Original article

An integrative study of *Pannaria hookeri* (Ascomycota lichenized) from Argentina and the update of the taxon circumscription based on specimens from the Northern and Southern Hemispheres

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ABSTRACT

Pannaria hookeri (Borrer ex Sm.) Nyl. is reported as a bipolar cyanolichen that occurs in more than twenty countries in both Hemispheres. In the Northern Hemisphere, a minor variation is attributed to the species, whereas larger morphological and chemical variations have been reported in specimens from the Southern Hemisphere. Six *P. hookeri* DNA sequences are available in GenBank, and only one (from Australia) is associated with a published morphological description. In this study, we performed a preliminary phylogenetic analysis including seven nuITS and mtSSU sequences from eight specimens collected in south Argentina. In addition, we provide an update of the taxon circumscription based on 50 specimens from 13 countries. Here, the molecular analyses revealed a well-supported monophyletic group formed by the *P. hookeri* sequences from Argentina, Australia, Norway and USA (Alaska). Regarding the morphological and anatomical characteristics, the Southern Hemisphere specimens did not present the historical reported variations, resulting in the proposition of a concise species description that accomplishes most of *P. hookeri* known geographical distribution.

Keywords: species circumscription, cyanolichen, lichenized fungi, nuITS, taxonomy, Tierra del Fuego

Introduction

    Pannaria Delise ex Bory is one of the largest genera within *Pannariaceae* (lichenized Ascomycota), in which the lichen-forming fungi can be associated with different photobiont partners (Ekman *et al*. 2014; Magain & Sérusiaux 2014). Usually, the thalli are bipartite and the mycobiont is associated with *Nostoc* cyanobacteria. Still, tripartite thalli are also found within the genus where the mycobiont is associated with green algae, as the main photobiont, and the cyanobacteria are confined in structures called cephalodia,

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for example in *Pannaria durietzii* (P. James & Henssen) Elvebakk & D.J. Galloway (Elvebakk *et al.* 2008). The *Pannaria* species are characterized by squamulose or foliose thalli, apothecia with thalline margins, an amyloid hymenium (I+ blue), asci without internal amyloid apical structures, secondary metabolism with pannarin and related substances, and occurrence in tropical, subtropical, temperate and polar regions from both Hemispheres (Ekman *et al.* 2014).

Pannaria hookeri (Borrer ex Sm.) Nyl. is a species with bipolar distribution (Jørgensen 1978; Kantvilas & Gueidan 2018), which has already been reported from Austria, Canada, Faeroes Island, Finland, France, Greenland, Iceland, Ireland, Italy, Norway, Russia, Sweden, Switzerland, and U.S.A. in the Northern Hemisphere (Jørgensen 1978; 2000b; 2003; 2007; Thomson 1984); as well as from the Antarctic Peninsula, Argentina, Australia, Chile, New Zealand, and for the subantarctic islands and archipelagos Bouvetøya, Kerguelen, Marion, South Georgia, South Orkney, and South Shetlands in the Southern Hemisphere (Lindsay 1974; 1976; Redón 1985; Jørgensen 1978; 1986; 2000a; 2007; Olech 1989; 2001; Øvstedal & Lewis Smith 2001; Calvelo & Fryday 2006; Kantvilas & Gueidan 2018; Passo *et al.* 2020). In addition, *P. hookeri* was recorded at high altitudes in Ecuador (5050 m. *a.s.l.*, Z. Palice 4598; Jørgensen & Palice 2010) and points from Kenya (4430 m *a.s.l.* and at 4640 m *a.s.l.*; Frisch & Hertel 1998). The species was originally described from Scotland (Smith & Sowerby 1811), and its first record in the Southern Hemisphere was made from a South African specimen, initially identified as *P. leucolepis* (Wahlenb.) Nyl. (Stizenberger 1890; Doidge 1950) and posteriorly included in the synonym list of *P. hookeri* (Jørgensen 2006). Nevertheless, there is no consensus about the identification of the South African material, and also from others localities from Southern Hemisphere, which can also be an extreme form of *P. rubiginosa* (Thunb.) Delise (Jørgensen 2003).

Regarding the morphological descriptions, a minor variation is reported to *P. hookeri* from Europe (Jørgensen 1978), while a larger variation has been described to *P. hookeri* from the Southern Hemisphere (Jørgensen 2000a). In the same way, the chemical profile differs according to the region studied. Traces of atranorin and ergosterol peroxide was found in specimens from Robert Island (Quilhot *et al.* 1989), the absence of secondary substances to Bouvetøya specimens (Jørgensen 1986), and pannarin or related substances were reported to specimens from Argentina (Passo *et al.* 2020), Australia (Kantvilas & Gueidan 2018), and Europe (Jørgensen 1978; 2007), inferring cryptic lineages and taxonomical problems in the morphological and chemical studies.

