

Diversity and interactions of lichen mycobionts and photobionts in the Fildes Region, King George Island, maritime Antarctica

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Abstract Lichens, as dual organisms comprising a major mycobiont and a major photobiont, exhibit remarkable survival capabilities in extreme conditions, such as those found in Antarctica. Despite their adaptability, the diversity and distribution of lichen photobionts in the ice-free areas of maritime Antarctica remain less understood compared to their mycobiont counterparts. In our study, we investigated the diversity of both lichen mycobionts and photobionts in 56 samples collected from the Fildes Region on King George Island, maritime Antarctica. Through sequencing of the nuclear ribosomal internal transcribed spacer regions and subsequent phylogenetic analysis, we examined the relationships and association patterns between mycobionts and photobionts. Our findings revealed 19 taxa of lichen mycobionts across 13 families and seven orders, along with nine photobiont species within the class Trebouxiophyceae. These photobionts encompassed six *Trebouxia* species, one *Asterochloris* species, one *Chloroidium* species, and one *Stichococcus* species. In particular, a new *Trebouxia* lineage (*Trebouxia* sp. OTU D08) and a new *Chloroidium* lineage were found. The analysis indicated that many mycobionts could be associated with multiple photobiont species, a pattern also observed among the photobionts. These results contribute significantly to our understanding of the complex diversity of lichen mycobionts and photobionts in the ice-free areas of maritime Antarctica.

Keywords ice-free area, lichenized fungi, symbiosis, algae, phylogenetic diversity

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1 Introduction

Lichens, demonstrating mutualistic symbiosis between fungi and algae, play a pivotal role in the vegetation of ice-free areas in maritime Antarctica. These communities consist of approximately 427 lichen species, with 40% identified as endemic to Antarctica (Øvstedal and Lewis-Smith, 2001). As a dominant vegetation group in these ice-free areas, lichens withstand various abiotic

stresses, such as low temperatures and prolonged periods of darkness and light, contributing significantly to carbon storage and nutrient cycling. Numerous lichen species have developed a range of physiological and morphological adaptations to thrive under the extreme environmental conditions prevalent in Antarctica (Armstrong, 2017).

Selectivity and specificity are key terms used to characterize the interactions among potential lichen symbionts (Yahr et al., 2004). Moreover, the environment in which these symbionts inhabit significantly influences these interactions, as demonstrated by Peksa and Škaloud (2011). The availability of compatible partners, alongside environmental conditions, plays a crucial role in the growth

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of individual lichen thalli. Photobionts exhibit diverse environmental preferences and develop specific ecophysiological traits, impacting their relationships with mycobionts (Peksa and Škaloud, 2011; Yahr et al., 2006).

Several studies have explored the diversity of lichen photobionts in extreme environments such as Antarctica (Beck et al., 2019; Engelen et al., 2016; Park et al., 2015; Pérez-Ortega et al., 2012; Romeike et al., 2002; Ruprecht et al., 2012; Wirtz et al., 2003). These studies reveal a trend of low selectivity in the associations between mycobionts and photobionts, though varying degrees of selectivity have been observed among some mycobionts towards their photobionts in the region (Pérez-Ortega et al., 2012). While there is an increasing understanding of the diversity of Antarctic lichen-forming fungi (Borchhardt et al., 2017; Lee et al., 2008; Øvstedal and Lewis-Smith, 2001), the diversity and distribution of lichen photobionts in the terrestrial ecosystems of Antarctica remain less comprehensively documented. The objectives of this study are to (1) characterize the diversity of lichen mycobionts and photobionts in

Antarctica and (2) clarify the relationships between these symbionts within this unique ecosystem.

2 Materials and method

2.1 Lichen samples collection

The sampling site is the Fildes Region on King George Island, maritime Antarctica, which comprises the Fildes Peninsula, Ardley Island, and the northern part of Nelson Island (Figure 1). This region is one of the largest ice-free areas in maritime Antarctica and boasts a relatively high level of biodiversity. The mean annual temperature here is $-2.2\text{ }^{\circ}\text{C}$ (Michel et al., 2014), and has witnessed an average increase of $0.7\text{ }^{\circ}\text{C}$ from 1969 to 2013 (Braun et al., 2017). Lichen samples were collected in January 2017 during the 33rd Chinese National Antarctic Research Expedition (CHINARE) and are deposited at the China Pharmaceutical Culture Collection (CPCC), Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences.

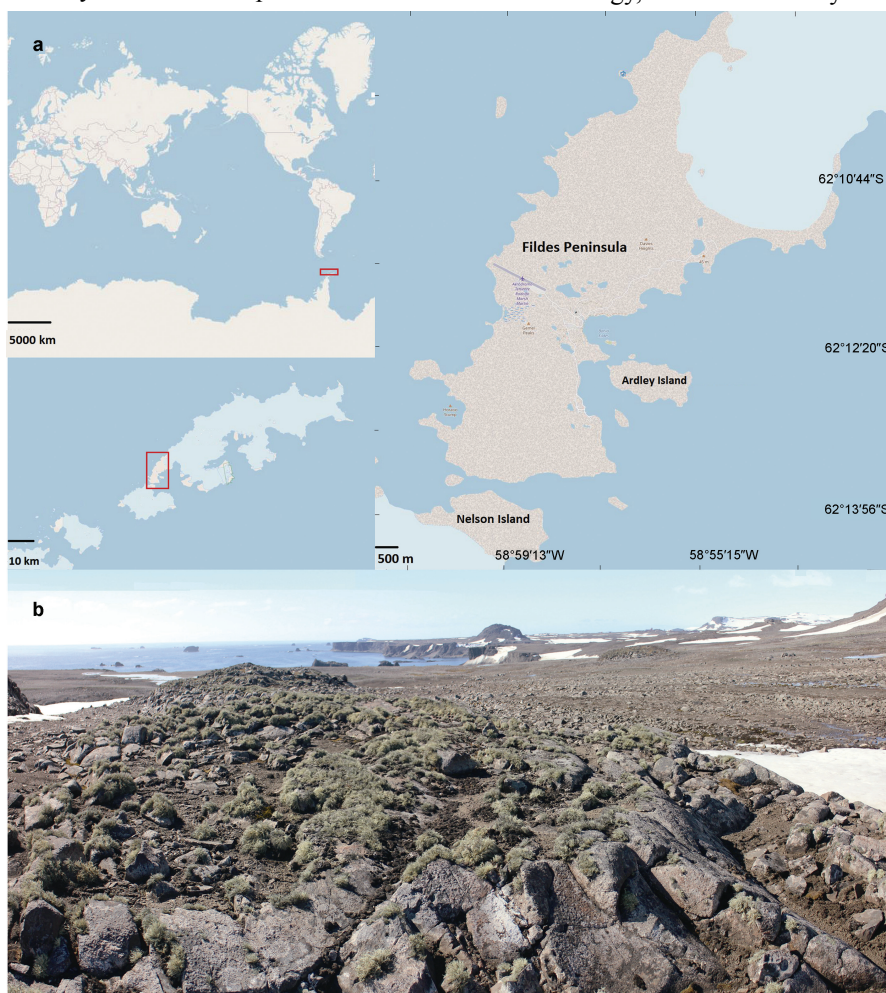


Figure 1 a, map of the Fildes Region (marine Antarctica) indicating the sample sites; b, views of lichens on soil in the Fildes Region.

