MULTIGENE ANALYSES RESOLVE EARLY DIVERGING LINEAGES IN THE RHODYMENIOPHYCIDAE (FLORIDEOPHYCEAE, RHODOPHYTA)[1]

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Abstract

Multigene phylogenetic analyses were directed at resolving the earliest divergences in the red algal subclass Rhodymeniophycidae. The inclusion of key taxa (new to science and/or previously lacking molecular data), additional sequence data (SSU, LSU, EF2, *rbc*L, COI-5P), and phylogenetic analyses removing the most variable sites (site stripping) have provided resolution for the first time at these deep nodes. The earliest diverging lineage within the subclass was the enigmatic *Catenellopsis oligarthra* from New Zealand (Catenellopsidaceae), which is here placed in the Catenellopsidales ord. nov. In our analyses *Atractophora hypnoides* was not allied with the other included Bonnemaisoniales, but resolved as sister to the Peyssonneliales, and is here assigned to Atractophoraceae fam. nov. in the Atractophorales ord. nov. Inclusion of *Acrothesaurum gemellifilum* gen. et sp. nov. from Tasmania has greatly improved our understanding of the Acrosymphytales, to which we assign three families, the Acrosymphytaceae, Acrothesauraceae fam. nov. and Schimmelmanniaceae fam. nov.

Keyword index words: Acrosymphytales, Acrothesauraceae, *Acrothesaurum*, Atractophoraceae, Atractophorales, Catenellopsidales, Schimmelmanniaceae

Abbreviations: COI-5P, 5' region of the mitochondrial cytochrome oxidase subunit 1 gene; EF2, nuclear elongation factor 2 gene; LSU, nuclear large subunit ribosomal DNA; *rbc*L, plastid ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene; SSU, nuclear small subunit ribosomal DNA

As with most lineages of living organisms, molecular data have come to play an essential role in reshaping our understanding of organismal relationships and providing new evolutionary perspectives for red algae (see Saunders and Hommersand 2004, Yoon et al. 2006, Verbruggen et al. 2010). Among the five or six classes comprising the phylum Rhodophyta (Saunders and Hommersand 2004, Yoon et al. 2006), the Florideophyceae is by far the most species-rich, containing upwards of 95% of the currently reported species (Guiry and Guiry 2015). The Florideophyceae consists of multicellular marine and freshwater species currently assigned to five subclasses on the basis of molecular and morphological analyses (Saunders and Hommersand 2004, Le Gall and Saunders 2007). The subclass Rhodymeniophycidae contains some 75% of the species currently assigned to the Florideophyceae (Guiry and Guiry 2015) including many that are well known to non-specialists, e.g., Irish moss (*Chondrus crispus* Stackhouse) and dulse [*Palmaria palmata* (Linnaeus) F.Weber & D.Mohr].

The Rhodymeniophycidae was established by Saunders and Hommersand (2004) on the basis of molecular data available at that time (e.g. Saunders and Bailey 1997, 1999, Harper and Saunders 2001), as well as the key ultrastructural synapomorphy of pit plugs that are covered by a cap membrane at their cytoplasmic faces (Pueschel and Cole 1982). Saunders et al. (2004) completed a relatively comprehensive molecular phylogenetic assessment of this subclass and established that all orders except the Gigartinales were largely monophyletic, however, relationships among most orders were unresolved. Saunders et al. (2004) included data for only the SSU, for which varied rates of change in divergent lineages likely confounded

phylogenetic determinations (Le Gall and Saunders 2007). Subsequent research included additional taxa and used the LSU and SSU in combination. As a result more orders were recognized (e.g. Withall and Saunders 2006), but the relationships among most of them remained equivocal. Indeed, of the 11 orders recognized in Withall and Saunders (2006), only the positioning of the Halymeniales as sister to the Sebdeniales and Rhodymeniales was consistently resolved. Studies using the *rbc*L have similarly failed to resolve interordinal relationships (e.g. Gavio et al. 2005, Krayesky et al. 2009).

