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# DNA Barcoding of Four Lichenized Fungi from Horseshoe Island (Antarctic Peninsula, Antarctica)

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*nrITS*

**Abstract:** In this study, it was aimed to identify four lichenized fungi species from Horseshoe Island which is located in the west of the Antarctic Peninsula and where the temporary Turkish Science Base is located, through their morphological and anatomical properties and DNA barcoding using the *nrITS* or *mtSSU* gene regions. In this direction, DNA barcoding of samples belonging to *Acarospora macrocyclos* Vain., *Rhizocarpon grande* (Flörke ex Flot.) Arnold, *Mastodia tessellata* (Hook. f. & Harv.) Hook. f. & Harv. and *Verrucaria tesselatula* Nyl. species carried out. Within the scope of the study, *nrITS* data were obtained for the first time for *A. macrocylos* and *R. grande* and *mtSSU* data was obtained for the first time for *M. tessellata* species and uploaded to genBANK.

# Horseshoe Adası (Antarktika Yarımadası, Antarktika)'ndan Bazı Likenleşmiş Mantarların DNA Barkodlaması

**Anahtar Kelimeler**
Antarktika
Biyoçeşitlilik
DNA barkodlama
Horseshoe Adası
Likenleşmiş Mantar
*mtSSU*
*nrITS*

**Öz:** Bu çalışmada Antarktika Yarımadası'nın batısında bulunan ve geçici Türk Bilim Üssü'nün bulunduğu Horseshoe Adası'nda yayılış gösteren dört likenleşmiş mantar türünün morfolojik ve anatomik özellikleri aracılığı ile teşhis edilmesi ve *nrITS* veya *mtSSU* gen bölgeleri kullanılarak DNA barkodlamalarının yapılması amaçlanmıştır. Bu doğrultuda ikinci yazar tarafından Horseshoe Adası'ndan toplanan *Acarospora macrocyclos* Vain., *Mastodia tessellata* (Hook. f. & Harv.) Hook. f. & Harv., *Rhizocarpon grande* (Flörke ex Flot.) Arnold ve *Verrucaria tesselatula* Nyl. species türlerine ait örneklerin DNA barkodlamaları gerçekleştirilmiştir. Çalışma kapsamında *A. macrocylos* ve *R. grande* için ilk defa *nrITS* verileri elde edilirken, *M. tessellata* türü için ilk defa *mtSSU* verileri elde edilmiş ve genBANK'a yüklenmiştir.

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## 1. Introduction

Lichens are the most dominant organisms of Antarctic terrestrial vegetation. Studies on lichens of Antarctica have a history of about two centuries, and especially recently, with the use of DNA-based molecular phylogeny stuides, lichen biodiversity determinaton of the continent has accelerated [1-8]. It is known that the number of lichenized fungi species reported from Antarctica today exceeds 500 [9].

Horseshoe Island is one of the largest islands in Marguerite Bay, west of the Antarctic Peninsula. The island is located at the entrance to Square Bay on the west coast of Graham Lands, occupying it is 12 km long and 6 km

wide. The total area of the island is about 60 km$^2$, two-thirds of which is covered by glaciers or semi-annual ice and snow. 29 lichenized fungi species and 15 moss species have been reported from Horseshoe Island. A temporary Science Camp was established on Horseshoe Island during the TAE III expedition in 2019. The preliminary work required to establish a Turkish Scientific Research Base in this region was completed in 2023 and one step further was taken in the process of establishing the base. Because of that determining lichen biodiversity of Horseshoe Island is important.

In this study, it was aimed to DNA barcoding some lichenized fungi samples collected from Horseshoe Island (Antarctic Peninsula, Antarctica) collected by the second author during the VI. National Antarctic Antarctic Expedition. Collected specimens identified at the species level using morphological characters and these identifications were compared with the species reported in the literature from Horseshoe Island and its surroundings. After DNA isolation from the samples of the studied species, *nrITS* or *mtSSU* gene regions are amplified and then sequence analyses were obtained. The obtained sequence analysis was compared with the sequence datas of other species belonging to the related genus in the GenBank and phylogenetic trees obtained. As a result, in the study, four lichenized fungi species from Horseshoe Island (Antarctic Peninsula, Antarctica) were identified by anatomical, morphological methods and DNA barcodings were made: *Acarospora macrocyclos* Vain., *Mastodia tessellata* (Hook. f. & Harv.) Hook. f. & Harv., *Rhizocarpon grande* (Flörke ex Flot.) Arnold ve *Verrucaria tesselatula* Nyl.

## 2. Material and Method

### 2.1. Materials and morphological observation

Lichen samples were collected by the second author during the 6th Turkish Antarctic Expedition. Lichen samples deposited in Erciyes University Lichen Herbarium (ERCH). Stereomicroscope was used for morphological determinations and light microscope was used for anatomical determinations. Macroscopic observations were conducted using an Olympus S2X7 dissecting microscope equipped with an OLYMPUS SC30 image capture system. Handmade sections of ascomata were analyzed using a Leica DM2500 light microscope, and microphotographs were captured with a Flexacam C1 digital camera.

### 2.2. Isolation, DNA extraction, amplification, and sequencing

For DNA isolation DNeasy Plant Mini Kit (Qiagen) used and isolation carried out according to the manufacturer's instructions. PCR amplifications for the "internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) gene or Mitochondrial small subunit (*mtSSU*) rDNA gene" were performed with total 50 µl standard reaction volume for each sample. Optimum amplification conditions were obtained with "25 µl 2 × Taq PCR MasterMix in each tube with 19 µl of distilled water, 2 µl of DNA extracts and 2 µl of the primers *ITS1F* and *ITS4* or *mrSSUI^* and *mrSSU2^"* [10-12]. The thermal cycling conditions included "an initial denaturation step of 95°C for 5min, followed by 35 cycles of 95°C for 45sec (denaturation), 54°C for 45sec (annealing), and 72°C for 60sec (extension) followed by a final extension period of 72°C for 10min". PCR products' sequence analysis made by the EPIGEN laboratories (Ankara, Türkiye). Thin-layer chromatography (TLC) for lichen substances made by following method of Orange et al. [13].

### 2.3. Phylogenetic analyses

*nrITS* or *mtSSU* sequence results of fungi samples were edited and aligned using the Clustal W option in the BioEdit V7.2.6.1 [14] program. Phylogenetic analysis of lichenized fungi samples were performed using the Maximum Likelihood (ML) method of the Mega 11 (Molecular Evolutionary Genetic Analysis) software program with a rapid 1000 bootstrap replications [15]. For the ML phylogeny analysis method kimura two-parameter was selected.

