



## Standard Paper

# *Psoroma capense* and *P. esterhuyseniae* (Pannariaceae), two new alpine species from South Africa

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## Abstract

The new species *Psoroma capense* and *P. esterhuyseniae* are described from four alpine localities in the Western Cape Province of South Africa and are the only known *Psoroma* species from Africa. The specimens were all collected from moist sites near watercourses, on cool and mostly south-facing cliffs. *Psoroma capense* resembles *P. tenue* in gross morphology but differs in the ascending thallus squamules, lack of secondary compounds and short-ellipsoid to ovoid ascospores. However, a phylogenetic analysis involving the markers ITS, nucLSU, mtSSU and *Mcm7*, comparing the only recent collection of *P. capense* with previously published sequences, shows that it belongs to the *P. hypnorum* lineage, with no known, closely related species. *Psoroma esterhuyseniae* resembles *P. hypnorum* but has subglobose to short-ellipsoid ascospores without apical perispore extensions. The two species are thought to have evolved from one or two long-distance dispersal events during the Pleistocene.

**Key words:** biodiversity, evolution, lichens, phylogeny, taxonomy

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## Introduction

Previously, the genus *Psoroma* was interpreted widely, and included almost all tripartite *Pannariaceae* species (Jørgensen & Galloway 1992), with green algae as major photobionts, and cyanobacteria located in smaller cephalodia. However, many foliose tripartite species were later transferred to the genera *Pannaria* (see review in Elvebakk & Elix (2017)) and the newly described *Gibbosporina* (Elvebakk *et al.* 2016). Some squamulose species were transferred to the new genus *Joergensenia* (Passo *et al.* 2008) and to *Psorophorus* and *Xanthopsoroma* (Elvebakk *et al.* 2010), whereas six bipartite, squamulose species were transferred from *Pannaria* and *Santessoniella* to *Psoroma* (Ekman *et al.* 2014). After these revisions, the genus *Psoroma* became much more homogeneous. Its species have squamules, densely distributed or more scattered, and connected by a distinct or indistinct hypothallus/prothallus. In most species, the squamules are brown from melanins and lack secondary compounds that can be detected by TLC, with the presence of pannaric acid and porphyritic acid or porphyritic acid methyl ester as the most common exceptions.

The lichen genus *Psoroma* is mostly terricolous, occasionally corticolous in austral forests, rarely saxicolous, and is concentrated in the Southern Hemisphere, with most of its species distributed in

southern South America, south-eastern Australia, and New Zealand (Galloway 2007). Park *et al.* (2018) showed that no less than 10 species are known from Antarctica and also listed the four species reaching the Northern Hemisphere. Since then, two rare species have been reported from Alaska and arctic Canada (Elvebakk & Tønsberg 2018; Fryday *et al.* 2019).

When Jørgensen (2003) reviewed the *Pannariaceae* flora of the African continent, he included *Psoroma asperellum* Nyl. and *P. fruticulosum* P. James & Henssen, both from South Africa. The report of the former from ‘Promontorio Bonæ Spei’ by Nylander (1863) was later shown to be a misinterpretation of ‘Montis Tabularis’, an old name for Mt Wellington in Tasmania (Galloway 2007). An unpublished record from South Africa of *Psoroma hypnorum* (Vahl) S.F. Gray has also been posted in the GBIF database. A report from South Africa of the austral species ‘*Psoroma sphinctrinum* Nyl.’ by van der Byl (1931: 9) and cited by Doidge (1950), possibly refers to material of the tropical genus *Gibbosporina*.

During studies of *Pannariaceae* in various herbaria, no African material of *Psoroma* has been discovered, except the two Esterhuysen collections from BG determined as *P. fruticulosum* and *P. hypnorum*, and a third Esterhuysen sample borrowed from BOL. The three Esterhuysen specimens were collected between 1943 and 1951, and a fourth specimen was collected by T. Rämä in 2018. The aim of the present study is therefore to describe the species, but also to search for related species through phylogenetic analyses. This was carried out by comparing the recent collection with other *Psoroma* species using four phylogenetic markers.

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## Materials and Methods

### Lichen material

Herbarium materials used for this study are housed at BG and BOL, and the species was not found during extensive studies of the *Pannariaceae* collections in herbaria such as B, BM, C, CANB, O, S, SGO, UPS and W. 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 c. 80 ascospores and copies of all original drawings have been included with the specimens. Several specimens of other species were studied specifically for comparison. Thin-layer chromatography of acetone extracts followed standardized procedures and used solvents A and C (Culberson 1972; Orange *et al.* 2001). Nomenclature of ascospore structures follows Nordin (1997).

