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Pannaria hookeri (lichenised Ascomycetes) – a remarkable new record for Australia

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Abstract

Pannaria hookeri (Borrer ex Sm.) Nyl., a bipolar lichen, is recorded for the first time for Australia (south-west Tasmania) where it grew on alpine limestone outcrops. Its identity was confirmed by morphological, anatomical and DNA-sequence data.

***Keywords*:** biodiversity, lichens, species discovery, Tasmania

Introduction

Additions to the list of lichens recorded for Australia are made almost continuously, as demonstrated very well by McCarthy (2017), whose checklist for Australia requires regular updates. It is no exaggeration to observe that, in Australia, a survey of almost any area, or a revision of any taxonomic group, will reveal previously overlooked novelties. The Bush Blitz Programme, conducted by the Australian Biological Resources Study since 2010, has been especially successful in this regard. This paper reports a particularly interesting discovery, *Pannaria hookeri* (Borrer ex Sm.) Nyl., a first record for Australia. This bipolar species is widespread in arctic-alpine areas of the Northern Hemisphere (Jørgensen 1978; Thomson 1984) with scattered occurrences in the Antarctic (Øvstedal & Lewis Smith 2001), Mt Kenya in Africa (Jørgensen 2007), southern South America (Jørgensen 1978) and New Zealand (Galloway 1985). It was discovered in south-western Tasmania during a Bush Blitz Expedition in 2016.

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Methods

Morphology, anatomy and chemistry

Morphological and anatomical investigations were undertaken on material collected in Tasmania and compared with reference herbarium material of *P. hookeri* as listed. Hand-cut sections were mounted in water, 10% KOH, 50% HNO_3 and Lactophenol Cotton Blue. Ascospore measurements are based on 75 observations and are presented in the format: least value–*average*–highest value; outlying values are given in brackets. Routine chemical analyses by thin-layer chromatography follow standard methods (Orange et al. 2001).

Selected comparative specimens examined: **SWEDEN.** Torne Lappmark, Jukkasjärvi par., along river Loktajohka, c. 14 km ESE of Riksgränsen, 4.vii.2015. M. Westberg VAR 192 (S); Lycksele Lappmark: par. Tärna, Rivovardo, 800-1000 m alt., 1924, A.H. Magnusson 7922 (S). **NORWAY.** Opland: Nord-Fron hd, Sikilsdalshö, c. 1620 m. 1620 m alt., 6.ix.1949, S. Ahlner (S). **AUSTRIA.** Nordtirol, Kluppescharte, 1960, H. Doppelbaur & J. Poelt (Lichenes Alpium 141) (MEL 1017675, S).

DNA extraction, amplification and sequencing

Part of the specimen GK102/16 was sampled for DNA extraction using a clean pair of tweezers. The sampled material was transferred to an Eppendorf tube and the DNA extracted using a DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany). The manufacturer's protocol was modified as follows: the material was ground in AP1 buffer using a sterile plastic pestle and then incubated with RNase A for an hour at 70ºC. The lengths of all centrifuge steps were doubled and the DNA was recovered in 50 µl of AE buffer.