## 3. Results

### 3.1. *Acarospora macrocyclos* Vain.

Thallus crustose, effigurate, areolate in the center and lobate in the margins, creamish brown, dark brown or almost black, matt or shiny, up to 13 cm diam. Lobes mostly flat, fan shaped or extends paralelly, 1–1.3 mm wide, 2–5 mm high. Prothallus and vegetative propagules absent. Apothecia present, common, aggregated especially on the center of thallus, roundish or sligthly angular, semi-immersed to the thallus, reddish brown, dark brown or black, (0.2–)0.3–*0.5*–0.7(–0.8) mm (n=10) diam. Apothecial margin, prominent, concolorous with thallus, thicker at the young ones. Epihymenium light brown, (20–)–*50*–(–75) µm (n=5). Hymenium hyaline, sometimes with brownish tinge, (65–)–*140*–(–170) µm (n=5). Hypothecium hyaline, sometimes with brownish tinge or beige coloured, (50–)–*120*–(–260) µm (n=5). Ascus poly-spored, more than 100, 130 × 25 µm. Ascospores simple, hyaline, ellipsoid, (3–)–*3.5*–(–4.5) µm (n=10). Ascospore l/w ratio: (1.2–)–*2.25*–(–1.7) µm (n=10). Paraphyses

simple, unbranched, septate, tips are not enlarged, 2-3 µm. Pycnidium not seen. Algae green, chlorococcoid, algal cells 9.5–12.5 × 8.5–12 µm (Figure 1).

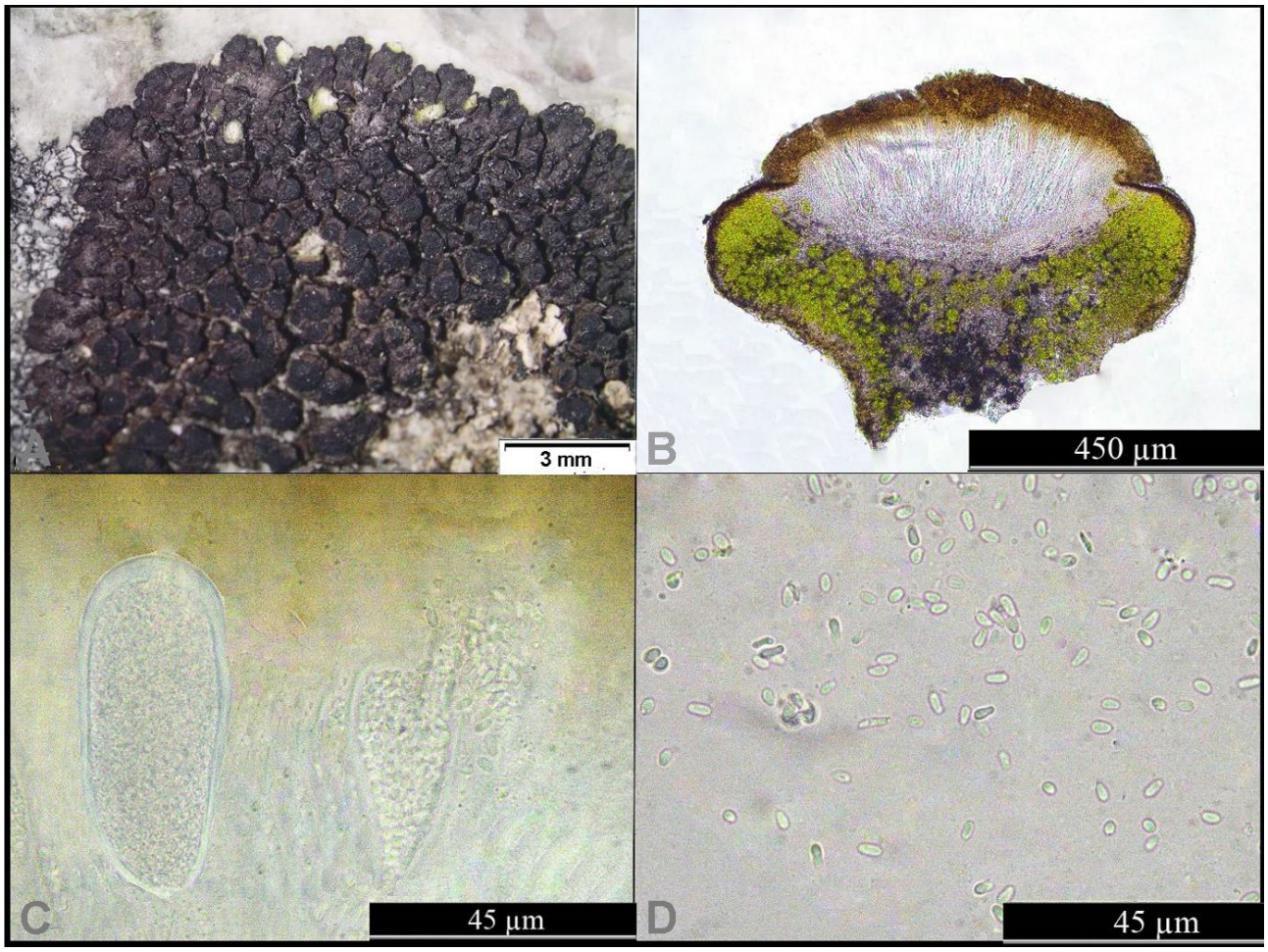


**Figure 1.** *Acarospora macrocyclos*, A. Habitus, B. Apothecia section, C. Ascus, D. Ascospores.

57 *nrITS* rDNA sequences were used for the phylogenetic analysis of *A. macrocyclos* specimens. Final alignment of the *nrITS* sequence of *A. macrocyclos* in the BLASTn search contained 560 bp after trimming. *Glypholecia scabra* (Pers.) Müll. Arg. is used as outgroup. It is a *Gylpholecia* Nyl. genus member which phylogenetically related to *Acarospora* A . Massal genus [16] (Figure 2).

