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DNA BARCODING OF SOME LICHENIZED AND LICHENICOLOUS FUNGI FROM GALINDEZ ISLAND (ANTARCTIC PENINSULA, ANTARCTICA)

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Abstract
Galindez Island (65° 15' S, 64° 15' W) is one of the Argentine Islands located in the West Antarctic Peninsula, 5-6 km away from the main continent. It has a total surface area of 0.8 km^2 and an annual temperature range of 9–13°C. There are not many studies providing information about lichenized fungi on Galindez Island. This study aimed to DNA barcode some lichenized and lichenicolous fungi from Galindez Island (Antarctic Peninsula, Antarctica). Lichenized fungi were collected from Galindez Island during the 2016-2017 Austral Summer. Stereo and light microscopes were used for the identification of lichen samples. After being anatomically and morphologically identified, DNA barcoding was carried out using the nrITS gene. As a result of the study, DNA barcoding of the following samples was successfully carried out: Lecanora intricata (Ach.) Ach., Mastodia tessellata (Hook. f. & Harv.) Hook. f. & Harv., Raesaenenia usneae (C.W. Dodge) Etayo & Pino-Bodas, Rhizocarpon grande (Flörke ex Flot.) Arnold, Umbilicaria umbilicarioides (Stein) Krog & Swinscow.

Keywords: DNA barcoding, Antarctica, Antarctic Peninsula, lichenized fungi, lichen.

1. INTRODUCTION

Lichens are the main floral elements in Antarctica. Their unique characteristics allow them to thrive in a variety of habitats and survive in extreme temperatures ranging from -50°C to 55°C. Lichens are of great interest for taxonomic and ecological studies due to their high level of adaptation to harsh climatic conditions (Nayaka and Upreti, 2008). In Antarctica, lichens are not significant in the food chain but are the dominant components of terrestrial vegetation (Øvstedal and Lewis-Smith, 2001).

Galindez Island (65°15' S, 64°15' W) is a 0.8 km long island located east of Winter Island, in the Argentine Islands, in the Wilhelm Archipelago, off the Graham Coast, on the west coast of Graham Land (Stewart, 2011).

Data on lichenized and lichenicolous fungi from Galindez Island is very limited (Øvstedal and Lewis-Smith, 2001; Güllü et al., 2017; Halıcı et al., 2017a, 2017b; Kılıç et al., 2017; Avcı et al., 2020; Kahraman Yiğit et al., 2020). Therefore, it is important to conduct research on lichenized and lichenicolous fungi from Galindez Island. Furthermore, thoroughly examining lichens and lichenicolous fungi on the Antarctic continent can lead to a better understanding of the vegetation, provide more accurate regional data, and allow for the evaluation of biogeographic affinities and

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evolutionary trends. In this study, it was aimed to DNA barcoding of some lichenized and lichenicolous fungi from Galindez Island (Antarctic Peninsula, Antarctica).

2. MATERIALS AND METHODS

Lichen samples were collected by the second author from Galindez Island (Antarctic Peninsula, Antarctica). The samples deposited in Erciyes University Lichen Herbarium (ERCH, Türkiye) Standard microscope techniques were used for identifying the specimens, with a standard light microscope and stereomicroscope used for morphological and anatomical examinations. Sections were taken in water and measurements were made from these sections.

Total DNA was extracted from six apothecia using a commercial DNA isolation kit as per the manufacturer's protocol. PCR amplification was carried out using the internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) gene (White et al., 1990). The samples were prepared using 50 µl of standard reaction mixture. Optimal amplification conditions were achieved by combining 25 µl of 2 × Taq PCR MasterMix, 2 µl of ITS1F and ITS4 primers, 2 µl of DNA extracts, and 19 µl of distilled water in each tube. The sequence analysis of the samples were performed by BM Labosis Laboratory located in Ankara, Turkey.

The Clustal W feature of the BioEdit V7.2.6.1 application (Hall, 1999) was utilized to modify and align the *nrITS* sequencing data from lichen samples. Phylogenetic analysis of lichenized fungi samples were conducted using the Maximum Likelihood (ML) method in the Mega 11 software program with 1000 bootstrap replications (Tamura et al., 2021). The Kimura 2-parameter analysis method was chosen for the ML phylogeny. The newly generated data from this study was uploaded to GenBank (Table 1).

Table 1. GenBank accession numbers for the newly generated data in this study.

Species	**Specimen**	**nrITS**
Lecanora intricata	ERCH ANT 0.041	PP829117
Mastodia tessellata	ERCH ANT 0.020	PP829118
Raesaenenia usneae	ERCH ANT 0.047	PP829119
Rhizocarpon grande	ERCH ANT 0.029	PP829120
Umbilicaria umbilicarioides	ERCH ANT 0.035	PP829121

3. RESULTS AND DISCUSSIONS

3.1. *Lecanora intricata* (Ach.) Ach.

