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ANATOMICAL-MORPHOLOGICAL AND ITS-BASED PHYLOGENETIC EVALUATION OF SELECTED LICHENIZED FUNGI FROM ROBERT ISLAND (ANTARCTIC PENINSULA, ANTARCTICA)

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Abstract
In this study, taxonomic investigations were conducted on six lichenized fungi species from Robert Island (Antarctic Peninsula). The anatomical and morphological characteristics of the specimens were examined, along with molecular data. Internal Transcribed Spacer (ITS) primers were used as molecular markers for six species. Phylogenetic analyses were performed using sequences obtained from both the collected specimens and reference data from GenBank. The phylogenetic relationships and evolutionary tree construction were based on the Maximum Likelihood method. As a result of the study, the following lichenized fungi species were identified: Austrolecia antarctica Hertel, Lecanora polytropa (Ehrh.) Rabenh., Placopsis antarctica (Nyl.) I.M.Lamb, Rhizocarpon geminatum Körb., Rhizocarpon polycarpum (Hepp) Th.Fr., Usnea aurantiacoatra (Jacq) Bory

Keywords: Antarctic lichens, ITS Region, Lichenized fungi, Robert Island, Phylogenetic analysis.

1. INTRODUCTION

Lichenized fungi are mutualistic associations between a fungal partner and a photosynthetic organism (typically green algae or cyanobacteria), and they are capable of thriving in a wide range of habitats due to their remarkable adaptation to extreme environmental conditions (Spribille et al., 2016; Widhelm, 2019). Lichens are commonly categorized into crustose, foliose, and fruticose forms, and are morphologically characterized based on features such as attachment to substrates, cortex structure, and vegetative reproductive structures (Nash, 2008; Widhelm, 2019).

Antarctica is one of the harshest environments on Earth, marked by extreme cold, dryness, high levels of radiation, and limited nutrient availability (Convey and Stevens, 2007; Kappen, 2000). In this challenging landscape, lichens constitute the dominant vegetation in terrestrial ecosystems and serve as ecologically important indicators due to their high stress tolerance and unique physiological adaptations (Ovstedal and Lewis-Smith, 2001; Green et al., 1999).

Robert Island, located in the South Shetland Islands of the Antarctic Peninsula, holds geological and microbial diversity significance; however, comprehensive systematic studies of its lichen flora are limited. Most lichen species reported from the island have been mentioned in broader biodiversity studies, and detailed morpho-anatomical and molecular characterizations remain scarce (Bellio et al., 2017; Beck et al., 2019).

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In recent decades, the integration of molecular data—especially the Internal Transcribed Spacer (ITS) regions of ribosomal DNA—has significantly enhanced traditional lichen taxonomy. ITS sequences have proven useful for species-level identification and for resolving phylogenetic relationships, as they are sufficiently variable, universally amplifiable, and supported by extensive reference databases (White et al., 1990; Bruns et al., 1991; Lee and Taylor, 1992; Madigan et al., 2003).

Previous reports from Robert Island have recorded species such as *Cladonia cornuta*, *Lecanora atra*, *Placopsis contortuplicata*, and *Psoroma hypnorum* as part of large-scale Antarctic lichen surveys (Quilhot et al., 1989; Øvstedal and Lewis-Smith, 2001; Beck et al., 2019). However, many of these records lack comprehensive taxonomic treatment, and DNA-based phylogenetic confirmation is largely absent.

In the present study, six lichenized fungi species collected from Robert Island were identified based on anatomical and morphological characteristics. To confirm the identifications at the molecular level, ITS sequence data were obtained and phylogenetic analyses were conducted. The results were compared with reference sequences from GenBank, with the aim of contributing to the taxonomic knowledge of Antarctic lichen flora.

2. MATERIALS AND METHODS

I. Sample Collection and Identification

Lichen specimens analyzed in this study were collected from Robert Island (62°24′S, 59°30′W), part of the South Shetland Islands of the Antarctic Peninsula, during the 2017–2018 austral summer. During fieldwork, substrate type, locality information, and GPS coordinates were recorded for each specimen. Samples were placed into paper herbarium envelopes and deposited in the Lichen Herbarium of Erciyes University, Faculty of Science (accession codes: ERCH ROB).

Morphological and anatomical characteristics were examined under a stereo microscope (Olympus SZX16, Leica EX4) and a compound light microscope (LEICA DM 1000). Lobe dimensions were measured with a millimeter scale, and ascospore and apothecial features were assessed using an ocular micrometer. Standard spot tests (K, C, PD, I) were conducted for chemical characterization.

II. DNA Extraction and ITS Amplification

Apothecial or thallus fragments from the specimens were ground using a sterile porcelain mortar, and genomic DNA was extracted using a commercial plant DNA extraction kit (e.g., NucleoSpin Plant II, Macherey-Nagel). The Internal Transcribed Spacer (ITS) region was amplified using the universal primers ITS1 and ITS4 (White et al., 1990). PCR protocols were optimized based on standard recommendations in the literature.

The amplification products were visualized via agarose gel electrophoresis (1.5%), and successfully amplified fragments were sequenced commercially. The resulting ITS sequences were compared with GenBank entries using BLASTn to assess sequence similarity.

