CAMBRIDGE
UNIVERSITY PRESS

Standard Paper

Gibbosporina cyanea (*Pannariaceae*), a new bipartite cyanolichen from Sri Lanka with comparisons to related palaeotropical cyanogenera

Arve Elvebakk [ORCID]

Arctic University Museum of Norway, UiT – the Arctic University of Norway, PO Box 6050 Langnes, N-9037 Tromsø, Norway

Abstract

Gibbosporina cyanea is described here as new to science, based on its holotype collected in Sri Lanka in the 1860s. The species is a bipartite cyanolichen in contrast to all other known *Gibbosporina* species, which are tripartites. The species appears to have evolved through cephalodia emancipation followed by divergence. Phyllidia indicate a cephalodiate evolutionary origin with a continued function similar to vegetative propagules. The species is considered to represent a relatively young speciation within an evolutionary old genus and *G. sphaerospora* is discussed as a possible candidate for being its closest known relative based on spore morphology. As the species appears to be so different from the remaining *Gibbosporina* species, it is compared here to representatives of other related palaeotropical cyanogenera, such as *Leightoniella*, *Lepidocollema*, *Pannaria* and *Physma*. Perispore structure is shown to be a useful character in separating these genera, including distinct clades of the non-monophyletic genera *Physma* and *Lepidocollema*. *Leightoniella zeylanensis* is reported here as new to Japan, and it is concluded that *Pannaria lurida* needs to be restudied in India and Sri Lanka.

Key words: biodiversity, evolution, Palaeotropics, photobionts, taxonomy, tripartite lichens, vegetative propagation

(Accepted 7 April 2021)

Introduction

The genus *Gibbosporina* was described by Elvebakk *et al.* (2016), comprising 13 species distributed through palaeotropical areas from Réunion, Mauritius and Sri Lanka eastwards to islands in the western part of the Pacific. All species look somewhat similar and are rather large foliose lichens dominated by their green major photobiont, but with conspicuous cephalodia. All species are normally abundantly fertile without vegetative dispersal units, except for brittle cyanobiont lobes in several species. The only exception was the sterile *G. phyllidiata* Elvebakk, which was recently transferred to *Pannaria* and renamed *P. melanesica* Elvebakk (Elvebakk & Sipman 2020). Although resembling tripartite austral *Pannaria* species, *Gibbosporina* clearly belongs in a clade with genera such as *Physma*, *Leightoniella* and *Lepidocollema*, as shown in phylograms by Elvebakk *et al.* (2016) and Weerakoon *et al.* (2018), and also by Magain & Sérusiaux (2014), prior to the description of the new genus. Elvebakk *et al.* (2016) also showed good molecular support for seven of the new species.

Concerning Sri Lanka, Elvebakk *et al.* (2016) reported on two studied *Gibbosporina* collections, both originally deposited as *Thwaites* 52 in S and determined as *G. mascarena* Elvebakk *et al.*, although they were found to differ in some characters.

The conclusion was that 'future studies might well discover more than one taxon from this island'. It was therefore exciting when another Thwaites collection of a *Gibbosporina* from Sri Lanka deposited in BM was discovered. At first glance, it appeared similar to a classic *Gibbosporina* with small-foliose cephalodia, but it soon proved to be a remarkable, bipartite cyanolichen, with cephalodia-like structures as phyllidia-like ramifications of the thallus. The aim of the present paper is to describe this collection as a new species. The new species is more likely to be confused with other cyanogenera within *Pannariaceae* than with other species of *Gibbosporina*. To provide clarification, the species is therefore compared here to related cyanolichen genera from the Palaeotropics based on perispore structure, a diagnostic but previously overlooked character.

Material and Methods

Herbarium material used for this study is housed in BG, BM, G, PC, TNS and TROM. Perispore structures were studied in water mounts and restricted to mature spores liberated from asci, and iodine reactions were tested in microscope sections by adding IKI to mounts pretreated with KOH. Ascospore morphology was studied in detail by drawing detailed sketches of *c.* 150 ascospores with a focus on perispore structures, and copies of the sketches were deposited with the herbarium specimens. The illustrations presented here aim to depict the variations in shape and size within and between the species studied, and, in addition to the new species, specimens were selected to represent

CrossMark

neighbouring genera of the genus *Gibbosporina*, as well as major clades of genera which have recently been shown not to be monophyletic. Thin-layer chromatography of acetone extracts follows standard procedures (Orange *et al.* 2001), and nomenclature of ascospore structures follows Nordin (1997).

Additional collections examined. Gibbosporina bifrons Elvebakk et al. **Malaysia:** *Sarawak*: Gunong Mulu National Park, 4th Division, Baram District, Long Pala, limestone hill, *c.* 2 km E of Base Camp, S side of Sungei Melinau Paku, 70–300 m alt., 1978, *G. Argent, B. Coppins* 5440, *C. Jermy* & *P. Chai* (BG L-32437).

Gibbosporina boninensis (Kurok.) Elvebakk & P. M. Jørg. **Japan:** *Bonin Islands*: US North Pacific exp. under command of Ringold and Rodgers 1893–96, *C. Wright* s. n. (PC0012753).

Leightoniella zeylanensis (Leight.) Henssen. **Sri Lanka:** *G. H. K. Thwaites* 1876 (G 00292260).—**Japan:** *Bonin Islands*: Chichijima Island, Takeda pastureland, 1930, *F. Fujikawa* s. n. (TNS).

Lepidocollema brisbanense (C. Knight) P. M. Jørg. **New Caledonia:** S part, Village de Prony, near the beach N of the village, 22°19′09″S, 166°49′37″E, on large trunk of dying mango tree, 3 m, 2005, *A. Elvebakk* 05:559 (TROM).

Lepidocollema marianum (Fr.) P. M. Jørg. **New Caledonia:** SE part, Touaourou, near the church, 22°11′10″S, 166°58′30″E, on *Araucaria columnaris*, 10 m, 2005, *A. Elvebakk* 05:618 (TROM).

Lepidocollema polyphyllinum (P. M. Jørg.) P. M. Jørg. **Solomon Islands:** *Guadalcanal Island*: central part, Mt Popomansiu, on ridge SE of Sutakiki River, montane rainforest, 5800–6600 ft, 1965, *D. Jackson Hill* 9485 (BM000731913).

