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DNA Barcoding of Some Lichenized Fungi from James Ross Island (Antarctic Peninsula, Antarctica)

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Abstract: Due to the harsh environmental conditions in Antarctica, the life of living organisms is very limited. Lichenized fungi, which can survive in harsh environmental conditions, form the dominant vegetation of Antarctica. Studies on the biodiversity of lichenized fungi in Antarctica have been conducted for many years based on anatomical and morphological observations. However, with the use of DNA barcoding methods, these studies have been accelerating in recent years and new species are being discovered day to day. On the other hand, DNA barcoding of known species from the past is also done and anatomical/morphological diagnoses are confirmed. In this study, *Catillaria contristans* (Nyl.) Zahlbr., *Gyalidea antarctica* Øvstedal & Vězda, *Physconia muscigena* (Ach.) Poelt, *Rhizocarpon geminatum* Körb., *Steinera intricata* (Øvstedal) Ertz and *Xanthocarpia tominii* (Savicz) Frödén, Arup & Søchting species based on nr*ITS* DNA barcoding were performed. In addition, detailed descriptions of the species are also included in the study.

James Ross Adası (Antarktika Yarımadası, Antarktika)'ndan Bazı Likenleşmiş Mantarların DNA Barkodlaması

Anahtar Kelimeler
Antarktika
Biyoçeşitlilik
DNA barkodlama
James Ross Adası
Likenleşmiş Mantar
nr*ITS*

Öz: Antarktika'da zorlu çevresel koşullar nedeniyle canlıların yaşamı oldukça sınırlanmıştır. Zorlu çevresel koşullarda hayatta kalabilen likenleşmiş mantarlar Antarktika'nın baskın vejetasyonunu oluşturmaktadır. Antarktika'da likenleşmiş mantarların biyoçeşitliliği ile ilgili çalışmalar anatomik ve morfolojik gözlemlere dayalı olarak uzun yıllardır devam etmektedir. Ancak DNA barkodlama yöntemlerinin kullanılmasıyla son yıllarda bu çalışmalar hız kazanmaktadır ve her geçen gün yeni türler keşfedilmektedir. Öte yandan geçmişten bu yana bilinen türlerin DNA barkodlamaları da yapılmakta ve anatomik/morfolojik teşhislerin doğrulanmaktadır. Bu bağlamda bu çalışmada James Ross Adası (Antarktika Yarımadası, Antarktika)'ndan daha önce rapor edilen likenleşmiş mantar türlerinden *Catillaria contristans* (Nyl.) Zahlbr., *Gyalidea antarctica* Øvstedal & Vězda, *Physconia muscigena* (Ach.) Poelt, *Rhizocarpon geminatum* Körb., *Steinera intricata* (Øvstedal) Ertz ve *Xanthocarpia tominii* (Savicz) Frödén, Arup & Søchting türlerinin nr*ITS*'e dayalı DNA barkodlamaları yapılmıştır. Ayrıca çalışmada türlere ait detaylı deskripsiyonlara da yer verilmiştir.

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1. Introduction

The dominant organisms of the Antarctic terrestrial vegetation are lichenised fungi. It constitutes the largest macroorganism group in terms of the number of species [1]. Studies on lichenized fungi in Antarctica have been going on for about 2 centuries. Today, with DNA-based research, the number of lichenized fungi known from Antarctica is nearly 500 [2].

James Ross Island, located in the north-east tip of Antarctic Peninsula "(64° 15' S, 57° 45' W)" is known as one of the most lichen-rich islands in Antarctica, with more than 140 species of lichenized fungi reported. As usually the anatomical and morphological characters were used in identification of lichenized fungi species on James Ross Island; it is thought that the lichens of the island should be examined by molecular methods to accurately [3]. For several years some researchers studied the island's lichenized fungi biodiversity; identified and/or reported new species from the island [3-15]. DNA barcoding plays an important role in making these identifications especially in the last years.
In this study, it is aimed to perform DNA barcoding of some previously known and reported species from James Ross Island. For this purpose, *Catillaria contristans* (Nyl.) Zahlbr., *Gyalidea antarctica* Øvstedal & Vězda, *Physconia muscigena* (Ach.) Poelt, *Rhizocarpon geminatum* Körb, *Steinera intricata* (Øvstedal) Ertz and *Xanthocarpia tominii* (Savicz) Frödén, Arup & Søchting are identified in species level based on nr*ITS* phylogeny.

2. Material and Method

2.1. Materials and morphological observation

Samples of lichenized fungi were collected by the third author from James Ross Island (Antarctic Peninsula). Collected specimens are deposited in "Erciyes University Herbarium (ERCH-Kayseri, Turkey)". The specimens were identified by using standard microscope methods. Sections were taken in Lugol's solution, potassium hydroxide (K) and water. However, measurements were made only from sections in water. The measurements are written in as "(smallest value) mean minus standard deviation-mean-mean plus standard deviation-(largest value)" format by calculating mean, standard deviation, maximum and minimum values. "n" was the total number of measurements for all samples of that species.

2.2. Isolation, DNA extraction, amplification, and sequencing

An average of five to six apothecia were taken for DNA isolation and a commercial DNA isolation kit ("DNeasy Plant Mini Kit; Qiagen") was used for DNA isolation. The isolation was carried out according to the instructions prepared by the manufacturer in the kit. "Internal transcribed spacer region (*ITS*1-5.8S-*ITS*2 rDNA)" genes were used for PCR amplification. Each sample was prepared for a total of 50 µl of standard reaction. Optimum amplification conditions were obtained with 25 µl of 2 × Taq PCR MasterMix in each tube with 2 µl of the primers *ITS*1F and *ITS*4, 2 µl of DNA extracts and 19 µl of distilled water [16-17]. The thermal cycling conditions are as follows: 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". Sequence analyzes of lichen samples from which PCR products were obtained were performed by BM Labosis Laboratory (Ankara, Turkey).

2.3. Phylogenetic analyses

ITS sequences of all species were aligned and optimized manually using ClustalW in BioEdit V7.2.6.1 for preparing the phylogenetic trees. In MEGA XI, only parsimony-informative regions were used for analysis. Indeterminate regions were excluded from the alignment. [18-19].

1000 bootstrap replications were performed by bootstrap analysis for the estimation of confidence levels of the clades. Phylogenetic relationships and support values were investigated using maximum likelihood (ML) bootstrapping, as implemented in MEGA XI. Kimura two-parameter model was used for the analysis of the ML method. Genbank numbers of used sequences in phylogenetic trees within this study are given in Appendix Table 1 (Appendix 1).

3. Results

3.1. *Catillaria contristans* (Nyl.) Zahlbr.

Thallus crustose, as clumps of warty granulose squamules, chalky white. Apothecia lecidein, plane or convex, angular, black, weakly whitish pruinose, clustered or dispersed as single ones, (0.2–)–*0.3*–(–0.6) mm (n=10). Epihymenium bluish-black, 50 µm. Hymenium hyaline sometimes with a blackish tinge, 80-90 µm. Hypothecium hyaline and 45-50 µm. Asci 8-spored. Ascospores hyaline, one-septate, ellipsoid or narrowly ellipsoid, sometimes slightly curved, (9–)9,6–10,6–11,5(–12) × (3,5–)–4,5–(–5) µm (n=20) and ascospores l/w ratio: (2–)–2,4–(–3) µm (n=20). Paraphyses are not branched, adnate, tips capitate with blackish pigment and 3–7 µm diam. Pycnidium was not observed (Figure 1). Thallus and medulla K-, C-, KC-, Pd-, KI-.