III. Phylogenetic Analysis

ITS sequences were aligned using BioEdit v7.2, and reference sequences of related taxa were retrieved from GenBank. Phylogenetic analyses were conducted using the Maximum Likelihood (ML) method implemented in MEGA X software. The best-fitting substitution model was selected using the Akaike Information Criterion (AIC). The robustness of the tree topology was assessed by 1000 bootstrap replicates.

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3. RESULTS AND DISCUSSIONS

A total of six lichenized fungal taxa were identified from Robert Island based on combined morphological, anatomical, and molecular evidence. All taxa exhibited distinct macroscopic and microscopic features that were consistent with descriptions in the relevant taxonomic literature.

The identified species are as follows: *Austrolecia antarctica* Hertel, *Lecanora polytropa* (Ehrh.) Rabenh., *Placopsis antarctica* (Nyl.) I.M.Lamb, *Rhizocarpon geminatum* Körb., *Rhizocarpon polycarpum* (Hepp) Th.Fr., *Usnea aurantiacoatra* (Jacq) Bory (Figure 3).

Macroscopic examination revealed variation in thallus morphology, ranging from crustose and foliose to fruticose forms. Anatomical analyses showed distinctive features in cortex type, medulla structure, and reproductive tissues (e.g., apothecia, ascospores), supporting species-level identifications.

ITS sequences were successfully obtained from six lichenized fungi species. BLASTn comparisons of these sequences with GenBank data confirmed the morphological identifications, showing ≥98% sequence similarity in all cases. Phylogenetic analysis based on the ITS region using the Maximum Likelihood method produced a well-resolved tree that supported the placement of each taxon within its expected clade. Bootstrap support values were generally high (>70%) for most nodes.

***Austrolecia antarctica* Hertel (Fig. 3A)**

Habitat / Substrate: Siliceous rock surfaces on Robert Island.

Morphological and Anatomical Characteristics: Thallus crustose, effuse, and white. Apothecia black, sessile, slightly convex, 0.25–0.35 mm in diameter. Epihymenium brownish (~15 µm), hymenium hyaline (55–60 µm), hypothecium brownish. Asci Catillaria-type, 8-spored, 65 × 10 µm. Ascospores simple, hyaline, 11.5–13.5 × 5.5–7 µm. Paraphyses simple, slightly swollen apices, 2–4 µm. No pycnidia observed.

Spot Test Results: Apothecia K–, C–, KC–; medulla K+ yellow-orange.

Molecular Findings (ITS/BLAST/ML-Fig. 1): BLASTn analysis revealed 99.3% sequence similarity with existing *Austrolecia antarctica* accessions in GenBank (e.g., MG925951.1). Phylogenetic analysis based on the Maximum Likelihood method placed the specimens within a strongly supported clade (bootstrap value 93%) containing reference *A. antarctica* sequences. The placement was consistent with the morpho-anatomical identification. No sequence-level ambiguity was observed for the *A. antarctica* group, supporting its species-level cohesion within the genus.

Notes: *Austrolecia antarctica* is the sole representative of its genus and is considered an Antarctic endemic species, with previous records from continental Antarctica, sub-Antarctic islands, Tasmania, and southern Australia. Its crustose habit and black apothecia may superficially resemble some species of *Lecidea* (e.g., *Lecidea lapicida*); however, *A. antarctica* is readily distinguished by its *Catillaria*-type asci, smaller ascospores, and a characteristic K+ yellow-orange medullary reaction, in contrast to the typically K– reaction observed in *L. lapicida*. In our Maximum Likelihood phylogenetic tree, *A. antarctica* formed a distinct clade, supporting its phylogenetic independence and consistent with earlier studies highlighting the uniqueness of its ascus structure and apothecial anatomy (e.g., Hertel, 1995).

Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Robert Island, 62°24′S 59°30′W, on siliceous rock, leg. Bülent Gözcelioğlu, ERCH ROB 0.001, ROB 0.009.

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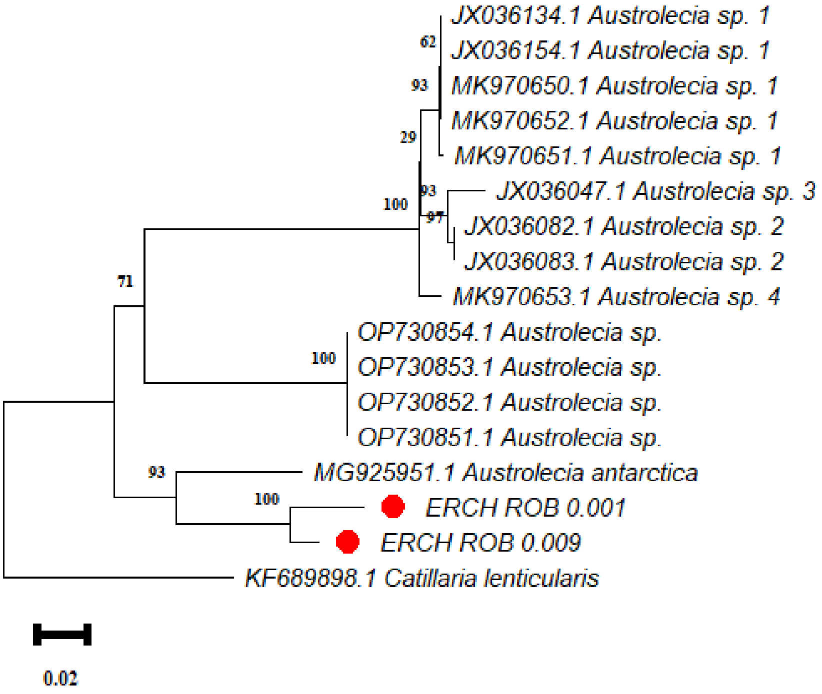