Lepidocollema stylophorum (Vain.) P. M. Jørg. **Réunion:** W of St Philippe, below Mare Longue Nature Reserve, 1.5 km up along the road towards the Reserve, 21°21′36″S, 55°44′28″E, 90 m, on trunks along the road, 2011, *A Elvebakk* 11:093 (TROM).

Pannaria complanata P. M. Jørg. **India:** Nilgiri Mts, Ootacamund, Dodabetta Peak, in eucalypt forest, *c.* 3000 m, 1972, *K. P. Singh* 72126 (BG—isotype).

Pannaria lurida (Mont.) Nyl. **USA:** *Hawaii* ('Sandwich Isl.'): without further data, Gaudichaud, Voy. 'Bonite' (BM—lectotype!); Kauai, mountain forest at Waimea Canyon, 21°57′60″N, 159°39′45″W, 900 m, epiphytic, 1988, *A. Elvebakk* 88:016 (TROM).—**Sri Lanka:** 'Ceylon, Central Highlands', *G. H. K. Thwaites*, undated, as C.L.9 (BM013392391b).

Physma cf. *boryanum* (Pers.) A. Massal. **Réunion:** W of St Philippe, below Mare Longue Nature Reserve, 2.5 km up along the road towards the Reserve, 21°20′30″S, 55°44′27″E, 120 m, on trunks along the road, 2011, *A. Elvebakk* 11:096, 11:098 (TROM, both previously determined as *P. byrsaeum* by A. Elvebakk).

Physma byrsaeum (Afzel. ex Ach.) Tuck. **Réunion:** W of St Philippe, below Mare Longue Nature Reserve, 2.5 km up along the road towards the Reserve, 21°20′30″S, 55°44′27″E, 120 m, on trunks along the road, 2011, *A. Elvebakk* 11:095, 11:097, 11:099 (TROM, previously determined as *P. radians* Vain. by A. Elvebakk); Takamaka, 2009, *F. Schumm* 15272 & *J.-P. Frahm* (TROM).—**Seychelles:** *Mahé*: Morne Seychellois National Park, 2008, *F. Schumm* 14658 & *J.-P. Frahm* (TROM, previously determined as *P. radians* Vain. by A. Elvebakk); Morne Blanc, 2008, *F. Schumm* 14644 & *J.-P. Frahm* (TROM).—**New Caledonia:** 10 km NE of Nouméa, Forêt de Koghi, 1.5 km above the auberge, near Belvedère, 22°10′20″S, 166°30′28″E, 590 m, on the trunk of an unidentified small Araliaceae tree in an open savannah community, 2005, *A.*

Elvebakk 05:694 (TROM, previously determined as *Physma* sp. by A. Elvebakk).

Physma sp. 1. **French Polynesia:** *Tahiti*: Papete, park at Gaugi Museum and Gardens, Papeaivi, 10 m, i 1996, *M. P. Jones* (BG L-32410; previously as *P. byrsaeum* (Afzel. ex Ach.) Tuck. by P. M. Jørgensen).

Results

The new species

Gibbosporina cyanea Elvebakk sp. nov.

MycoBank No.: MB 839600

Differs from all other known *Gibbosporina* species by being a bipartite, phyllidiate cyanolichen, and in addition from *G. sphaerospora* Elvebakk & Hong by having slightly longer ascospores and nodulose pycnidia.

Type: Sri Lanka, 'Ceylon, Central Highlands', *G. H. K. Thwaites*, undated, as C.L.9 (BM013392391a—holotype).

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

Thallus a cyanomorph 3–7 cm diam., foliose, corticolous; *lobes* irregularly and shallowly divided, 150–200 μm thick, *c.* 2 mm wide, lobes discrete only in peripheral parts, soon becoming coalescent. *Upper surface* glabrous and matt, with depressions and ridges when dry, probably gelatinous when moist; old herbarium specimens brown, fresh specimens probably greyish. *Phyllidia* 2–3 mm wide, formed along thallus margins, convex, decumbent and geotropically arranged, 0.2–0.3 mm thick, often with pale rhizomorphs on the lower side, of a bluish grey colour contrasting with the brown main thallus. *Upper cortex* 10–15 μm thick in the lobules, 30–40 μm in the main thallus, plectenchymatic, lumina up to 5 × 10 μm, globose or irregularly subellipsoid and then arranged perpendicularly to the surface, surface weakly sclerenchymatic; *lower cortex* absent. *Cyanobacterial layer c.* 100 μm thick; of *Nostoc* cells, 3–7 × 2–5 μm, globose to irregular and short-ellipsoid, bluish violet, forming distinct chains, in the main lobes in long chains without glomeruli, elsewhere coiled within 20–80 μm large glomeruli or goniocysts. *Medulla* of loosely interwoven hyphae, 30–50 μm thick, in lowermost part with brown-pigmented rhizines, fibrous, black, 0.5–1.5 mm long, simple or in bundles. *Lower cortex* absent.

Apothecia subsessile, laminal, 1–2 mm diam., disc orange-brown; *thalline excipulum c.* 0.2 mm, indistinctly crenate-striate; *epithecium* pale brown, 10–20 μm thick, IKI–; *hymenium* colourless, but strongly IKI+ blue, 80–120 μm thick; *hypothecium* pale brown, IKI–, 20–30 μm thick; *cyanobacterial layer* extending below the hypothecium; *paraphyses* simple to sparingly branched, with slightly swollen apices, septate and much adglutinated; *asci* clavate, 8-spored, with pronounced, internal, apical, IKI+ blue, amyloid tube structures, 90–110 × 15–20 μm. *Proper ascospores* colourless, simple, subglobose to short-ellipsoid, 12–15 × 9–12 μm; *perispores* 15–20 × 13–15 μm, walls mostly 1–1.5 μm thick with scattered small gibbae when mounted in water.