Two gene regions were amplified: the region including the 5.8S subunit of the nuclear ribosomal RNA gene and the internal transcribed spacers 1 and 2 (ITS) and the small subunit of the mitochondrial ribosomal RNA gene (mtSSU). The primers used were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) for ITS and mtSSU1 and mtSSU3R (Zoller et al. 1999) for mtSSU. DNA extracts were checked with a gel electrophoresis and for each sample the band intensity was used to choose the appropriate genomic DNA dilution for amplification. One micro-litre (µl) of a 1, 1/10, or 1/100 dilution of genomic DNA was added to the following PCR mix: 2.5 µl PCR buffer 10 × NH_4 (Bioline, London, U. K.), 1.5 µl of $MgCl_2$ (50 mM), 0.5 µl dNTP (100 mM), 2.5 µl of BSA (10 mg/ml), 1 µl of each primer (10 µM), 0.25 µl DNA polymerase Bioline BioTaq (5 U μl^{-1}), and water to a total volume of 25 µl. The PCR reactions were performed on a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). The PCR program for ITS was as follow: 5 min at 94°C, followed by 35 cycles of the three steps 1 min at 94°C (denaturation), 1 min at 53°C (annealing), 2 min at 72°C (extension), and a final extension time of 10 min at 72°C. For mtSSU, the program was: 3 min at 94°C, followed by 35 cycles of the three steps 1 min at 94°C, 1 min at 52°C, 1.5 min at 72°C, and a final extension time of 7 min at 72°C. Cloning was conducted on PCR products using a TOPO-TA Cloning kit (Invitrogen, Carlsbad, California), as instructed by the manufacturer. PCR product clean-up and sequencing were carried out by Macrogen (Seoul, South Korea) using BigDye chemistry and an ABI 3730xl sequencer (Applied Biosystems, Carlsbad, California).

Phylogenetic analysis

New *Pannaria* sequences were edited using Sequencher v. 5.4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Published ITS and mtSSU *Pannaria* sequences were obtained from GenBank (https://www.ncbi.nlm.nih.gov/) and manual alignments of all sequences were done using Mesquite v. 3.04 (Maddison & Maddison 2011). The final alignments included 24 ingroup taxa from the genus *Pannaria* and three outgroup species (*Psoroma implexum*, *Staurolemma oculatum* and *S. omphalarioides*) were chosen, based on a previous phylogenetic study (Ekman et al. 2014). Taxon and gene sampling are shown in Table 1. Ambiguous regions were delimited as described in Lutzoni et al. (2000) and were excluded from the phylogenetic analyses. To test for congruence, each locus (ITS and mtSSU) was first subjected to a bootstrap analysis separately using maximum likelihood (ML) (RAxML VI-HPC v.8.2.9; Stamatakis et al. 2005, 2008), as implemented on the CIPRES Web Portal (http://www.phylo.org; Miller et al. 2010). A GTRCAT model was applied to the two markers. Support values were obtained using a fast bootstrap analysis of 1,000 pseudoreplicates. Resulting topologies

were compared for a potential conflict among loci using a 70% reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996). Because no conflict was detected, the two gene regions were concatenated and the combined dataset analysed using RAxML as described above. Trees were visualized in PAUP* (Swofford 2002) and edited with Illustrator (Adobe Systems, San Jose, CA, USA). The dataset was deposited in TreeBase (22176).

Results

Phylogenetic results

The dataset included 27 taxa (24 ingroup and 3 outgroup taxa) and 1,114 characters (385 from ITS and 729 from mtSSU). The concatenated alignment had 228 distinct alignment patterns and a proportion of gaps and completely undetermined characters of 21.70%. The most likely tree is presented in Figure 1 with bootstrap support values. As in Ekman et al. (2014),

Table 1. Taxon and gene sampling for the phylogenetic analysis. Missing sequences are indicated by a dash. The accession numbers of the two newly generated sequences are highlighted in bold.