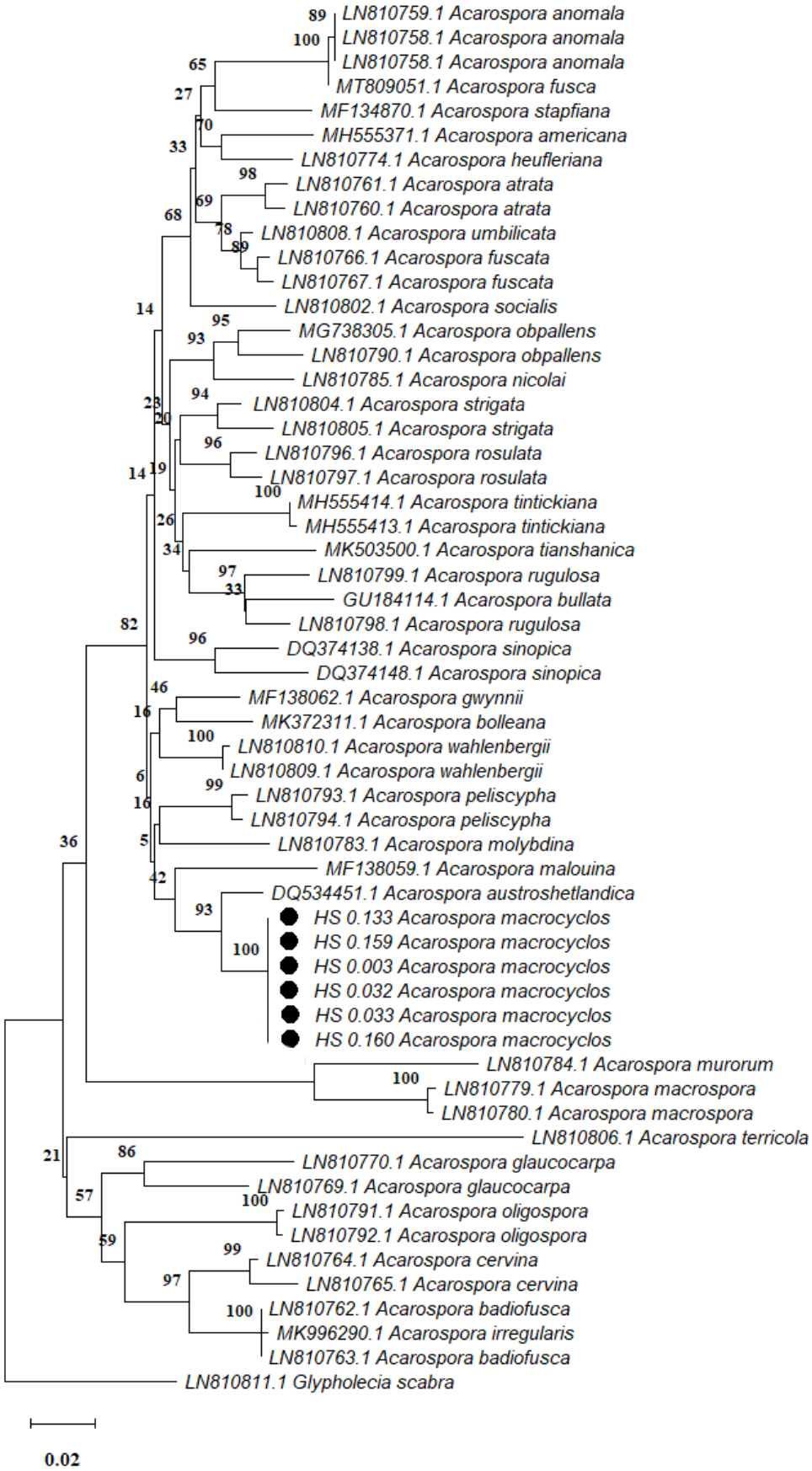


**Figure 2.** ML phylogeny based on *nrITS* gene region of *Acarospora macrocyclos.*

*A. macrocylos* is a common species on the rocks in the shore and less common on the morens and rocks at inner places. It is especially very common near the penguin colonies and bird nests [9]. It is an Antarctic endemic species. In Antarctica; it has been reported from South Georgia, Bouvetoya, South Sandwich Islands, South Orkney Islands, South Shetland Islands [9].

Specimen examined: "Antarctica, Antarctic Peninsula, Sally Cove, Horsehoe Island: Bourgeois Fjord, Marguerite Bay, near Y base, 67°48′30″S 67°17′39″W, alt. 10 m, 17 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.003, ERCH HS 0.159, ERCH HS 0.160; Lystad Bay, near Temporary Turkish Base, 67°50′09″S 67°14′18″W, alt. 14 m, 11 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.032, ERCH HS 0.033; coast of Gaul Bay, 67°49′07″S 67°12′21″W, alt. 10 m, 23 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.133."

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

Thallus foliose, usually as a form of particles up to 2 cm with brownish black ascending and curved lobes. Lobes 4–8 mm thick and 10–20 mm wide. Perithecia present, usually on the lower surface, sessile. Ascospores simple, hyaline, ellipsoid, 12–13.5 × 3–4 µm, usually without septa, rarely 3-septate. No pycnidium observed (Figure 3).


**Figure 3.** Thalli of *Mastodia tessellata*.

22 *mtSSU* rDNA sequences was used for the phylogenetic analysis of *M. tessellata* specimen. Final alignment of the *mtSSU* sequence of *M. tessellata i*n the BLASTn search contained 800 bp after trimming. *Heteroplacidium imbricatum* (Nyl.) Breuss is used as outgroup belonging to the same family with *M. tessellata* [17] (Figure 4).