Pycnidia scattered, marginal, brown and nodulose, 0.1 × 0.1 mm, surface finely papillose; spermatia not seen.
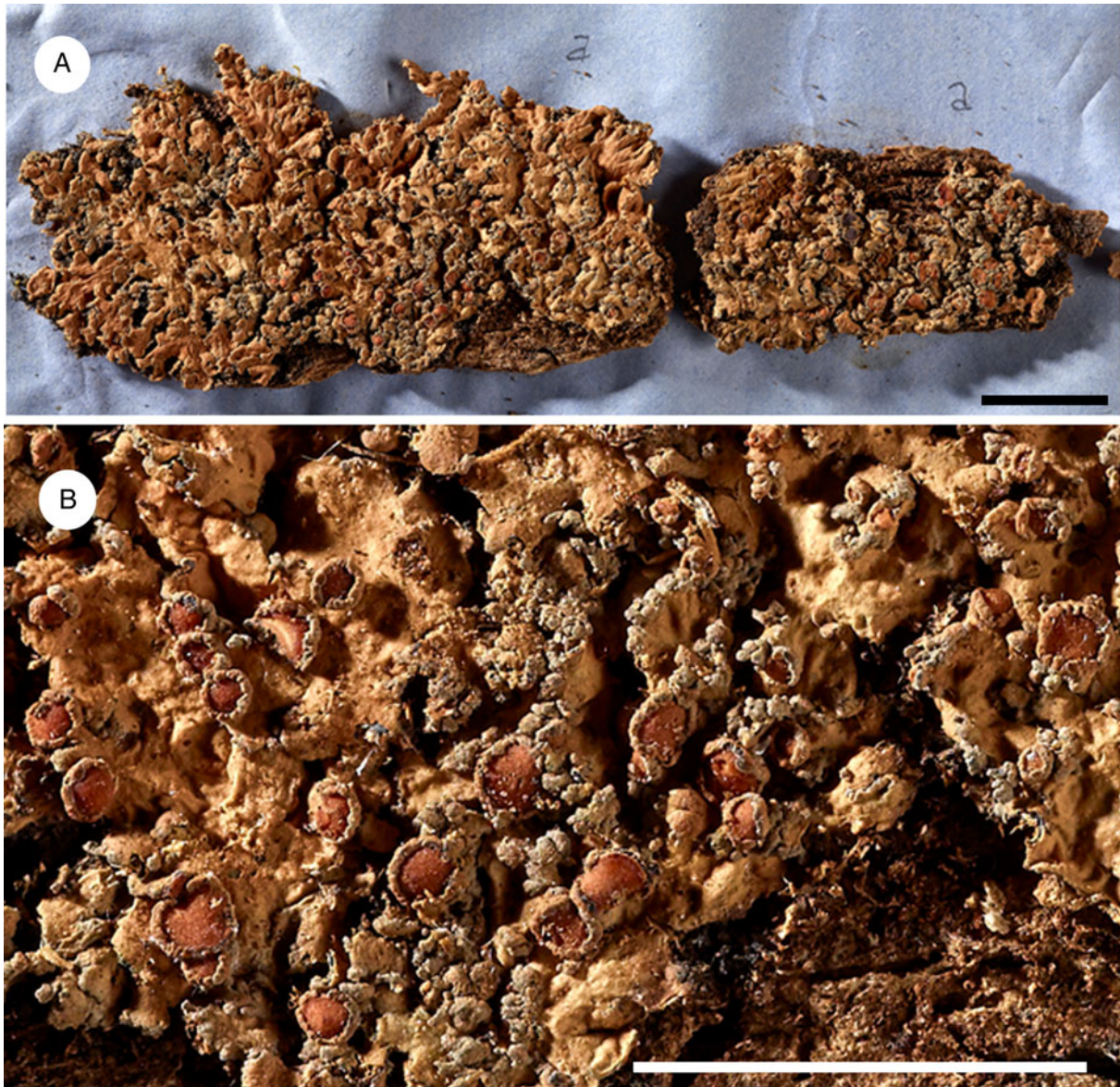
Chemistry. No TLC-detectable compounds found.

Fig. 1. *Gibbosporina cyanea* (holotype). A, the entire holotype. B, details of the phyllidia. Scales = 5 mm. In colour online.

Etymology. The species is named after its deviating photobiont structure.

Distribution and ecology. Known only from the type collection.

Perispore structures in members of neighbouring Pannariaceae *genera*

Leightoniella zeylanensis. Proper spores are regularly ellipsoid and 10–13 × 5–7 μm in size, and perispores are 13–18 × 6.5–8 μm. The latter are thin and even and 0.5–1 μm thick in central parts, but swell gradually into *c.* 2 μm thick, even, nodulose apical extensions, maintaining a roughly ellipsoid outline (Fig. 4A). The spores of the collections from Sri Lanka and Japan have the same pattern, and the species is reported here as new to Japan.

Lepidocollema polyphyllinum. Both the proper spores (15–17 × 7–8 μm) and the perispores (16.5–19.5 × 8–9.5 μm) are regularly ellipsoid, and the perispores are smooth and evenly thick, 0.5–1 μm (Fig. 4B).

Lepidocollema stylophorum. Proper spores are regularly ellipsoid (13–17 × 8–10 μm), perispores 16–21 × 10–12.5 μm with gradual transitions to weak apical extensions (Fig. 4C). The ascospores of *L. brisbanense* are slightly smaller, and with weak apical extensions seen only in some of the spores, but very distinct in immature spores. *Lepidocollema marianum* has distinct perispore extensions but the spores are shorter and slightly wider, 12–15 × 8.5–10.5 μm.