### Phylogenetic analyses

In order to determine the phylogenetic position of the undescribed species from South Africa, the phylogenetic relationships of 12 species of the genera *Psoroma*, *Psorophorus*, *Xanthoprosoma* and *Pannaria* were reconstructed. *Protopannaria pezizoides* (G. H. Web.) P. M. Jørg. & S. Ekman was used as an outgroup. The reference materials were selected from those used in a previous study (Park *et al.* 2018). Four phylogenetic markers, 5.8S-ITS2 rRNA (ITS), the nuclear large subunit rRNA (nuLSU), the mitochondrial small subunit rRNA (mtSSU) and minichromosome maintenance component 7 (*Mcm7*), were used for phylogenetic reconstruction. Sequence information for ITS, nuLSU and mtSSU of the reference materials was retrieved from a previous study (Park *et al.* 2018). Sequence information for ITS, nuLSU and mtSSU of the new material was obtained following procedures described by Park *et al.* (2018). *Mcm7* was amplified using the primers mcm7-709for and mcm7-1348rev (Schmitt *et al.* 2009). Touchdown PCR amplifications were performed in a T-gradient thermocycler (Biometra, Göttingen, Germany) with the following cycling parameters: 1 min initial denaturation at 95 °C, 6 touchdown cycles of 30 s denaturation at 95 °C, 50 s annealing at 60–56 °C at the ramp of 1° per cycle and 1 min extension at 72 °C, followed by 38 cycles of 45 s denaturation at 94 °C, 50 s annealing at 56 °C, and 1 min extension at 72 °C, with a 5 min final extension at 72 °C. The new sequences, including the holotype of *Psoroma capense* (cited as NK-1080 in Fig. 5 and Supplementary Material Table S1, available online) and the additional *Mcm7* sequences of the samples analyzed previously were deposited in the GenBank database under the accession numbers MT316196 to MT316208 (Supplementary Material Table S1).

Sequence alignments of ITS, nuLSU, mtSSU and *Mcm7* were conducted using the software ClustalX (Larkin *et al.* 2007) and manually adjusted. The size variation and ambiguous alignment of the ITS1 domain resulted in it being excluded from the phylogenetic analyses, as were other ambiguously aligned sites. Phylogenetic trees were inferred from each genetic locus and the combined dataset by maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses. MP trees were obtained using the Tree-Bisection-Regrafting (TBR) algorithm of MEGA X (Kumar *et al.* 2018) with search level 5 in which the initial trees were obtained by the random addition of sequences (1000

replicates). ML trees were constructed using MEGA X based on the GTR + I + G evolutionary model (Lanave *et al.* 1984), the search options of best tree topology finding by branch swapping of NNIs and SPRs, and random addition of sequences (1000 replicates). Aligned sites with less than 95% coverage by alignment gaps, missing data, or ambiguous bases were excluded. The Bayesian tree was generated using a search approach 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% of trees as burn-in.

### Taxonomy

#### *Psoroma capense* Elvebakk, S. G. Hong & Rämä *sp. nov.*

Mycobank No.: MB 836049

Superficially similar to *Psoroma tenue* var. *tenue* Henssen but with ascending thallus squamules, regularly short-ellipsoid to ovoid spores, and lacking TLC-detectable secondary compounds.

Type: South Africa, Western Cape, Witzenberg municipality, Hex River Mountains/Hexrivierberge, Matroosberg, Spekrievierskloof, 33°21'13"S, 19°37'42"E, 1310 m, S-exposed slope 50 m NW of a small dam in the river, 1–2 m high rock outcrop located 30 m NE of the river channel, on soil in a vertical rock cavity, apothecia occurring in an area of c. 3 × 5 cm, 18 March 2018, T. Rämä 1–2018 (BOL 59675—holotype). GenBank Accession nos.: MT316196, MT316197, MT316208.

(Figs 1, 2A, B & D, 3)

*Thallus* squamulose, tripartite, terricolous, forming 3–5 cm wide patches. *Chloromorph squamules* 150–250 µm thick, starting as small, 0.1–0.3 mm wide, circular and appressed squamules peripherally, developing into a dense mat of irregularly lobate and mostly ascending squamules, 0.5–2 mm tall. *Upper surface* pale chestnut brown, darker at apices, glabrous and weakly glossy. *Upper cortex* 30–50 µm thick, sclerenchymatic, upper third dark brown, pale brown below, paraplectenchymatic, lumina mostly isodiametric, 6–12 µm wide, walls 2–3.5 µm thick. *Chlorobiont layer* c. 60–100 µm thick, of cf. *Trebouxia* cells, globose to irregularly globose, 8–20 µm diam., with papillose chloroplasts. *Medulla* 80–130 µm thick; *lower cortex* absent. *Prothallus/hypothallus* indistinct, but visible in peripheral parts as a pale, byssoid network.

*Cephalodia* common, blackish, forming coralloid cushions in between or on chlorobiont squamules, 0.5–2.5 mm wide, cortex as in the chlorobiont squamules. *Cyanobiont Nostoc*, small-celled, greenish blue, obtusely angular, 3–6 × 3–7 µm, arranged in indistinct glomeruli, 30–50 µm wide, and without visible chain structures.

