



Psoroma antarcticum, a new lichen species from Antarctica and neighbouring areas

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Abstract

Icefree, terrestrial, Antarctic ecosystems have a polar desert-like appearance with a scarce vegetation cover, completely dominated by bryophytes and lichens. Knowledge of the rich Antarctic lichen biodiversity, including c. 400 species, is therefore necessary, also for studies of other ecosystem components. The genus *Psoroma* is partly dominant there, and own ongoing research indicates that many of its members have been misunderstood. The aim of the present study is to describe *Psoroma antarcticum* as new to science, study its habitat ecology and total distribution, and include a genetic analysis with respect to its internal variation and relationship to other species. The species differs from the closely related species *Psoroma hypnorum*, in having distinctly cup-shaped apothecia with thalline excipuli without squamules and tomentose lower sides, in having shorter ascospores without apical nodulose extensions and thalli with grey-to-black melanins. It is shown to form a monophyletic clade based on an analysis of the ITS, LSU, and mtSSU loci, and this clade is included in the *Psoroma* s. str. clade, which includes *P. hypnorum*, *Psoroma paleaceum*, *Psoroma buchananii*, and *Psoroma fruticulosum* with high statistical support. The new species has its main distribution in the maritime South Shetland and South Orkney Islands of Antarctica, and most samples originate from King George Island, where it is common and an important component in polar desert-like vegetation. Scattered occurrences have also been found in Chilean Tierra del Fuego, South Georgia, and Bouvet Island.

Keywords King George Island · Taxonomy · Phylogeny · Pannariaceae

Introduction

The genus *Psoroma* is defined by its generitype *Psoroma hypnorum* (Vahl) S.F. Gray (Jørgensen 1978). This is a tripartite, terricolous to muscicolous species consisting of squamules with chlorobionts interconnected by an inconspicuous hypothallus, with nodulose to coralloid cephalodia

inbetween the chlorobiont squamules. The genus has traditionally been interpreted to comprise practically all tripartite species within Pannariaceae. However, several squamulose species have been transferred to the new genera *Joergensenia*, *Psorophorus*, and *Xanthopsoroma*, described with molecular support (Passo et al. 2008; Elvebakk et al. 2010), some also to *Pannaria* (Elvebakk and Bjerke 2005; Passo and Calvelo 2011). The austral foliose and tripartite species have been transferred to *Pannaria*, e.g. by Jørgensen (2000b) and Elvebakk and Galloway (2003), whereas foliose and tripartite Palearctic species were described as a new genus *Gibbosporina* (Elvebakk et al. 2016). On the other hand, several species previously treated in *Pannaria* and *Santesoniella* were recently transferred to *Psoroma* (Ekman et al. 2014).

Some of the species remaining in *Psoroma* s. str. are corticolous in austral forests (e.g. *Psoroma asperellum* and *Psoroma aphthosum*). However, a great majority of species have similar habitat ecology as *P. hypnorum*, and are also mainly distributed in alpine and polar ecosystems and in adjacent cold areas. There is a strong concentration of species in the

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Southern Hemisphere; however, a few species, such as *P. hypnorum*, *Psoroma cinnamomeum*, *Psoroma paleaceum*, and *Psoroma tenue*, also reach the Northern hemisphere. As shown by Elevebakk (2012), the latter group does not only concern four species, but also a fifth taxon, with a more temperate distribution in southern Scandinavia. This taxon and its potentially related species in the Southern Hemisphere are not yet understood.