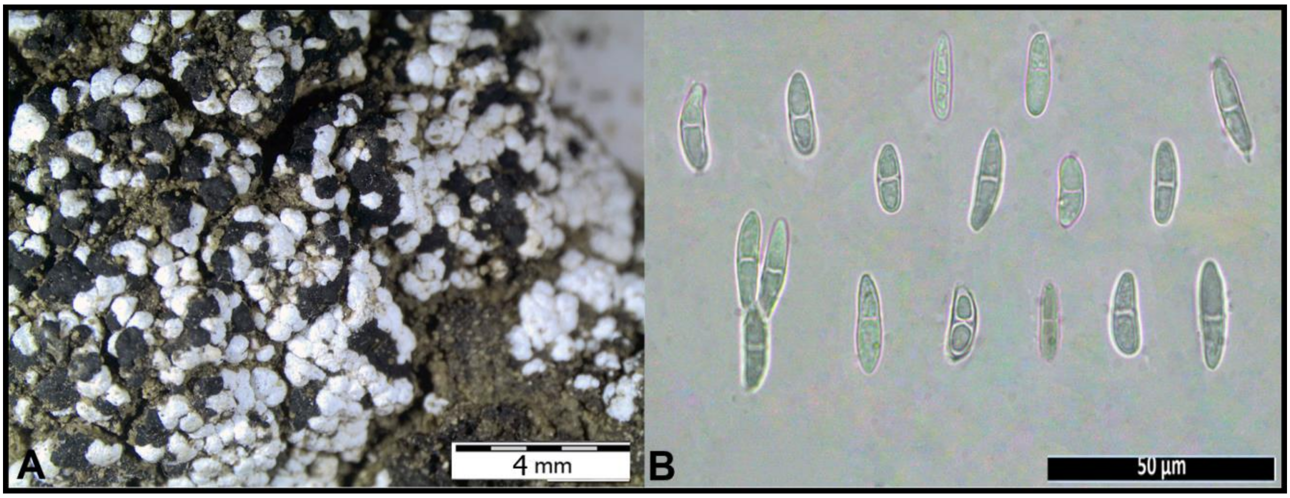

Figure 1. *Catillaria contristans*, A. Habitus, B. Ascospores.

For the phylogenetic analysis of the species *C. contristans*, a total of 16 *ITS* rDNA sequences were analyzed. In the BLASTn search, the final alignment of the *ITS* sequence of *Catillaria contristans* contained 550 bp after trimming. Altogether, 273 nucleotides were found to be conserved sites (C), and 270 nucleotides were found to be variable sites (V) in the *ITS* gene region. *Bryobilimbia diapensiae* (Th. Fr.) Fryday, Printzen & S. Ekman is used as an outgroup which is a member of the genus *Bryobilimbia* Fryday, Printzen & S. Ekman, phylogenetically related to the genus *Catillaria* A . Massal [20] (Figure 2).

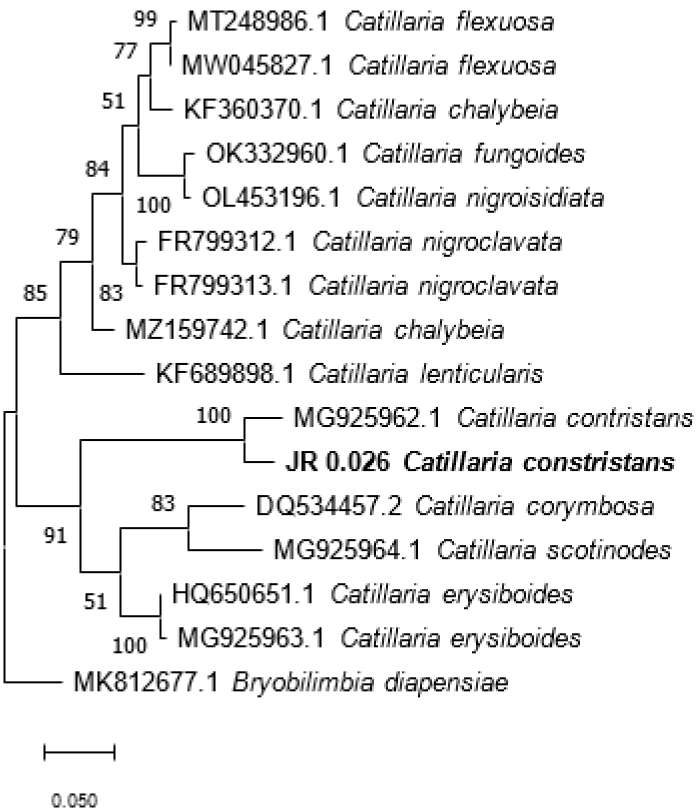

Figure 2. ML phylogeny based on *ITS* gene region of *Catillaria contristans.*

C. contristans grows on gravelly soil and terricolous crustose lichens in stony fellfield [21]. It can grow over bryophytes (*Andreaea* spp.) on sheltered, vertical rock face with no calcareous influence [22], and also it has been reported from plant debris on rock or bryophytes in acid habitats, in higher mountains or on the ground [23]. In James Ross Island, it grows on soil near streams at low altitudes.

It is a bipolar species [21]. It has been reported from Tasmania [21], Australia (24), New Zealand, North America (22), Poland (23), Germany (25), Scotland (26), Panarctic (27), England (28), Ireland (29), Iceland (30), USA (31), France (32), North Norway (33), British Isles (26), Greenland (34) and Antarctica.

In Antarctica; it has been reported from Antarctic Peninsula, South Shetland and Orkney Islands [21, 35], King George Island [36, 37], Singy Island (38). *C. contristans* is a new record for James Ross Island.

Specimen examined: "Antarctica, Antarctic Peninsula, James Ross Island, Solorina Valley 63° 52′ 39.0″ S, 57° 46′ 51.6″ W, alt. 2 m., on soil, 26 January 2017, Leg. M. G. Halıcı (JR 0.026)".

3.2. *Gyalidea antarctica* Øvstedal & Vězda

Thallus granulate, mostly not conspicuous. Apothecia dispersed in various parts of the substrate, immersed to the thallus, 0.2–0.25 mm diam. Mature apothecia porous, honey-brown. Epihymenium light yellowish brown, 25–30 µm. Hymenium honey brown, 75–110 µm, Hypothecium hyaline, 30–40 µm. Asci 8-spored, 80–91 × 28–36 µm. Ascospores wide ellipsoid, simple, hyaline, with many oil droplets, (16–)16.5–*21.5*–27.5(–28) × (11–)11.5–*13.5*–15.5(–16) µm (n=10). Ascospores l/w ratio: (1.14–)1.25–1.6–1.95(–2) µm. Paraphyses are branched, slender, 1–1.5 µm. Pycnidium was not observed (Figure 3). All spot tests are negative.