Despite the observed inconsistencies, the wide geographical distribution of *P. hookeri* was confirmed when the sequences of the nuITS and mtSSU regions were generated from an Australian specimen and clustered with *P. hookeri* sequences from Norway (Kantvilas & Gueidan 2018).

Nowadays, only six specimens have available sequences in the GenBank: one from Australia, three from Norway, one from USA (Alaska) and one of unknown origin (Ekman & Jørgensen 2002; Spribille & Muggia 2013; Kantvilas & Gueidan 2018; Park *et al.* 2018; Marthinsen *et al.* 2019). Therefore, better knowledge is still needed for the circumscription characters that belong to *P. hookeri*, including genetic analysis with Southern Hemisphere specimens.

The aim of this study was to a) perform a phylogenetic analysis of *P. hookeri* from Argentina and sequences available on GenBank, generating the first molecular sequences from the South America; and b) to update the species circumscription based on specimens from the Northern (including topotypes) and Southern Hemispheres, establishing a unified morphological description.

Material and methods

Sampling

Fresh samples were collected in the National Park of Tierra del Fuego, Ushuaia, extreme south of the Argentina, during the summer of 2016-2017 (Proyecto Número 105-CPA-2016, supported by CONICET and PROANTAR), and specimens were found on rock and saxicolous mosses.

Phylogenetic analysis

Eight fresh-collected specimens were used in the molecular analysis. DNA extraction, amplification, and sequencing of the nuITS (nuclear internal transcribed spacer) and mtSSU (mitochondrial small subunit) regions were performed according to Kitaura *et al.* (2018). However, the specimens requested as loans have more than 10 years, and the DNA extraction was not performed. In addition, four sequences of *P. hookeri* of each nuITS and mtSSU regions were retrieved from GenBank. These sequences were generated from Australian, Norwegian, U.S.A., and unknown origin specimens (Tab. 1).

Sequences of *Staurolemma oculatum* P.M. Jørg. & Aptroot (GenBank accession numbers KC618738 and GQ259045) and *S. omphalarioides* (Anzi) P.M. Jørg. & Henssen (KJ533487 and KJ533439) were used as outgroups according to Kantvilas & Gueidan (2018) (Tab. 1).

The alignments were performed in Geneious v9.1.2 (Kearse *et al.* 2012) with the MAFFT v7.308 algorithm (Katoh *et al.* 2002) using default settings, and the Gblocks web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used to exclude unreliable aligned sites using the less stringent options. The alignments were used to infer trees from nuITS, mtSSU, and concatenated regions. Phylogenetic trees were estimated using the Bayesian (BA) and Maximum Likelihood (ML) approaches, and analyses for each region separately and for a combined data matrix were performed.

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Table 1. Dataset used in the present study for the phylogenetic analysis. Species, geographical origin, voucher information, nuITS and mtSSU GenBank accession codes and references. Sequences generated in this study are marked in bold.