The genus is well represented in the lichen flora of Antarctica, and Øvstedal and Smith (2001) included six species, one of them (*Psoroma ciliatum* (Ach. ex Fr.) Nyl. ex Hue), now treated as *P. paleaceum* (Timdal and Tønsberg 2006). Later, *Psoroma saccharatum* was added from King George Island by Olech (2004), and *Psoroma pannarioides* from Deception Island by Øvstedal and Smith (2011). The number also increased when Ekman et al. (2014) transferred four bipartite *Pannaria* Sect. *Cryopannaria* species from the area, to *Psoroma*, including the Antarctic *Psoroma orphnium* (Hue) P.M. Jørg. Thus, the genus can be summarized to include nine accepted species, *Psoroma buchananii*, *P. cinnamomeum*, *Psoroma fruticulosum*, *P. hypnorum*, *P. orphnium*, *P. paleaceum*, *P. pannarioides*, *P. saccharatum*, and *P. tenue*, from the Antarctic. Two additional species (*Psoroma bryantii* Dodge and *Psoroma follmannii* Dodge) described from the area have rarely been accepted as separate taxa, and are being studied separately by us.

During the field work on King George Island, Antarctica, to understand the biodiversity and distribution of the genus *Psoroma*, we discovered a common *Psoroma* sharing some morphological characters with *P. hypnorum*, which after closer studies appeared to differ significantly from it. The species has been analysed by molecular methods and with regard to morphology and anatomy and has been found to represent an undescribed species. Also the holotypes of *P. bryantii* and *P. follmannii* and the subantarctic *Psoroma absconditum* Øvstedal have been studied, and the new species has been found not to correspond to any of these. Thus, the aim of the present paper is to describe this new species, compare it with the existing species, also genetically, and present its known distribution and habitat ecology.

Materials and methods

Taxon sampling and identification

Thirty samples collected from King George Island are housed at Korean Polar Research Institute, Incheon, Republic of Korea (KOPRI) and Tromsø University Museum, University of Tromsø – the Arctic University of Norway, Tromsø, Norway (TROM). Additional nine samples of the species were discovered after search in the herbaria at British Antarctic Survey, Cambridge, U.K. (AAS), University

of Bergen, Bergen, Norway (BG), and Universidad Complutense, Madrid, Spain (MAF). The species has been searched for *in vain* also at Natural History Museum, Department of Botany, London, U.K. (BM), Swedish Museum of Natural History, Department of Botany, Stockholm, Sweden (S), and Uppsala University, Museum of Evolution, Botany Section, Uppsala, Sweden (UPS). Herbarium acronyms follow Global Registry of Biodiversity Repositories at <http://grbio.org/find-biorepositories>. We could not find the species in our large *Psoroma* collections from Southern Chile near Punta Arenas and Falkland Islands, which are housed at KOPRI and TROM. A list of samples from King George Island at KOPRI and TROM can be supplied on request. In microscope sections, iodine reactions were tested by adding IKI to mounts pretreated with KOH (Orange et al. 2001). Perispore structures were studied in water mounts and restricted to spores liberated from the asci. Ascospore morphology was studied in detail by drawing detailed sketches of ascospores, and copies of all original drawings have been included with the samples. Thin-layer chromatography of acetone extracts followed standardized procedures and used solvents A and C (Culbertson 1972; Orange et al. 2001). Nomenclature of ascospore structures follows Nordin (1997).

DNA extraction and sequencing

A total of 30 specimens that were included for phylogenetic analyses were collected from various locations on the Barton and Weaver Peninsulas of King George Island and Ardley Island (Fig. 1; Online Resource 1). Freeze-dried lichen specimens were ground using TissueLyser (Qiagen, Hilden, Germany). DNA was extracted using a Wizard[®] Genomic DNA Purification Kit (Promega, Madison, WI). The procedures of further purification of DNA were described in a previous study (Park et al. 2015). Amplification and sequencing of the ITS1-5.8S-ITS2-partial LSU rDNA were conducted using the ITS1F, ITS4, LR0R, and LR5 primers, by the procedures described in a previous study (Park et al. 2012). The mitochondrial small subunit rRNA gene was amplified using the primers, mrSSU1 and mrSSU3R (Zoller et al. 1999). PCR amplification was performed in a T-gradient thermocycler (Biometra, Germany) with the following cycling parameters: 5-min initial denaturation at 94 °C, 5 cycles of 30-s denaturation at 94 °C, 30-s annealing at 55 °C, and 1-min extension at 72 °C; followed by 30 cycles of 30-s denaturation at 94 °C, 30-s annealing at 52 °C, 1-min extension at 72 °C, and 10-min final extension at 72 °C. Sequences of mtSSU region were determined with primers mrSSU1 and mrSSU3R using ABI 3730XL automated sequencer (Applied Biosystems, USA). Sequences were deposited at GenBank database under accession KY350562–KY350638 (specific information is provided in Online Resource 1).