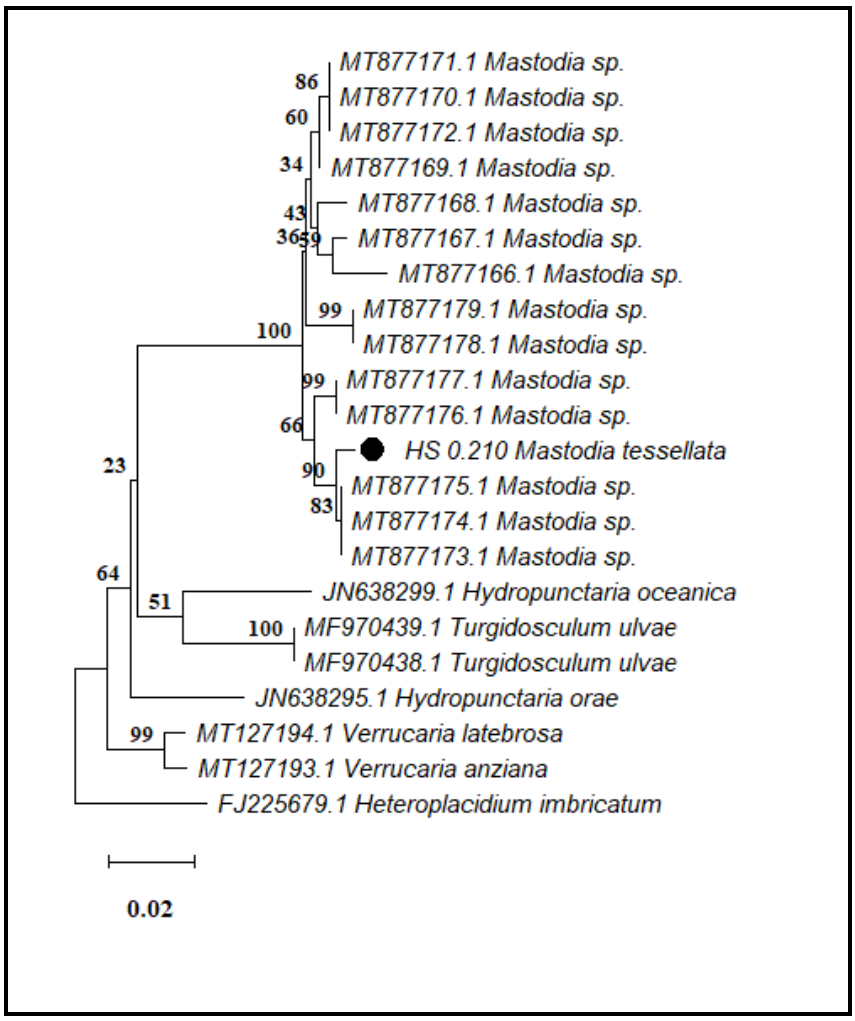


**Figure 4.** ML phylogeny based on *nrnrITS* gene region of *Mastodia tessellata.*

*M. tessellata* is usually abundant and widely distributed on rocks close to the shore and occurs on rock surfaces exposed to sea spray and near bird nests. It can also be seen in moisty concrete parts of buildings. It has a bipolar distribution. It has a wide distribution especially in Arctic Canada and North America. In Antarctica it has been reported from South Georgia, Marion and Prince Edward Islands, Kerguelen Island, Macquarie Island, South Sandwich Islands, South Orkney Islands, South Sandwich Islands, Antarctic Peninsula and Continental Antarctica [9].

Specimens examined: "Antarctica, Antarctic Peninsula, Sally Cove, Horsehoe Island: Bourgeois Fjord, Marguerite Bay, near Y base, 67°48′30″S 67°17′39″W, alt. 10 m, 17 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.210."

### 3.3. *Rhizocarpon grande* (Flörke ex Flot.) Arnold

Thallus crustose, rimose-areolate, brown or brownish gray. Prothallus present, both around at the thallus and areoles, very thick, black. Apothecia present, immersed between areoles or sessile on areoles, angular or roundish, flat, 0.25–0.7 mm diam. Apothecial disc black. Apothecial margin present, prominent, black. Epihymenium brown, 25–60 µm. Hymenium hyaline, lower part brownish, 100–135 µm. Hypothecium brown, 75–140 µm. Ascus 8-spored. Ascospores brown, muriform (22–)–*27*–(–41) × (11–)–*15*–(–18) µm (n=20). Paraphyses simple, unbranched, oil droplets present, tips are slighlty enlarged, brown pigmented, 2–3 µm. Algae green, chlorococcoid. Pycnidium not seen. Gyrophoric acid detected by TLC (Figure 5).

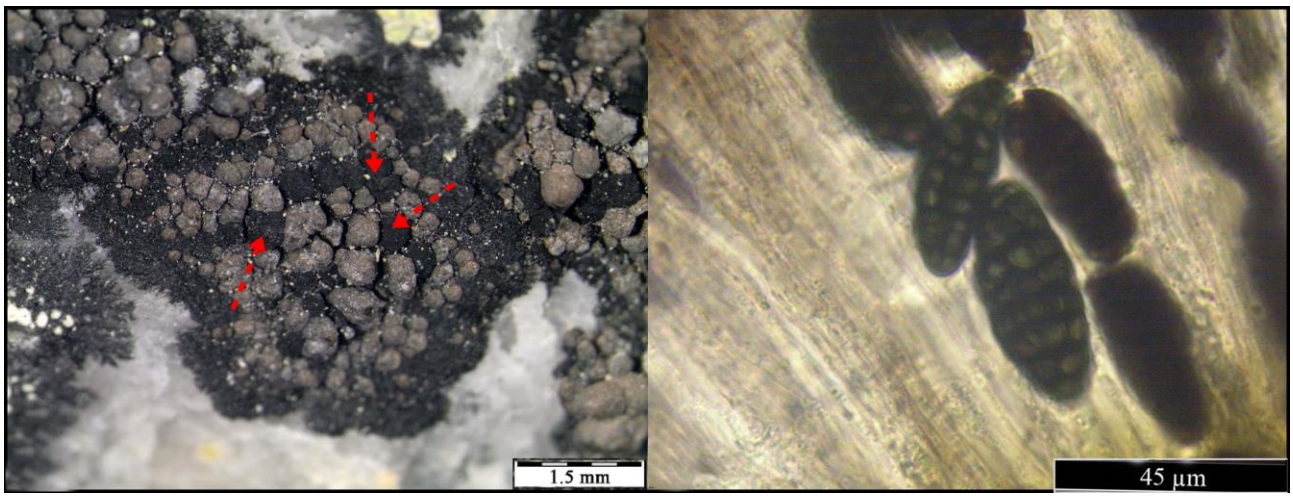


**Figure 5.** *Rhizocarpon grande*, A. Thallus and apothecia, B. Ascospores.

25 *nrITS* rDNA sequences was used for the phylogenetic analysis of *R. grande* specimen. Final alignment of the *nrITS* sequence of *R. grande i*n the BLASTn search contained 550 bp after trimming. *Catolechia wahlenbergii* (Ach.) Flot. is used as outgroup belonging to the same family with *R. grande* [18] (Figure 6).