Lichens collected for this study were identified using standard morphological, anatomical, and chemical

characteristics. Øvstedal and Lewis-Smith (2001) served as the starting point for most identifications, although

additional literature was also consulted for most groups. Thin-layer chromatography was employed to detect secondary lichen metabolites in some groups, according to Orange et al. (2001).

2.2 DNA extraction, PCR, and sequencing

A modified CTAB method was utilized for the extraction of total DNA from lichen thalli (Cubero et al., 1999). The internal transcribed spacer (ITS) regions (ITS1, 5.8S rRNA gene, ITS2) were amplified using PCR with DNA extracts. Fungal-specific primers, ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), were employed to amplify the ITS regions of the lichen mycobionts. The photobiont ITS regions were amplified using algal-specific primer pairs, ITS1T (5'-GGAAGGATCATTGAATCTATCGT-3') and ITS4T (5'-GTTTCGCTCGCCGCTACTA-3') (Kroken and Taylor, 2000). PCR reactions were conducted in 50 μ L volumes consisting of 3 μ L DNA extract, 2 μ L MgCl₂ (1.5 mmol·L⁻¹), 3 μ L dNTPs (1 mmol·L⁻¹), 1.5 μ L of each primer (10 pmol·L⁻¹), 1.5 μ L Taq DNA-polymerase, and 37.5 μ L H₂O. The amplification conditions for primers ITS1F and ITS4 were as follows: an initial denaturation at 95 °C for 3 min; followed by 37 cycles of 94 °C for 30 s, annealing at 52 °C for 30 s, and elongation at 72 °C for 30 s; with a final extension at 72 °C for 10 min. For primers ITS1T and ITS4T, the conditions were as follows: an initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 1 min, 56 °C for 1 min, and 72 °C for 1 min 30 s; followed by a final extension at 72 °C for 10 min. The PCR products were examined on a 0.8% agarose gel, purified, and sequenced by Sangon Biotech Co., Ltd (Beijing, China). The ITS sequence data of lichen mycobionts were deposited in GenBank with accession numbers PP337093–PP337148, and the lichen photobionts with accession numbers PP337150–PP337205.

2.3 Sequence alignment and phylogenetic analysis

To identify the closest matches in the GenBank database, the ITS sequences of lichen mycobionts and photobionts were analyzed using the BLASTN online tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). For phylogenetic analysis, the sequences were aligned using the MAFFT online service, employing the G-INS-i alignment algorithm (<http://mafft.cbrc.jp/alignment/server/>) (Katoh et al., 2005), and then manually refined with MEGA software version 7.0 (Kumar et al., 2016). The dataset included 74 sequences of lichen mycobionts and 406 sequences of photobionts, among which 18 sequences of mycobionts and 350 sequences of photobionts were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov>) based on previous studies on *Trebouxia* (De Carolis et al., 2022; Kosecka et al., 2022; Medeiros et al., 2021; Muggia et al., 2020; Xu et al., 2020), *Asterochloris* (Kim et al., 2020; Škaloud et al., 2015),

Chloroidium (Darienko et al., 2018), *Stichococcus* (Hodač et al., 2016). The optimal substitution model was determined via FindModel (Posada and Crandall, 1998). Phylogenetic trees for both mycobionts and photobionts were generated through maximum likelihood (ML) analysis in RAxML version 8.2.10 (Stamatakis, 2006), using the GTR + G + I model with 1000 bootstrap replicates, and Bayesian inference (BI) in MrBayes version 3.2.6 (Ronquist et al., 2012). Posterior probabilities were calculated by Markov Chain Monte Carlo (MCMC) sampling, running two simultaneous runs of four chains (one cold and three heated) for 10000000 generations, with a temperature setting of 0.2, sampling every 100th tree until the standard deviation of split frequencies fell below 0.01. The burn-in was assessed with Tracer v1.7.1 (Rambaut et al., 2018), discarding the first 10% of trees. Phylogenetic trees were visualized using FigTree version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

2.4 Statistic analysis

The delineation of photobiont operational taxonomic units (OTUs) was conducted using the Assemble Species by Automatic Partitioning (ASAP) method (Puillandre et al., 2021) and phylogenetic analysis revealing the evolutionary relationships among species. The parameters set for the ASAP analysis included identifying split groups with probabilities below 0.001, highlighting results for genetic distances between 0.005 and 0.05, and employing the Jukes-Cantor model (JC69). Furthermore, the relationships between lichen and photobiont species were visualized through network analysis employing Gephi version 0.9.2 software (<https://gephi.org>).

3 Results

3.1 Diversity of lichen mycobionts

In this study, 56 lichen samples were classified into 19 distinct taxa (Table 1). These species are distributed across 13 families, namely Parmeliaceae, Tephromelataceae, Ramalinaceae, Catillariaceae, Sphaerophoraceae, Stereocaulaceae, Cladoniaceae, Pannariaceae, Trapeliaceae, Mniaceae, Ochrolechiaceae, Umbilicariaceae and Caliciaceae, and are grouped into seven orders (Lecanorales, Peltigerales, Baecomyetales, Leotiales, Pertusariales, Umbilicariales and Caliciales) (Figure 2).

3.2 Diversity of lichen photobionts

In this study, 56 photobiont ITS sequences were analyzed using a combined approach of ASAP and phylogenetic analysis, which led to the identification of nine candidate species, as detailed in Table 1. These species are categorized under the class Trebouxiophyceae and span four genera: *Trebouxia*, with 47 sequences distributed across six species; *Asterochloris*, with four sequences

Table 1 Information on samples used in the Fildes Region (maritime Antarctica) with species name and the accession numbers for the ITS sequences of mycobionts and photobionts