Thallus crustose, rimose-areolate. Areoles have crenulate margins, yellowish white. Apothecia abundant, clustered or dispersed through areoles, black, sessile, flat, roundish or mostly angular, 0.4–0.65 mm. Apothecial margin distinct and prominent, pale yellow-green. Epihymenium brown, 100 µm. Hymenium and hypothecium hyaline. Ascospores simple, hyaline, ellipsoid, oil droplets present, 10–12 × 5.5–7 µm. Pycnidium not seen.

Specimen examined: "Antarctica, Antarctic Peninsula, Argentine Islands, Galindez Island, Marina Point, near Vernadsky Research Base, 65°14′45″S 64°15′28″W, alt. 10 m, 17 February 2016, on andesite rock, leg. M. G. Halıcı, ERCH ANT 0.041.

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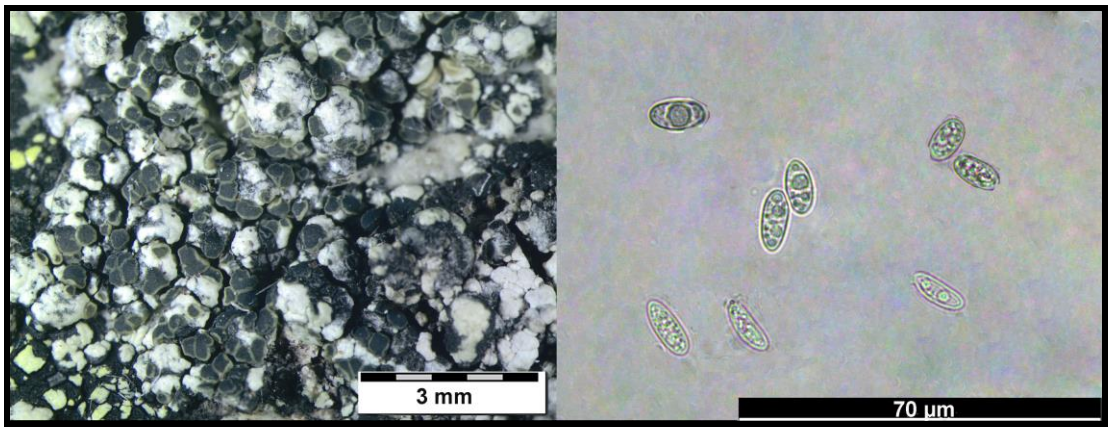

Figure 1. Lecanora intricata, A. Habitus, B. Ascospores.

39 *nrITS* rDNA sequences were used for the phylogenetic analysis of *L. intricata* specimens. Final alignment of the *nrITS* sequence of *L. intricata* in the BLASTn search contained 450 bp after trimming. *Lecidea rubrocastanea* T. Sprib. & Printzen is used as outgroup (Figure 2).

