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Standard Paper

Independent, structurally distinct transitions to microfruticose growth in the crustose genus *Porina* (*Ostropales, Lecanoromycetes*): new isidioid species from south-western Florida

William Sanders[1], Roberto De Carolis[2], Damien Ertz[3], Asunción de los Ríos[4] and Lucia Muggia[2]

[1]Department of Biological Sciences, Florida Gulf Coast University, Ft Myers, FL 33965-6565, USA; [2]Department of Life Sciences, University of Trieste, 34127 Trieste, Italy; [3] Department Research, Meise Botanic Garden, BE-1860 Meise, Belgium; and Fédération Wallonie-Bruxelles, Service Général de l'Enseignement Supérieur et de la Recherche Scientifique, BE-1080 Bruxelles, Belgium and [4]Departamento de Biogeoquímica y Ecología Microbiana, Museo Nacional de Ciencias Naturales (CSIC), E-28006, Madrid, Spain

Abstract

Porina is a widely distributed, species-rich genus of crustose, lichen-forming fungi, some with thalline outgrowths that have been recognized as isidia. We studied three taxa with thalli consisting chiefly of ascending isidioid structures occurring on trunks and branches of *Taxodium* in southwestern Florida, and provide details of their structure with light and electron microscopy. Two of these taxa we describe as new species: *P. microcoralloides* and *P. nanoarbuscula*. Genetic sequences (mtSSU) suggest that they are closely related to each other, yet they differ markedly in the size, morphology and anatomical organization of their isidioid branches as well as in the length of their ascospores. In the three Floridian taxa studied, the crustose portion of the thallus is partly endophloeodic and partly superficial, the latter often patchy, evanescent or inconspicuous, and completely lacks the differentiated anatomical organization characteristic of the isidioid structures arising from it. In *Porina microcoralloides*, the ascendant thallus consists of branched, coralloid inflated structures with phycobiont (*Trentepohlia*) unicells arranged at the periphery of a loose central medulla. Sparse fungal cells are interspersed and overlie the algal layer in places, but no differentiated cortex is present, leaving phycobiont cells more or less exposed at the surface. In the closely related *Porina nanoarbuscula*, the isidioid structures are much finer, more densely branched, and composed of a single, central file of roughly spherical *Trentepohlia* cells surrounded by a jacket of subglobose fungal cells. The ascospores of *P. microcoralloides* are more than twice the length of those of *P. nanoarbuscula*. Although thalli of these two *Porina* species occur in the same habitats and are sometimes found growing alongside each other, phylogenetic analysis of *rbcL* sequences suggest that they partner with distinct clades of *Trentepohlia* phycobionts. A third taxon examined, *Porina* cf. *scabrida*, is morphologically rather similar to *P. microcoralloides*, but the ascendant branches are bright yellow-orange, more cylindrical, and corticated by a thin layer of agglutinated fungal hyphae; perithecia were not seen. Analysis of mtSSU sequences places it distant from *P. microcoralloides* and *P. nanoarbuscula* phylogenetically. None of the Floridian taxa studied was particularly close to the European isidiate species *Porina hibernica* and *P. pseudohibernica*, which appeared as sister to each other in the analysis. While a particular type of isidiose structure may be reliably characteristic of specific taxa, similarities or differences in these structures do not seem to be useful indicators of phylogenetic proximity or distances among taxa. The morphological trends evident in *Porina* suggest that multiple transitions from crustose to isidioid or microfruticose growth have arisen repeatedly and in quite different ways within this single genus. At least some of the diverse structures treated within the broad concept of isidia may be representative of the developmental pathways by which fruticose growth forms may arise.

Keywords: epiphytic; isidia; lichens; mycobiont; phylogeny; *Trentepohlia*

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Introduction

A majority of lichens develop within the mainly two-dimensional confines of crustose or appressed foliose growth forms closely associated with the substratum surface. Others exploit three-dimensional space by growing and branching upward and outward as fruticose forms. While still only partially explored, there can be significant ecological implications associated with lichen growth forms and overall thallus morphology (e.g. Larson & Kershaw 1976; Pintado *et al.* 1997; Sojo *et al.* 1997; Esseen *et al.* 2015). For example, ascending forms may overgrow and outcompete lower-growing crustose lichens and bryophytes for light (Jahns 1988), as occurs in vascular plant communities. The more extensive surface area of fruticose forms may be more efficient in condensing and absorbing moisture from fog

Corresponding author: William Sanders; Email: wsanders@fgcu.edu

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and dew, but will lose moisture more readily when drying conditions prevail (Larson 1981). The energetic costs associated with extensive, supportive mycobiont tissues may also limit the practicality of such thallus forms in warm, humid climates where high respiration rates result in substantial carbon loss at night and under low light conditions (Zotz & Winter 1994). On a broad scale, evolutionary transitions between crustose and fruticose growth forms have occurred many times among lichen-forming fungi, and in both directions. In a few remarkable cases, they may be observed within a single species (e.g. *Lecanora swartzii* (Ach.) Ach.; Poelt 1989), and apparently correlated with environmental gradients (Weber 1967; Kunkel 1980; Pérez-Ortega *et al.* 2012). Additionally, most species of the hyperdiverse genus *Cladonia* have dimorphic thalli, where fruticose axes arising from the horizontal thallus are thought to be homologous with apothecial stipe tissue that later acquired an algal layer and assimilative function (Krabbe 1891; Jahns 1970; Ahti 1982). Nonetheless, a single basic growth form is usually characteristic of a given genus, with relatively few exceptions (e.g. Tehler & Irestedt 2007; Sohrabi *et al.* 2013). Indeed, lichen growth form may sometimes indicate phylogenetic relationships better than other characters, even at higher taxonomic levels, such as the alectorioid clade within the *Parmeliaceae* (Crespo *et al.* 2007). Thus, it appears that where growth form and its strategic implications are concerned, lichen lineages have tended to be conservative, at least at the lower taxonomic levels.

From a principally two-dimensional thallus, however, some degree of vegetative upgrowth and branching in three-dimensional space may also occur. This is evident in the formation of isidia, appendicular organs of diverse structure, development, and phylogenetic origin (Beltman 1978). They are common in foliose and fruticose lichens, occurring more rarely in crustose forms (Jahns 1973), and have long been considered useful as a distinguishing character at species-level (Poelt 1973). Isidia are integral components of the thallus that arise as protuberances of the upper cortex, incorporating fungal and algal tissue as they develop (Hale 1983; Barbosa *et al.* 2009). The concept of isidia is very broad. Some are transitional with soredia, erumpent symbiotic propagules arising from below the cortex, but isidia are usually distinguished from soredia by their possession of a cortex (Jahns 1973; Beltman 1978). Those isidia that are easily detached may serve, like soredia, as vegetative diaspores (Honegger 1987*a*; Scheidegger 1995; Zoller *et al.* 2000), while the basal scars left upon the thallus may aid in CO_2 diffusion, as do pseudocyphellae. Sturdier, less easily detached isidia increase thallus surface area for photosynthesis and condensation, absorption or external storage of moisture (Jahns 1984; Rikkinen 1997; Tretiach *et al.* 2005); they may also allow more efficient assimilation of CO_2 (Tretiach *et al.* 2005). Such isidia permit the lichen to take at least partial advantage of three-dimensional space for additional access to light, carbon and/or moisture resources, on a more limited scale than fully fruticose thalli but without relinquishing the extensive contact with substratum resources enjoyed by the underlying thallus. Thus, a lichen's adoption of two-dimensional versus three-dimensional growth patterns has functional significance for its success. Repeated transitions between these growth strategies within a single genus are therefore likely to indicate substantial environmental selection pressures exerted upon morphology.

In south-western Florida, recent examination of lichen communities on *Taxodium* bark revealed several phenotypically different types of minutely fruticose thallus structures containing *Trentepohlia* photobionts, often without a well-developed basal crustose thallus. The identities of the lichens, which were initially found without sexual structures, remained mysterious until molecular sequences and eventually perithecia indicated their affinities within the genus *Porina*. As one of the larger lichen-forming fungal genera, *Porina* currently includes some 140–300 species, depending on circumscription (McCarthy 2013; Lücking *et al.* 2017). They occur on bark, rock and leaf substrata, with the highest diversity in humid subtropical and tropical regions. *Porina* is known as a genus of crustose lichens, with several species described as isidiate (Swinscow 1962; James 1971; Harris 1995; Cáceres *et al.* 2013; Tretiach 2014; Diederich *et al.* 2017; Orange *et al.* 2020; Ertz & Diederich 2022). In the present work, we attempt to better understand the structural and phylogenetic context of such morphological transitions within the genus *Porina*. We describe and compare the structure of our isidiate/microfruticose *Porina* collections using light and scanning electron microscopy, and examine molecular markers to determine phylogenetic placement among other *Porina* species for which sequences are available. Additionally, since unstratified crustose lichens commonly show intracellular haustorial penetrations while fruticose lichens are usually found to have non-intrusive fungal-algal contacts (Tschermak 1941; Honegger 1986), we examine symbiont interfaces with TEM to see how this paradigm might apply in our structurally transitional lichen collections.

Materials and Methods

Sample collection and microscopy

Lichens were collected on *Taxodium* bark within and near the margins of seasonally flooded groves on the Florida Gulf Coast University campus, at a nearby residential community, at Corkscrew Swamp Sanctuary, and at Corkscrew Regional Ecosystem Watershed (CREW) in Lee County and Collier County, Florida. Voucher/type specimens will be deposited at FLAS (University of Florida), with duplicates at BR and TSB herbaria.

Thalli were examined and photographed with an Olympus SZX12 dissecting microscope equipped with an Infinity 3S camera. Fruticose branches and hand sections of perithecia were wet mounted in tap water, 10% KOH (K), or in Lugol's iodine solution (1% I_2) without (I) or with K pre-treatment (KI), and photographed through an Olympus BX51 compound microscope. Colour reactions of the thallus were studied using K, common household bleach (C), crystals of para-phenylenediamine dissolved in ethanol (PD) and long wave UV (366 nm). Ascospores measurements are indicated as (minimum value–)mean(–maximum value), followed by the number of measurements (*n*).

Specimens were affixed to SEM stubs with carbon adhesive, coated with gold, and examined with an FEI Inspect scanning electron microscope (Thermo Fisher Scientific, Waltham, Massachusetts).

Specimens selected for embedding were hydrated in Petri dishes with moist filter paper 24 h prior to further processing according to de los Ríos & Ascaso (2002). The samples were then fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) for 3 h at 4–5 °C, with vacuum infiltration for three 10-min periods inside a desiccator during the initial portion of the fixation period. Specimens were then washed three times, 30 min each, with phosphate buffer at room temperature, followed by post-fixation in 1% osmium tetroxide for 5 h in the dark. The post-fixed material was then washed three times in buffer, dehydrated in an ethanol series followed by propylene oxide, then

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Table 1. *Porina* specimens newly sequenced and included in the phylogenetic analyses of the present study, with their collection and DNA extraction numbers and NCBI Accession codes for the ITS, mtSSU and *rbcL* marker sequences obtained. NAS and WBS refer to collection numbers of N. A. Sanderson and the first author, respectively; TSB refers to collections accessioned at the University of Trieste Herbarium.