Lichen species	Sample code	Mycobiont ITS	Photobiont ITS	Photobiont taxa	Identification	Reference
<i>Buellia anisomera</i> [#]	ZT2017117	PP337093	PP337201	OTU05	<i>Trebouxia</i> sp. OTU I01	This study
Catillariaceae sp.1	ZT2017055	PP337094	PP337157	OTU05	<i>Trebouxia</i> sp. OTU I01	This study
Catillariaceae sp. 2	ZT2017086	PP337095	PP337177	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Cladonia carneola</i>	ZT2017099	PP337096	PP337188	OTU06	<i>Asterochloris pseudoirregularis</i>	This study
<i>Epithamnolia xanthoriae</i>	ZT2017065	PP337097	PP337163	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Himantormia lugubris</i> [#]	ZT2017004	PP337100	PP337151	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Himantormia lugubris</i> [#]	ZT2017071	PP337102	PP337167	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Himantormia lugubris</i> [#]	ZT2017077	PP337101	PP337170	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Himantormia lugubris</i> [#]	ZT2017080	PP337103	PP337172	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Himantormia lugubris</i> [#]	ZT2017091	PP337104	PP337182	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Himantormia lugubris</i> [#]	ZT2017116	PP337098	PP337200	OTU04	<i>Trebouxia</i> sp. OTU D08*	This study
<i>Himantormia lugubris</i> [#]	ZT2017118	PP337099	PP337202	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Lecania brialmontii</i> [#]	ZT2017062	PP337105	PP337162	OTU08	<i>Chloroidium</i> sp.*	This study
<i>Lecania glauca</i> [#]	ZT2017059	PP337106	PP337160	OTU05	<i>Trebouxia</i> sp. OTU I01	This study
<i>Ochrolechia frigida</i>	ZT2017090	PP337107	PP337181	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Placopsis antarctica</i> [#]	ZT2017052	PP337108	PP337155	OTU07	<i>Stichococcus antarcticus</i>	This study
<i>Placopsis contortuplicata</i>	ZT2017053	PP337109	PP337156	OTU07	<i>Stichococcus antarcticus</i>	This study
<i>Placopsis contortuplicata</i>	ZT2017087	PP337110	PP337178	OTU07	<i>Stichococcus antarcticus</i>	This study
<i>Psoroma antarcticum</i> [#]	ZT2017056	PP337111	PP337158	OTU03	<i>Trebouxia</i> sp. OTU D01	This study
<i>Psoroma antarcticum</i> [#]	ZT2017109	PP337112	PP337196	OTU03	<i>Trebouxia</i> sp. OTU D01	This study
<i>Psoroma cinnamomeum</i>	ZT2017002	PP337113	PP337150	OTU01	<i>Trebouxia</i> sp. OTU A12	This study
<i>Psoroma cinnamomeum</i>	ZT2017068	PP337114	PP337166	OTU01	<i>Trebouxia</i> sp. OTU A12	This study
<i>Ramalina terebrata</i>	ZT2017108	PP337115	PP337195	OTU09	<i>Trebouxia</i> sp. OTU A13	This study
<i>Sphaerophorus globosus</i>	ZT2017098	PP337116	PP337187	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Sphaerophorus globosus</i>	ZT2017105	PP337117	PP337192	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Stereocaulon alpinum</i>	ZT2017049	PP337121	PP337153	OTU08	<i>Chloroidium</i> sp.*	This study
<i>Stereocaulon alpinum</i>	ZT2017067	PP337118	PP337165	OTU06	<i>Asterochloris pseudoirregularis</i>	This study
<i>Stereocaulon alpinum</i>	ZT2017088	PP337119	PP337179	OTU06	<i>Asterochloris pseudoirregularis</i>	This study
<i>Stereocaulon alpinum</i>	ZT2017089	PP337120	PP337180	OTU06	<i>Asterochloris pseudoirregularis</i>	This study
<i>Tephromela atra</i>	ZT2017072	PP337122	PP337168	OTU04	<i>Trebouxia</i> sp. OTU D08*	This study
<i>Umbilicaria antarctica</i> [#]	ZT2017066	PP337123	PP337164	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017014	PP337148	PP337152	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017050	PP337124	PP337154	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017058	PP337125	PP337159	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017061	PP337126	PP337161	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017074	PP337127	PP337169	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017079	PP337128	PP337171	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017081	PP337129	PP337173	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017082	PP337130	PP337174	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017083	PP337131	PP337175	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017085	PP337132	PP337176	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017093	PP337133	PP337183	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017094	PP337134	PP337184	OTU02	<i>Trebouxia</i> sp. OTU S02	This study

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Lichen species	Sample code	Mycobiont ITS	Photobiont ITS	Photobiont taxa	Identification	Reference
<i>Usnea aurantiaco-atra</i>	ZT2017095	PP337135	PP337185	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017097	PP337136	PP337186	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017101	PP337137	PP337189	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017102	PP337138	PP337190	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017104	PP337139	PP337191	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017106	PP337140	PP337193	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017107	PP337141	PP337194	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017110	PP337142	PP337197	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017112	PP337143	PP337198	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017114	PP337144	PP337199	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017119	PP337145	PP337203	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017120	PP337146	PP337204	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Usnea aurantiaco-atra</i>	ZT2017122	PP337147	PP337205	OTU02	<i>Trebouxia</i> sp. OTU S02	This study
<i>Umbilicaria antarctica</i> [#]	-	-	JX509839	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Umbilicaria antarctica</i> [#]	-	-	JX509855	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Umbilicaria antarctica</i> [#]	-	-	JX509856	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Umbilicaria antarctica</i> [#]	-	-	JX509857	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Umbilicaria antarctica</i> [#]	-	-	JX509858	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Umbilicaria antarctica</i> [#]	-	-	JX509859	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea antarctica</i>	-	-	JX509853	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea antarctica</i>	-	-	JX509854	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509840	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509841	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509842	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509844	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509845	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509846	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509847	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509848	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509849	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509850	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509851	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509852	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509860	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509861	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	JX509843	OTU02	<i>Trebouxia jamesii</i>	Li et al., 2013
<i>Usnea aurantiaco-atra</i>	-	-	KX147254	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147255	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147256	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147257	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147258	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147259	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147260	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147261	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147262	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018

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Lichen species	Sample code	Mycobiont ITS	Photobiont ITS	Photobiont taxa	Identification	Reference
<i>Usnea aurantiaco-atra</i>	-	-	KX147263	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147264	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147265	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147266	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147267	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147268	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KX147269	OTU02	<i>Trebouxia jamesii</i>	Cao et al., 2018
<i>Usnea aurantiaco-atra</i>	-	-	KT187269	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187270	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187271	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187272	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187273	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187274	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187275	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187276	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187277	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187278	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187279	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017
<i>Usnea aurantiaco-atra</i>	-	-	KT187280	OTU02	Uncultured <i>Trebouxia</i> isolate	Cao et al., 2017

Notes: * indicating the newly discovered lineage; # indicating the Antarctic endemic lichen species; - indicating data not available in previous studies.

representing one species; *Chloroidium*, with two sequences representing one species; and *Stichococcus*, with three sequences representing one species.

Within the genus *Trebouxia*, four main clades were identified: clade A (*arboricola/gigantea* group), clade I (*impressa/gelatinosa* group), clade S (*simplex/letharii/jamesii* group), and clade D (*delisei* group). Clade A included three sequences across two species-level lineages, with *Trebouxia* sp. OTU A12 identified from two sequences and *Trebouxia* sp. OTU A13 from one (Figure S1a). Clade D comprised four sequences and two species-level lineages, introducing a new lineage named *Trebouxia* sp. OTU D08 (Figure S1b). Clade I disclosed that three sequences belong to a previously identified *Trebouxia* species, specifically OTU I01 (Figure S1c). Clade S, the most prominent within *Trebouxia*, consisted of 88 sequences (37 sequences in this study and 51 sequences in three previous studies) representing a single species-level lineage, *Trebouxia* sp. OTU S02 (Figure S1d).