	Species	Voucher	ITS	mtSSU
1	*Pannaria andina*	Elvebakk 06-245	GQ927268	-
2	*Pannaria athroophylla*	Passo 181	EU885295	EU885317
3	*Pannaria athroophylla*	Passo 251	EU885303	EU885325
4	*Pannaria calophylla*	Passo 101	EU885296	EU885318
5	*Pannaria conoplea*	Ekman 3188	AF429281	-
6	*Pannaria contorta*	Passo 142	EU885297	EU885319
7	*Pannaria farinosa*	Passo 119	EU885299	EU885321
8	*Pannaria hookeri*	Jørgensen s.n.	AF429282	KC608083
9	*Pannaria hookeri*	GK102/16	**MG786563**	**MG792317**
10	*Pannaria immixta*	Elvebakk 02-352b	-	KC608084
11	*Pannaria insularis*	Kashiwadani 43760	KC618716	KC608085
12	*Pannaria leucosticta*	Hur 041227	EU266107	-
13	*Pannaria lurida subsp. lurida*	Kashiwadani 43861	-	KC608086
14	*Pannaria lurida subsp. russellii*	Tønsberg 22565	-	KC608087
15	*Pannaria microphyllizans*	Passo 264	EU885300	EU885322
16	*Pannaria multifida*	Schumm & Frahm s.n.	KC618717	KC608088
17	*Pannaria pallida*	Passo 249	EU885301	EU885323
18	*Pannaria rubiginella*	Tønsberg 32508	KC618718	KC608089
19	*Pannaria rubiginella*	Thor 10050	-	GQ259037
20	*Pannaria rubiginosa*	Anonby 870/Purvis s.n.	AF429280	AY340513
21	*Pannaria sphinctrina*	Passo 221	EU885302	EU885324
22	*Pannaria subfusca*	Tønsberg 33592	KC618719	-
23	*Pannaria tavaresii*	Schumm s.n.	KC618720	-
24	*Pannaria tavaresii*	Passo 122	EU885294	EU885316
25	*Psoroma implexum*	Passo 84	-	EU885333
26	*Staurolemma oculatum*	Aptroot 55941	KC618738	GQ259045
27	*Staurolemma omphalarioides*	Tibell s.n.	KJ533487	KJ533439

the genus *Pannaria* is supported as monophyletic (82% bootstrap support). The specimen of *Pannaria* collected in Tasmania (GK102/16) is highly supported as sister taxon to *Pannaria hookeri* (99% bootstrap support). The relatively short branch lengths between the Tasmanian and Northern Hemisphere specimens agree with their conspecific nature.

Taxonomy

***Pannaria hookeri* (Borrer ex Sm.) Nyl.,** *Mém. Soc. Natn. Sci. Nat. Cherbourg* **5: 109 (1858)**

Lichen hookeri Borrer ex Sm., in J.E. Smith & J. Sowerby, *Engl. Bot.* 32: 2283 (1811).

Type: SCOTLAND. Ben Lawers and Meall Greigh, *W. Borrer* (lectotype, *fide* Jørgensen 1978: BM!).

