

Научная статья

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**ДНК-КОДИРОВАНИЕ НЕКОТОРЫХ ЛИХЕНИЗИРОВАННЫХ
ГРИБОВ, СОБРАННЫХ НА ОСТРОВЕ ЛИВИНГСТОН,
И ВСТРЕЧАЮЩИХСЯ В РОССИИ (СЕВЕРНАЯ ЕВРАЗИЯ)**

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Аннотация. Были изучены пять видов лишайников (грибов) с биполярным ариалом, обитающих как на Антарктическом полуострове, так и на севере России. Полученные данные свидетельствуют об их широкой экологической толерантности и способности к расселению на большие расстояния, а также дают ключевую информацию о биоразнообразии и биогеографии полярных регионов.

Ключевые слова: штрихкодирование ДНК, лишайники, лихенизированные грибы, остров Ливингстон, биполярное распределение

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Original article

**DNA CODING OF SOME LICHENIZED FUNGI COLLECTED
ON LIVINGSTON ISLAND FOUND IN RUSSIA (NORTHERN
EURASIA)**

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Abstract. Five species of bipolar lichen native to both the Antarctic Peninsula and Northern Russia were studied. The obtained data indicate their broad ecological tolerance and ability to disperse over long distance and also provide key information on the biodiversity and biogeography of polar regions.

Keywords: DNA barcoding, lichen, lichenized fungi, Livingston Island, bipolar distribution

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Introduction

Antarctica, with about 98% of its 14 million km² surface covered by ice, hosts limited vascular plants but a rich diversity of lichens, the dominant terrestrial organisms adapted to extreme conditions [1]. Although lichenological research spans nearly two centuries, taxonomic uncertainties and limited molecular data leave biodiversity only partly resolved [2–4]. DNA-based studies, especially of nrITS regions, have clarified species identities and revealed new records for the continent [5].

Livingston Island harbors around 250 lichenized and lichenicolous fungi [6–8], with genera such as *Amandinea*, *Buellia*, *Lecanora*, *Umbilicaria*, and *Usnea*. However, identifications have relied solely on morphology and anatomy, and no molecular studies have yet been conducted. This lack of genetic data limits knowledge of phylogeny, biogeography, and potential cryptic species, as well as links with Northern Eurasian floras.

The present study addresses this gap by DNA barcoding lichenized fungi collected from Livingston Island during the 6th Turkish Antarctic Expedition (TAE 6, 2022), including taxa also found in Russia. Integrative analyses—morphological, anatomical, and molecular—were applied, with nrITS sequences generated for each species. These sequences provide a valuable reference for phylogenetic and biogeographical research, and enable direct comparison with Eurasian conspecifics. By contributing baseline molecular data, this study supports biodiversity assessment, conservation planning, and ecological monitoring in the Antarctic region.

Materials and Methods

Lichen samples were collected from Livingston Island (South Shetland Islands, Antarctic Peninsula) during the 6th Turkish Antarctic Expedition (TAE 6, 2022) by the first author, and are preserved in the ERCH Lichen Herbarium, Erciyes University. Species identification combined morphological, anatomical, and molecular analyses.

Morphological features were studied with a stereomicroscope, while anatomical structures were examined under a compound light microscope. Polarized light microscopy was used to detect crystals and granules in apothecia. Thallus dimensions were measured, and identifications were compared with previous records for Livingston Island [6–8].

Genomic DNA was extracted from dried thallus or ascomata tissues (20–100 mg) using the DNeasy Plant Mini Kit (Qiagen). The nrITS region was amplified with primers ITS1-F and ITS4 [5]. PCR (50 µl) consisted of 2 µl template DNA, 2 µl of each primer, 25 µl 2× Taq MasterMix, and 19 µl water. The program included: 95 °C 5 min; 35 cycles of 94 °C 1 min, 55 °C 1 min, 72 °C 1 min; final extension 72 °C 10 min. Amplicons were checked on 1.6% agarose gels, purified, and sequenced bidirectionally by a commercial service. Forward and reverse reads were assembled and edited in BioEdit v7.2.5 [9]. Reference sequences from Russian collections were included for comparative analysis. Alignments were generated and analyzed in MEGA11 [10]. The Kimura 2-parameter model was selected, and Maximum Likelihood trees were built with 1000 bootstrap replicates. Outgroups were chosen based on related clades. Nodes with $\geq 70\%$ bootstrap support were considered reliable. Final alignments were deposited in GenBank (Table).