Lichen species	WBS / TSB ID	Samples ID	mtSSU	ITS	*rbcL*
Porina hibernica	NAS 2894	L4415	OR036953	-	OR053633
P. hibernica	NAS 2895	L4416	-	-	OR053634
P. hibernica	NAS 2896	L4417	OR036954	OR036908	OR053635
P. microcoralloides	WBS 20423.11b	L3124	OR036926	-	OR053611
P. microcoralloides	WBS 20424.5	L3125	OR036927	-	OR053612
P. microcoralloides	WBS 20511.2	L3126	OR036928	-	OR053622
P. microcoralloides	WBS 20423.9	L3127	OR036929	-	OR053613
P. microcoralloides	WBS 20423.6	L3128	OR036930	-	OR053614
P. microcoralloides	WBS 20510.3	L3131	OR036933	-	OR053617
P. microcoralloides	WBS 20425.1	L3134	OR036935	-	OR053619
P. microcoralloides	WBS 20506.3	L3136	OR036937	-	OR053621
P. microcoralloides	WBS 20510.2	L3137	-	-	OR053623
P. microcoralloides	WBS 21312.16	L4243	OR036944	-	OR053628
P. microcoralloides	WBS 21312.17	L4244	OR036945	-	-
P. microcoralloides	WBS 21320.5	L4245	OR036946	-	-
P. microcoralloides	WBS 21502.2	L4246	OR036947	-	OR053629
P. microcoralloides	WBS 21410.1	L4247	OR036948	-	OR053630
P. microcoralloides	WBS 21425.6	L4248	OR036949	-	OR053631
P. nanoarbuscula	WBS 20511.3	L3129	OR036931	-	OR053615
P. nanoarbuscula	WBS 20423.1	L3130	OR036932	OR036909	OR053616
P. nanoarbuscula	WBS 20424.4	L3133	OR036934	-	OR053618
P. nanoarbuscula	WBS 21320.1	L4236	-	OR036914	-
P. nanoarbuscula	WBS 21403.5	L4237	OR036938	OR036913	OR053624
P. nanoarbuscula	WBS 21421.5	L4238	OR036939	OR036912	OR053625
P. nanoarbuscula	WBS 21421.8	L4239	OR036940	OR036911	-
P. nanoarbuscula	WBS 21502.2	L4240	OR036941	OR036910	OR053626
P. pseudohibernica	TSB 44450	L4360	OR036950	OR036902	-
P. pseudohibernica	TSB 44451	L4361	OR036951	OR036906	OR053632
P. pseudohibernica	TSB 44452	L4362	-	OR036907	-
P. pseudohibernica	TSB 44453	L4363	-	OR036903	-
P. pseudohibernica	TSB 44454	L4364	-	OR036905	-
P. pseudohibernica	TSB 44455	L4365	OR036952	OR036904	-
P. cf. *scabrida*	WBS 21212.7	L4241	OR036942	-	OR053627
P. cf. *scabrida*	WBS 21320.3	L4242	OR036943	-	-
Porina sp.	WBS 20509.4	L3135	OR036936	-	OR053620

infiltrated in a 1:1 mixture of propylene oxide and Spurr's low-viscosity resin. The following day, the specimens were infiltrated with fresh resin, left for three days in the refrigerator, then polymerized at 60 °C.

Semi-thin sections were cut 1–2 µm thick and stained with toluidine blue. Ultrathin sections 79 nm thick were stained with uranyl acetate followed by lead citrate.

Molecular analyses: DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from a total of 35 lichen thalli (Table 1), following the CTAB protocol according to Cubero *et al.* (1999). The Floridian samples used for DNA extraction corresponded to those that were morphologically studied; two samples of *Porina hibernica* P. James & Swinscow and three of *P.*

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pseudohibernica Tretiach, collected in their type localities, were also included. Mycobiont sequences were compared with those available in GenBank. Part of the DNA coding for the small subunit of the mitochondrial ribosome (mtSSU) was amplified using the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999). The ITS locus was amplified using the forward primer ITS1F (Gardes & Bruns 1993) and the specific *Porina* reverse primer ITSPoR (5′ - CCT TGC CTG ATC CGA AGT GAA ACC G - 3′; Orange *et al.* 2020). Chloroplast DNA corresponding to the large subunit of ribulose-1,5-biphosphate carboxylase (*rbcL*) was amplified with the primers rbcL803rev and rbcl320 (Nozaki 1995) to check the identity of the photobiont. The PCR conditions for the amplification of the mtSSU locus followed Orange *et al.* (2020), while those for the *rbcL* locus followed Muggia *et al.* (2008, 2010). The PCR products were purified with Mag-Bind® Total Pure NGS; Sanger sequencing was performed by Macrogen Europe, Inc. (Amsterdam) using the forward primers for all loci. The identity of the sequences was checked with a BLAST search (Altschul *et al.* 1990) in the NCBI database.

Phylogenetic analyses

The phylogenetic analyses of *Porina* mycobionts included the newly generated mtSSU and ITS sequences, plus 139 sequences retrieved from NCBI for the mtSSU locus and 38 for the ITS locus. All sequences were aligned in a comprehensive dataset individually constructed for each locus. For those samples for which both ITS and mtSSU sequences were available (i.e. those 15 retrieved from NCBI and nine new samples we collected), a multilocus concatenated dataset was prepared in MEGA11 (Tamura *et al.* 2021). *Coenogonium leprieurii* (Mont.) Nyl., *C. luteum* (Dicks.) Kalb & Lücking, *C. pineti* (Ach.) Lücking & Lumbsch, *Gyalidea praetermissa* Foucard & G. Thor and *Sagiolechia protuberans* (Ach.) A. Massal. were selected as outgroups, according to Orange *et al.* (2020) and Ertz & Dieterich (2022), for the mtSSU dataset; *Porina austroatlantica* P.M. McCarthy & Fryday and *Porina multipuncta* (Coppins & P. James) Ertz, *et al.* were selected as outgroups for the ITS dataset, according to Orange *et al.* (2020), and *P. austroatlantica* was also set as outgroup for the concatenated ITS + mtSSU dataset. The phylogenetic analyses of the photobiont included the newly generated *rbcL* sequences and 444 sequences retrieved from NCBI, including the genera *Trentepohlia* and *Printzina*, while as outgroups the species *Batophora oerstedii*, *Bornetella nitida, Bryopsis hypnoides*, *Caulerpa prolifera, Halimeda discoidea*, *H. opuntia, Polyphysa peniculus* and *Ulva linta* were selected (according to Rindi *et al.* (2009) and Borgato *et al.* (2022)). The alignments were performed using MAFFT v.7 (Katoh *et al.* 2002) with MSA algorithm set on 100 bootstrap replicates and G-ins-I as the substitution model, and then manually adjusted in BioEdit v.7.2.5 (Hall 1999).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were run for both the *Porina* and photobiont datasets on the CIPRES web portal (Miller *et al.* 2010), using the programs RaxML v. 8.2.12 (Stamatakis 2014) and MrBayes v. 3.2.7a (Huelsenbeck & Ronquist 2001), respectively. The ML analysis used the GTRGAMMA substitution model, with 1000 bootstrap replicates; the BI was carried out setting two parallel runs with six chains over five million generations, starting with a random tree and sampling every 100th step. We discarded the first 25% of the data as burn-in, and the corresponding posterior probabilities (PPs) were calculated from the remaining trees.

The phylogenetic trees were visualized in TreeView v. 1.6.6 (Page 1996). Species level lineages were recognized as those clades that were monophyletic, fully supported, and represented by more than two samples; they were named according to Orange *et al.* (2020), Ertz & Diederich (2022) and Borgato *et al.* (2022).

Results

Structural features of the material examined

Porina microcoralloides. The ascending thallus consisted of somewhat irregularly swollen, branching, coralloid microfruticose structures, ranging in colour from yellowish brown to dark brown or dark olive green (Fig. 1A–F & K). The crustose thallus at the base of these structures appeared patchy, often inconspicuous or evanescent (Fig. 1B, C & E). Basal thallus portions comprising a superficial crust consisted of a mixture of scattered fungal hyphae and individual rounded cells or short filaments of *Trentepohlia* phycobionts, without any discernable organization into discrete tissue layers (Fig. 2A). Material associated with the cell walls of some of the superficial fungal cells formed a chiefly acellular epilayer at the surface of the crustose thallus (Fig. 2A). In other places, the basal thallus consisted of mycobiont hyphae and *Trentepohlia* cells growing loosely over the substratum (Fig. 3E) and/or occupying empty cell lumina of the bark substratum (Fig. 2B, C & F), from which isidioid structures emerged directly (Figs 2B, 3G & H).

In contrast with substratic thallus portions, the ascending isidioid structures had a distinctly stratified anatomy. They were composed of a central region of sparse fungal hyphae surrounded by a peripheral layer of subspherical algal cells (Fig. 2A, C & D). Some fungal cells were exterior to, as well as interspersed among, the algal symbionts, and a largely acellular epilayer of material associated with these fungal cells was sometimes evident in sections (Fig. 2E), but no organized cortex was present (Fig. 3A–D). Indeed, algal cells were often visible at the exterior surface in scanning electron micrographs (Fig. 3C, D & F). Within zones of intimate symbiont contact, algal cell walls were invaginated to a modest degree around an intrusive protuberance of a fungal cell whose wall often appeared reduced in thickness at the contact point (Fig. 2G).

Perithecia were approximately globose, sometimes somewhat pyriform, and mostly immersed in the plant substratum; they possessed a thick, carbonized wall (Figs 2H, 4A & B). Ascospores were long-bacilliform to needle-shaped, averaging 66 µm × 3.7 µm, with *c.* 11–13 transverse septa (Fig. 4C–F).

Porina nanoarbuscula. Crustose thallus portions on the substratum surface were often patchy and of limited extent (Fig. 1G & I). They consisted of subglobose to short filamentous photobiont cells and scattered mycobiont hyphae without stratification or any indication of cortical development (Fig. 5A). A chiefly acellular epilayer of material associated with the cell walls of some superficial mycobiont cells often formed at the upper surface (Fig. 5A). In other places, the surface crustose layer was not developed, and ascending isidioid structures arose from a disorganized mixture of mycobiont and photobiont cells colonizing dead, superficial cells within the plant substratum (Figs 1J, 5B & C). The isidioid structures were exceptionally fine and densely branched, the branches breaking and detaching readily upon mechanical contact (Figs 1H, 6A, B & H). They were each composed of a single central file of more or less globose *Trentepohlia* cells, surrounded

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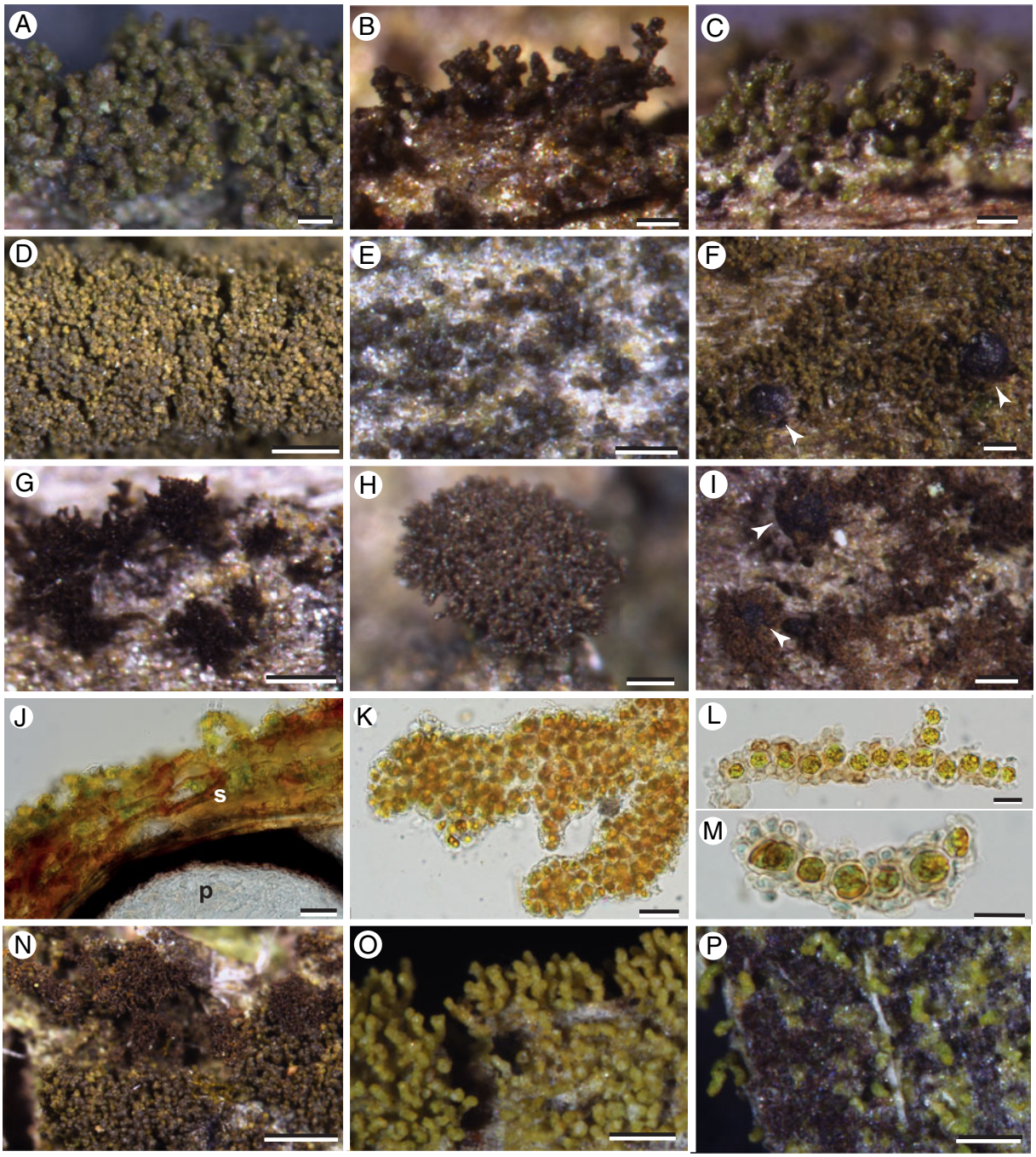