Figure 1. Maximum Likelihood phylogenetic tree based on ITS rDNA sequences showing the placement of *Austrolecia antarctica* specimens collected from Robert Island (ERCH ROB 0.001, ERCH ROB 0.009; highlighted in red). The tree includes closely related *Austrolecia* species and environmental isolates retrieved from GenBank. Bootstrap support values (≥50%) are shown at branch nodes. *Catillaria lenticularis* (KF689898.1) was used as the outgroup. Scale bar represents 0.02 substitutions per site.

Lecanora polytropa **(Ehrh.) Rabenh. (Fig. 3B)**

Habitat / Substrate: Collected from exposed siliceous rock outcrops on Robert Island. The species was found forming small, scattered colonies in sunlit, wind-exposed microhabitats.

Morphological and Anatomical Characteristics: Thallus crustose, thin to moderately developed, whitish-gray to pale greenish-gray, areolate to rimose. Apothecia numerous, sessile, up to 0.6 mm in diameter, with flat, pale yellow to orange discs and a persistent, slightly raised margin. Epihymenium light yellow-brown, hymenium hyaline (~60–75 µm), hypothecium pale brown. Asci Lecanora-type, 8-spored. Ascospores simple, hyaline, ellipsoid, 9–13 × 4–6 µm. Paraphyses septate, branched, slightly capitate. Pycnidia not observed.

Spot Test Results: Thallus K+ yellow, C–, KC–, P+ orange; cortex and medulla contain usnic and atranorin as secondary compounds, consistent with published chemistry of *L. polytropa*.

Molecular Findings (ITS/BLAST/ML-Fig. 2): BLASTn analysis showed 98.72–99.18% similarity with *Lecanora polytropa* sequences in GenBank (e.g., PQ228290, PQ228291). Maximum Likelihood analysis placed the ERCH ROB 0.011 specimen within a monophyletic clade with confirmed *L. polytropa* accessions (bootstrap value 98%). The specimen was clearly separated from

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closely related taxa such as *Lecanora fuscobrunnea*, *L. swartzii*, and *L. symmicta*, further supporting the morphological identification as *L. polytropa*.

Notes: *Lecanora polytropa* is a widespread, cosmopolitan saxicolous lichen frequently found in arctic, alpine, and Antarctic regions. Its thallus and apothecial variability have historically led to confusion with members of the *L. dispersa* group. However, its identification is supported by consistent chemical reactions (K+ yellow, P+ orange), the presence of atranorin, and yellow-brown apothecia with persistent margins. In our study, although superficially similar crustose taxa such as *Austrolecia antarctica* were present in the same habitat, *L. polytropa* was clearly differentiated by its pigment chemistry and *Lecanora*-type asci. Morphologically, it closely resembles *L. intricata*; however, the latter has crenulate areole margins and a smoother surface, while *L. polytropa* displays straight margins and a flat areolate thallus (Øvstedal & Lewis-Smith, 2001). Molecular data further corroborated this distinction, and the species' detection on Robert Island contributes to our understanding of its distribution within the maritime Antarctic.

Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Robert Island, 62°24′S, 59°30′W, on siliceous rock, leg. Bülent Gözcelioğlu, ERCH ROB 0.011.

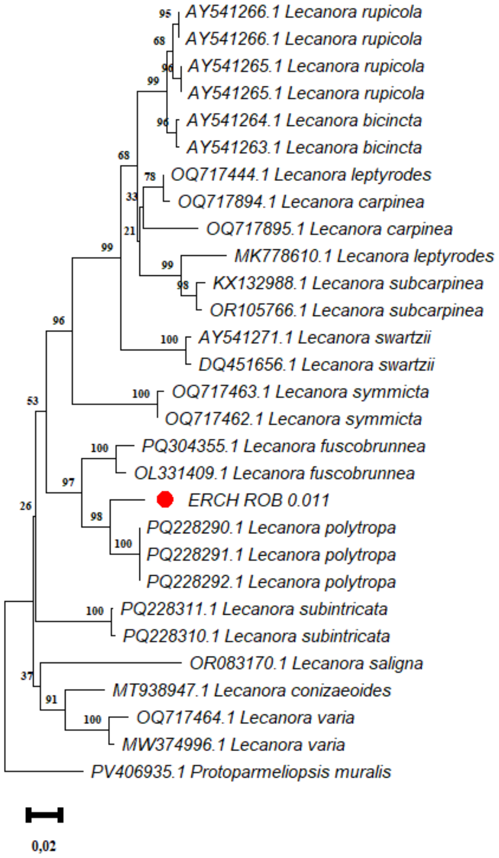

Figure 2. Maximum Likelihood phylogenetic tree of *Lecanora* species based on ITS rDNA sequences. The Robert Island specimen (ERCH ROB 0.011; highlighted in red) is placed within a well-supported clade containing other *Lecanora polytropa* accessions. Bootstrap values (≥50%) are shown at branch points. *Protoparmeliopsis muralis* (PV406935.1) was used as the outgroup. Scale bar indicates 0.02 substitutions per site.