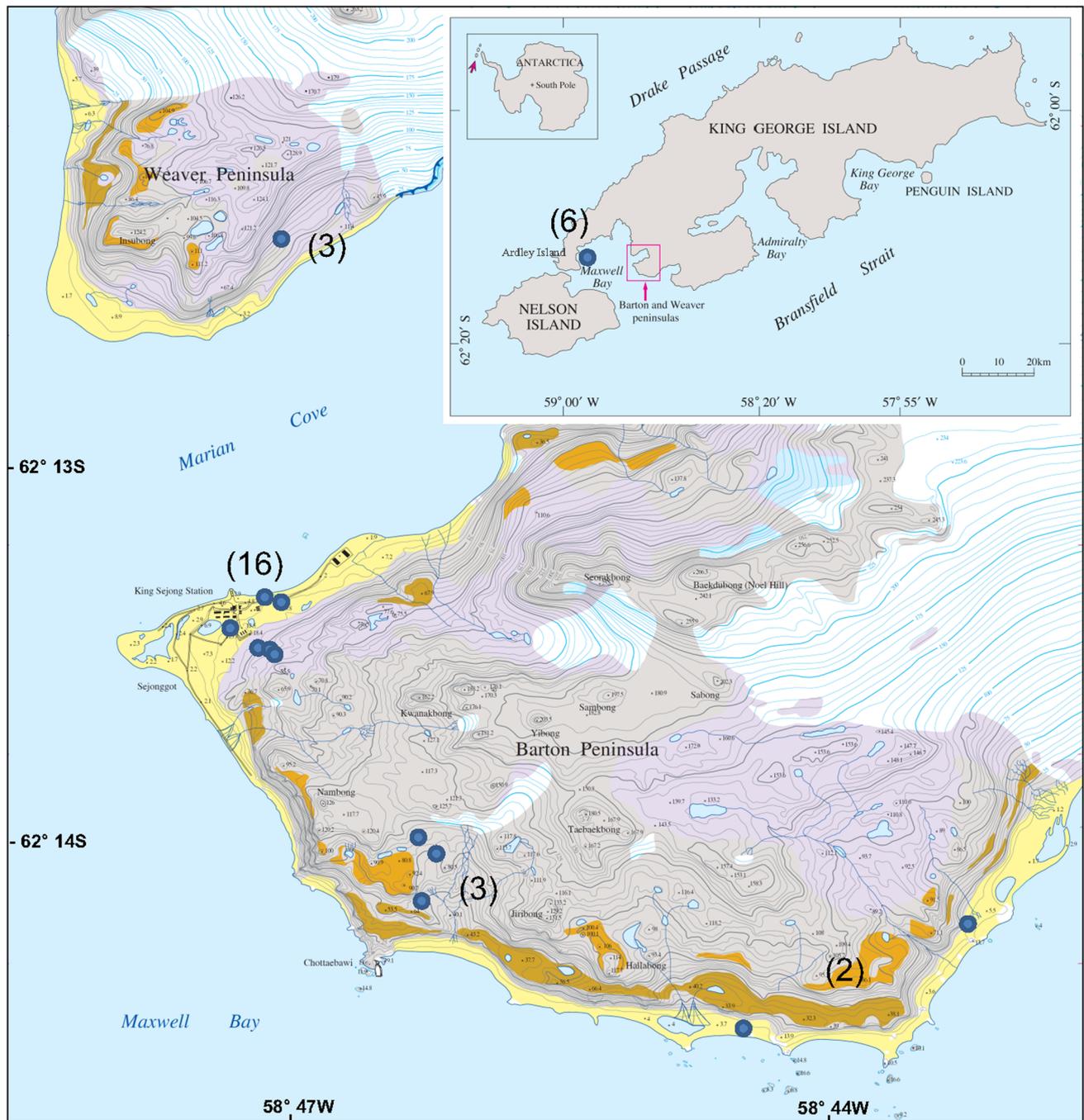


Fig. 1 Geographical distribution of *Psoroma antarcticum* in Weaver and Barton Peninsulas of King George Island and Ardley Island. Numbers in parentheses are numbers of specimens collected

Phylogenetic analyses

To determine phylogenetic relationships among Pannariaceae, reference sequences presented by Passo et al. (2008) and Elvebakk et al. (2010) were included, and *Protopanaria pezizoides* was included as an outgroup. Sequence alignments of ITS1-5.8S-ITS2-partial LSU rDNA and

mtSSU were conducted by the program ClustalX (Larkin et al. 2007) and manually adjusted. Ambiguously aligned sites were excluded from phylogenetic analyses. Phylogenetic trees were inferred for each gene and for combined datasets by maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm of

MEGA7 (Kumar et al. 2016) with search level 5, in which the initial trees were obtained by the random addition of sequence (1000 replicates). The ML tree was also constructed using MEGA7 based on the GTR+I+G evolutionary model (Lanave et al. 1984) and the search options of best tree topology finding by branch swapping of NNIs and SPRs, random addition of sequences (1000 replicate). All positions containing gaps were treated as missing data. The Bayesian tree was searched for by MrBayes ver. 3.2. (Ronquist et al. 2012) with the GTR+I+G model. Two parallel Markov Chain Monte Carlo (MCMC) runs were performed for 1,000,000 cycles, each with one cold and three heated chains and the temperature parameter set to 0.1; trees were sampled every 100 generations. A consensus tree was calculated after discarding the first 25% trees as burn-in. All analyses involved 38 nucleotide sequences including 8 reference sequences which had only ITS rDNA and mtSSU.

