


Three new records of lichenised fungi for Antarctica

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Research Note

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Abstract

As part of a project aiming to determine the lichenised fungal biodiversity of James Ross Island (Eastern coast of Antarctic Peninsula), we identified three infrageneric taxa which were previously not reported from Antarctica: *Farnoldia micropsis* (A. Massal.) Hertel, *Gyalolechia epiphyta* (Lynge) Vondrák and *Placidium squamulosum* var. *argentinum* (Räsänen) Breuss. Detailed morphological and anatomical properties of these species along with photographs based on the Antarctic specimens are provided here. In addition, the nrITS, mtSSU and/or RPBI gene regions of the selected specimens are studied and the phylogenetic positions of the species are discussed. The DNA sequence data for *Farnoldia micropsis* are provided for the first time. *Farnoldia micropsis* and *Gyalolechia epiphyta* are also new to the Southern Hemisphere.

Introduction

Lichens are considered key elements of the Antarctic flora. Their distribution ranges from coastal regions of maritime Antarctica (Schmitz et al., 2021) to climatically harsh areas of continental Antarctica (Wagner et al., 2021). They occupy various environments and substrates and exhibit region- and site-dependent biodiversity determined mainly by macro- and micro-climatic characteristics. The Antarctic peninsula and the adjacent island on the eastern (e.g. Seymour Islands, Vega Island, James Ross Island) and western side of the Antarctic peninsula (e.g. South Shetlands Isl., Livingstone Isl., Deception Isl., Argentine Islands, etc.) belong to the regions with highest reported lichen biodiversity (Green, Sancho, Pintado, & Schroeter, 2011; Halıcı, Barták, & Güllü, 2018; Sancho, Schulz, Schroeter, & Kappen, 1999; Singh et al., 2015). Thanks to the relatively good accessibility of these islands for scientists during austral summer season, these locations belong among the most studied Antarctic regions.

According to long-term climatic characteristics, James Ross Island has a cold, polar-continental climate (Martin & Peel, 1978). The climate of the island is relatively dry because of the Trinity Peninsula mountains (Antarctic Peninsula) that shield the island from precipitation (Davies et al., 2013). Precipitation estimates range from 200 to 500 mm per year (Van Lipzig, King, Lachlan-Cope, & Van den Broeke, 2004) and, therefore, James Ross Island is considered a semi-arid environment.

Conversely, ecological studies (e.g. Cannone & Guglielmin, 2008) rank James Ross Island as among those having a maritime climate. Recent biological studies (see e.g. Kopalová, Nedbalová, Elster, & Van de Vijver, 2013 for biodiversity of diatoms) suggest that James Ross Island might be considered a transitional zone between maritime and continental Antarctica. The lichen biota supports such view because some species such as *Usnea aurantiaco-atra* are quite frequent in the South Shetland Islands (maritime Antarctica) but absent from James Ross Island.

Taxonomic studies of lichens from James Ross Island have been carried out for several decades. Thanks to a large deglaciated area on the northern part of the Island of about 250 km² (Jennings et al., 2021), a great variety of microhabitats are available there for biological colonisation (Barták, Váczi, Stachoň, & Kubešová, 2015). Some ice-free parts of the island have not yet been inspected for lichens, which is why a lichen biodiversity list for the island is far from being complete. Several new species for the island and/or Antarctica have been described, mainly in last decade (e.g. Halıcı et al., 2018; Halıcı, Kahraman, Scur, & Kitaura, 2022). In this study, we report three infrageneric taxa of lichens which were previously not known from Antarctica collected at James Ross Island. Morphological and anatomical descriptions of these taxa are provided along with original photographs and phylogenetic positions of these taxa are discussed as well.

Materials and methods

Collection sites

Lichen specimens were collected from three different locations on James Ross Island. Locality No.1 is a long-term research plot (LTRP) located close to the J.G-Mendel station on the northern

Table 1. List of species used in phylogenetic trees. The newly generated sequences are in bold.