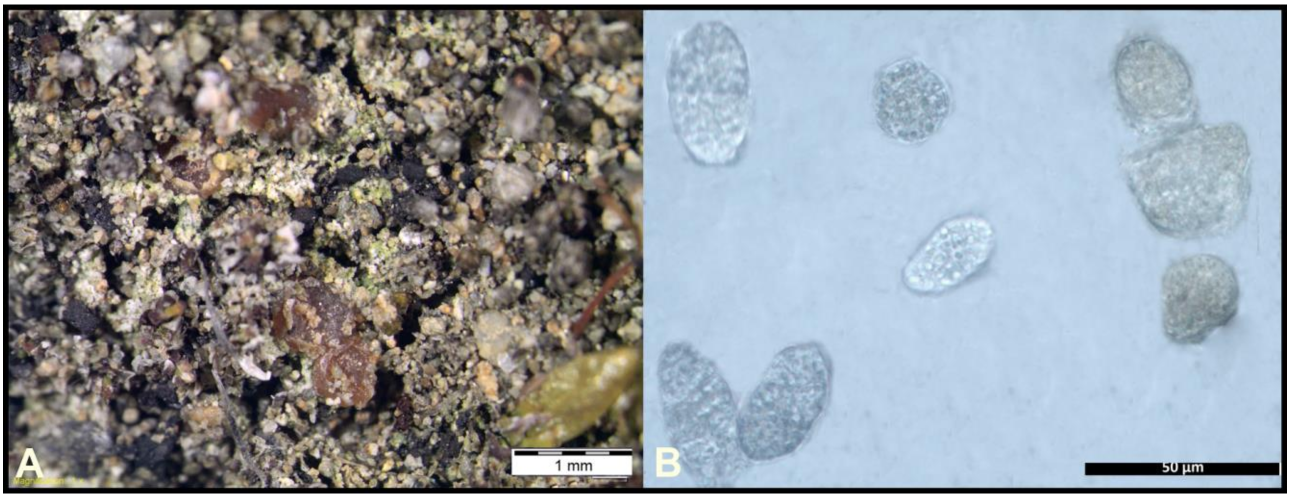

Figure 3. *Gyalidea antarctica*, A. Habitus, B. Ascospores.

For the phylogenetic analysis of the species *G. antarctica*, a total of 4 *ITS* rDNA sequences were analyzed. In the BLASTn search, the final alignment of the *ITS* sequence of *G. antarctica* contained 512 bp after trimming. Altogether, 200 nucleotides were found to be conserved sites (C), and 304 nucleotides were found to be variable sites (V) in *ITS* gene region. *G. antarctica* is classified in the family Gomphillaceae. There are 23 genera and about 450 species in this family but in GenBANK there is only two other *ITS* data from family members (*Gyalidea fritzei* (Stein) Vězda and *Gyalidea* aff. *lecideopsis* var. *eucarpa* (Servít) Vězda). Therefore, *Diploschistes scruposus* (Schreb.) Norman, belonging to the family Thelotrematacea which is phylogenetically closely related to the family Gomphillaceae [39] was used as an outgroup (Figure 4).

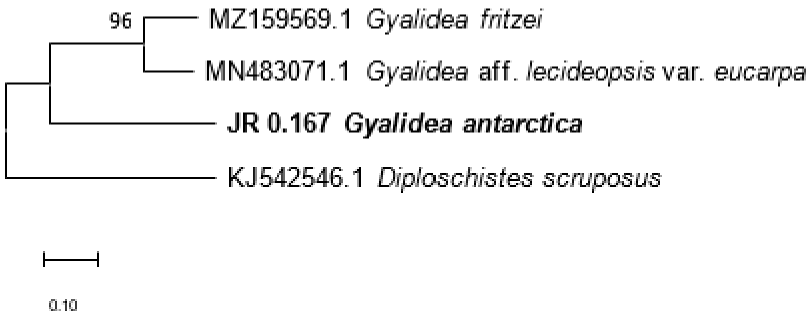

Figure 4. ML phylogeny based on *ITS* gene region of *Gyalidea antarctica*

It is an Antarctic endemic species and it has only been reported from James Ross Island so far and it grows at an altitude of 10-35 m on moist *Bryum pseudotriquetrum* located on the banks of the stream [21].

Specimen examined: "Antarctica, Antarctic Peninsula, James Ross Island, Long Term Research Spot 7-8, 63° 48′ 03″ S, 57° 52′ 50″ W, alt. 3 m., on moss, 24 January 2017, Leg. M. G. Halıcı (JR 0.167)".

3.3. *Physconia muscigena* (Ach.) Poelt

Thallus foliose, up to 4 cm diam, upper surface grey and margins has purple tinge. Whitish pruinose patches are present. Lobes regular or irregular, 1–2 mm broad and 2–4 mm long. Margins of the lobes sometimes upturned. Vegetative propagules not present. Lower surface i whitish brown or white and black rhizines present. Apothecia and pycnidia not observed (Figure 5). All spot tests are negative.

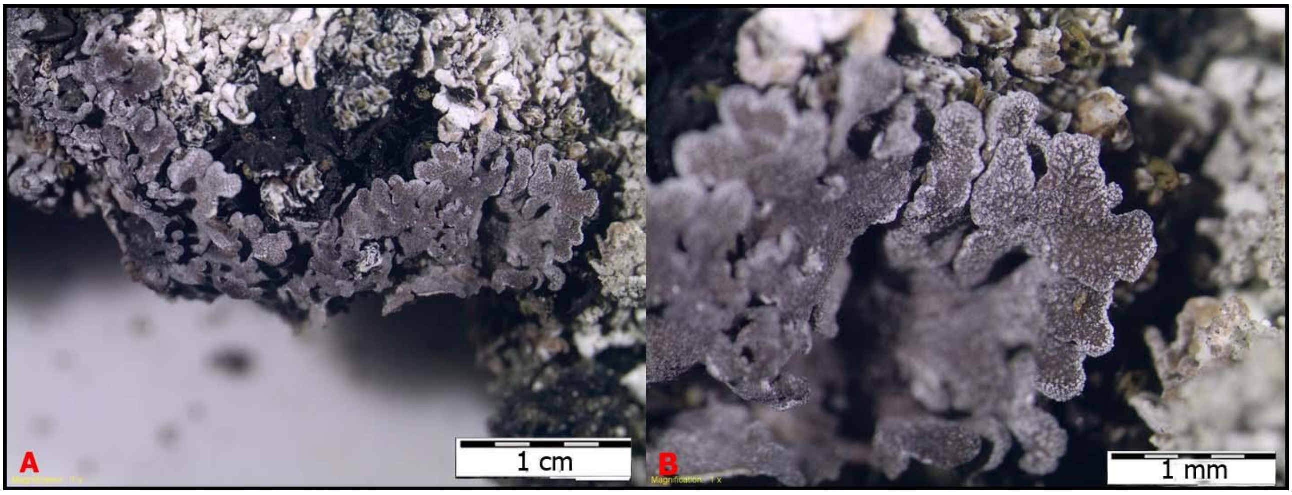

Figure 5. *Physconia muscigena*, A. Thallus, B. Lobes.

For the phylogenetic analysis of the species *P. muscigena*, a total of 44 *ITS* rDNA sequences were analyzed. In the BLASTn search, the final alignment of the *ITS* sequence of *P. muscigena* contained 544 bp after trimming. Altogether, 343 nucleotides were found to be conserved sites (C), and 149 nucleotides were found to be variable sites (V) in *ITS* gene region. *Anaptychia ciliaris* (L.) Körb. ex A. Massal, a species belonging to the same family with *P. muscigena* [40] was used as an outgroup (Figure 6).