*Apothecia* common, substipitate, 1–3.5 mm wide; *disc* dark chestnut brown, weakly concave; *thalline excipulum* 1–3 mm wide when viewed from above, irregularly crenulated, occasionally with small verrucose or scale-like thalline outgrowths but generally non-squamulose, lower half of the sides thickly covered by a dense, thin and white tomentum, sometimes eroded. *Epithecium* c. 20–25 µm thick, sclerenchymatic, pale brown, upper third hyaline. *Hymenium* 100–120 µm thick, colourless, but strongly IKI+ blue. *Asci* clavate, 70–80 × 15 µm, 8-spored,





**Fig. 1.** *Psoroma capense*. A, holotype. B, Esterhuysen 19747 (BOL). Images by M. Karlstad. Scales: A = 5 mm; B = 1 mm. In colour online.

with an internal apical structure shown as a distinct tube in moderate concentration IKI. *Proper ascospores* hyaline, non-septate, short-ellipsoid to ovoid,  $15\text{--}21 \times 9\text{--}13\ \mu\text{m}$ . *Perispores* of the same shape,  $16\text{--}24 \times 10\text{--}16\ \mu\text{m}$ , with large, distinct verrucae, mostly with distinct, nodulose apical extensions, up to  $2.5 \times 3\ \mu\text{m}$ . *Paraphyses* septate, simple to sparingly branched, c.  $2.5\ \mu\text{m}$  wide, apices slightly swollen. *Hypothecium* pale brownish, 0–50  $\mu\text{m}$  thick, IKI–. An algal layer, 60–100  $\mu\text{m}$  thick, is distributed uniformly below the hypothecium.

*Pycnidia* scattered, black and verrucose, 150–200  $\mu\text{m}$  wide, ostiole fissure-like, c. 50  $\mu\text{m}$  long, spermatia  $1.5\text{--}2 \times 0.5\ \mu\text{m}$ , mostly curved.

**Chemistry.** Brownish melanins present, of a chestnut-coloured type, appearing similar to those of *P. tenue* Henssen. No TLC-detectable components found.

**Etymology.** Named after its occurrence in the Western Cape Region.

**Distribution and ecology.** Only known as three collections from the Western Cape Province of South Africa, found at moist sites at high altitudes in cool, mostly south-facing sites, often near water.

**Additional specimens examined (paratypes).** **South Africa:** Western Cape Province: Worcester Div., Mt Waaihoek (= 'Waaihoekpiek'), 5000 ft, damp southern cliffs above ravine, 1943, *E. Esterhuysen* 8929 (BOL 155421); Tulbagh Div., Sneeuwgat Peak (= 'Sneeuwgatpiek'), 6000 ft, on mossy, sandy bank on cliffs, near seasonal watercourse, 1951, *E. Esterhuysen* 19747 (BOL 155420; BG L-71578; LD not seen).

***Psoroma esterhuyseniae* Elvebakk sp. nov.**

Mycobank No.: MB 836050

Similar to *Psoroma hypnorum* but ascospores subglobose to short-ellipsoid without apical perispore extensions, and tomentum of the lower parts of thalline excipuli less prominent.

**Type:** South Africa, Western Cape, Hexriver Mts (= Hexriverberge), mountain ridge peak, 4500 ft, damp cliffs, S side, 11 November 1943, *E. Esterhuysen* 9419 (BG L-71579—holotype).

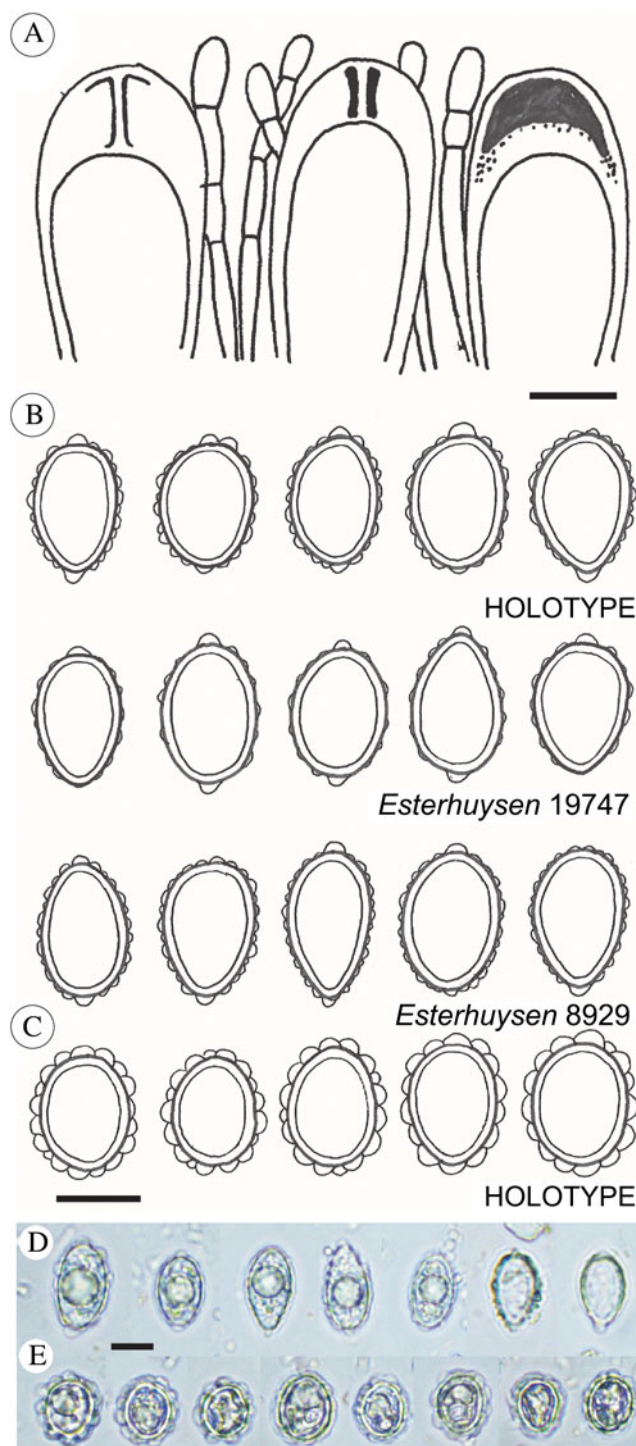
(Figs 2C & E, 4)

