






Standard Paper

Further expansion of morphological variability in the *Porinaceae* (*Ascomycota*, *Ostropales*) with the placement of the enigmatic genus *Gallaicolichen*

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Abstract

The foliicolous lichen *Gallaicolichen pacificus* exhibits unique goniocystangia-like structures named peltidiangia and peltidia. Its taxonomic classification within the *Ascomycota* has been unclear due to the absence of ascomata and lack of molecular data. Here we clarify the phylogenetic affinities of *Gallaicolichen pacificus* by analyzing mitochondrial small subunit ribosomal RNA (mtSSU) sequences obtained from specimens collected in New Caledonia. Ascomata and ascospores of *G. pacificus*, previously unknown, are described and illustrated for the first time. The results from the molecular and morphological analyses clearly indicate that *Gallaicolichen pacificus* belongs to the *Porinaceae* and is closely related to *Porina guianensis*. This is a remarkable extension of the already known, wide morphological diversity of thalli and diaspores produced within this family.

Keywords: Australasia; foliicolous; goniocystangia; lichen; New Caledonia; peltidiangia; phylogeny

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Introduction

In a Festschrift volume of *Bibliotheca Lichenologica* dedicated to the late lichenologist Dr David Galloway, the enigmatic monospecific lichen genus *Gallaicolichen* Sérus. & Lücking was described from several locations in the Pacific Ocean, viz. the Hawaiian archipelago (USA), Queensland (Australia), Vanuatu and New Caledonia (Sérusiaux & Lücking 2007). It is also documented from the Philippines (Grant *et al.* 2022), based on a gathering by the American botanist Adolph Daniel Edward Elmer, possibly the oldest known collection of the taxon. Recently, the species was detected in the Ryukyu Islands (Iriomote) in southern Japan (Miyazawa *et al.* 2022).

The asexual reproductive structures produced by this species, somewhat resembling the goniocystangia described for opegraphoid lichens (Sérusiaux 1985), were named peltidiangia, since they are morphologically unique: they produce distinctive, disc-shaped diaspores, known as peltidia, which are made of small arms of dichotomously branched cells of the photosynthetic partner (assumed to represent *Phycopeltis*). These arms are linked

to a central foot and coiled up inwards to encapsulate a tiny, irregular layer of mycobiont hyphae. This pattern of co-dispersal of a fungus, wrapped by a photobiont diaspore, has no equivalent throughout the fungal kingdom; in other superficially similar structures, it is the fungal hyphae that enclose the photobiont cells.

In the present study, the systematic position of *Gallaicolichen pacificus* Sérus. & Lücking was resolved, due to the discovery of fertile specimens found on living leaves in New Caledonia, and to phylogenetic analyses of mitochondrial small subunit ribosomal RNA (mtSSU) sequences.

Material and Methods

Sampling material

This study is based on specimens of *Gallaicolichen pacificus* collected by EL and AS during a field trip dedicated to foliicolous lichen species in New Caledonia, in November and December 2022 (for specimen information see Supplementary Material File 1, available online). Specimens of *Coenogonium luteum* (Dicks.) Kalb & Lücking s. lat., *Porina alba* (R. Sant.) Lücking and *P. fusca* Lücking were collected by EL, ES and Nicolas Magain during a collecting trip focused on foliicolous lichens in Guadeloupe (Lesser Antilles), in January 2022. The leaves bearing the specimens were carefully dried for three weeks using a plant

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press with absorbent paper and placed in a box containing silica gel beads, then stored at -20°C until DNA extraction. Collection of specimens was carried out under collection permit no. 395-2022/ARR/DDDT (EL) for the South Province of New Caledonia and nos 609011-18/2022/JJC (EL) and 609011-51/2022/JJC (AS) for North Province. For Guadeloupe, collection of specimens was carried out under the collection permit no. 2022-03 (EL) issued by the Guadeloupe National Park.

Morphological analysis

Specimens were examined with an Olympus SZX12 stereomicroscope. Macroscopic images were captured using a Keyence VHX-5000 digital microscope and a VH-Z20R/W/T lens. Hand-cut sections and squash preparations of thallus were mounted in water, a 5% aqueous potassium hydroxide solution (K), or in Lugol's iodine solution (1% I_2) or with K pretreatment (KI) and studied using an Olympus BX51 compound microscope. The presence of crystals was investigated using polarized light. Measurements refer to dimensions in water and are given as follows: $(\text{min})-\bar{x}(-\text{max})$ (n = number of measurements). Microscopic images were captured using an Olympus BX51 compound microscope fitted with an Olympus SC50 digital camera.

Molecular analysis

Well-preserved specimens of *Coenogonium luteum* s. lat. (Lebreton 1584a), *Gallaicolichen pacificus* (Lebreton 1811a, Lebreton 1812b), *Porina alba* (Lebreton 879a, Lebreton 880a1) and *P. fusca* (Lebreton 879b, Lebreton 880a2), lacking any visible symptoms of fungal infection, were selected for DNA extraction. Specimens of *G. pacificus* were possessing peltidiangia (*sensu* Sérusiaux & Lücking 2007). Due to the small size, the material was extracted using the Phire Plant Direct PCR Kit from Thermo Fisher Scientific® (Massachusetts, USA), following the manufacturer's instructions. DNA was extracted at the laboratories of the Botanical Institute, University of Liège, Belgium. The primer pairs mrSSU1 and mrSSU3R (Zoller *et al.* 1999) were used to amplify the mtSSU, with the following PCR conditions: initial denaturation for 5 min at 98°C , followed by 40 cycles of denaturation for 5 s at 98°C , annealing for 5 s at 58°C , elongation for 20 s at 72°C and a final elongation for 1 min at 72°C . PCR products were visualized by electrophoresis on a 2% agarose gel, purified with ExoSAP-IT™ (IT PCR clean-up protocol) and sequenced by Macrogen® (Maastricht, the Netherlands). Sequence fragments were assembled with Geneious Prime v. 2022.2.2 (Biomatters, Auckland, New Zealand). Sequences were then subjected to a BLAST search (Altschul *et al.* 1997) in GenBank, using megaBLAST, to detect potential contamination.

