*(1), 88–100. https://doi.org/10.1111/1365-2435.12783

Bascompte, J. (2009). Disentangling the web of life. *Science*, *325*(5939), 416–419. https://doi.org/10.1126/science.1170749

Bascompte, J. (2010). Structure and dynamics of ecological networks. *Science*, *329*(5993), 765–766. https://doi.org/10.1126/science.1194255

Bascompte, J., & Jordano, P. (2007). Plant-animal mutualistic networks: The architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *38*(May), 567–593. https://doi.org/10.1146/annurev.ecolsys.38.091206.095818

Bascompte, J., & Jordano, P. (2014). *Mutualistic Networks*. Princeton University Press.

Bascompte, J., Jordano, P., Melián, C. J., & Olesen, J. M. (2003). The nested assembly of plant-animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(16), 9383–9387. https://doi.org/10.1073/pnas.1633576100

Batstone, R. T., Carscadden, K. A., Afkhami, M. E., & Frederickson, M. E. (2018). Using niche breadth theory to explain generalization in mutualisms. *Ecology*, *99*(5), 1039–1050. https://doi.org/10.1002/ecy.2188

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*(4), 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Blázquez, M., Hernández-Moreno, L. S., Gasulla, F., Pérez-Vargas, I., & Pérez-Ortega, S. (2022). The Role of Photobionts as Drivers of Diversification in an Island Radiation of Lichen-Forming Fungi. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.784182

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, *6*. https://doi.org/10.1186/1472-6785-6-9

Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., & Blüthgen, N. (2007). Specialization, Constraints, and Conflicting Interests in Mutualistic Networks. *Current Biology*, *17*(4), 341–346. https://doi.org/10.1016/j.cub.2006.12.039

Boucher, D. H., James, S., & Keeler, K. H. (1982). The ecology of mutualism. *Annual Review of Ecology and Systematics*, *13*, 315–347.

Brosi, B. J. (2016). Pollinator specialization: From the individual to the community. *New Phytologist*, *210*(4), 1190–1194. https://doi.org/10.1111/nph.13951

Bunnell, F. L., & Huggard, D. J. (1999). Biodiversity across spatial and temporal scales: Problems and opportunities. *Forest Ecology and Management*, *115*(2–3), 113–126. https://doi.org/10.1016/S0378-1127(98)00392-2

CaraDonna, P. J., Petry, W. K., Brennan, R. M., Cunningham, J. L., Bronstein, J. L., Waser, N. M., & Sanders, N. J. (2017). Interaction rewiring and the rapid turnover of plant–pollinator networks. *Ecology Letters*, *20*(3), 385–394. https://doi.org/10.1111/ele.12740

Cardós, J. L. H., Prieto, M., Jylhä, M., Aragón, G., Molina, M. C., Martínez, I., & Rikkinen, J. (2019). A case study on the re-establishment of the cyanolichen symbiosis: Where do the compatible photobionts come from? *Annals of Botany*, *124*(3), 379–388. https://doi.org/10.1093/aob/mcz052

Casano, L. M., Del Campo, E. M., García-Breijo, F. J., Reig-Armiñana, J., Gasulla, F., Del Hoyo, A., … Barreno, E. (2011). Two Trebouxia algae with different physiological performances are ever-present in lichen thalli of Ramalina farinacea. Coexistence versus Competition? *Environmental Microbiology*, *13*(3), 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x

Chagnon, P.-L., Magain, N., Miadlikowska, J., & Lutzoni, F. (2018). Strong specificity and network modularity at a very fine phylogenetic scale in the lichen genus Peltigera. *Oecologia*, *187*(3), 767–782. https://doi.org/10.1007/s00442-018-4159-6

Chagnon, P.-L., U'Ren, J. M., Miadlikowska, J., Lutzoni, F., & Elizabeth Arnold, A. (2016). Interaction type influences ecological network structure more than local abiotic conditions: evidence from endophytic and endolichenic fungi at a continental scale. *Oecologia*, *180*(1), 181–191. https://doi.org/10.1007/s00442-015-3457-5

Chave, J. (2013). The problem of pattern and scale in ecology: What have we learned in 20 years? *Ecology Letters*, *16*(SUPPL.1), 4–16. https://doi.org/10.1111/ele.12048

Clavel, J., Julliard, R., & Devictor, V. (2011). Worldwide decline of specialist species: Toward a global functional homogenization? *Frontiers in Ecology and the Environment*, *9*(4), 222–228. https://doi.org/10.1890/080216

Connell, J. H. (1978). Diversity in Tropical Rain Forests and Coral Reefs. *Science*, *199*(4335), 1302–

1310. https://doi.org/10.1126/science.199.4335.1302

Dal Grande, F., Rolshausen, G., Divakar, P. K., Crespo, A., Otte, J., Schleuning, M., & Schmitt, I. (2018). Environment and host identity structure communities of green algal symbionts in lichens. *New Phytologist*, *217*(1), 277–289. https://doi.org/10.1111/nph.14770

Dal Grande, F., Widmer, I., Wagner, H. H., & Scheidegger, C. (2012). Vertical and horizontal photobiont transmission within populations of a lichen symbiosis. *Molecular Ecology*, *21*(13), 3159–3172. https://doi.org/10.1111/j.1365-294X.2012.05482.x

Dalsgaard, B., Magård, E., Fjeldså, J., González, A. M. M., Rahbek, C., Olesen, J. M., … Svenning, J. C. (2011). Specialization in plant-hummingbird networks is associated with species richness, contemporary precipitation and quaternary climate-change velocity. *PLoS ONE*, *6*(10). https://doi.org/10.1371/journal.pone.0025891