Species	Origin	Voucher	GenBank Accession numbers		Reference
			nuITS	mtSSU	
Pannaria andina	Chile	Elvebakk 06-245	GQ927268	-	(Elvebakk *et al.* 2010)
Pannaria athroophylla	Argentina	Passo 181	EU885295	EU885317	(Passo *et al.* 2008)
Pannaria athroophylla	Argentina	Passo 251	EU885303	EU885325	(Passo *et al.* 2008)
Pannaria calophylla	Argentina	Passo 101	EU885296	EU885318	(Passo *et al.* 2008)
Pannaria conoplea	Norway	Ekman 3188	AF429281	-	(Ekman & Jørgensen 2002)
Pannaria contorta	Argentina	Passo 142	EU885297	EU885319	(Passo *et al.* 2008)
Pannaria farinosa	Argentina	Passo 119	EU885299	EU885321	(Passo *et al.* 2008)
Pannaria hookeri	Australia	GK102/16	MG786563	MG792317	(Kantvilas & Gueidan 2018)
Pannaria hookeri	Norway	Jørgensen s.n.	AF429282	KC608083	(Ekman & Jørgensen 2002)
Pannaria hookeri	Norway	NK-325	KY350562	-	(Park *et al.* 2018)
Pannaria hookeri	Norway, NO, Nordland	JT Klepsland	MK812291	-	(Marthinsen *et al.* 2019)
Pannaria hookeri	USA, Alaska	Spribille 29292 (KLGO)	-	JX464134	(Spribille & Muggia 2013)
Pannaria hookeri	Unknown	Jørgensen s.n. (BG)	-	KC608083	Unpublished
Pannaria hookeri	**Argentina**	**MJK3314**	**MT755913**	**MN63424**	**This study**
Pannaria hookeri	**Argentina**	**MJK3319**	**MT755912**	**MN634242**	**This study**
Pannaria hookeri	**Argentina**	**MJK3323**	**MT755911**	**MN634243**	**This study**
Pannaria hookeri	**Argentina**	**MJK4022**	**-**	**MN634244**	**This study**
Pannaria hookeri	**Argentina**	**MJK4028**	**MT755910**	**MN634245**	**This study**
Pannaria hookeri	**Argentina**	**MJK4036**	**MT755909**	**MN634246**	**This study**
Pannaria hookeri	**Argentina**	**MJK4041**	**MT755908**	**MN634247**	**This study**
Pannaria hookeri	**Argentina**	**MJK4045**	**MT755907**	**-**	**This study**
Pannaria immixta	Unknown	Elvebakk 02-352b	-	KC608084	(Kantvilas & Gueidan 2018)
Psoroma implexa	Argentina	Passo 84	-	EU885333	(Passo *et al.* 2008)
Pannaria insularis	Japan	Kashiwadani 43760	KC618716	KC608085	(Kantvilas & Gueidan 2018)
Pannaria leucosticta	Unknown	Hur 041227	EU266107	-	(Kantvilas & Gueidan 2018)
Pannaria lurida subsp. *lurida*	Unknown	Kashiwadani 43861	-	KC608086	(Kantvilas & Gueidan 2018)
Pannaria lurida subsp. *russellii*	Unknown	Tønsberg 22565	-	KC608087	(Kantvilas & Gueidan 2018)
Pannaria microphyllizans	Argentina	Passo 264	EU885300	EU885322	(Passo *et al.* 2008)
Pannaria multifida	Unknown	Schumm & Frahm s.n.	KC618717	KC608088	(Kantvilas & Gueidan 2018)
Pannaria pallida	Argentina	Passo 249	EU885301	EU885323	(Passo *et al.* 2008)
Pannaria rubiginella	Canada	Thor 10050	-	GQ259037	(Wedin *et al.* 2009)
Pannaria rubiginella	Unknown	Tønsberg 32508	KC618718	KC608089	(Kantvilas & Gueidan 2018)
Pannaria rubiginosa	Unknown	Anonby 870/Purvis s.n.	KC618717	AY340513	(Kantvilas & Gueidan 2018)
Pannaria sphinctrina	Argentina	Passo 221	EU885302	EU885324	(Passo *et al.* 2008)
Pannaria subfusca	Unknown	Tønsberg 33592	KC618719	-	(Kantvilas & Gueidan 2018)
Pannaria tavaresii	Argentina	Passo 122	EU885294	EU885316	(Passo *et al.* 2008)
Pannaria tavaresii	Unknown	Schumm s.n.	KC618720	-	(Kantvilas & Gueidan 2018)
Staurolemma oculatum	China	Aptroot 55941	KC618738	GQ259045	(Kantvilas & Gueidan 2018)
Staurolemma omphalarioides	Turkey	Tibell s.n.	KJ533487	KJ533439	(Bendiksby *et al.* 2014)

The nucleotide substitution and site heterogeneity models were inferred following the Bayesian Inference Criterion in jModelTest2 on XSEDE, CIPRES Science Gateway V.3.3 (Guindon & Gascuel 2003; Miller *et al.* 2010; Darriba *et al.* 2012). The BA was performed in MrBayes v.3.2.7a on XSEDE, available in the CIPRES Science Gateway V.3.3 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Miller *et al.* 2010) using TrNef+I+G as substitution model for nuITS, TPM2uf+I+G for mtSSU regions and TIM1+I+G for the concatenated dataset, and their respective base frequencies were settled. The settings were: two independent runs with four chains (length of 10,000,000 generations), with trees sampled every 1,000 steps. The first 25 % of the generated trees were discarded as burn-in, and a 50 %-majority-rule tree was built. The ML trees were built with the RaxML-HPC2 v.8.2.12 on XSEDE available in the CIPRES Science Gateway V.3.3 (Miller *et al.* 2010; Stamatakis 2014), using GTR+G+I as nucleotide substitution model and 1,000 bootstrap replicates. FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) was used to edit the trees. Branches with posterior probability values above 0.95 (BA) and bootstrap above 75 (ML) were considered significantly supported.