Sequence alignment and phylogenetic analysis

After confirmation of the newly generated sequences as members of the *Porinaceae*, a dataset of mtSSU sequences was assembled for resolving the exact placement of the newly sequenced taxa in a broader phylogeny of the family *Porinaceae*. Sequences retrieved from GenBank were selected mainly from previous studies of the family (Sobreira *et al.* 2018; Lücking *et al.* 2020; Ertz & Diederich 2022; Sanders *et al.* 2023), for a total of 122 sequences in the matrix. Four accessions of *Coenogonium* were selected as outgroup. Sequences were aligned with MAFFT v. 7 online (Kato *et al.* 2019) and the alignment was checked manually

using Mesquite v. 2023.3.81 (Maddison & Maddison 2023). Ambiguous regions were delimited using the online version of Gblocks v. 0.91b (Castresana 2000) at <http://phylogeny.lirmm.fr/>, allowing for gap positions within the final blocks, and carefully checked manually (Supplementary Material Files 2 & 3, available online). Consequently, a total of 568 positions were conserved for the phylogenetic analysis. We inferred the best-scoring maximum likelihood tree and bootstrap support values based on 1000 pseudoreplicates in the same run using RAxML-HPC2 v. 8.2.12 (Stamatakis 2006; Stamatakis *et al.* 2008), with the GTRGAMMA model and default settings as implemented on the CIPRES portal (Miller *et al.* 2010) (Supplementary Material File 4, available online). Phylogenetic trees were visualized using FigTree v. 1.4.4 (Rambaut 2018). Maximum likelihood bootstrap values (BS) > 70% were considered significant.

Results

Phylogenetic analyses

The backbone topology of the molecular phylogeny of *Porinaceae* is poorly supported (Fig. 1), which is consistent with recent phylogenetic reconstructions (Sobreira *et al.* 2018; Lücking *et al.* 2020; Orange *et al.* 2020; Ertz & Diederich 2022; Sanders *et al.* 2023). The 14 lineages highlighted in Sobreira *et al.* (2018) are supported, along with five new clades added by Lücking *et al.* (2020), Ertz & Diederich (2022) and Sanders *et al.* (2023).

Nineteen main clades can be recognized within the *Porinaceae*. The largest one, with 87% BS, includes the lineages named *Phyllophiale*, *Phylloporina*, the *Porina dolichophora* clade, the *Porina guianensis* clade, and *Porina* s. str. Additionally, there is another well-supported clade with 97% BS, comprising *Clathroporina* and *Myeloconis*. A separate clade with 98% support includes *Porina hibernica* P. James & Swinscow and *P. pseudohibernica* Tretiach, closely related to the *P. collina* and *P. byssophila* clades (*sensu* Sobreira *et al.* 2018). Another clade containing *P. leptalea* (Durieu & Mont.) A. L. Sm. forms a well-supported clade with the *P. fusca* clade, and finally, a clade with *P. rupicola* Ertz & Diederich, *P. internigrans* (Nyl.) Müll. Arg. s. lat. and *P. nuculoides* Ertz & Diederich is closely related to the recently described genus *Saxiloba* (Lücking *et al.* 2020).

However, several lineages lack robust support in the deeper nodes. The sister relationship between the clade *Porina covidii* Ertz & Diederich and '*Porina cf. malmei*', the clade *P. nanoarbuscula* Ertz *et al.* and *P. microcoralloides* Ertz *et al.* is weakly supported (BS = 48). The placement of some species remains unresolved, for example *Porina mamillosa* (Th. Fr.) Zahlbr., *P. rivalis* Orange and *P. pacifica* Brodo.

Based on the named species in the tree, the genus *Porina* appears to be resolved as paraphyletic because the lineages corresponding to recently accepted or suggested genera (e.g. *Clathroporina*, *Flabelloporina*, *Myeloconis*, *Phragmopelthea*, *Phyllophiale*, *Phylloporina*, *Pseudosagedia*, *Segestria*, *Saxiloba*, *Trichothelium*, *Zamenhofia*, etc.) are nested within a backbone of species still classified within *Porina*. However, this depends on the definition of what constitutes the genus *Porina*. In a narrower definition, *Porina* could be circumscribed to include all species with a crystallostratum, a clade supported in the analyses by Sobreira *et al.* (2018) and Lücking *et al.* (2020) but not for instance in Ertz & Diederich (2022). The inconsistencies include two specimens of *P. internigrans* and one specimen of *Porina nuculoides*, both species that have a crystallostratum, placed