Results

We are now revising material of this genus, and show here that the new species *Psoroma antarcticum* is quite easily distinguished from *P. hypnorum* which has been considered to be the most common of the bipolar species in the lichen genus *Psoroma*.

Taxonomy

Psoroma antarcticum Hong & Elvebakk, sp. nov.

Mycobank No.: MB 822519

Differs from *Psoroma hypnorum* *in having thalli which predominantly have black-to-dark grey instead of brown melanins, by lacking tomentum on the external sides of the thalline excipulum, by having strongly urn-shaped apothecia without squamulose margins, and with short-ellipsoid spores, mostly without nodulose apical extensions* (Fig. 2a, b, c).

Type: ANTARCTICA: South Shetland Islands, King George Island, SW part, Barton Peninsula, just S of the Korean King Sejong Station, 62°13.37'S–58°47.02'W, 20 m elev., over mosses, 11 Jan. 2008, S. G. Hong HSG080111-09 (KOPRI–holotype; TROM–isotype).

Thallus squamulose, 2–7 cm wide, terricolous over soil, detritus, other lichens and bryophytes. *Chlorobiont squamules* c. 300 µm thick, loosely appressed to the substratum, 0.2–1.5 mm wide, weakly and irregularly incised, becoming confluent, suberect and subcoralloid centrally, mostly pale brownish-grey when young, becoming almost black-pigmented on age, occasionally uniformly brownish. *Upper surface* glabrous and weakly glossy. *Epicortex* 10 µm thick,