Figure 1. *Porina microcoralloides*, *P. nanoarbuscula* and *P.* cf. *scabrida* from south-west Florida. Dissecting microscope and whole mounted compound microscope images. A–F, *P. microcoralloides.* G–I, *P. nanoarbuscula*. Arrowheads: perithecia. J, section through plant substratum (s) with embedded thallus and underlying perithecium (p) of *P. nanoarbuscula*. K, *P. microcoralloides*, isidioid structure whole-mounted in water. L & M, *P. nanoarbuscula*, isidioid structure whole-mounted in water and aniline blue, respectively. N, *P. microcoralloides* (lower half of image) and *P. nanoarbuscula* (upper half of image) growing intermixed. O & P, *P.* cf. *scabrida*. Scales: A, B, C & H = 100 µm; D & N = 500 µm; E & G = 200 µm; F, I, O & P = 250 µm; J & K = 25 µm; L & M = 10 µm. (A, WBS 20425.1; B, WBS 20424.6; C, WBS 20424.6; D, WBS 20423.9; E, WBS 20425.4; F, WBS 21501.5; G, WBS 20424.4; H, WBS 20423.2; I, WBS 21421.8; J, WBS 20424.6; L & M, WBS 20423.9a; N, WBS 20423.2; O, WBS 20506.1; P, WBS 21212.7). In colour online.

peripherally by relatively swollen, subglobose mycobiont cells (Figs 1L & M, 5B & D, 6C–H). Short chains of these cells were also occasionally seen running along the substratum surface in the vicinity of the isidioid thallus (Fig. 6I). Within differentiated symbiont contact zones, algal cell walls were invaginated to a modest degree around the intrusive protuberance of a fungal cell whose wall often appeared reduced in thickness at the contact point (Fig. 5E).

Perithecia were approximately globose with a thick, carbonized wall, mostly immersed in the plant substratum (Fig. 4G). Ascospores were bacilliform, averaging 29 × 3.3 µm, with 3–5 transverse septa (Fig. 4H–M).

Porina *cf.* scabrida. The thallus resembled that of *P. microcoralloides*, but was orangey yellow with somewhat more vertical, cylindrical branches (Figs 1O & P, 7A & B) containing clusters

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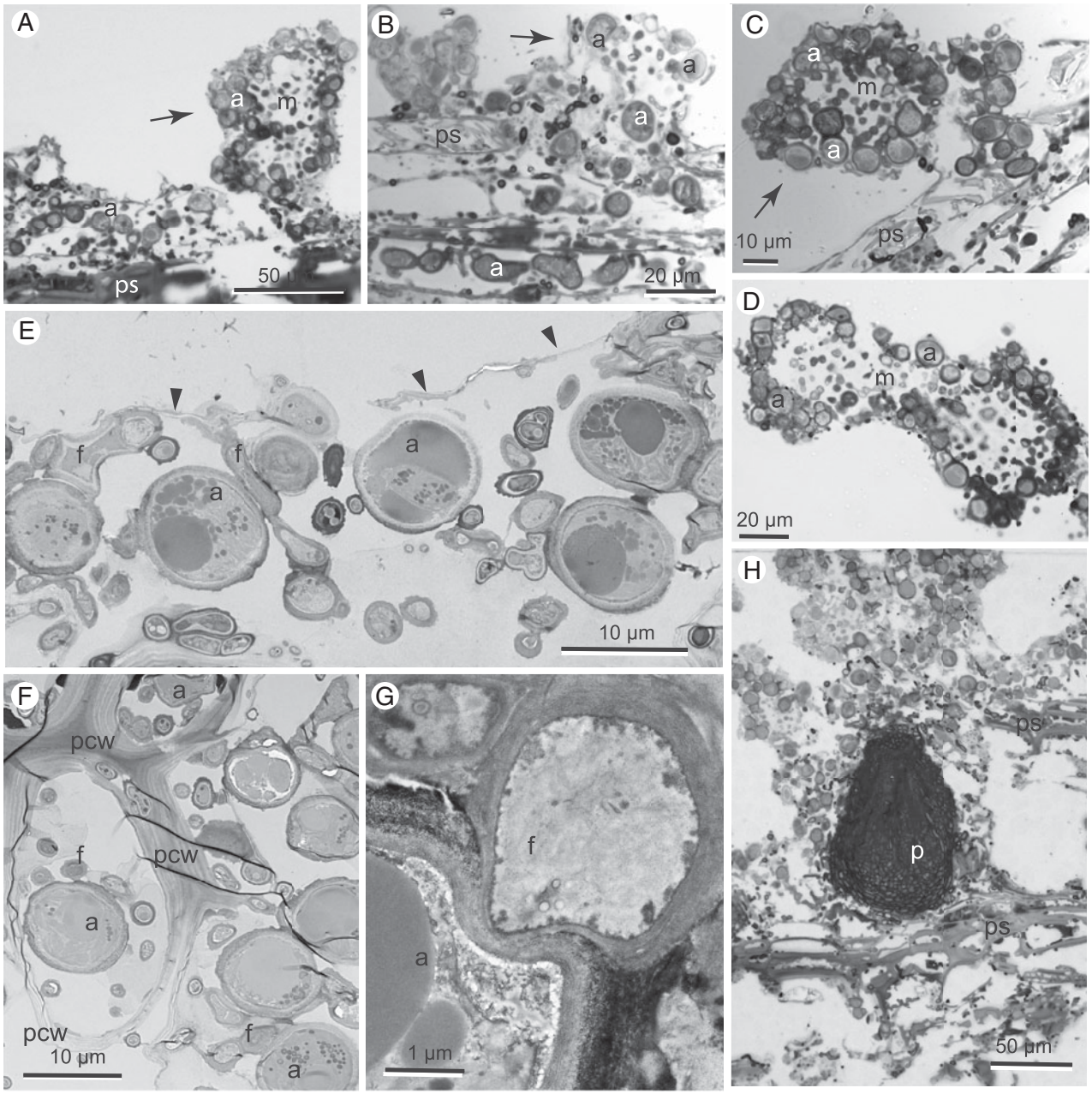

Figure 2. Sections of resin-embedded thalli of *Porina microcoralloides*, examined with light microscopy (A–D) and TEM (E–G). A, unstratified crustose thallus on surface of plant substratum (ps) at left; isidioid structure (arrow) with heteromerous anatomy at right, showing algal layer (a) surrounding medulla (m). B, isidioid primordium (arrow) emerging from plant substratum (ps); phycobiont (a) unicells and filaments in primordium and within lumen of dead plant cells below. C, later stage of emergence directly from plant substratum: note stratification of primordium into algal layer (a) and medulla (m). D, section through portion of a mature isidioid structure. E, periphery of isidioid structure, with mycobiont cells (f) interspersed among algal symbionts (a) and partial epilayer of material associated with fungal cell walls (arrowheads). F, lichen symbionts associated within the confines of substratum plant cell walls (pcw). G, intrusive symbiotic contact between mycobiont (f) and phycobiont (a), showing local invagination of algal cell wall and thinning of fungal wall in contact zone. H, perithecium (p) developing with delaminated layers or plant substratum (ps). Scales: A & H = 50 µm; B & D = 20 µm; C, E & F = 10 µm; G = 1 µm.

of calcium oxalate crystals. As in *P. microcoralloides*, phycobiont unicells were arranged in a distinct layer at the periphery of a lax central medulla of mycobiont hyphae (Fig. 7C). Exterior to the algal cells, however, a more developed layer of agglutinated fungal hyphae formed at the surface (Fig. 7D–G); exposed phycobiont cells were not observed at the surface, in contrast with *P. microcoralloides*. At symbiont contact zones, limited invagination of the algal cell wall was evident, with thinning of both algal and fungal cell walls visible at the point of 'haustorial' intrusion (Fig. 7H). Perithecia were not observed in the two collections of this taxon.

Porina hibernica *sample from Great Britain.* For comparison, the widely reported isidiate *Porina hibernica* was also studied. In the collection examined, irregular isidia-like upgrowths emerged from a crustose thallus (Fig. 8A–C). In section, these upgrowths appeared to lack stratification or differentiation of symbionts into discrete layers (Fig. 8C). Mycobiont and