Specimen	ITS	Locality	mtSSU	Locality
JR 0.386 <i>Gyalolechia epiphyta</i>	ON028635	Antarctica	ON042224	Antarctica
JR 0.302 <i>Placidium squamulosum</i> var. <i>argentinum</i>	ON042213	Antarctica	–	–
<i>Blastenia ammiospila</i>	KC179413	Austria	MF114972	Turkey
<i>Catapyrenium daedaleum</i>	JX000099 MZ244171	– USA	–	–
<i>Gyalolechia allochroa</i>	HQ415800	South Korea	–	–
<i>Gyalolechia arizonica</i>	–	–	KC179529	USA
<i>Gyalolechia aurea</i>	KC179434	Austria	KC179350	South Africa
<i>Gyalolechia bassiae</i>	KC179435	Austria	–	–
<i>Gyalolechia bracteata</i>	– –	– –	MT952926 JQ301502	Austria Estonia
<i>Gyalolechia canariensis</i>	KC179436	Spain	MT952927	Spain
<i>Gyalolechia cranfieldii</i>	KC179437	Australia	KJ021294	Australia
<i>Gyalolechia ehrenbergii</i>	KC179438	Israel	–	–
<i>Gyalolechia epiphyta</i>	JN813383 KU360123 KT804977 KU360122	Turkey China Iran China	– – – –	– – – –
<i>Gyalolechia flavorubescens</i>	KU360121 AF279887 KT804981	Russia Sweden Iran	KC179531 AY143403 –	Estonia Sweden –
<i>Gyalolechia flavovirescens</i>	KT804971 KT804972	Iran Russia	– –	– –
<i>Gyalolechia fulgens</i>	KC179440 –	Italy –	JQ301503 EU680864	Sweden Ukraine
<i>Gyalolechia gomerana</i>	KC179441	Spain	KC179534	Spain
<i>Gyalolechia lenae</i>	KC179442	Russia	–	–
<i>Gyalolechia persimilis</i>	KT804978 KC179444	USA USA	– –	– –
<i>Gyalolechia stantonii</i>	KC179445	USA	KC179535	USA
<i>Gyalolechia stipitata</i>	KC179446	Mexico	KT291490	Mexico
<i>Gyalolechia subflavorubescens</i>	KT804960	South Korea	–	–
<i>Gyalolechia ussuriensis</i>	KT804988 KT804991	USA Russia	– –	– –
<i>Gyalolechia xanthostigmoidea</i>	KT804992	Canada	–	–
<i>Placidium adami-borosi</i>	GU228986 GU228985	Italy Italy	– –	– –
<i>Placidium</i> aff. <i>lichenoides</i>	GU228972	Spain	–	–
<i>Placidium arboreum</i>	KY769559 GU228995	– USA	– –	– –
<i>Placidium fingens</i>	GU228989	Spain	–	–
<i>Placidium imbecillum</i>	GU228979 GU228980	Spain Spain	– –	– –
<i>Placidium lachneum</i>	MZ244182 MZ244186	Spain USA	– –	– –
<i>Placidium lachneum</i> var. <i>oleosum</i>	GU228981	Spain	–	–
<i>Placidium lacinulatum</i> var. <i>atrans</i>	GU228958	Mexico	–	–
<i>Placidium lacinulatum</i> var. <i>lacinulatum</i>	GU228964	USA	–	–
<i>Placidium lacinulatum</i> var. <i>erythrostratum</i>	GU228965	USA	–	–

(Continued)

Table 1. (Continued)

Specimen	ITS	Locality	mtSSU	Locality
<i>Placidium michelii</i>	GU228992	Spain	–	–
<i>Placidium norvegicum</i>	GU228973	Spain	–	–
<i>Placidium pilosellum</i>	KY981585 KY981584	Slovakia Slovakia	– –	– –
<i>Placidium pseudorufescens</i>	GU228963 GU228966	France USA	– –	– –
<i>Placidium rufescens</i>	GU228970 GU228971	Spain Spain	– –	– –
<i>Placidium semaforonense</i>	GU228961	Spain	–	–
<i>Placidium squamulosum</i>	GU228994 GU228969	Spain Spain	– –	– –
<i>Placidium squamulosum</i> var. <i>argentinum</i>	GU228991	Spain	–	–
<i>Placidium subrufescens</i>	GU228974	Spain	–	–
<i>Placidium tenellum</i>	GU228990	Spain	–	–
<i>Placidium umbrinum</i>	GU228962	Mexico	–	–
<i>Placidium velebiticum</i>	GU228976 GU228978	Spain Slovenia	– –	– –
<i>Placidium yoshimurae</i>	GU228984	Japan	–	–
<i>Protoparmelia badia</i>	KF562191	Austria	–	–
Specimen	RPB1	Locality	mtSSU	Locality
JR 0.016 <i>Farnoldia micropsis</i>	ON042761	Antarctica	ON042213	Antarctica
<i>Farnoldia jurana</i> subsp. <i>jurana</i>	MK684889	Austria	GU074511	Austria

coast of the island. The LTRP is close to the coast (geographical coordinates: 63°48'03" S, 57°52'50" W, altitude of 5 m a.s.l.) in between the confluence of the Bohemian and Algal streams. The area is dominated by *Bryum pseudotriquetrum* that forms small-area carpets, with smaller areas rich in microbial mats formed mainly by *Nostoc* sp. colonies. Several seepages located on the LTRP are rich in algae (e.g. *Zygnema* sp.) and cyanobacteria. Mean annual temperature of the site is –4.6°C and annual relative air humidity varies within the range of 60–100% (Láska, Barták, Hájek, Prošek, & Bohuslavová, 2011). Locality No. 2 is a group of boulders (each of several cubic metres dimension) of volcanic rock located on the lower slopes (63°48'22.5" S, 57°51'00" W, altitude 140 m a.s.l.) of the Berry Hill mesa. The upper surface of the boulders is, due to nutrient availability from the occasionally resting skuas (*Catharacta macormicki*), rich in lichens. Typically, *Umbilicaria decussata* can be found together with nitrophilous lichens *Caloplaca* sp., *Candelaria* sp. and *Candelariella* sp. (personal observations, not yet published). Locality No. 3 is located at the SE margin of the Johnson Mesa, 63°49'46.2" S, 57°54'21.6" W, at the altitude of 292 m a.s.l. The locality represents a vegetation-rich, sorted stony surface formed by the activity of an active layer of permafrost on the table mountain (mesa). An organo-mineral substrate is available, mainly at the margins of the stony polygons, which typically form shallow depressions with enhanced snow accumulation. Therefore, water availability in such places is higher than that in the polygon centres, which is beneficial for the development of lichen-dominated communities at the margins of polygons. At locality No. 3, *Dermatocarpon polyphyllum* and *Xanthoria elegans* are found quite frequently.