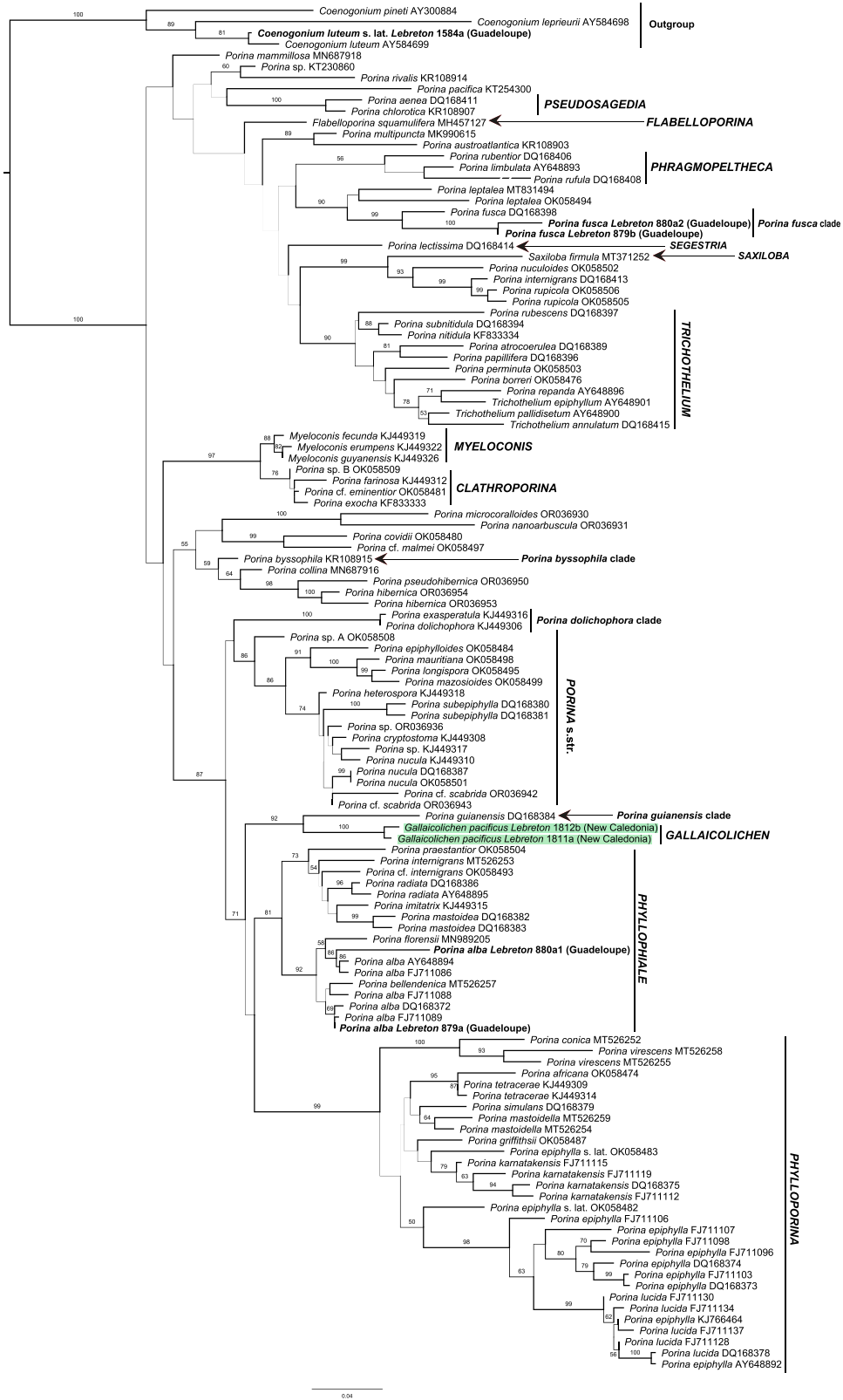


Figure 1. Best-scoring maximum-likelihood (ML) tree of *Porinaceae* based on the mtSSU marker, showing the position of *Galliaicolichen pacificus* highlighted in green and the new sequences produced in the present study in bold. Potential genus-level lineages *sensu* Sobreira *et al.* (2018) are indicated. Scores for branches with ML bootstrap values < 50 are not shown. In colour online.

outside this clade. *Porina* could alternatively be defined as the subclade including the *P. nucula*, *P. dolichophora*, *Phyllophiale* and *Phylloporina* clades (i.e. excluding *Clathroporina*, *Myeloconis* and the rogue samples of *P. internigrans* and *P. nuculoides*). In its most narrow definition, it would correspond to the *P. nucula* clade, containing the type species. One of the main challenges regarding genus-level classification is the notion that gross morphology generally correlates with phylogeny, but some oddly placed taxa challenge this assumption.

The placement of the *Coenogonium luteum*, *Porina fusca* and *P. alba* accessions recently collected in Guadeloupe falls with sequences of the same species; however, the long branches suggest that some of these are species complexes. Both accessions of *Gallaicolichen pacificus* are resolved as sister to *Porina guianensis* with strong support (BS = 92) (Fig. 1). The clade formed by *Gallaicolichen* and *P. guianensis* is closely related to the larger clades of *Phylloporina* and *Phyllophiale* (BS = 71), all representing largely foliicolous taxa or species growing on smooth bark. Furthermore, all these taxa share the production of a crystallostratum, a thick layer of crystals in the ascromatal wall, as also found in the related *P. dolichophora* and *P. nucula* clades, in addition to *Clathroporina* and *Myeloconis*.

Taxonomy

A detailed description of *Gallaicolichen pacificus* with only peltidiangia (i.e. without ascromata) was provided by Sérusiaux & Lücking (2007; Fig. 2); it can now be completed with the description of its perithecia and ascospores (Figs 3 & 4).

Gallaicolichen pacificus Sérus. & Lücking

In Sérusiaux & Lücking, *Bibliotheca Lichenologica* **95**, 510 (2007); type: USA, Hawaii, Hamakua, Kolekole Park, on leaves of *Syzygium cuminii*, 11 vii 1995, C. W. Smith s. n. (LG12004-RTR00A).

Description provided by Sérusiaux & Lücking (2007): ‘Thallus foliicolous, epiphyllous, composed of rounded patches, 0.5–1.2 (–1.7) mm in diam., pale greenish yellow to pale yellowish gray, or very pale yellowish, with a slightly lobulate margin, surface somewhat shiny, smooth or very slightly uneven (under high magnification), coalescing when contiguous, up to 20 µm thick, formed of a loose network of interwoven hyphae and large, regular plates of photobiont cells, containing large (up to c. 25 × 10 µm) oxalate crystals, mostly present in mature parts and absent near the margins; cortex sometimes developed, sometimes completely absent, formed of a single layer of polygonal cells, sometimes with a slightly brownish wall, 5–7 × 2–3 µm; prothallus usually present, membranaceous and colourless, rarely bluish or brownish. Photobiont: a species of *Phycopeltis* (*Trentepohliaceae*) with greenish brown, c. 8–10(–11) × 4–5 µm cells, regularly and radiately arranged rows in plates. Goniocystangia-like structures always present (here named peltidiangia), 1–5(–8) per thallus patch, the first one developing at the center, almost perfectly circular, 0.1–0.15(–0.2) mm in diam. and c. 0.13–0.15 mm high, formed by a rather thick erect margin with its inner part typically whitish and made of raised, not agglutinated hyphae and its outer part usually covered by the thallus. Diaspores (here named