Morphological and anatomical studies

The AAS, C, COLO, E, H, M, O, and S herbaria sent 35 specimens from the Northern Hemisphere and seven from the Southern Hemisphere. The specimens received as loan were morphologically examined, and the determinations were confirmed (Text S1), when compared with eight fresh *P. hookeri* specimens.

The *P. hookeri* description was built according to Jørgensen (1978). Then, our description was compared with other available in the literature (Lindsay 1974; Redón 1985; Jørgensen 1986, 2007; Øvstedal & Lewis Smith 2001; Kantvilas & Gueidan 2018; Passo *et al.* 2020), and the variable characters were highlighted in Table 2 and discussed here. The specimens were tested chemically by applying PD spot tests, and orange reactions were interpreted as indicating the presence of pannarin. Therefore, TLC and HPLC analyses are necessary to confirm the substances.

Results

Molecular analysis

The dataset of the present study was composed of sequences of the nuITS and mtSSU regions generated from eight specimens of *Pannaria hookeri* collected in southern Argentina. Furthermore, six *P. hookeri* sequences retrieved from GenBank composed the dataset together with other *Pannaria* species sequences and the selected outgroups (Tab. 1). The final dataset comprised 31 sequences and an alignment 1105 base long, with 423 of the nuITS and 682 of the mtSSU regions.

Trees of both BA and ML approaches, among genes and concatenated datasets, showed congruent topology, so only the concatenated tree is presented, with the values of the posterior probabilities and bootstrap values shown (Fig. 1). The sequences of the specimens identified as *P. hookeri,* newly produced and those obtained from GenBank, presented 0.89/95 of posterior probability and bootstrap values, respectively, grouping the specimens from Argentina, Australia, Norway, and the USA in a well-supported monophyletic group. The divergence in the nuITS region, considered the universal barcode DNA of fungi (Schoch *et al.* 2012), revealed a low variation, with the mean percentage of identity of 99.34 % (98.25 % to 100 %) among the sequences, without deeply divergent populations or cryptic lineages.

The morphological descriptions of *Pannaria hookeri* from the literature were gathered and used for the circumscription of the species and for the observation of inconsistencies in the species descriptions and identifications, with the differences found highlighted in the sections "Description of Species" and "Discussion" below (Tab. 2).

Description of species

Pannaria hookeri (Borr. ex Sm.) Nyl., Mém. Soc. Sci. Nat. Math. Cherbourg 5: 109 (1857). (Fig. 2 A-F)