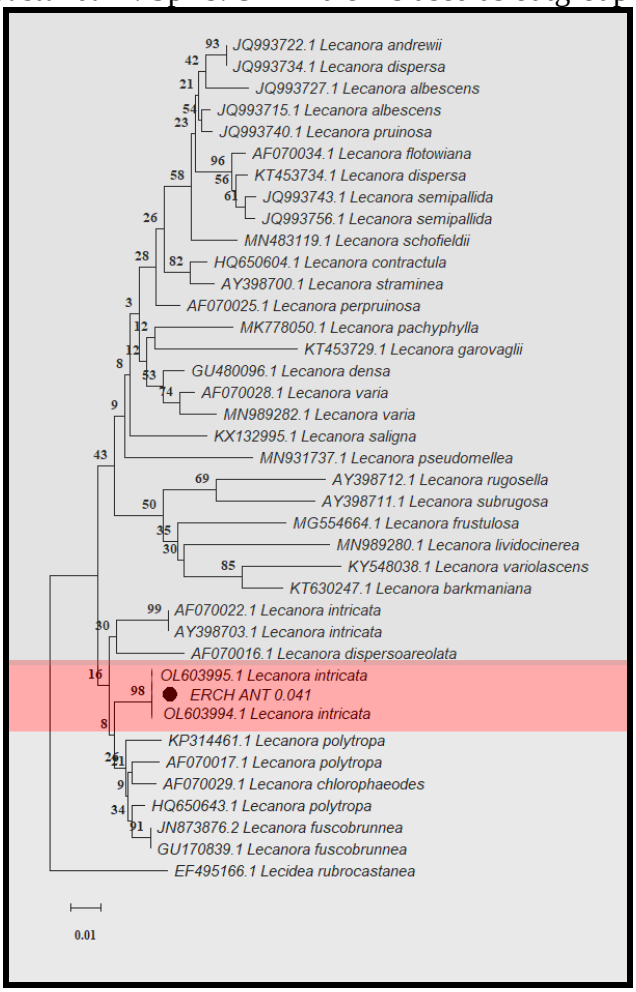

Figure 2. ML phylogeny based on nrITS gene region of Lecanora intricata

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L. intricata is a species that inhabits rocks. It has been reported from Holarctic-highland, boreal-arctic, arctic and highland regions in South America, North America, Europe, and Antarctica (De la Rosa et al. 2016). In Antarctica it has been known from South Shetland Islands (Øvstedal and Lewis-Smith, 2001) and Antarctic Peninsula (De la Rosa et al. 2016).
Lecanora intricata is morphologically very similar to *Lecanora polytropa* (Hoffm.) Rabenh. A According to nrITS gene region analysis shown in Figure 2, the two species are related phylogenetically too. *Lecanora polytropa* is characterized by granules and areoles distributed on the substrate and sessile, ascending and convex apothecia with smooth edges. Usnic acid, rangiformic acid, zeorin, and eulecanoral can be found in *L. polytropa*. *L. intricata* is distinguished from *L. polytropa* by its more continuous thallus, mostly blackish apothecia that are sessile or almost immersed. It also contains especially usnic acid and zeorin in addition to rangiformic acid (Śliwa and Flakus, 2011). In poorly developed specimens, it is challenging to differentiate between the two species based on morphology alone. However, the presence of blackish-dark green, almost immersed apothecia of *L. intricata* helps in distinguishing the two species. In Antarctic samples, *L. intricata* exhibits significant variation in shape and color, with apothecia that may be flat, convex, or very convex in yellowish, blackish, bluish, grayish, or greenish colors. Molecular methods are necessary for accurate differentiation between *L. intricata* and *L. polytropa* (De la Rosa et al., 2016).

3.2. *Mastodia tessellata* (Hook. f. & Harv.) Hook. f. & Harv.

Thallus foliose, in the form of particles up to 4 cm with greenish brown or blackish brown ascending and curved lobes. Perithecia and pycnidium were not observed (Figure 3).
Specimen examined: "Antarctica, Antarctic Peninsula, Argentine Islands, Galindez Island, Marina Point, near Vernadsky Research Base, 65°14′45″S 64°15′28″W, alt. 7 m, 19 February 2016, on rock, leg. M. G. Halıcı, ERCH ANT 0.020".

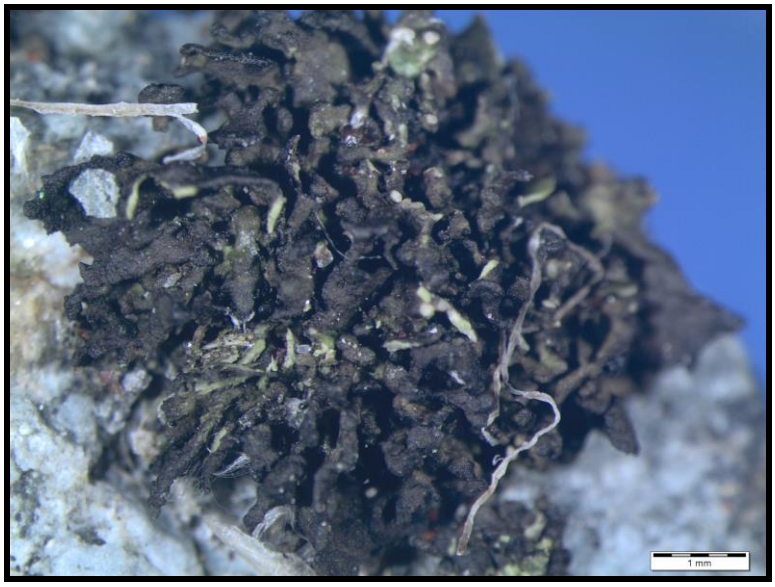

Figure 3. Mastodia tessellata

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19 *nrITS* rDNA sequences were used for the phylogenetic analysis of *M. tessellata* specimens. The final alignment of the nrITS sequence of *M. tessellata* in the BLASTn search contained 575 base pairs after trimming. *Heteroplacidium imbricatum* (Nyl.) Breuss is used as an outgroup (Figure 4).

