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Molecular and Phylogenetic Characterization of Lichens from Dismal Island (Antarctic Peninsula, Antarctica) Using nrITS Marker

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Abstract

Purpose: Lichenized fungi are the most dominant macro-organisms in Antarctic terrestrial ecosystems. Studies on lichens in Antarctica have a history of approximately two centuries. Especially with the recent use of DNA-based techniques in taxonomy studies, lichen biodiversity studies in Antarctica has accelerated. Dismal Island is the largest of the Faure Islands, 1.9 kilometers long and 60 meters high, mostly covered with ice in Marguerite Bay on the west coast of Graham Land. In the literature, there is no study directly addressing or examining lichen biodiversity on Dismal Island. In this context, the aim of this study is to examine anatomically-morphologically and to perform molecular and phylogenetic characterization of some lichenized fungi species from Dismal Island..
Method: Lichen samples were collected from Dismal Island during the 6th Turkish National Antarctic Scientific Expedition by the second author. The collected samples were identified by anatomical and morphological examinations and molecular analyses were performed using the nrITS gene region.
Findings: As a result of the study, molecular and phylogenetic characterization of 11 lichen species was successfully conducted. This species are *Austroplaca hookerii* (C.W. Dodge) Søchting, Frödén & Arup, *Buellia russa* (Hue) Darb., *Candelariella flava* (C.W. Dodge & Baker) Castello & Nimis, *Mastodia tessellata* (Hook & Harv.) Hook & Harv., *Polycauliona candelaria* (L.) Frödén, Arup & Søchting, *Rhizoplaca aspidophora* (Vain.) Follmann, *Rhizocarpon geographicum* (L.) DC., *Tephromela atra* (Huds.) Hafellner ex Kalb., *Tetramelas anisomerus* (Vain.) Elix., *Umbilicaria antarctica* Frey & I.M. Lamb., *Usnea antarctica* Du Rietz.
Conclusion: The study provides the first direct investigation of lichen biodiversity on Dismal Island through anatomical, morphological, molecular, and phylogenetic approaches, contributing novel data on Antarctic lichenized fungi.
Keywords: Lichenized fungi, biodiversity, Antarctica, Dismal Island, nrITS

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Dismal Adası (Antarktika Yarımadası, Antarktika)'ndan toplanan likenlerin nrITS markırı kullanılarak moleküler ve filogenetik karakterizasyonu

Özet
Amaç: Likenleşmiş mantarlar, Antarktika karasal ekosistemlerinde en baskın makro organizmalardır. Antarktika'daki likenler üzerine yapılan çalışmalar yaklaşık iki yüzyıllık bir geçmişe sahiptir. Özellikle son yıllarda taksonomi çalışmalarında DNA temelli tekniklerin kullanılmaya başlanmasıyla birlikte, Antarktika'daki liken biyoçeşitliliği üzerine yapılan çalışmalar hız kazanmıştır. Dismal Adası, Graham Land'in batı kıyısında, Marguerite Körfezi'nde yer alan ve büyük ölçüde buzla kaplı, 1,9 kilometre uzunluğunda ve 60 metre yüksekliğinde, Faure Adaları'nın en büyüğüdür. Literatürde, Dismal Adası'ndaki liken biyoçeşitliliğini doğrudan ele alan ya da inceleyen herhangi bir çalışma bulunmamaktadır. Bu bağlamda, bu çalışmanın amacı Dismal Adası'ndan elde edilen bazı likenleşmiş mantar türlerini anatomik ve morfolojik olarak incelemek ve moleküler ve filogenetik karakterizasyonunu gerçekleştirmektir.

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Metod: Dismal Adası'ndan toplanan liken örnekleri, İkinci yazar tarafından 6. Ulusal Türk Antarktika Bilim Seferi sırasında toplanmıştır. Toplanan örnekler, anatomik ve morfolojik incelemelerle tanımlanmış, ayrıca nrITS gen bölgesi kullanılarak moleküler analizler gerçekleştirilmiştir.
Bulgular: Çalışma sonucunda, 11 liken türünün moleküler ve filogenetik karakterizasyonu başarıyla gerçekleştirilmiştir. Bu türler *Austroplaca hookerii* (C.W. Dodge) Søchting, Frödén & Arup, *Buellia russa* (Hue) Darb., *Candelariella flava* (C.W. Dodge & Baker) Castello & Nimis, *Mastodia tessellata* (Hook & Harv.) Hook & Harv., *Polycauliona candelaria* (L.) Frödén, Arup & Søchting, *Rhizoplaca aspidophora* (Vain.) Follmann, *Rhizocarpon geographicum* (L.) DC., Tephromela atra (Huds.) Hafellner ex Kalb., *Tetramelas anisomerus* (Vain.) Elix., *Umbilicaria antarctica* Frey & I.M. Lamb., *Usnea antarctica* Du Rietz'dır.
Sonuç: Çalışma, Dismal Adası'ndaki liken biyoçeşitliliğine yönelik anatomik, morfolojik, moleküler ve filogenetik yaklaşımlar aracılığıyla yapılan ilk doğrudan araştırmayı sunmakta ve Antarktika'daki likenleşmiş mantarlara ilişkin yeni veriler sağlamaktadır.

Anahtar kelimeler: Likenleşmiş mantar, biyoçeşitlilik, Dismal Adası, nrITS

1. Introduction

Dismal Island is an island mostly covered with glaciers, located at the entrance to Marguerite Bay on the west coast of Graham Lands within the Antarctic Peninsula, between Adelaide Island in the northeast and Alexander Island in the southwest, and is the largest of the Faure Islands, 1.9 kilometers long and 60 meters high; located at 68°6'S, 68°50'W coordinates [1].

In the literature, there is no study that directly addresses or examines lichen biodiversity on Dismal Island. However, In the British Antarctic Survey (BAS) database, it is seen that there are 17 lichen species collected by various researchers from Dismal Island. These are; *Buellia russa* (Hue) Darb., *Candelariella vitellina* (Hoffm.) Mull. Arg., *Cladonia galindezii* Ovst., *Mastodia tessellata* (Hook. f. & Harv.) Hook. f. & Harv., *Ochrolechia frigida* (Sw.) Lynge, *Physcia caesia* (Hoffm.) Furnr., *Polycauliona candelaria* (L.) Th. Fr., *Pseudephebe pubescens* (L.) Choisy, *Psoroma cinnamomeum* Malme, *Psoroma hypnorum* (Vahl) Gray, *Rhizoplaca aspidophora* (Vain.) Redon, *Rhizoplaca melanophthalma* (Ram.) Leuck. & Poelt, *Thamnolecania brialmontii* (Vain.) Gyeln. *Umbilicaria antarctica* Frey & Lamb, *Umbilicaria decussata* (Vill.) Zahlbr., *Usnea antarctica* Du Rietz, *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt [2].