The lichen species referred to in this study were collected from the following sites: *Farnoldia micropsis* (stone surfaces at locality

No. 1), *Gyalolechia epiphyta* (boulder surface at locality No. 2) and *Placidium squamulosum* var. *argentinum* (sorted stony soils of a table mountain at locality No. 3)

Handling of specimens

Samples of lichenised fungi were collected from James Ross Island which, according to bioecological characteristics, belongs to the North-East Antarctic Peninsula Region (Terauds & Lee, 2016). The specimens detailed below are deposited in the Erciyes University Herbarium Kayseri, Turkey (ERCH). Before transport from Antarctica, the specimens were dried in the field and stored for 3 days in a deep freezer. They were numbered starting with "JR" and added to the database of the herbarium under those numbers. All the lichen specimens were examined by standard microscopic techniques. Hand-cut sections were studied in water, potassium hydroxide (KOH) and Lugol's solution (I). Measurements of anatomical structures such as ascospores were made in water. Standard spot tests were carried out to determine the lichen secondary metabolites present. Ascospores were measured from five different ascomata for each species. The measurements are reported in the format: (minimum) mean minus standard deviation – mean – mean plus standard deviation (maximum), from N measurements. The descriptions of the lichen species are based on the specimens collected from James Ross Island by the authors.

DNA isolation, PCR and sequencing

Genomic DNA extraction was performed directly using fresh apothecia, perithecia (fruiting bodies) or small-area thallus fragments. DNA was extracted using the protocol of the Dneasy Plant Mini Kit (Qiagen). The nuclear rDNA ITS gene region was amplified by

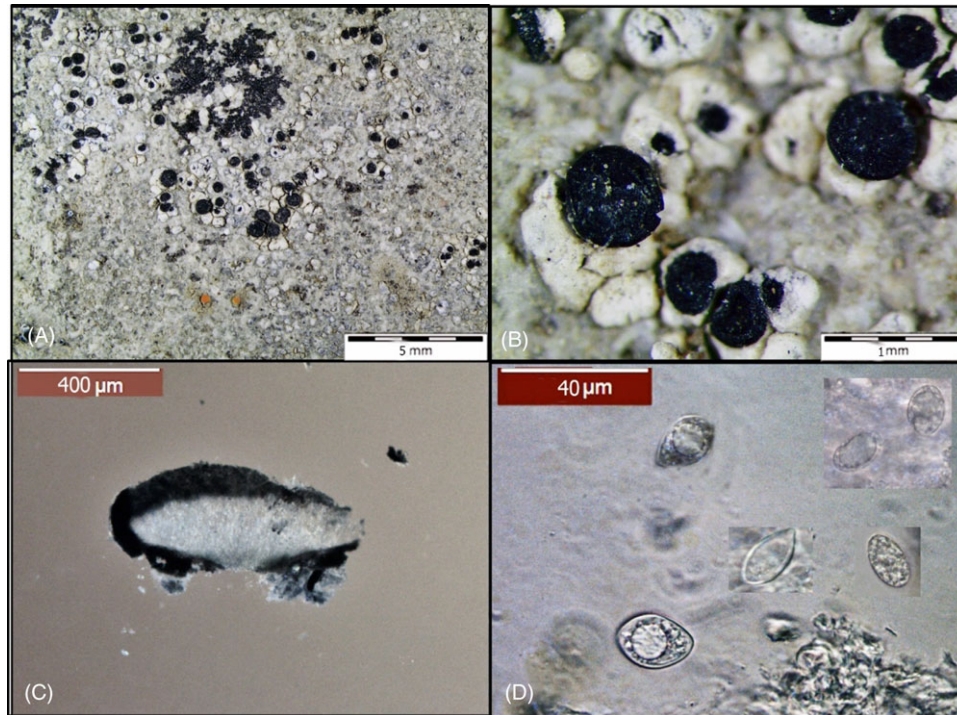


Fig. 1. *Farnoldia micropsis*. A. Thallus overview. B. Areolles and apothecia in close view. C. Apothecial section. D. Ascospores.

Notes: To date, the genus *Farnoldia* Hertel was represented in Antarctica by only one species, *F. dissipabilis* (Nyl.) Hertel (Øvstedal & Lewis Smith, 2001). That species has ochre-yellow to ochre-brown verrucose areolate to subsquamulose thallus, whereas *F. micropsis* has whitish areolate thallus. Of the other species of the genus, *F. jurana* (Schaer.) Hertel subsp. *jurana* has a very poorly developed thallus and *F. similigena* (Nyl.) Hertel has narrowly ellipsoid ascospores $\leq 7 \mu\text{m}$ wide (Nimis, 2016).