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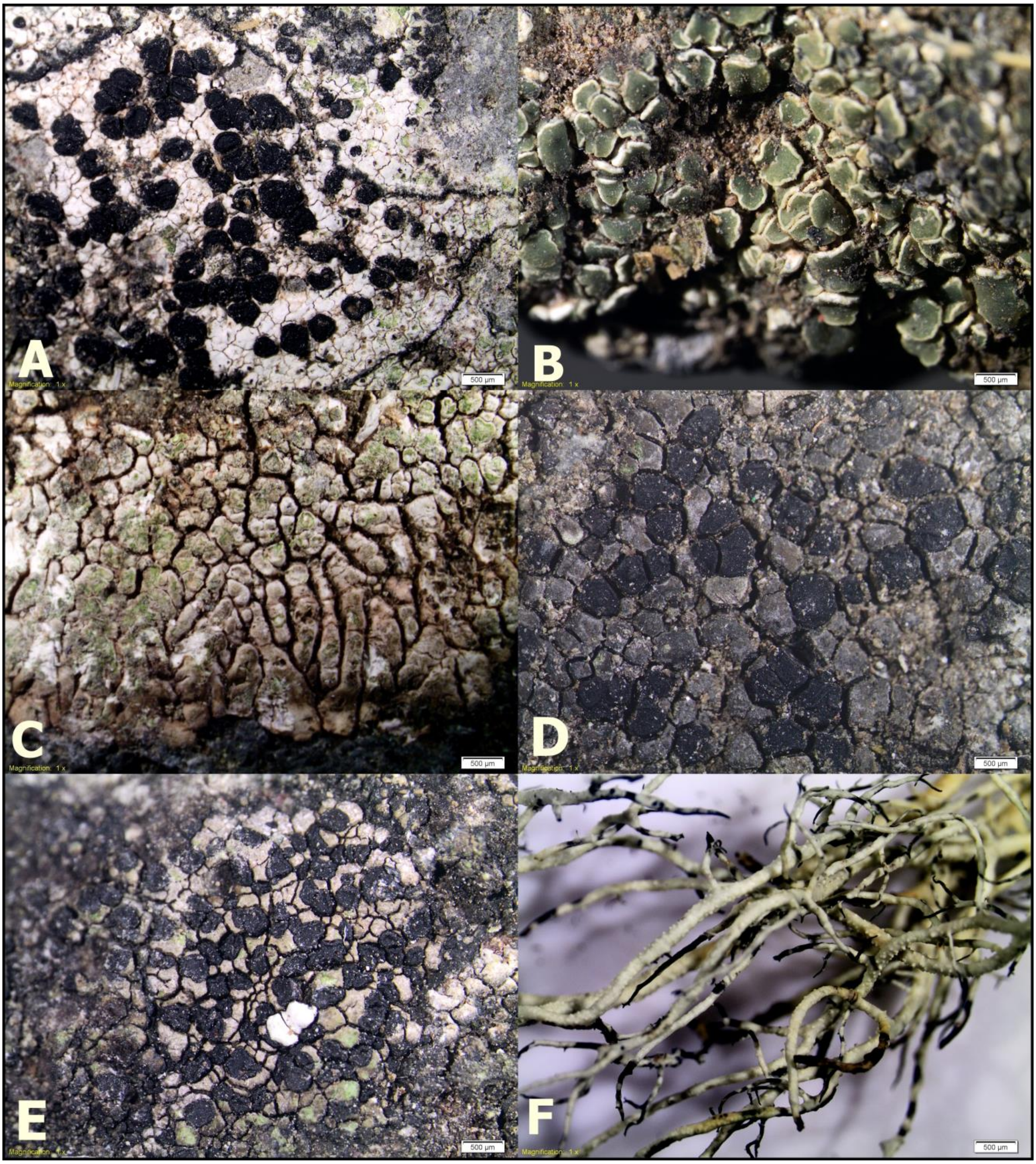

Figure 3. Macroscopic morphology of representative lichenized fungi collected from Robert Island: (a) *Austrolecia antarctica*, (b) *Lecanora polytropa*, (c) *Placopsis antarctica*, (d) *Rhizocarpon geminatum*, (e) *Rhizocarpon polycarpum*, (f) *Usnea aurantiacoatra*. Scale bars = 0.5 mm.

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***Placopsis antarctica* (Nyl.) I.M.Lamb (Fig. 3C)**

Habitat / Substrate: Found on siliceous boulders and exposed rock surfaces on Robert Island. Frequently observed in open, high-humidity microhabitats and occasionally co-occurring with *Umbilicaria antarctica*.