Pannaria complanata. Proper spores are ellipsoid, 13–16 × 7–8.5 μm, perispores 17–22 × 9–12 μm (Fig. 4E). Mature perispores have acuminate apical extensions, in semi-mature spores they are more diffusely triangular. Perispores have uneven sizes of verrucae, some up to 2.5 μm tall and appearing almost as gibbose structures.
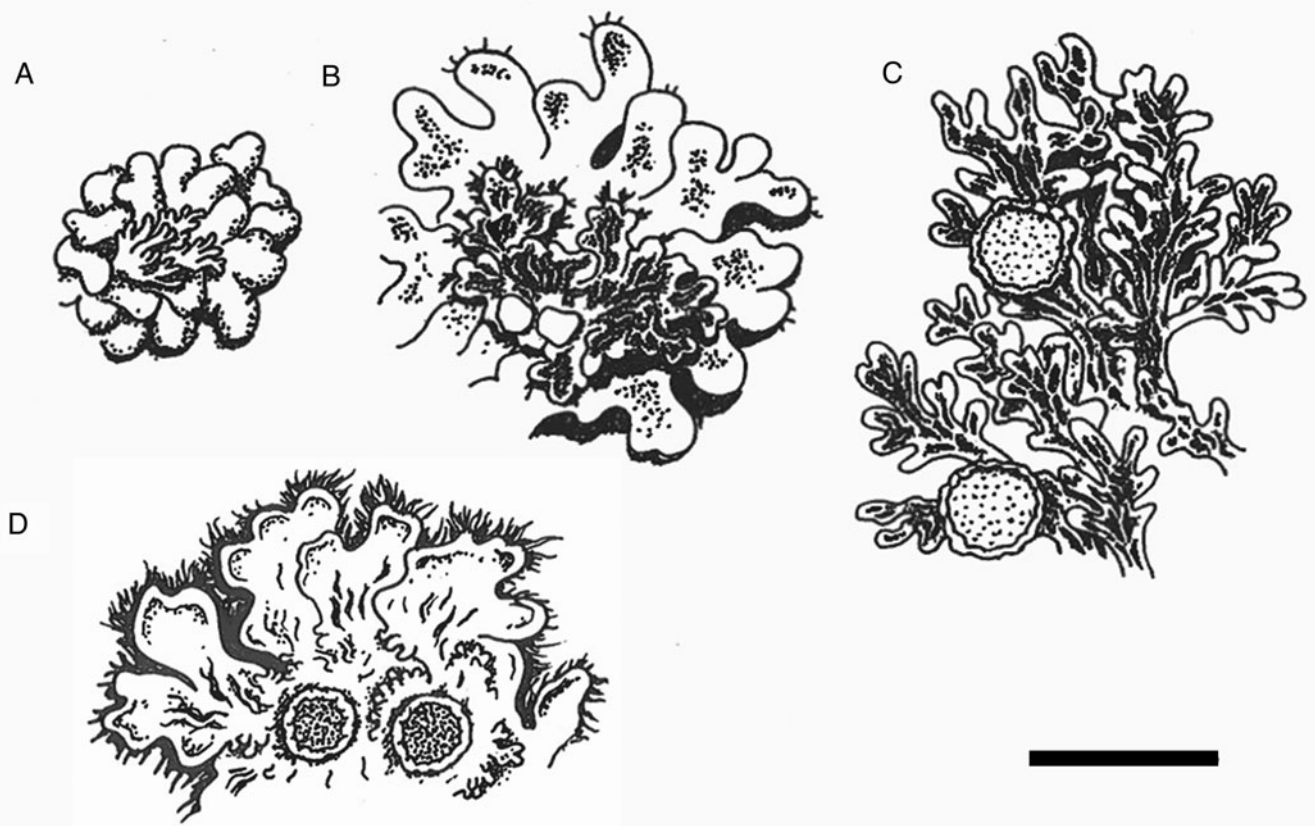
Fig. 2. Sketches of parts of thalli. A, cephalodia on chlorobiont thallus of *Gibbosporina boninensis* (*Wright*, PC 0012753). B, cephalodia (with epiphytic chlorobiont squamules) on chlorobiont thallus of *G. bifrons* (*Coppins* 5440 et al., BG L-32437). C, cyanothallus of *Leightoniella zeylanensis* (*Fujikawa* s. n., TNS). D, cyanothallus of *G. cyanea* (holotype). Scale = 1 cm.

Pannaria lurida. *Pannaria lurida* is considered a pantropical species, except for ssp. *quercicola* P. M. Jørg. and ssp. *russellii* (Tuck.) P. M. Jørg. which occur further northwards, the latter extending into both Canada and easternmost Russia (Jørgensen 2000; Ezhkin & Jørgensen 2018). The lectotype of *P. lurida* ssp. *lurida* from Hawaii has been studied but is sterile. Other samples from Hawaii have also been studied and correspond in general morphology to the sample from Sri Lanka admixed with the holotype of *Gibbosporina cyanea* and illustrated in Fig. 4D. Its spores are ellipsoid to ovoid, proper spores 15–19 × 7.5–9 µm, perispores 18–23 × 8.5–10 µm. Perispores have regularly small verrucae and small, nodulose apical extensions. In half of the cases the apical extensions are replaced by normal verrucae. The Sri Lankan specimen deviates from normal *P. lurida* by having a thick thallus with white margins and with apothecia that are coronate from protruding basal rhizohyphae.

Physma cf. boryanum. One sample from Réunion previously included in the phylogeny by Elvebakk *et al.* (2016) as '*Physma byrsaeum*' has now been redetermined together with one additional sample from the same locality. The ascospores are illustrated in Fig. 5A. The proper spores are 13–17 × 8–9 µm and regularly ellipsoid. Perispores are 28–42 × 10–12 µm, almost always with two 10–15 µm long, apiculate apical extensions, tapering gradually from an 8–12 µm wide base, equally as broad as the proper spores. In central parts, perispores are only 0.5–2 µm thick and very distinctly gibbose.

Physma sp. 1 Jones *s. n. (Tahiti).* Figure 5B illustrates the spores of this unidentified species. Proper spores are 12–17 × 8–10 µm long and regularly ellipsoid. Perispores are 17–23 × 10.5–12.5 µm long, always with swollen and obtuse apical extensions and 0.5–2 µm thick, gibbose central parts.

Physma byrsaeum. Figure 5C is based on 100 spore sketches of six specimens collected from Réunion and the Seychelles. Proper spores are 10–16 × 8–12 µm long, regularly subglobose to short-ellipsoid. Perispores are 21–35 × 13–20 µm long, often 1–2.5 µm thick and mostly even, with short acicular, often curved apical extensions, mostly 2–5 µm long, occasionally 10 µm long. Among the perispores studied from the three Mare Longue collections from Réunion, 80% were of this type, whereas only 40% of the three Schumm & Frahm collections from Réunion and the Seychelles were of this category, some also with uneven central parts of the perispores. The remaining perispores were thick, irregularly gibbose, occasionally up to 6 µm thick, without any structures developed in regular apical positions. Figure 5D shows spores from the collection *Elvebakk* 05:694 from New Caledonia. The frequency of acicular perispores is lower and proper spores are shorter, but there is overlap with the spores studied from islands in the Indian Ocean.