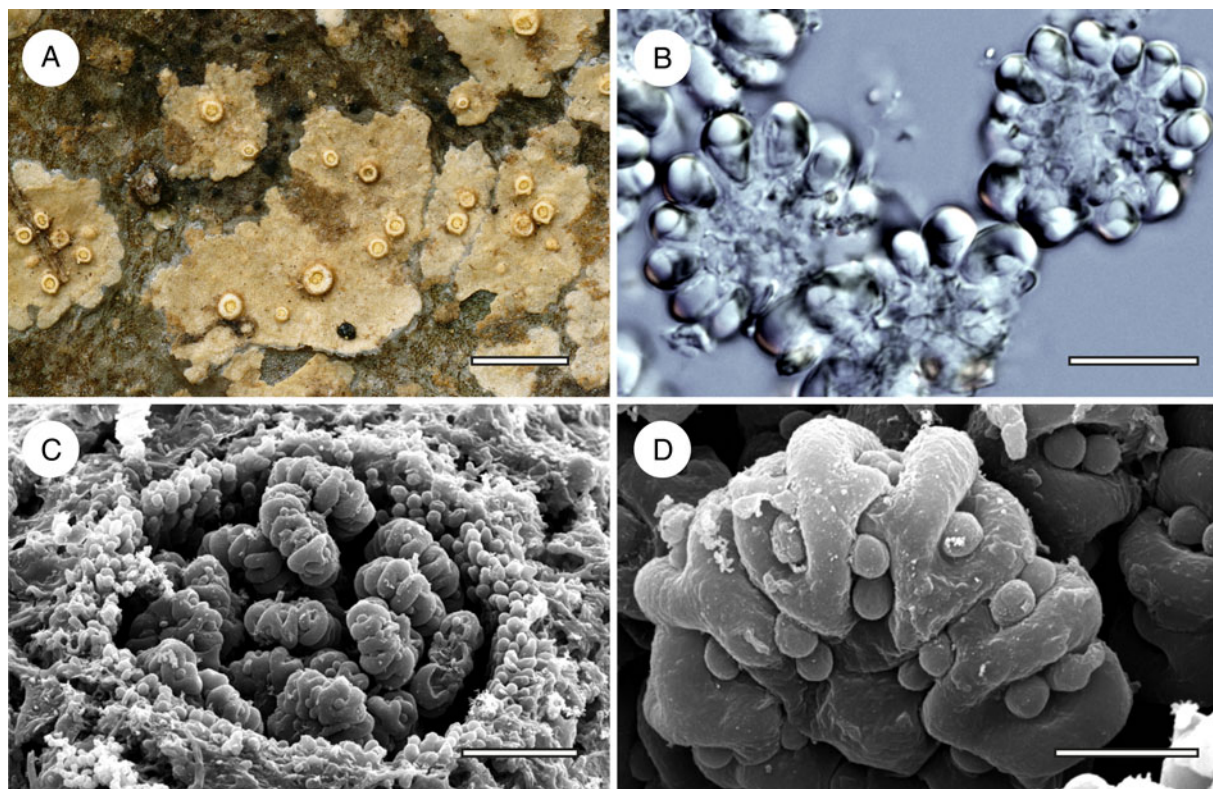


Figure 2. *Gallaicolichen pacificus*; historical collections studied in Sérusiaux & Lücking (2007). A, holotype collected in Hawaii by C. W. Smith in 1995 (LG12004-RTR00A). B, peltidia mounted in water. C, lateral view of a young peltidiangia with its inner margin made of raised, not agglutinated hyphae. D, mature peltidia with tips of mycobiont hyphae developed on the other side and emerging through the dichotomously branched *Phycopeltis* cells. Scales: A = 500 µm; B & C = 20 µm; D = 5 µm. Images by Damien Ertz & Elise Lebreton (A) and Emmanuël Sérusiaux (B–D) (*Bibliotheca Lichenologica* **95**, 510 (2007)). In colour online.

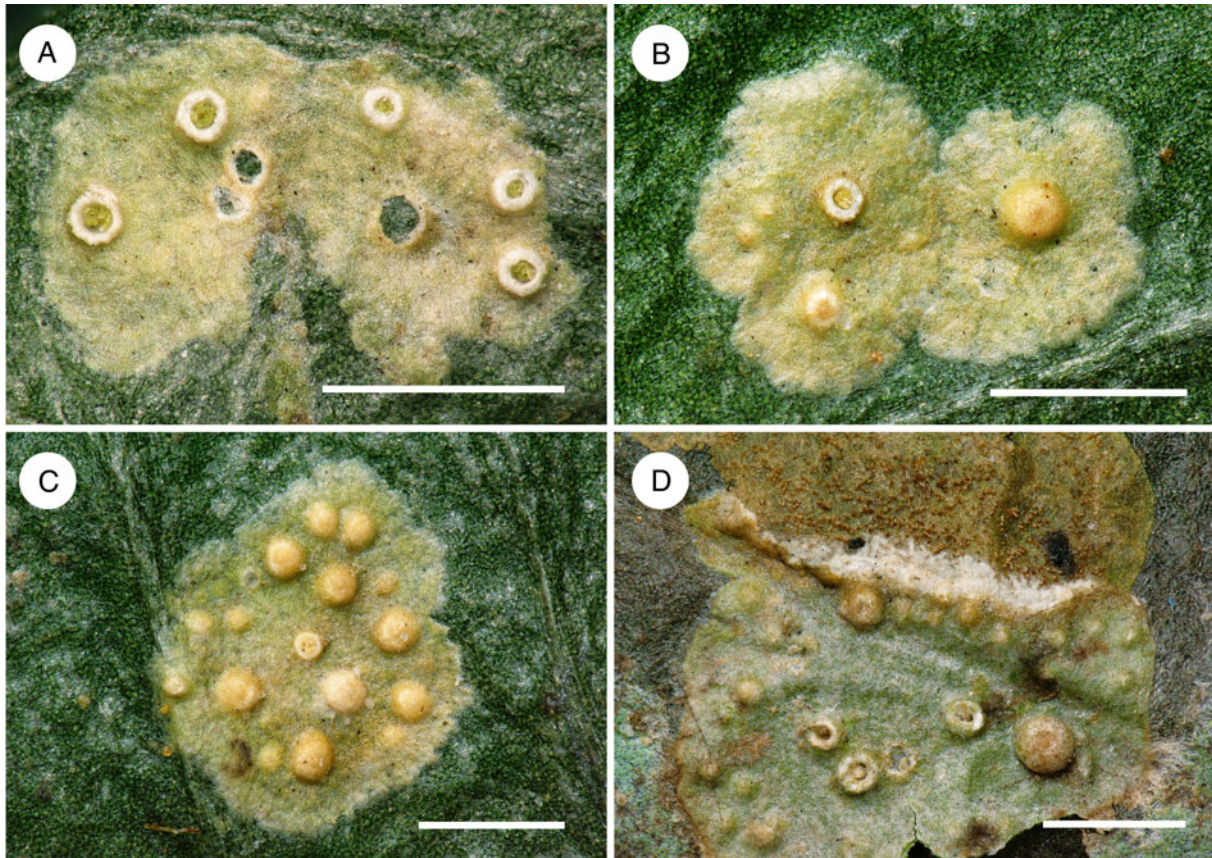


Figure 3. *Gallaicolichen pacificus* (Lebreton 1886c; LG12002-PTR00A). A, specimen with only peltidiangia. B, two agglutinated thalli, one with only perithecia and the other with only peltidiangia. C & D, specimens with both peltidiangia and perithecia. Scales = 0.5 mm. In colour online.

peltidia) numerous, usually filling up the peltidiangia cavity, disc-like, c. 25–55 μm in diam. and c. 10–15 μm thick, with a regular pattern of organization: small arms of dichotomously branched (1–2 branching points) cells of *Phycopeltis*, all linked to a central foot-like structure and coiled up inwards on the other side where they encompass a tiny, regularly arranged layer of mycobiont cells; 1–2 extremities of hyphal filaments usually present between algal arms and easily seen on the diaspores outer surface; extremities of algal arms slightly but distinctly inflated, and refringent.