**Thallus** squamulose, tripartite, terricolous, 3–5 cm wide. **Chloromorph squamules** c. 150 µm thick, 0.1–0.3 mm wide, horizontal to weakly ascending, irregularly lobate, 1–2 mm tall. **Upper surface** chestnut brown, glabrous and glossy. **Upper cortex** c. 30 µm thick, sclerenchymatic, upper third dark brown, pale brown below, paraplectenchymatic, lumina mostly isodiametric, 6–12 µm wide, walls 2–3.5 µm thick. **Chlorobiont layer** c. 50 µm thick, of cf. *Trebouxia* cells, globose to irregularly globose, 7–15 µm diam., with angular chloroplasts. **Medulla** 60–100 µm thick; **lower cortex** absent. **Prothallus/hypothallus** indistinct, but visible in peripheral parts as a pale, byssoid network.

**Cephalodia** rare, pale, forming a coarse coralloid cushion in between or on chlorobiont squamules, c. 1 mm wide, cortex as in the chlorobiont squamules. **Cyanobiont Nostoc**, cells greenish blue, obtusely angular, 3–6 × 3–7 µm, arranged in indistinct glomeruli, 30–50 µm wide, and without visible chain structures.

**Apothecia** common, substipitate, 1–3 mm wide; **disc** dark chestnut brown, weakly concave; **thalline excipulum** 2–3 mm wide when viewed from above, irregularly crenulated, squamulose, external parts glabrous or occasionally with a tomentum-like mycelium in lower parts. **Epithecium** c. 20–25 µm thick, sclerenchymatic, pale brown, upper third hyaline. **Hymenium** 100–120 µm thick, colourless, but strongly IKI+ blue. **Asci** clavate, 70–80 × 15 µm, 8-spored, with an internal apical structure shown as a distinct tube in moderate concentration IKI. **Proper ascospores** hyaline, non-septate, subglobose to short-ellipsoid, 15–19 × 11–15 µm. **Perispores** of the same shape, 18–23 × 14–17 µm, with up to 2.5 × 3 µm wide, distinct verrucae, appearing inflated, and without apical perispore extensions. **Paraphyses** septate, simple to sparingly branched, c. 2.5 µm wide, apices slightly swollen. **Hypothecium** pale brownish, 40–50 µm thick, IKI–. An algal layer, 60–100 µm thick, is distributed uniformly below the hypothecium.

**Pycnidia** not seen.



**Fig. 2.** *Psoroma capense* and *P. esterhuyseniae*. A, ascus structures of *P. capense*. B, ascospore sketches of *P. capense* (from three collections). C, ascospore sketches of *P. esterhuyseniae*. D, ascospore micrographs of *P. capense*, from the holotype. E, ascospore micrographs of *P. esterhuyseniae*, from the holotype. Scales: A–C = 10 µm; D & E = 15 µm. In colour online.

**Chemistry.** Brownish melanins present, appearing similar to those of *P. hypnorum*. No TLC-detectable components found.

**Etymology.** Named after the South African botanist Elsie Elizabeth Esterhuysen (1912–2003), who collected three of the four samples of *Psoroma* known from South Africa.





**Fig. 3.** *Psoroma capense*. A, habitat of the holotype, in the crevice immediately to the right of the backpack. B, the holotype specimen prior to being collected. In colour online.