In this context, the aim of this study is to examine anatomically and morphologically, and to perform molecular and phylogenetic characterization of some lichenized fungi species from Dismal Island.

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2. Materials and methods

2.1. Collection of Lichen Material and Identification as Species Level

Lichen samples studied were collected from Dismal Island by the second author, during the sixth Turkish Antarctic Expedition (TAE VI). Lichen samples were collected from different habitats of the island with tools such as hammers, spatulas, chisels, along with their substrates, moss and rocks. After wrapping them in a napkin or paper, the location information of the area where they were collected was written on the paper bag and numbered along with their photographs and placed in paper bags. A stereomicroscope was used to determine the external morphological characters and a light microscope was used to determine the anatomical features of studied lichen samples. Standard lichen spot tests were used for determining secondary metabolites. Molecular methods were used for the molecular and phylogenetic characterization of the studied specimens.

2.2. Molecular Methods Used in Lichenized Fungi Identification

2.2.1. DNA Isolation

Apothecia, thallus, or ascocarps of the studied lichen samples were used for DNA isolation. DNA isolation was performed using the Qiagen DNeasy Plant Mini Kit (Catalog No: 69104), following the manufacturer's protocol. DNA was eluted from the column. The isolated genomic DNA was stored at −20 °C until use.

2.2.2. Polymerase Chain Reaction

The gene regions were amplified from the isolated DNA using the primer(s) listed in Table 1 and the PCR protocol provided in Table 2. The PCR reaction mixture consisted of 200 ng DNA, 10X reaction buffer, 25 mM $MgCl_2$,

25 µM dNTPs, 10 µM forward and reverse primers, 0.5 U Taq DNA polymerase, and PCR-grade water to bring the total volume to 50 µl.

Table 1. Primers Used in the Study

Primer	Targeted Gene Region	Primer Sequence	References
ITS1-F (Forward)	ITS	5' CTTGGTCATTTAGAGGAAGTAA 3'	[3]
ITS4 (Reverse)	ITS	3'-TCCTCCGCTTATTGATATGC-5'	[4]

Table 2. PCR Protocol

Gene Region	Predenaturation	Denaturation	Annealing	Extension	Last extension	Reference
ITS	95 °C – 4 min	94 °C – 3 min (35 cycle)	55 °C – 1 min	72 °C – 1 min	72 °C – 8 min	[5]

2.2.3. Agarose Gel Electrophoresis

Gene regions amplified by PCR from the samples were analyzed using 1.2% agarose gel electrophoresis. A 20 µl aliquot of each sample was mixed with loading buffer (containing 40% sucrose and 0.25% bromophenol blue) and loaded onto the gel. The loaded samples were run for 1 hour in 1X TAE buffer (Tris, acetic acid, EDTA) at 100 V. The gel was stained with ethidium bromide (0.5 µl/ml), and imaging was performed under UV light using a gel documentation system. If no bands were observed at the end of agarose gel electrophoresis and the target gene regions were not successfully amplified, the PCR was repeated using the same primers.

2.2.4. Sequence Analysis

The sequence analysis of the amplified gene regions obtained by PCR was performed externally by a commercial sequencing service. The sequences obtained from the analyzed samples were compared with reference sequences available in GenBank (NCBI) using the BLAST tool to determine species-level similarity. Alignment and editing of the raw sequence data were conducted using the BioEdit 7.2.5 (Biological Sequence Alignment Editor) software. Sequence alignment was performed using the Clustal W module in BioEdit. Each sample was processed separately, and each base was manually checked for accuracy. The dataset was compared with ITS gene region sequences of species previously deposited in GenBank. To infer phylogenetic relationships and construct evolutionary trees among the samples, the Maximum Likelihood (ML) method was employed with 1000 bootstrap replicates in the MEGA 11 (Molecular Evolutionary Genetics Analysis) software. The results were evaluated accordingly [11].

Table 3. The specimens' herbarium numbers and GenBank accession numbers

Specimen	*nrITS* GenBank Number
Austroplaca hookeri (ERCH DIS 0.085)	PV030391
Buellia russa (ERCH DIS 0.051-0.152)	PV030389-PV030396
Candelariella flava (ERCH DIS 0.005)	PV030386
Mastodia tessellata (ERCH DIS 0.140)	PV030392
Polycauliona candelaria (ERCH DIS 0.141)	PV030393
Rhizocarpon geographicum (ERCH DIS 0.007)	PV030387
Rhizoplaca aspidophora (ERCH DIS 0.004)	PV030397
Tephromela atra (ERCH DIS 0.142)	PV030394
Tetramelas anisomerus (ERCH DIS 0.083)	PV030390
Umbilicaria antarctica (ERCH DIS 0.147)	PV030395
Usnea antarctica (ERCH DIS 0.008)	PV030388

3. Results

3.1. *Austroplaca hookerii* (C.W. Dodge) Søchting, Frödén & Arup

Description: Thallus crustose, well developed, lobate, orange or yellow-orange in some places. Apothecia and pycnidia not observed. Thallus K+ purple (Figure 2).

Ecology and distribution: on rocks near seaside especially where penguin colonies are located. It has been known from Argentina, Antarctica and Falkland Islands so far [8-10]

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near Turkish Dismal Global Navigation Satellite System, 68°6′ S 68°50′ W, alt. 10 m. 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.085.