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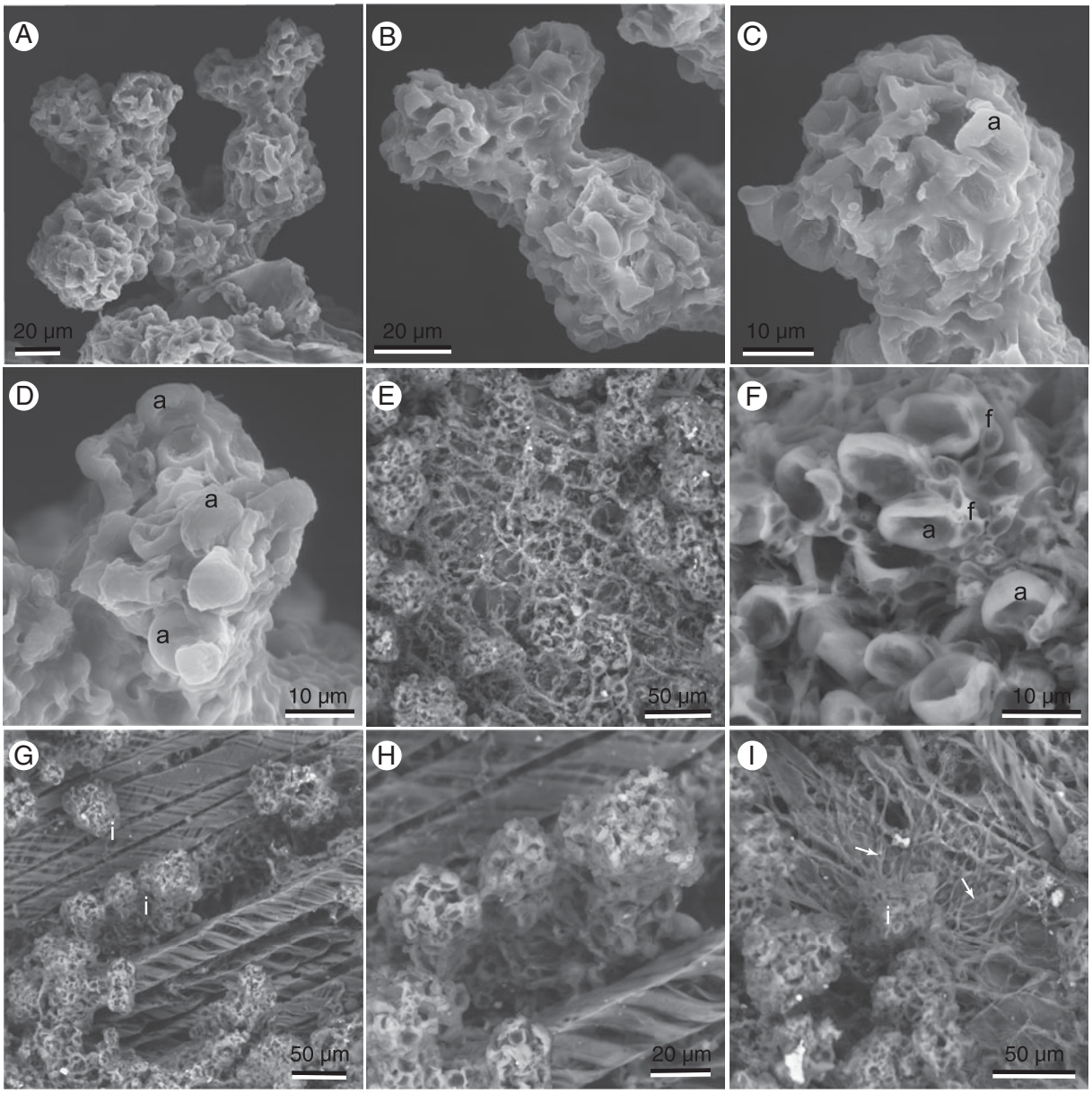

Figure 3. Scanning electron micrographs of *Porina microcoralloides*. A–D, views of branching isidioid structures, with algal cells (a) visible at surface. E, crustose mat of loosely organized symbionts (centre) with isidioid structures arising at periphery. F, detail of E showing *Trentepohlia* phycobionts (a) and associated mycobiont cells (f). G & H, isidioid structures emerging directly from plant substratum: note absence of any crustose thallus upon substratum surface. I, isidioid fragment (i) establishing on substratum; note radiating attachment hyphae (arrows). Scales: A, B & H = 20 µm; C, D & F = 10 µm; E, G & I = 50 µm.

phycobionts inhabited dead cells of the plant substratum (Fig. 8C–D), and dead cell walls of the plant substratum were incorporated into the isidia-like upgrowth (Fig. 8C). Slight invagination and substantial thinning of both phycobiont and mycobiont cell walls was evident at sites of symbiont contact (Fig. 8E).

Phylogenetic analyses

A total of 29 mtSSU and 13 ITS sequences for *Porina* and 25 *rbcL* sequences for their *Trentepohlia* photobionts were newly obtained in this study. The phylogenetic inferences based on the individual loci ITS and mtSSU of the mycobiont (Figs 9 & 10) were congruent with the recent phylogenetic reconstructions presented by Orange *et al.* (2020) and Ertz & Diederich (2022) for the genus *Porina*. The multilocus (ITS + mtSSU) phylogenetic reconstruction (see Supplementary Material Fig. S1, available online) was concordant with the single locus phylogenies.

Among *Porina* species for which mtSSU sequences were available, *P. microcoralloides* and *P. nanoarbuscula* were placed as well-supported sister clades (Fig. 9). The European isidiate taxa *P. hibernica* and *P. pseudohibernica* were well-supported clades sister to each other, and not very closely related to *P. microcoralloides* and *P. nanoarbuscula*. Rather, they were placed closely to *P. collina* Orange *et al.* and *P. byssophila* (Körb. ex Hepp) Zahlbr. This result is also corroborated by the two-loci analysis (Supplementary Material Fig. S1). The two sequenced samples of *Porina* cf. *scabrida* were quite distant from the four aforementioned taxa in an unresolved clade with *P. nucula* Ach. One last sample of *Porina*, namely L3135, was placed close to *Porina cryptostoma* Mont. and two sequences of *Porina nucula*. This single specimen appeared similar

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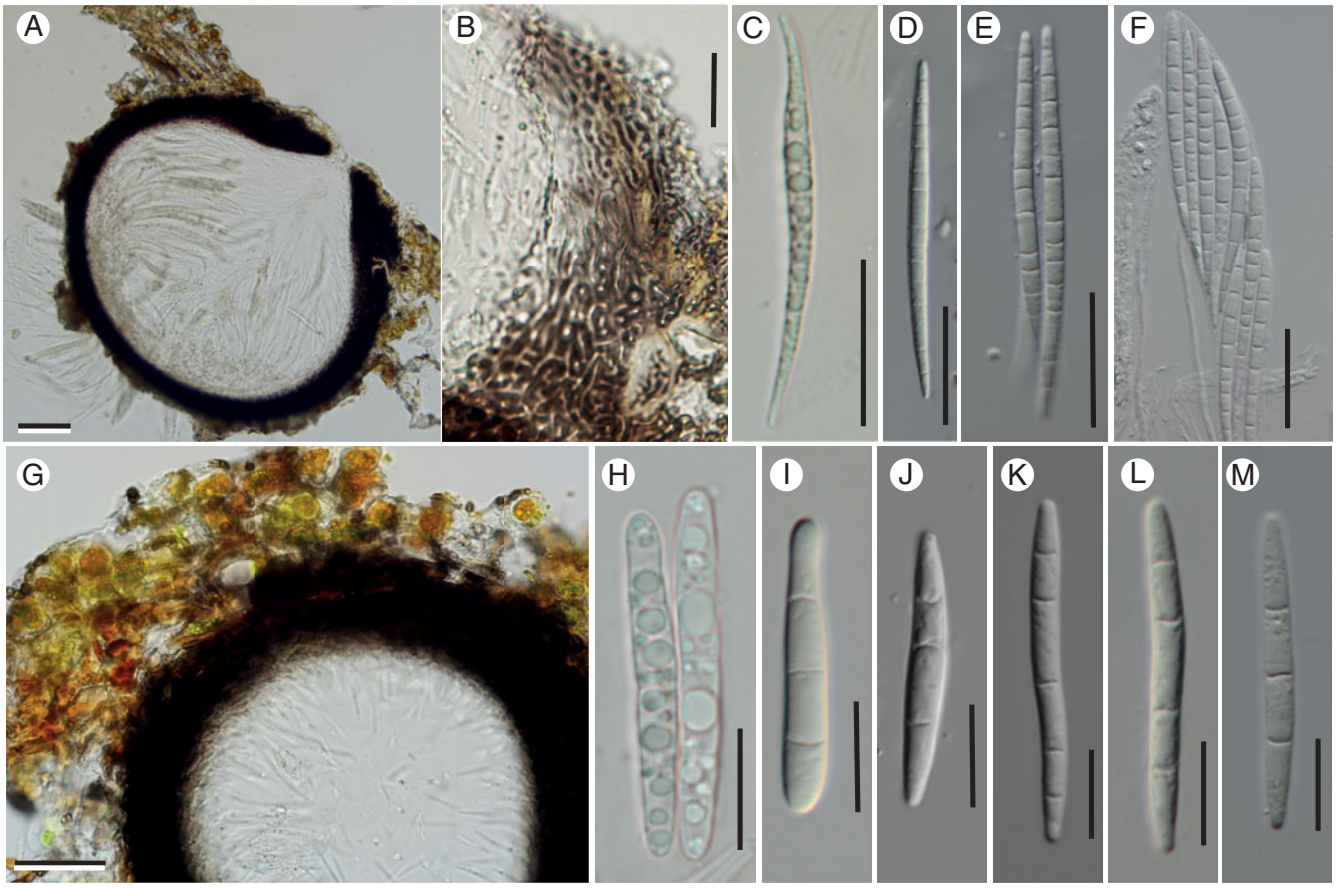

Figure 4. Perithecium and ascospores of *Porina microcoralloides* (A–F) and *P. nanoarbuscula* (G–M). A, perithecium. B, melanized perithecial wall tissue. C–E, free ascospores. F, ascospores in ascus (C, live cell, bright field; D–F, in KOH, DIC optics). G, perithecium. H–M, free ascospores (H, live cell, bright field; I–M, in KOH, DIC optics). Scales: A = 50 μm; B & G = 20 μm; C–F = 25 μm; H–M = 10 μm. In colour online.

to *P.* cf. *scabrida*, and its placement elsewhere was unexpected. We were unable to study it further in the present work.

The ITS phylogeny (Fig. 10) confirmed the sister relationship between *P. hibernica* (represented by a single sequence) and *P. pseudohibernica*, and their distance from *P. nanoarbuscula*, which was sister to *P. sorediata* Aptroot *et al.* (represented by a single sequence). However, relatively few ITS sequences were available from GenBank, nor were we able to obtain many from our collections. No ITS data could be obtained from *P. microcoralloides*, for which several attempts at PCR amplification were unsuccessful.

The *Trentepohlia* sequences we obtained from the isidiate *Porina* phycobionts segregate well into separate monophyletic clades. They are all relatively distant from the other *Trentepohliaceae*, forming a clearly defined major clade in the phylogeny. Phycobiont *rbcL* sequences from *Porina microcoralloides* and *P. nanoarbuscula* thalli indicated that these two mycobionts were each consistently associated with a distinct pool of *Trentepohlia* strains (Fig. 11; Supplementary Material Fig. S2, available online). Only one sequence was obtained for the *Porina* cf. *scabrida* photobiont, and it was placed close to two sequences from free-living *Trentepohlia* cf. *annulata* and *T.* cf. *umbrina*. A single sequence was also obtained for *Trentepohlia* sp. from *Porina pseudohibernica*, which was placed close to one from free-living *Trentepohlia annulata* and others (OL956825, OL956907) obtained from the lichen *Enterographa zonata*. The three new sequences obtained from *Porina hibernica* formed a single, well-supported clade.

The *Trentepohlia* sequence obtained from the sample L3135 was placed in Clade 31 *sensu* Borgato *et al.* (2022). This clade also included sequences obtained from lichen samples (species of *Enterographa*, *Opegrapha* and *Porina leptalea*) from European temperate forests.

Taxonomy

Porina microcoralloides Ertz, W. B. Sanders, R. Carolis, A. Ríos & Muggia sp. nov.

MycoBank No.: MB 848937

This species resembles *Porina coralloidea* P. James in its isidiate thallus and its small black perithecia but differs by having (8–)11–13(–15)-septate ascospores of (55–)66.3(–92) × (3–)3.7(–4.5) μm that are less septate (9–11 septa), shorter (40–57 μm) and much broader (9–13 μm) in *P. coralloidea*.

Type: USA, Florida, Lee County, Fort Myers, Florida Gulf Coast University campus, Cypress swamp north of Parking Garage 3, on *Taxodium* bark, 20 March 2021, *W. B. Sanders* 21320.5 (FLAS—holotype).