GenBank accession numbers for lichen specimens from Livingston Island

Species	Specimen Code (ERCH LV)	GenBank Accession No.
<i>Candelariella vitellina</i>	0,101	–
<i>Lecanora polytropa</i>	0,095	–
<i>Rhizocarpon geographicum</i>	0,017	PX112972
<i>Rusavskia elegans</i>	0,078	–
<i>Umbilicaria decussata</i>	0,104	–

Results and Discussion

The results of morphological, anatomical, and molecular analyses for each taxon are presented below. To provide a comprehensive interpretation, morphological descriptions and ecological notes are integrated with molecular phylogenetic findings. This combined approach confirms species identities, reveals new distributional records for Livingston Island, and clarifies phylogenetic relationships among taxa. Species-specific results are detailed in subsections, while the general discussion emphasizes diversity patterns, biogeographical significance, and taxonomic implications. Special attention is given to the five taxa shared between Antarctica and Russia (*Rhizocarpon geographicum*, *Candelariella vitellina*, *Lecanora polytropa*, *Rusavskia elegans*, and *Umbilicaria decussata*), allowing comparison of their inter-regional distributions and phylogenetic placements.

Candelariella vitellina (Hoffm.) Müll. Arg.

Description: Thallus almost reduced, appearing as scattered very small granules on various parts of the substrate, especially concentrated at the base of apothecia, egg-yolk yellow in color. Apothecia lecideine, 0.2–0.4 mm in diameter. Disc orangish yellow, roundish, with a light yellow margin; margin very thin and sometimes crenulate. Epihymenium dark brown, 30–40 μm . Hymenium hyaline, 75 μm . Hypothecium hyaline to slightly brownish, 40–50 μm . Paraphyses branched, septate, containing oil droplets, 3–4.5 μm wide. Asci 12–16-spored, 65 \times 15 μm . Ascospores simple, ellipsoid, 10–14 \times 3–6 μm . Pycnidia not observed (fig. 1).

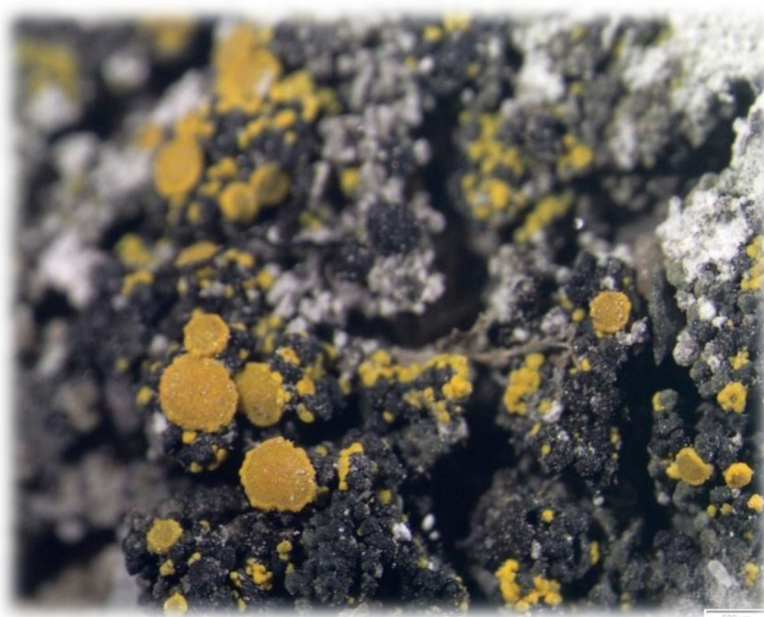


Fig. 1. *Candelariella vitellina* from Livingston Island (LV 0.101). The black thallus visible beneath the apothecia belongs to another lichen species

Specimen Examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Livingston Island, near St. Kliment Ohridski Base, 62°38'27"S 60°21'53"W, alt. 15 m, 17 February 2022, on rock, leg. M.G. Halıcı, ERCH LV 0.101.

Ecology and Distribution: This species has a broad ecological amplitude. While it predominantly grows on siliceous rocks, it also occurs on bricks, roof tiles, mosses, soil, and occasionally tree bark. It is cosmopolitan in global distribution. In Antarctica, it has been reported from South Georgia, South Orkney Islands, South Shetland Islands, the Antarctic Peninsula [1], and continental Antarctica (Victoria Land) [11]. It is also known from Russia (Northern Eurasia).