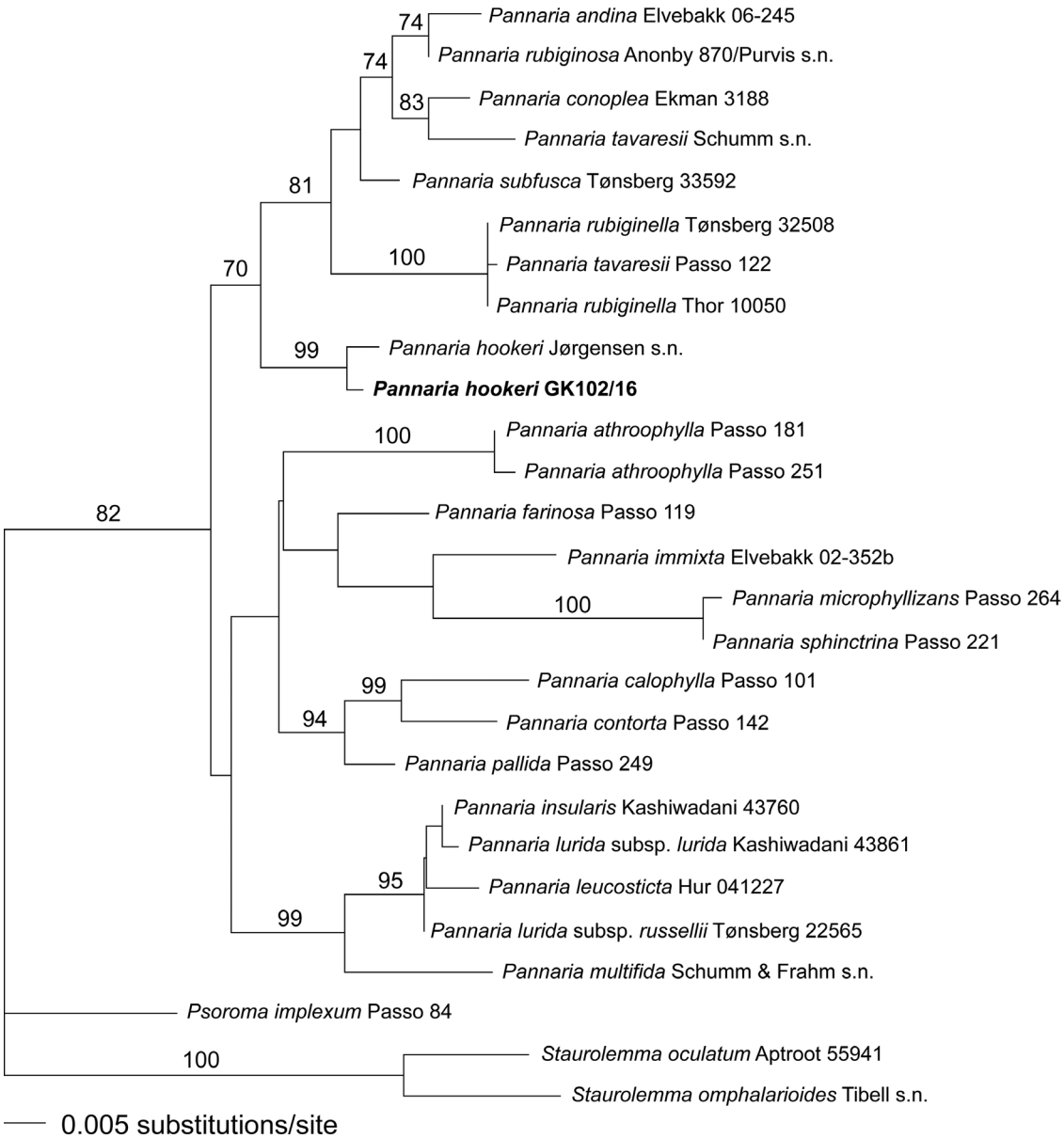

Figure 1. Most likely tree showing the phylogenetic placement of the Tasmanian *Pannaria* specimen (GK102/16). The phylogeny was obtained using a combined ITS-mtSSU dataset and analysed with maximum likelihood using RAxML. Bootstrap support values are reported above the branches. The scale bar represents the number of nucleotide substitutions/site.

Thallus subcrustose to squamulose; squamules plane to convex, sometimes rather gnarled, 0.3–1 mm wide, 250–500 µm thick, dispersed or clustered and overlapping, tightly adnate or loosely attached over a black, effuse prothallus, irregularly rhomboidal and delimited by deep cracks in the centre of the thallus, ± effigurate and spathulate at the margins; upper surface pale brownish grey to smoky bluish grey, ± scabrid-maculate and faintly whitish striate, especially at the thallus margins; photobiont *Nostoc*, comprising single, roundish cells, 5–10 µm wide. *Apothecia* lecanorine, 0.4–1.3 mm diam., scattered, basally constricted when mature; disc dark brown to black, mostly plane but becoming convex and puckered with age; thalline margin mostly entire, crenulate, somewhat inrolled, in section 80–150 µm thick; proper margin prosplectenchymatous, highly reduced to a dark brown band *c.* 10–20 µm wide between the hymenium and the thalline margin. *Hypothecium* colourless to pale yellowish, lacking photobiont cells. *Hymenium* (65–)70–90(–120) µm thick, colourless, I+ brown, K/I+ blue, overlain by an epithecial band of blue-green pigment, K+ intensifying greenish, N+ crimson. *Paraphyses* simple, 1.5–2.5 µm thick, with the terminal cell frequently enlarged to 4–5 µm and internally blue-green pigmented. *Asci* 8-spored but usually with a few ascospores deformed or aborted, (50–)55–65 x 14–20 µm, clavate, with a well developed, non-amyloid or very weakly amyloid tholus lacking any internal discernible structures, and an intensely amyloid, thin outer sheath. *Ascospores* broadly ellipsoid to ovate, smooth-walled, (10–)12–*14.0*–16(–18) x 6–*7.6*–9(–10) µm, when young with a distinct wall *c.* 1 µm thick and a large vacuole. *Pycnidia* not found. *Chemistry:* traces of pannarin sometimes found by t.l.c. but not detectable by spot tests. Figs 2–3.