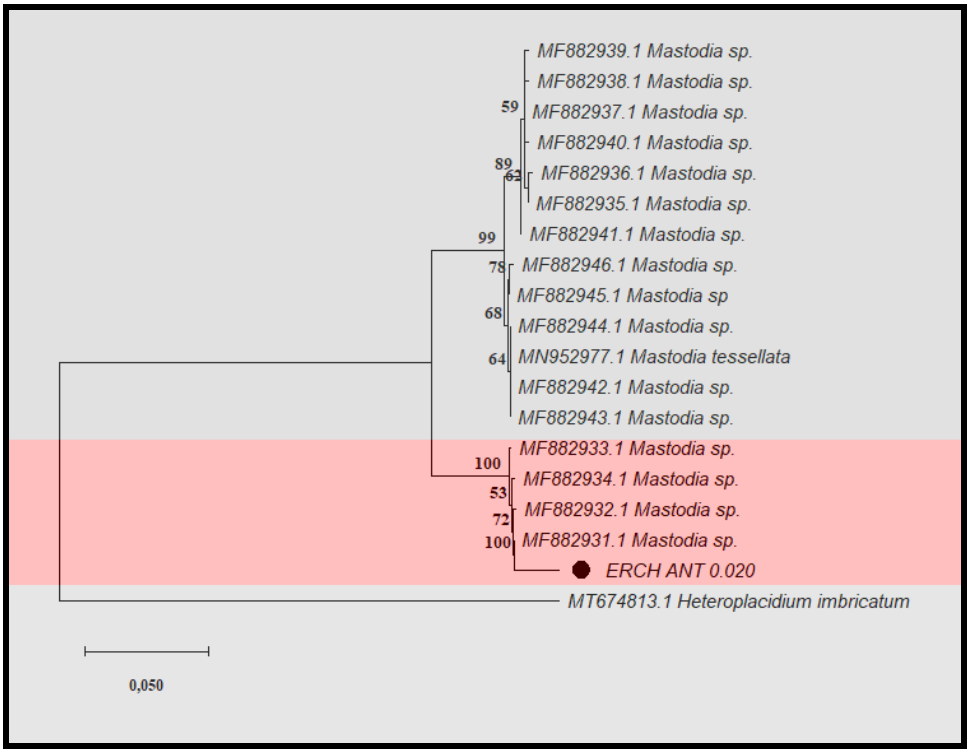

Figure 4. ML phylogeny based on nrITS gene region of Mastodia tessellata

Mastodia tessellata is a very common species especially on rocks near the shore and near bird nests. It also occurs on rocks exposed to sea spray. This species is well-known in North America and Arctic Canada. In Antarctica it has been reported from Continental Antarctica, Antarctic Peninsula and some subantarctic ıslands (Øvstedal and Lewis-Smith, 2001).
M. tessellata is the type species of the *Mastodia* genus and is the only lichenized fungus species known to establish a symbiotic relationship with macro green algae of the *Prasiola* genus (Garrido-Benavent et al., 2020). There is only one nrITS data of *M. tessellata* in GenBank. As seen in Figure 4, there are other *Mastodia* sp. datas in GenBank. ERCH ANT 0.041 matches with four of these data. We believe that our specimen is *M. tessellata* and that these other *Mastodia* species data probably belong to *M. tessellata* as well.

3.3. *Raesaenenia usneae* (C.W. Dodge) Etayo & Pino-Bodas

Lichenicolous on *Usnea* sp. Apothecia present on host branches, black, dull, 0.3–0.8 mm. (Figure 5).
Specimen examined: “Antarctica, Antarctic Peninsula, Argentine Islands, Galindez Island, Marina Point, near Vernadsky Research Base, 65°14′45″S 64°15′28″W, alt. 12 m, 19 February 2016, on rock, leg. M. G. Halıcı, ERCH ANT 0.047”.

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Figure 5. Apothecia of Raesaenenia usneae on host branches

7 *nrITS* rDNA sequences were used for the phylogenetic analysis of *R. usneaea* specimens. The final alignment of the nrITS sequence of *R. usneaea* in the BLASTn search contained 520 base pairs after trimming. *Usnea florida* (L.) F.H. Wigg. is used as an outgroup (Figure 6).