Fig. 2 *Psoroma antarcticum*, **a** Holotype (HSG080111-09), scale bar: 1 cm; **b** Engelskjøn BG L79374 from Bouvet Island, scale bar: 5 mm; **c** Ascospores, showing the verrucose perispore and its absence of apical extensions, scale bar: 10 µm

sclerenchymatic and hyaline; *upper cortex* c. 30 µm thick, paraplectenchymatic, lumina globose and 3–5 µm diam., or elongate 3–6 × 4–10 µm, walls 2.5–4 µm thick. *Chlorobiont layer* 150–180 µm thick, of cf. *Myrmecia* cells, globose-to-irregularly globose, 8–14 µm diam., with reduced concentrations in the lower part. *Medulla* 70–80 µm thick; *lower cortex* 15 µm thick, dark-pigmented, present in distal parts of horizontal squamules and on suberect ones. *Prothallus/hypothallus* dark and fragmentarily developed, mostly invisible.

Cephalodia common, 0.3–1.5 mm, forming coarsely branched, pulvinate cushions, sometimes cracked, often becoming coarsely coralloid, and packed between chlorobiont squamules, black. *Upper cortex* paraplectenchymatic, appearing prosoplectenchymatic on the sides of convex parts, 10–15 µm thick, lumina 2–3 × 3–15 µm, walls black-pigmented. *Cyanobiont Nostoc*, cells sea-green, 3–4 × 3–6 µm, without chain structures, densely packed and arranged in mostly indistinct 10–40 µm large glomeruli.

Apothecia common, substipitate, distinctly urn-shaped, 1.5–4 mm wide; *disc* blackish-brown, concave; *thalline excipulum* blackish, with anatomy as in the squamules, forming a thick ring of incurved, obtuse and partly fused lobules, occasionally developing into weakly coralloid structures, but never producing squamules; surface of lower sides glabrous, and partly pruinose. *Epithecium* 15–20 µm thick, sclerenchymatic, dark brown. *Hymenium* c. 100 µm thick, colourless, but strongly IKI+blue. *Asci* clavate, 15 × 70–80 µm, with 8 ascospores and a tube-like IKI+blue, apical structure. *Proper ascospores* hyaline, non-septate, ellipsoid, 10–12.5 × 15–22 µm. *Perispores* of the same shape, 13–16 × 18–26 µm, coarsely verrucose, verrucae 2–3 µm wide, 1–2 µm tall, no apical extensions seen. *Parafyses* septate, simple to sparingly branched, c. 2 µm thick, apices not distinctly swollen. *Hypothecium* light brown, 40–50 µm thick, IKI negative, with a chlorobiont layer below.

Pycnidia not seen.

Chemistry: Brownish, brownish-grey-to-black melanins present, but no TLC-detectable components found.

Habitat ecology: Grows on the ground over bryophytes, other lichens and soil and detritus in polar desert-like Antarctic and subantarctic areas.

Distribution: Known from West Antarctica from the South Orkney Islands, South Shetland Islands, and Bouvet Island, in addition to single collections from South Georgia and from the alpine zone of Isla Navarino in Chilean Tierra del Fuego.

Selected additional samples studied:

ANTARCTICA: Maritime Antarctic Zone, South Orkney Islands, Signy Island, Knife Point, Feb. 1980, *K. Richards* s.n. (BG L83451); South Shetland Islands: Isla Livingston, Península de Byers, El Retsal, 30 Jan. 1990, *L.G. Sancho* s.n. (MAF Lich4139); Bahía Sur, 20 m, 22 Jan. 1991, *L.G.*

Sancho s.n. (MAF Lich4142). South Shetland Islands: King George Islands, Weaver and Barton Peninsulas (62°12'–14'S, 58°43'–47'W) and Ardley Island (62°12'S, 58°54'–55'W) Jan. 2008, *Park, Hong, Hong & Lee*, Feb. 2010. (mapped on Fig. 1).