***Specimen examined*: TASMANIA.** North East Ridge of Mt Anne, at the western rim of Annakanada sinkhole, 42°55′57″ 146°26′29″E, 1050 m alt., on sheltered limestone outcrops, 5.ii.2016, G. Kantvilas 102/16 (HO).

Discussion

The above description is based solely on Tasmanian material but compares favourably with published descriptions (e.g. Jørgensen 1978; Thomson 1984; Galloway 1985; Stenroos et al. 2016), and with reference herbarium material. Further confirmation of our determination was provided by molecular data.

Together with three other species that have highly restricted, circum-Antarctic distributions, *Pannaria hookeri* was placed in the subgenus *Chryopannaria* (Jørgensen 2000). However, subsequent phylogenetic research (Ekman & Jørgensen 2002; Ekman et al. 2014) did not support this classification and simply places *P. hookeri* within *Pannaria s. str.*

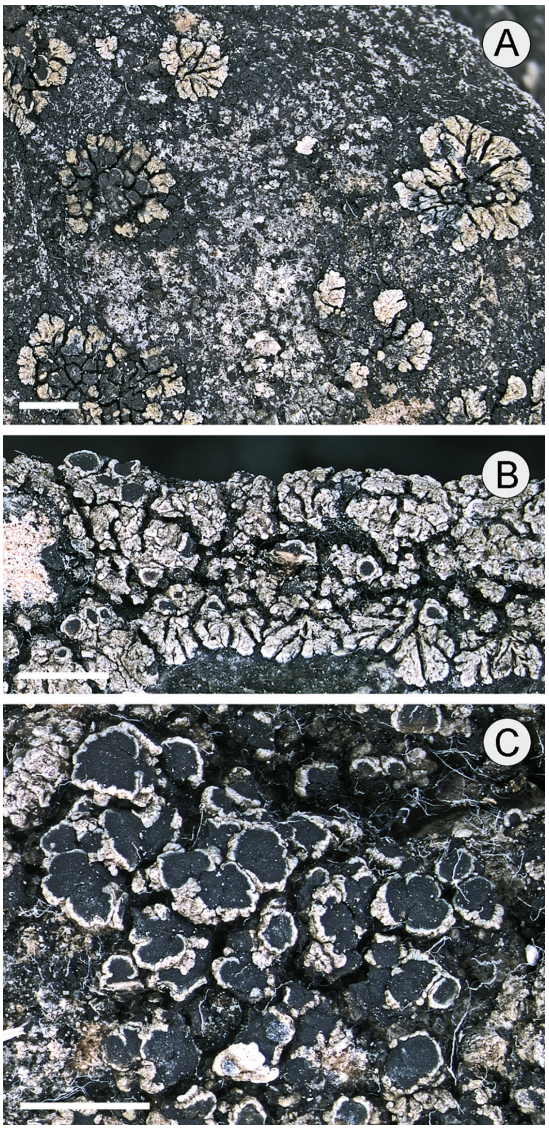

Figure 2. Morphology of *Pannaria hookeri* (GK 102/16). **A.** Habit; **B.** Detail of effigurate squamules with maculate, striate margins; **C.** Detail of apothecia. Scale = 2 mm