Phylogeny: In the nrITS phylogenetic tree (fig. 2), the specimen from this study (LV 0.101) clusters with previously sequenced *C. vitellina* specimens from various regions (e.g., AJ460085.1, MG271776.1, EF535200.1) with high bootstrap support (85%), confirming its identity. *C. vitellina* forms a clade closely

related to *Candelariella faginea* and *Candelariella granuliformis*. *C. faginea* differs in occurring on tree bark and having a blastidiate yellowish-green thallus [12], whereas *C. granuliformis* has a paler yellow thallus composed of granules resembling blastidia [13].

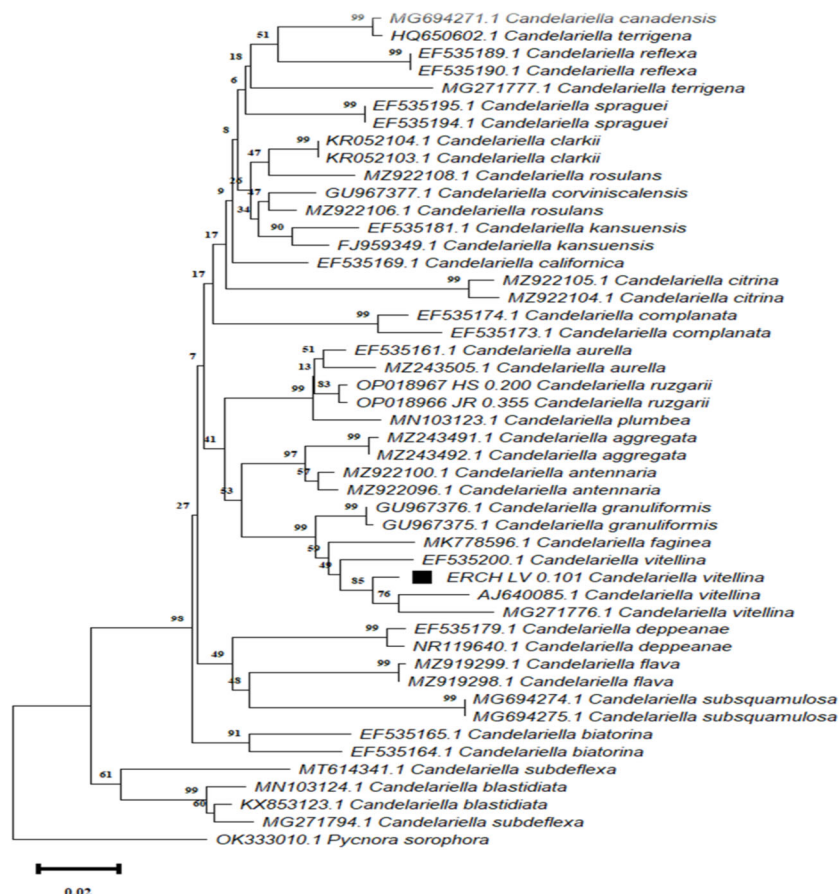


Fig. 2. Maximum Likelihood phylogenetic tree of *Candelariella vitellina* and related taxa based on nrITS sequences

Discussion: *Candelariella vitellina* is morphologically close to *C. rosulans* and can be difficult to distinguish in poorly developed specimens. *C. vitellina* typically shows a crenulate apothecial margin and smaller, squamulose thalli, whereas *C. rosulans* has a more lobate thallus. It may also resemble *C. coralliza*, but the latter forms coralloid cushions with immersed apothecia, unlike the flattened, squamulose thallus of *C. vitellina*. When sterile, it can be mistaken for *C. kuusamoënsis*, which has a thicker, more orange, coralloid thallus.

C. vitellina shows a broad bipolar distribution, occurring in both Antarctica and Russia (Northern Eurasia) [1, 14]. Its presence in these distant regions reflects wide ecological tolerance and dispersal ability. While Russian records (e.g., Sakha, Siberia, Caucasus) confirm its distribution, no ITS sequence data from Russian specimens are yet available in GenBank, limiting direct molecular comparisons.

Lecanora polytropa Hoffm.) Rabenh.

Description: Thallus crustose, cream, almost reduced, present only as yellow-cream remnants at the base of apothecia. Apothecia sessile, flat, disc greenish yellow, or cream-yellow; margin distinct, persistent, concolorous with the thallus, cream-yellowish. Epihymenium brown. Hymenium hyaline. Hypothecium hyaline to brownish hyaline. Asci 8-spored. Ascospores simple, hyaline, ellipsoid, $6\text{--}15 \times 4\text{--}7 \mu\text{m}$. Pycnidia not observed. Thallus medulla and cortex K-, K/I-, C--, KC- (fig. 3).