SOUTH GEORGIA: North shore of North Bay, Prince Olav Harbour, 54°04'S, 37°08'E, 100 ft., on scree slopes near waterfall, 2 Feb. 1961, *S.W. Greene 1744* (AAS).

BOUVET ISLAND: Nunatak E of Rustadkollen, SE side, 54°25'S, 03°18'E elev. 200 m, in a soaked depression, snow-bed influenced. 1 Jan 1979, *T. Engelskjøn 103.2* (BG L079374; O—not seen; BIRM—not seen); Nunatak N of Rustadkollen, 54°25'S, 8°8'E, 28 Feb. 1985, *T. Engelskjøn* (BG L501043; L501044; L501045).

CHILE: XII Región de Magallanes y de la Antártica Chilena, Isla Navarino, parte alta del Valle del Róbalo, camino hasta Dientes de Navarino, pared y piedras del torrente, suelo, 54°59'54"S, 67°40'53"W, 700 m, 19 Jan. 2005, *J. Etayo 22758*, *A. Gómez-Bolea*, *U. Sjøchting & R. Vilch* (MAF Lich15860).

Phylogeny

All the 30 specimens of *P. antarcticum* were homogeneous in ITS1-5.8S-ITS2-partial LSU rDNA and mtSSU rDNA sequences, except one specimen which had one different base in the LSU sequence. Therefore, phylogenetic trees were constructed by using six specimens of *P. antarcticum*. This species formed a monophyletic lineage in all phylogenetic analyses and was statistically supported by the MP, ML, and Bayesian analyses based on combined sequences of ITS1-5.8S-ITS2-partial LSU rDNA and mtSSU rDNA (Fig. 3). When phylogenetic trees were reconstructed using the ITS1-5.8S-ITS2-partial LSU rDNA and mtSSU separately, monophyly of *P. antarcticum* was also strongly supported by high bootstrap values by MP and ML analyses and high posterior probability by Bayesian analysis. Phylogenetic relationships with other species of the genus *Psoroma* were not clearly resolved, and the relationships were not robust. However, it was maintained in a monophyletic lineage comprised by *P. hypnorum*, *P. palaeceum*, *P. buchananii*, and *P. fruticosum* as confirmed by means of MP, ML, and Bayesian analyses for single locus and combined dataset. It was also clear that *P. antarcticum* formed a distinct phylogenetic lineage from the other *Psoroma* species. Although bootstrap support was not high, close relationship with *P. hypnorum* was maintained as confirmed in all MP, ML, and Bayesian analyses.