(Figs 1A–F, K & N, 2, 3, 4A–F)

Thallus consisting of a crustose basal portion from which isidioid structures emerged directly; basal thallus mostly

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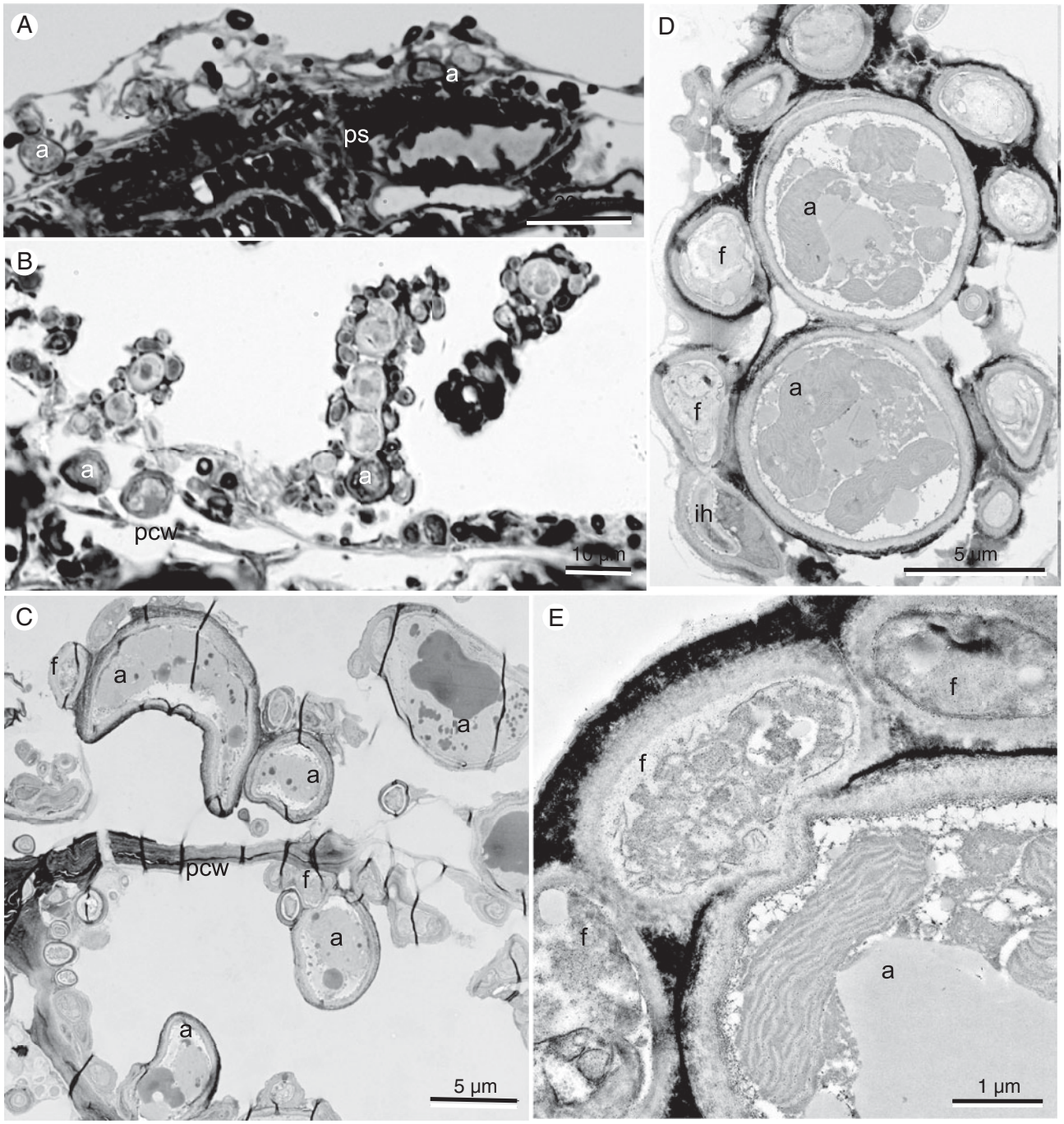

Figure 5. Sections of resin-embedded thalli of *Porina nanoarbuscula*, examined with light microscopy (A & B) and TEM (C–E). A, thin unstratified thallus crust on surface of plant substratum (ps). B, isidioid structure emerging from symbionts within substratum; pcw, plant cell wall. C, fungal (f) and algal (a) symbionts among cell walls of plant substratum (pcw). D, portion of isidioid structure showing uniseriate central strand of algal symbiont (a) and surrounding mycobiont cells (f); ih, intrahyphal hypha. E, intrusive symbiotic contact between mycobiont (f) and phycobiont (a), showing local invagination of algal cell wall and thinning of fungal wall in contact zone. Scales: A = 20 µm; B = 10 µm; C & D = 5 µm: E = 1 µm.

endophloeodal or rarely growing loosely over the bark substratum, ecorticate, consisting of a mixture of individual rounded cells or short filaments of trentepohlioid photobiont cells and scattered mycobiont hyphae without stratification; isidioid structures often abundant, forming dense clusters *c.* 0.1–1 mm diam. or covering more or less evenly larger areas of the substratum, ascending, richly branched-coralloid, irregularly swollen, 30–45(–50) µm broad and up to *c.* 350 µm long, easily breaking and detaching upon mechanical contact, yellowish brown to dark brown or dark olive green, each composed of a central region of sparse fungal hyphae surrounded by a peripheral layer consisting of subspherical algal cells *c.* (5–)7–11(–14) µm diam. interspersed or sometimes covered with some fungal hyphae *c.* 2–4 µm diam.; without crystals; *soralia* absent; *prothallus* inconspicuous, or presence of a black borderline *c.* 0.2–0.5 mm wide.

Ascomata perithecioid, scattered, rarely two contiguous, subglobose or rarely somewhat pyriform, black, smooth to slightly

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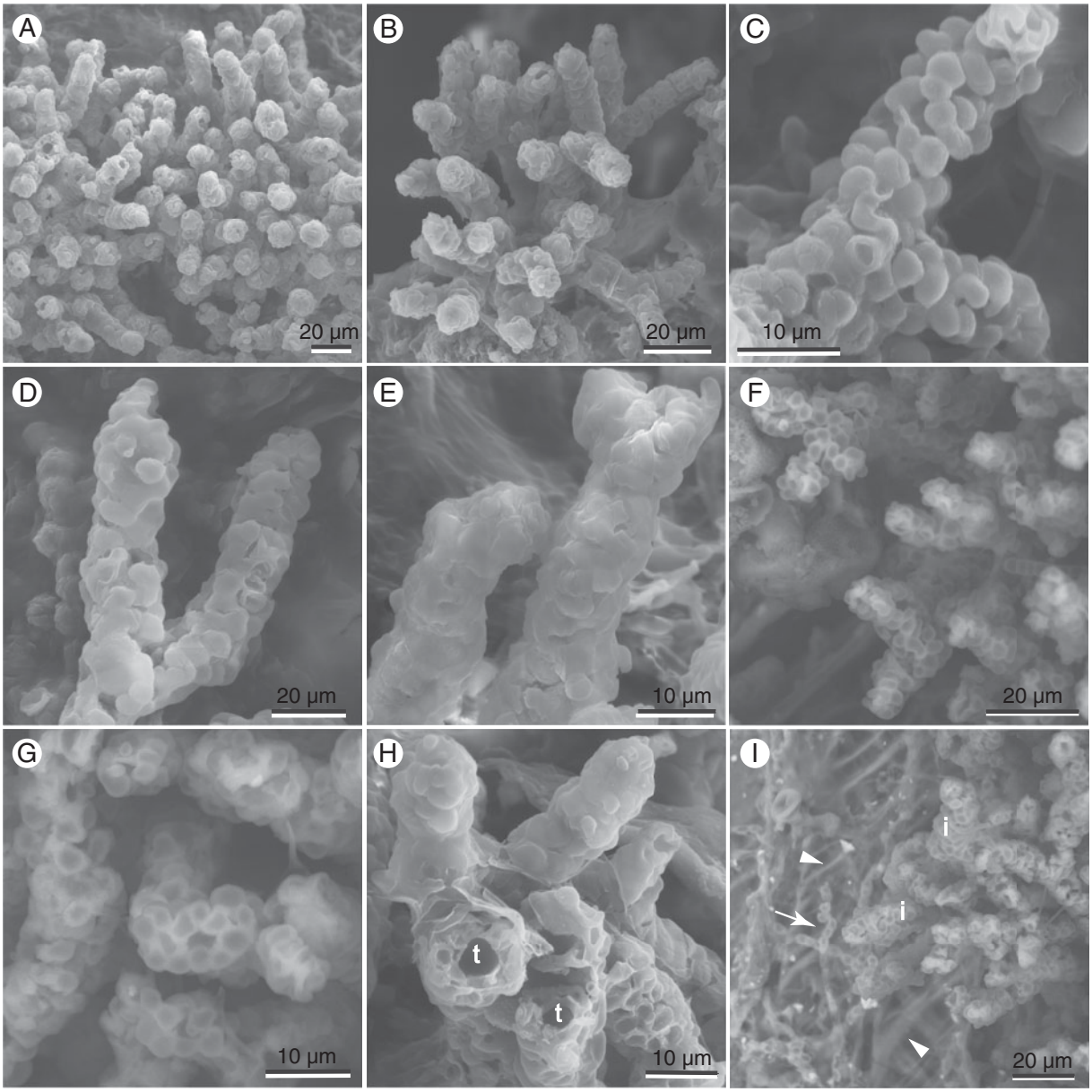

Figure 6. Scanning electron micrographs of *Porina nanoarbuscula.* A & B, densely branching isidioid structures. C, surface layer of subglobose fungal cells. D & E, surface layer, with some deposition of wall associated substances somewhat obscuring the individual fungal cells. F–I, backscattered electron detector images highlighting individual fungal cells of surface layer. H, broken ends of isidioid structures showing central zone (t) normally occupied by a single central file of *Trentepohlia* cells. I, low-magnification image showing isidioid structures (i) arising from substratum in absence of basal crust; arrows, mycobiont cells overrunning substratum; arrowheads, wall thickenings of plant substratum. Scales: A, B, D, F & I = 20 µm; C, E, G & H = 10 µm.

rugulose, (150–)234(–290) µm diam. (n = 16), *c.* one third to almost entirely immersed in the substratum, without thallus cover and setae; crystallostratum absent; ostiole apical, usually visible by a tiny black pore or inconspicuous. *Proper excipulum* dark reddish brown to carbonized all around the hymenium, K± olivaceous black, *c.* 20–45 µm thick. *Involucrellum* reduced, appearing as a thickening of the upper part of the excipulum, dark reddish brown or ±purplish brown to carbonized, K± olivaceous black, *c.* 45–70 µm thick. *Periphyses* numerous, 10–22 × 1.5–2 µm. *Hamathecium* hyaline, clear, of thin, simple or sparingly furcate-branched, (1–)1.5–2 µm diam.; *paraphyses c.* 110–150(–170) µm tall; *subhymenium* hyaline to pale fawn, 8–15 µm thick. *Asci* cylindrical-clavate to ±fusiform, *c.* (75–)100–118 × 13–15 µm (n = 11), 8-spored; ascus apex rounded, without a ring structure. *Ascospores* hyaline, transversely (8–)11–13(–15)-septate, long-bacilliform to needle-shaped, (55–)66.3(–92) × (3–)3.7(–4.5) µm (n = 25); usually with a gelatinous sheath *c.* 0.5 µm thick.

Pycnidia not observed.

Chemistry. Thallus and isidia K+ blackish, C–, PD–, UV–. TLC not performed.

Etymology. The epithet refers to the micro-coralloid habit of the thallus consisting mostly of ascending isidioid structures.