**Distribution and ecology.** Known only from the holotype collected from damp cliffs in Western Cape, South Africa.

## Results

### Molecular analysis and phylogeny

The phylogeny based on the concatenated multi-locus dataset of ITS, nucLSU, mtSSU and *Mcm7* indicates that *Psoroma capense* forms a well-supported monophyletic group (referred to here as the *Psoroma hypnorum* lineage) with *P. antarcticum* Hong & Elvebakk, *P. buehnerii* (Knight) Nyl., *P. fruticulosum*, *P. hypnorum* and *P. paleaceum* (Fr.) Timdal & Tønsberg (Fig. 5). The monophyletic group was consistently recovered by MP, ML and Bayesian methods, and also based on single-locus analyses (data not shown). The group was clearly separated from the *Psoroma tenue* lineage, including *P. cinnamomeum* Malme and *P. tenue*, and from the genera *Psorophorus*, *Xanthopsores* and *Pannaria*. The phylogenetic position of *P. capense* within the *Psoroma hypnorum* lineage was not clearly resolved and the relationship was poorly supported by bootstrap and posterior probability. *Psoroma capense* was grouped with *P. buehnerii*, *P. fruticulosum* and *P. paleaceum* in the ML tree based on the combined dataset (Fig. 5), but the relationship was not always recovered by MP, ML, and Bayesian methods with single-locus datasets. Branch lengths from the common ancestor of the group leading to terminal taxa were generally very short and statistical support for bifurcation was generally very low. Sequence similarity of the combined dataset between *P. capense* and the other species of the *Psoroma hypnorum* lineage ranged between 97 and 98%, which is close to similarity values among the other species of the group.

## Discussion

In recent phylograms, the genus *Psoroma* has either appeared as polyphyletic (Ekman *et al.* 2014) or paraphyletic (Park *et al.*

2018; the present study), with species of the *P. hypnorum* and *P. tenue* groups forming separate lineages. The possible recognition of these two lineages as separate genera has not been proposed due to insufficient taxon sampling. The *P. hypnorum* and *P. tenue* lineages both clearly have evolutionary histories featuring adaptations to cold climates, probably initiated in or near Antarctica, where glaciation occurred at c. 34 Ma (Pollard & DeConto 2020). Data from thermophilous *Psoroma* species from austral forests should be incorporated in future phylogenies, since they are potential members of older lineages needed in analyses to define the genus.

From its gross morphology alone, *Psoroma capense* resembles *P. tenue*. The latter species is distributed in Antarctica and subantarctic areas, but also in the Northern Hemisphere by a taxon considered to represent a separate variety (Henssen & Renner 1981; Jørgensen 2004b), a concept which has recently been challenged by Marthinsen *et al.* (2019). *Psoroma capense* and *P. tenue* share a related melanin colour and strongly sessile apothecia with crenate-lobate margins, but without the excipulum squamules typical of *P. hypnorum*. However, *P. capense* differs from *P. tenue* by ascending thallus squamules, short-ellipsoid to ovoid ascospores and a lack of TLC-detectable compounds.

*Psoroma esterhuyseniae* resembles *P. hypnorum*, although the characteristic regular tomentum on the apothecia of *P. hypnorum* (see Elvebakk & Tønsberg 2018) is lacking; replaced by some mycelium-like cover in only the least exposed apothecia. The few cephalodia seen in *P. esterhuyseniae* are regularly coarsely coralloid, whereas they are irregular in *P. hypnorum*. The ascospores of *P. esterhuyseniae* are very different from those of both *P. hypnorum* and *P. capense* (Fig. 2C), in being subglobose to short-ellipsoid, and very rarely ovoid. The apical perispore extensions present in both these species are absent in *P. esterhuyseniae*.

Phylogenetically, *P. capense* is very distinct from *P. tenue*, and is instead positioned within the *Psoroma hypnorum* lineage based



**Fig. 4.** *Psoroma esterhuyseniae* (holotype). Image by M. Karlstad. Scale bar = 5 mm. In colour online.

on a concatenated dataset of ITS, nucLSU, mtSSU and *Mcm7* sequences. This is very well supported by all phylogenetic methods and by all datasets examined in the present study. Within the *P. hypnorum* lineage, *P. capense* is in a poorly supported sister group position to a clade including *P. paleaceum*, *P. fruticulosum* and *P. buchananii*. None of these have any resemblance to *P. capense*. The former has characteristic long scales along apothecium margins (Elvebakk & Tønsberg 2018) and the two latter species were previously considered to form a subgroup within *Psoroma* by Henssen *et al.* (1983), a conclusion confirmed by our ongoing studies, as well as by the present phylogram. The Esterhuysen 19947 specimen (erroneously cited by Jørgensen (2003) as *Esterhuysen* 9419) was determined as *P. fruticulosum* because of its 'erect, isidioid lobules, which are partly flattened' (Jørgensen 2003), a character resembling *P. capense*. However, both *P. fruticulosum* and *P. buchananii* have conspicuous black pycnidia, prominent apothecia almost appearing stipitate, and spores deviating from those of the remaining *Psoroma* species.