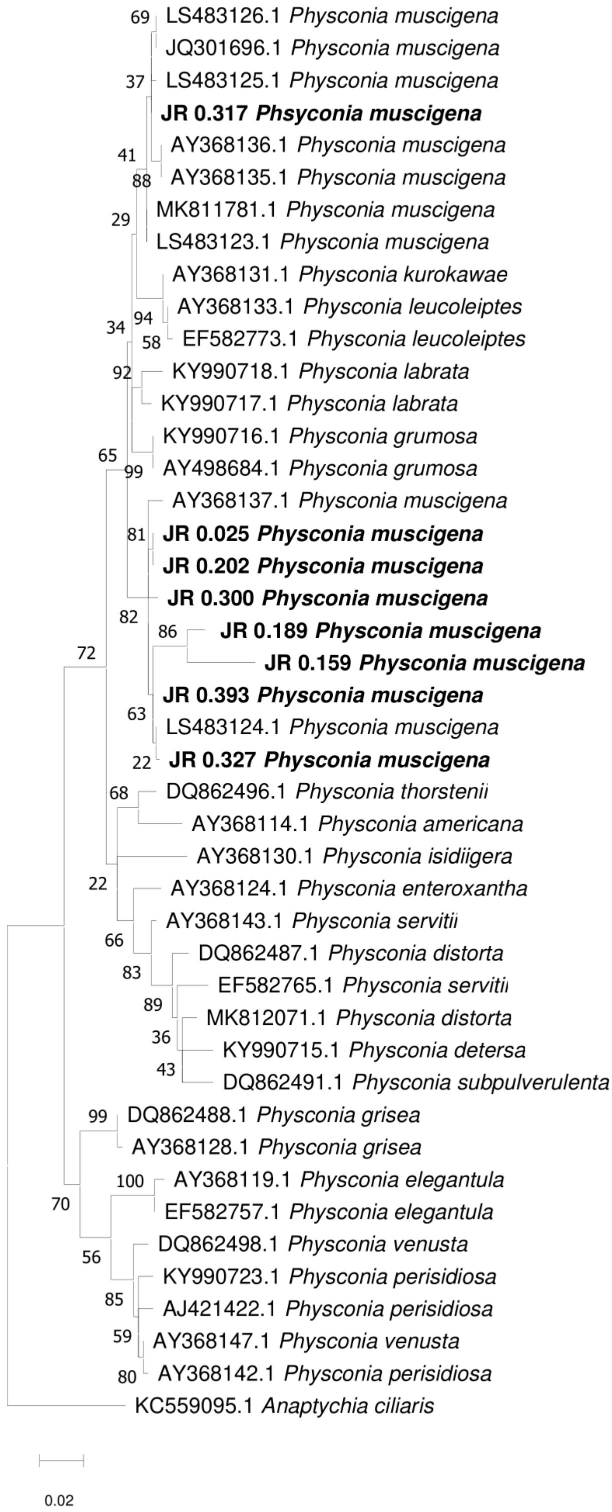

Figure 6. ML phylogeny based on *ITS* gene region of *Physconia muscigena*

P. muscigena is a bipolar cosmopolite species and has a wide distribution in the world. In Antarctica it has been known from, South Shetland Islands (King George Island), South Orkney Islands [21], James Ross Island (41), Cape Lion (42), King George Island (43), Dronning Maud Land (44), Alexander Island, Cockburn Island (45), South Shetland Island (46), Half Moon Island (47) on soil or mosses.

Specimens examined: "Antarctica, Antarctic Peninsula, James Ross Island: Berry Hill Point, 63° 48′ 42.0″ S, 57° 50′ 5,4″ W, alt. 300 m., on soil (JR 0.025); Neck of Lachman, 63° 47′ 22.5″ S, 57° 48′ 12″ W, alt. 36 m., on moss (JR

0.089); Lachman Bay, 63° 47′ 22.5″ S, 57° 48′ 12″ W, alt. 36 m., on soil (JR 0.159, JR 0.202); SE Tip of Johnson Mesa, 63° 49′ 46.2″ S, 57° 54′ 21.6″ W, alt. 292 m., on soil (JR 0.300); Panoramic Pass, 63° 48' 56" S, 57° 50' 36" W, alt. 220 m., on soil (JR 0.317); Berry Hill Mesa, 63° 48′ 42.0″, 57° 50′ 5.4″ W, alt. 345 m., on moss (JR 0.327); Puchau, 63° 48′ 24.9″ S, 57° 50′ 27.6″ W, alt. 142 m., on soil (JR 0.397); Leg. M. G. Halıcı."

3.4. *Rhizocarpon geminatum* Körb.

Thallus crustose, well developed, areolate verrucose, blackish dark brown. Areoles convex and swollen, circular, dispersed through thallus. Apothecia black, immersed between areoles, (0.1–)0.15–0.2–0.35(–0.5) mm (n=90). Epihymenium dark brown to black, (10–)21–35–49(–70) µm (n=25). Hymenium hyaline, (40–)78–107–136(–170) µm (n=25). Hypothecium dark brown, (10–)32.5–67.5–102.5(–130) µm (n=25). Asci 2-spored, (42–)56.5–76.5–96.5(–113) × (10–)17–27–37(–54) µm (n=25). Ascospores greenish to dark brown, ellipsoid, murriform, (23–)37–48–59(–90) × (11–)18–24–30(–40) µm (n=85). Ascospores l/w ratio: (1.17–)1.49–2.1–2.71(–4.82) µm (n=85). Paraphyses are simple, branched or not branched, tips are slightly enlarged, (2–)2.5–3–3.5(–4) µm (n=30). Pycnidium not observed (Figure 7). All spot tests are negative.

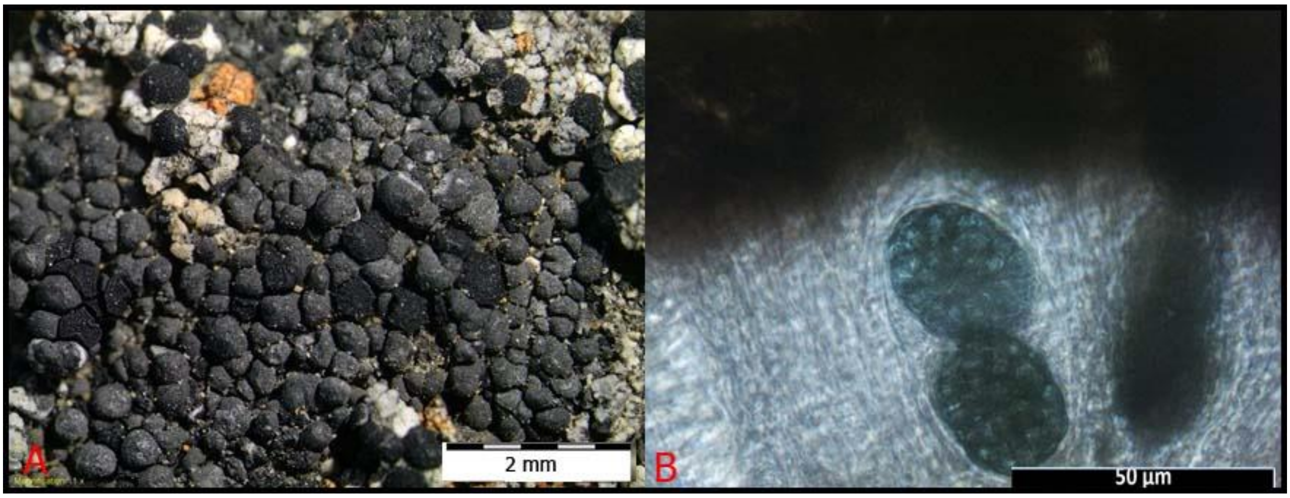

Figure 7. *Rhizocarpon geminatum*, A. Thallus and apothecia, B. Ascus and ascospores