Distribution and ecology. The species is known from several localities in south-west Florida (Collier and Lee Co.), where it

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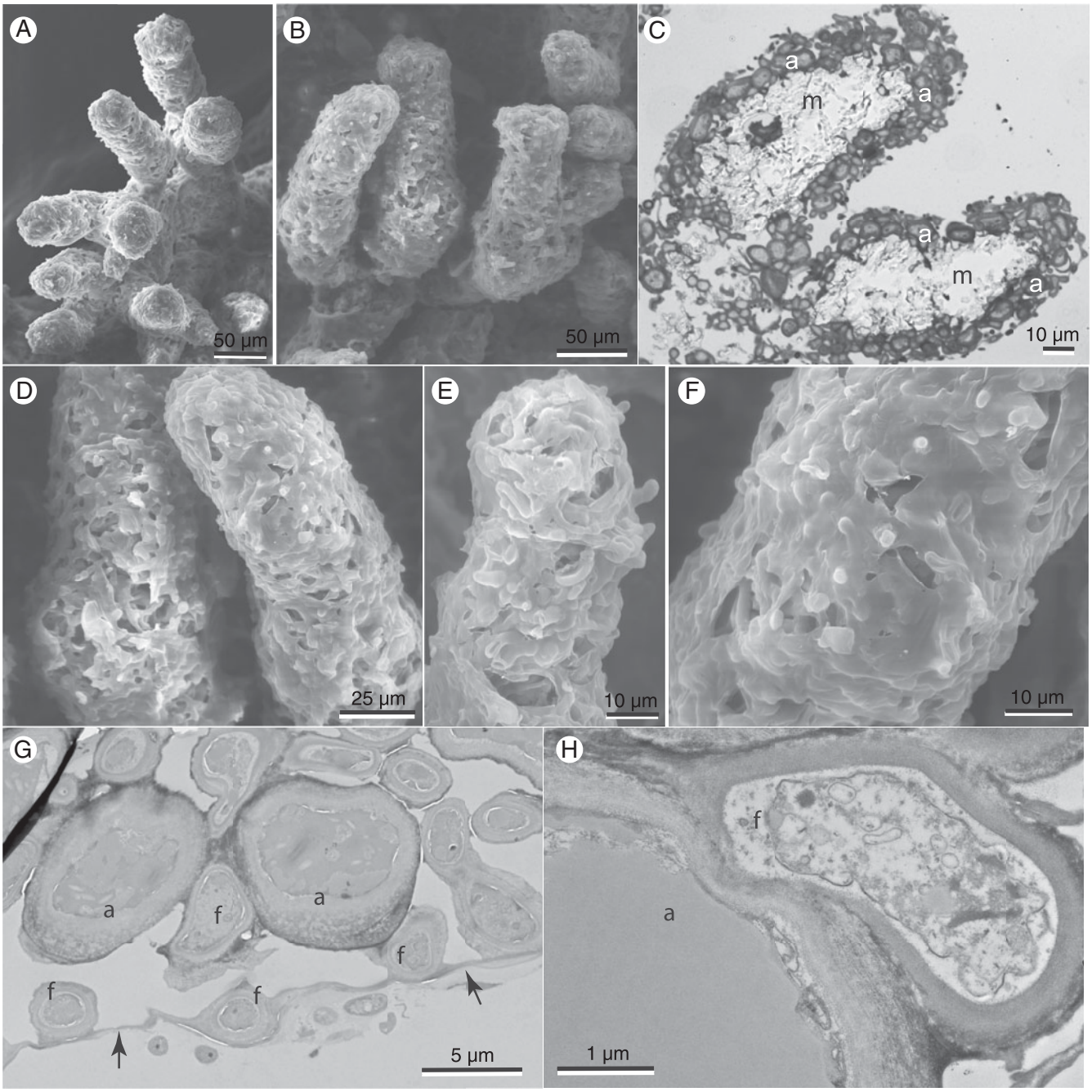

Figure 7. Light and electron micrographs of isidioid structures in *Porina* cf. *scabrida*. A & B, SEM images showing morphology of branches. C, resin-embedded semi-thin section showing algal layer (a) at periphery of a central medullary cavity (m). D–F, surface layer of agglutinated mycobiont hyphae; no exposed algal cells evident. G & H, TEM images of peripheral portion of structure. G, algal layer (a) and associated mycobionts cells (f), with wall-derived materials (arrows) forming an agglutinating layer among mycobiont cells at the surface. H, symbiont contact showing invagination of algal cell ahead of intruding mycobiont haustorium, and the walls of both symbionts substantially thinned at contact zone. Scales: A & B = 50 µm; D = 25 µm; C, E & F = 10 µm; G = 5 µm; H = 1 µm.

inhabits the bark of *Taxodium* within and near the margins of seasonally flooded groves, at low elevation (*c.* 3–6 m).

Notes. The long, almost needle-shaped ascospores distinguish *P. microcoralloides* from most isidiate species of *Porina*, although two non-isidiate taxa with spores at least as long and half the width have been recently described (Aptroot & Cáceres 2014). The British taxon *P. hibernica* has spores of similar length, but about twice as wide; its isidia, at least in the material we examined, were highly irregular in shape and lacked the stratified anatomy of *P. microcoralloides* (Swinscow 1962). Phylogenetic distance between these taxa was also evident in their mtSSU sequences (Fig. 9). In Florida, the isidiate taxon *Clathroporina isidiifera* has a black hypothallus and much shorter, wider ascospores (Harris 1995). A few species of *Porina* described recently by Ertz & Diederich (2022) from paleotropical Mauritius have isidia that somewhat resemble those of *P. microcoralloides*; however, they arise from well-developed crustose thalli, have quite different ascospore dimensions, and are placed distantly from *P. microcoralloides* in the mtSSU sequence analysis (Fig. 9).

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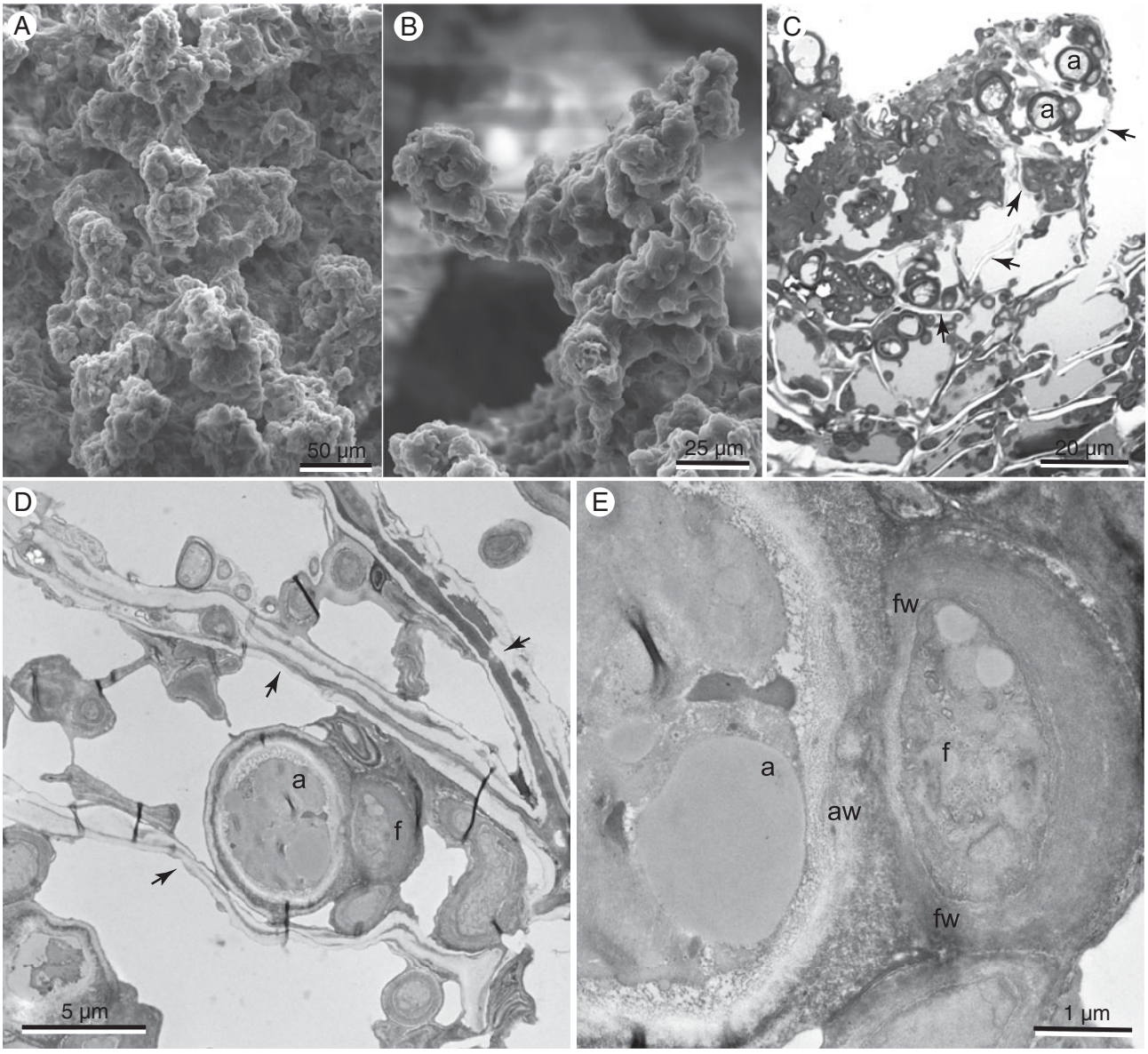

Figure 8. Light and electron micrographs of isidioid structures in *Porina hibernica* from Great Britain. A & B, SEM images showing outgrowth of irregularly shaped isidia from well-developed crustose thallus. C, semi-thin section of resin-embedded material; emerging isidium with unstratified algal cells (a) and incorporating the cell wall lattice (arrows) of the underlying plant substratum. D & E, TEM images. D, associated mycobiont (f) and phycobiont (a) within empty cells of plant substratum (arrows). E, detail of symbiont contact zone, showing slight invagination of algal cell wall and substantial thinning of fungal cell wall at contact point. Scales: A = 50 µm; B = 25 µm; C = 20 µm; D = 5 µm; E = 1 µm.

Additional specimens examined (all on Taxodium *bark).* **USA:** *Florida*: Lee County, Fort Myers, Florida Gulf Coast University (FGCU) campus, Cypress dome north of FGCU Blvd near Parking Garage 3, 2020, *W. B. Sanders* 20423.6 (FLAS, BR), 20423.11b (FLAS); *ibid*., north of *Thalia* swamp at centre of dome, 2020, *W. B. Sanders* 20423.9 (FLAS, BR); *ibid*., Cypress swamp north of Parking Garage 3, 2021, *W. B. Sanders* 21501.5 (TSB 44459); *ibid*., Cypress dome south of campus, near parking garage 2, 2020, *W. B. Sanders* 20511.2 (FLAS); *ibid*., Cypress dome between FGCU Blvd and Aquatic Center, 2020, *W. B. Sanders* 20424.5 (FLAS); *ibid*., Cypress dome near solar field, FGCU main entrance, 2020, *W. B. Sanders* 20425.1 (FLAS); *ibid*., Cypress swamp between swimming pool and FGCU Blvd, 2021, *W. B. Sanders* 21410.1, 21410.2 (FLAS); *ibid*., Cypress dome north of main entrance road, 2021, *W. B. Sanders* 21425.6 (FLAS); *ibid*., Estero, Grandezza, Cypress grove behind 'The Studio' sales centre, 2020, *W. B. Sanders* 20506.3 (FLAS); *ibid*., woods between Villa Grande and Grande Estates, 2020, *W. B. Sanders* 20510.3 (FLAS, BR); Collier County, Naples, Audubon Corkscrew Swamp Sanctuary, 2021, *W. B. Sanders* 21312.16, 21312.17 (FLAS).

Porina nanoarbuscula Ertz, W. B. Sanders, R. Carolis, A. Ríos & Muggia sp. nov.

MycoBank No.: MB 848938

This species resembles *Porina coralloidea* P. James by its isidiate thallus and its small black perithecia but differs by having 3–5 (–6)-septate ascospores, (20–)28.8(–37) × (3–)3.3(–4) µm;

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Figure 9. Phylogenetic hypothesis based on the mtSSU locus; 50% majority-rule consensus tree obtained by Bayesian analysis. ML bootstrap values > 70% shown in bold branches; Bayesian PP values > 0.8 are reported above branches. DNA extraction numbers of the new sequences obtained from *P. microcoralloides*, *P. nanoarbuscula*, *P.* cf. *scabrida* and an additional south Floridian collection (L3531), as well as *P. hibernica* and *P. pseudohibernica* are highlighted in bold. '*Porina chlorotica*' appears as several distinct clades labeled with letters in parentheses.