Dalsgaard, B., Schleuning, M., Maruyama, P. K., Dehling, D. M., Sonne, J., Vizentin-Bugoni, J., … Rahbek, C. (2017). Opposed latitudinal patterns of network-derived and dietary specialization in avian plant–frugivore interaction systems. *Ecography*, *40*(12), 1395–1401. https://doi.org/10.1111/ecog.02604

Dalsgaard, B., Timmermann, A., Martín González, A. M., Olesen, J. M., Ollerton, J., & Andersen, L. H. (2010). Heliconia-hummingbird interactions in the Lesser Antilles: A geographic mosaic? *Caribbean Journal of Science*, *46*(2–3), 328–331. https://doi.org/10.18475/cjos.v46i2.a22

Dalsgaard, B., Trøjelsgaard, K., Martín González, A. M., Nogués-Bravo, D., Ollerton, J., Petanidou, T., … Olesen, J. M. (2013). Historical climate-change influences modularity and nestedness of pollination networks. *Ecography*, *36*(12), 1331–1340. https://doi.org/10.1111/j.1600-0587.2013.00201.x

Danieli-Silva, A., de Souza, J. M. T., Donatti, A. J., Campos, R. P., Vicente-Silva, J., Freitas, L., & Varassin, I. G. (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? *Oikos*, *121*(1), 35–43. https://doi.org/10.1111/j.1600-0706.2011.19089.x

Darwin, C. R. (1862). *On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing.* London: John Murray.

Dáttilo, W., Guimarães, P. R., & Izzo, T. J. (2013). Spatial structure of ant-plant mutualistic networks. *Oikos*, *122*(11), 1643–1648. https://doi.org/10.1111/j.1600-0706.2013.00562.x

Davis, B. J., Phillips, R. D., Wright, M., Linde, C. C., & Dixon, K. W. (2015). Continent-wide distribution in mycorrhizal fungi: Implications for the biogeography of specialized orchids. *Annals of Botany*, *116*(3), 413–421. https://doi.org/10.1093/aob/mcv084

DePriest, P. T. (2004). Early molecular investigations of lichen-forming symbionts: 1986-2001. *Annual Review of Microbiology*, *58*, 273–301. https://doi.org/10.1146/annurev.micro.58.030603.123730

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., … Mouquet, N. (2010). Defining and measuring ecological specialization. *Journal of Applied Ecology*, *47*(1), 15–25. https://doi.org/10.1111/j.1365-2664.2009.01744.x

Devoto, M., Medan, D., & Montaldo, N. H. (2005). Patterns of interaction between plants and pollinators along an environmental gradient. *Oikos*, *109*(3), 461–472. https://doi.org/10.1111/j.0030-1299.2005.13712.x

Dupont, Y. L., & Olesen, J. M. (2009). Ecological modules and roles of species in heathland plant-

insect flower visitor networks. *Journal of Animal Ecology*, *78*(2), 346–353. https://doi.org/10.1111/j.1365-2656.2008.01501.x

Duran-Nebreda, S., & Valverde, S. (2023). Composition, structure and robustness of Lichen guilds. *Scientific Reports*, *13*(1), 1–14. https://doi.org/10.1038/s41598-023-30357-w

Dyer, L. A., Singer, M. S., Lill, J. T., Stireman, J. O., Gentry, G. L., Marquis, R. J., … Coley, P. D. (2007). Host specificity of Lepidoptera in tropical and temperate forests. *Nature*, *448*(7154), 696–699. https://doi.org/10.1038/nature05884

Egan, S. P., & Funk, D. J. (2006). Individual advantages to ecological specialization: Insights on cognitive constraints from three conspecific taxa. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1588), 843–848. https://doi.org/10.1098/rspb.2005.3382

Ehrlich, P. R., & Raven, P. H. (1964). Butterflies and Plants: A Study in Coevolution. *Evolution*, *18*(4), 586–608. https://doi.org/10.2307/2406212

Ellis, C. J., Asplund, J., Benesperi, R., Branquinho, C., Di Nuzzo, L., Hurtado, P., … Giordani, P. (2021). Functional traits in lichen ecology: A review of challenge and opportunity. *Microorganisms*, *9*(4), 1–27. https://doi.org/10.3390/microorganisms9040766

Elvebakk, A., Papaefthimiou, D., Robertsen, E. H., & Liaimer, A. (2008). Phylogenetic patterns among Nostoc cyanobionts within bi- and tripartite lichens of the genus Pannaria. *Journal of Phycology*, *44*(4), 1049–1059. https://doi.org/10.1111/j.1529-8817.2008.00556.x

Ertz, D., Guzow-Krzemińska, B., Thor, G., Łubek, A., & Kukwa, M. (2018). Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the Arthoniomycetes. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-23219-3

Estes, L., Elsen, P. R., Treuer, T., Ahmed, L., Caylor, K., Chang, J., … Ellis, E. C. (2018). The spatial and temporal domains of modern ecology. *Nature Ecology and Evolution*, *2*(5), 819–826. https://doi.org/10.1038/s41559-018-0524-4

Fedrowitz, K., Kaasalainen, U., & Rikkinen, J. (2012). Geographic mosaic of symbiont selectivity in a genus of epiphytic cyanolichens. *Ecology and Evolution*, *2*(9), 2291–2303. https://doi.org/10.1002/ece3.343