Description. Thallus placodioid. Lobes at the circumference narrow, 0.5-1.0(-2.0) mm broad and 1.0-2.5 mm long, convex, enlarged in the apices, centrally verrucose and fragmented or often partially detached. Upper surface whitish beige to brownish-grey under fluorescent light, whitish beige to whitish brown under the stereomicroscope. Hypothallus inconspicuous at marginal branches. Apothecia frequent, 0.4-1.5(-2.0) mm diam., with black discs. Thalline margin conspicuous, usually crenulate, concolorous with the thallus. Thallus 200-400 µm thick. Upper cortex 10-50 µm (2-12 layers of cells) thick, proso- to colloparaplectenchymatous tissue; cells thick-walled cells, 2.5-5.0 µm diam. Photobiont layer dense and massive, probably *Nostoc* clusters, spherical cells *ca.* 5 µm diam.; numerous vertically orientated fascicles of hyphae derived from the medulla penetrating into the photobiont layer. Medulla is hyaline with some photobionts. Lower cortex 12.5-25.0 µm (*ca.* 3 cells) thick when on the moss, blackish. Apothecia with a thick thalline margin to 250 µm, containing a large number of algal cells; cortex of thalline exciple colloplectenchymatous or with amorphous cells, 5.0-10.0 µm (2 cells) thick at the apices, 7.5-35.0 µm (2-8 cells) thick at the middle, indistinct at the base. Proper exciple *ca.* 50 µm thick, colloplectenchymatous to elongated cells. Subhymenial layers yellowish to hyaline, 25-65 µm thick, usually prosoplectenchymatous. Hymenium 100-200 µm thick, partially I+ persistently deep blue, mainly in the vicinity of the asci. Simple paraphyses, up to 2.5 µm thick, slightly clavate at the apices. Asci 50-65 × *ca.* 20 µm.
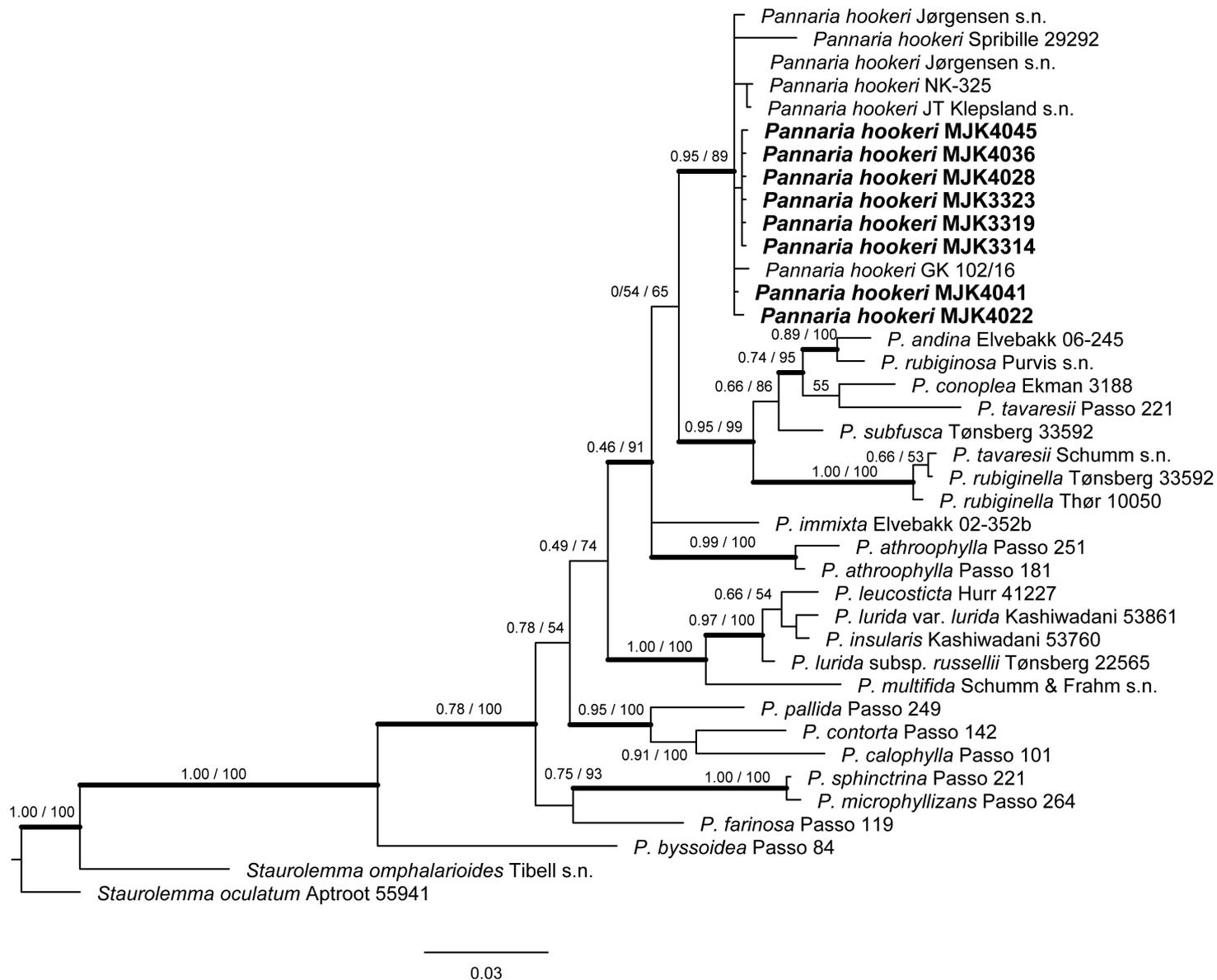
Figure 1. Bayesian Maximum Clade Credibility tree based on nuITS and mtSSU datasets showing the phylogenetic relationships within the *Pannaria* and *P. hookeri* sequences from Argentina, Australia, Norway and USA (Alaska). Posterior probabilities and Bootstrap values are above branches, respectively. Bold branches have posterior probabilities (PP) >0.95 % or bootstrap values >75. Sequences obtained in this study are marked in bold. Text after species names corresponds to voucher information (Tab. 1).

Table 2. Morphological descriptions of *Pannaria hookeri* reported in the literature and in the present study.