Perithecia solitary, dispersed, hemispherical to \pm subglobose, 0.2–0.3 mm diam., 0.1–0.2 mm high, glabrous, surface smooth, with applanate top. *Ostiole* apical, rather inconspicuous, periostiole area pale orange. *Crystallostratum* of calcium oxalate crystals, yellowish, K+ orange-red, c. 30–45 μm thick, covered by a 15–20 μm layer of algeriferous tissue. *Involucrellum* fused with excipulum, densely covered with the same crystals 10–15 μm thick. *Hamathecium* hyaline, not interspersed, paraphyses simple, non-branched and non-anastomosing, 1–1.5 μm . *Asci* narrowly obclavate to cylindrical clavate, I–, c. 92.5 \times 12 μm ($n=2$); ascus apex slightly truncate with a ring structure. *Ascospores* 8 per ascus, (4–)6–7-septate, fusiform, (24.5–)26(–28) \times (4–)5(–5.5) μm ($n=30$), 4–7 times as long as wide, colourless with c. 3.5 μm thick gelatinous sheath.

Pycnidia not observed.

Chemistry. TLC not performed.

Ecology and distribution. *Gallaicolichen pacificus* is reported from various regions of the Pacific Ocean: Hawaiian archipelago,

Queensland in Australia, Vanuatu and New Caledonia (Sérusiaux & Lücking 2007). Additionally, it has been observed in the Philippines and the Ryukyu Islands, Japan (Miyazawa *et al.* 2022). In New Caledonia, *G. pacificus* has been observed in several locations in the South and North Provinces of Grande Terre, in high numbers on phorophytes in forests at mid-altitude (150–600 m elev.), as well as along forest edges and in riparian habitats. The majority of specimens exhibit only peltidiangia without ascomata. The occurrence of both perithecial ascomata and peltidiangia on the same thallus is uncommon. When perithecia are present, peltidiangia are reduced (Fig. 3B). A similar phenomenon has also been observed in *Porina alba*, where the thallus, when producing perithecia, exhibits few disc-shaped isidia, and vice versa (Lücking 2008).

Notes. *Porina atriceps* (Vain.) Vain., *P. epiphylla* Fée, *P. lucida* R. Sant, *P. karnatakensis* Makhija *et al.* and *P. subepiphylla* Lücking & Vězda share similar perithecia morphology and 7-septate ascospores. However, they differ in spore size and the presence of a reddish or blackish brown spot around the ostiole (Lücking & Vězda 1998). In the absence of peltidiangia, *Gallaicolichen pacificus* could be considered an intermediate species between *P. epiphylla* and *P. lucida*. *Gallaicolichen pacificus* has broader spores than *P. epiphylla* and shorter spores than *P. lucida*. *Porina subepiphylla* also develops larger spores.

Porina epiphylla belongs to a taxonomically difficult group of species, with intermediate forms being common, and should be treated as *P. epiphylla* s. lat. when identification is uncertain (Lücking 2008).

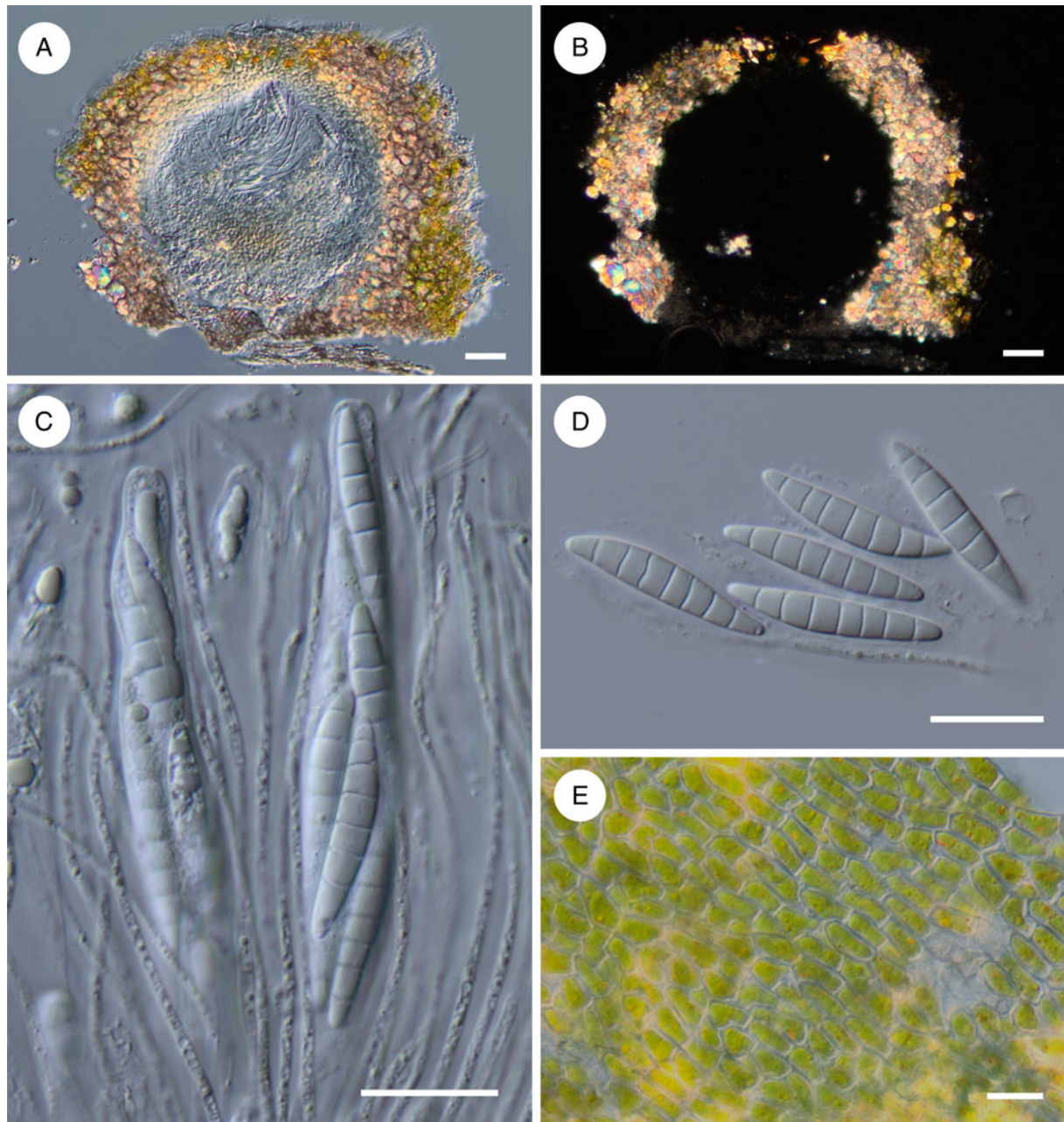


Figure 4. *Gallaicolichen pacificus* (Lebreton 1886c; LG12002-PTR00A), mounted in water. A, cross-section of perithecia showing crystallostratum and algeriferous layer covering the yellowish involucrellum. B, crystallostratum visible with polarized light. C, ascus with ascospores. D, ascospores (with gelatinous sheath). E, thallus with trepophloid algal cells arranged in rows in plates. Scales: A & B = 20 μ m; C–E = 10 μ m. In colour online.