Attempts to resolve ordinal relationships among florideophycean subclasses then took two divergent approaches. Le Gall and Saunders (2007) attempted to improve resolution by adding taxa and generating sequence data for an additional nuclear marker, EF2, whereas Verbruggen et al. (2010) used a data-mining approach to prepare a supermatrix for phylogenetic analyses. Although support for monophyly of some orders was improved and the subclass Corallinophycidae was recognized as distinct from the Nemaliophycidae (Le Gall and Saunders 2007), relationships among orders of the Rhodymeniophycidae remained poorly resolved. Verbruggen et al. (2010) identified ordinal relationships among Rhodymeniophycidae as one of five poorly supported regions in the red algal tree of life that were in need of further study. They noted that "data availability for (this subclass) is meager to poor", but provided compelling evidence that resolution would be possible with the addition of more data (Verbruggen et al. 2010, fig. 3). In the most recent effort, Yang et al. (2015) analyzed mitochondrial genomes for 21 Rhodymeniophycidae. Again few novel interordinal relationships were resolved with meaningful support except for an early divergence of

the Bonnemaisoniales, Gigartinales and Peyssonneliales relative to the remaining orders (94% support in maximum likelihood analyses, Yang et al. 2015, fig. 1).

To improve the resolution of ordinal relationships within the Rhodymeniophycidae we have generated data from more taxa, including some not previously included in phylogenetic analyses, e.g. *Catenellopsis oligarthra* (J.Agardh) V.J.Chapman, and more genes combining the five markers SSU, LSU, EF2, *rbc*L, and COI-5P. We additionally completed analyses on alignments of progressively more conservative characters (site stripping) in an effort to reduce the effects of saturation and thus improve phylogenetic signal (Verbruggen 2012).

MATERIALS AND METHODS

Molecular methods: Samples for molecular investigation (Table S1) were processed and DNA extracted following Saunders and McDevit (2012). Sequence data were generated for the SSU, LSU, EF2, *rbc*L and CO1-5P following Saunders and Moore (2013). Sequences were aligned using the ClustalW plugin for Geneious R7 version 7.1.5 (http://www.geneious.com; Kearse *et al.* 2012) and included data from GenBank (Table S1). We generated five individual gene alignments: SSU (68 taxa, 1702 of 1895 sites included in analyses; 93% complete); LSU (72 taxa, 2605 of 3434 sites included in analyses; 99% complete); EF2 (67 taxa, 1681 sites; 92% complete); *rbc*L (69 taxa, 1358 sites; 95% complete) and CO1-5P (65 taxa, 664 sites; 89% complete). In addition, a concatenated alignment for all taxa and regions (73 taxa, 8010 aligned sites) was generated. Most taxa were at least 75% complete except for

115 *Acrosymphyton purpuriferum* (J.Agardh) G.Sjöstedt (54% complete, data only for SSU
116 and LSU) and *Pihiella liagoraciphila* Huisman, A.R.Sherwood & I.A.Abbott (21%
117 complete, data only for SSU), which together accounted for ~26% of the missing data.
118 Single-gene alignments were analyzed with a GTR+I+G model, with
119 partitioning by codon for the three protein-coding genes, in the web-server program
120 RAxML (Stamatakis 2014) and robustness determined with 500 bootstrap replicates.
121 There were no strong inconsistencies noted among the single-gene trees and the five
122 genes were combined for phylogenetic analyses. Bayesian analysis was performed on
123 the full dataset using the MrBayes plugin for Geneious R7 version 7.1.5 under a
124 GTR+I+G model with parameter settings unlinked and the rates prior set to allow rate
125 differentiation across partitions (by gene and then by codon for protein-coding genes =
126 full partitioning scheme). This analysis was run twice for 1,000,000 generations with
127 sampling every 1000 generations. Plotting the overall likelihood against the number of
128 generations identified the stationary phase to determine the burn-in for each run.
129 Maximum likelihood analysis was performed under a GTR+I+G model with the data
130 fully partitioned using the RAxML plugin for Geneious R7 version 7.1.5 with 1000
131 bootstrap replicates.
132 Owing to the paucity of data for *Pihiella* (SSU only) the combined Bayesian
133 analyses were repeated after removing this taxon. There were no significant changes in
134 topology and only minor variations in posterior probability support indicating that its
135 inclusion was not negatively impacting our phylogenetic results.
136 To assess phylogenetic inference problems due to substitution saturation,
137 quickly evolving sites were calculated and systematically removed from the full