	Lindsay (1974)	Redón (1985)	Jørgensen (1986)	Jørgensen (2000a)	Øvstedal & Lewis Smith (2001)	Jørgensen (2007)	Kantvilas & Gueidan (2018)	Passo *et al.* (2020)	This study
Upper surface color	Greenish grey, streaked with white towards tips of squamules	Greenish grey	Brownish, white-grey striated and with distinctly white-grey margins which are sometimes blackened	Brownish grey, but dominated by the whitish margins and the laminal white striae	Grey to brownish, also to Pannaria caespitosa P.M. Jørg	Grey-brown though whitish striate	Pale brownish grey to smoky bluish grey	Bluish grey to brownish	Whitish beige to brownish-grey
Branches	Not mentioned	Up to 3 mm broad	Up to 4 mm broad	To 2 mm	3–4 mm, also to Pannaria caespitosa P.M. Jørg	Up to 3 mm	0.3–1.0 mm wide	0.5–1.0 mm diam.	0.5–1.0(–2.0) mm broad
Marginal lobes		Enlarged	Enlarged	Deeply divided, finger like peripheral lobes	Fan-shaped	Radiated	± effigurate and spathulate	Discrete	Enlarged in the apices
Central part		Areolate to granulose	Not mentioned	Disintegrating in the subcrustose	Not mentioned	Centrally often verrucosely fragmented and partially detached	Irregularly rhomboidal and delimited by deep cracks	Grouped	Verrucose and fragmented or often partially detached
Apothecia	Up to 5 mm diam.	0.5–2.0 (–5.0) mm diam.	Up to 3 mm broad	To at most 2 mm diam	Up to 3.5 mm, also to Pannaria caespitosa P.M. Jørg	Up to 2 mm	0.4–1.3 mm diam.	0.4–1.3 mm diam.	0.4–1.5(–2.0) mm diam.
Discs	Some shade of brown	Brown to black	Black or dark brown	Usually with black	Pale brown	Black to rarely dark brown	Dark brown to black	Black to dark brown	Black discs
Ascospores	Ellipsoid, 14–16 × 7–8 µm	Ellipsoid, (11.5–)14–16(–17) × 7–8(–11) µm	Ellipsoid to globular, 12–14 × 10–12 µm	Nearly globular, 12–15 × 8–11 µm	Subglobose, 12–15 × 10–13 µm	Subglobose, 12–15 × 8–11 µm	Ellipsoid to ovate, (10–)12–14.0–16(–18) × 6–7.6–9(–10) µm	Ellipsoid to ovate, 12–16 × 6–9 µm	Ellipsoid to spherical, (7.5–)12.5–15.0(–17.5) × 5.0–12.5 µm
Examined specimens	South Georgia	South Shetland Islands	Bouvetøya Island	Canada, Greenland, Argentina, Chile, Kerguelen, Marion I., New Zealand, and South Shetland Isl.	Antarctica and South Georgia Island	Nordic Region	Tasmania	Argentina	14 localities from the northern and southern Hemispheres
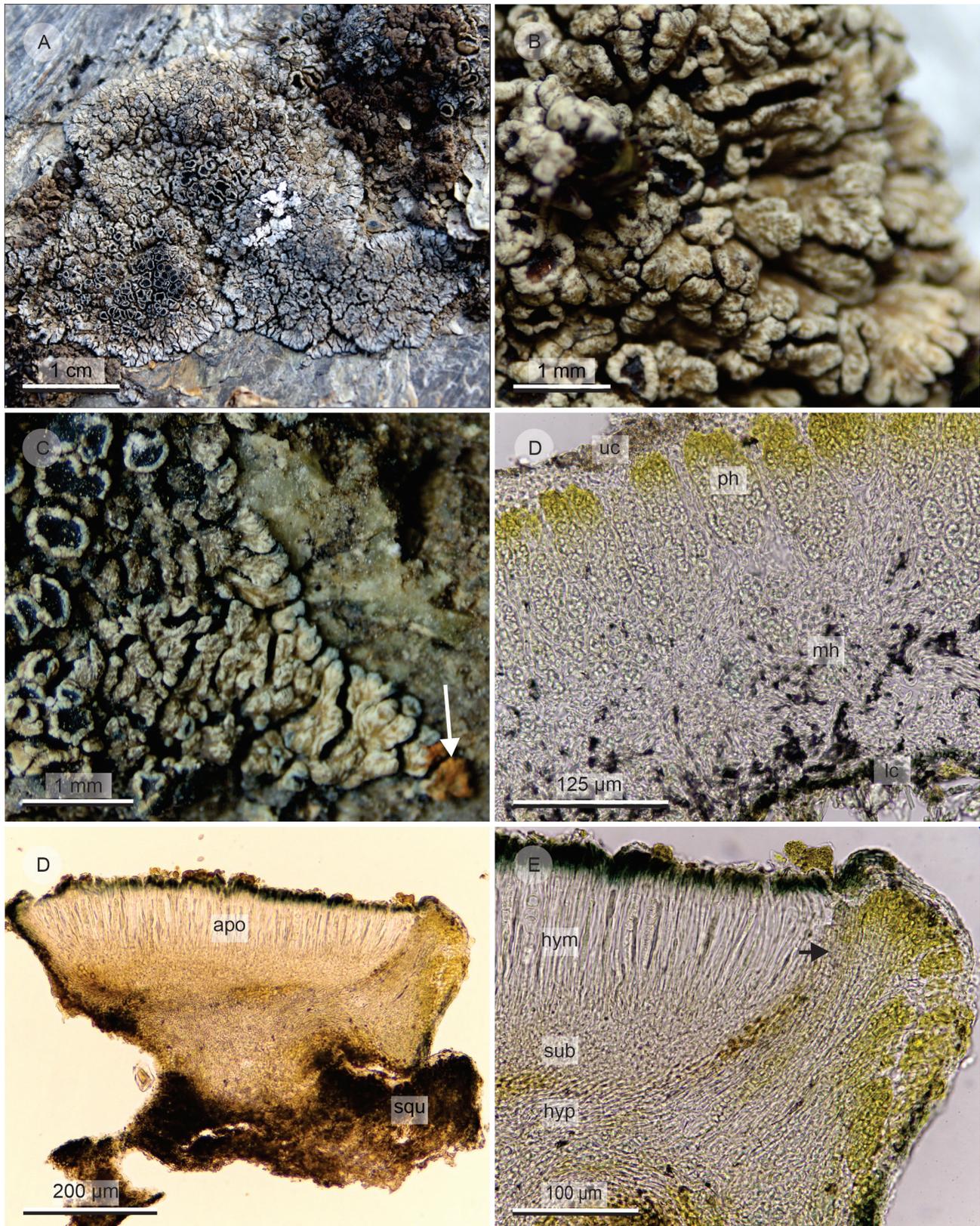
Figure 2. *Pannaria hookeri* from Argentina. (**A**): Specimen on the habitat. (**B**): Detail of laciniate branches on moss (MJK3314). (**C**): Saxicolous specimen (MJK4041). (**D**): Transversal section of the thallus. (**E**): Section of apothecium on the squamule. (**F**): Diametral section of apothecium. Symbols. apo = apothecium; black arrowhead = parahymenial tissue; black arrow = moss; hym = hymenium; hyp = hypothallum; lc = lower cortex; mh = medullar hyphae; ph = photobiont layer; squ = squamule; sub = subhymenium; uc = upper cortex; white arrow = P+ orange (pannarin).