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, *35*, 375–403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Fernández-Mendoza, F., Domaschke, S., García, M. A., Jordan, P., Martín, M. P., & Printzen, C. (2011). Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. *Molecular Ecology*, *20*(6), 1208–1232. https://doi.org/10.1111/j.1365-294X.2010.04993.x

Forister, M. L., Dyer, L. A., Singer, M. S., Stireman III, J. O., & Lill, J. T. (2012). Revisiting the evolution of ecological specialization, with emphasis on insect–plant interactions. *Ecology*, *93*(5), 981–991. https://doi.org/10.1890/11-0650.1

Fox, L. R., & Morrow, P. A. (1981). Specialization: Species property or local phenomenon. *Science*, *211*(4485), 887–893. https://doi.org/10.1126/science.211.4485.887

Friedl, T. (1987). Thallus Development and Phycobionts of the Parasitic Lichen Diploschistes Muscorum. *The Lichenologist*, *19*(2), 183–191. https://doi.org/10.1017/S002428298700015X

Futuyma, D. J., & Moreno, G. (1988). The Evolution of Ecological Specialization. *Annual Review of Ecology and Systematics*, *19*(1988), 207–233. https://doi.org/10.1146/annurev.es.19.110188.001231

Gleason, H. A. (1926). The Individualistic Concept of the Plant Association. *Bulletin of the Torrey Botanical*, *53*(1), 7–26. https://doi.org/10.2307/2479933

Götzenberger, L., de Bello, F., Bråthen, K. A., Davison, J., Dubuis, A., Guisan, A., … Zobel, M. (2012). Ecological assembly rules in plant communities-approaches, patterns and prospects. *Biological Reviews*, *87*(1), 111–127. https://doi.org/10.1111/j.1469-185X.2011.00187.x

Grube, M., & Spribille, T. (2012). Exploring symbiont management in lichens. *Molecular Ecology*, *21*(13), 3098–3099. https://doi.org/10.1111/j.1365-294X.2012.05647.x

Grube, M., & Wedin, M. (2016). Lichenized Fungi and the Evolution of Symbiotic Organization. *Microbiology Spectrum*, *4*(6), 1–17. https://doi.org/10.1128/microbiolspec.funk-0011-2016

Guimarães, P. R. J., Rico-Gray, V., Oliveira, P. S., Izzo, T. J., dos Reis, S. F., & Thompson, J. N. (2007). Interaction intimacy affects structure and coevolutionary dynamics in mutualistic networks. *Current Biology : CB*, *17*(20), 1797–1803. https://doi.org/10.1016/j.cub.2007.09.059

Hagen, M. K., WD, R., C, C., DW, D., YL, K.-B., CN, O., … JM, T. (2012). *Biodiversity, species interactions and ecological networks in a fragmented world*. *Advances in Ecological Research,* (Vol. 46).

Harley, C. D. G. (2011). Climate Change, Keystone Predation, and Biodiversity Loss. *Science*, *334*(6059), 1124–1127. https://doi.org/10.1126/science.1210199

Herrera, P., Suárez, J. P., Sánchez-Rodríguez, A., Molina, M. C., Prieto, M., & Méndez, M. (2019). Many broadly-shared mycobionts characterize mycorrhizal interactions of two coexisting epiphytic orchids in a high elevation tropical forest. *Fungal Ecology*, *39*, 26–36. https://doi.org/10.1016/j.funeco.2018.11.003

Hillebrand, H. (2004). On the generallity of the latutinal diversity gradient. *American Naturalist*, *163*(2), 192–211. https://doi.org/10.1086/381004

Hoz, C. J. P. D. La, Magain, N., Lutzoni, F., Goward, T., Restrepo, S., & Miadlikowska, J. (2018). Contrasting Symbiotic Patterns in Two Closely Related Lineages of Trimembered Lichens of the Genus Peltigera. *Frontiers in Microbiology*, *9*, 1–14. https://doi.org/10.3389/fmicb.2018.02770

Hughes, J. B. (2000). The Scale of Resource Specialization and the Distribution and Abundance of Lycaenid. *Oecologia*, *123*(3), 375–383. https://doi.org/10.1007/s004420051024

Iglesias-Prieto, R., Beltrán, V. H., LaJeunesse, T. C., Reyes-Bonilla, H., & Thomé, P. E. (2004). Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1549), 1757–1763. https://doi.org/10.1098/rspb.2004.2757

Irschick, D., Dyer, L., Sherry, T. W., & Ranta, E. (2005). Phylogenetic Methodologies for Studying Specialization. *Oikos*, *110*(2), 404–408. https://doi.org/10.1111/j.0030-1299.2005.13927.x

Jocque, M., Field, R., Brendonck, L., & De Meester, L. (2010). Climatic control of dispersal-ecological specialization trade-offs: A metacommunity process at the heart of the latitudinal diversity gradient? *Global Ecology and Biogeography*, *19*(2), 244–252. https://doi.org/10.1111/j.1466-8238.2009.00510.x

Johnson, S. D., & Steiner, K. E. (1997). LONG-TONGUED FLY POLLINATION AND EVOLUTION OF

FLORAL SPUR LENGTH IN THE DISA DRACONIS COMPLEX (ORCHIDACEAE). *Evolution; International Journal of Organic Evolution*, *51*(1), 45–53. https://doi.org/10.1111/j.1558-5646.1997.tb02387.x

Johnson, S. D., & Steiner, K. E. (2000). Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution*, *15*(4), 140–143. https://doi.org/10.1016/S0169-5347(99)01811-X

Joppa, L. N., Bascompte, J., Montoya, J. M., Solé, R. V., Sanderson, J., & Pimm, S. L. (2009). Reciprocal specialization in ecological networks. *Ecology Letters*, *12*(9), 961–969. https://doi.org/10.1111/j.1461-0248.2009.01341.x

Jordano, P. (1987). Patterns of Mutualistic Interactions in Pollination and Seed Dispersal: Connectance, Dependence Asymmetries, and Coevolution. *The American Naturalist*, *129*(5), 657–677.