For the phylogenetic analysis of the species *R. geminatum*, a total of 26 *ITS* rDNA sequences were analyzed. In the BLASTn search, the final alignment of the *ITS* sequence of *R. geminatum* contained 652 bp after trimming. Altogether, 301 nucleotides were found to be conserved sites (C), and 257 nucleotides were found to be variable sites (V) in *ITS* gene region. *Catolechia wahlenbergii* (Ach.) Flot., a species belonging to the same family with *R. geminatum* [48] was used as an outgroup (Figure 8).

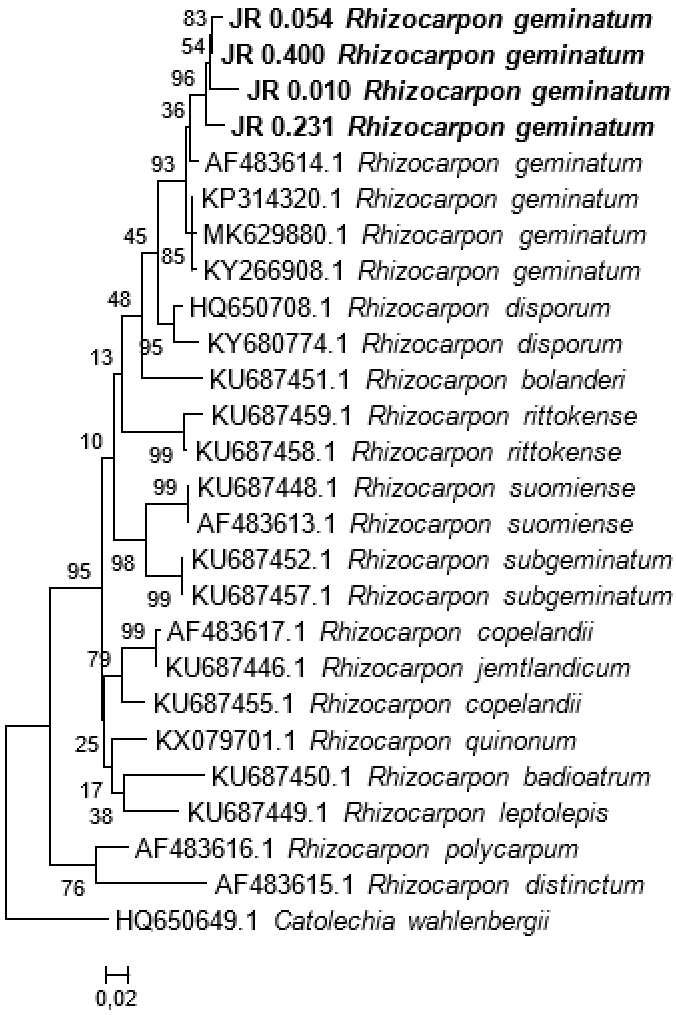

Figure 8. ML phylogeny based on *ITS* gene region of *Rhizocarpon geminatum*

R. geminatum is a bipolar cosmopolite species. It has been reported from Europe, North America, Antarctica [21], Australia (49), Turkey (50), Northern Hemisphere (51). In Antarctica; it has been reported from James Ross Island, South Shetland Islands, Argentina Island [21], King George Island (52), Livingstone Island (53) Botany-Bay (54), Redcastle Ridge, Cape King, Gondwana, Finger Point (55), Victoria Land (56), Terra Nova Bay (57), Schirmacher Oasis- Larsemann Hill (58) , Cape Lion (42), Ross Sea Region (59).

Specimens examined: "Antarctica, Antarctic Peninsula, James Ross Island: British Navy Point, on rock (JR 0.010); Peters Collection, on rock (JR 0.054); Solorina Valley 63° 52′ 39.0″ S, 57° 46′ 51.6″ W, alt. 2 m., on rock (JR 0.231, JR 0.400); Leg. M. G. Halıcı."

3.5. *Steinera intricata* (Øvstedal) Ertz

Thallus brown, foliose, 3 cm wide, straight and circular, lobate. Lobes terete, branched, 1–2 cm diam and 0.5–1 cm length. Cortex of lobes up to 7 µm thick, composed of densely conglutinated isodiametric cells. Medulla dense and full of photobiont. Photobiont cyanobacteria. Isidia present. Apothecia not observed (Figure 11). All spot tests are negative.

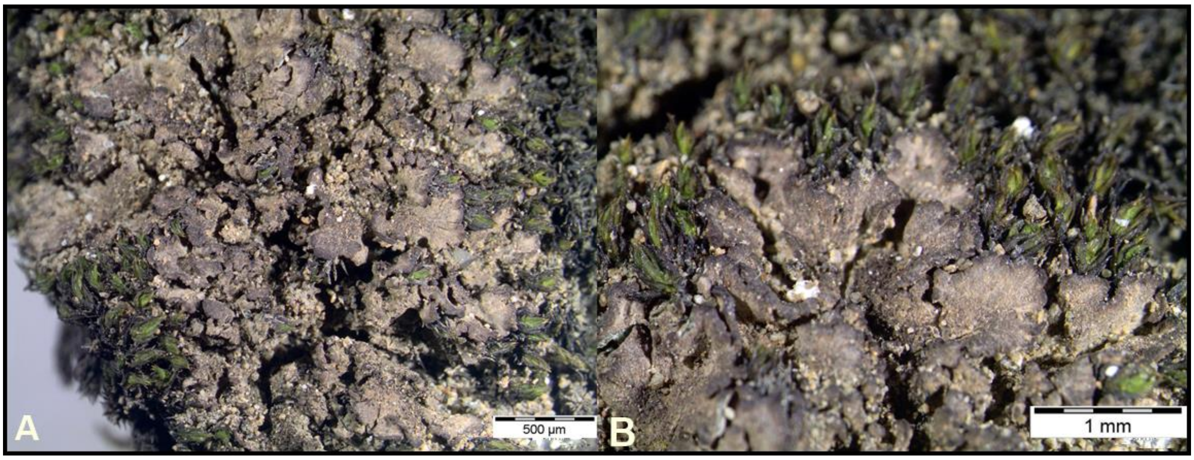

Figure 9. *Steinera intricata,* A. Thallus, B. Lobes.