Discussion

Gibbosporina cyanea is remarkable in being a cyanolichen within a genus so far known only to include tripartite species dominated
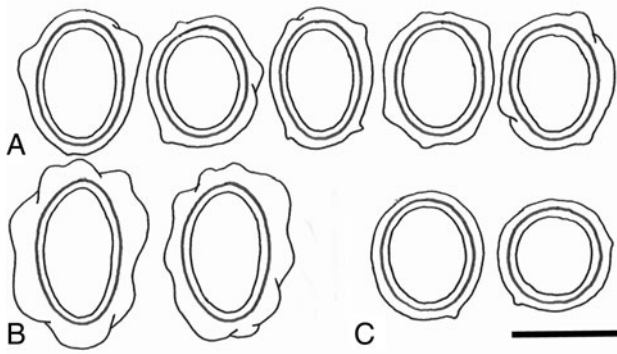
Fig. 3. Ascospores of *Gibbosporina* species (B & C based on spore sketches used by Elvebakk *et al*. (2016)). A, *G. cyanea*. B, *G. mascarena*. C, *G. sphaerospora*. Scale = 10 μm.
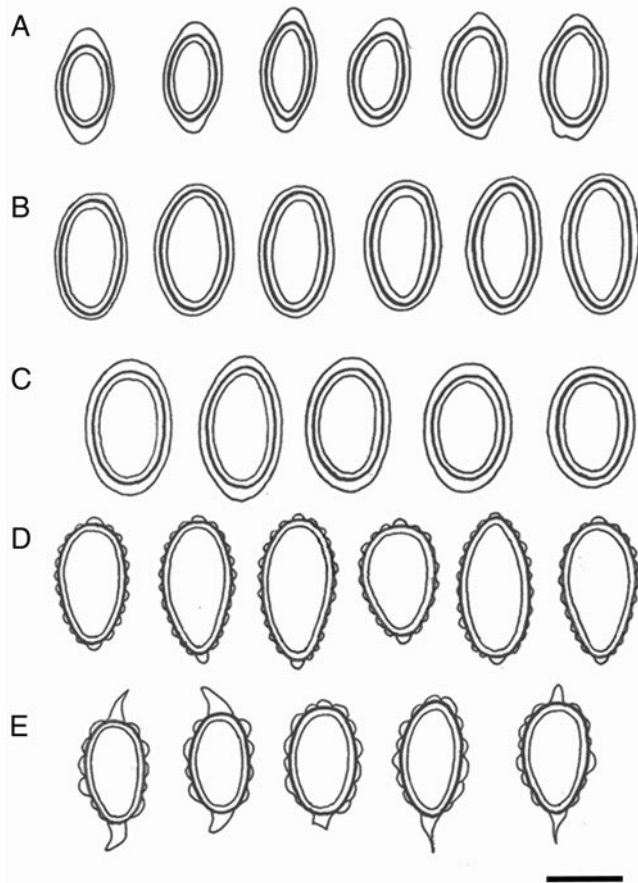

Fig. 4. Ascospores of *Leightoniella*, *Lepidocollema* and *Pannaria*. A, *Leightoniella zeylanensis*, Sri Lanka (*Thwaites* 1876, G) and Japan (*Fujikawa* s. n., TNS). B, *Lepidocollema polyphyllinum*, Solomon Islands (*Jackson Hill* 9485, BM). C, *L. stylophorum*, Réunion (*Elvebakk* 11:093, TROM). D, *Pannaria lurida*, Sri Lanka (*Thwaites* C.L.9, BM013392391b). E, *P. complanata*, India (*Singh* 72126, BG). Scale = 10 μm.

Fig. 5. Ascospores of *Physma* s. lat. species. A, *P*. cf. *boryanum*, Réunion (*Elvebakk* 11:096). B, *Physma* sp., Tahiti (*Jones* s. n., BG L-32410). C, *P. byrsaeum*, Réunion and the Seychelles (based on several cited specimens). D, *P. byrsaeum*, New Caledonia (*Elvebakk* 05:694). Scale = 10 μm.

by the chlorobiont. Its thick but still heteromerous medulla of chain-forming *Nostoc* explains why its surface is wrinkled when dry, changing to gelatinous when moist, similar to several species of Pannariaceae and Collemataceae members in general. This might explain why the specimen was determined and published
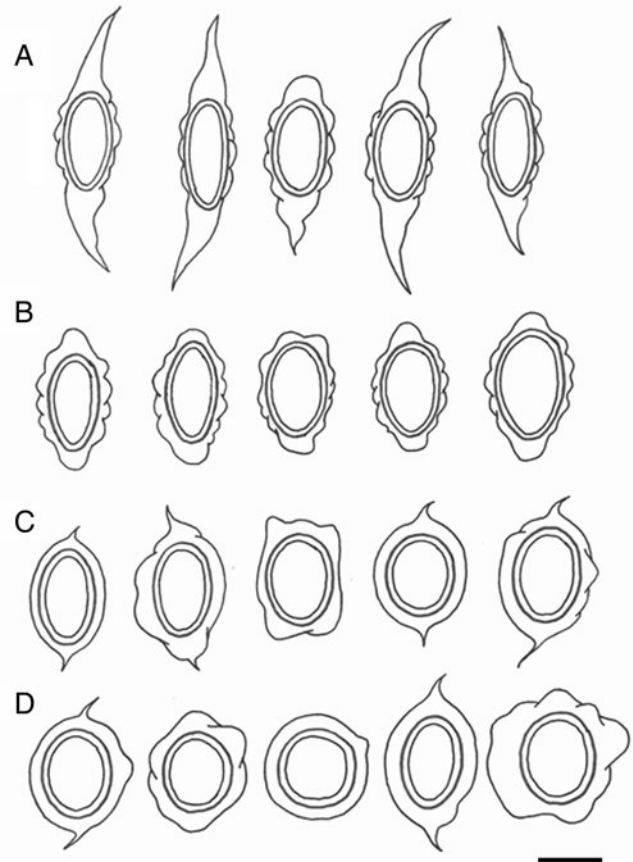
as *Leptogium cyanescens* Nyl. by Leighton (1871). The marginal phyllidia look quite similar to the cephalodia of other *Gibbosporina* species in being pale and bluish grey-brown, contrasting the uniform, brown colour of the main thallus (Figs 1B & 2D). However, this is because the upper cortex of the phyllidia of *G. cyanea* is much thinner than in the main thallus, making the phyllidia brittle, thus probably enhancing their functioning as vegetative propagules. Still, they are ramifications of the major thallus and not simply partially attached to it, like cephalodia in tripartite *Gibbosporina* species.