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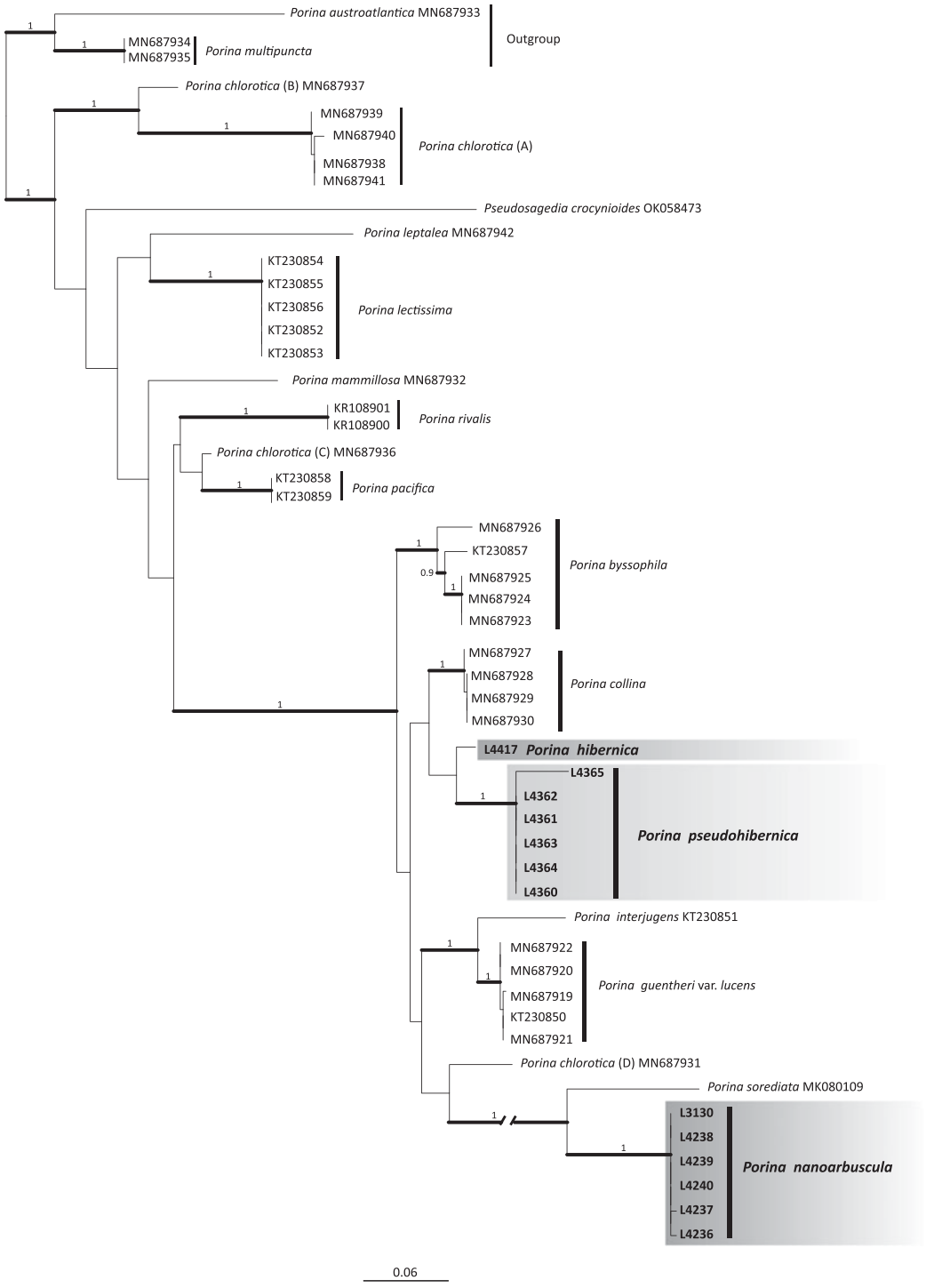

Figure 10. Phylogenetic hypothesis based on the ITS locus; 50% majority-rule consensus tree obtained by Bayesian analysis. ML bootstrap values > 70% shown in bold branches; Bayesian PP values > 0.8 are reported above branches. DNA extraction numbers of the new sequences obtained from *Porina nanoarbuscula, P. hibernica* and *P. pseudohibernica* are highlighted in bold. '*Porina chlorotica*' appears as several distinct clades labeled with letters in parentheses.

ascospores of *P. coralloidea* are more septate (9–11 septa) and much larger (40–57 × 9–13 µm).

Type: USA, Florida, Lee County, Fort Myers, Florida Gulf Coast University campus, Cypress swamp north of Parking Garage 3, on *Taxodium* bark, 3 April 2021, *W. B. Sanders* 21403.5a (FLAS—holotype).

(Figs 1G–J, L–N, 4G–M, 5 & 6)

Thallus consisting of a crustose basal portion from which isidioid structures emerged directly; basal thallus endophloeodal, ecorticate, consisting of subglobose to short filamentous trentepohlioid photobiont cells and scattered mycobiont hyphae without stratification; isidioid structures often abundant, forming dense clusters *c.* 0.2–0.6 mm diam. or covering more evenly larger areas of the substratum, ascending, densely branched, cylindrical, fine, 12–16(–20) µm broad and up to *c.* 200 µm long, easily breaking

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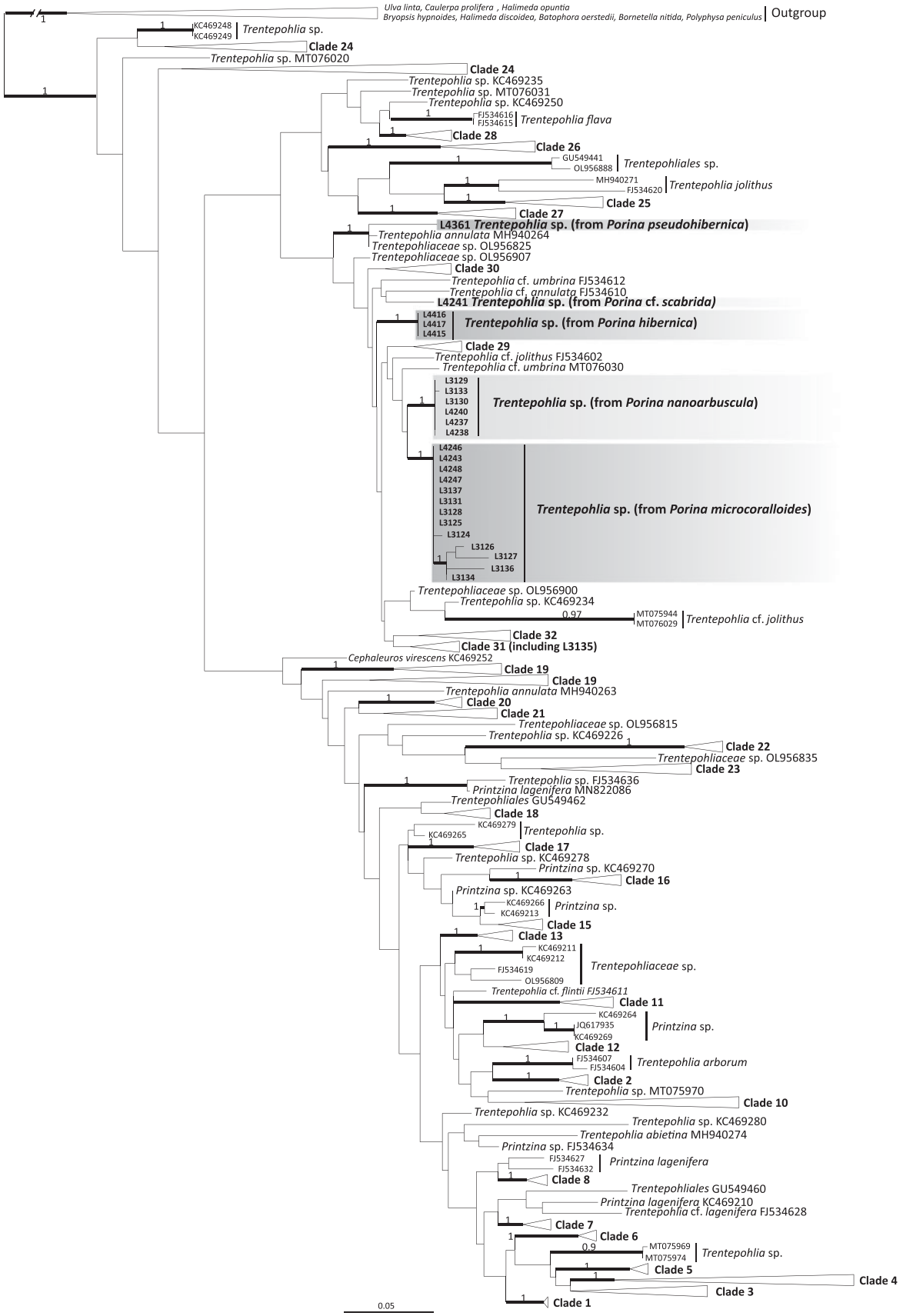

Figure 11. Phylogenetic hypothesis based on phycobiont plastidial *rbcL* locus. 50% majority-rule consensus tree from Bayesian analysis. ML bootstrap values > 70% are shown in bold branches; Bayesian PP values > 0.8 are reported above branches. DNA extraction numbers of the new sequences obtained for *Trentepohlia* sp. are in bold. Clade numbers in the phylogeny correspond to those assigned in Borgato *et al.* (2022).

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and detaching upon mechanical contact, orange-brown to dark brown, each composed of single central file of more or less globose *Trentepohlia* cells *c.* (6–)8–11 µm diam., surrounded peripherally by relatively swollen, subglobose to slightly elongated mycobiont cells (3–)4–5 µm diam. or 5–6 × 3–4 µm, without crystals; *soralia* absent; *prothallus* inconspicuous.

Ascomata perithecioid, scattered, rarely two contiguous, subglobose, black, smooth to slightly rugulose, 155–264.4–350 µm diam. ($n = 46$), *c.* two fifths to almost entirely immersed in the substratum, without thallus cover and setae; crystallostratum absent; ostiole apical, visible by a tiny black pore or inconspicuous. *Proper excipulum* dark reddish brown to carbonized all around the hymenium, K± olivaceous black, *c.* 18–25 µm thick. *Involucrellum* reduced, appearing as a thickening of the upper part of the excipulum, sometimes extending slightly laterally when the perithecia is almost entirely immersed in the substratum, dark reddish brown to carbonized, K± olivaceous black, *c.* 40–50 µm thick. *Periphyses* numerous, *c.* 5–30 × 1.5–2 µm. *Hamathecium* hyaline, clear, of thin, simple, (1–)1.5–2 µm diam.; *paraphyses c.* 125–130 µm tall; *subhymenium* hyaline to pale fawn, 14–20 µm thick. *Asci* cylindrical-clavate to ±fusiform, *c.* (75–)80–122 × 9–11 µm ($n = 12$), 8-spored; ascus apex rounded, without a ring structure. *Ascospores* hyaline, transversely 3–5(–6)-septate, elongate-fusiform to bacilliform, (20–)28.8(–37) × (3–)3.3(–4) µm ($n = 54$); gelatinous sheath not seen.

Pycnidia not observed.

Chemistry. Thallus and isidia K+ blackish, C−, PD−, UV−. TLC not performed.

Etymology. The epithet refers to the microfruticose habit of the thallus consisting mostly of ascending isidioid structures.