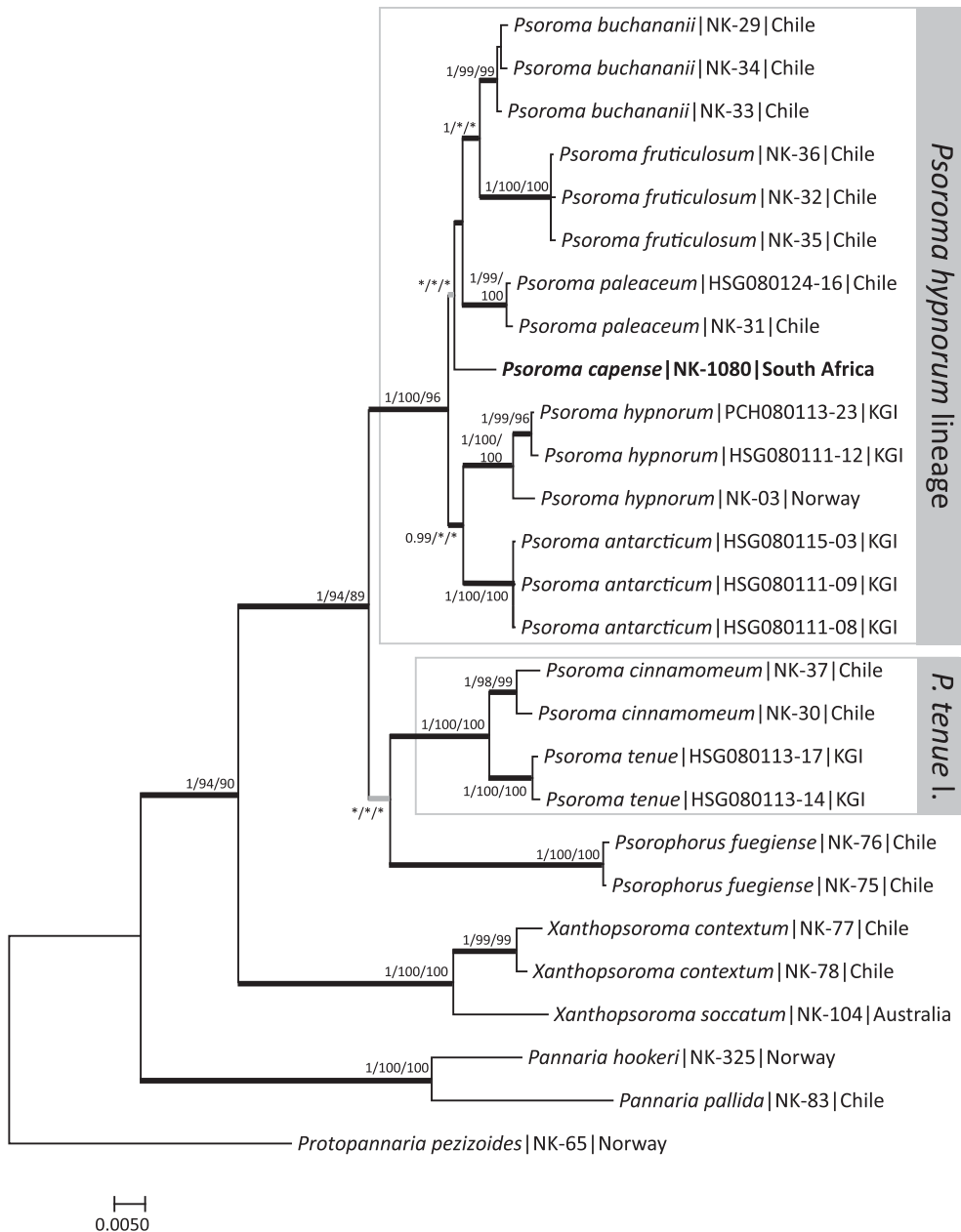
Subantarctic islands of the Indian Ocean are the *Psoroma* sites closest to the distribution area of *P. capense* and *P. esterhuyseniae*. These areas house endemic species such as *Psoroma absconditum* Øvstedal and *P. xanthorioides* (P. M. Jørg.) P. M. Jørg., and represent the major distribution area of *P. dichroum* (Hooker f. & Taylor) P. M. Jørg. (Jørgensen 2000, 2004c; Øvstedal & Gremmen 2008; Ekman *et al.* 2014). *Psoroma absconditum* is the most similar to the South African species; however, it is not well understood since it was not compared to other members of the *P. hypnorum* lineage, but instead to the very different species *P. asperellum* Nyl. (Øvstedal & Gremmen 2008). *Psoroma esterhuyseniae* has shorter spores and apothecia with squamulose margins compared to

*P. absconditum*, the latter named after its sunken apothecia, partly hidden by squamules.

Fresh material of *P. capense* appeared to have a yellowish brown melanin colour where the pigments were not strongly concentrated, which in combination with the chlorobiont cells gave the lichen a peculiar 'grass green' colour, even in a dried specimen two years after collection. A similar colour, contrasting with most other *Psoroma* species, has been observed in fresh specimens of the New Zealand species *P. cyanosorediatum* P. M. Jørg. (A. Elvebakk, unpublished data), which has very different, long and narrowly ellipsoid ascospores according to Jørgensen (2004a). Among the rather few *Psoroma* species described with short ascospores, *P. antarcticum* Elvebakk & S. G. Hong, *P. saccharatum* Scutari & Calvelo and *P. pannarioides* Henssen lack other similarities with the two new species from South Africa (Henssen 1983; Scutari & Calvelo 1995; Park *et al.* 2018).