Figure 1. *Austroplaca hookeri*

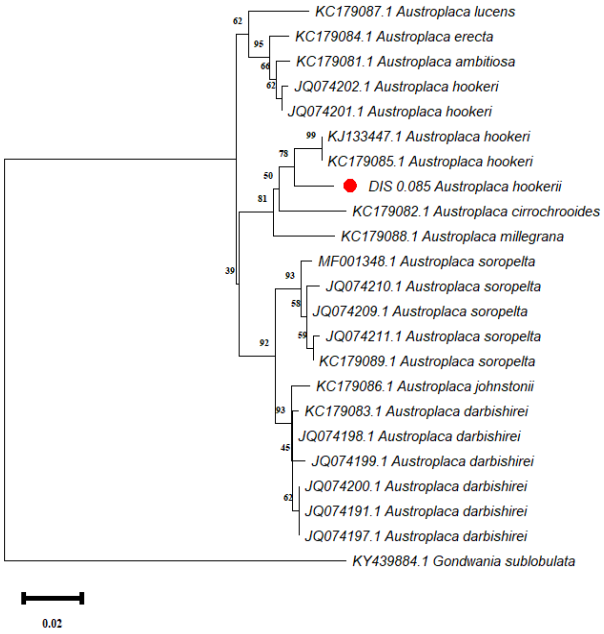

Figure 2. *nrITS* ML dendrogram of *Austroplaca hookeri.*

3.2. *Buellia russa (Hue) Darb.*

Description: Thallus crustose, rimose-areolate, creamish-brown. Apothecia present, very common, lecideine, smaller ones almost immersed to areoles, mature ones sessile, flat, roundish or angular, 0.1–0.5 mm in diam. Epihymenium brown, 70 µm. Hymenium hyaline, 95 µm. Hypothecium brown, 150–260 µm. Algae green, chlorococcoid. Ascus 8-spored, 65 × 20 µm. Ascospores brown, one septate, *Buellia* and/or *Physconia* type, 16.5–23.5 × 8–10 µm. Paraphyses slender, simple, tips clavate, 2–4 µm. Pycnidia not observed. The immersed black spots are undeveloped apothecia. Thallus K+ red (Figure 3).

Ecology and distribution: Occurs on rocks near coast. *B. russa* is an Antarctic and sub-Antarctic endemic species [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near Turkish Dismal Global Navigation Satellite System, 68°6′ S 68°50′ W, alt. 10 m. 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.051, ERCH DIS 0.152.

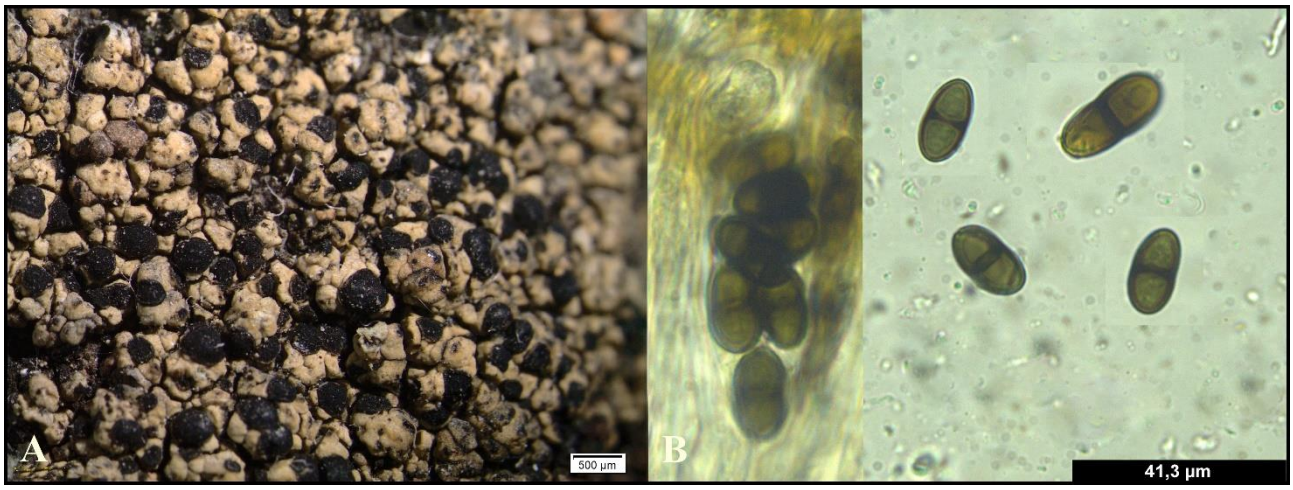

Figure 3. *Buellia russa* A. Habitus, B. Ascospores

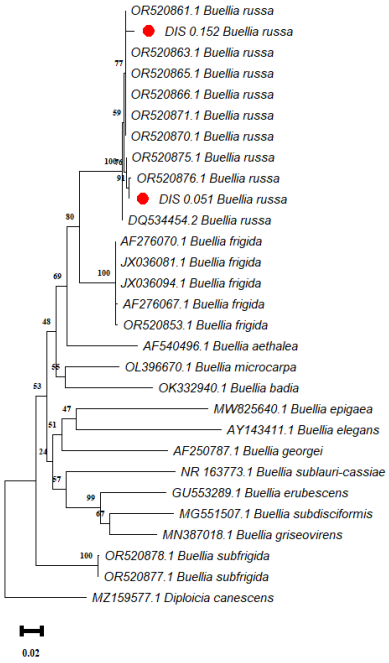

Figure 4. *nrITS* ML dendrogram of *Buellia russa*

3.3. *Candelariella flava* (C.W. Dodge & Banker) Castello & Nimis

Description: Thallus forms a thin layer consisting of small granules. Granules yellow, 3-4 µm in diam. Apothecia not observed (Figure 5).

Ecology and distribution: *C. flava* occurs on mosses and other lichens. It is an Antarctic endemic species. It has been reported from South Georgia, South Orkney Islands, South Shetland Islands, and Antarctic Peninsula [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.005

Figure 5. *Candelariella flava*

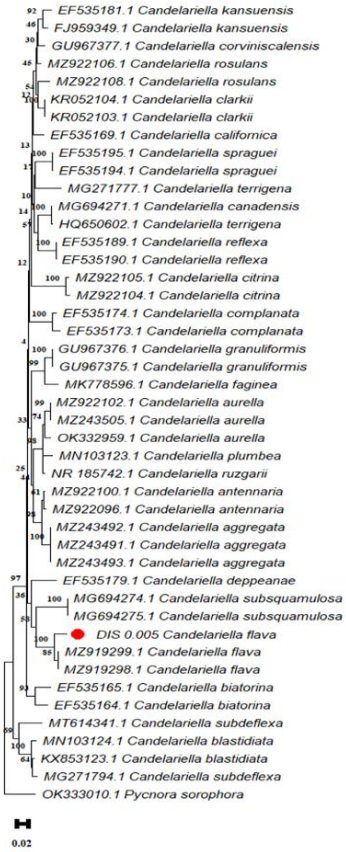

Figure 6. *nrITS* ML dendrogram of *Candelariella flava*

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

Description: Thallus foliose, brownish-black; lobes ascending and curved, usually forming aggregates up to 2 cm in diameter. Lobes thick, measuring 4–8 mm wide and up to 10–20 mm in diameter. Apothecia and pycnidia not observed (Figure 7).