alignment. Site-specific rates were determined using the "substitution rates" analysis tool in HyPhy (Pond et al. 2005) under the JC69 model with a Bayes phylogram as a guide tree. To generate a series of progressively more conservative alignments the program SiteStripper (Verbruggen 2012) was used to order the sites by rate then remove the more rapidly evolving sites in increments of 5%. Maximum likelihood analysis (ML) was performed on each "stripped" alignment (fully partitioned) using RAxML version 7.3.5 with a command line script available through SiteStripper under a GTR+I+G model and 1000 bootstrap replicates.

To assess how partitions might impact phylogenetic inference, the original alignment and "stripped" alignments were analyzed by running PartitionFinder (Lanfear et al. 2012) using the "greedy" algorithm under the BIC model selection method with linked branch-length estimation. The best partitioning scheme and most appropriate model of evolution as determined by PartitionFinder were subsequently used to reanalyze the alignments with RAxML again with 1000 bootstrap replicates.

Anatomical methods. For anatomical observations, whole-mounts of gelatinous species were made from liquid-preserved thallus fragments, and cross-sections of more robust species were prepared from rehydrated or formalin-fixed samples by hand-sectioning or in a cryostat (CM1850, Leica). Tissues were stained with 1% aniline blue and mounted in 40-50% corn syrup. Observations were made with a light microscope and documented with digital photography.

Morphological development of *Atractophora hypnoides* P.Crouan & H.Crouan was followed in cultured material fixed in formalin-seawater (4%) and stained with aniline blue acidified in 1% HCl. Cell nuclei were visualized by staining formalin-fixed material in a drop of Hoechst 33258 solution (10 µg mL^{-1}) and examined with an epifluorescence microscope (Leitz Dialux), or by staining with aceto-iron-haematoxylin-chloral hydrate (Wittmann 1965) and photographed using Nomarski interference, as described by Maggs (1989). Photographs were taken using Technical Pan film developed in Kodak HC110 liquid developer.

Culture studies. *Atractophora hypnoides* was isolated from tetraspores released by *Rhododiscus* tetrasporophytes collected at Finavarra, Co. Clare, Ireland, and Cloghy Rocks, Strangford Lough, N. Ireland (multiple cultures were isolated from 1982-1986, several of which were maintained long-term; see Maggs 1988). Cultures were grown in half-strength modified von Stosch medium (Guiry and Cunningham 1984), at 15°C, in a regime of 16:8 h light: dark (LD), under a photon irradiance of c. 20 µmol photons m^{-2} s^{-1}, and subject to changes in photoperiod as described in the text.

RESULTS

Molecular phylogenetic analyses. Topologies were congruent for all four analyses of the full combined alignment (Bayes and maximum likelihood under full and PartitionFinder partitioning) and the Bayesian result with partitioning by gene and codon is presented (Fig. 1; with support values for all analyses summarized in Table 1).

Tree scores and branch support were typically slightly better for the fully partitioned analyses, i.e. not using partitions identified by PartitionFinder (Table 1). To assess the effects of substitution saturation a series of progressively more conservative alignments were analyzed with maximum likelihood, again fully partitioned and with the schemes determined by PartitionFinder. Consistent with the full combined alignment, tree scores and overall support were typically better for analyses in which the data were fully partitioned and only those values are presented (Table 1).