Jüriado, I., Kaasalainen, U., Jylhä, M., & Rikkinen, J. (2019). Relationships between mycobiont identity, photobiont specificity and ecological preferences in the lichen genus Peltigera (Ascomycota) in Estonia (northeastern Europe). *Fungal Ecology*, *39*, 45–54. https://doi.org/10.1016/j.funeco.2018.11.005

Kaasalainen, U., Tuovinen, V., Mwachala, G., Pellikka, P., & Rikkinen, J. (2021). Complex Interaction Networks Among Cyanolichens of a Tropical Biodiversity Hotspot. *Frontiers in Microbiology*, *12*(June), 1–12. https://doi.org/10.3389/fmicb.2021.672333

Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science*, *3*(2), 157–164. https://doi.org/10.2307/3235676

Kinlock, N. L., Prowant, L., Herstoff, E. M., Foley, C. M., Akin-Fajiye, M., Bender, N., … Gurevitch, J. (2018). Explaining global variation in the latitudinal diversity gradient: Meta-analysis confirms known patterns and uncovers new ones. *Global Ecology and Biogeography*, *27*(1), 125–141. https://doi.org/10.1111/geb.12665

Krasnov, B. R., Mouillot, D., Khokhlova, I. S., Shenbrot, G. I., & Poulin, R. (2008). Scale-invariance of niche breadth in fleas parasitic on small mammals. *Ecography*, *31*(5), 630–635. https://doi.org/10.1111/j.0906-7590.2008.05522.x

Krause, A. E., Frank, K. A., Mason, D. M., Ulanowicz, R. E., & Taylor, W. W. (2003). Compartments revealed in food-web structure. *Nature*, *426*(6964), 282–285. https://doi.org/10.1038/nature02115

Laenen, B., Patiño, J., Hagborg, A., Désamoré, A., Wang, J., Shaw, A. J., … Vanderpoorten, A. (2018). Evolutionary origin of the latitudinal diversity gradient in liverworts. *Molecular Phylogenetics and Evolution*, *127*, 606–612. https://doi.org/10.1016/j.ympev.2018.06.007

Lara-Romero, C., Seguí, J., Pérez-Delgado, A., Nogales, M., & Traveset, A. (2019). Beta diversity and specialization in plant–pollinator networks along an elevational gradient. *Journal of Biogeography*, *46*(7), 1598–1610. https://doi.org/10.1111/jbi.13615

Le Bagousse-Pinguet, Y., Gross, N., Maestre, F. T., Maire, V., de Bello, F., Fonseca, C. R., … Liancourt, P. (2017). Testing the environmental filtering concept in global drylands. *Journal of Ecology*, *105*(4), 1058–1069. https://doi.org/10.1111/1365-2745.12735

Leavitt, S. D., Kraichak, E., Nelsen, M. P., Altermann, S., Divakar, P. K., Alors, D., … Lumbsch, T. (2015). Fungal specificity and selectivity for algae play a major role in determining lichen partnerships across diverse ecogeographic regions in the lichen-forming family Parmeliaceae (Ascomycota). *Molecular Ecology*, *24*(14), 3779–3797.

https://doi.org/10.1111/mec.13271

Lehr, H., Galun, M., Ott, S., Jahns, H. M., & Fleminger, G. (2000). Cephalodia of the lichen Peltigera aphthosa (L.) Willd. Specific recognition of the compatible photobiont. *Symbiosis*, *29*(4), 357–365.

Levin, S. A. (1992). The problem of pattern and scale in ecology. *Ecology*, *73*(6), 1943–1967. https://doi.org/10.2307/1941447

Lu, J., Magain, N., Miadlikowska, J., Coyle, J. R., Truong, C., & Lutzoni, F. (2018). Bioclimatic factors at an intrabiome scale are more limiting than cyanobiont availability for the lichen-forming genus Peltigera. *American Journal of Botany*, *105*(7), 1198–1211. https://doi.org/10.1002/ajb2.1119

MacArthur, R. H. (1972). *Geographical Ecology.* Princeton, NJ: Princeton University Press.

Macarthur, R., & Levins, R. (1967). The Limiting Similarity, Convergence, and Divergence of Coexisting Species. *The American Naturalist*, *101*(921), 377–385. https://doi.org/10.1086/282505

Magain, N., Miadlikowska, J., Goffinet, B., Sérusiaux, E., & Lutzoni, F. (2017). Macroevolution of Specificity in Cyanolichens of the Genus Peltigera Section Polydactylon (Lecanoromycetes, Ascomycota). *Systematic Biology*, *66*(1), 74–99. https://doi.org/10.1093/sysbio/syw065

Martín González, A. M., Allesina, S., Rodrigo, A., & Bosch, J. (2012). Drivers of compartmentalization in a Mediterranean pollination network. *Oikos*, *121*(12), 2001–2013. https://doi.org/10.1111/j.1600-0706.2012.20279.x

May, R. M. (1972). Will a Large Complex System be Stable? *Nature*, *238*(5364), 413–414. https://doi.org/10.1038/238413a0

May, R. M. (1982). Mutualistic interactions among species. *Nature*, *296*(5860), 803–804. https://doi.org/10.1038/296803a0