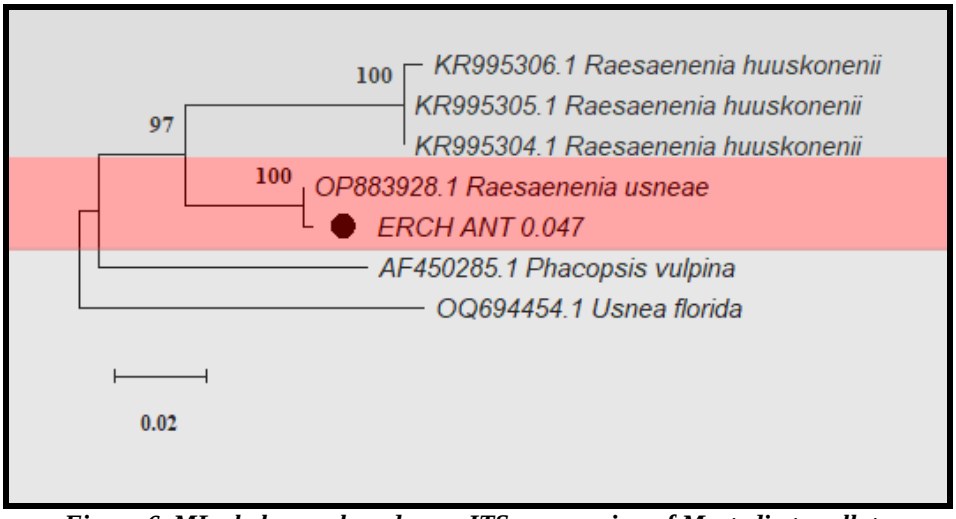

Figure 6. ML phylogeny based on nrITS gene region of Mastodia tessellata

R. usneaea is a lichenicolous fungi that grows on *Usnea* and *Protousnea* species. It has variable apothecia, ranging from effuse on the host's laciniae to subspherical, lobate, or irregular due to aggregation. It has been on various species such as *Protousnea magellanica* (Mont.) Krog, *Usnea antarctica* Du Rietz, *U. aurantiacoatra* (Jacq.) Bory, *U. fasciata* Torr., *U. lethariiformis* Motyka, *U. sphacelata*, *U. trachycarpa* (Stirt.) Müll. Arg. (Etayo and Sancho, 2006).

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R. usneaea is very similar to *Nesolechia falcispora* (Triebel & Rambold) Diederich and *Phacopsis lethariellae* Hafellner & Rambold. *N. falcispora* also grows on *Usnea* species, but it has falciform ascospores, smaller apothecia, and smaller thallus. On the other hand, *P. lethariellae* grows on *Lethariella intricata* (Moris) Krog and has less anastomosing and branched paraphyses and smaller ascomata (Etayo and Sancho, 2006). As seen in Figure 6, ANT 0.047 matches with *R. usneaea* GenBank data with a highly BS support (<95). It is phylogenetically related to *Raesaenenia huuskonenii* (Räsänen) D. Hawksw., Boluda & H. Lindgr, another lichenicolous species. *R. usneaea* grows on *Usnea* species, while *R. huuskonenii* grows on *Bryoria* species.

3.4. *Rhizocarpon grande* (Flörke ex Flot.) Arnold.

Thallus crustose, rimose-areolate, brown. Prothallus present, black. Apothecia present, nearly immersed within areoles or in cracks between areoles, flat, black, 0.4-0.6 mm. Epihymenium brown, 40 µm. Hymenium hyaline, 80-100 µm. Hypothecium brown, 110 µm. Ascus 8-spored. Ascospores muriform, brown, 26–34.5 × 12–16 µm. Pycnidium not observed. Medulla KI+ blue.
Specimen examined: "Antarctica, Antarctic Peninsula, Argentine Islands, Galindez Island, Marina Point, near Vernadsky Research Base, 65°14′45″S 64°15′28″W, alt. 10 m, 17 February 2016, on andesite rock, leg. M. G. Halıcı, ERCH ANT 0.029."