The genus *Asterochloris* was represented by *A. pseudoirregularis* through four sequences (Figure S2). The genus *Chloroidium* included 19 sequences in the phylogenetic tree, revealing one new lineage represented by two sequences (Figure S3). Lastly, three sequences within the genus *Stichococcus* were identified as *S. antarcticus* (Figure S4). For example, two sequences of *Chloroidium* was identified as one species using ASAP and these two sequences were also formed a new clade in the phylogenetic tree of all known *Chloroidium* species (Figure S3).

Therefore, one new *Chloroidium* species was identified.

3.3 Lichen mycobionts and photobionts association patterns

A network diagram (Figure 3) illustrated the relationships between 20 taxa of lichen mycobionts and nine photobiont species found in the Fildes Region (identified in this study and three previous studies in Table 1 and Figures S1–S2). It was found that several lichen taxa share identical photobiont species. For example, Catillariaceae sp. 2, *Epithamnolia xanthoriae*, *Himantormia lugubris*, *Ochrolechia frigida*, *Sphaerophorus globosus*, *Umbilicaria antarctica*, and *Usnea aurantiaco-atra* were all associated with the same photobiont, *Trebouxia* sp. OTU S02. Similarly, *Placopsis antarctica* and *Placopsis contortuplicata* were both linked to the photobiont *Stichococcus antarcticus*. Furthermore, certain photobionts, such as *Chloroidium* sp. and *Asterochloris pseudoirregularis*, were found to associate with the same lichen species, *Stereocaulon alpinum*.

4 Discussion

Lichens play a crucial role in the flora of Antarctica's ice-free marine areas. Numerous studies have explored the diversity of lichen-forming fungi (mycobionts) in Antarctica, which is now estimated to comprise at least 500 species (Øvstedal and Lewis-Smith, 2001, 2009, 2011). However, research focusing specifically on the lichen

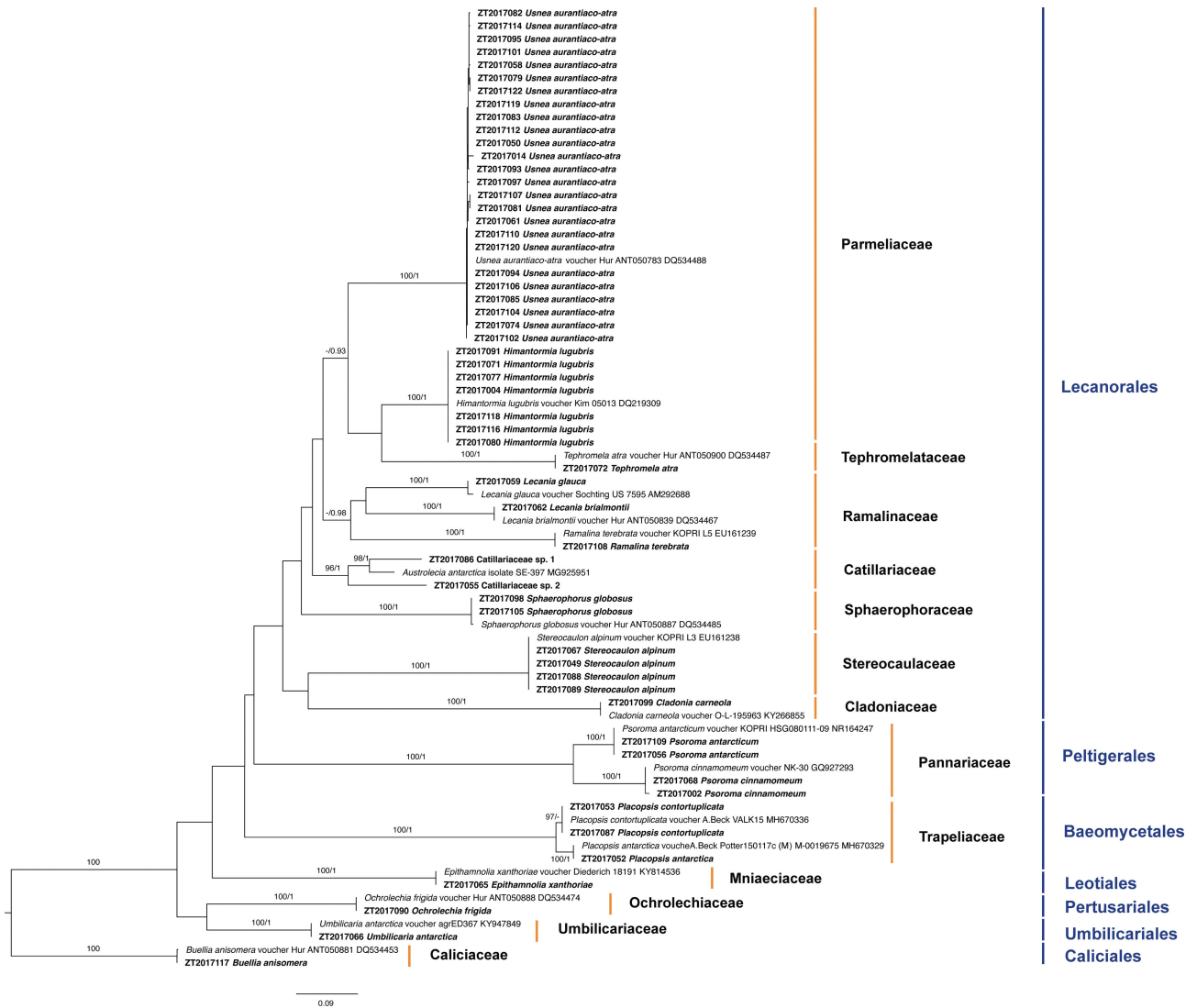


Figure 2 A ML tree was constructed from the ITS sequences of lichen mycobionts under the GTR + G + I model in RAxML. Along branches (ML/PP), ML bootstrap values (50%) and Bayesian posterior probability (0.9) are displayed.

photobionts associated with certain mycobiont taxa in Antarctica remains limited.

In a previous study, Andreev (1988) reported 119 taxa from the Fildes Peninsula. In this study, 19 lichen species represent approximately 16% of the lichen diversity in the study area. This study primarily identified the lichen photobionts as species within the class Trebouxiophyceae, including genera *Trebouxia*, *Asterochloris*, and *Chloridium*. *Trebouxia* has been recognized as the most common and abundant photobiont genus, with comprehensive taxon sampling conducted by Muggia et al. (2020). This work provided a genus-wide, multi-locus phylogenetic hypothesis, serving as a guideline for future research. Subsequently, four main clades within the *Trebouxia* genus were established: clade A (*arboricola/gigantea* group), clade C (*corticola/galapagensis* group), clade I (*impressa/gelatinosa* group), and clade S

(*simplex/letharii/jamesii* group), based on reassessments by Beck et al. (1998), Hauck et al. (2007), Leavitt et al. (2015), and Muggia et al. (2020) using molecular phylogenetics and systematics. Additionally, Xu et al. (2020) identified a fifth monophyletic lineage, the *delisei* group (clade D).