Ecology and distribution: This species has a bipolar distribution and usually occurs on rocks near the shore, particularly in moist concrete-like layers found near bird nests. It has a wide distribution, especially in Arctic Canada

and North America. In Antarctica, it has been reported from Kerguelen Island, South Georgia, South Sandwich Islands, Marion and Prince Edward Islands, Macquarie Island, South Orkney Islands, the Antarctic Peninsula, and continental Antarctica [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.140.

Figure 7. *Mastodia tessellata*

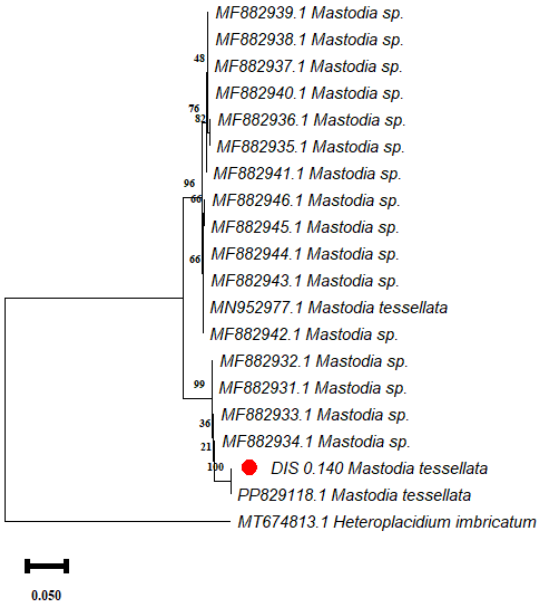

Figure 8. *nrITS* ML dendrogram of *Mastodia tessellata*

3.5. *Polycauliona candelaria* (L.) Frödén, Arup & Søchting

Description: Thallus fruticose; branches composed of squamules, yellow-orange in color. Soralia present on the squamules. Apothecia not observed. Thallus K+ red (Figure 9).

Ecology and distribution: *P. candelaria* is an ornithophilous lichen that occurs on mosses, in rock crevices and edges, on soil, and occasionally on timber or concrete surfaces. It has a bipolar and alpine distribution. In Antarctica, it

has been reported from South Georgia, Bouvet Island, South Sandwich Islands, South Orkney Islands, South Shetland Islands, and the Antarctic Peninsula [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.141.

Figure 9. *Polycauliona candelaria*

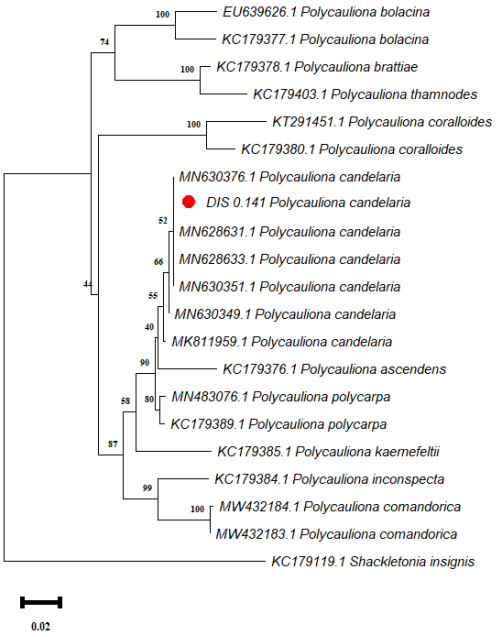

Figure 10. *nrITS* ML dendrogram of *Megaspora verrucosa*

3.6. *Rhizocarpon geographicum* (L.) DC.

Description: Thallus crustose, areolate, shiny yellow. Prothallus present, prominent, black. Apothecia common, dispersed throughout the areoles, mostly immersed or occasionally almost sessile, angular, 0.2–0.5 mm in diameter. Epihymenium brown, 60 µm. Hymenium hyaline, 160 µm. Hypothecium brown, 130 µm. Ascus 8-spored, 120 × 60 µm. Ascospores muriform, brown, ellipsoid, 25–36 × 14–21 µm. Paraphyses simple, 3–4.5 µm. Pycnidia not observed. All spot tests negative.

Ecology and distribution: A cosmopolitan species that occurs on siliceous rocks. It has a very wide distribution, especially in Europe and North America. In Antarctica, it has been reported from many localities in the Antarctic Peninsula and continental Antarctica [12].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.007