using the fungi-specific primer ITS1-F (5'-CTTGGTCAT TTAGAGGAAGTAA-3') and the universal primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Gardes & Bruns, 1993; White, Bruns, Lee, & Taylor, 1990). The mtSSU gene region was amplified by using the primers mtSSU1F (GATGATGG CTCTGATTGAAC) (Shiguo & Stanosz, 2001) and mtSSU3R (ATGTGGCACGTCTATAGCCC) (Zoller, Scheidegger, & Sperisen, 1999). The RPB1 gene region was amplified using the primers RPB1-5F pelt 5'-TTCAACAARCTBACVAARGATGT-3' (Denton, McConaughy, & Hall, 1998) and fRPB1-11aR 5'-GCRTGGATCTTRTCRTCSACC-3' (Liu, Whelen, & Hall, 1999). PCR amplification was carried according to the following protocol. The final volume of 25 μL consisted of 12.5 μL of 2 \times Power Taq PCR Master Mix, 1 μL of each primer (10 μM), 4 μL of extract DNA and 7.5 μL of deionised water. The PCR cycling parameters included initial denaturation at 95 $^{\circ}\text{C}$ for 5 min, followed by 35 cycles at 95 $^{\circ}\text{C}$ (30 s), 1 cycle at 52 $^{\circ}\text{C}$ (30 s), another 1 at 72 $^{\circ}\text{C}$ (1 min), followed by a final elongation at 72 $^{\circ}\text{C}$ for 8 min. The PCR products were visualised on 1.2% agarose gel as a band of approximately 550–600 base pairs (bp) (ITS), 700–800 bp (mtSSU) or 750 bp (RPB1). All amplified products were electrophoresed on a 1.2 % agarose gel and compared with a 1 Kb Plus DNA Ladder for size estimation. The PCR amplification products were sequenced by the BM Labosis Laboratory (Ankara).

Phylogenetic analyses

Phylogenetic analyses were performed based on ITS, mtSSU and RPB1 sequence data (Table 1). Newly generated sequences were subjected to a BLAST search to assess their affinities and aid in taxon sampling for the phylogeny. The sequences were aligned

using Clustal W. The resulting alignment was further adjusted manually in a BioEdit v.7.2 sequence alignment editor (Hall, 1999). Ambiguous regions were delimited and excluded from the alignment. Phylogenetic relationships between taxa were investigated using a MEGA 7 software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The dataset was analysed using the maximum likelihood method and support values were obtained using a bootstrap analysis of 1,000 pseudoreplicates. The out-groups used in the phylogenetic trees were chosen to be phylogenetically related with the in-groups. When necessary, the variable sites of the gene regions of nrITS, mtSSU or RPB1 were shown using BioEdit v.7.2 programme (Hall, 1999).

Results and discussion

After morphological and molecular examination of the specimens collected from James Ross Island, we report here three infrageneric taxa which were previously not reported from Antarctica: *Farnoldia micropsis*, *Gyalolechia epiphyta* and *Placidium squamulosum* var. *argentinum*. Morphological descriptions based on Antarctic specimens and comparisons with the related species are provided below along with photographs. mtSSU and RPB1 gene regions of *Farnoldia micropsis*, nrITS and mtSSU gene regions of *Gyalolechia epiphyta* and nrITS gene region of *Placidium squamulosum* var. *argentinum* were sequenced and the phylogenetic positions of the species are discussed.

Farnoldia micropsis (A. Massal.) Hertel

Thallus crustose, areolate, areoles well developed, flat to convex, contiguous or scattered, up to 1.5 mm thick, dirty white, prothallus not visible. Medulla white, I + blue. Apothecia lecideine, black,

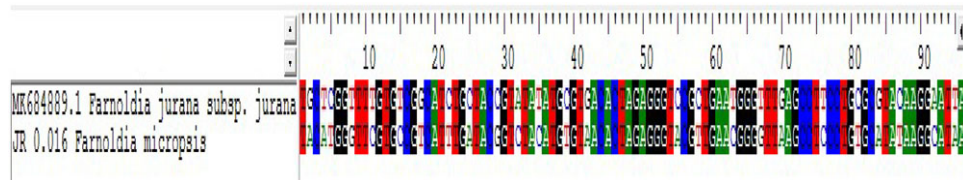


Fig. 2. Shortened RPB1 alignment, including only variable positions of *Farnoldia jurana* subsp. *jurana* and *F. micropsis*.

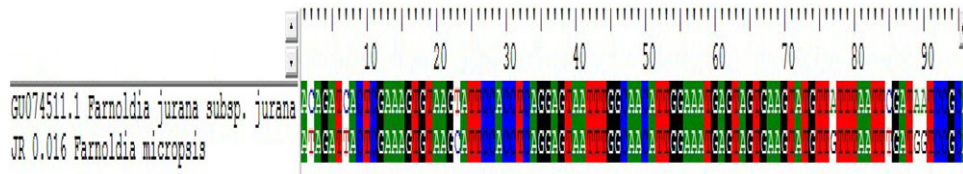


Fig. 3. Shortened mtSSU alignment, including only variable positions of *Farnoldia jurana* subsp. *jurana* and *F. micropsis*.



Fig. 4. *Gyalolechia epiphyta*. Blastidiate/granulose thallus.

epruinose, 0.3–0.6 mm in diam, flat to slightly convex or concave disc. Proper exciple black, 30–50 μm thick. Epiphytenium dark greenish-blue; hymenium colourless, slightly greenish tinge present in the upper part, 80–100 μm tall; paraphyses branched and anastomosing, apical cells $\sim 3 \mu\text{m}$ wide. Hypothecium grayish. Asci 8-spored; ascospores simple, colourless, ellipsoid, (13–)15.5–18–20.5(–23) \times (6–)8.5–11–13.5,5(–16) μm , length/width ratio (1–)1.4–1.7–2(–2.5) (n = 44) (Fig. 1).