McGill, B. J., Enquist, B. J., Weiher, E., & Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution*, *21*(4), 178–185. https://doi.org/10.1016/j.tree.2006.02.002

Melián, C. J., & Bascompte, J. (2002). Complex networks: Two ways to be robust? *Ecology Letters*, *5*(6), 705–708. https://doi.org/10.1046/j.1461-0248.2002.00386.x

Morris, R. J., Gripenberg, S., Lewis, O. T., & Roslin, T. (2014). Antagonistic interaction networks are structured independently of latitude and host guild. *Ecology Letters*, *17*(3), 340–349. https://doi.org/10.1111/ele.12235

Moya, P., Molins, A., Chiva, S., Bastida, J., & Barreno, E. (2020). Symbiotic microalgal diversity within lichenicolous lichens and crustose hosts on Iberian Peninsula gypsum biocrusts. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-71046-2

Moya, P., Molins, A., Martínez-Alberola, F., Muggia, L., & Barreno, E. (2017). Unexpected associated microalgal diversity in the lichen Ramalina farinacea is uncovered by pyrosequencing analyses. *PLOS ONE*, *12*(4), e0175091. https://doi.org/10.1371/journal.pone.0175091

Muggia, L., Pérez-Ortega, S., Kopun, T., Zellnig, G., & Grube, M. (2014). Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Annals of Botany*, *114*(3), 463–475. https://doi.org/10.1093/aob/mcu146

Muggia, L., Vancurova, L., Škaloud, P., Peksa, O., Wedin, M., & Grube, M. (2013). The symbiotic playground of lichen thalli - a highly flexible photobiont association in rock-inhabiting lichens. *FEMS Microbiology Ecology*, *85*(2), 313–323. https://doi.org/10.1111/1574-6941.12120

Myllys, L., Stenroos, S., Thell, A., & Kuusinen, M. (2007). High cyanobiont selectivity of epiphytic lichens in old growth boreal forest of Finland. *New Phytologist*, *173*(3), 621–629. https://doi.org/10.1111/j.1469-8137.2006.01944.x

Nadyeina, O., Dymytrova, L., Naumovych, A., Postoyalkin, S., Werth, S., Cheenacharoen, S., & Scheidegger, C. (2014). Microclimatic differentiation of gene pools in the Lobaria pulmonaria symbiosis in a primeval forest landscape. *Molecular Ecology*, *23*(21), 5164–5178. https://doi.org/10.1111/mec.12928

Nash, T. H. (1996). *Lichen Biology.* (Cambridge). Cambridge University Press.

Nelsen, M. P., Leavitt, S. D., Heller, K., Muggia, L., & Lumbsch, H. T. (2021). Macroecological diversification and convergence in a clade of keystone symbionts. *FEMS Microbiology Ecology*, *97*(6). https://doi.org/10.1093/femsec/fiab072

Novotny, V. (2009). Beta diversity of plant-insect food webs in tropical forests: A conceptual framework. *Insect Conservation and Diversity*, *2*(1), 5–9. https://doi.org/10.1111/j.1752-4598.2008.00035.x

Novotny, V., & Basset, Y. (2005). Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1568), 1083–1090. https://doi.org/10.1098/rspb.2004.3023

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

Olesen, J. M., Bascompte, J., Dupont, Y. L., & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(50), 19891–19896. https://doi.org/10.1073/pnas.0706375104

Olesen, J. M., & Jordano, P. (2002). Geographic Patterns in Plant-Pollinator Mutualistic Networks. *Ecology*, *83*(9), 2416. https://doi.org/10.2307/3071803

Ollerton, J., Alarcon, R., Waser, N. M., Price, M. V., Watts, S., Cranmer, L., … Rotenberry, J. (2009). A global test of the pollination syndrome hypothesis. *Annals of Botany*, *103*(9), 1471–1480. https://doi.org/10.1093/aob/mcp031

Ollerton, J., & Cranmer, L. (2002). Latitudinal trends in plant-pollinator interactions: Are tropical plants more specialised? *Oikos*, *98*(2), 340–350. https://doi.org/10.1034/j.1600-0706.2002.980215.x

Ollerton, J., Johnson, S., & Hingston, A. (2006). Geographical variation in diversity and specificity of pollination systems. In N.M. Waser and J. Ollerton (Ed.), *Plant- Pollinator Interactions: From Specialization to Generalization* (pp. 283–308). Chicago: University of Chicago Press.

Otálora, M. A. G., Martínez, I., O'Brien, H., Molina, M. C., Aragón, G., & Lutzoni, F. (2010). Multiple origins of high reciprocal symbiotic specificity at an intercontinental spatial scale among gelatinous lichens (Collemataceae, Lecanoromycetes). *Molecular Phylogenetics and Evolution*, *56*(3), 1089–1095. https://doi.org/10.1016/j.ympev.2010.05.013

Otálora, M. A. G., Salvador, C., Martínez, I., & Aragón, G. (2013). Does the Reproductive Strategy

Affect the Transmission and Genetic Diversity of Bionts in Cyanolichens? A Case Study Using Two Closely Related Species. *Microbial Ecology*, *65*(2), 517–530. https://doi.org/10.1007/s00248-012-0136-5

Palmqvist, K. (2002). Cyanolichens: Carbon Metabolism. In Amar N Rai, B. Bergman, & U. Rasmussen (Eds.), *Cyanobacteria in Symbiosis* (pp. 73–96). Dordrecht: Springer Netherlands. https://doi.org/10.1007/0-306-48005-0_5