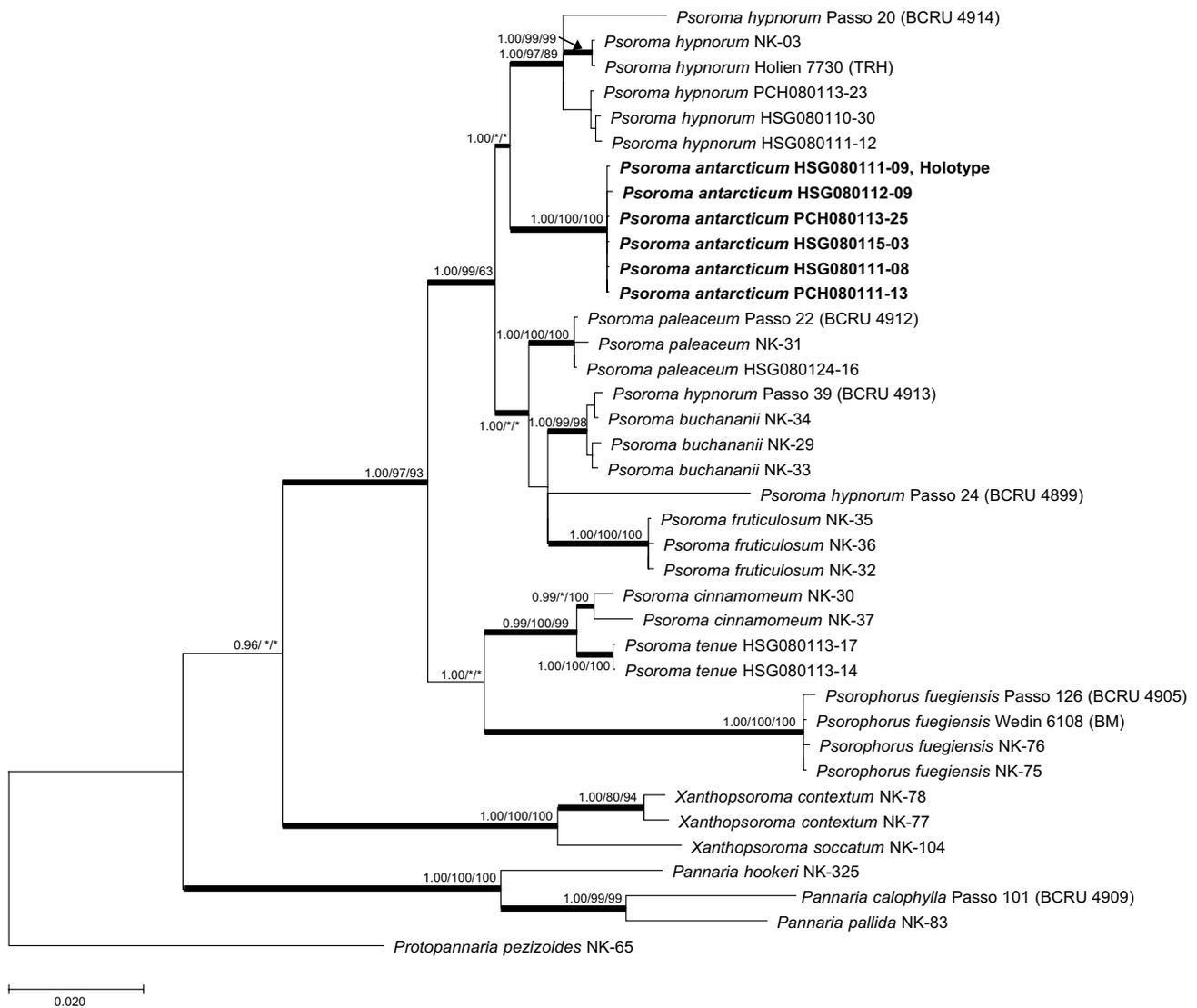


Fig. 3 Bayesian tree based on combined sequences of ITS1-5.8S-ITS2-partial LSU and mtSSU. Thick branches indicate those that were conserved in MP and ML. Bayesian posterior probabilities

(PP ≥ 0.95) and bootstrap values in ML and MP trees (≥ 90%) are indicated above the branches (PP/ML/MP)

Discussion

Psoroma antarcticum is very common on King George Island in lowlands, where dense vegetation is dominated by bryophytes and lichens (Lee et al. 2008; Kim et al. 2006, 2007; Shin et al. 2014; Kim 2016). This vegetation is mostly referred to as polar desert vegetation (Shin et al. 2014; Williams et al. 2017), although moss and lichen can form continuous carpets. Here, *P. antarcticum* forms rather large colonies over bryophytes, sometimes on other lichen species. The lichen genus *Psoroma* is a species-rich genus in this community, and the bipolar species *P. hypnorum* has been considered to be the most common of these (Thomson 1984). The new species *P. antarcticum* is quite easily

distinguished from *P. hypnorum* and also easily recognized in the field although material representing the new species has been included in this species by previous studies. A striking character is the consistently urn-shaped apothecia with in-rolled thalline excipulum margins, always lacking the squamules found in *P. hypnorum* (Fig. 2a, b). Those of the latter may be urn-shaped at times, but this is a consistent character of *P. antarcticum*. A reliable character is the lack of tomentum on the outer lower sides of the apothecia, a distinctive character of *P. hypnorum*. The thalline squamules of *P. antarcticum* are very thick and compact. Specimens of *P. antarcticum* are also strongly grey-to-black-pigmented (Fig. 2a, b) in colour, with the exception of the cited AAS specimen, which is uniformly brown. Antarctic