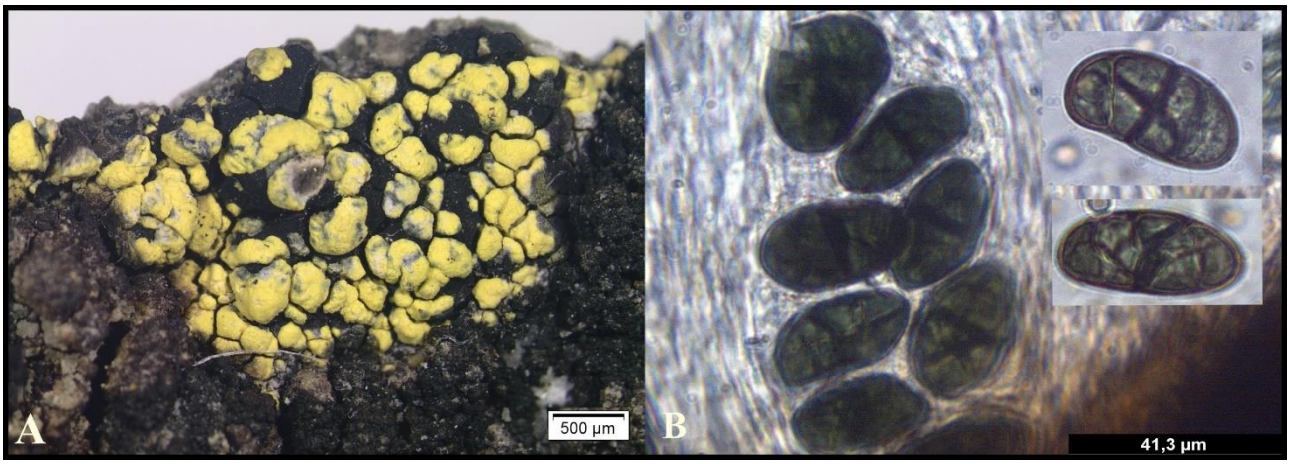

Figure 11. *Rhizocarpon geographicum* A. Thallus, B. Ascospores

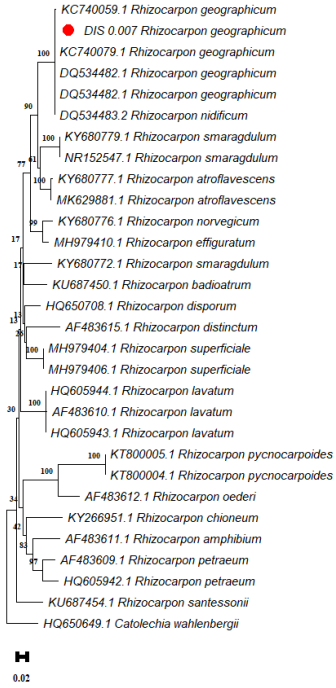

Figure 12. *nrITS* ML dendrogram of *Rhizocarpon geographicum*

3.7. *Rhizoplaca aspidophora* (Vain.) Follmann

Description: Thallus crustose, rimose-areolate, yellow, almost pulvinate, not well developed. Apothecia present, lecanorine, sessile, usually aggregated, and flat or rarely concave, 0.5–0.7 mm in diameter. Apothecial disc greenish-yellow. Apothecial margin present, distinct, prominent, and concolorous with the thallus. Epihymenium light brown-yellowish, 35 µm. Hymenium hyaline, 50 µm. Hypothecium hyaline, 70–115 µm. Ascus 8-spored. Ascospores simple, hyaline, ellipsoid, 9–12.5 × 5–7 µm. Pycnidia not observed (Figure 13).

Ecology and distribution: *Rhizoplaca aspidophora* occurs on rocks. It is an Antarctic endemic species. In Antarctica, it has been reported from the South Sandwich Islands, South Orkney Islands, South Georgia, and the South Shetland Islands [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.004.

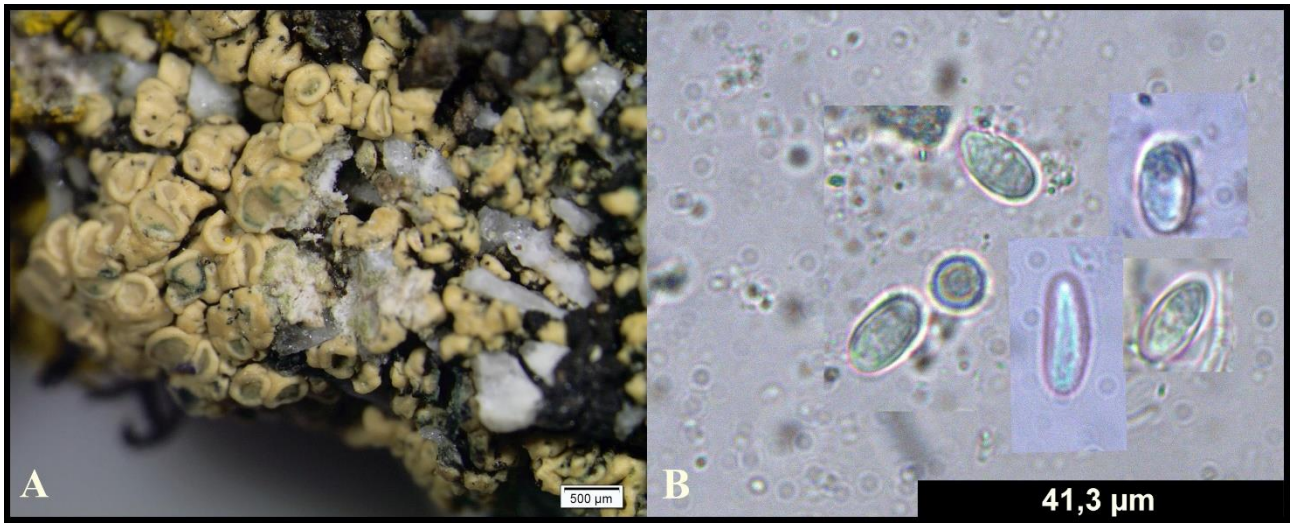

Figure 13. *Rhizoplaca aspidophora*. A. Thallus, B. Ascospores

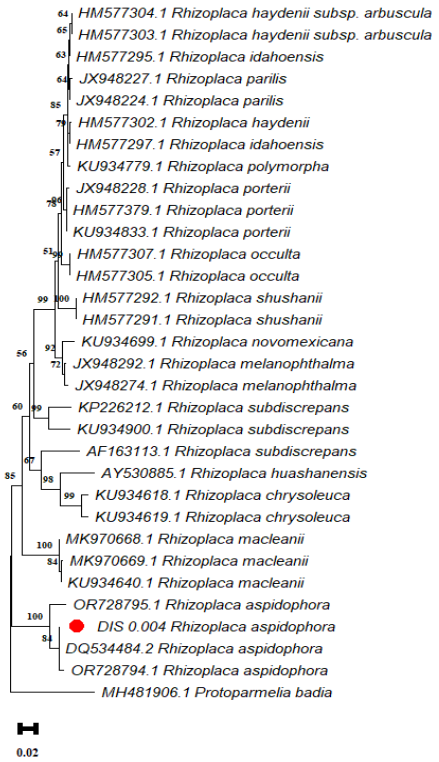

Figure 14. *nrITS* ML dendrogram of *Rhizoplaca aspidophora*

3.8. *Tephromela atra* (Huds.) Hafellner ex Kalb.

Description: Thallus crustose, areolate, almost pulvinate, creamish-white. Prothallus absent. Apothecia present, lecanorine, 0.3–0.7 mm in diameter. Apothecial disc roundish, black, rarely white-pruinose. Apothecial margin present but very thin, concolorous with the thallus. Epihymenium purple-brown, 25 µm. Hymenium hyaline, 70 µm. Hypothecium hyaline, 110 µm. Paraphyses simple, with enlarged tips, 12 µm. Ascus 8-spored. Ascospores simple, hyaline, 11–13 × 6–9 µm. Pycnidia present, immersed in the thallus, very small. Conidiospores cylindrical to filiform, 9 × 1.5 µm. Thallus K+ yellow (Figure 15).