Paulsrud, P., & Lindblad, P. (1998). Sequence variation of the tRNA(Leu) intron as a marker for genetic diversity and specificity of symbiotic cyanobacteria in some lichens. *Applied and Environmental Microbiology*, *64*(1), 310–315. https://doi.org/10.1128/aem.64.1.310-315.1998

Paulsrud, P., Rikkinen, J., & Lindblad, P. (2001). Field investigations on cyanobacterial specificity in Peltigera aphthosa. *New Phytologist*, *152*(1), 117–123. https://doi.org/10.1046/j.0028-646x.2001.00234.x

Peay, K. G. (2016). The Mutualistic Niche: Mycorrhizal Symbiosis and Community Dynamics. *Annual Review of Ecology, Evolution, and Systematics*, *47*, 143–164. https://doi.org/10.1146/annurev-ecolsys-121415-032100

Peay, K. G., Kennedy, P. G., Davies, S. J., Tan, S., & Bruns, T. D. (2010). Potential link between plant and fungal distributions in a dipterocarp rainforest: Community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. *New Phytologist*, *185*(2), 529–542. https://doi.org/10.1111/j.1469-8137.2009.03075.x

Pedraza, F., & Bascompte, J. (2021). The joint role of coevolutionary selection and network structure in shaping trait matching in mutualisms. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1957). https://doi.org/10.1098/rspb.2021.1291

Peksa, O., & Škaloud, P. (2011). Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae). *Molecular Ecology*, *20*(18), 3936–3948. https://doi.org/10.1111/j.1365-294X.2011.05168.x

Piercey-Normore, M. D., & DePriest, P. T. (2001). Algal switching among lichen symbioses. *American Journal of Botany*, *88*(8), 1490–1498. https://doi.org/10.2307/3558457

Pinheiro, R. B. P., Félix, G. M. F., Chaves, A. V., Lacorte, G. A., Santos, F. R., Braga, É. M., & Mello, M. A. R. (2016). Trade-offs and resource breadth processes as drivers of performance and specificity in a host-parasite system: A new integrative hypothesis. *International Journal for Parasitology*, *46*(2), 115–121. https://doi.org/10.1016/j.ijpara.2015.10.002

Pires, M. M., & Guimarães, P. R. J. (2013). Interaction intimacy organizes networks of antagonistic interactions in different ways. *Journal of the Royal Society, Interface*, *10*(78), 20120649. https://doi.org/10.1098/rsif.2012.0649

Poisot, T., Canard, E., Mouillot, D., Mouquet, N., & Gravel, D. (2012). The dissimilarity of species interaction networks. *Ecology Letters*, *15*(12), 1353–1361. https://doi.org/https://doi.org/10.1111/ele.12002

Poisot, T., Canard, E., Mouquet, N., & Hochberg, M. E. (2012). A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, *3*(3), 537–544. https://doi.org/10.1111/j.2041-210X.2011.00174.x

Rai, A. N. (2002). Cyanolichens: Nitrogen Metabolism. In Amar N Rai, B. Bergman, & U. Rasmussen (Eds.), *Cyanobacteria in Symbiosis* (pp. 97–115). Dordrecht: Springer Netherlands. https://doi.org/10.1007/0-306-48005-0_6

Rai, A. N., Söderbäck, E., & Bergman, B. (2000). Tansley Review No. 116. Cyanobacterium–plant symbioses. *New Phytologist*, *147*(3), 449–481. https://doi.org/10.1046/j.1469-8137.2000.00720.x

Ramos-Jiliberto, R., Domínguez, D., Espinoza, C., López, G., Valdovinos, F. S., Bustamante, R. O., & Medel, R. (2010). Topological change of Andean plant-pollinator networks along an altitudinal gradient. *Ecological Complexity*, *7*(1), 86–90. https://doi.org/10.1016/j.ecocom.2009.06.001

Rezende, E. L., Lavabre, J. E., Guimarães, P. R., Jordano, P., & Bascompte, J. (2007). Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature*, *448*(7156), 925–928. https://doi.org/10.1038/nature05956

Rikkinen, J. (2003). Ecological and evolutionary role of photobiont-mediated guilds in lichens. *Symbiosis*, *34*(2), 99–110.

Rikkinen, J. (2013). Molecular studies on cyanobacterial diversity in lichen symbioses. *MycoKeys*, *6*(April 2013), 3–32. https://doi.org/10.3897/mycokeys.6.3869

Rikkinen, J., Oksanen, I., & Lohtander, K. (2002). Lichen guilds share related cyanobacterial symbionts. *Science*, *297*(5580), 357. https://doi.org/10.1126/science.1072961

Rolshausen, G., Dal Grande, F., Sadowska-Deś, A. D., Otte, J., & Schmitt, I. (2018). Quantifying the climatic niche of symbiont partners in a lichen symbiosis indicates mutualist-mediated niche expansions. *Ecography*, *41*(8), 1380–1392. https://doi.org/10.1111/ecog.03457

Rolshausen, G., Hallman, U., Grande, F. D., Otte, J., Knudsen, K., & Schmitt, I. (2020). Expanding the mutualistic niche: Parallel symbiont turnover along climatic gradients. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1924). https://doi.org/10.1098/rspb.2019.2311

Sacristán, M., Vivas, M., Millanes, A. M., Fontaniella, B., Vicente, C., & Legaz, M. E. (2007). The recognition pattern of green algae by lichenized fungi can be extended to lichens containing a cyanobacterium as photobiont. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, (July 2014), 213–219.