This study did not detect the clade C of *Trebouxia*, which according to Nelsen et al. (2021), predominantly inhabits warm and wet environments. However, clade D of *Trebouxia* was identified in this study, based on lichen photobionts found in Iceland and Svalbard (Arctic) (Xu et al., 2020). The remaining three *Trebouxia* clades—A, I, and S—are distributed globally, including in Antarctica. Notably, in the studied Antarctic region, clade S (encompassing the *simplex/letharii/jamesii* group) was the most prevalent. Clade S members are adapted to drier and cooler habitats (Nelsen et al., 2021). Within clade S, the lineage *Trebouxia* sp. OTU S02, which has been associated with *Pseudephebe*



Figure 3 A network diagram showing the relationships between 20 lichen mycobionts and nine photobiont species (identified in this study and three previous studies in Table 1). The nodes are colored according to lichen mycobiont (orange) and photobiont (green).

species in Svalbard (Arctic) (Garrido-Benavent et al., 2020), was the most commonly detected *Trebouxia* species in this study.

Asterochloris has traditionally been considered the exclusive photobiont for the lichen genus *Cladonia* (Piercey-Normore and DePriest, 2001; Pino-Bodas and Stenroos, 2021), with 18 species of *Asterochloris* identified to date (Kim et al., 2020; Škaloud et al., 2015). This study confirms *Asterochloris*'s association with *Cladonia* and *Stereocaulon* species, aligning with previous researches (Škaloud and Peksá, 2010; Steinová et al., 2019; Vančurová et al., 2018). Notably, *Stereocaulon* species were found to associate with *Asterochloris* and other algal genera, including *Vulcanochloris* and *Chloroidium*, expanding the known diversity of photobiont relationships (Vančurová et al., 2018, 2021). In maritime Antarctica, the monophyletic sister species *Placopsis antarctica* and *P. contortuplicata* share photobionts, forming associations with *Stichococcus antarcticus* and *S. allas* (Beck et al., 2019).

We found that several lichen species shared the same photobiont species. Moreover, there were no significant differences in the relationships between mycobionts and

photobionts in lichens endemic to Antarctica compared to other lichens. In previous studies, lichen mycobionts across different species, genera, and families can associate with the same photobiont species (Ahmadjian, 1993; Beck, 1999), yet some show varied selectivity towards different photobiont species (Guzow-Krzemińska, 2006; Muggia et al., 2008). In extreme environments such as deserts, alpine regions, and polar habitats, mycobionts exhibit lower selectivity for photobionts (Muggia et al., 2008; Pérez-Ortega et al., 2012; Wirtz et al., 2003). Photobiont diversity significantly decreases towards the Antarctic, suggesting ecological factors play a limited role in Antarctic photobiont diversity (Domaschke et al., 2012).

In summary, this study reveals the diversity of lichen mycobionts and photobionts across 56 lichen samples collected from the Fildes Region in maritime Antarctica, utilizing ITS region sequence analysis. This study identifies a total of 19 taxa of lichen mycobionts, spanning 13 families and seven orders, alongside nine candidate photobiont species. These photobiont species are classified under one class Trebouxiophyceae, and distributed across four genera: *Trebouxia*, with eight species; *Asterochloris*,

with one species; *Chloroidium*, with one species; and *Stichococcus*, with one species. Furthermore, this study reveals the relationships between mycobionts and photobionts, including the level of selectivity observed within their symbiosis. The findings provide a crucial reference dataset for characterizing the diversity of both lichen mycobionts and photobionts in the ice-free regions of maritime Antarctica.