Morphological and Anatomical Characteristics: Thallus crustose, thick, rosette-forming, often lobate at margins, pale to whitish gray with orange-pink pigmented patches (due to anthraquinones). Surface areolate to rimose. Soralia abundant, marginal or laminal, round and powdery. Apothecia rare; when present, immersed to slightly raised, disc dark red to black. Epihymenium reddish brown, hymenium hyaline (~80 µm), hypothecium brown. Ascospores simple, hyaline, ellipsoid, 10–14 × 5–6 µm. Paraphyses septate, branched, apices slightly thickened.

Spot Test Results: Thallus K+ red (due to anthraquinones), C–, KC–, P+ orange to red; consistent with presence of gyrophoric and lecanoric acids.

Molecular Findings (ITS/BLAST/ML-Fig. 4): BLASTn results showed >99% similarity with *Placopsis antarctica* sequences in GenBank (e.g., MH670330, MH670317). Maximum Likelihood phylogenetic analysis placed the specimen (ERCH ROB 0.003) within a strongly supported monophyletic clade (bootstrap value 99%) containing confirmed *P. antarctica* accessions. The sequence was clearly separated from *Placopsis contortuplicata* and other related taxa, confirming its identity as *P. antarctica*.

Notes: *Placopsis antarctica* is one of the most frequently encountered species of the genus in the maritime Antarctic. Its pigmented thallus, characterized by well-developed soralia and a K+ red reaction due to anthraquinones, facilitates reliable identification. In our study, *P. antarctica* was readily distinguished from *Xanthoria borealis*, which also exhibits orange pigmentation, by its crustose thallus (vs. foliose in *Xanthoria*), reproductive structures (soralia vs. apothecia), and contrasting chemical reactions (*P. antarctica*: K+ red; *Xanthoria*: K–, C+). Among congeners, *P. antarctica* is morphologically distinct from *P. contortuplicata* due to the presence of finger-like laminal dactyls, which promote soralia formation as thalli mature. This distinction is also supported by molecular evidence, which places *P. antarctica* in a well-supported, phylogenetically distinct clade.

Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Robert Island, 62°24′S, 59°30′W, on siliceous rock, leg. Bülent Gözcelioğlu, ERCH ROB 0.003.

***Rhizocarpon geminatum* Körb. (Fig. 3D)**

Habitat / Substrate: Collected from sun-exposed siliceous rock surfaces on Robert Island, often found in close proximity to *Rhizocarpon geographicum* and *Lecidea lapicida*. Typically grows in dry microhabitats at moderate elevation.

Morphological and Anatomical Characteristics: Thallus crustose, continuous to areolate, pale yellow-green to yellow-gray. Margins well-defined, surrounded by prothallus. Areoles angular to irregular, often with scattered apothecia. Apothecia black, lecideine, sessile, 0.4–0.8 mm in diameter. Disc flat to slightly convex, margin persistent. Epihymenium brown, hymenium hyaline (70–90 µm), hypothecium brownish. Asci *Teloschistes*-type, 8-spored. Ascospores 2-celled (muriform in older), hyaline to brown, ellipsoid, 14–22 × 6–9 µm.

Spot Test Results: Thallus K+ yellow, C–, KC–, P+ orange; presence of rhizocarpic and psoromic acids confirmed via literature correlation.

Molecular Findings (ITS/BLAST/ML-Fig. 5): The ITS sequence ERCH ROB 0.002 was placed within a strongly supported clade (bootstrap value 99%) containing reference *Rhizocarpon geminatum* accessions (e.g., KP314320.1, KY266908.1). This clade was clearly separated from

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phylogenetically proximate taxa such as *R. grande* and *R. polycarpum*. The molecular data fully support the morphological assignment of the specimen as *R. geminatum*.

Notes: *Rhizocarpon geminatum* is a relatively uncommon species that occasionally co-occurs with *R. geographicum* in Antarctic saxicolous habitats. It is morphologically distinguishable by its paler, more uniform areolate thallus, smaller apothecia, and narrower, shorter ascospores (up to 22 µm). Unlike *R. polycarpum*, it lacks elongate muriform spores. Additionally, it closely resembles *R. disporum*, but can be separated by ascus structure: *R. geminatum* typically possesses two-spored asci, whereas *R. disporum* has one-spored asci (Øvstedal and Lewis-Smith, 2001). The species shows a consistent K+ yellow and P+ orange reaction, further aiding in field identification. Phylogenetic analysis placed *R. geminatum* in a distinct, well-supported clade, separate from other *Rhizocarpon* taxa, corroborating its morphological and chemical distinctiveness.

Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Robert Island, 62°24′S, 59°30′W, on siliceous rock, leg. Bülent Gözcelioğlu, ERCH ROB 0.002.

Rhizocarpon polycarpum **(Hepp) Th. Fr. (Fig. 3E)**

Habitat / Substrate: Collected from siliceous rock outcrops on Robert Island, generally occurring in more sheltered microsites compared to *R. geographicum*, and sometimes found in partial shade or mossy rock crevices.