Specimens examined with ascomata. New Caledonia: *Prov. Sud:* Mts Koghis, Pic Malaoui tropical rainforest in a creek, collected on *Cyathea* sp. leaves, 540 m elev., 22°10'57.468"S, 166°30'34.667"E, 2022, *E. Lebreton* 1886c (LG12002-PTR00A); Mts Koghis départ du sentier de randonnée juste après la guérite, 500 m elev., 22°10.774'S, 166°30.576'E, 2022, *E. Lebreton* 1901e (LG11997-KTR00A).

Specimens examined without ascomata. New Caledonia: *Prov. Sud:* Mts Koghis-Dumbéa, tropical rainforest with *Cryptocarya macrocarpa*, *Bureavella wakere*, *Hernandia cordigera*, *Cyathea intermedia*, 15 km NNE of Nouméa, 22°14'S, 166°30'E, 550 m elev., 23

viii 1994, K. & A. Kalb s. n. (LG12003-QTR00A); Mts Koghis vallée des fougères, 530 m elev., 22°10.631'S, 166°30.519'E, 2022, *E. Lebreton* 1811a (LG14602-PPV00A; GenBank Accession no. [PP080177]), *E. Lebreton* 1812b (LG14601-OPV00A; GenBank Accession no. PP080178); Mt Dore, sentier de randonnée du Pic Malaoui par Yahoué, près de la rivière, 138 m elev., 22°11.742'S, 166°30.072'E, 2 xi 2022, *E. Lebreton* s. n. (LG12001-OTR00A); Sarraméa, sentier de randonnée du plateau du Dogny, 662 m elev., 21°37.273'S, 165°52.189'E, 2022, *E. Lebreton* 1925b (LG11993-GTR00A). *Prov. Nord:* Pouébo, Mt Amos, patch de forêt humide, 573 m elev., 20°18.135'S, 164°26.278'E, 13 xi 2022, *E. Lebreton* s. n. (LG11992-FTR00A); Koné, Massif du

Koniambo, Vallée de la Confiance, foliicolous in lowland rainforest, along the river, 21.0298°S, 164.8218°E, 2022, A. Simon 1204 (O, O-L-400621) with A. Evankow, R. Haugan, E. Möller & E. Timdal.—**Philippines:** Luzon: Sorsogon Province, Irosin, Mt Bulusan, A. D. E. Elmer 15932 (F).

Discussion

In the absence of ascomata or molecular data, *Gallaicolichen pacificus* would probably not be assigned to the *Porinaceae*, a similar case being the recently described genus *Saxiloba* (Lücking *et al.* 2020). As for *Gallaicolichen pacificus*, several hypotheses have been proposed based on morphological similarities, such as the resemblance of the peltidiangia to the goniocystangia found in the *Opegrapha lambinonii* aggr. (Sérusiaux 1985), and the resemblance of the thallus to *Porina alba* (Lücking *et al.* 2001) or species of *Opegraphaceae* (*Opegrapha*) and *Roccellaceae* (*Mazosia*) (Lücking & Kalb 2001; Sérusiaux & Lücking 2007). The discovery of ‘fertile’ thalli, producing both peltidiangia and perithecia, together with studies of the perithecial structure and molecular data with the mtSSU marker, strongly support its assignment in the *Porinaceae*.

This family is characterized by perithecioid ascomata with unbranched paraphyses, thin-walled unitunicate asci, and hyaline ascospores with thin walls and septa, all features displayed by *G. pacificus*. Furthermore, the ascomata features support the close relationship of this species with the *Porina epiphylla* aggr., characterized by perithecia with a yellowish involucrellum turning orange-red when treated with K, the presence of a crystallostratum covering algiferous thallus tissue, and septate ascospores (Hafellner & Kalb 1995; Lücking & Vězda 1998; Lücking & Cáceres 1999; Lücking 2008; Ertz & Diederich 2022).

Gallaicolichen pacificus can be recognized easily based on the unique thallus structure and the co-dispersal structures named peltidiangia. These propagules share morphological characteristics with those produced by *Porina alba*, *P. fusca*, *P. mirabilis* and the *Opegrapha lambinonii* aggr. Their morphology is characterized by a disc-like structure, supposedly developed by the plate-forming alga *Phycopeltis* within the thallus. In the case of *P. alba* and its relatives, the disc-shaped propagules extend into a sucker that requires inversion for attachment, and they are produced individually. Within the propagule, a singular young discoid thallus of the algal symbiont is present, featuring radial, dichotomously branched filaments (Sanders 2002). The branching pattern of the algae is consistent with morphological descriptions provided by Karsten (1891) and Thompson & Wujek (1997) for non-lichenized *Phycopeltis*. In the *Opegrapha lambinonii* aggr., multiple propagules, referred to as goniocysts, are produced together within a single, dedicated structure similar to that produced by *G. pacificus* (Sérusiaux & Lücking 2007). The propagules in both cases remain small and coin-like. However, in *G. pacificus*, the mycobiont cells are predominantly embedded or nearly so by algal cells, whereas it is the reverse for species in the *Opegrapha lambinonii* aggr. where the alga is trapped within the hyphal envelope of the propagules (Sérusiaux 1985). The distinctive feature observed in *G. pacificus*, where the photobiont partner organizes the propagules and envelops its mycobiont partner, is unique among structures containing both the photobiont and mycobiont of lichenized fungi.

Its closest relative in our phylogeny (Fig. 1), *Porina guianensis*, has a different thallus morphology. While both species have a non-carbonaceous perithecial wall, a crystallostratum and transverse-

septate ascospores, this is also the case for most other taxa within *Porina* s. str., *Phyllophiale* and *Phylloporina*. A unique feature of *P. guianensis* is the finely verrucose thallus. This, combined with a radiating photobiont, is otherwise also known in *P. mazosioides* Lücking & Vězda, *P. epiphyloides* Vězda and *P. longispora* Vězda, species belonging to another clade. Thus, although these two taxa form a separate clade within the *P. nucula* complex, the genus name *Gallaicolichen* is available for this clade. However, no morphological or anatomical characters can be used as autapomorphies to support the genus *Gallaicolichen* with these two species. Similar problems are found with other morphologically unique taxa, such as *Flabelloporina*, *Myeloconis* and *Saxiloba*, which form parts of phylogenetically separate clades that, however, also include species with other morphologies (Nelsen *et al.* 2014; Sobreira *et al.* 2018; Lücking *et al.* 2020; Ertz & Diederich 2022).