Ecology and distribution: A cosmopolitan species that occurs especially on moist, non-calciferous and/or calciferous rocks. In Antarctica, it has been reported from South Georgia, South Orkney Islands, South Shetland Islands, and the Antarctic Peninsula [11]

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.142.

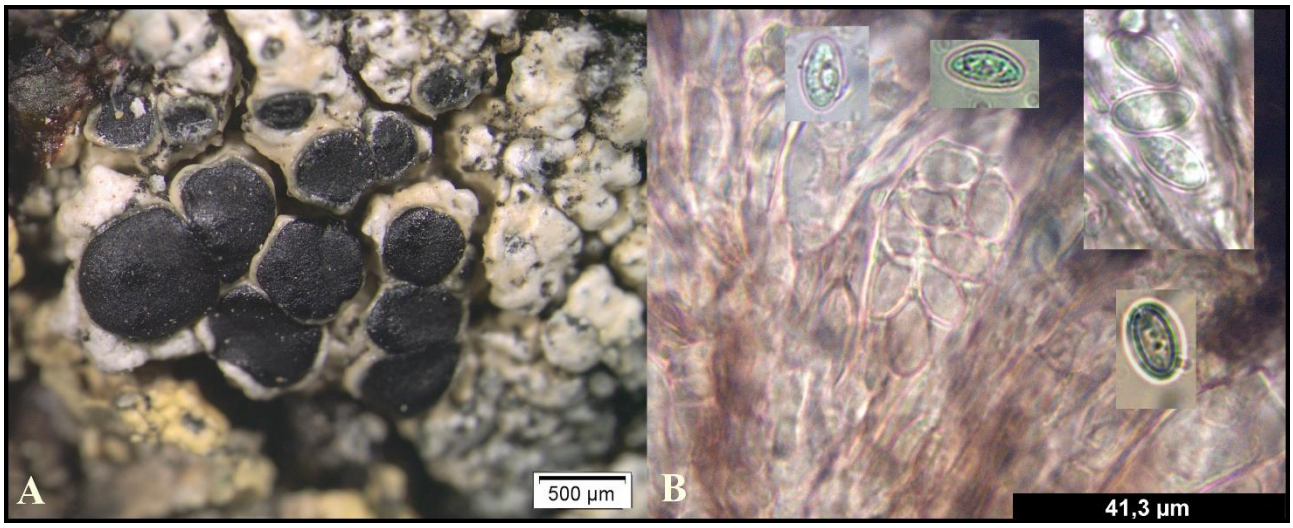

Figure 15. *Tephromela atra* A. Thallus, B. Ascospores

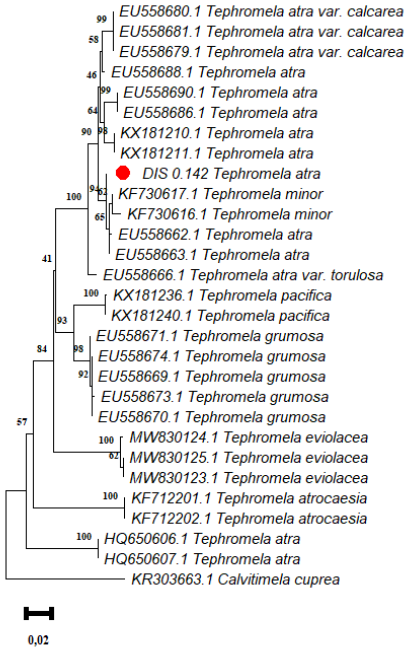

Figure 16. *nrITS* ML dendrogram of *Tephromela atra*

3.9. *Tetramelas anisomerus* (Vain.) Elix.

Description: Thallus crustose, rimose-areolate, effuse, pulvinate, yellow. Apothecia present, sessile, black, 0.2–0.8 mm in diameter. Epihymenium brown, 70 µm. Hymenium hyaline, 55–110 µm. Hypothecium dark brown, 200 µm. Ascus 8-spored. Ascospores 1–3-septate, brown, 16–24 × 7.5–9 µm. Paraphyses simple, unbranched, tips clavate, 4.5 µm. Thallus K–, C–, KC–; medulla KI+ blue (Figure 17).

Ecology and distribution: *T. anisomerus* occurs on rocks. It has been reported from Antarctica, Argentina, and Chile [13].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.083.