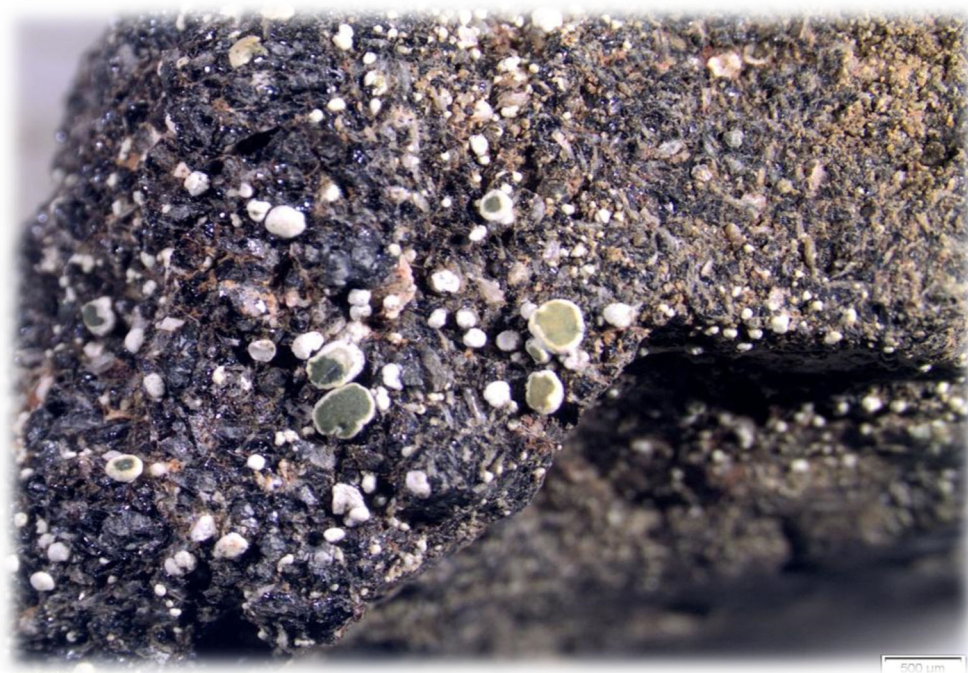


Fig. 3. *Lecanora polytropa* from Livingston Island (LV 0.095)

Ecology and Distribution: Often found on bird-manured rocks, particularly under nesting sites of storm petrels, ornithophilous, occurring at 2–400 m a.s.l. [1]. It has been known from Bipolar; Europe, Antarctica [1]. In Antarctica, reported from South Georgia, South Orkney Islands, South Shetland Islands, Antarctic Peninsula [1], Schirmacher Oasis, King George Island [15], and Victoria Land.

Specimen Examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Livingston Island, near St. Kliment Ohridski Base, $62^{\circ}38'27''\text{S}$ $60^{\circ}21'53''\text{W}$, alt. 10 m, 17 February 2022, on rock, leg. M.G. Halıcı, ERCH LV 0.095.

Phylogeny: In the nrITS phylogenetic tree (fig. 4), the specimen from this study (LV 0.095) groups with *L. polytropa* reference sequences from GenBank (PP104700.1, LC742638.1, PP104706.1, PQ279286.1) with high bootstrap support (100%), confirming its identification. *L. polytropa* is phylogenetically close to *Lecanora fuscobrunnea*, from which it differs morphologically by the presence of

pigmented paraphyses apices and paler thallus. It also shows morphological similarity to *L. intricata*, but differs in having smooth areole margins (vs. crenulate in *L. intricata*) and a more regular surface [1].