Sahli, H. F., & Conner, J. K. (2006). Characterizing ecological generalization in plant-pollination systems. *Oecologia*, *148*(3), 365–372. https://doi.org/10.1007/s00442-006-0396-1

Sandel, B., & Smith, A. B. (2009). Scale as a lurking factor: Incorporating scale-dependence in experimental ecology. *Oikos*, *118*(9), 1284–1291. https://doi.org/10.1111/j.1600-0706.2009.17421.x

Schemske, D. W., Mittelbach, G. G., Cornell, H. V., Sobel, J. M., & Roy, K. (2009). Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics*, *40*, 245–269. https://doi.org/10.1146/annurev.ecolsys.39.110707.173430

Schleuning, M., Fründ, J., Klein, A. M., Abrahamczyk, S., Alarcón, R., Albrecht, M., … Blüthgen, N. (2012). Specialization of mutualistic interaction networks decreases toward tropical latitudes. *Current Biology*, *22*(20), 1925–1931. https://doi.org/10.1016/j.cub.2012.08.015

Schleuning, M., Ingmann, L., Strauß, R., Fritz, S. A., Dalsgaard, B., Matthias Dehling, D., … Dormann, C. F. (2014). Ecological, historical and evolutionary determinants of modularity in weighted seed-dispersal networks. *Ecology Letters*, *17*(4), 454–463. https://doi.org/10.1111/ele.12245

Shipley, B., De Bello, F., Cornelissen, J. H. C., Laliberté, E., Laughlin, D. C., & Reich, P. B. (2016).

Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*, *180*(4), 923–931. https://doi.org/10.1007/s00442-016-3549-x

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

Swarts, N. D., & Dixon, K. W. (2009). Terrestrial orchid conservation in the age of extinction. *Annals of Botany*, *104*(3), 543–556. https://doi.org/10.1093/aob/mcp025

Swarts, N. D., Sinclair, E. A., Francis, A., & Dixon, K. W. (2010). Ecological specialization in mycorrhizal symbiosis leads to rarity in an endangered orchid. *Molecular Ecology*, *19*(15), 3226–3242. https://doi.org/10.1111/j.1365-294X.2010.04736.x

Thompson, J. N. (2005). *The geographic mosaic of coevolution.* University of Chicago Press.

Traveset, A., Tur, C., Trøjelsgaard, K., Heleno, R., Castro-Urgal, R., & Olesen, J. M. (2016). Global patterns of mainland and insular pollination networks. *Global Ecology and Biogeography*, *25*(7), 880–890. https://doi.org/10.1111/geb.12362

Tripp, E. A., & Manos, P. S. (2008). Is floral specialization an evolutionary dead-end? Pollination system transitions in Ruellia (Acanthaceae). *Evolution*, *62*(7), 1712–1737. https://doi.org/10.1111/j.1558-5646.2008.00398.x

Trøjelsgaard, K., Jordano, P., Carstensen, D. W., & Olesen, J. M. (2015). Geographical variation in mutualistic networks: Similarity, turnover and partner fidelity. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1802). https://doi.org/10.1098/rspb.2014.2925

Trøjelsgaard, K., & Olesen, J. M. (2013). Macroecology of pollination networks. *Global Ecology and Biogeography*, *22*(2), 149–162. https://doi.org/10.1111/j.1466-8238.2012.00777.x

Tylianakis, J. M., & Morris, R. J. (2017). Ecological Networks Across Environmental Gradients. *Annual Review of Ecology, Evolution, and Systematics*, *48*(June), 25–48. https://doi.org/10.1146/annurev-ecolsys-110316-022821

Vančurová, L., Muggia, L., Peksa, O., Řídká, T., & Škaloud, P. (2018). The complexity of symbiotic interactions influences the ecological amplitude of the host: A case study in Stereocaulon (lichenized Ascomycota). *Molecular Ecology*, *27*(14), 3016–3033. https://doi.org/10.1111/mec.14764

Vargas Castillo, R., & Beck, A. (2012). Photobiont selectivity and specificity in Caloplaca species in a fog-induced community in the Atacama Desert, northern Chile. *Fungal Biology*, *116*(6), 665–676. https://doi.org/10.1016/j.funbio.2012.04.001

Vázquez, D. P., & Aizen, M. A. (2004). Asymmetric specialization: a pervasive feature of plant–pollinator interactions. *Ecology*, *85*(5), 1251–1257. https://doi.org/10.1890/03-3112

Vázquez, D. P., J. Melián, C., M. Williams, N., Blüthgen, N., R. Krasnov, B., & Poulin, R. (2007). Species abundance and asymmetric interaction strength in ecological networks. *Oikos*, *116*(7), 1120–1127. https://doi.org/10.1111/j.2007.0030-1299.15828.x

Vázquez, D. P., Poulin, R., Krasnov, B. R., & Shenbrot, G. I. (2005). Species abundance and the distribution of specialization in host-parasite interaction networks. *Journal of Animal Ecology*, *74*(5), 946–955. https://doi.org/10.1111/j.1365-2656.2005.00992.x

Vázquez, D. P., & Stevens, R. D. (2004). The latitudinal gradient in niche breadth: concepts and evidence. *The American Naturalist*, *164*(1), 0–19. https://doi.org/10.1086/421445

Ventre Lespiaucq, A., Jacquemyn, H., Rasmussen, H. N., & Méndez, M. (2021). Temporal turnover in mycorrhizal interactions: a proof of concept with orchids. *New Phytologist*, *230*(5), 1690–1699. https://doi.org/10.1111/nph.17291