The continuous expansion of morphological diversity within the *Porinaceae*

Hafellner & Kalb (1995) were the first to separate *Porinaceae* from the order *Pyrenulales*, a large and heterogeneous order at the time, and to assign it to its own order, the *Trichotheliales* (since the name *Trichotheliaceae* was used for the family). Autapomorphic characters for this order included hamathecial elements made of unbranched paraphyses and periphyses; an ascus wall with a single functional layer; apical structures with an external chitinous ring structure; poroid ascus dehiscence; hyaline ascospores with thin septa. Based on pigment chemistry and ascus tip structure, as well as the presence of perithecial setae, these authors distinguished five genera at the time: *Clathroporina*, *Porina*, *Pseudosagedia*, *Trichothelium* and *Zamenhofia*. Later, McCarthy (2003) published an extensive catalogue of all species of *Porinaceae*, recognizing only three genera (*Polycornum*, *Porina* and *Trichothelium*) and 394 species, especially diverse in the tropics and subtropics.

More recently, Nelsen *et al.* (2014) demonstrated the placement of *Myeloconis* within *Porinaceae*. Sobreira *et al.* (2018), providing a first larger phylogeny of the family, suggested distinguishing at least 15 genus-level lineages, including the new genus *Flabelloporina*, with *F. squamulifera* (Breuss *et al.*) Sobreira *et al.*, presenting a squamule-bearing thallus (see Lücking *et al.* (2013) for original description as *Porina squamulifera*). Another new genus, *Saxiloba*, was described based on a unique, rock-dwelling thallus with photobiont chambers separated by crystalline walls, with two species, *S. firmula* (Müll. Arg.) Lücking *et al.* from the Caribbean and *S. hawaiiensis* Lücking *et al.* from Hawaii (Lücking *et al.* 2020).

The *Porinaceae* are widely distributed worldwide, colonizing various substrata, such as living leaves, rocks and tree trunks or tiny branches. Most members exhibit a crustose thallus with a smooth or verrucose surface, along with the presence or absence of a dark, shiny, or white prothallus (Figs 5 & 6). An impressively diverse range of forms, colours and structures have been identified within this family and their taxonomic relevance has been debated (McCarthy & Malcolm 1997; Baloch & Grube 2006; Nelsen *et al.* 2014; Lücking *et al.* 2020; Ertz & Diederich 2022). *Gallaicolichen pacificus* now adds a further morphological dimension to the *Porinaceae*.

Together with the assignment of the genera *Myeloconis* and *Trichothelium*, and the recent recognition of *Flabelloporina* (Sobreira *et al.* 2018), *Saxiloba* (Lücking *et al.* 2020) and *Gallaicolichen* (this paper) within the *Porinaceae*, a pattern of generic delimitation seems to be emerging. However, as

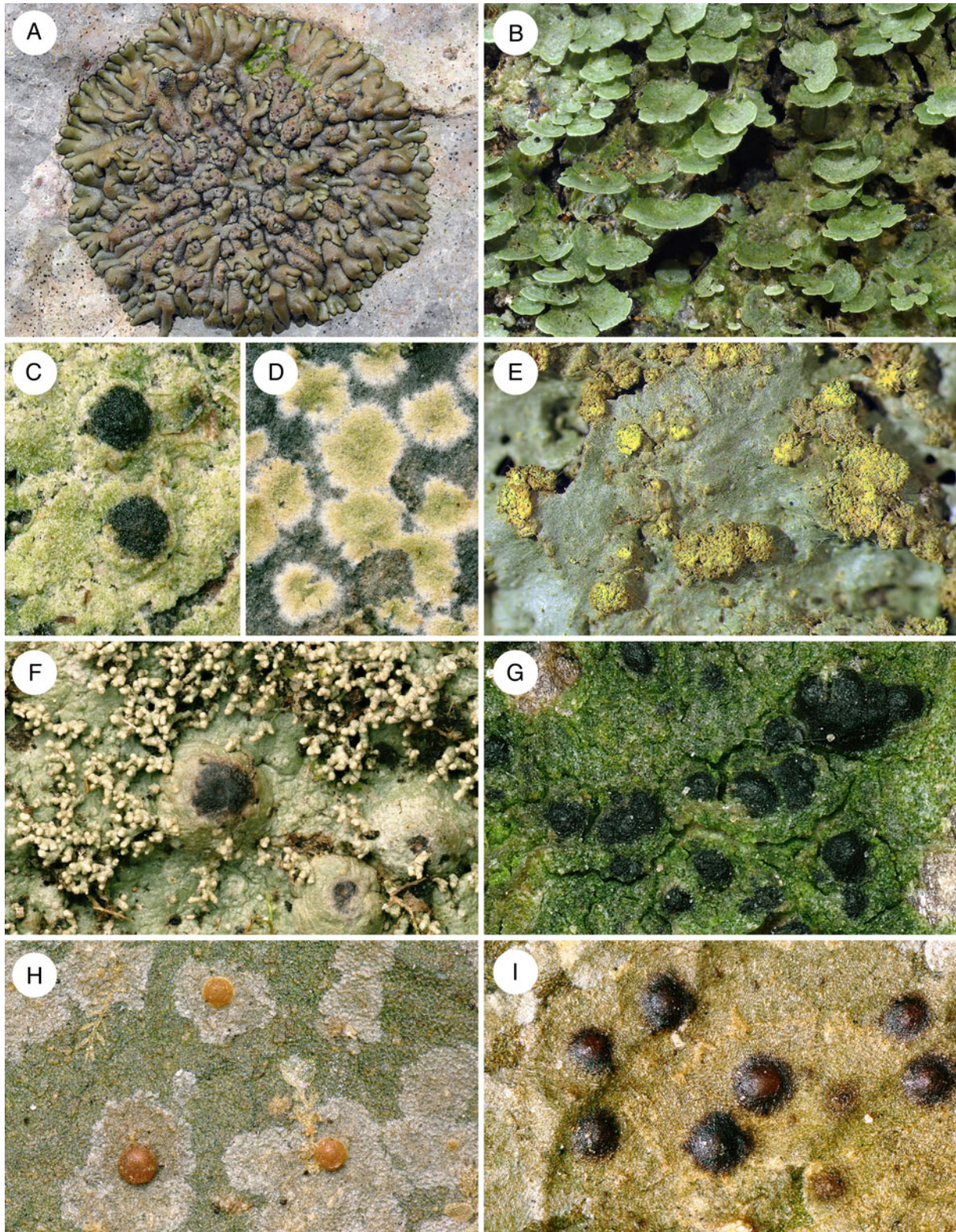


Figure 5. Morphological diversity of *Porinaceae*. A, *Saxiloba firmula* (Lücking et al. 43034), thallus placodioid. B, *Flabelloporina squamulifera* (Lücking et al. 39729), thallus bearing flabelliform squamules. C & D, *Pseudosagedia crocynioides* (R. C. Harris) R. C. Harris (Ertz 24301), byssoid thallus and black perithecia. E, *Myeloconis guyanensis* P. M. McCarthy & Elix (Cáceres 324), thallus with yellow medulla. F, *Porina florensis* Diederich & Ertz (Ertz 24283), thallus bearing cylindrical and branched isidia. G, *Porina byssophila* (Körb. ex Hepp) Zahlbr. (Ertz 26674), black perithecia. H, *Porina leptosperma* Müll. Arg. (Lebreton 184a), orange perithecia. I, *Porina rubentior* (Stirt.) Müll. Arg. (Lebreton 188a), cherry red perithecia. Note: specimens are not presented at the same scale. In colour online.