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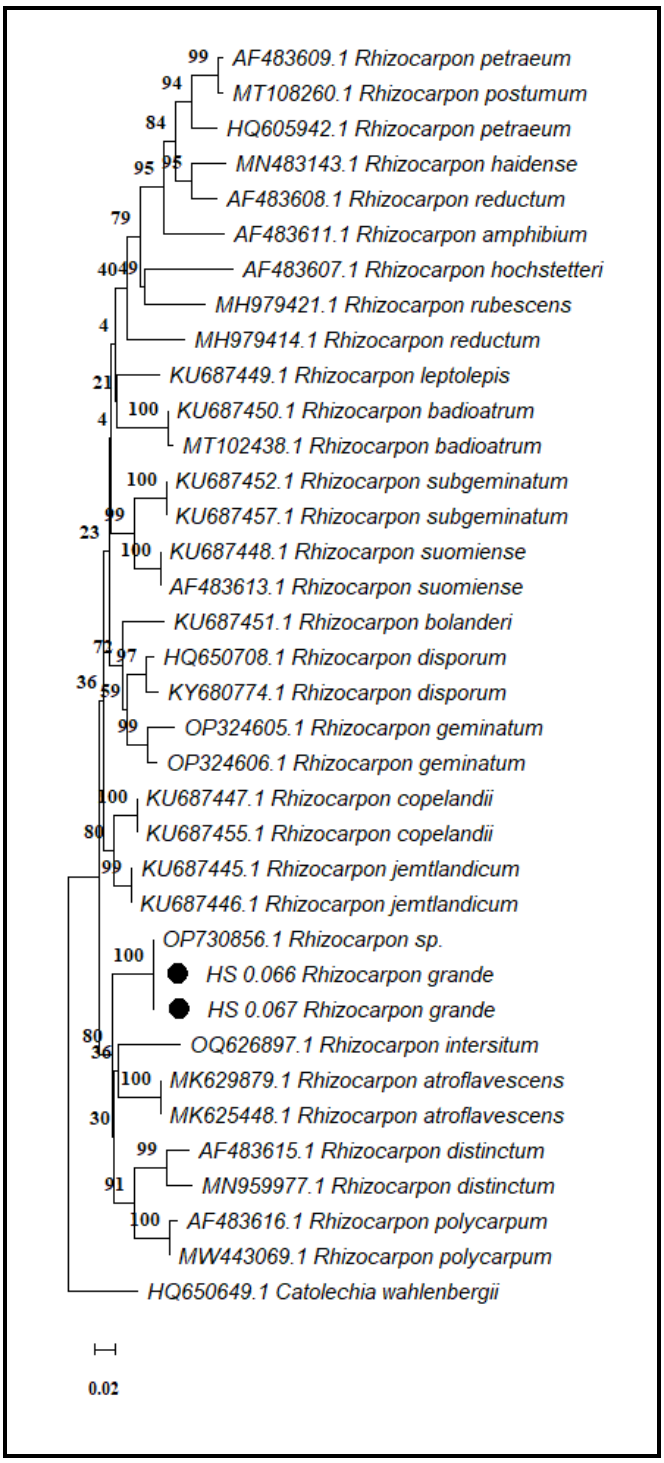


**Figure 6.** ML phylogeny based on *nrITS* gene region of *Rhizocarpon grande.*

*R. grande* occurs on rocks and stones. It has a bipolar distribution. It has been known from North Europe, North America and Antarctica. Recently it has been reported from South Korea and China too. In Antarctica, it has been reported from South Orkney Islands, South Shetland Islands and Antarctic Peninsula [9].

Specimens examined: Antarctica, Antarctic Peninsula, Horseshoe Island, coast of Gaul Bay, 67°49′07″S 67°12′21″W, alt. 10 m, 23 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.066; Southeast of Sally Cove, 67°48′58″S 67°18′9″W, alt. 65 m, 17 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.067."

### 3.4. *Verrucaria tesselatula* Nyl.

Thallus effuse, matt grenish brown, rimose, medium thick, up to 3 cm diam. Cracks irregular, whitened, divides thallus into small pieces. Perithecia immersed or semi-immersed, black 0.1–0.25 mm diam. Involucrum only at upper parts, blackish brown, edges of the involucrum are hyaline. Ascus 8-spored. Ascospores ellipsoid, hyaline, oil droplets present,, 9–12 × 5–7 µm. Pycnidia not seen (Figure 7).

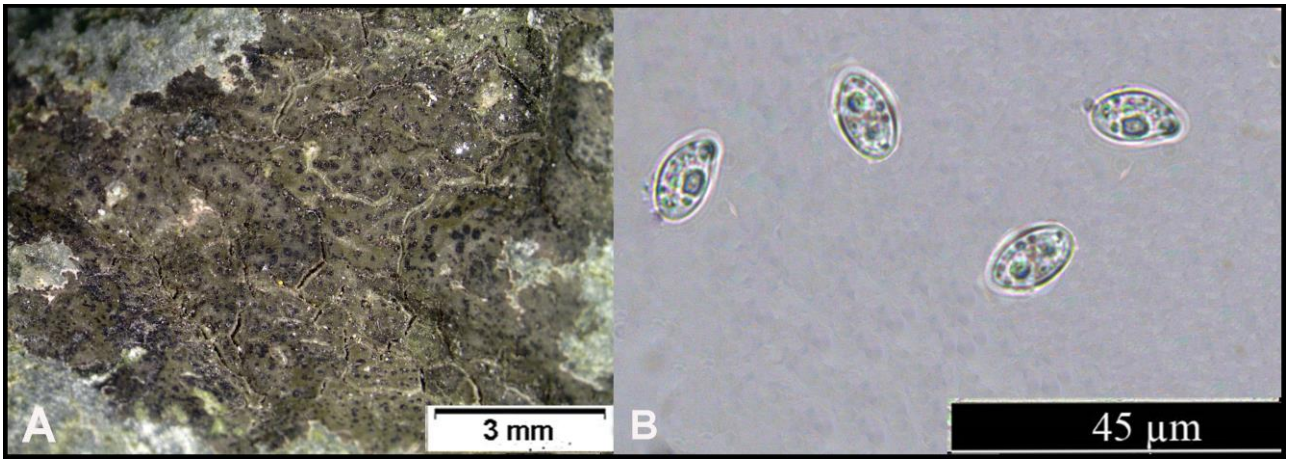


**Figure 7.** *Verrucaria tesselulata,* A. Thallus, B. Ascospores.

42 *nrITS* DNA sequences was used for the phylogenetic analysis of *V. tesselulata* specimen. Final alignment of the *nrITS* sequence of *V. tesselulata* in the BLASTn search contained 665 bp after trimming. *Heteroplacidium imbricatum* (Nyl.) Breuss is used as outgroup belonging to the same family with *V. tesselulata* [17] (Figure 8).