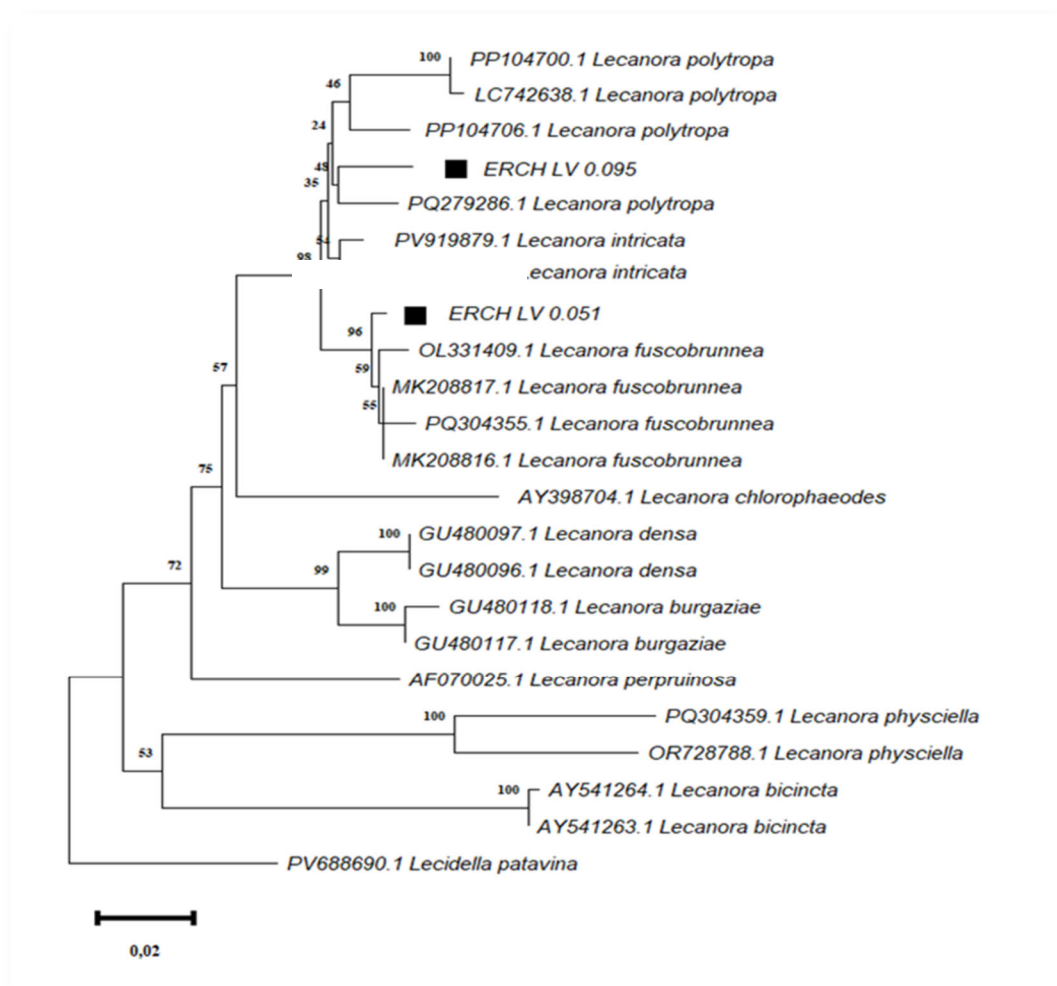


Fig. 4. Maximum Likelihood phylogenetic tree of *Lecanora polytropa* and related taxa based on nrITS sequences

Rhizocarpon geographicum (L.) DC

Description: Thallus crustose, areolate, sometimes effigurate, yellow-green in colour, with a very thin black prothallus. Apothecia abundant, scattered among areoles, circular or angular, 0.15–0.30 mm in diameter (n=10). In young apothecia, the margins are distinct; in mature and convex apothecia, the margins are reduced. Epihymenium brown, 35–40 µm thick; hymenium hyaline, 60–70 µm high; hypothecium brown, 52–75 µm thick. Asci 8-spored, 65–70 × 14–25 µm. Ascospores muriform or submuriform, brown (immature spores hyaline and 1-septate), 20–30 × 10–19 µm (n=15). Length/width ratio 1.18–2.50. Medulla K/I+ blue; epihymenium K+ red (fig 5).