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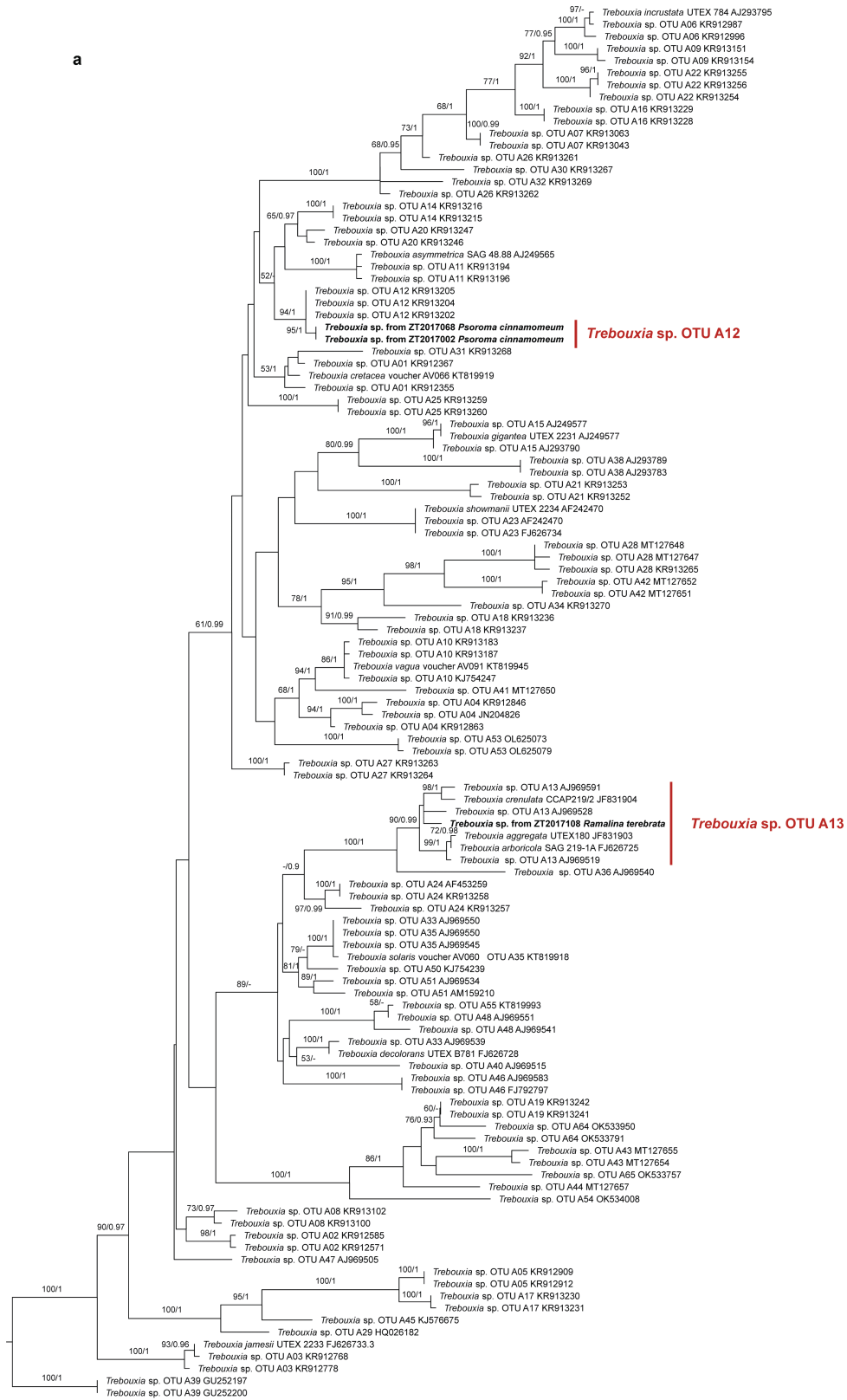
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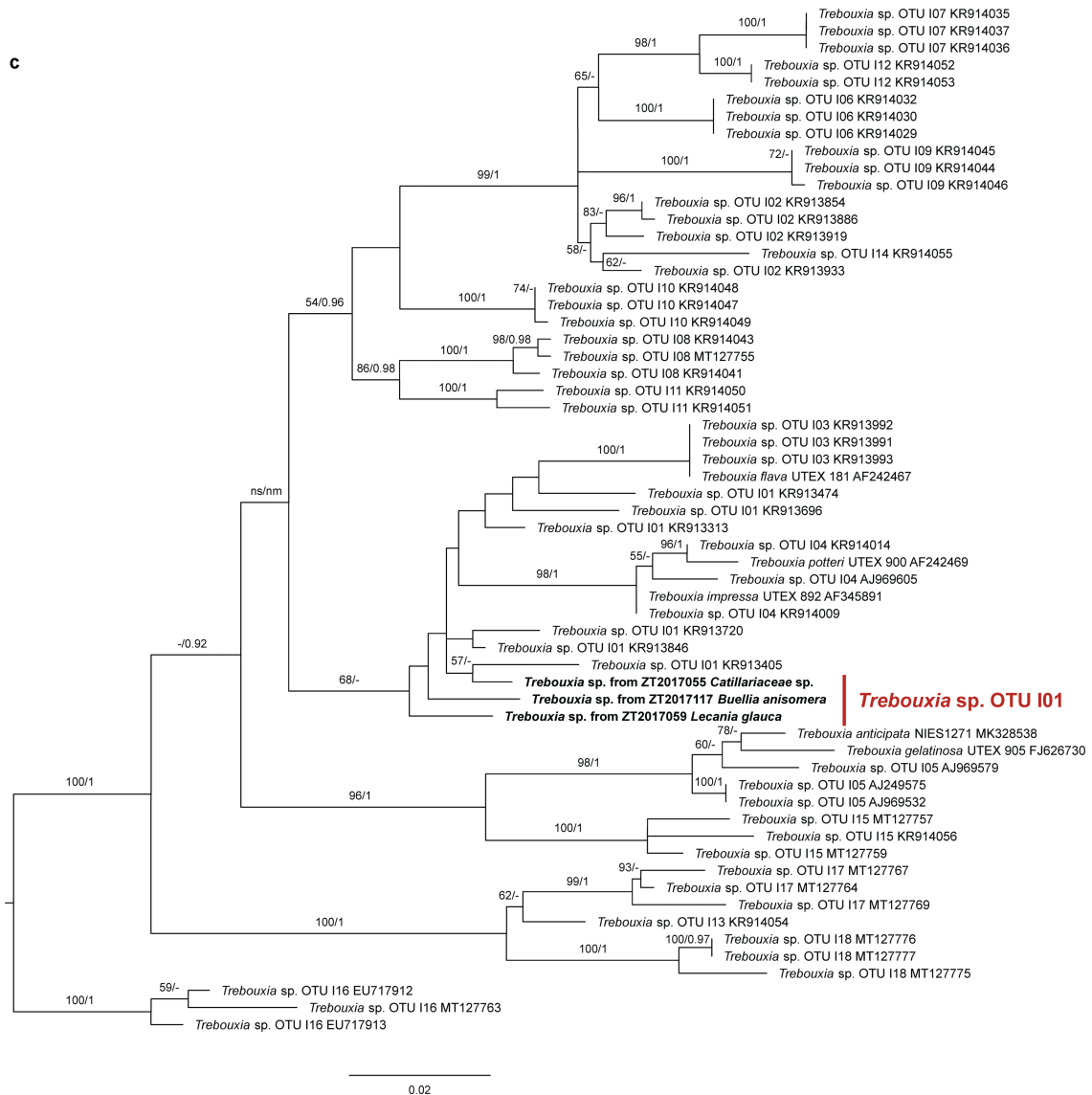
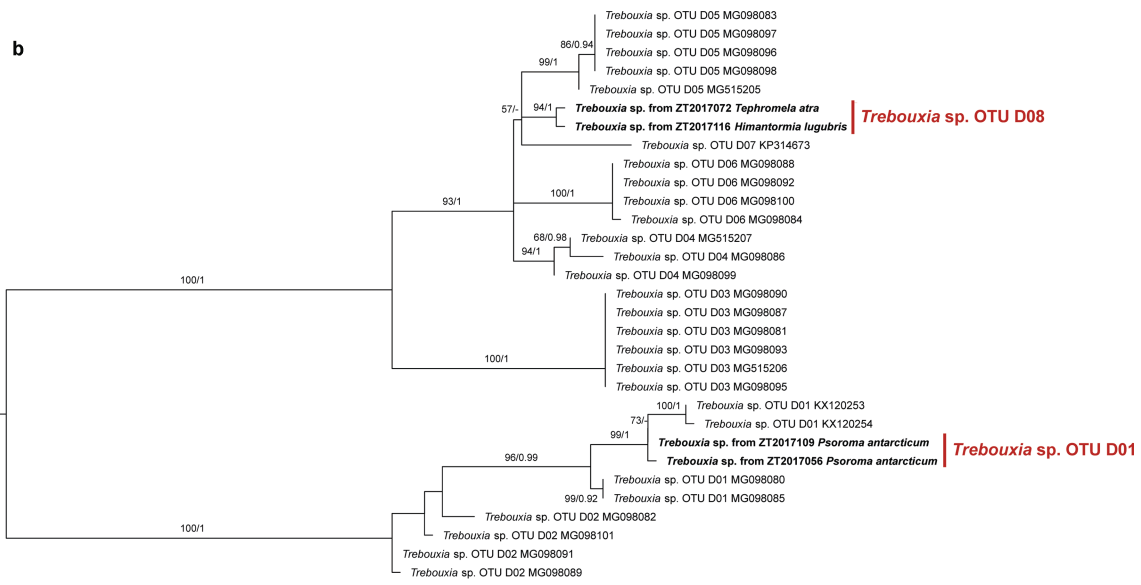
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Supplementary Figures