All the specimens of the new species were collected from moist and S-facing sites at altitudes between 1300 and 1800 m at Sneeuwatpiek, Waaihoekpiek, and Hexrivierberge in the Western Cape Province, only 140–200 km NNE of Cape Town. A search for the species by TR in Spekrivierskloof, on 18 March and 17 November in 2018, revealed no additional findings. At higher altitudes in this area the habitats were drier, and the species might be truly rare here due to a scarcity of moist, suitable habitats. There is a clear need to search in the Western Cape for more populations of these species, which appear as Red List candidates, and to determine if more species are present in South African mountains. South Africa features extreme speciation in many groups of organisms, for example in the plant genus *Erica* which has evolved no less than 690 endemic species in the Cape Region during the last 15 million years (Pirie *et al.* 2016).





**Fig. 5.** Bayesian tree based on concatenated sequences of ITS, nuLSU, mtSSU and *Mcm7*. Black thick branches indicate those that were conserved in maximum likelihood (ML) and maximum parsimony (MP). Grey thick branches indicate those that were conserved in ML or MP. Asterisks indicate that branches were conserved but not supported by high bootstrap values. Bayesian posterior probabilities (PP  $\geq 0.90$ ) and bootstrap values in ML and MP trees ( $\geq 80\%$ ) are indicated above or below the nearest branches (PP/ML/MP). The geographical origins of the specimens are shown after the voucher number. KGI = King George Island, Antarctica; *P. tenue* l. = *P. tenue* lineage.


Chlorobiont acquisition is an important evolutionary feature in lichens but has not yet been studied in *Pannariaceae*. Previously, *Myrmecia* was the most commonly identified chlorobiont in *Psoroma*, but recent studies instead identify it as *Trebouxia* (Park *et al.* 2016; Muggia *et al.* 2018). In *Psoroma esterhuyseniae* the chlorobiont has cells with angular chloroplasts, whereas they are differently shaped and papillose in *P. capense*.

In conclusion, there are currently no candidates closely related to *P. capense* and *P. esterhuyseniae*, from phylogenetic analysis or by comparison of taxonomic characters. In this context, it should be added that a high proportion of specimens collected throughout the distribution area of *Psoroma* represent misunderstood or undescribed species (A. Elvebakk & S. G. Hong, unpublished data). For these reasons, it is difficult to hypothesize on the migration history of the ancestors of these two species into Africa, where they are the only known members of the genus *Psoroma*.

The bipolar element within the genus is most easily explained by migrations along American mountain chains during the Pleistocene; several *Psoroma* species occur in the Central Andes (Jørgensen & Palice 2010) where rapid diversification has taken place during this period in *Lobariaceae* lichens, as shown by Widhalm *et al.* (2019). The dramatic cooling during the Pleistocene probably represented a scenario of expansion of the cold-adapted groups within *Psoroma*, and our hypothesis is that *P. esterhuyseniae* and *P. capense* obtained their isolated and shared geographical positions as a result of one or two long-distance dispersal events during this period.

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**Supplementary Material.** To view supplementary material for this article, please visit <https://doi.org/10.1017/S0024282920000377>.