Chemistry: Spot tests: Epiphytenium N + red.

The specimen of *F. micropsis* was collected on small pebbles in the coastal zone of James Ross Island. This species was previously reported from Murmansk region of Russia (Melechkin, 2015), SE Alaska, Montana, Colorado, and Utah of USA (McCune, Glew, Nelson, & Villella, 2007), France (Sussey, 2012), China (Zhao, Hu, & Zhao, 2016), Sweden (Westberg et al., 2016), Slovenia

(Batic et al., 2003), Siberia, Finland, Turkey, Canada (Hertel, 2001), Alaska (Hertel & Andreev, 2003), Arctic (Hertel, 1991), Italy (Ravera et al., 2020), Germany (Wirth et al., 2011), Greenland, Svalbard (Kristinsson, Hansen, & Zhurbenko, 2015), Macedonia, Romania, Bulgaria, Montenegro (Oukarroum, Strasser, & Schansker, 2012), Australia (Reiter & Türk, 2001) and Spain (Gómez-Bolea et al., 2021) on rocks with intermediate carbonate content, being rare on pure limestone (McCune et al., 2007), on calcareous boulders (Westberg et al., 2016) and various calcareous rock types (Hertel & Andreev, 2003). This is the first report of this species from the Southern Hemisphere and Antarctica.

Unfortunately, only one species of *Farnoldia* (*F. jurana* subsp. *jurana*) has RPB1 and mtSSU sequence data in GenBank and none have nrITS sequence. 665 nucleotides in the RPB1 gene region and

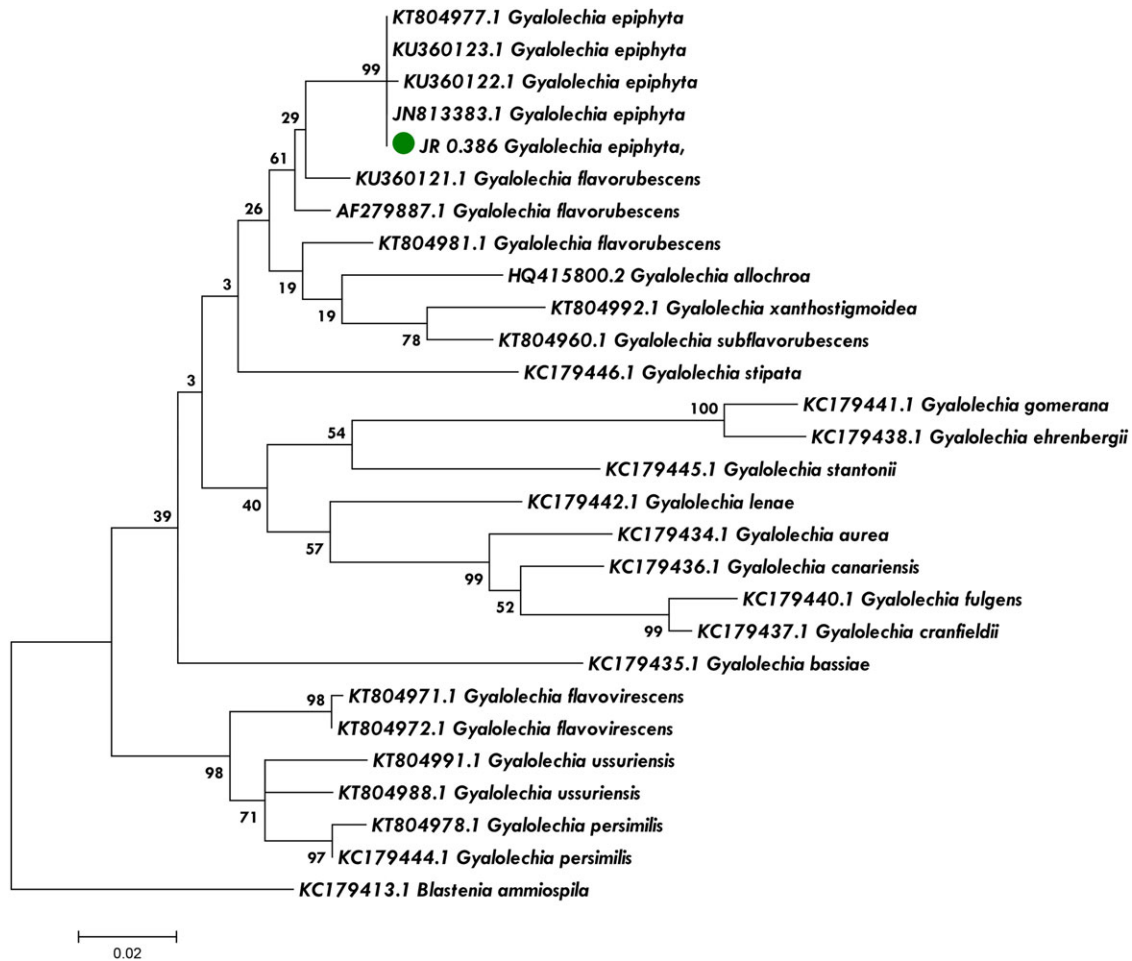


Fig. 5. Maximum likelihood (ML) analysis inferred from nrITS sequences of *Gyalolechia epiphyta* and related species.