Gibbosporina cyanea is compared with other *Gibbosporina* species in Table 1, with thalli of *G. boninensis*, *G. bifrons* and *Leightoniella zeylanensis* in Fig. 2, and with ascospores of *G. mascarena* and *G. sphaerospora* in Fig. 3. The latter illustrations originate from the spore sketches used by Elvebakk *et al*. (2016). The selected *Gibbosporina* species in Table 1 all have mini-foliose to foliose cephalodia, and in three of the species the cephalodia are frequently rhizinate, indicating the potential for a free-living state. The spores are most similar to those of *G. sphaerospora*, a species which is widely distributed in SE Asia and NE Australia and which has broader, black pycnidia. The overall similarity appears to be closer to *G. sphaerospora* than to *G. mascarena*, the latter known from Sri Lanka, although this observation can only be verified by future molecular studies. So far, no *Gibbosporina* specimens appear to have been collected in Sri Lanka during the last 150 years.

Table 1. A comparison of characters of the bipartite *Gibbosporina cyanea* vs selected tripartite *Gibbosporina* species. Character states of the tripartite species from Elvebakk *et al.* (2016).

Species	Morphology of cephalodia or thallus fringes	Lower side of cephalodia or thallus fringes	Upper lobe surfaces	Ascospore outlines	Perispore structures
G. cyanea	small-foliose	rhizinate	matt	short-ellipsoid	thin and even
G. bifrons	foliose	rhizinate	glossy	short-ellipsoid	thick and gibbose
G. didyma	foliose	erhizinate	matt	short-ellipsoid	very thick and even
G. mascarena	mini-foliose	erhizinate	glossy	ellipsoid	thick and gibbose
G. sphaerospora	mini-foliose	rhizinate	matt	subglobose	thin and even

Magain & Sérusiaux (2014) showed that photobiont switches and cephalodia emancipation followed by divergence are evolutionary drivers in *Pannariaceae*, and in the *Pannaria* group of genera a tripartite morphology was postulated to be the most likely ancestral state. As understood today, *Pannaria* consists of 30% tripartite species, with the remaining ones being cyanolichens. In most cases the morphology of the dominating morphs within these two groups is similar. Furthermore, *Nostoc* phylotypes in cyanospecies of *Pannaria*, are different to those in cephalodia of tripartites (Magain & Sérusiaux 2014). This could suggest that photobiont switch rather than cephalodium emancipation was a more likely driver of this evolutionary development.

In the case of *Pannariaceae*, Magain & Sérusiaux (2014) proposed that only photobiont switch occurred between the two major phototypes of cyanolichen. *Fuscopannaria viridescens* P. M. Jørg. & Zhurb., the only tripartite species in a large cyanolichen genus (Jørgensen & Zhurbenko 2002; Nelson & Wheeler 2013), could be postulated to have been derived from photobiont switch, as also proposed to have taken place in the genus *Peltigera* (Miadlikowska *et al.* 2006). Another remarkable example is from *Gibbosporina didyma* Elvebakk *et al.*, where cephalodia form small chlorobiont thalli which produce apothecia, almost like the adverse situation of a cephalodium on a green thallus (Elvebakk *et al.* 2016). This transition from cyanomorph to chloromorph is hard to explain, except that the cephalodium apparently cannot produce apothecia itself.

In *Gibbosporina*, cephalodia emancipation appears more likely. Magain & Sérusiaux (2014) analyzed one mycobiont sample of *G. mascarena*, then named 'Pannaria tripartite R969', and included it as a member of the 'Physma group', a major clade within their phylogenetic study of *Pannariaceae*. This group is dominated by cyanospecies, but the cyanobiont of the genus *Physma* is genetically almost identical to a cephalodiate *Nostoc* of their analyzed sample of *G. mascarena*, together forming 'Nostoc Phylotype E'. Their hypothesis is, therefore, that *Physma* has originated through emancipation of *Gibbosporina* cephalodia followed by divergence.

Gibbosporina is an old genus and diverged from *Physma* at 75 Ma, although this is a cautious estimate (Elvebakk *et al.* 2016). Another genus, *Xanthopsoroma*, was described with two austral species (Elvebakk *et al.* 2010). Apart from the major clade of parmotrichelloid genera (i.e. containing the genus *Parmeliella*), *Gibbosporina* occupies a basal position in recent *Pannariaceae* phylogenies (Elvebakk *et al.* 2020; Spribille *et al.* 2020). It is tripartite, and its isolated position as a basal group

to the remaining members of their *Physma* group was one reason why Magain & Sérusiaux (2014) hypothesized that the ancient state of *Physma* was most likely tripartite.

Several *Gibbosporina* species have foliose cephalodia superficially resembling cyanospecies within the *Physma* clade, such as *Leightoniella zeylanensis* (Fig. 2C), although the latter is genetically distinct (Weerakoon *et al.* 2018). The proposed long evolutionary history of *Gibbosporina*, and its dominant and probably ancestral tripartite structure, suggests that *Gibbosporina cyanea* represents a relatively recent speciation. Its phyllidia have maintained the brittle character and thin cortex of its putative ancestor, and its function as a vegetative dispersal unit, and could be considered homologous to the small-foliose lobules of cephalodia of a tripartite ancestral species, although it has diverged in several characters compared with tripartite *Gibbosporina* species (Table 1). Such patterns are not known among other members of the *Physma* clade, except for a possible transitory state in *Lepidocollema polyphyllinum*, a cyanolichen which like *Gibbosporina cyanea* produces smaller lobules, probably also serving as vegetative propagules. Elvebakk *et al.* (2016) showed that there is a morphological distinction between the cyanobiont of the cephalodium-like lobules versus those of the main thallus, a difference which is not evident in *G. cyanea*, although these morphotypes have not been compared genetically.

A comparison of *Gibbosporina cyanea* with related palaeotropical cyanolichen genera within *Pannariaceae* is summarized in the key below. The taxa included here are primarily divided by perispores being either gibbose, verrucose or smooth. A challenge in comparing *G. cyanea* with related *Pannariaceae* genera is that two of the latter, *Lepidocollema* and *Physma*, are not monophyletic according to recent phylogenies (Magain & Sérusiaux 2014; Elvebakk *et al.* 2016; Rangsiruji *et al.* 2016). The approach here was to focus on specimens already included in phylogenies and to name their clades *Lepidocollema* I and II and *Physma* I and II. Perispores from species of these clades are illustrated here while taxonomic conclusions are left for future studies.