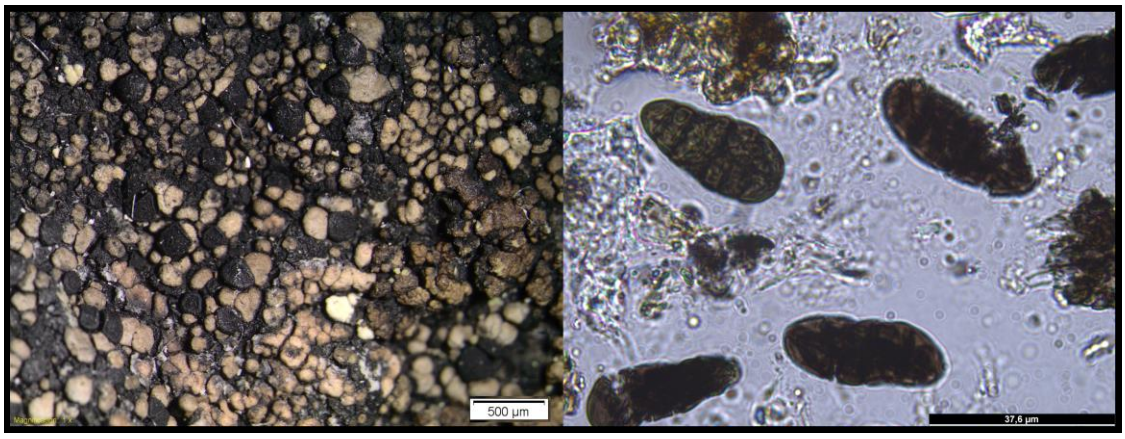

Figure 7. Rhizocarpon grande, A. Habitus, B. Ascospores

31 *nrITS* rDNA sequences were used for the phylogenetic analysis of *R. grande* specimens. The final alignment of the nrITS sequence of *R. grande* in the BLASTn search contained 590 base pairs after trimming. *Catolechia wahlenbergii* (Ach.) Körb. is used as an outgroup (Figure 8).

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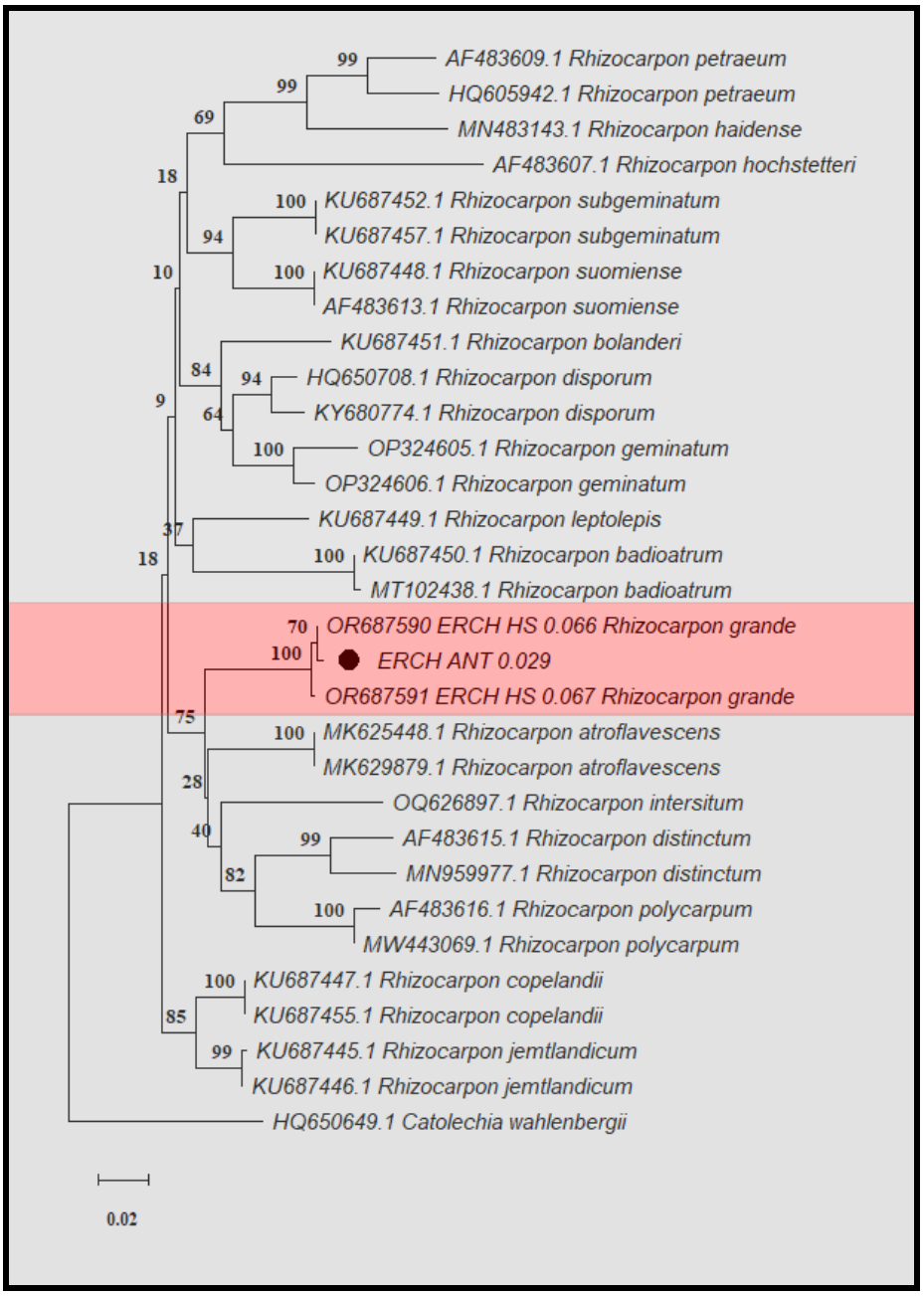