## References

- Culberson CF (1972) Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatography method. *Journal of Chromatography* **72**, 113–125.
- Doidge EM (1950) The South African fungi and lichens to the end of 1945. *Bothalia* **5**, 1–1094.
- Ekman S, Wedin M, Lindblom L and Jørgensen PM (2014) Extended phylogeny and a revised generic classification of the Pannariaceae (Peltigerales, Ascomycotina). *Lichenologist* **46**, 627–656.
- Elvebakk A and Elix JA (2017) A trio of endemic New Zealand lichens: *Pannaria aotearoana* and *P. gallowayi*, new species with a new chemosyndrome, and their relationship with *P. xanthomelana*. *Nova Hedwigia* **105**, 167–184.
- Elvebakk A and Tønsberg T (2018) *Psoroma spinuliferum* (Pannariaceae), a new corticolous lichen species from Alaska with two different types of cephalodia. *Bryologist* **121**, 166–173.
- Elvebakk A, Robertsen EH, Park CH and Hong SG (2010) *Psorophorus* and *Xanthopsoroma*, two new genera for yellow-green, corticolous and squamulose lichen species, previously in *Psoroma*. *Lichenologist* **42**, 563–585.
- Elvebakk A, Hong SG, Park CH, Robertsen EH and Jørgensen PM (2016) *Gibbosporina*, a new genus for foliose and tripartite, Palaeotropic Pannariaceae species previously assigned to *Psoroma*. *Lichenologist* **48**, 13–52.
- Fryday AM, Elvebakk A, Anderson FL and Gagnon JY (2019) *Psoroma nivale* (Pannariaceae, lichenized Ascomycota) a new species with dark, elongate squamules and bacilliform ascospores from arctic Québec, Canada. *Lichenologist* **51**, 419–429.
- Galloway DJ (2007) *Flora of New Zealand Lichens. Revised Second Edition Including Lichen-forming and Lichenicolous Fungi*. Lincoln, New Zealand: Manaaki Whenua Press.
- Henssen A (1983) Studies in the lichen genus *Psoroma* 3. *Psoroma pannarioides* and *Psoroma internectens*. *Mycotaxon* **18**, 97–111.
- Henssen A and Renner B (1981) Studies in the lichen genus *Psoroma* I. *Psoroma tenue* and *Psoroma cinnamomeum*. *Mycotaxon* **13**, 433–449.
- Henssen A, Renner B and Marton K (1983) Studies in the lichen genus *Psoroma* 2. *Psoroma fruticulosum* and *Psoroma rubromarginatum*. *Mycotaxon* **18**, 29–48.
- Jørgensen PM (2000) Studies in the lichen family Pannariaceae IX. A revision of *Pannaria* subg. *Chryopannaria*. *Nova Hedwigia* **71**, 405–414.
- Jørgensen PM (2003) Notes on African Pannariaceae (lichenized ascomycetes). *Lichenologist* **35**, 11–20.
- Jørgensen PM (2004a) Further contributions to the Pannariaceae (lichenized Ascomycetes) of the Southern Hemisphere. *Bibliotheca Lichenologica* **88**, 229–253.
- Jørgensen PM (2004b) *Psoroma tenue* var. *boreale*, an overlooked, widespread, arctic-alpine lichen. *Graphis Scripta* **15**, 60–64.
- Jørgensen PM (2004c) The first yellow *Pannaria* species (lichenized ascomycetes). *Nova Hedwigia* **79**, 537–539.
- Jørgensen PM and Galloway DJ (1992) Pannariaceae. *Flora of Australia* **54**, 246–293.
- Jørgensen PM and Palice Z (2010) Additions to the lichen family Pannariaceae in Ecuador. *Nordic Journal of Botany* **28**, 623–628.
- Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.
- Lanave C, Preparata G, Saccone C and Serio G (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20**, 86–93.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. (2007) ClustalW and ClustalX version 2.0. *Bioinformatics* **23**, 2947–2948.
- Marthinsen G, Rui S and Timdal E (2019) OLICH: a reference library of DNA barcodes for Nordic lichens. *Biodiversity Data Journal* **7**, e36252.
- Muggia L, Leavitt S and Barreno E (2018) The hidden diversity of lichenized *Trebouxioiophyceae*. *Phycologia* **57**, 503–524.
- Nordin A (1997) Ascospore structures in *Physciaceae*: an ultrastructural study. *Symbolae Botanicae Upsalienses* **32**(1), 195–208.
- Nylander W (1863) *Synopsis Methodica Lichenum Omnium Hucusque Cognitorum Praemissa Introductione Linguae Gallica Tractata*. Fasc. II. Paris: Martinet.
- Orange A, James PW and White FJ (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.
- Øvstedal DO and Gremmen NJM (2008) Additions and corrections to the lichens of Heard Island. *Lichenologist* **40**, 233–242.
- Park CH, Kim EH, Noh H-J, Elvebakk A and Hong SG (2016) Diversity and biogeography of symbiotic microalgae of the lichen genus *Psoroma*. Abstracts of the 8th International Association for Lichenology Symposium, 1–5 August 2016, Helsinki, Finland, p. 54.
- Park CH, Hong SG and Elvebakk A (2018) *Psoroma antarcticum*, a new lichen species from Antarctica and neighbouring areas. *Polar Biology* **41**, 1083–1090.
- Passo A, Stenroos S and Calvelo S (2008) *Joergensenia*, a new genus to accommodate *Psoroma cephalodinum* (lichenized Ascomycota). *Mycological Research* **112**, 1465–1474.
- Pirie MD, Olivier EGH, Mugrabi de Kuppler A, Gehrke B, Le Maitre NC, Kandziara M and Bellstedt DU (2016) The biodiversity hotspot as evolutionary hot-bed: spectacular radiation of *Erica* in the Cape Floristic Region. *Evolutionary Biology* **16**, 190.
- Pollard D and DeConto RM (2020) Continuous simulation over the last 40 million years with a coupled Antarctic ice sheet-sediment model. *Palaeogeography, Palaeoclimatology, Palaeoecology* **537**, 109374.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.
- Schmitt I, Crespo A, Divakar PK, Fankhauser JD, Herman-Sackett E, Kalb K, Nelsen MP, Nelson NA, Rivas-Plata E, Shimp AD, et al. (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* **23**, 35–40.
- Scutari NC and Calvelo S (1995) A new species of *Psoroma* (Pannariaceae, lichenized Ascomycotina) from Tierra del Fuego, Argentina. *Annales Botanici Fennici* **32**, 55–61.
- van der Byl PA (1931) In Lys van Korsmosse (Lichenes) versamel in die Unie van Suid-Afrika en in Rhodesie gedurende die tydperk 1917–1929 [List of lichens collected in the Union of South Africa and in Rhodesia from 1917–1929]. *Annale van die Universiteit van Stellenbosch* **9A**(3), 1–17.
- Widhelm TJ, Grewe F, Huang JP, Mercado-Díaz JA, Gofinet B, Lücking R, Moncada B, Mason-Gamer R and Lumbsch T (2019) Multiple historical processes obscure phylogenetic relationships in a taxonomically difficult group (Lobariaceae, Ascomycota). *Scientific Reports* **9**, 8968.