Morphological and Anatomical Characteristics: Thallus crustose, continuous to slightly areolate, yellow-green to olive-green, lacking a distinct prothallus. Areoles irregular, less sharply delimited than in *R. geographicum*. Apothecia lecideine, black, 0.4–0.9 mm in diameter, flat to slightly convex, with a distinct, persistent margin. Epihymenium dark brown, hymenium hyaline (80–100 µm), hypothecium brownish to dark brown. Asci Teloschistes-type, 8-spored. Ascospores muriform, dark brown when mature, broadly ellipsoid to elongate, 30–45 × 12–20 µm.

Spot Test Results: Thallus K+ yellow, C–, KC–, P–; consistent with rhizocarpic acid presence. No unique chemical differentiation from closely related *Rhizocarpon* species was observed via spot tests.

Molecular Findings (ITS/BLAST/ML-Fig. 5): The ITS sequence obtained from ERCH ROB 0.007 showed 98.7% similarity to *Rhizocarpon polycarpum* sequences in GenBank. Maximum Likelihood analysis clustered the sequence with confirmed *R. polycarpum* accessions (e.g., AF483616.1, OR021810.1, OR021811.1) in a strongly supported clade (bootstrap value 99%). This clade was clearly separated from other taxa, including *R. geographicum* and *R. geminatum*. The observed sequence divergence was moderate, yet consistent with interspecific variation reported within the genus *Rhizocarpon*.

Notes: *Rhizocarpon polycarpum* is morphologically similar to *R. geographicum*, but can be distinguished by its broader, more elongate muriform spores and a slightly more diffuse thallus structure. It also differs from *R. geminatum* by having larger, darker spores and broader areoles. Among morphologically similar taxa, *R. richardii* shows an I+ blue medulla and apothecial crystals with a green K– epihymenium, features absent in *R. polycarpum*. *R. umense* is distinguishable by its significantly smaller spores and reduced thallus, while *R. badioatrum*, though molecularly related, differs in spore and epihymenium characteristics and K/I reactions (Øvstedal & Lewis-Smith, 2001; Wang et al., 2015). In our study, although its spot test results were similar to other *Rhizocarpon* species, the integration of spore morphology, apothecial traits, and ITS sequence divergence confirmed its identity. Ecologically, *R. polycarpum* appears to prefer slightly more humid or shaded microhabitats compared to its congeners, suggesting possible niche partitioning on Robert Island.

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Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Robert Island, 62°24′S, 59°30′W, on siliceous rock, leg. Bülent Gözcelioğlu, ERCH ROB 0.007, ERCH ROB 0.008.

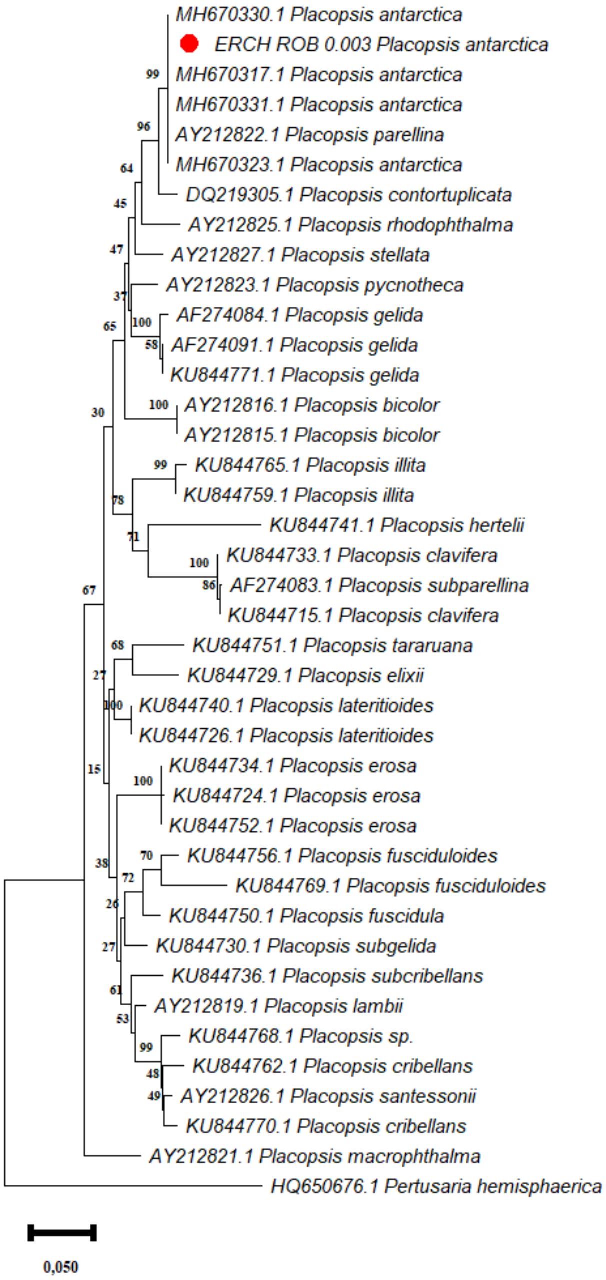

Figure 3. Maximum Likelihood phylogenetic tree of *Placopsis* species based on ITS rDNA sequences. The Robert Island specimen (ERCH ROB 0.003; highlighted in red) was clustered within a strongly supported clade of *Placopsis antarctica* (bootstrap support 99%). Bootstrap values above 50% are shown near branches. *Pertusaria hemisphaerica* (HQ650676.1) was used as the outgroup. The scale bar represents 0.05 substitutions per nucleotide position.