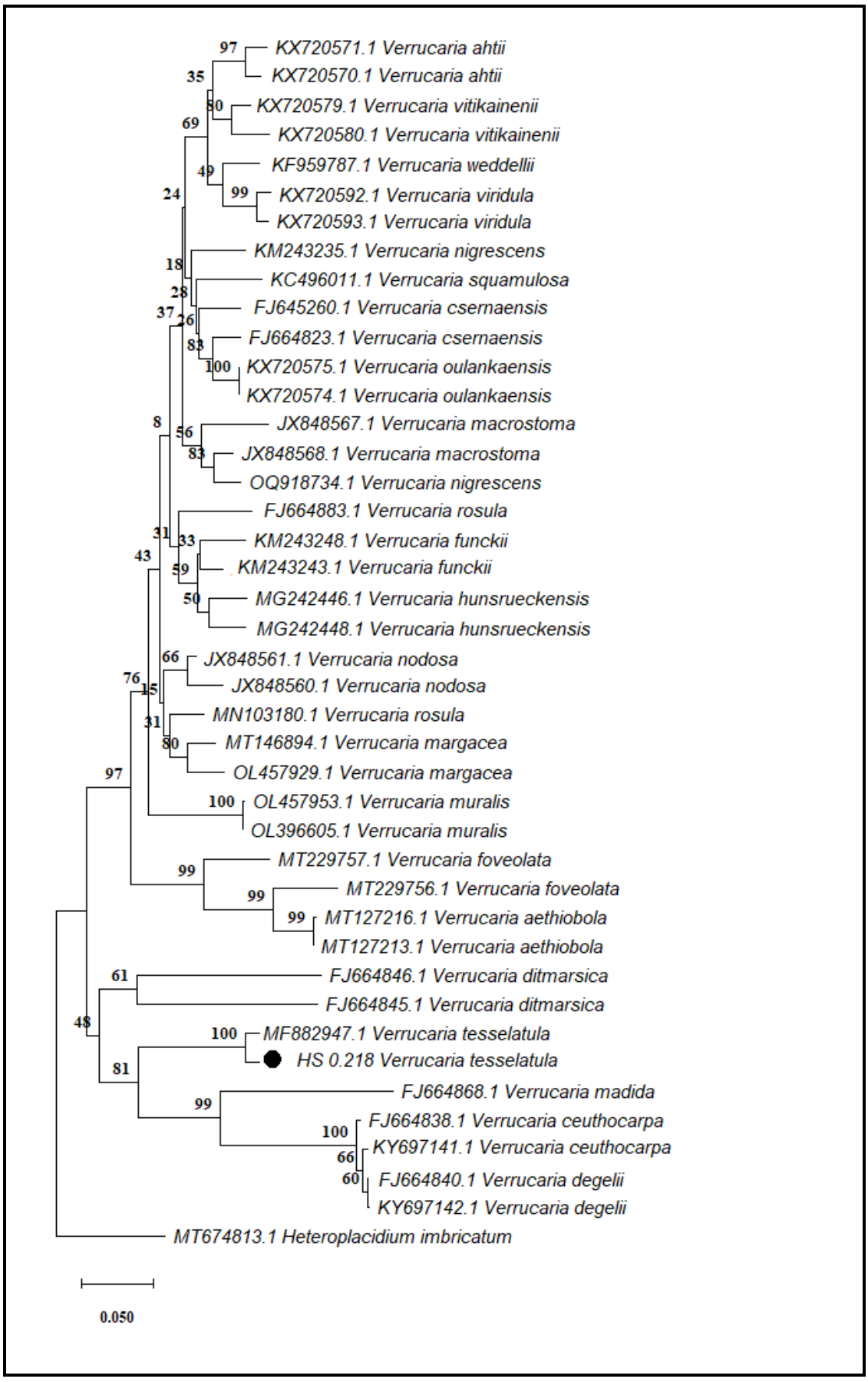


**Figure 8.** ML phylogeny based on *nrITS* gene region of *Verrucaria tesselulata.*

*V. tesselatula* occurs on on rocks close to the shore and exposed to sea spray. It is Antarctic endemic species. In Antarctica it has been reported from South Georgia, Marion and Prince Edward Island, Kerguelen Island, Bouvetoya, South Orkeny Islands, South Shetland Islands [9].

Specimen examined: "Antarctica, Antarctic Peninsula, Horseshoe Island, Lystad Bay, North of the Temporary Turkish Base, 67°49′49″S 67°14′03″W, alt. 14 m, 16 February 2022, on rock, leg. M. G. Halıcı, ERCH HS 0.218.".

## 4. Discussion and Conclusion

In this study, we performed DNA barcoding of some previously known and reported species from Horsehoe Island. For this purpose, we studied *Acarospora macrocyclos* Vain., *Mastodia tessellata* (Hook. f. & Harv.) Hook. f. & Harv., *Rhizocarpon grande* (Flörke ex Flot.) Arnold and *Verrucaria tesselatula* Nyl. species based on *nrITS* or *mtSSU* phylogeny.

*A. macrocyclos* is a species characterized by its large, effigurate, dark brown thallus and semi-immersed apothecia in the areoles. According to ITS phylogeny (Figure 2) six specimen of *A. macrocyclos* (ERCH HS 0.003, ERCH HS 0.032, ERCH HS 0.033, ERCH HS 0.133, ERCH HS 0.159 and ERCH HS 0.160) matches with each other with a high BS support (BS>95). In GenBANK, there is no molecular data of *A. macrocyclos* [19]. Within this study, *nrITS* gene data of *A. macrocylos* obtained and uploaded to GenBANK for the first time in the literature. In Figure 2, six specimens belong to *A. macrocylos* is in the same clade with *A. austroshetlandica* (C.W. Dodge) Øvstedal. *A. macrocyclos* and *A. austroshetlandica* is also similar morphologically and anatomically. The two species are distinguished from each other whether the lobes are convex or flat and the length of the ascospores. *A. macrocyclos* has flat lobes and ascospores are mostly 4 µm, but *A. austroshetlandica* has convex lobes and ascospores are mostly up to 3 µm [9]. There is only one record in GenBANK for *A. austroshetlandica* species, and the specimens we collected from Horseshoe Island are separated from this record. according to *nrITS* gene phylogeny. Although the morphological difference between these two species is very controversial, we think that the samples we collected from the study area belong to the *A. macrocyclos* species and molecular data confirms this finding.

*M. tessellata* is the type species of the *Mastodia* genus and is known to be the only lichenized fungus species that establishes a symbiotic relationship with macro green algae of *Prasiola* genus [20]. In the literature, this species was also called a lichen-like organism in the past and with studies conducted over the years, it was decided that it i a lichenized fungus. And also it was decided that *M. tessallata* is synonymous with *Mastodia mawsonii* Dodge, another species described by Dodge from Antarctica [9, 21]. Studies based on the *nrITS* gene region were carried out on *M. tessellata* specimen (ERCH HS 0.210), but despite long efforts *nrITS* gene data could not be obtained. According to *mtSSU* phylogeny (Figure 4) HS 0.210 matches with Mastodia sp. datas in GenBANK. There is no data on the *mtSSU* gene region of the *M. tessellata* species in GenBANK. The datas matching our specimen uploaded to GenBANK as *Mastodia* sp. is probably are *M. tessellata*. With this study, the first *mtSSU* data of *M. tessellata* species has been obtained and uploaded to GenBANK.