For the phylogenetic analysis of the species *S. intricata*, a total of 19 *ITS* rDNA sequences were analyzed. In the BLASTn search, the final alignment of the *ITS* sequence of *S. intricata* contained 494 bp after trimming. Altogether, 338 nucleotides were found to be conserved sites (C), and 147 nucleotides were found to be variable sites (V) in *ITS* gene region. *Gregorella humida* (Kullh.) Lumbsch, a species belonging to the same family with *S. intricata* [60] was used as an outgroup (Figure 10).

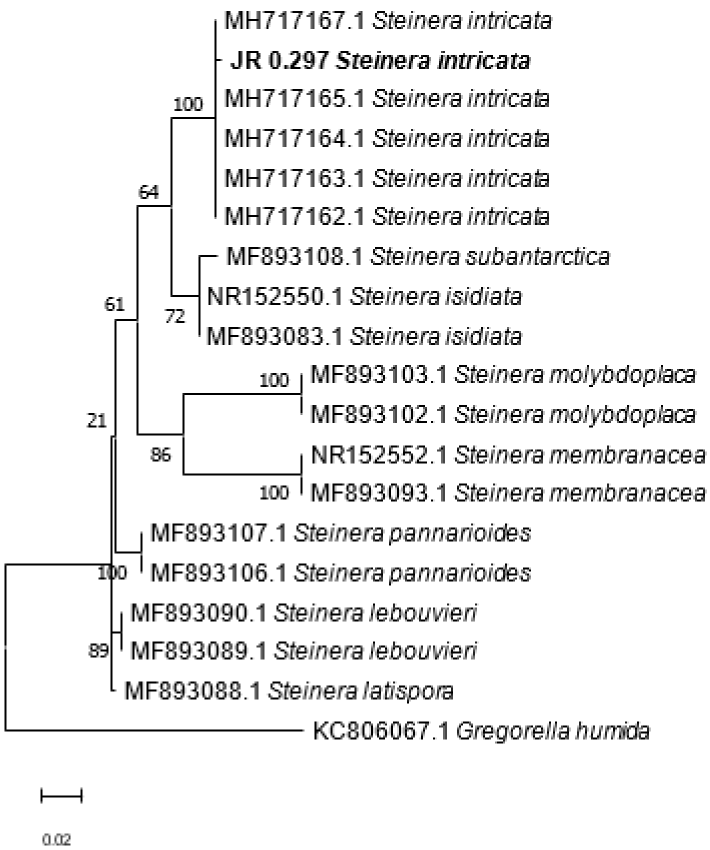

Figure 10. ML phylogeny based on *ITS* gene region of *Steinera intricata*

S. intricata occurs on mosses and rocks [21]. It has been reported from South Shetland Islands, James Ross Island, Charcot Island, Southern South America (Terra del Fuego)[61]. In James Ross Island, it was collected on mosses in high altitudes.

Specimen examined: "Antarctica, Antarctic Peninsula, James Ross Island, SE Tip of Johnson Mesa, 63° 49′ 46.2″ S, 57° 54′ 21.6″ W, alt. 292 m., on moss (JR 0.297), Leg. M. G. Halıcı".

3.6. *Xanthocarpia tominii* (Savicz) Frödén, Arup & Søchting

Thallus crustose, squamulose, yellow-orange. Soredia present. Especially the margins of the thallus are covered with dense soralia. Soralia are lighter yellow than thallus, almost lemon yellow. Apothecia was not observed (Figure 11). Thallus and soralia K+ purple.

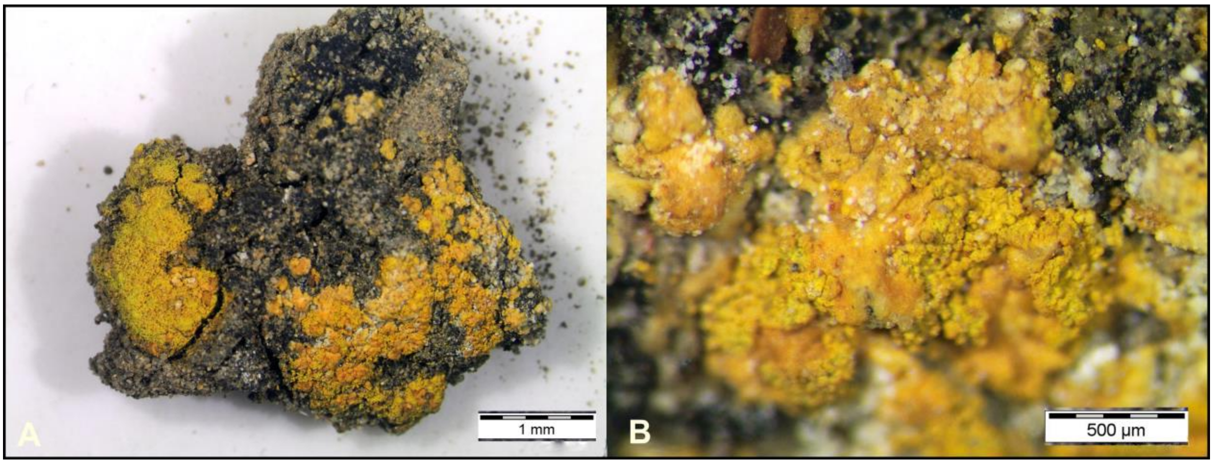

Figure 11. *Xanthocarpia tominii,* A. Thallus, B. Soralia.

For the phylogenetic analysis of the species *X. tominii*, a total of 26 *ITS* rDNA sequences were analyzed. In the BLASTn search, the final alignment of the *ITS* sequence of *X. tominii* contained 588 bp after trimming. Altogether, 353 nucleotides were found to be conserved sites (C), and 158 nucleotides were found to be variable sites (V) in *ITS* gene region. *Calogaya saxicola* (Hoffm.) Vondrák, a species belonging to the same family with *X. tominii* [62] was used as an outgroup (Figure 12).

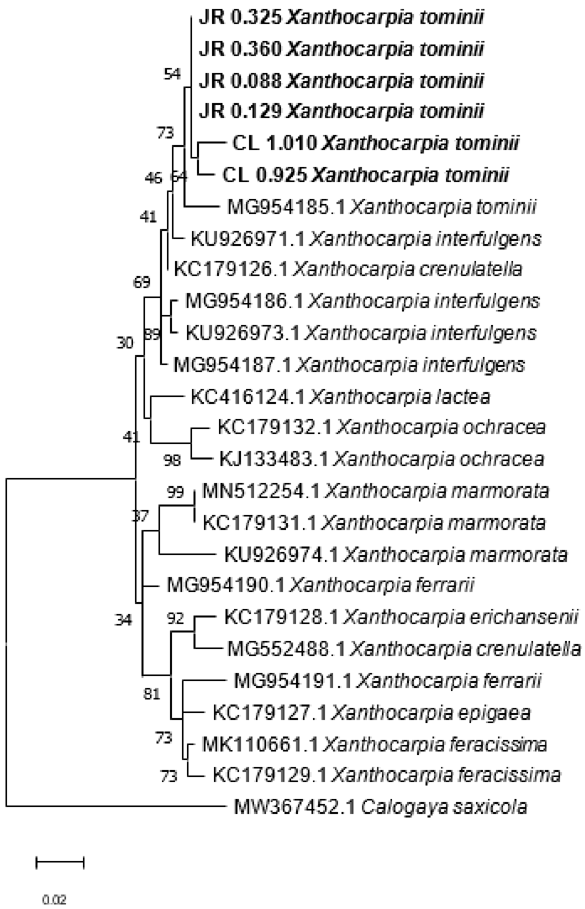

Figure 12. ML phylogeny based on *ITS* gene region of *Xanthocarpia tominii.*

X. tominii is a species that develops widely on calcareous and non-calcareous soils and plant residues (63). It has been known from North America, Greenland, Norway, Austria, Ukraine, Central Asia, Peru and Antarctica [21]. In Antarctica, it has been reported from Victoria Land (63), Utsteinen Nunatak (60) and Enderbyland (64). It is a new record for James Ross Island.