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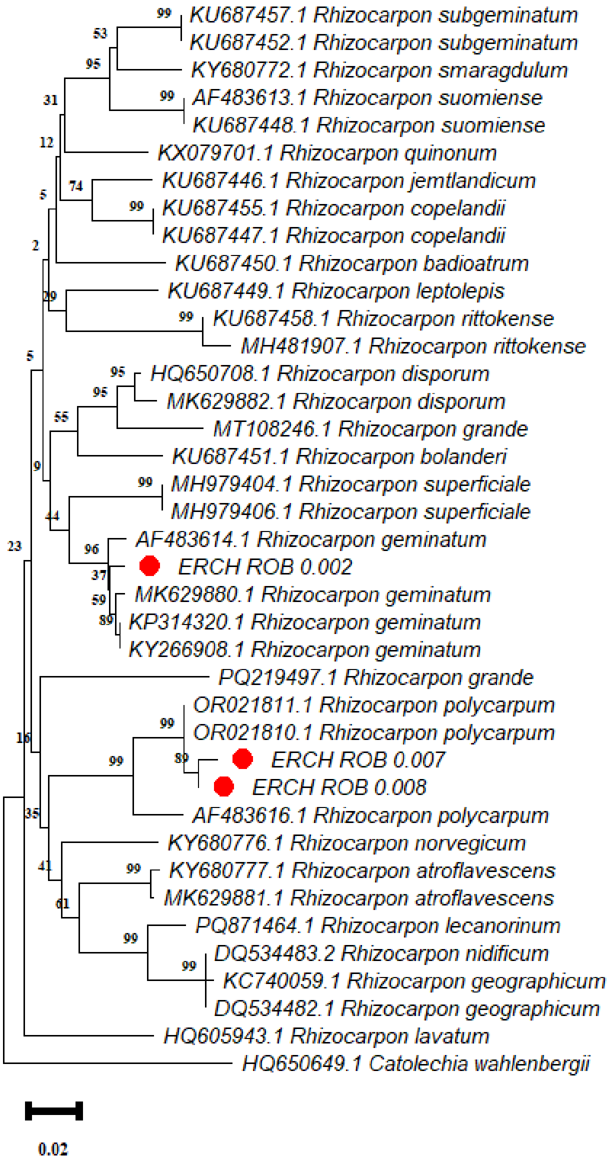

Figure 5. Maximum Likelihood phylogenetic tree of *Rhizocarpon* species based on ITS rDNA sequences. Specimens from Robert Island (ERCH ROB 0.002 and ERCH ROB 0.007–0.008; highlighted in red) are clustered respectively with *R. geminatum* and *R. polycarpum* reference accessions. Bootstrap values (≥50%) are indicated at branch nodes. *Catolechia wahlenbergii* (HQ650649.1) was used as the outgroup. Scale bar represents 0.02 substitutions per site.

***Usnea aurantiacoatra* (Jacq) Bory (Fig. 3F)**

Habitat / Substrate: Collected from rock surfaces on Robert Island. Frequently observed growing on bare rock as well as over other lichens (e.g., *Umbilicaria antarctica*), indicating a degree of substrate flexibility. Found in moderately exposed, cool and humid microhabitats.

Morphological and Anatomical Characteristics: Thallus fruticose, shrubby, erect to decumbent, typically 2–6 cm in height. Branches cylindrical, thick, and matte greenish-yellow to yellow. Soredia abundant, covering much of the thallus surface. No apothecia observed. Medulla white; cortex well-developed. Lacks isidia and cilia.

Spot Test Results: Thallus K–, C–, KC–, P–; chemical analysis suggests presence of usnic acid, although standard spot tests were negative, which is consistent with low concentration levels reported in some populations.

Molecular Findings (ITS/BLAST/ML-Fig 6): BLASTn comparison revealed 99.3% similarity with *Usnea aurantiacoatra* sequences in GenBank (e.g., JQ314840.1, JQ314842.1). Maximum Likelihood analysis placed the ERCH ROB 0.005 sequence within a monophyletic clade of confirmed *U. aurantiacoatra* accessions, with moderate bootstrap support (62%). This clade was clearly separated from other morphologically similar fruticose taxa such as *U. antarctica* and *U. sphacelata.* No signs of cryptic divergence or polyphyly were observed within the *U. aurantiacoatra* group, and the molecular findings were consistent with the morphological identification.

Notes: *Usnea aurantiacoatra* is a widespread fruticose lichen in the maritime Antarctic, particularly common on rocky substrates in both coastal and inland exposed habitats. It exhibits high tolerance to desiccation, subzero temperatures, and UV radiation. The thallus is typically sorediate and may or may not develop apothecia. Usnic acid is present as a major secondary metabolite. A well-developed central medullary axis distinguishes *U. aurantiacoatra* from superficially similar taxa such as *Sphaerophorus globosus*, which lacks such an axis and has a different cortical organization. It is further differentiated from the closely related *U. antarctica* based on several characters: while both share a thick central axis and similar pigmentation, *U. antarctica* develops extensive soredia over the entire thallus and has smoother branches, whereas *U. aurantiacoatra* exhibits heavily warted branches and typically lacks soredia. Radiating fibrils are rare in *U. aurantiacoatra* but absent in *U. antarctica*. Thallus pigmentation ranges from pale yellow in *U. aurantiacoatra* to dark brown in *U. antarctica*. Molecular data supported this morphological separation. Additionally, *U. aurantiacoatra*'s occasional epiphytic growth on other lichens (e.g., *Umbilicaria* spp.) may reflect ecological adaptation to limited substrate availability in Antarctic lithic environments (Clerc and Herrera-Campos, 1997).

Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Robert Island, 62°24′S, 59°30′W, on siliceous rock, leg. Bülent Gözcelioğlu, ERCH ROB 0.005.

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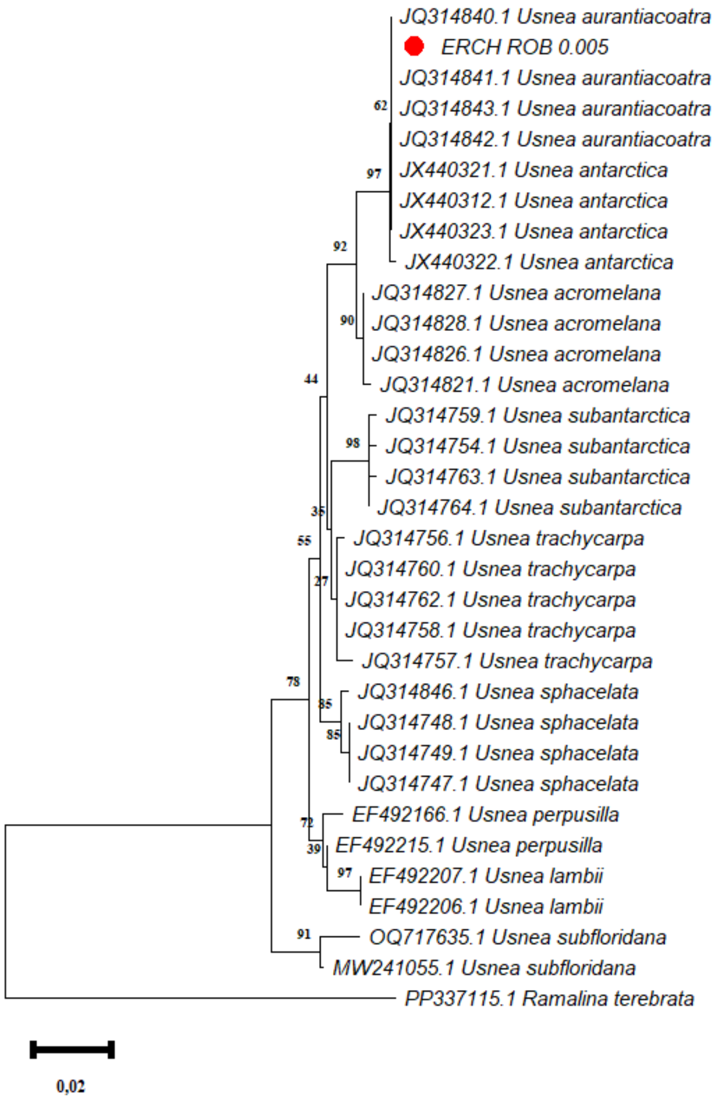

Figure 6. Maximum Likelihood phylogenetic tree of *Usnea* species based on ITS rDNA sequences. The Robert Island specimen (ERCH ROB 0.005; highlighted in red) clustered with *Usnea aurantiacoatra* accessions, forming a distinct clade separate from *U. antarctica* and other morphologically similar fruticose taxa. Bootstrap values ≥50% are shown at nodes. *Ramalina terebrata* (PP337115.1) was used as the outgroup. Scale bar indicates 0.02 substitutions per site.

4. CONCLUSIONS

This study presents an integrative taxonomic evaluation of six lichenized fungi species from Robert Island (Antarctic Peninsula), combining classical morphological and anatomical analyses with ITS-based molecular data. A total of six taxa were identified, encompassing representatives from crustose, foliose, and fruticose growth forms, all of which are known components of Antarctic terrestrial ecosystems.

Morphological and anatomical investigations provided the primary framework for species identification, while ITS sequencing and phylogenetic analysis served to confirm taxonomic

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placements and clarify species boundaries. The application of BLAST comparison and Maximum Likelihood phylogenetics effectively validated the identifications of taxa such as *Lecanora polytropa*, and *Usnea aurantiacoatra*, while also confirming the distinctiveness of morphologically similar species (e.g., *Rhizocarpon geminatum* vs. *R. polycarpum*).

The results contribute to the growing knowledge of Antarctic lichen biodiversity, particularly from the underexplored Robert Island. The confirmation of known species and their precise delimitation enhances the baseline data required for future biodiversity monitoring, climate response studies, and ecological assessments in the region.

Overall, this study demonstrates the value of integrative taxonomy in polar mycology and highlights the importance of combining field-based morphological observations with molecular tools to accurately assess species diversity in remote and ecologically sensitive environments.

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