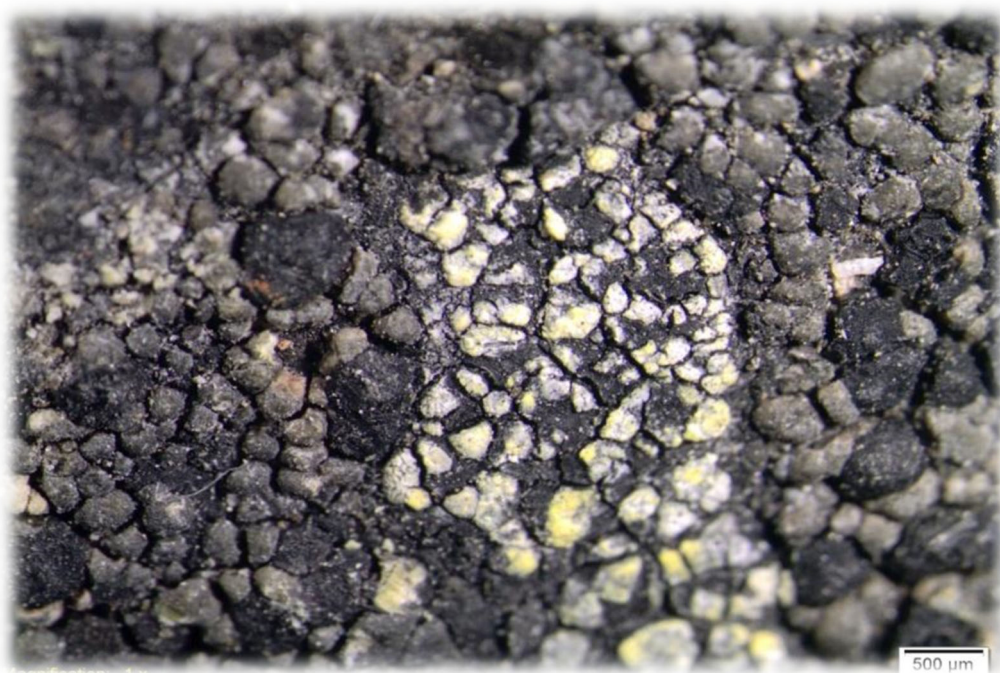


Fig. 5. *Rhizocarpon geographicum* from Livingston Island (ERCH LV 0.017).
The thallus is bordered by surrounding areoles and apothecia of *R. grande* (ERCH LV 0.016)

Ecology and Distribution: Cosmopolitan in cold regions on rocks [1]. In Antarctica it has been reported from South Georgia, Bouvetøya, South Orkney Islands, South Shetland Islands (King George Island; [15]), Antarctic Peninsula, Continental Antarctica [1], Botany Bay–Granite Harbour–Ross Sea, Schirmacher Oasis, Victoria Land (Cape Sastrugi, Football Saddle, Harrow Peaks, Stefania Cirque, Teall Nunatak, Vegetation Island). The species has previously been reported from Livingston Island, Antarctica.

Specimen examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Livingston Island, near St. Kliment Ohridski Base, 62°38'27"S 60°21'53"W, alt. 2 m, 17 February 2022, on rock, leg. M.G. Halıcı, ERCH LV 0.017.

Phylogeny: The phylogenetic analysis based on nrITS sequences placed the Livingston Island specimen (ERCH LV 0.017) within a well-supported clade (ML bootstrap = 100%) together with other *R. geographicum* sequences from various Antarctic localities, including ERCH HS 0.199 and GenBank accessions KC740059, DQ534482, and DQ534481. This clade also included *R. nidificum* (DQ534483.2) as a closely related taxon, but with strong bootstrap separation, confirming its distinct species status. The topology supports the monophyly of *R. geographicum* and its clear distinction from other Antarctic *Rhizocarpon* species such as *R. aff. atrofusces* and *R. smaragdulum* (fig. 6).