Species of *Gibbosporina*, *Physma* I and *Physma* II all share gibbose perispores. Apical perispore extensions are absent in all *Gibbosporina* species but are present in both *Physma* I and *Physma* II. Species of the genus *Physma* s. lat. were positioned in two paraphyletic clades by Magain & Sérusiaux (2014) and in three polyphyletic clades by Elvebakk *et al.* (2016). Two species in two different clades are from Réunion but have unreliable identifications, as do many other *Physma* species represented in recent

phylograms, as indicated by Elvebakk *et al.* (2016) and the present study.

'*Physma* I' is defined here as the clades in both studies containing the sample *Jones* s. n. of '*Physma byrsaeum*' from Tahiti, '*P. byrsaeum* R1121' from Réunion in Magain & Sérusiaux (2014) and '*P. byrsaeum* NK-273' also from Réunion in Elvebakk *et al.* (2016). Both the latter and *Jones* s. n. have been studied here (Fig. 5) and show gibbose perispores with wide apical extensions, and additionally with long apiculi in NK-273. It is most likely that the identifications of these specimens, as well as R1121 by Magain & Sérusiaux (2014), are incorrect. Hue (1906) reported *P. boryanum* from Réunion, and his perispore descriptions (the first ones of this species) correspond to those shown for NK-273 here (Fig. 5A). If this determination can be confirmed from type studies, then '*Physma* I' might represent *Physma* s. str., since *P. boryanum* is the generitype of the genus. NK-273 is referred to here as *P.* cf. *boryanum*, and the sample *Jones* s. n. is named *Physma* sp. These two species have very distinct perispores and their clade *Physma* I formed a well-supported sister group to all remaining specimens of *Physma* s. lat., *Lepidocollema* and *Gibbosporina* in the phylogram by Elvebakk *et al.* (2016).

Specimens from '*Physma* II' are shown here to have a considerably high percentage of thick, more even perispores with short, filiform apiculi and fewer, but distinct, gibbae (Fig. 5C & D). One of these illustrated spores lacks gibbae, as do those of *P. byrsaeum* illustrated by Schumm & Aptroot (2010). This species is the only widespread pantropical *Physma* species (according to, for example, GBIF), and was listed as the only one occurring in Réunion by van den Boom *et al.* (2011). Three specimens collected by Schumm & Frahm in the Seychelles and in Réunion as *P. byrsaeum*, matching the species concept in Schumm & Aptroot (2010), Magain & Sérusiaux (2014) and Diederich *et al.* (2017), have been examined for the present study and correspond to specimens identified as '*P. radians*' by Elvebakk *et al.* (2016). These identifications are corrected to *P. byrsaeum* here. The *Physma* sp. from New Caledonia, analyzed as NK-162 by Elvebakk *et al.* (2016), has the same kind of spores and is also identified as *P. byrsaeum* here, although a higher percentage of the perispores lack apical extensions and therefore resemble *Gibbosporina* spores. Nevertheless, the short and filiform diagnostic apical extensions are present, and the specimen was positioned close to the specimens now determined as *P. byrsaeum* in the phylogram by Elvebakk *et al.* (2016). Their clade of *P. byrsaeum*, here named '*Physma* II', is shown as a well-supported clade by Elvebakk *et al.* (2016) but is closer to *Gibbosporina* than '*Physma* I'. '*Physma* II' needs a different genus affiliation, and the name *Dichodium* has already been proposed for *P. byrsaeum* by Nylander (1888), a topic to be investigated further.

A third *Physma* clade shown by Elvebakk *et al.* (2016) included a sample of *Physma radians* Vain. from Japan and one of *P. pseudoisidiatum* Aptroot & Sipman from the USA, both first analyzed phylogenetically by Wedin *et al.* (2009). Specimens from this clade have not been studied further here.

The genus *Pannaria* is characterized by verrucose perispores in the key below. Perispore structures are incompletely known for many species of this genus, now being studied by the current author, including reviews of the *P. rubiginosa* and *P. lurida* groups. Two representatives from India and Sri Lanka characterizing these groups are included here, with perispore illustrations shown in Fig. 4D and 4E. The studied sample of *P. lurida* from Sri Lanka deviates from other samples of the species but has its characteristic nodulose apical perispore extensions. The only sample of *P. lurida* accepted from India by Upreti *et al.* (2005) is from the Andaman Islands and is described as apiculate on both ends. Both these collections indicate a need to restudy *P. lurida* in India and Sri Lanka.

The genera *Leightoniella* and *Lepidocollema* share smooth and even perispores with gradual and often poorly defined pulvinate apical extensions, which are sometimes absent. The monotypic genus *Leightoniella* has a perispore described by Weerakoon *et al.* (2018) as 'thick and gelatinous with pointed ends', matching its illustration as lenticular by Leighton (1871, fig. 36:1). Personal studies of *Leightoniella zeylanensis* (Fig. 4A) show a smooth, almost ellipsoid perispore with swollen and obtuse ends, corresponding to the structure of the lower part of the spore illustrated by Weerakoon *et al.* (2018). This structure is quite similar to the perispores of the austral genus *Xanthopsoroma* (see illustrations by Elvebakk *et al.* (2010)), a small genus with isolated phylogenetic positions as referred to above.

Ekman *et al.* (2014) included only two specimens of the *Lepidocollema marianum* group in their phylogeny when they redefined the genus *Lepidocollema* to include tropical members of *Parmeliella* with thalline excipuli. Magain & Sérusiaux (2014) included 13 *Parmeliella* specimens of this category in their phylogram, which rendered *Lepidocollema* polyphyletic. *Lepidocollema borbonicum* was positioned in a different but neighbouring clade to the nine specimens of the *L. marianum* group. These were united in a monophyletic clade by Weerakoon *et al.* (2018), but here the taxon sampling was aimed at defining the position of *Leightoniella*. Three specimens of *L. polyphyllinum* were positioned phylogenetically with the differently positioned members of *Parmeliella* s. str. by Magain & Sérusiaux (2014), indicating that the identity of the specimens may need to be confirmed.

The *L. marianum* group is defined here as '*Lepidocollema* I', and has thin perispores with smooth walls which are gradually thickened apically (Fig. 4C), corresponding to the patterns shown by Schumm & Aptroot (2010). '*Lepidocollema* II' is represented here by a specimen of *L. polyphyllinum* from the Solomon Islands, and is the same specimen published by Elvebakk (2016). It has similar perispores to '*Lepidocollema* I' but without apical expansions (Fig. 4B), although the material is too scarce to reach a general conclusion.