Figure 8. ML phylogeny based on nrITS gene region of Rhizocarpon grande

R. grande is a bipolar species. It has been reported from North America, South Korea, China and Antarctica. R. grande is characterized by large ascospores and its secondary chemistry. It contains norstictic acid, barbatic acid and gyrophoric acid. Anatomically and morphologically, it is similar to *Rhizocarpon intersitum* Arnold. *R. intersitum* generally differs from *R. grande* by its I- medulla, K- epihimenium and smaller ascospores. Also *R. intersitum* contains atranorine unlike *R. grande* (Yiğit and Halıcı, 2024). In GenBank there are two datasets belonging to *R. grande.* They were recently uploaded by Yiğit and Halıcı (2024) from James Ross Island. As seen in Figure 8, ANT 0.029 matches with these two datasets with a high BS support (<95). Phylogenetically *R. grande* is related

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to *Rhizocarpon atroflavescens* Lynge. But *R. atroflavescens* has yellowish thallus and even smaller ascospores (Timdal, 2017).

3.5. *Umbilicaria umbilicarioides* (Stein) Krog & Swinscow

Thallus foliose. Upper surface brown-gray, rimose or not. Margins densely covered with black rhizines. Lower surface grayish brown. Thallospores are present in patches on the lower surface, multicellular, 15–25 µm. No apothecia were observed.
Specimen examined: "Antarctica, Antarctic Peninsula, Argentine Islands, Galindez Island, Marina Point, near Vernadsky Research Base, 65°14′45″S 64°15′28″W, alt. 10 m, 17 February 2016, on andesite rock, leg. M. G. Halıcı, ERCH ANT 0.035".

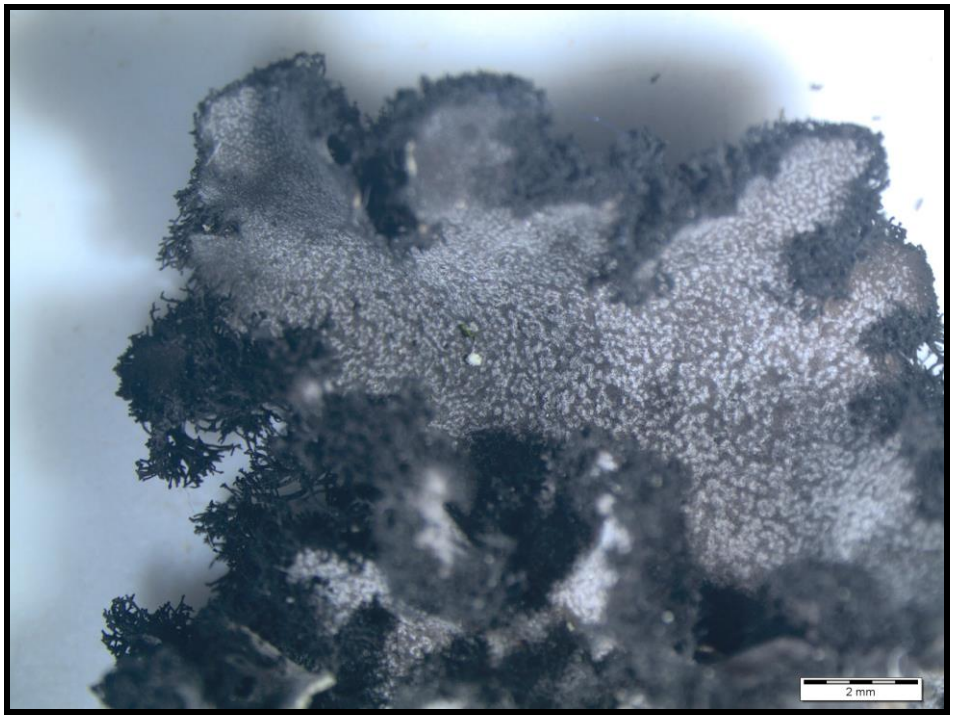

Figure 9. Umbilicaria umbilicarioides

23 *nrITS* rDNA sequences were used for the phylogenetic analysis of *U. umbilicarioides* specimens. The final alignment of the nrITS sequence of *U. umbilicarioides* in the BLASTn search contained 510 base pairs after trimming. *Lasallia pustulata* (L.) Mérat is used as an outgroup (Figure 10).