Lichens may be notoriously widespread, displaying cosmopolitan, bipolar, pan-temperate and pan-tropical distribution patterns, as well as having more localised ranges. However, applying names across wide geographic areas carries some risks, and there are numerous instances where a species perceived to be the same in both hemispheres has been subsequently found to comprise two distinct entities. For example, Australasian populations of what was once referred to as *Menegazzia terebrata* (Hoffm.) A.Massal. are now called *M. subpertusa* P.James & D.J.Galloway, and the lichen once called *Parmelia omphalodes* (L.) Ach. in Australasia is now correctly called *Notoparmelia signifera* (Nyl.) A.Crespo, Ferencova & Divakar. In this context, Robert Brown's list of lichens native to both Australia and Europe (Brown 1814) makes interesting reading.

In the case of *Pannaria hookeri*, the molecular investigation was critical. When first observed in the field and collected (by GK), the species was immediately recognised as truly novel for Tasmania. However, initial misinterpretation of some ambiguous apothecial characters led to assumptions that it was new to science and of uncertain generic affinity. It was the molecular investigation, essentially to explore its generic relationships within the Pannariaceae, which redirected us towards *P. hookeri* and to making the necessary anatomical and morphological comparisons to confirm our identification.

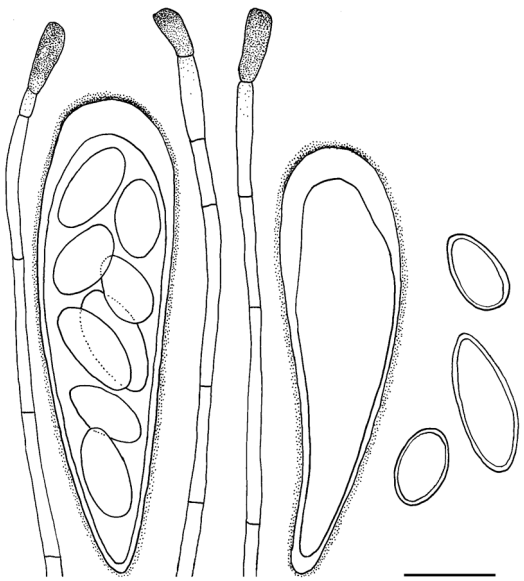

Figure 3. Anatomy of *Pannaria hookeri* (GK 102/16). Asci (amyloid parts stippled), paraphyses and ascospores. Note the typical *Pannaria*-type ascus with a well developed, non-amyloid tholus that lacks internal differentiation. Scale = 10 µm

Ecology and distribution

The ecology of *Pannaria hookeri* is indeed unusual, for it occurs on alpine limestone, an extremely uncommon habitat in Tasmania. Interestingly, several European authors (e.g. Jørgensen 2000; Stenroos et al. 2016; James & Purvis 2009) likewise note its predilection for calcareous substrata. The species was part of a highly depauperate community on relatively sheltered aspects where lichens were patchy and extensive areas of bare rock prevailed. Other lichens recorded included *Baeomyces heteromorphus* Nyl. ex C.Bab. & Mitt., *Catillaria lenticularis* (Ach.) Th.Fr., *Lepraria vouauxii* (Hue) R.C.Harris, *Paraporpidia leptocarpa* (C.Bab. & Mitt.) Rambold & Hertel, *Placopsis brevilobata* (Zahlbr.) I.M.Lamb, *P. subcribellans* (I.M.Lamb) D.J.Galloway, *Porpidia crustulata* (Ach.) Hertel & Knoph, *P. umbonifera* (Müll.Arg.) Rambold, *Rhizocarpon petraeum* (Wulfen) A.Massal., *R. reductum* Th.Fr., *Staurothele succedens* (Rehm ex Arnold) Arnold, *Stereocaulon ramulosum* (Sw.) Räusch. and a putative new species of *Trapelia* M.Choisy. Several of these taxa are themselves new or interesting records for Tasmania.

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