Perispore structure appears to be a very useful character among these *Pannariaceae* genera, where phylogenies coupled with thorough studies of samples are required to obtain an improved understanding.

A key for determining *Gibbosporina cyanea* and related palaeotropical cyanolichen groups within *Pannariaceae*

1	Perispores gibbose	2
	Perispores verrucose or smooth	4
2(1)	Perispores without apical extensions	**Gibbosporina cyanea**
	Perispores with apical extensions	3

3(2)   Apical extensions broad, either nodulose to pulvinate or tapering and long-apiculate . . . **Physma I** (incl. P. cf. boryanum)
            Apical extensions narrow, short, filiform and often curved, although absent in some spores . . . .
            . . . . **Physma II** (incl. P. byrsaeum)

4(1)   Perispores verrucose 5
            Perispores smooth 6

5(4)   Perispores with triangular to long-acuminate apical extensions . . . . **Pannaria rubiginosa**
            Perispores with nodulose extensions . . . . **Pannaria lurida** group

6(4)   Perispores with distinct pulvinate extensions and proper spores < 8 µm wide . . . . **Leightoniella zeylanensis**
            Perispores with very weak and gradual apical extensions or without such structures; proper spores > 8 µm wide 7

7(6)   Perispores with very weak apical extensions; thalli with radiating laciniae on a very distinct blackish hypo- and
            prothallus . . . . **Lepidocollema** I
            Perispores without apical extensions, laciniae different and prothallus not very distinct . . . . **Lepidocollema** II

Author ORCID. ⓘD Arve Elvebakk, 0000-0002-7682-3797.

References

Diederich P, Lücking R, Aptroot A, Sipman HJM, Braun U, Ahti T and Ertz D (2017) New species and new records of lichens and terricolous fungi from the Seychelles. *Herzogia* **30**, 182–236.

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 (2016) *Lepidocollema polyphyllinum* (*Pannariaceae*) from the Solomon Islands: cephalodium-like structure with two different *Nostoc* symbionts in dimorphous thalli. *Lichenologist* **48**, 339–341.

Elvebakk A and Sipman HJM (2020) *Gibbosporina* revisited: new records from Fiji, Indonesia, New Caledonia, Papua New Guinea, and Queensland, with one species from the Solomon Islands transferred to *Pannaria*. *Australasian Lichenology* **87**, 52–57.

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* **47**, 13–52.

Elvebakk A, Park CH and Hong SG (2020) *Hispidopannaria* and *Phormopsora*, two new and small, but evolutionary old *Pannariaceae* genera from southern South America. *Mycological Progress* **19**, 1353–1364.

Ezhkin AK and Jørgensen PM (2018) New records of *Pannariaceae* (lichenized Ascomycota) from Sakhalin and the Kuril Islands, Russian Far East. *Evansia* **35**, 43–52.

Hue A (1906) *Physma* unum e familiae Collemacearum generibus morphologice et anatomice descripsit. *Bulletin de la Société Linnéenne de Normandie, 5e Série* **9**, 119–138.

Jørgensen PM (2000) Survey of the lichen family *Pannariaceae* on the American continent, north of Mexico. *Bryologist* **103**, 670–704.

Jørgensen PM and Zhurbenko M (2002) Two new, remarkable, arctic species in the lichen genus *Fuscopannaria* (*Pannariaceae*, lichenized Ascomycetes). *Bryologist* **105**, 465–469.

Leighton WA (1871) The lichens of Ceylon collected by G. H. K. Thwaites. *Transactions of the Linnean Society of London* **27**, 161–185.

Magain N and Sérusiaux E (2014) Do photobiont switch and cephalodia emancipation act as evolutionary drivers in the lichen symbiosis? A case study in the *Pannariaceae*. *PLoS ONE* **9**, e89876.

Miadlikowska J, Kauff F, Hofstetter V, Fraker E, Grube M, Hafellner J, Reeb V, Hodkinson BP, Kukwa M, Lücking R, et al. (2006) New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia* **98**, 1088–1103.

Nelson PR and Wheeler T (2013) Cephalodia found on *Fuscopannaria viridescens*. *Lichenologist* **45**, 694–696.

Nordin A (1997) Ascospore structures in *Physciaceae*: an ultrastructural study. *Symbolae Botanicae Upsalienses* **32**, 195–208.

Nylander W (1888) *Lichenes Novae Zelandiae*. Paris: Paul Schmidt.

Orange A, James PW and White FJ (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Rangsiruji A, Boonpragob K, Mongkolsuk P, Sodamuk M, Buaruang K, Binchai S, Lumbsch HT and Parnmen S (2016) Diversity and phylogenetic survey of cyanobacterial lichens (*Collematineae*, Ascomycota) in mangrove forests of eastern Thailand. *Bryologist* **119**, 123–130.

Schumm F and Aptroot A (2010) *Seychelles Lichen Guide*. Süssen: Beck, OHG.

Spribille T, Fryday AM, Pérez-Ortega S, Svensson M, Tønsberg T, Ekman S, Holien H, Resl P, Schneider K, Stabentheiner E, et al. (2020) Lichens and associated fungi from Glacier Bay National Park, Alaska. *Lichenologist* **52**, 61–181.

Upreti DK, Divakar PK and Nayaka S (2005) Notes on some Indian pannariaceous lichens. *Nova Hedwigia* **81**, 97–113.

van den Boom PPG, Brand M, Ertz D, Kalb K, Magain N, Masson D, Schiefelbein U, Sipman HJM and Sérusiaux E (2011) Discovering the lichen diversity of a remote tropical island: working list of species collected on Réunion (Mascarene Archipelago, Indian Ocean). *Herzogia* **24**, 325–349.

Wedin M, Wiklund E, Jørgensen PM and Ekman S (2009) Slippery when wet: phylogeny and character evolution in the gelatinous cyanobacterial lichens (*Peltigerales*, Ascomycetes). *Molecular Phylogenetics and Evolution* **53**, 862–871.

Weerakoon G, Aptroot A, Wedin M and Ekman S (2018) *Leightoniella zeylanensis* belongs to the *Pannariaceae*. *Nordic Journal of Botany* **36**, e01880.