550 nucleotides in mtSSU gene region of two *Farnoldia* species (*F. micropsis* and *F. jurana* subsp. *jurana*) were compared. 640 nucleotides were found to be conserved sites (C) and 25 nucleotides were found to be variable sites (V) in RPB1 gene region. The light coloured parts in Figure 2 show the nucleotide differences. According to this comparison, there is a 3.75 % difference between *F. micropsis* and *F. jurana* subsp. *jurana*.

542 nucleotides were found to be conserved sites (C) and 7 nucleotides were found to be variable sites (V) in mtSSU gene region. The light coloured parts show the nucleotide differences (Fig. 3). According to this comparison, there is a 1.27 % difference between *F. micropsis* and *F. jurana* subsp. *jurana*.

Specimen examined: Antarctica, Antarctic Peninsula, the James Ross Island, Long Term Research Plot (subplot No.7), 63° 48' 03" S, 57° 52' 50" W, alt. 3 m., on small calcareous pebbles, 13.02.2017, leg. M. G. Halıcı & M. Bartak (JR 0.016).

Gyalolechia epiphyta (Lynge) Vondrák

Thallus crustose, blastidiate/granulose (without true soredia), lemon yellow, areoles 0.1–0.2 mm in diam. Apothecia and pycnidia not observed (Fig. 4).

Chemistry: Thallus K+ red, C-, KC-, P-.

This species is typical in the genus with its blastidiate/granulose thallus and absence of true soralia but also quite similar to the sorediate taxa of the genus (*G. persimilis* (Wetmore) Søchting, Frödén & Arup, *G. ussuriensis* (Oxner, S.Y. Kondr. & Elix) Vondrák and *G. xanthostigmoidea* (Räsänen) Søchting, Frödén & Arup) (Vondrák

et al., 2016). In the nrITS tree (Fig. 5), it is clearly seen that the nrITS sequence of the Antarctic specimen collected by us is placed in the supported clade of *G. epiphyta* and differs from *G. persimilis*, *G. ussuriensis* and *G. xanthostigmoidea*. There is no sequence of *G. epiphyta* and fewer data of the genus for the mtSSU gene region in GenBank. In the mtSSU tree (Fig. 6), the closest relatives to our Antarctic specimen are *G. flavorubescens* s. lat. (Huds.) Søchting, Frödén & Arup and *G. arizonica* (H. Magn.) Søchting, Frödén & Arup.

The Antarctic specimen was collected on soil at 140 m altitude on James Ross Island. This species is usually reported on bark or moss cushions but also occurring on calcareous rock (Vondrák, Ismailov, & Urbanavichus, 2017). The type specimen of *G. epiphyta* (Lynge) Vondrák is from Greenland and it has a wide distribution in the Northern Hemisphere including Russia, China, Iran, USA and Canada (Esslinger, 2016; Vondrák et al., 2016). This is the first report of this species from the Southern Hemisphere and Antarctica.

Specimen examined: Antarctica, Antarctic Peninsula, James Ross Island, Puchau, 63° 48' 25" S, 57° 50' 28" W, alt. 142 m., on soil, 07.02.2017, leg. M. G. Halıcı & M. Bartak (JR 0.016).

Placidium squamulosum* var. *argentinum (Räsänen) Breuss.

Thallus consisting of squamules, to 2–4 mm diam., lobed, not overlapping, upper surface grayish and bluish pruinose, margins paler (almost white) and curled up, lower surface creamish brown to brown (Fig. 7). Thallus 150–250 µm thick; upper cortex 25–