A neighbor-joining tree constructed with the HKY model was generated in Geneious R7 (Supplementary Fig. S1) to determine if the starting tree employed by HyPhy to determine individual site rates had biased downstream analyses. These NJ-based site rates were used by SiteStripper to generate a series of subalignments, which were analyzed in RAxML. The results of a Shimodaira-Hasegawa test indicated that the neighbor-joining tree (likelihood -144264.31) was significantly different ($p<0.01$) from the Bayesian Inference tree (likelihood -144682.75) used in the initial site-stripping analyses; however, use of the neighbor-joining topology for calculating sites rates did not impact downstream site-stripping analyses (data not shown).

In general terms support was moderate to strong at many key ordinal and interordinal nodes, with some deeper nodes seeing enhanced support values at 10% site removal (Table 1). Neither the Bonnemaisoniales nor Gigartinales was monophyletic (Fig. 1, Table 1). *Catenellopsis oligarthra*, not previously included in phylogenetic analyses, resolved as an independent lineage sister to the remainder of the Rhodymeniophycidae and was not associated with other taxa assigned to the

Gigartinales (Fig. 1). The next diverging lineage was the fully supported Bonnemaisoniales *sensu stricto* (Fig. 1), i.e. excluding *Atractophora hypnoides*, which was resolved as sister to the strongly supported Peyssonneliales in a lineage with the remaining Gigartinales (Fig. 1, Table 1). The remaining orders were resolved as a large clade subdivided into two well-supported groups. The first of these consisted of Acrosymphytales + Ceramiales + *Schmitzia* (Calosiphoniaceae). The novel Australian taxon *Acrothesaurum gemellifilum* resolved within a fully supported lineage encompassing species of the genera *Acrosymphyton* and *Schimmelmannia*, both currently assigned to a single family in the Acrosymphytales (Fig. 1, Table 1). This expanded Acrosymphytales was sister to the Ceramiales, which included with moderate support the genus *Inkyuleea* (Fig. 1). Relationships among the Gelidiales, Gracilariales, Nemastomatales, Plocamiales and the Halymeniales+Rhodymeniales+Sebdeniales lineage remained largely unresolved, although some support for an alliance of the Rhodymeniales+Sebdeniales was recognized (Fig. 1, Table 1). Finally, moderate support was acquired for the continued inclusion of the Sarcodiaceae in the Plocamiales (Fig. 1, Table 1).

Taxonomic changes

Catenellopsidales K.R.Dixon, Filloramo & G.W.Saunders, **ord. nov.**

Description: Thalli develop from triaxial apices. Gonimoblasts numerous, arising from an extensive conjugated reticulum with associated nutritive tissue. Tetrasporangia cruciate or decussate, terminal, embedded in outer cortical tissue.

Type and only family: Catenellopsidaceae Robins 1990, p. 698.

Atractophorales Maggs, L.Le Gall, Filloramo & G.W.Saunders, **ord. nov.**

Description: Gametangial thallus consisting of erect axes arising from a basal disc; lubricous with erect branches spirally arranged; uniaxial with four periaxial cells per whorl. Monoecious; carpogonial branches 3-celled; procarpic, supporting cell functioning as auxiliary cell, fusing with the fertilized carpogonium, from which the gonimoblast arises. Gonimoblast a diffuse system of loose descending filaments, forming a covering around one or more cells of the main axis; pericarp absent. Mature cystocarps spindle-shaped. Spermatangia in superficial clusters. Tetrasporangial thallus crustose; tetrasporangia regularly cruciate, terminal.

Type and only family: Atractophoraceae Maggs, L.Le Gall & G.W.Saunders, fam. nov.

Atractophoraceae Maggs, L.Le Gall & G.W.Saunders, **fam. nov.**

Description: as for Atractophorales.

Type genus: *Atractophora* P.Crouan & H.Crouan 1848: 371.

Additional genus: *Liagorothamnion* Huisman, D.L.Ballantine & M.J.Wynne, 2000: 507, 508 (discussed below).