a





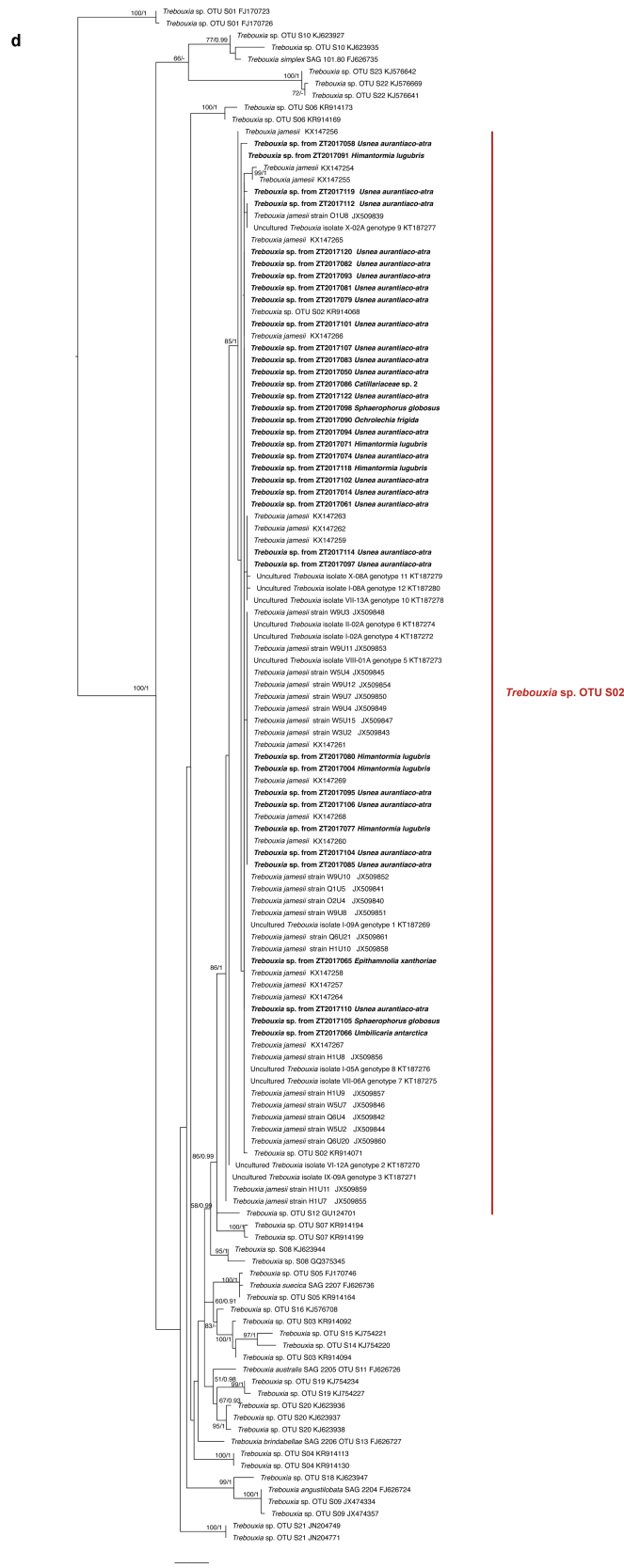


Figure S1 A ML tree was constructed from the ITS sequences of lichen photobiont *Trebouxia* species under the GTR + G + I model in RAXML. **a**, clade A; **b**, clade D; **c**, clade I; **d**, clade S. Along branches (ML/PP), ML bootstrap values (50%) and Bayesian posterior probability (0.9) are displayed.

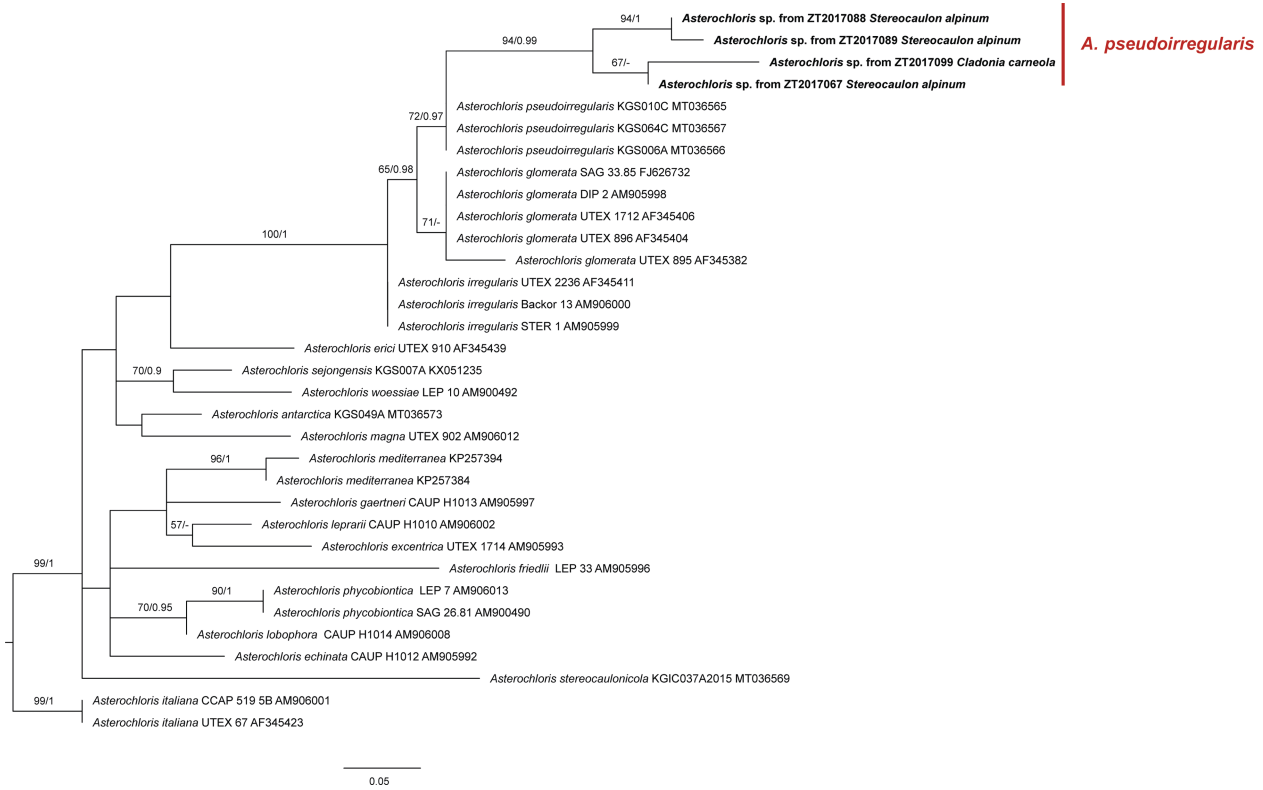


Figure S2 A ML tree was constructed from the ITS sequences of lichen photobiont *Asterochloris* species under the GTR + G + I model in RAxML. Along branches (ML/PP), ML bootstrap values (50%) and Bayesian posterior probability (0.9) are displayed.