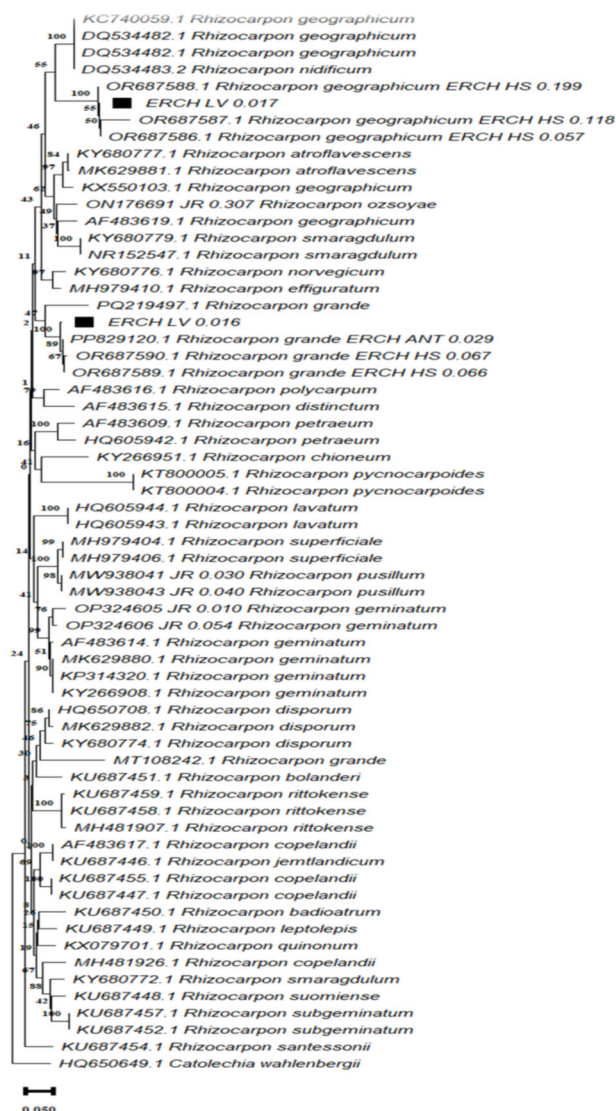


Fig. 6. Maximum Likelihood phylogenetic tree of *Rhizocarpon geographicum*, *R. grande* and related taxa based on nrITS sequences

Discussion: The specimen LV 0.017 from Livingston Island matches the description of *Rhizocarpon geographicum* with its yellow-green areolate thallus, abundant apothecia, and muriform brown ascospores ($20\text{--}30 \times 10\text{--}19 \mu\text{m}$) [1]. Found at 2 m a.s.l., it extends the Antarctic distribution of the species, which spans from coastal to high-altitude habitats (up to 2400 m). Its wide ecological range reflects strong environmental tolerance. Phylogenetic analysis (fig. 6) places LV 0.017 within the *R. geographicum* clade (ML bootstrap = 100 %), including Antarctic and non-Antarctic sequences, with minimal divergence across distant populations. This congruence of morphology, ecology, and genetics confirms its persistence in maritime Antarctica. The species also occurs in Northern Eurasia, including Russia, where its type was collected in the Ural Mountains. However, no ITS sequences with precise Russian metadata are available in GenBank, limiting direct molecular comparison.

Rusavskia elegans (Link) S.Y. Kondr. & Kärnefelt

Description: Thallus foliose, bright orange, 3–5 cm in diameter, lobate. Lobes flat to slightly convex. Apothecia absent. Both thallus and apothecia K+ purple (fig. 7).



Fig. 7. *Rusavskia elegans* from Livingston Island (ERCH LV 0.078)

Distribution and Ecology: This species commonly grows on bird-manured rocks and stones, and occasionally on lichens or mosses. It has a bipolar distribution, occurring in Antarctica, New Zealand, South America, North Africa, North America, and Northern Europe. Within Antarctica, it has been reported from the Peninsula, Continental Antarctica, Bouvetoya, South Shetland and South Orkney Islands, as well as South Georgia [1].

Specimen Examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Livingston Island, near St. Kliment Ohridski Base, 62°38'27"S 60°21'53"W, alt. 10 m, 17 February 2022, on rock, leg. M.G. Halıcı, ERCH LV 0.078.

Phylogeny: The nrITS phylogeny places the Livingston Island specimen (ERCH LV 0.078) within the *R. elegans* clade, clustering with sequences from Russia (PP061092, KC179406) and other regions with moderate to high support values. This close genetic relationship between Antarctic and Russian specimens supports the species' broad bipolar distribution and suggests potential long-distance dispersal events (fig. 8).

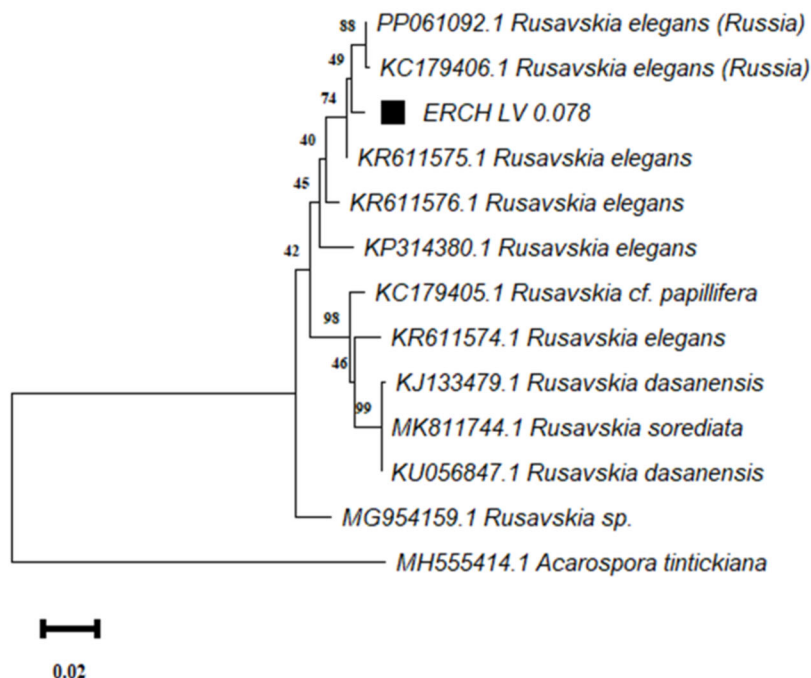


Fig. 8. Maximum Likelihood phylogenetic tree of *Rusavskia elegans* and related taxa based on nrITS sequences