and subantarctic material of *P. hypnorum* is also greyer compared to the brown colour of thalli of *P. hypnorum* from the Northern Hemisphere; however, we have not seen any *Psoroma* species with such a blackish pigmentation as that of *P. antarcticum*. Also the cephalodia of *P. antarcticum* are much more black-pigmented than any tripartite Pannariaceae species we have studied so far.

The ascospores of *P. antarcticum* have the coarse and large perispore verrucae shared by *P. hypnorum* (Jørgensen 1978, p. 91); however, they are more short-ellipsoid and lack the common nodulose apical extensions found in *P. hypnorum* (Fig. 2b).

Phylogenetic analyses demonstrate that *P. antarcticum* is included in a *Psoroma* lineage that consists of *P. hypnorum*, *P. paleaceum*, *P. buchananii*, and *P. fruticulosum*, but clearly distinct from other *Psoroma* species. The most closely related species is *P. hypnorum*. These results support the distinct morphological characteristics of *P. antarcticum* and also explain why it has been regarded as *P. hypnorum* by their similar phenotypes for a long time.

At present it appears that the distribution of the species is restricted to Antarctic and subantarctic areas encompassing South Shetland Islands (King George Island and Livingston Island), South Orkney Islands, South Georgia, Bouvet Island, and southernmost Chile. We could not find the species during thorough searches of *Psoroma* species in anti-boreal heathlands and steppes in southern Chile near Punta Arenas and in the Falkland Islands. The fact that the only locality in South America was in a mountain area of the far south of Chile indicates its preference for Antarctic habitats. It is obviously well adapted to long-distance dispersal as it is found on Bouvet Island, said to be the most isolated island on earth (Arntz et al. 2006). In the present study, we could determine sequences only from King George Island as the other samples were old herbarium specimens. The nucleotide sequences of 30 specimens from King George Island are notably homogeneous compared to the heterogeneous sequences of *Cladonia borealis* analysed from the same location (Park et al. 2012). Although we need to obtain sequence information for specimens from other geographical areas to understand the evolution of *P. antarcticum* more clearly, we may make the preliminary conclusion on the origin and evolution of the species on King George Island that it was introduced there quite recently and did not have enough time to evolve to have a heterogeneous genetic population. Alternatively, it has a restricted sexual reproduction rate, and pycnidia have not yet been observed on this species.

Phylogeographically, *P. antarcticum* can be placed in a West Antarctic element within the genus *Psoroma*, including also *P. orphninum*, *P. pannarioides*, and *P. saccharatum* (Jørgensen 2000a; Olech 2004; Øvstedal and Smith 2001, 2011). The genus also includes an endemic subantarctic

element from the Southern Indian Ocean, centred on Kerguelen Island and Heard Island with species such as *Psoroma obscurium* (Ekman et al. 2014, recombined then as '*Psoroma obscurius*', corrected here), *Psoroma xanthorioides* (Jørgensen 2004) and *P. absconditum* (Øvstedal and Gremmen 2008), in addition to *Psoroma dichroum*, which has surprisingly disjunct localities in Campbell Island (Jørgensen 2000a). In addition, there is a well-known panaustral (*P. buchananii*, *P. cinnamomeum*, *P. dichroum*, and *P. fruticulosum*), and a bipolar element (*P. hypnorum*, *P. paleaceum*, and *P. tenue* s.l.). This confirms the evidently long evolutionary history of *Psoroma* in Antarctica, being the largest Pannariaceae genus there. Our own ongoing studies indicate that the *Psoroma* flora of this area is still not sufficiently known, and the taxa and their phylogenetic and phylogeographical properties are now being studied.

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