*R. grande* is a species characterized by containing gyrophoric acid, barbatic acid, norstictic acids, stictic acid and not containing rhizocarpic acid as secondary metabolite. According to ITS phylogeny (Figure 6), two specimens (ERCH HS 0.066 and ERCH HS 0.067) matches with each other and seperated from other known *Rhizocarpon* species. In GenBANK there is not data of *R. grande*. Within this study, ITS gene data of *R. grande* obtained for the first time and uploaded to GenBANK. These two specimens also match with *Rhizocarpon* sp. data in GenBANK. This data is uploaded to GenBANK by La Torre et al. [22]. Their specimen has a gray or light green thallus on a notorious black prothallus, areoles scattered on the substrate, and lecideine and sessile apothecia. The gray, submuriform ascospores were 28–32 µm long, with up to four transverse and one longitudinal septa. They said their specimen similar to *R. grande* species but comparison of characteristics to confirm the identity of the sample uploaded to GenBANK was not possible. But we believe, it is probably *R. grande* too. According to ITS phylogeny *R. grande* is closely related to *Rhizocarpon intersitum* Arnold species. Both species is also similar by their large ascospores. Both species contain gyrophoric acid. However, *R. grande* generally differs from *R. intersitum* by its I+ blue medulla, K+ red epihymenium, and larger ascospores compared to *R. intersitum*. Additionally, *R. intersitum* contains atranorin in addition to gyrophoric acid [23]. Anatomically and morphologically *R. grande* is similar to *R. distinctum* Th. Fr. and *R. obscuratum* (Ach.) A. Massal species, which are distributed in Antarctica too and have 8-spored ascus like *R. grande*. However, the ascospores in *Rhizocarpon grande* are considerably larger than the other two species (9).

*V. tesselatula* is an Antarctic endemic species. According to ITS phylogeny (Figure 8) HS 0.218 matches with *V. tesselatula*data in GenBANK. Phylogenetically *V. tesselatula* closely related to *Verrucaria madida* Orange. *V. madida* is the only species which has 4-spored asci in *Verrucaria* genus. So it can be easily distinguished from other *Verrucaria* genus members. Also *V. tesselulata* is similar to *Verrucaria ceuthocarpa* Wahlenb. ex Ach. and

*Verrucaria psycrophila* I. M. Lamb. In all three species, involucrum is only at upper part and there are cracks on the thallus. While these cracks are black or white in *V. tesselatula*, they are concolorous with thallus in *V. ceuthocarpa* and *V. psychrophila* [9].

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## Appendices

**Appendix A.** Genbank numbers of used sequences in phylogenetic trees within this study. **Newly generated data within this study given in bold.**

| Species | *nrITS* | *mtSSU* | Locality |
|---|---|---|---|
| **ERCH HS 0.003 *Acarospora macrocyclos*** | **OR687574** | | **Horseshoe Island, Antarctica** |
| **ERH HS 0.032 *Acarospora macrocyclos*** | **OR687575** | | **Horseshoe Island, Antarctica** |
| **ERH HS 0.033 *Acarospora macrocyclos*** | **OR687576** | | **Horseshoe Island, Antarctica** |
| **ERH HS 0.133 *Acarospora macrocyclos*** | **OR687579** | | **Horseshoe Island, Antarctica** |
| **ERH HS 0.159 *Acarospora macrocyclos*** | **OR687577** | | **Horseshoe Island, Antarctica** |
| **ERH HS 0.160 *Acarospora macrocyclos*** | **OR687578** | | **Horseshoe Island, Antarctica** |
| **ERCH HS 0.210 *Mastodia tessellata*** | | **PP397045** | **Horseshoe Island, Antarctica** |
| **ERCH HS 0.066 *Rhizocarpon grande*** | **OR687590** | | **Horseshoe Island, Antarctica** |
| **ERCH HS 0.067 *Rhizocarpon grande*** | **OR687591** | | **Horseshoe Island, Antarctica** |
| **ERCH HS 0.218 *Verrucaria tesselatula*** | **OR728797** | | **Horseshoe Island, Antarctica** |
| *Acarospora anomala* | LN810758 | | Sweden |
| | LN810759 | | Sweden |
| *Acarospora atrata* | LN810760 | | Seden |
| | LN810761 | | Norway |
| *Acarospora badiofusca* | LN810762 | | Sweden |
| | LN810763 | | Sweden |
| *Acarospora bullata* | GU184114 | | Italy |
| *Acarospora americana* | MH555371 | | U.S.A |
| *Acarospora austroshetlandica* | DQ534451 | | King George Island, Antarctica |
| *Acarospora bolleana* | MK372311 | | U.S.A |
| *Acarospora cervina* | LN810764 | | Switzerland |
| *Acarospora cervina* | LN810765 | | Sweden |
| *Acarospora fusca* | MT809051 | | Germany |
| *Acarospora fuscata* | LN810766 | | Sweden |
| | LN810767 | | Sweden |
| *Acarospora glaucarpa* | LN810769 | | Sweden |
| | LN810770 | | Sweden |
| *Acarospora gwynii* | MF138062 | | China |
| *Acarospora heufleriana* | LN810774 | | Switzerland |
| *Acarospora irregularis* | MK996290 | | Türkiye |
| *Acarospora macrospora* | LN810779 | | Norway |
| | LN810780 | | Sweden |
| *Acarospora malouina* | MF138059 | | China |
| *Acarospora molybdina* | LN810783 | | Sweden |
| *Acarospora murorum* | LN810784 | | Spain |
| *Acarospora nicolai* | LN810785 | | U. S. A |
| *Acarospora obpallens* | LN810790 | | U. S. A |
| | MG738305 | | Falkland Islands |
| *Acarospora oligospora* | LN810791 | | Norway |
| | LN810792 | | Sweden |
| *Acarospora peliscypha* | LN810793 | | Sweden |
| | LN810794 | | Norway |
| *Acarospora rosulata* | LN810796 | | U.S.A |
| | LN810797 | | Norway |
| *Acarospora rugulosa* | LN810798 | | Norway |
| | LN810799 | | Sweden |
| *Acarospora sinopica* | DQ374138 | | Sweden |
| | DQ374148 | | Sweden |
| *Acarospora socialis* | LN810802 | | U.S.A |
| *Acarospora stapfiana* | MF134870 | | China |
| *Acarospora strigata* | LN810804 | | U.S.A |
| | LN810805 | | U.S.A |
| *Acarospora terricola* | LN810806 | | Sweden |
| *Acarospora tianshanica* | MK503500 | | U.S.A |
| *Acarospora tintickiana* | MH555413 | | U.S.A |
| | MH555414 | | U.S.A |