Discussion: *Rusavskia elegans* closely resembles *R. sorediata* (Vain.) S.Y. Kondr. & Kärnefelt morphologically and anatomically; the key distinction lies in the absence of soredia in *R. elegans*, whereas *R. sorediata* produces soredia [1]. It is also morphologically similar to *R. papillifera*, which, like *R. sorediata*, has soredia and can thus be separated from *R. elegans* [1].

This species is confirmed to occur in both Antarctica and Russia (Northern Eurasia), as supported by both herbarium records and molecular data. Notably, ITS sequences derived from Russian specimens are available in GenBank (PP061092, KC179406), enabling direct molecular comparisons with Antarctic populations. The presence of genetically verified records from geographically distant regions highlights the broad ecological tolerance and dispersal capacity of *R. elegans*.

Umbilicaria decussata (Vill.) Zahlbr.

Description: Thallus umbilicate, thick, up to 2 cm in diameter, upper surface dark grey to blackish, with apical parts black and central parts light brownish. Apothecia absent. Lower surface lacking rhizines (fig. 9).



Fig. 9. *Umbilicaria decussata* from Livingston Island (ERCH LV 0.104)

Distribution and Ecology: Occurs in relatively dry areas on sun-exposed surfaces of large rocks. Rare in the South Orkney and South Shetland Islands, becoming more common with increasing altitude, and abundant in areas surrounding the southern Antarctic Peninsula and continental Antarctica. Found from sea level up to 2000 m a.s.l., typically accompanied by *Buellia frigida*, *Pseudophebe minuscula* and *Usnea sphacelata* [1]. Cosmopolitan in cold regions [1]. In Antarctica, South Orkney Islands, South Shetland Islands, Antarctic Peninsula and continental Antarctica [1].

Specimen Examined: Antarctica, Antarctic Peninsula, South Shetland Islands, Livingston Island, near St. Kliment Ohridski Base, 62°38'27"S 60°21'53"W, alt. 10 m, 17 February 2022, on rock, leg. M.G. Halıcı, ERCH LV 0.104.

Phylogeny: Maximum Likelihood phylogenetic analysis places our specimen of *U. decussata* (ERCH LV 0.104) within the same clade as *U. decussata* sequences in GenBank. This clade includes KY948001, KY947790, KY947785 and AF096214, which are reported from Russia. This indicates that the species has a confirmed morphological presence in both Antarctica and Northern Eurasia, including Russia. However, as GenBank records do not always include precise geographical metadata, additional Russian-origin sequences may exist without explicit location tags.



Fig.10. Maximum Likelihood phylogenetic tree of *Umbilicaria decussata* and related taxa based on nrITS sequences

Discussion: *Umbilicaria decussata* closely resembles *U. nylanderiana*. The key difference is that *U. decussata* has a cracked upper surface with white necrotic patches, while *U. nylanderiana* is verrucose. Thallospores are smaller in *U. decussata* (up to 9 μm) than in *U. nylanderiana* (up to 12 μm). Phylogenetic clustering with Russian samples indicates possible genetic affinity between Antarctic and Northern Hemisphere populations, though this requires confirmation with well-documented ITS sequences.

Conclusion

This study provides an integrative taxonomic evaluation of selected lichenized fungi from Livingston Island using DNA barcoding (nrITS) in combination with morphological and anatomical analyses. The approach enabled precise species identification and updated the lichen diversity records for the Island, including new distributional reports for *Lecanora fuscobrunnea* and *Rhizocarpon grande*. Phylogenetic analyses, which also included comparative sequences from Russia, confirmed the distinct genetic identities of all examined taxa and revealed low intra-

specific variation among geographically distant Antarctic and Russian populations. The results contribute to the molecular reference library for Antarctic lichens, enhance the basis for future phylogenetic and biogeographical research, and offer a reference point for long-term biodiversity monitoring in the maritime Antarctic.

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