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Spores 8 per ascus, (7.5-)12.5-15.0(-17.5) × 5.0–12.5 μm, colorless, ellipsoid to spherical, smooth-walled. Pycnidia were not observed.

Spot tests: K-, C-, KC-, P+ orange (probably pannarin).

Notes: *Pannaria hookeri* is characterized by presence of thallus placodioid with marginal lobes enlarged (less than 2 mm), central part often verrucose, fragmented and partially detached, and by presence of pannarin (P+ orange). The lecanorine apothecia have 0.4-1.5(-2.0) mm diam., with the blackish discs, subhymenium of prosoplectenchymatous tissue, and hypothecium and parahymenium of colloplectenchymatous tissue and elongated cells. The ascospores have always smooth epispores.

We examined specimens are from Austria (4 specimens), Canada (2), Denmark (Faroe Islands, 1), Greenland (1), Iceland (1), Italy (1), Norway (16), Russia (1), Scotland (4), and Sweden (4) from the Northern Hemisphere; and Argentina (11), Chile (1), Kerguelen Island (2), and New Zealand (1) from the Southern Hemisphere. The determination of the specimens received on loan from herbaria was confirmed only through morphological approach. Unfortunately, most of the received material was collected more than 10 years ago (Text S1), and we were not able to generate genetic sequences. When the *P. hookeri* characters were not observed in the material, the specimen was excluded of the study.

Discussion

In this study, we re-confirmed the wide geographical distribution of *Pannaria hookeri* through phylogenetic and morphological evidence, now including the first *P. hookeri* sequences from the southern South America, Argentina. The phylogenetic analysis resulted in a well-supported clade with *P. hookeri* sequences from Argentina, Australia, Norway, and the USA, whereas the morphological analysis included specimens from Argentina, Austria, Canada, Chile, Denmark (Faroe Islands), Greenland, Iceland, Italy, Kerguelen Island, New Zealand, Norway, Russia, Scotland and Sweden, generating a concise morphological description. The materials from Scotland are topotypes (Text S1) and were compared with fresh specimens.