Lectotypification: *Atractophora hypnoides* was provisionally lectotypified by Dixon and Irvine (1977) in CO. The collection of the brothers Crouan contains five specimens of *Atractophora hypnoides*, as well as an illustration (IC BOT/Herb. CO/0001) with a fragment of a plant (CO00287). None of the specimens accord with

252 the protologue, which mentioned a specimen dredged at 8-10 m depth on August 20th
253 1848 in the Rade de Brest and growing on *Melobesia polymorpha* (Linnaeus) Harvey
254 and on *Ceramium rubrum* C.Agardh. Among the five specimens, one lacks collection
255 information (CO00289), one is from Noirmoutier (CO00291), and the remainder are
256 from the Rade de Brest. Among the last, one has no collection date (CO00290) and the
257 other two were collected at Baie Sainte Anne in 1847. Specimen CO00288 was
258 growing on *Ceramium*, a host mentioned in the protologue. We therefore designate
259 specimen CO00288 as the lectotype of *Atractophora hypnoides* (Fig. 2).
260 *Gametophyte observations in culture:*. *Atractophora hypnoides* tetraspores form
261 small multicellular loosely coherent discs that give rise centrally to an erect axis. Erect
262 axes consist initially of a single filament produced by transverse divisions of a more or
263 less isodiametric apical cell. Beginning at about the sixth cell from the apex, each axial
264 cell cuts off a periaxial cell from a lateral protuberance. Alternate axial cells give rise to
265 two periaxial cells/whorl branch initials at 180° to each other, initially forming a
266 distichous arrangement of branchlets (Fig. 3a). As axes develop further, periaxial cells
267 are also cut off at 90° to the first branchlets, resulting in whorls of four branchlets of
268 limited growth in a cruciate arrangement (Fig. 3b). Axial cells enlarge greatly in length
269 and diameter, mainly below the insertion of the whorl, so that the whorl is eventually
270 positioned around the distal part of the axial cell, all enclosed in a thin (<10 µm)
271 mucilaginous sheath (Fig. 3b, d). Whorl branchlets consist of inflated cells 10 µm in
272 diameter, tapering to cylindrical/conical apical cells that often bear hairs up to 100 µm
273 long (Fig. 3c). Occasional whorl branchlets are replaced by axes of indeterminate

274 growth, of the same construction as the primary axis, but generally forming the cruciate
275 arrangement of whorl branchlets within the apical 10-12 axial cells (Fig. 4a).
276 When about one month old, thalli start to produce a distichous arrangement of
277 lateral ramuli from the whorl branchlets, and axes develop a filamentous cortication
278 formed by down-growing rhizoidal filaments that originate from the basal cell of every
279 whorl branchlet (Fig. 3d). All vegetative cell types are uninucleate and contain irregular
280 ribbon-like to reticulate chloroplasts; neither secondary pit connections nor cell fusions
281 are formed.
282 At about 1.5 months old, thalli form spermatangia and carpogonial branches just
283 below the apices of axes (Figs 3e-j, 4a-e). Spermatangia develop in dense clusters all
284 around axes, arising from modified whorl branchlets, each cell of which cuts off small
285 spermatangial mother cells in all directions, singly or in chains. Spermatangial mother
286 cells are rectangular/pyriform and by oblique divisions cut off 2-3 uninucleate
287 spermatangia 1.5-2 µm long (Figs 3e, 4b). Released spermatia are spherical,
288 uninucleate and 2.5-3 µm in diameter (Fig. 4c).
289 Carpogonial branches usually develop from the basal cell of a modified whorl
290 branch, which is thus the supporting cell (Figs 3f-j, 4d-e). The carpogonial branch is 3-
291 celled, the carpogonium and hypogynous cell lying at right angles to the first branch
292 cell, which brings the carpogonium close to the supporting cell (Fig. 4d, e). The
293 supporting cell bears a 1-celled and a 2-celled lateral branch, and the first cell of the
294 carpogonial branch also bears a lateral cell, forming together an 8-celled structure (Figs
295 3j, 4e). The carpogonium is triangular as one side lies along the hypogynous cell, and
296 another along the first carpogonial branch cell. The trichogyne develops from the third