Specimens examined: "Antarctica, Antarctic Peninsula, James Ross Island: Neck of Lachman, 63° 47′ 22.5″ S, 57° 48′ 12″ W, alt. 36 m., on soil (JR 0.088); Dirty Valley 63° 48′ 38.1″ S, 57° 51′ 36″ W, alt. 92 m., on soil (JR 0.129); Panoramic Pass 63° 48' 56" S, 57° 50' 36" W, alt. 220 m., on soil (JR 0.325, JR 0.360), Leg. M. G. Halıcı."

4. Discussion and Conclusion

In this study, we performed DNA barcoding of some previously known and reported species from James Ross Island. For this purpose, we studied *Catillaria contristans* (Nyl.) Zahlbr., *Gyalidea antarctica* Øvstedal & Vězda, *Physconia muscigena* (Ach.) Poelt, *Rhizocarpon geminatum* Körb, *Steinera intricata* (Øvstedal) Ertz and *Xanthocarpia tominii* (Savicz) Frödén, Arup & Søchting species based on nr*ITS* phylogeny.

According to ITS phylogeny (Figure 2), it is clear that JR 0.026 belongs to *C. contristans.* It matches 100% with the species data from Genbank. Phylogenetically *C. contristans* is closely related to *Catillaria corymbosa* (Hue) I.M. Lamb. *C. corymbosa* has fruticose thallus, while *C. constristans* has crustose thallus. *C. contristans* is anatomically and morphologically similar to *Bilimbia lobulata* (Sommerf.) Hafellner & Coppins. Two species differ from each other by ascus types. *C. contristans* has *Catillaria*-type ascus, while *B. lobulata* has *Biatoria*-type ascus [21].

G. antarctica is classified under the genus *Gyalidea* in the family Gomphillaceae. There are 23 genera in the Gomphilaceae family. These 23 genera contain about 450 species [indexfungorum.org]. However, GenBANK does have only two *ITS* data for family species (*G. fritzei* and *G.* aff. *lecideopsis* var. *eucarpa*). These two data were used for phylogenetic tree. It is very clear in the phylogenetic tree (in Figure 4) that the species is *G. antarctica*, as well as with its anatomical and morphological features. Its data was uploaded to GenBANK within this publication for the first time.

According to ITS phylogeny (Figure 6), all eight JR specimens (JR 0.025, JR 0.089, JR 0.159, JR 0.202, JR 0.300, JR 0.317, JR 0.327, and JR 0.393) clearly match with *P. muscigena*. *P. muscigena* is anatomically and morphologically similar to *Physconia distorta* (With.) J.R. Laundon. It is quite similar in that it does not contain isidia and soredia in two species and *P. muscigena* differs from *P. distorta* with irregular and ascending lobes [21].

According to ITS phylogeny (Figure 8), all four JR specimens (JR 0.010, JR 0.054, JR 0.231 and JR 0.400) clearly match with *R. geminatum. R. geminatum* is similar to *Rhizocarpon disporum* (Nägeli ex Hepp) Müll. Arg. both phylogenetically and morphologically. While there is one ascospore in the ascus in *R. disporum*, there are two ascospores in *R. geminatum* [21].

According to ITS phylogeny (Figure 10), it is clear that JR 0.297 belongs to *S. intricata.* It matches 100% with the species data from Genbank. *S. intricata* is anatomically and morphologically similar to *Steinera isidiata* Ertz & R.S. Poulsen. Both specimens are isidiate. But *S. isidiata* has a thallus which is usually well visible lobed margin and it is endemic to Crozet and Kerguelen islands. *S. intricata* has a thallus with ramified lobes and it has distribution around Antarctic Peninsula and surrounding islands [60]. On the other hand, when there is no apothecium development in Antarctic material, it can be thought that isidia development has not started yet when the sample is young and it can be confused with a sorediate species, *Massalongia carnosa* (Dicks.) Körb. [21]. However, the *ITS* region data clearly shows that the species is *S. intricata*. Molecularly, it is seen that *Steinera subantarctica* (Øvstedal) Ertz and *S. isidiata* are in the same clade in the *ITS* tree and these species are closely related to *S. intricata.* In *S. subantarctica* species it has 5–7 septate ascospores*; S. intricata* predominantly has three septate ascospores [60]. This comparison could not be made because there was no ascospore development in this sample.

According to ITS phylogeny (Figure 12), all four specimens (JR 0.088, JR 0.129, JR 0.325 and JR 0.360) clearly match with *X. tominii.* Phylogenetically, the closest species to this species is *Xanthocarpia interfulgens* (Nyl.) Frödén, Arup & Søchting. It is distinguished morphologically by the absence of vegetative diaspores. On the other hand, it is extremely difficult to distinguish phenotypically from the other sorediate species *Xanthocarpia erichansenii* (S.Y. Kondr., A. Thell, Kärnefelt & Elix) Frödén, Arup & Søchting in the same genus [62].

As a result of this study, the specimens belonging to *Catillaria contristans*, *Gyalidea antarctica*, *Physconia muscigena*, *Rhizocarpon geminatum*, *Steinera intricata* and *Xanthocarpia tominii* were studied, nr*ITS* DNA barcoding were performed on these specimens and descriptions along with photographes were given for each species. With this study, the lichen biodiversity studies of James Ross Island have been taken one step further and it is thought that it will shed light on future studies in this area by providing molecular data.

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Appendices

Appendix A.

Table 1. Genbank numbers of used sequences in phylogenetic trees within this study.