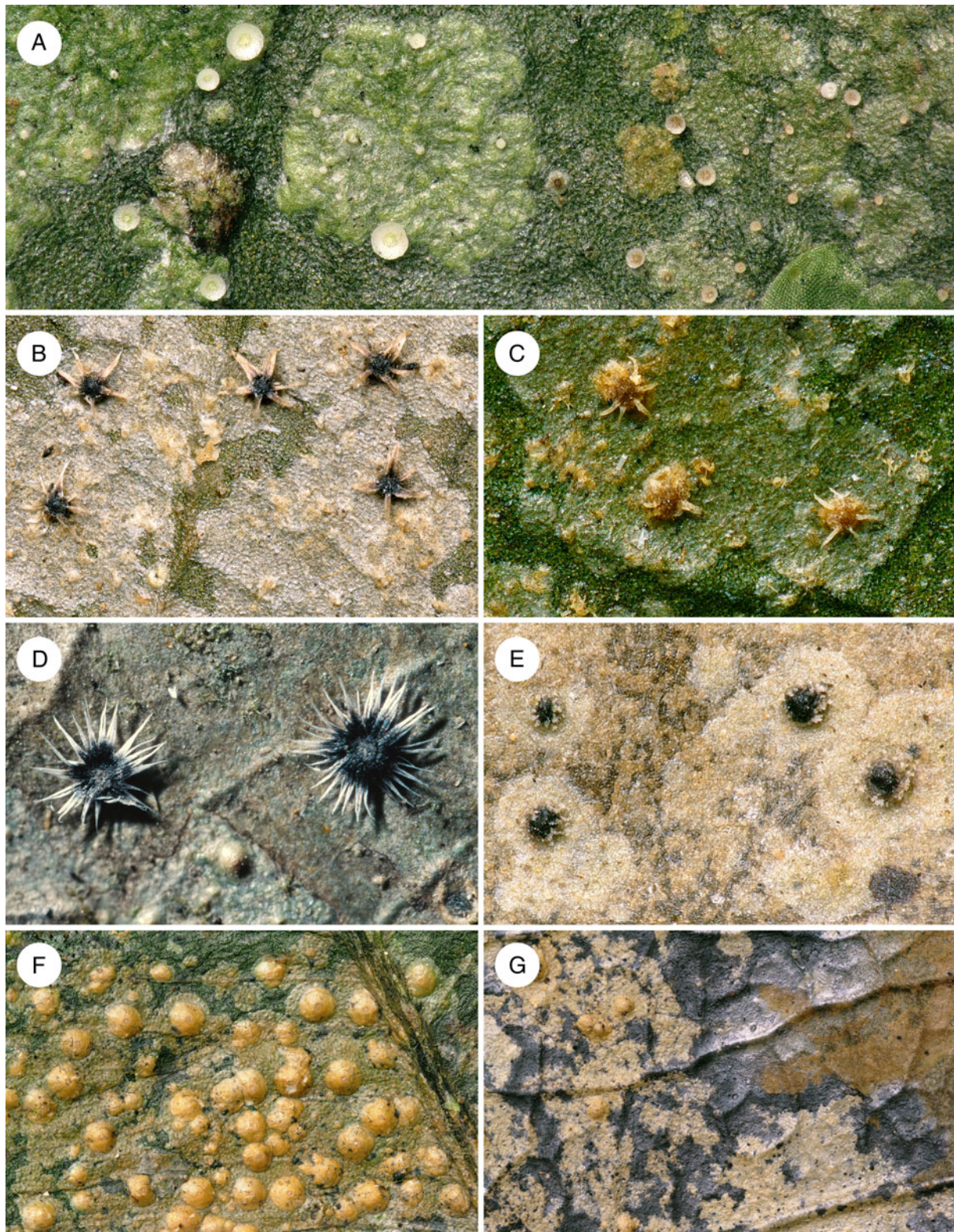



Figure 6. Morphological diversity of *Porinaceae*. A, *Porina alba* (left) (Lebreton 880a1) and *Porina fusca* (right) (Lebreton 880a2) growing side by side, both thalli bearing disc-shaped isidia. B, *Trichothelium minus* Vain. (Lebreton 196b), black perithecia with 4–6 pale perithecial setae. C, *Porina rubescens* (Lücking) Hafellner & Kalb (Lebreton 193c), brownish red perithecia with 5–8 pale perithecial setae. D, *Trichothelium argenteum* Lücking & L. I. Ferraro (Lücking 91-1374), black perithecia with numerous perithecial setae. E, *Porina nitidula* Müll. Arg. (Lebreton 72a), black perithecia, shortly pilose to tomentose. F, *Porina mazosioides* (Ertz 24342), perithecia yellow with a crystallostratum. G, *Porina subinterstes* (Nyl.) Müll. Arg. (Lebreton 108a), thallus with glossy dark prothallus. Note: specimens are not presented at the same scale. In colour online.

mentioned above, the otherwise seemingly clear correlations of phylogeny with morphology are being challenged by some oddly placed taxa and so no strongly supported consensus throughout the whole family can be obtained with the data currently available. Therefore, several authors opted to retain *Porina* s. lat. in their recent studies (Orange et al. 2020; Diederich & Ertz 2022; Sanders et al. 2023) waiting for wider sampling and, in particular, sequences from more loci.

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Supplementary Material File 1. Species names, voucher specimens and GenBank Accession numbers of mtSSU sequences used in this study.

Supplementary Material File 2. Matrix with *Porinaceae* mtSSU sequences before exclusion of ambiguous regions.

Supplementary Material File 3. Matrix with *Porinaceae* mtSSU sequences after exclusion of ambiguous regions.

Supplementary Material File 4. Phylogenetic tree of the *Porinaceae* in newick format.

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