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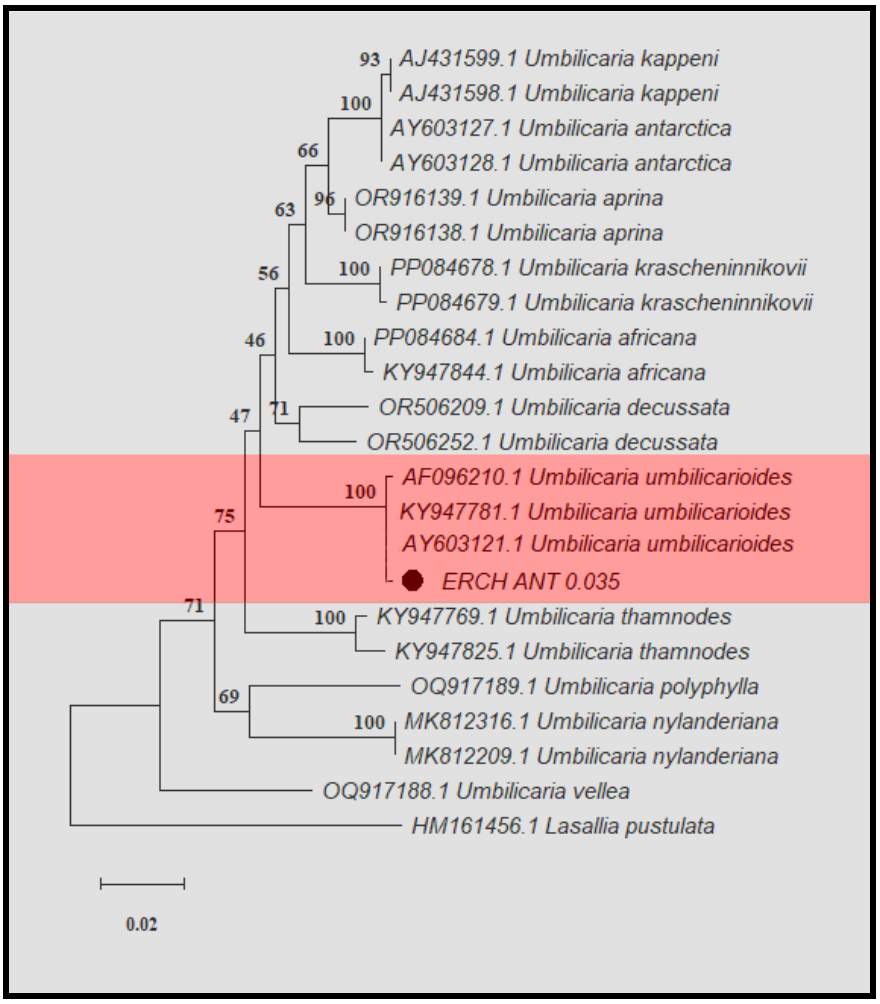

Figure 10. ML phylogeny based on nrITS gene region of Umbilicaria umbilicarioides

U. umbilicarioides is a species known to be present in Northern Europe, East Africa, New Zealand and Antarctica. It is commonly found in the mid-southern Antarctic Peninsula on dry rocks (Øvstedal and Lewis-Smith, 2001).

U. umbilicarioides is morphologically similar to *Umbilicaria cristata* C.W. Dodge & G.E. Baker. *U. cristata* has paler rhizines grouped on its lower surface while *U. umbilicarioides* has black rhizines concentrated on its margins (Øvstedal and Lewis-Smith, 2001). As seen in Figure 10 ANT 0.035 matches with *U. umbilicarioides* datas in GenBank with a highly BS support (<95). *U. umbilicarioides* is related to *U. decussata* phylogenetically. *U. umbilicarioides* has multicellular thallospores while *U. decussata* has unicellular ascospores and dense ridges on its upper surface as distinguishing characteristics (Øvstedal and Lewis-Smith, 2001).

4. CONCLUSIONS

In this study, it was aimed to DNA barcoding of some lichenized and lichenicolous fungi from Galindez Island (Antarctic Peninsula, Antarctica). As a result of the study, DNA barcoding of the following samples was successfully carried out: *Lecanora intricata* (Ach.) Ach., *Mastodia*

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tessellata (Hook. f. & Harv.) Hook. f. & Harv., *Raesaenenia usneae* (C.W. Dodge) Etayo & Pino-Bodas, *Rhizocarpon grande* (Flörke ex Flot.) Arnold, *Umbilicaria umbilicarioides* (Stein) Krog & Swinscow. This article provides detailed descriptions, localities, phylogenetic trees, and discussion of similar species.

5. ACKNOWLEDGEMENTS

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Note: This article has been improved by ChatGPT's GrammarCheck AI to correct grammar mistakes and ensure better word choices after being written by the authors.

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