The bipolar distribution has been concepted as species that are disjunctly distributed in both hemispheres, mostly in polar and subpolar regions, and are largely absent in the tropics. However, many lichens reported as bipolar are also distributed in temperate and tropical latitudes - usually in high mountains, where their ecological requirements are met (Garrido-Benavent & Pérez-Ortega 2017), which is the case of *P. hookeri*, and should be carefully revised and possibly considered as (sub)cosmopolitan. Nowadays, the species is mentioned in high altitudes of Ecuador and Kenya (material not revised in the present study) (Frisch & Hertel 1998; Jørgensen & Palice 2010), but efforts are still needed to confirm or not the species distribution through the tropical latitudes. Studies including DNA analysis of species reported as bipolar has confirmed this remarkable distribution, for example in *Austroplaca*, *Cetraria* and *Cladonia* (Myllys *et al.* 2003; Søchting & Castello 2012; Fernández-Mendoza & Printzen 2013), while others has resulted in the discovery of cryptic lineages or restricted distribution, such as in *Parmelia*, *Sphaerophorus* and *Rusavskia* (Crespo *et al.* 2002; Högnabba & Wedin 2003; Scur *et al.* 2022), indicating that phylogeographical studies should be performed to revise the distribution stated to *P. hookeri*.

Historically, *Pannaria hookeri* specimens from the Southern Hemisphere were reported with a larger morphological plasticity than specimens from Northern Hemisphere (Jørgensen 2000a), and the taxonomic problems has invalited other results, *e.g.* the chemical study (Quilhot *et al.* 1989). The variations in the color of the thallus, branching, diameter of apothecia, and color of the apothecium discs, which were reported in the literature from Antarctic specimens (Lindsay 1974; Redón 1985; Jørgensen 1986; 2000a; Øvstedal & Lewis Smith 2001), were not observed in the material confirmed by us and can be characters of other cryptic species. For example, apothecia up to 5.0 mm diam. were reported to the specimens from South Georgia and South Shetland Islands (Lindsay 1974; Redón 1985), apothecia up to 3.0 mm diam. in specimens from Bouvetøya Island (Jørgensen 1986), but only apothecia up to 2 mm diam. were observed during the present study.

In the same way, Øvstedal & Lewis Smith (2001) reported the species to South Georgia, Bouvetøya, South Orkney, South Shetland Islands, and Antarctic Peninsula, but posteriorly transferred the most of examined specimens within *P. caespitosa* P.M. Jørg. (Jorgensen 2000a). Only two specimens, SJA 168 and RILS 8347, were kept within *P. hookeri*, but they have the presence of pale brown discs in the apothecia (Øvstedal & Lewis Smith 2001) which do not match with the dark brown to blackish discs of *P. hookeri* (Tab. 2). Therefore, a revision is still required to the both specimens.

The material received as loan that were not confirmed as *P. hookeri* are from Antarctic and subantarctic Islands (Antarctica Peninsula and Kerguelen, Marion, South Georgia, South Orkney and King George islands - Text S1), highlighting the taxonomic problem in the region. In addition, fresh specimens collected during four Brazilian Antarctic Expeditions in the Antarctic Peninsula (near to Esperanza and Primavera stations), James Ross Island, Marambio Island, and South Shetland Islands (Deception, Greenwich, King George, Livingston, Nelson, Robert, and Snow Islands) did not cluster with the *P. hookeri* sequences and they were also excluded from our analysis. An integrative revision of the *Psoroma*-complex is also required to Antarctica as well as a complete identification key of the group; *P. hookeri* in Antarctica can be more restricted than previously thought.

Previously, *P. hookeri* was considered as strictly saxicolous (Jørgensen 2000a). However, we observed in Argentina that the species can grow on the rock (Fig. 1A), and also on saxicolous moss (Fig. 1B), expanding the species' known ecology. Furthermore, the species were usually found on the mountains in Argentina, Australia and Chile (Jørgensen 2000a; Kantvilas & Gueidan 2018). Calvelo & Fryday (2006) reported *P. hookeri* on rock outcrops along a stream the sea-level in Argentina, but the material has not been reviewed by us.

Our description was based on 50 specimens from 13 countries, corroborating with the descriptions provided by Jørgensen (1978; 2000a; 2007), Kantvilas & Gueidan (2018) and Passo *et al.* (2020). Therefore, the large morphological variations reported for specimens from the Southern Hemisphere are potentially characters of different *Psoroma* species. Studies including more genetic sequences, chemical and morphological variation analysis should be performed to dept the evolutionary history and concept of *Pannaria hookeri*.

Acknowledgments

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