Distribution and ecology. The species is known from several localities in south-west Florida (Lee Co.), where it inhabits the bark of *Taxodium* within and near the margins of seasonally flooded groves, at low elevation (*c.* 3–6 m).

Notes. Other isidiate species described in *Porina* appear to differ from this taxon in significant ways. *Porina coralloidea* P. James [=*Zamenhofia coralloidea* (P. James) Clauz. et Roux] has longer (40–57 µm), wider (9–13 µm) ascospores with considerably more numerous septa (9–11) and a very thick lateral wall (James 1971); its isidia are contorted chains of *Trentepohlia* surrounded by compacted hyphae (James 1971), rather than uniseriate structures surrounded by subglobose fungal cells. *Porina rosei* Sérusiaux has similar isidia corticated with globose cells, but the algal cells are not consistently uniseriate and the crustose thallus from which the isidia arise also has a cellular cortex of isodiametric fungal cells; its ascospores are wider (4–6(7) µm) and with only three septa (Sérusiaux 1991). *Porina collina* has fine fragile isidia, but they are not corticated nor is the phycobiont uniseriate. *Porina isidioambigua* M. Cáceres *et al.* has ascospores of about the same length as those of *P. nanoarbuscula* but twice as wide; the isidia incorporate many algal cells in width rather than a single file, and lack globose corticating mycobiont cells (Cáceres *et al.* 2013). *Porina pseudohibernica* has fine, abundantly branched, easily detached isidia, but their algal cells are not uniseriate, and their surface is ecorticate or covered with appressed hyphae rather than globose fungal cells; ascospores are longer (34–43 µm), wider (7–9 µm) and with more septa (7–8) compared to those of *P. nanoarbuscula* (Tretiach 2014). The mtSSU sequences furthermore indicate that *P. nanoarbuscula* and *P. pseudohibernica* are phylogenetically distinct (Fig. 9). The Floridian taxon *P. scabrida* (Harris 1995) is described as having isidia covered with a single layer of mycobiont cells, but its ascospores are longer (35–47 um), 8-celled, and almost twice as wide (5.5–7.5 um).

Additional specimens examined (all on Taxodium *bark).* **USA:** *Florida*: Lee County, Fort Myers, Florida Gulf Coast University (FGCU) campus, Cypress grove along boardwalk to Parking Garage 3, 2020, *W. B. Sanders* 20423.1 (FLAS); *ibid.*, Cypress swamp north of Parking Garage 3, 2021, *W. B. Sanders* 21320.1 (TSB 44456); *ibid.*, along nature trail to laurel oak hammock, 2021, *W. B. Sanders* 21421.5 (FLAS, BR); *ibid.*, along nature trail near laurel oak hammock, *W. B. Sanders* 21421.8 (FLAS); *ibid.*, Cypress dome south of campus, near parking garage 2, 2020, *W. B. Sanders* 20511.3 (FLAS); *ibid.*, 2021, *W. B. Sanders* 21502.2 (FLAS, BR); *ibid.*, Cypress dome between FGCU Blvd and Aquatic Center, 2020, *W. B. Sanders* 20424.4 (FLAS); Estero, Grandezza, Cypress grove behind 'The Studio' sales centre, 2020, *W. B. Sanders* 20506.1 (TSB 44457); *ibid.*, woods between Villa Grande and Grande Estates, 2020, *W. B. Sanders* 20510.2 (TSB 44458).

Discussion

Fruticose or isidiate crustose thallus?

Fruticose lichens, in the usual sense, bear their ascomata on their ascendant branches. In the *Porina* species examined here, perithecia develop on basal thallus portions embedded within the dead tissue layers of the plant substratum (Fig 1F & I–J, 2H). In this regard, their growth form corresponds to that of a crustose/endophloeodic lichen. Although true isidia are uncommon in crustose lichens, thalline structures of considerable anatomical, morphological, developmental and functional diversity are encompassed within this concept; previous authors have clearly found it broad enough to accommodate the appendages produced by certain species of *Porina*. However, in significant ways, the structural characteristics of the *Porina* species studied here call into question the utility of considering their ascendant structures as isidia. Isidia are supposed to be appendicular upgrowths of a corticated underlying thallus, such that there is continuity of the cortex and anatomy of the isidium with that of the thallus from which it arises (Jahns 1988). In our collections, the fruticose portions of the thallus predominate, while the basal crust is often evanescent, reduced, or largely integrated within the substratum. More significantly, the anatomical complexity of the corticated and/or stratified ascendant structures is nowhere evident in the diffuse, loosely organized substratic thallus from which they arise. The organized algal layer and medulla of *P. microcoralloides* and *P.* cf. *scabrida*, the corticating hyphae of the latter and the corticating layer of sub-globose fungal cells in *P. nanoarbuscula* are observed only in the ascending, isidia-like branches, without any counterpart in the unstratified basal portions. They therefore cannot be easily categorized as mere orthotropic outgrowths of the underlying crustose or endophloeodic thallus. We employ the term isidioid to describe the form of the ascendant vegetative thallus in the taxa described here.

Diversity of isidiate/isidioid taxa in Porina

Although isidia are not common in *Porina*, a number of species have now been described with such structures in this large genus.

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Our study suggests the presence of substantial genetic diversity among such taxa in south-west Florida, and amplifies previous indications that structures treated as isidia in this genus can be very different from each other anatomically. Other recent studies of *Porina* species have likewise found considerable genetic diversity (Orange *et al.* 2020), with many new taxa in the tropics (Ertz & Diederich 2022), including isidiate forms. In contrast, a number of isidiate collections worldwide, including from tropical Asia and South America, have been attributed to *Porina hibernica* (e.g. Aptroot 2003; Aptroot *et al.* 2017), a taxon originally described from Great Britain (Swinscow 1962). The seemingly cosmopolitan distribution of this species is thus noteworthy, but molecular data from such collections are needed to ensure that multiple, cryptic taxa are not involved. Structural and mtSSU sequence data (Fig. 9) from the present study indicated that none of the south-western Florida taxa examined correspond to *P. hibernica*.

Our sequence data provide further support to previous molecular studies (Orange *et al.* 2020; Ertz & Diederich 2022) that suggest multiple independent origins of isidiate morphology within *Porina*, showing this trait to be of little or no value as an indicator of biosystematic relationships. Strikingly, we found that the two isidioid taxa, *P. microcoralloides* and *P. nanoarbuscula*, appeared as sister taxa in the mtSSU analysis, yet their ascendant structures are so different in morphology and anatomical organization that one must suppose they originated independently of each other. On the other hand, the isidioid structures in *P. microcoralloides* were rather similar anatomically to those of *P.* cf. *scabrida*, the latter differing only in the presence of a better developed cortical layer. Yet these two taxa appear quite distant from each other in their mtSSU sequences. In at least some taxa, the presence or absence of isidia may also be variable. Ertz & Diederich (2022) found that isidiate morphs in certain species were scarcely different in nucleotide sequence from non-isidiate ones. In the case of our Floridian collections, where most of the vegetative thallus consists of the isidioid structures, it would be difficult to imagine conspecific morphotypes that lacked them. The extensive elaboration of the ascendant thallus component and concomitant reduction of the crustose portion suggests a trend by which fruticose thalli may evolve from crustose ones. Indeed, a transient basal crust is reported to precede the development of conventional fruticose structures in lichens such as *Usnea* (Lallemant 1984). While the biosystematic utility of isidia in *Porina* may be low, there is as yet no indication that more than one type of isidium or isidioid structure could occur within the very same taxon. It therefore seems reasonable to tentatively assume that details of their structure, at least when expressed, are characteristic of the taxon that possesses them, even if the trait tells us little or nothing about relationships to other taxa.

Also included within the concept of isidia is a distinctive structure produced by certain other *Porina* species (formerly *Phyllophiale*) that colonize leaves in tropical forests, forming symbioses with the multicellular discoid phycobiont *Phycopeltis* (Santesson 1952; Lücking & Cáceres 1999; Lücking 2008). The disc-shaped propagules are positioned on a very short central stalk above the thallus, from which they are easily detached. This type of isidium develops when a phycobiont filament (or filaments) from the margin turns upwards to emerge from the lichen surface and then branches radially in a plane parallel to the thallus below, accompanied by investing mycobiont hyphae on its upper and lower surfaces. The miniature lichenized disc perched above the main thallus can resume growth directly after detachment and dispersal (Sanders 2002). These isidia are unbranched, determinate structures (at least prior to detachment) with a highly uniform morphology likely adapted to water dispersal; they have little in common with the fruticose structures studied here. It has been pointed out that virtually identical discoid isidia are produced by foliicolous taxa of *Porina* representing different phylogenetic clades (Lücking & Vězda 1998; Lücking & Cáceres 1999), an interpretation supported by the positions of two such taxa (*Porina alba* (R. Sant.) Lücking and *P. fusca* Lücking) in gene-based cladograms (Orange *et al.* 2020; Ertz & Diederich 2022; see also Fig. 9). Thus, the discoid and the coralloid 'isidia' known in *Porina* share at least one feature: both appear to have arisen more than once independently. Although unreliable as indicators of biosystematic relatedness, such remarkable convergences do tell us something biologically important: that natural selection in these cases is almost certainly shaping morphologies with real and direct functional significance to the organisms involved.

Symbiont interactions

The consistent association of *Porina microcoralloides* and *P. nanoarbuscula*, each with a distinct clade of *Trentepohlia* phycobionts (Figs 11; Supplementary Material Fig. S2, available online), suggests a high degree of selectivity in these lichen-forming fungi. The correspondence of sister-clade pairs in the mycobiont and phycobiont trees suggests a possible occurrence of parallel cladogenesis in these symbiont lineages. It is noteworthy that both taxa occur in the same habitats and are even intermixed (Fig. 1N), where they are likely to encounter, and presumably reject, the algal strain preferred by the other species. A much larger dataset would be needed, however, before one may be confident that this is consistently the case.

It has been asserted that specialized penetrative contacts generally do not occur between symbionts in trentepohliaceous lichens (Nienburg 1926; Grube & Lücking 2002). However, this viewpoint was challenged by Tschermak (1941), who reported and illustrated deeply penetrating haustoria in numerous taxa of such lichens. Later TEM studies documented extensive haustorial development in almost every trentepohliaceous lichen examined (Withrow & Ahmadjian 1983; Matthews *et al.* 1989; Tucker *et al.* 1991; but see Lambright & Tucker 1980). These haustoria appear to finely invaginate the algal cell wall rather than actually traverse it. They would thus be classified as intraparietal (Type 2) in the scheme presented by Honegger (1986), although they often reach deeply into the algal cell (Tschermak 1941; Matthews *et al.* 1989). The haustoria observed in the *Porina* lichens examined here were all intraparietal, but with very limited intrusion causing only slight invagination of the algal cell wall (Figs 2G, 5E, 7H & 8E), as in Type 1 of Honegger (1986). They thereby contrast with the much deeper penetration/invagination of algal symbionts observed in the more typically crustose trentepohliaceous lichens as cited above. Why stratified and morphologically more complex foliose and fruticose lichens should have more superficial symbiont contacts than unstratified crustose lichens is unclear. One possibility is that mycobiont growth must be more closely coordinated with algal cell division to achieve more complex levels of organization (Greenhalgh & Anglesea 1979; Honegger 1987*b*), requiring more superficial attachments that can stimulate but not obstruct division and distribute its products. Another notable feature of the symbiont contact zones observed here is the very substantial thinning of the fungal cell wall at the point of its intrusion into the algal wall. Although lichen haustoria are not believed

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to play any central role in transfer of carbon, which is leaked by the alga across its walls in symbiosis, the reduction in mycobiont wall thickness does suggest a modification that serves to streamline exchange of substances. Symbiont contact zones of this type will be explored in more detail in a future work.

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Author ORCIDs. William Sanders, 0000-0001-9572-4244; Damien Ertz, 0000-0001-8746-3187.

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