| | | | |
|---|---|---|---|
| *Acarospora umbilicata* | LN810808 | | Sweden |
| *Acarospora wahlenbergii* | LN810809 | | Sweden |
| | LN810810 | | Sweden |
| *Catolechia wahlenbergii* | HQ650649 | | - |
| *Glypholecia scabra* | LN810811 | | Norway |
| *Heteroplacidium imbricatum* | MT674813 | FJ225679 | - |
| *Hydropunctaria oceanica* | | JN638299 | United Kingdom |
| *Hydropunctaria orae* | | JN638298 | Faroe Islands |
| *Mastodia* sp. I129 | | MT877166 | Chile |
| *Mastodia* sp. I930 | | MT877167 | Kerguelen Islands, France |
| *Mastodia* sp. I880 | | MT877168 | Kerguelen Islands, France |
| *Mastodia* sp. I902 | | MT877169 | Kerguelen Islands, France |
| *Mastodia* sp. I910 | | MT877170 | Australia |
| *Mastodia* sp. I911 | | MT877171 | Australia |
| *Mastodia* sp. I959 | | MT877172 | Livingston Island, Antarctica |
| *Mastodia* sp. I973 | | MT877173 | Rothera Point, Antarctica |
| *Mastodia* sp. I884 | | MT877174 | Bounty Islands, New Zealand |
| *Mastodia* sp. I927 | | MT877175 | King George Island, Antarctica |
| *Mastodia* sp. I937 | | MT877176 | Kerguelen Islands, France |
| *Mastodia* sp. I891 | | MT877177 | Antipodes Island, New Zealand |
| *Mastodia* sp. I866 | | MT877178 | Princess Island, New Zealand |
| *Mastodia* sp. I867 | | MT877179 | Princess Island, New Zealand |
| *Rhizocarpon amphibium* | AF483611 | | Norway |
| *Rhizocarpon atroflavescens* | MK625448 | | China |
| | MK629879 | | China |
| *Rhizocarpon badioatrum* | KU687450 | | Norway |
| | MT102438 | | China |
| *Rhizocarpon bolanderi* | KU687451 | | Norway |
| *Rhizocarpon copelandii* | KU687447 | | Norway |
| *Rhizocarpon copelandii* | KU687455 | | Norway |
| *Rhizocarpon disporum* | HQ650708 | | U.S.A |
| | KY680774 | | Russia |
| *Rhizocarpon distinctum* | AF483615 | | Norway |
| | MN959977 | | - |
| *Rhizocarpon geminatum* | OP324605 | | James Ross Island, Antarctica |
| | OP324606 | | James Ross Island, Antarctica |
| *Rhizocarpon haidense* | MN483143 | | U.S.A |
| *Rhizocarpon hochstetteri* | AF483607 | | Norway |
| *Rhizocarpon intersitum* | OQ626897 | | China |
| *Rhizocarpon jemtlandicum* | KU687445 | | Norway |
| | KU687446 | | Norway |
| *Rhizocarpon leptolepis* | KU687449 | | Finland |
| *Rhizocarpon petraeum* | AF483609 | | Norway |
| | HQ605942 | | Türkiye |
| *Rhizocarpon polycarpum* | AF483616 | | Norway |
| | MW443069 | | China |
| *Rhizocarpon postumum* | MT108260 | | China |
| *Rhizocarpon reductum* | AF483608 | | Norway |
| | MH979414 | | - |
| *Rhizocarpon rubescens* | MH979421 | | - |
| *Rhizocarpon* sp. | OP730856 | | King George Island, Antarctica |
| *Rhizocarpon subgeminatum* | KU687452 | | Norway |
| | KU687457 | | Norway |
| *Rhizocarpon suomiense* | AF483613 | | Norway |
| | KU687448 | | Norway |
| *Turgidosculum ulvae* | | MF970438 | U.S.A |
| | | MF970439 | U.S.A |
| *Verrucaria aethiobola* | MT127213 | | Finnmark |
| | MT127216 | | Norway |
| *Verrucaria ahtii* | KX720570 | | Finland |
| | KX720571 | | Finland |

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|---|---|---|---|
| *Verrucaria anziana* | | MT127193 | United Kingdom |
| *Verrucaria ceuthocarpa* | FJ664838 | | Iceland |
| | KY697141 | | Iceland |
| *Verrucaria csernaensis* | FJ645260 | | United Kingdom |
| *Verrucaria csernaensis* | FJ664823 | | United Kingdom |
| *Verrucaria degelii* | FJ664840 | | Iceland |
| | KY697142 | | Iceland |
| *Verrucaria ditmarsica* | FJ664845 | | United Kingdom |
| | FJ664846 | | United Kingdom |
| *Verrucaria foveolata* | MT229757 | | Finland |
| *Verrucaria funckii* | KM243243 | | Germany |
| | KM243248 | | Austria |
| *Verrucaria hunsrueckensis* | MG242446 | | Germany |
| | MG242448 | | Germany |
| *Verrucaria latebrosa* | | MT127194 | United Kingdom |
| *Verrucaria macrostoma* | JX848567 | | United Kingdom |
| *Verrucaria madida* | FJ664868 | | United Kingdom |
| *Verrucaria margacea* | MT146894 | | Germany |
| | OL457929 | | Czech Republic |
| *Verrucaria muralis* | OL396605 | | Czech Republic |
| | OL457953 | | Czech Republic |
| *Verrucaria nigrescens* | JX848568 | | United Kingdom |
| | KM243235 | | Germany |
| *Verrucaria nodosa* | JX848560 | | United Kingdom |
| | JX848561 | | United Kingdom |
| *Verrucaria oulankaensis* | KX720574 | | Finland |
| | KX720575 | | Finland |
| *Verrucaria rosula* | FJ664883 | | United Kingdom |
| | MN103180 | | China |
| *Verrucaria squamulosa* | KC496011 | | Italy |
| *Verrucaria tesselaluta* | MF882947 | | Chile |
| *Verrucaria viridula* | KX720592 | | Finland |
| | KX720593 | | Finland |
| *Verrucaria vitikainenii* | KX720579 | | Finland |
| | KX720580 | | Finland |
| *Verrucaria weddelii* | KF959787 | | France |