Genbank Number	Species	Locality
OP324602	**JR 0.026 *Catillaria constristans***	**James Ross Island, Antarctica**
OP324601	**JR 0.167 *Gyalidea antarctica***	**James Ross Island, Antarctica**
OP324609	**JR 0.025 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324610	**JR 0.089 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324611	**JR 0.159 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324612	**JR 0.202 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324613	**JR 0.300 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324614	**JR 0.317 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324615	**JR 0.327 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324616	**JR 0.393 *Physconia muscigena***	**James Ross Island, Antarctica**
OP324605	**JR 0.010 *Rhizocarpon geminatum***	**James Ross Island, Antarctica**
OP324606	**JR 0.054 *Rhizocarpon geminatum***	**James Ross Island, Antarctica**
OP324607	**JR 0.231 *Rhizocarpon geminatum***	**James Ross Island, Antarctica**
OP324608	**JR 0.400 *Rhizocarpon geminatum***	**James Ross Island, Antarctica**
OP324671	**JR 0.297 *Steinera intricata***	**James Ross Island, Antarctica**
OP324618	**JR 0.088 *Xanthocarpia tominii***	**James Ross Island, Antarctica**
OP324619	**JR 0.129 *Xanthocarpia tominii***	**James Ross Island, Antarctica**
OP324620	**JR 0.325 *Xanthocarpia tominii***	**James Ross Island, Antarctica**
OP324621	**JR 0.360 *Xanthocarpia tominii***	**James Ross Island, Antarctica**
OP324603	**CL 0.925 Xanthocarpia tominii**	**Turkey**
OP324604	**CL 1.010 *Xanthocarpia tominii***	**Turkey**
KC559095	*Anaptychia ciliaris*	Spain
MK812677	*Bryobilimbia diapensiae*	Norway
MW367452	*Calogaya saxicola*	Canada
KF360370	*Catillaria chalybeia*	Norway
MZ159742	*Catillaria chalybeia*	United Kingdom
MG925962	*Catillaria contristans*	Norway
DQ534457	*Catillaria corymbosa*	King George Island, Antarctica
HQ650651	*Catillaria erysiboides*	Sweden
MG925963	*Catillaria erysiboides*	Ireland
MT248986	*Catillaria flexuosa*	Netherlands
MW045827	*Catillaria flexuosa*	Netherlands
OK332960	*Catillaria fungoides*	Czech Republic
KF689898	*Catillaria lenticularis*	Slovakia
FR799312	*Catillaria nigroclavata*	United Kingdom
FR799313	*Catillaria nigroclavata*	United Kingdom
OL453196	*Catillaria nigroisidiata*	Czech Republic
MG925964	*Catillaria scotinodes*	Norway
HQ650649	*Catolechia wahlenbergii*	-
KJ542546	*Diploschistes scruposus*	Spain
KC806067	*Gregorella humida*	-
MZ159569	*Gyalidea fritzei*	United Kingdom
MN483071	*Gyalidea* aff. *lecideopsis* var. *eucarpa*	USA
AY368114	*Physconia americana*	Spain
KY990715	*Physconia detersa*	USA
DQ862487	*Physconia distorta*	Portugal
MK812071	*Physconia distorta*	Norway
AY368119	*Physconia elegantula*	USA
EF582757	*Physconia elegantula*	USA
AY368124	*Physconia enteroxantha*	Spain
AY368128	*Physconia grisea*	Spain
DQ862488	*Physconia grisea*	Spain
AY498684	*Physconia grumosa*	Spain
KY990716	*Physconia grumosa*	USA

AY368130	*Physconia isidiigera*	USA
AY368131	*Physconia kurokawae*	USA
KY990717	*Physconia labrata*	Canada
KY990718	*Physconia labrata*	USA
AY368133	*Physconia leucoleiptes*	USA
EF582773	*Physconia leucoleiptes*	USA
AY368135	*Physconia muscigena*	USA
AY368136	*Physconia muscigena*	Canada
AY368137	*Physconia muscigena*	USA
JQ301696	*Physconia muscigena*	Canada
LS483123	*Physconia muscigena*	Slovakia
LS483124	*Physconia muscigena*	Slovakia
LS483125	*Physconia muscigena*	Slovakia
LS483126	*Physconia muscigena*	Slovakia
MK811781	*Physconia muscigena*	Norway
AJ421422	*Physconia perisidiosa*	Germany
AY368142	*Physconia perisidiosa*	USA
KY990723	*Physconia perisidiosa*	USA
AY368143	*Physconia servitii*	Portugal
EF582765	*Physconia servitii*	Somalia
DQ862491	*Physconia subpulverulenta*	Spain
DQ862496	*Physconia thorstenii*	Spain
AY368147	*Physconia venusta*	Spain
DQ862498	*Physconia venusta*	Spain
KU687450	*Rhizocarpon badioatrum*	Norway
KU687451	*Rhizocarpon bolanderi*	Norway
AF483617	*Rhizocarpon copelandii*	Norway
KU687455	*Rhizocarpon copelandii*	Norway
HQ650708	*Rhizocarpon disporum*	-
KY680774	*Rhizocarpon disporum*	Russia
AF483615	*Rhizocarpon distinctum*	Norway
AF483614	*Rhizocarpon geminatum*	Norway
KP314320	*Rhizocarpon geminatum*	Svalbard
KY266908	*Rhizocarpon geminatum*	Norway
MK629880	*Rhizocarpon geminatum*	China
KU687446	*Rhizocarpon jemtlandicum*	Norway
KU687449	*Rhizocarpon leptolepis*	Finland
AF483616	*Rhizocarpon polycarpum*	Norway
KX079701	*Rhizocarpon quinonum*	USA
KU687458	*Rhizocarpon rittokense*	Norway
KU687459	*Rhizocarpon rittokense*	Norway
KU687452	*Rhizocarpon subgeminatum*	Norway
KU687457	*Rhizocarpon subgeminatum*	Norway
AF483613	*Rhizocarpon suomiense*	Norway
KU687448	*Rhizocarpon suomiense*	Norway
MH717162	*Steinera intricata*	King George Island, Antarctica
MH717163	*Steinera intricata*	Livingston Island, Antarctica
MH717164	*Steinera intricata*	King George Island, Antarctica
MH717165	*Steinera intricata*	King George Island, Antarctica
MH717167	*Steinera intricata*	Livingston Island, Antarctica
MF893083	*Steinera isidiata*	Crozet Island, Subantarctica
NR152550	*Steinera isidiata*	Crozet Island, Subantarctica
MF893088	*Steinera latispora*	Crozet Island, Subantarctica
MF893089	*Steinera lebouvieri*	Crozet Island, Subantarctica
MF893090	*Steinera lebouvieri*	Kerguelen Island, Subantarctica
MF893093	*Steinera membranacea*	Kerguelen Island, Subantarctica
NR152552	*Steinera membranacea*	-
MF893102	*Steinera molybdoplaca*	Kerguelen Island, Subantarctica
MF893103	*Steinera molybdoplaca*	Kerguelen Island, Subantarctica
MF893106	*Steinera pannarioides*	Crozet Island, Subantarctica
MF893107	*Steinera pannarioides*	Crozet Island, Subantarctica

MF893108	*Steinera subantarctica*	Prince Edward Island, Subantarctica
KC179126	*Xanthocarpia crenulatella*	Austria
MG552488	*Xanthocarpia crenulatella*	Pakistan
KC179127	*Xanthocarpia epigaea*	Spain
KC179128	*Xanthocarpia erichansenii*	Greenland
KC179129	*Xanthocarpia feracissima*	USA
MK110661	*Xanthocarpia feracissima*	-
MG954190	*Xanthocarpia ferrarii*	Russia
MG954191	*Xanthocarpia ferrarii*	Russia
KU926971	*Xanthocarpia interfulgens*	Russia
KU926973	*Xanthocarpia interfulgens*	Russia
MG954186	*Xanthocarpia interfulgens*	Russia
MG954187	*Xanthocarpia interfulgens*	Russia
KC416124	*Xanthocarpia lactea*	Italy
KC179131	*Xanthocarpia marmorata*	Italy
KU926974	*Xanthocarpia marmorata*	Russia
MN512254	*Xanthocarpia marmorata*	Greece
KC179132	*Xanthocarpia ochracea*	France
KJ133483	*Xanthocarpia ochracea*	Ukraine
MG954185	*Xanthocarpia tominii*	Russia