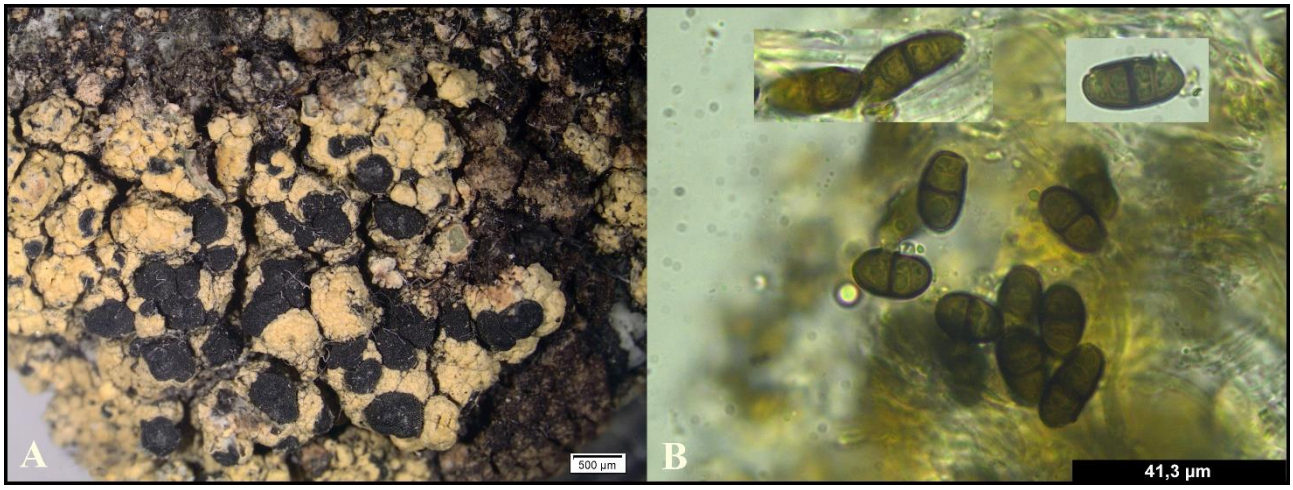

Figure 17. *Tetramelas anisomerus* A. Thallus, B. Ascospores

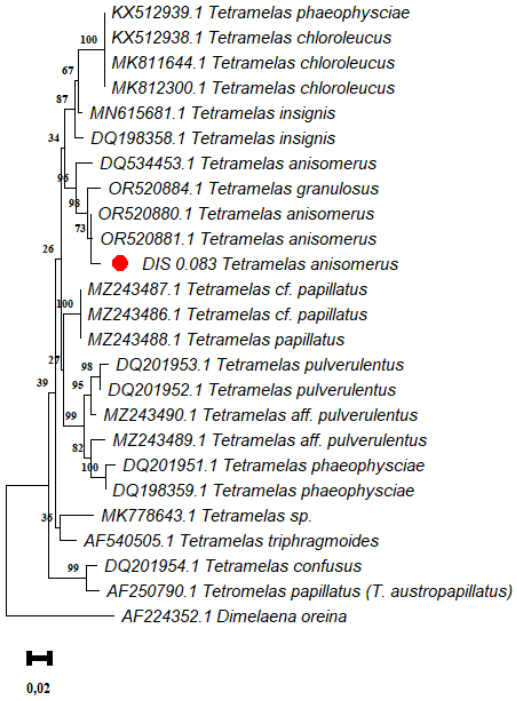

Figure 18. *nrITS* ML dendrogram of *Tetramelas anisomerus*

3.10. *Umbilicaria antarctica* Frey & I.M. Lamb.

DescriptionThallus foliose, 5–15 cm in diameter. Upper surface brownish-grey; lower surface black. Rhizines present. Rhizines simple or bifurcated, flat. Thallospores simple or rarely two-celled, 5–8 µm in diameter. Apothecia not observed (Figure 19).

Ecology and distribution: *U. antarctica* occurs on rocks and stones. It is very common on moist rock surfaces and occasionally found on mosses. It is an Antarctic endemic species and has a wide distribution throughout the continent [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.147.

Figure 19. *Umbilicaria antarctica*

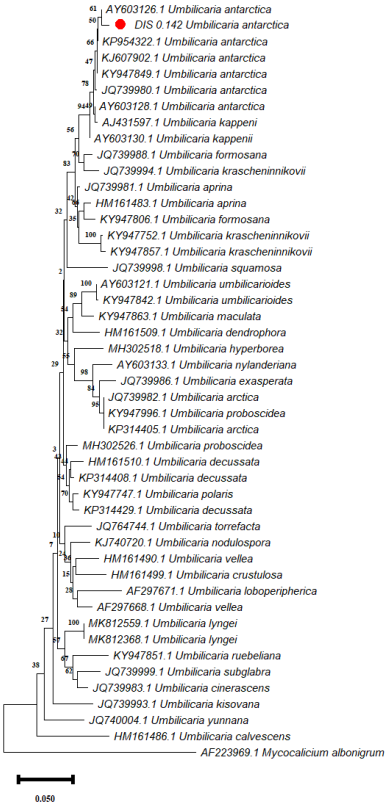

Figure 20. *nrITS* ML dendrogram of *Umbilicaria antarctica*

3.11. *Usnea antarctica* Du Rietz

Description: Thallus fruticose, yellow, with green and black tones present in the branches. Black patches are also present on the branches. Tips of branches completely black. Papillae present. Branches flat. Apothecia not observed. No annulations detected (Figure 21).

Ecology and distribution: Occurs in various habitats, including moist, dry, open, and sheltered surfaces. Mostly found on rocks, but rarely seen on mosses as well. It has been reported from the southernmost parts of South America, the Falkland Islands, New Zealand, and Antarctica [11].

Specimens examined: Antarctica, Antarctic Peninsula, Dismal Island, near the Turkish Dismal Global Navigation Satellite System, 68°6′ S, 68°50′ W, alt. 10 m, 12 February 2022, leg. M. G. Halıcı, ERCH DIS 0.008.

Figure 21. *Usnea antarctica*

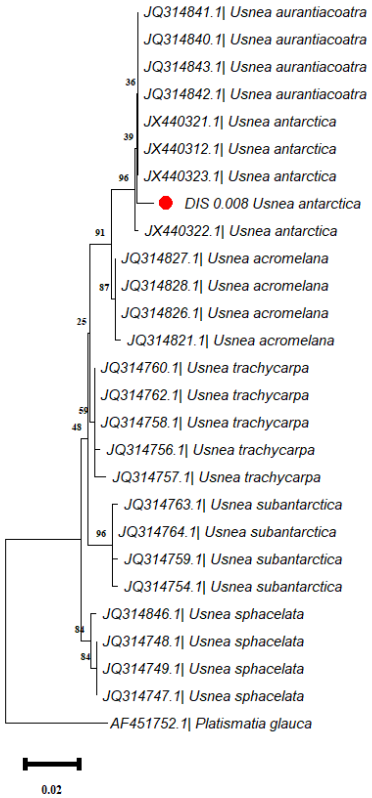

Figure 22. *nrITS* ML dendrogram of *Usnea antarctica*

4. Conclusions and discussion

In this study, lichen samples collected from Dismal Island during TAE VI by the second author were morpho-anatomically examined, and molecular and phylogenetic characterization was performed. A total of 12 lichen samples were examined in the study; morpho-anatomical examination and molecular and phylogenetic characterization based on the ITS gene region were successfully conducted. As a result, the 12 lichen samples were determined to belong to 11 different species: *A. hookerii, B. russa, C. flava, M. tessellata, P. candelaria, R. aspidophora, R. geographicum, T. atra, T. anisomerus, Umbilicaria antarctica, Usnea antarctica.*

According to the BAS database, 17 lichen species have been recorded from Dismal Island by various researchers. Among the species identified in this study, *B. russa*, *M. tessellata*, *P. candelaria, R. aspidophora, Usnea antarctica*, and *Umbilicaria antarctica*, for which molecular and phylogenetic characterization was performed in this study, had been previously reported from Dismal Island under BAS records. In contrast, *A. hookerii*, *C. flava*, *R. geographicum*, *T. atra*, and *T. anisomerus* are newly reported from Dismal Island in the present study.