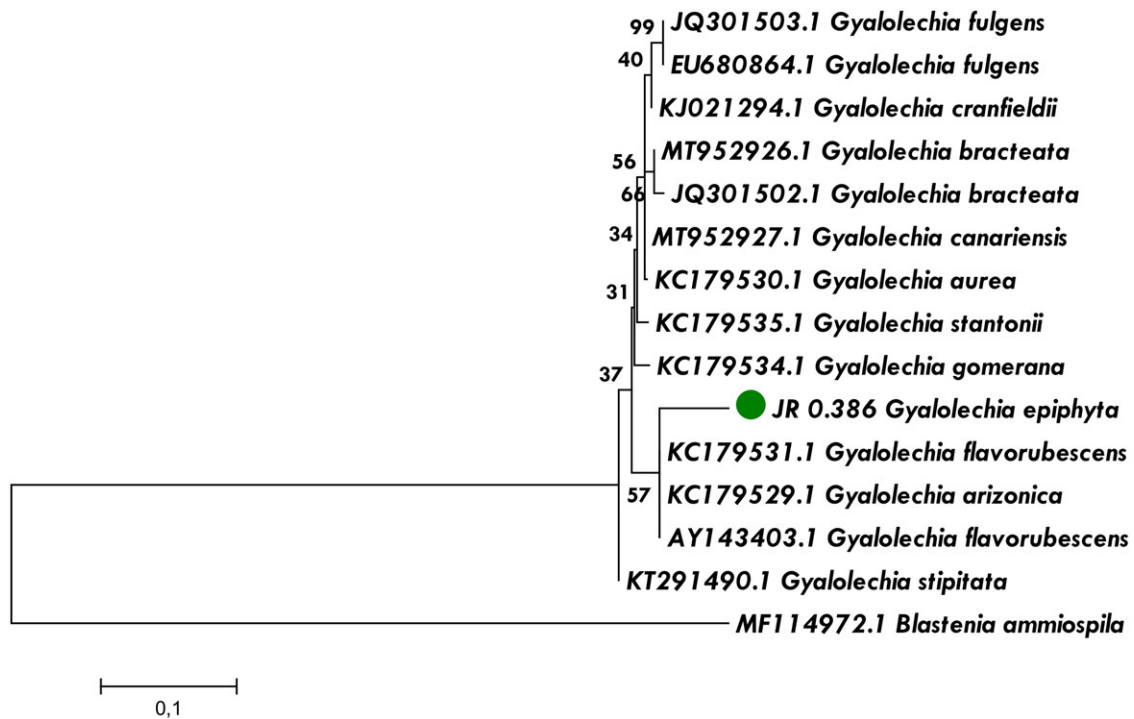


Fig. 6. Maximum likelihood (ML) analysis inferred from mtSSU sequences of *Gyalolechia epiphyta* and related species.

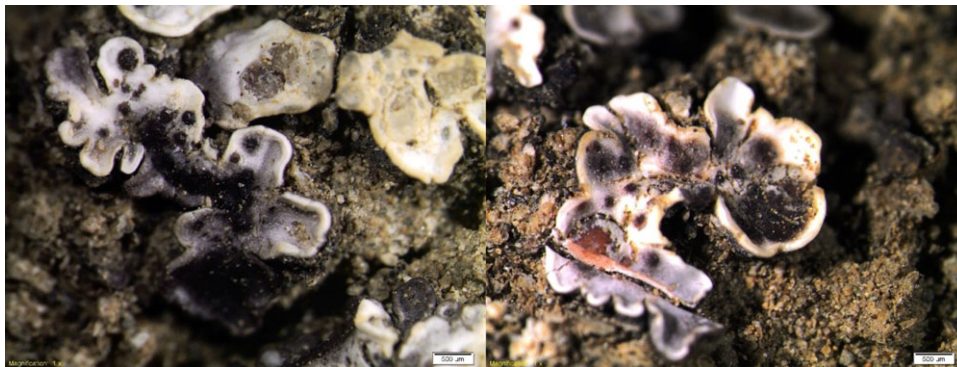


Fig. 7. *Placidium squamulosum* var. *argentinum*. Squamules on soil.

50 µm thick, paraplectenchymatous with an epinecral layer ~ 30 µm thick. Rhizohyphae hyaline, 5–6.5(–7.5) µm thick. Perithecia immersed usually in the marginal parts of the squamules, pyriform, exciple hyaline. Asci 8-spored, cylindrical; ascospores uniseriately arranged. Ascospores colourless, simple, (11.5–)13–15–17(–19) × (5.5–)6.5–8–9.5(–16) µm (n = 20). Pycnidia laminal, conidia oblong ellipsoid, 2.5–5 × 1.5–3 µm.

Chemistry: Spot tests all negative.

Breuss (2001) reported *Placidium squamulosum* (Ach.) Breuss as a cosmopolitan species that grows on soil and humus in all continents except of Antarctica (Breuss, 2001). Here we report this species as new to Antarctica; so the species is now known from all continents. The Antarctic specimen collected on soil at the altitude of 292 m a.s.l. from James Ross Island has bluish grey squamules that are not appressed by the whole underside. *Placidium squamulosum* (Ach.) Breuss has a dull or subnitid, pale to dark brown upper surface, and squamules densely aggregated, mostly

appressed to the substratum by the whole underside, occasionally with slightly raised margins (Breuss, 1993). However, *P. squamulosum* var. *argentinum* differs from the type in having broader ascospores (12–16 × 7.5–8.5 µm vs. 12–16 × 5.5–7.5 µm) and thinner rhizohyphae (4–5 µm vs. 5–6.5 µm) (Breuss, 1993; Prieto, Aragón, Martínez, & Breuss, 2008). Actually, the Antarctic specimen has wider ascospores as in *P. squamulosum* var. *argentinum* but wider rhizohyphae as var. *squamulosum*. In the nrITS tree (Fig. 8), it is clearly seen that the nrITS sequence of the Antarctic specimen places it in the supported clade of *P. squamulosum* var. *argentinum*. 593 nucleotides in the nrITS gene region of *P. squamulosum* (Ach.) Breuss, *P. squamulosum* var. *argentinum* from Argentina and Antarctica were compared. When the Antarctic specimen was compared with *P. squamulosum* (Ach.) Breuss, 431 nucleotides were found to be conserved sites (C) and 40 nucleotides were found to be variable sites (V) in nrITS gene region. The light coloured parts in Figure 9 show the nucleotide