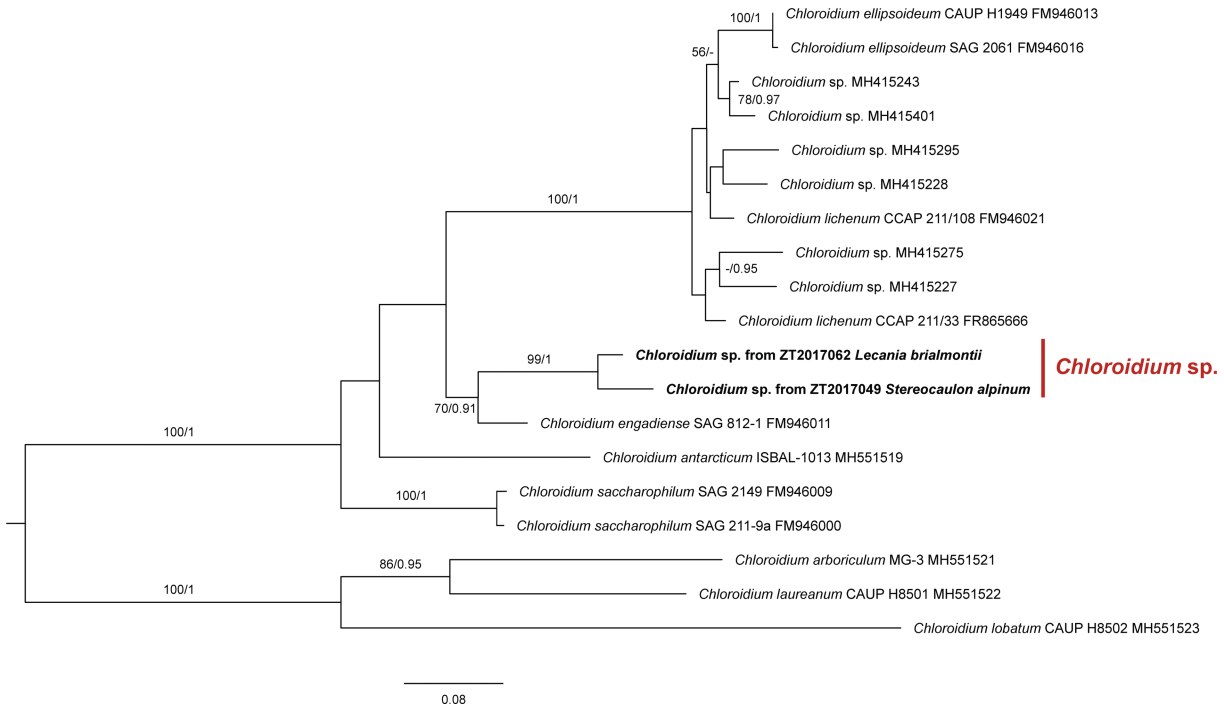


Figure S3 A ML tree was constructed from the ITS sequences of lichen photobiont *Chloroidium* species under the GTR + G + I model in RAxML. Along branches (ML/PP), ML bootstrap values (50%) and Bayesian posterior probability (0.9) are displayed.

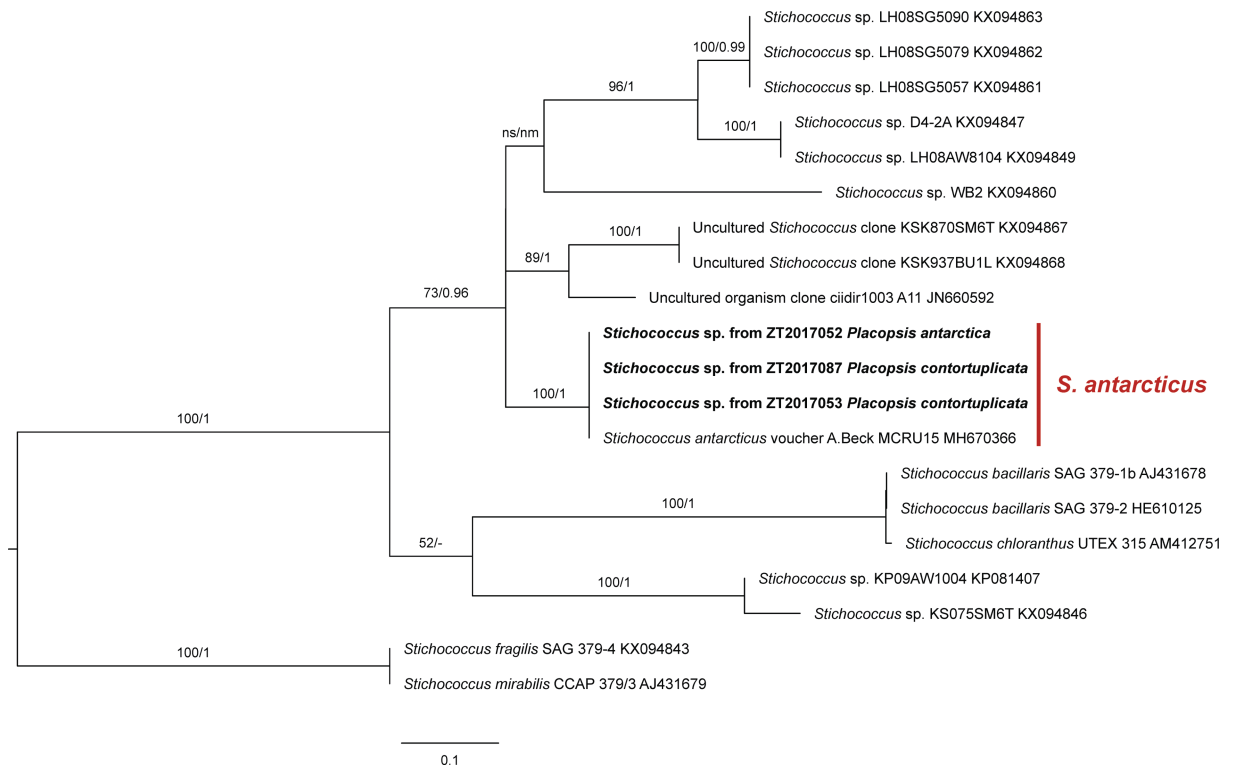


Figure S4 A ML tree was constructed from the ITS sequences of lichen photobiont *Stichococcus* species under the GTR + G + I model in RAxML. Along branches (ML/PP), ML bootstrap values (50%) and Bayesian posterior probability (0.9) are displayed.