Austroplaca hookeri is morphologically and anatomically very similar to *Gondwania sublobulata* (Nyl.) S.Y. Kondr., Kärnefelt, Elix, A. Thell, Jung Kim, M.H. Jeong, N.N. Yu, A.S. Kondr. & Hur, which also has a wide distribution in Antarctica. The most reliable distinction between the two species can be made through molecular analysis. According to the ITS data obtained in this study, sample DIS 0.085 matches *A. hookeri* with a high BS value (BS > 75). Phylogenetically, *A. hookeri* is closely related to *A. cirrochrooides* (Vain.) Søchting, Frödén & Arup and *A. millegrana* (Müll. Arg.) Søchting, Frödén & Arup. However, unlike *A. hookeri*, *A. millegrana* has dense papillae, while *A. cirrochrooides* has soredia [11].

Buellia russa is anatomically and morphologically similar to *Buellia pycnogonoides* Darb. The thallus is continuous in *B. russa*, while it appears as scattered patches in *B. pycnogonoides*. *B. pycnogonoides* is distinguished from *B. russa* by its densely immersed pycnidia. Phylogenetically, it is very closely related to *Buellia frigida* Darb. Both species contain norstictic acid. However, *B. frigida* is distinguished from *B. russa* by its characteristic effigurate black-and-white thallus [11].

Candelariella flava is anatomically and morphologically similar to sorediate species such as *Austroplaca darbishirei* (C.W. Dodge & G.E. Baker) Søchting, Frödén & Arup and *A. soropelta* (E.S. Hansen, Poelt & Søchting) Søchting, Frödén & Arup, especially in samples without apothecia as in this study. These two species give positive reactions due to the secondary compounds they contain, while the thallus of *C. flava* gives negative results with KOH solution. Phylogenetically, *C. flava* is closely related to *C. subsquamulosa* D. Liu & Hur. While *C. flava* occurs on mosses and other lichens, *C. subsquamulosa* occurs on tree bark and wood. Although the thalli of the two species are quite similar morphologically; the thallus of *C. subsquamulosa* s notable for its green color [14].

Mastodia tessellata is the type species of the genus *Mastodia* and is the only lichenized fungal species that forms a symbiotic relationship with macro green algae of the *Prasiola* genus [15]. The only ITS sequence of *M. tessellata* available in GenBank matches the ITS sequence of sample DIS 0.140 obtained in this study with a high BS value. We believe that the other data uploaded as "*Mastodia* sp." in GenBank also belong to *M. tessellata*.

Polycauliona candelaria is morphologically similar to *Xanthoria mawsonii* C. W. Dodge. While the conidiospores are longer than 3 µm in *X. mawsonii*, they are shorter than 2.5 µm in *P. candelaria*. In addition, the septa in ascospores are wider in *X. mawsonii.* Phylogenetically, *P. candelaria* is closely related to *Polycauliona ascendens* (S.Y. Kondr.) Frödén, Arup & Søchting. *P. ascendens* is distinguished from *P. candelaria* by its helmet-shaped lobe tips and powdery soralia [16].

Rhizocarpon geographicum is a very common species worldwide and Antarctica. *R. geographicum* is morphologically very similar to *Rhizocarpon nidificum* (Hue) Darb. Furthermore, the two species are phylogenetically very closely related. In *R. geographicum*, the ascospores are muriform, whereas in *R. nidificum,* they are 3-septate or submuriform [11].

Rhizoplaca aspidophora is distinguished from other species distributed in Antarctica by its lighter yellow apothecia and thallus. Phylogenetically, *R. aspidophora* is closely related to *Rhizoplaca mcleanii* (C.W. Dodge) Castello. *R. macleanii* is distinguished from *R. aspidophora* by its dark brown, black, or gray thallus and apothecia [17].

Tephromela atra is anatomically and morphologically very similar to *Tephromela minor* Øvstedal. In addition, the two species are phylogenetically very closely related. *T. minor* is distinguished from *T. atra* by its smaller ascospores, light-colored hymenium, narrower paraphyseal tips, and secondary chemistry. *T. minor*, unlike *T. atra*, contains atranorin and norstictic acid [11].

Tetramelas anisomerus is anatomically and morphologically similar to *Tetramelas granulosus* (Darb.) A. Nordin. The two species are also very closely related phylogenetically. The distinction between these two species is based on the secondary compounds they contain. While *T. anisomerus* contains arthothelin, *T. granulosus* contains granulosin [11].

Umbilicaria antarctica is anatomically and morphologically very similar to *Umbilicaria africana* (Jatta) Krog - Swinscow. While the lower surface is black in *U. africana*, it is mottled, grayish-black or brownish in *U. antarctica*. Phylogenetically, *U. antarctica* is very closely related to *U. kappeni* Sancho, B. Schroet. & Vallad. *U. kappeni* has soredia, and thallospores are not observed [11].

Usnea antarctica has a very wide distribution in Antarctica. Anatomically and morphologically, it is very similar to *Usnea acromelana* Stirt. and *Usnea subantarctica* F.J. Walker. It is also phylogenetically very closely related to *U. acromelana*. *U. antarctica* lacks annulations and has papillae, whereas *U. acromelana* has annulations and no papillae. *U. subantarctica* has apothecia, but apothecia are rarely observed in *U. antarctica* [11].

Ethical statement: This research is based on non-invasive sampling of lichenized fungi conducted during the 6th Turkish National Antarctic Scientific Expedition, which was carried out under the official permissions granted by the Scientific and Technological Research Council of Turkey (TÜBİTAK) and the Presidency of the Republic of Turkey, Polar Research Institute (KARE). The study did not involve any endangered or protected species, vertebrate animals, or human participants. All procedures were conducted in accordance with relevant national and international guidelines for Antarctic scientific research.

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The grammar of the manuscript was reviewed using OpenAI's ChatGPT-4.0. No AI-generated content was included in the final version.

Conflicts of interest: The authors declare that they have no conflict of interest.

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Author contributions: Dilara BİŞGİN performed the microscopic examinations of the samples, participated in DNA isolation and laboratory analyses within the scope of the TÜBİTAK 2209-A project. M. Gökhan HALICI collected the samples during the 6th Turkish National Antarctic Scientific Expedition, supervised identifications, and validated the taxonomic determinations. Merve YİĞİT conducted the phylogenetic analyses, wrote the manuscript, and provided data interpretation and critical revisions. All authors read and approved the final version of the manuscript.

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