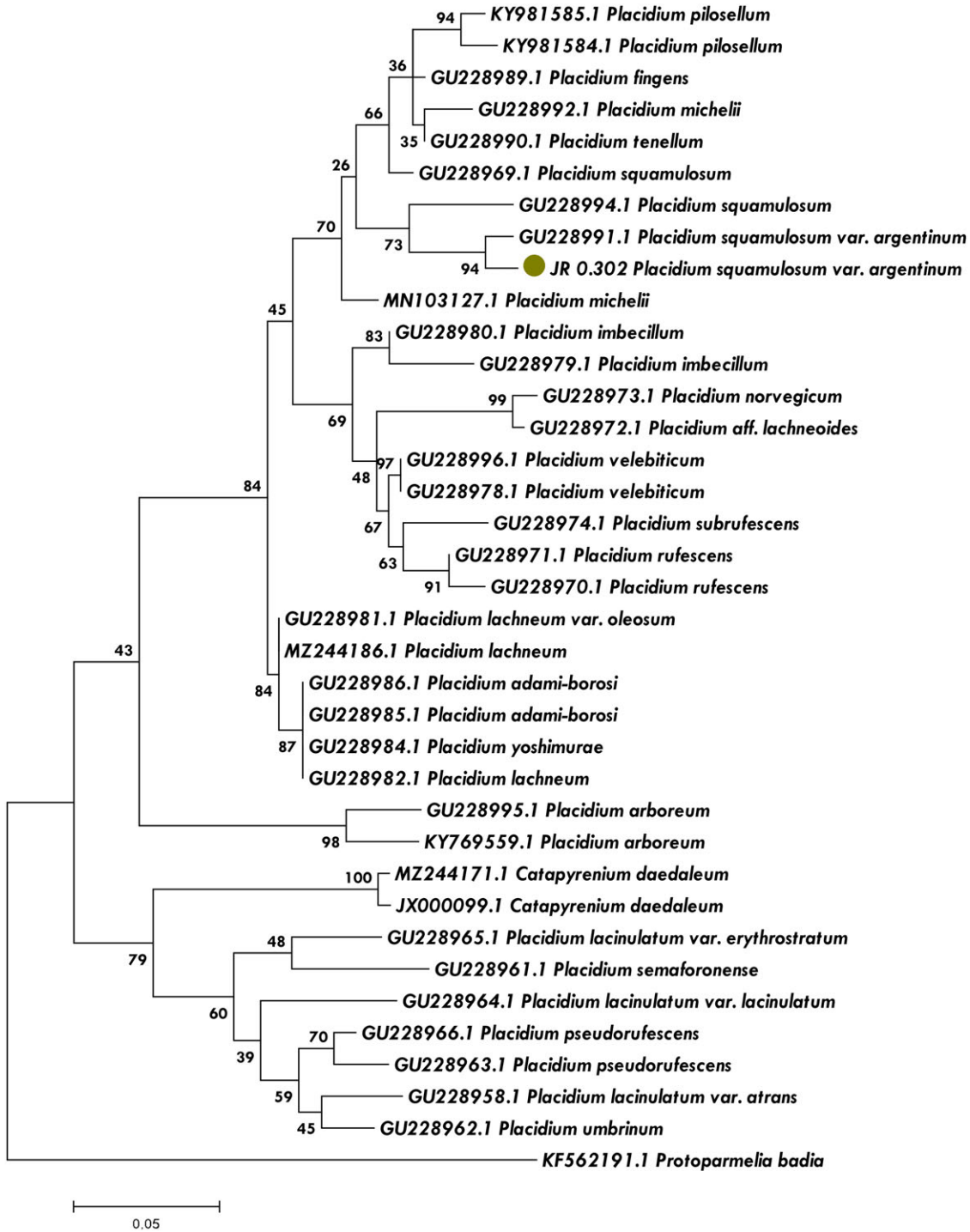


Fig. 8. Maximum likelihood (ML) analysis inferred from nrITS sequences of *P. squamulosum* var. *argentinum* and related species.

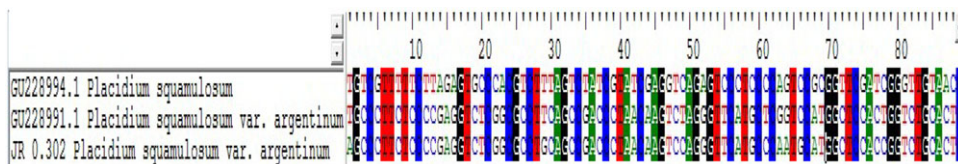


Fig. 9. Shortened nrITS alignment, including only variable positions of *P. squamulosum* and *P. squamulosum* var. *argentinum*.

differences. According to this comparison, there is a 6.74 % difference between *P. squamulosum* (Ach.) Breuss and *P. squamulosum* var. *argentinum* from Antarctica. When the Antarctic specimen was compared with *P. squamulosum* var. *argentinum* (Räsänen) Breuss from Argentina, 471 nucleotides were found to be conserved sites (C) and 5 nucleotides were found to be variable sites (V) in nrITS gene region. According to this comparison, there is a 0.84 % difference between the Antarctic and Argentina samples. Two species of the related genus *Catapyrenium*: *C. daedaleum* (Körb.) Stein and *C. lachneoides* Breuss were reported from Antarctica by Øvstedal & Lewis Smith (2001), but these species differ morphologically from our collection, and nrITS sequences of *C. daedaleum* from GenBank are phylogenetically distinct.

Specimen examined: Antarctica, Antarctic Peninsula, James Ross Island, Southeast of Johnson Mesa, 63° 49' 46" S, 57° 54' 21" W, alt. 292 m., on soil, 26.01.2017, leg. M. G. Halıcı & M. Bartak (JR 0.302).

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