Wang, J., Vanderpoorten, A., Hagborg, A., Goffinet, B., Laenen, B., & Patiño, J. (2017). Evidence for a latitudinal diversity gradient in liverworts and hornworts. *Journal of Biogeography*, *44*(3), 487–488. https://doi.org/10.1111/jbi.12909

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, *77*(4), 1043–1060. https://doi.org/10.2307/2265575

Wedin, M., Maier, S., Fernandez-Brime, S., Cronholm, B., Westberg, M., & Grube, M. (2016). Microbiome change by symbiotic invasion in lichens. *Environmental Microbiology*, *18*(5), 1428–1439. https://doi.org/10.1111/1462-2920.13032

Wiens, J. A. (1989). Spatial Scaling in Ecology. *Functional Ecology*, *3*(4), 385–397. https://doi.org/10.2307/2389612

Williams, L., Colesie, C., Ullmann, A., Westberg, M., Wedin, M., & Büdel, B. (2017). Lichen acclimation to changing environments: Photobiont switching vs. climate-specific uniqueness in Psora decipiens. *Ecology and Evolution*, *7*(8), 2560–2574. https://doi.org/10.1002/ece3.2809

Zúñiga-Reinoso, A., Muñoz-Escobar, C., & Hernández, C. E. (2013). Patrones y causas de estructuración geográfica latitudinal de los oribátidos (Acari: Oribatida) en Patagonia y Antártica. *Revista Chilena de Historia Natural*, *86*(3), 279–290. https://doi.org/10.4067/s0716-078x2013000300005

Zúñiga, C., Leiva, D., Carú, M., & Orlando, J. (2017). Substrates of Peltigera Lichens as a Potential Source of Cyanobionts. *Microbial Ecology*, *74*(3), 561–569. https://doi.org/10.1007/s00248-017-0969-z

CONCLUSIONES GENERALES

GENERAL CONCLUSIONS

The current research allows us to draw the following conclusions:

1. Lichens interacting with cyanobacteria lack of a consistent pattern defining the symbiotic interaction. This result is found at different biological scales: individuals, species and community levels. Thus, the interactions show varying levels of specialization, even at the individual level, and range from reciprocal specialization to generalization of both partners at the species level. At the community level, local networks show modular, nested, and random architectures.
2. Specialization is a scale-dependent concept that varies with the extension of the scale considered. At the spatial scale, the results show a tendency towards a more generalized pattern when the scale is broadened at different biological scales, the species and the community level. At the species level, this increase in generalization at larger spatial scales is explained by different turnover scenarios. At the community level, the metanetwork encompassing the whole latitudinal gradient studied shows a highly nested structure, which confers stability and robustness to the lichen community.
3. The results also show that different factors explain the variability of interactions depending on the biological scale. At the species level, the function of the cyanobiont explains part of the variability of the specialization measured as with diversity indices such as partner richness and Simpson's index, whereas the mycobiont identity explains most of the variability of specialization measured with a niche breadth index (d' index). At the community level, different environmental factors and functional traits influence the variability of network structures. Modularity increases with a decrease in latitude, with networks being more modular (specialized) at higher temperatures. The opposite pattern is found with connectance, which is also affected by the reproductive mode. Thus, less connected networks are those with lower proportion of species with reproductive structures.

4. Environmental factors related with temperature and forest quality (DBH) determine the composition of the cyanobiont communities along the latitudinal gradient studied.
5. Mycobionts' flexibility to interact with different cyanobionts enables them to switch their partner to other cyanobacteria that could be better adapted to the local environmental conditions, which could improve the fitness of the association.
6. Wide distributional ranges of lichens come from 1) the association with several partners in different localities or 2) the association with partners with broad distributional ranges.
7. Cyanolichens are more specialized than cephalolichens when measuring specialization with partner richness and Simpson's index. This result is supported by a higher presence of cyanolichens in isolated modules of the interacting networks and cephalolichens with roles of connectors and hubs inside the networks. Cephalolichens also show low specialization even at the intrathalline level, with different cyanobionts in different cephalodia within the same thallus. Therefore, cephalolichens may act as core species in the lichen communities due to their low specificity and their ability to interact with many cyanobionts. The diversity of cyanobionts they contain, even within a single thallus, may represent a source of compatible cyanobionts to other coexisting lichen species.
8. Neither functional traits, nor phylogenetic relationships (i.e., genus and/or family) determine the composition of the different modules inside the networks. Thus, in the lichen symbiosis, there is no evidence of modules being coevolutionary units.
9. Two different groups of cyanobacteria are found, which could belong to the previously described *Peltigera* and *Nephroma* guilds. These guilds have been characterized by their ecological requirements related to the substrate. However, some *Peltigera* are found interacting with cyanobacteria belonging to the *Nephroma* guild, suggesting that these facultative epiphytes that can grow on trees are able to use cyanobacteria from epiphytic lichens when changing the substrate.
10. An integrative framework is proposed to consider specialization, which combines three complementary aspects (i.e., *specificity*, *preference,* and *selectivity)* of the

interactions. This framework allows to determine specialization from a more objective and integrative perspective. With this proposal, specialization between and within different interacting systems can be compared. Such tool may help to develop conservation strategies to prevent diversity loss within networks: it permits to identify specialized species that may be more vulnerable due to their scarce number of compatible partners as well as supergeneralized species which could cause extinction cascades if they disappear from the community.

11. The framework proposed was tested in five natural interaction systems revealing a tendency to specialization in both, antagonistic and mutualistic systems. However, it was a lax specialization mainly defined by a high *specificity* of the interactions, but with *preference* and *selectivity* more equally distributed.