297 side, towards the axis at first, and then bending outwards and growing to about 250 µm
298 in length (Figs 3f-i, 4d, e). The cytoplasm of the trichogyne is constricted near the
299 carpogonium and then expands to about 2 µm wide, surrounded by a mucilage sheath 2
300 µm thick. Numerous spermatia are observed on hairs and trichogynes, forming
301 cytoplasmic continuity with the trichogynes (Figs 3k, 4c).
302 Following fertilization, the carpogonium and supporting cell fuse to form a
303 dumb-bell shaped cell in some cases, with the hypogynous cell remaining separate (Fig.
304 4h). In other examples the carpogonium and hypogynous cell appear to fuse before
305 joining with the supporting cell. It appears that the first carpogonial branch cell
306 sometimes becomes part of the fusion cell (Fig. 4f, g). An additional fusion can occur
307 between two of the lateral cells, but this fusion cell is separate from the one involving
308 the carpogonium (Fig. 4f, g). Early post-fertilization development is apparently quite
309 variable but is obscured by the production of dense clusters of small “nutritive” cells by
310 the first carpogonial branch cell, its lateral cell, and the hypogynous cell (Figs 3j, 4f-h).
311 These persist as a small group of cells attached to the fusion cell(s). The fusion cell
312 formed from the carpogonium and the supporting cell cuts off a gonimoblast initial from
313 the carpogonium end (Figs 3l, 4f, g). The gonimoblast initial quickly gives rise to
314 several non-pigmented branched gonimoblast filaments, which surround the axis,
315 weaving amongst the whorl branchlets and giving rise to radiating filaments (Fig. 4i).
316 Deeply pigmented carposporangia 12-13 µm in diameter are borne terminally on these
317 outward-growing filaments (Figs 3m, 4i). Approximately 1.5 months after the first
318 appearance of gametangia, globular mature cystocarps about 250 µm in diameter are

present, often arranged in series on an axis due to its continued growth and formation of carpogonial branches.

Tetrasporophyte observations: Carpospores of about 20 µm diameter released in culture and grown under the same conditions as field-collected tetraspores (i.e. 15°C; 16:8h LD) germinate in the same manner as the tetraspores to produce cohesive discs (Fig. 5a). Basal layer filaments of crusts branch pseudodichotomously (Fig. 5c), forming a polyflabellate pattern due to the cessation of growth of most filaments soon after branching, causing considerable variation in cell dimensions. This pattern is also seen in field material of *Rhododiscus pulcherrimus* P.Crouan & H.Crouan (Fig. 5f), and results in a lobed or irregular margin. No cell fusions or secondary pit connections are formed; calcification is absent. Three months after germination, crusts are up to 2 mm in diameter and 30 µm thick, including a thick mucilage layer on the upper and lower surfaces. They consist of basal layer cells, each bearing one or two 5-6 celled erect filaments 8-14 µm in diameter (Fig. 5d). The cell cut off behind the apical cell of a basal layer filament immediately divides periclinally (Fig. 5b) to form crust margins two cells thick. No erect axes developed from these crusts. Crusts transferred to 15°C; 8:16h LD and then to 10°C; 8:16h LD formed tetrasporangia across the surface, developing from the darkly pigmented apical cells of the erect filaments (Figs 5d, e). Tetrasporangia are regularly cruciately divided (Fig. 5d), c. 16-20 µm in length and diameter, smaller and rounder than in field-collected material (Fig. 5f), in which they are 16-37 x 10-20 µm. In culture, tetrasporangia release tetraspores about a month after formation.

Acrosymphytales Withall & G.W.Saunders 2006, p. 389-390.

Type family: Acrosymphytaceae S.C.Lindstrom 1987, p. 52.

Type genus: *Acrosymphyton* G.Sjöstedt 1926, 8-9.

Acrothesauraceae G.W.Saunders & Kraft, **fam. nov.**

Description: Thalli uniaxial, apical cell division transverse, the central-axial cells each bearing nodal whorls of sub-/pseudodichotomous determinate laterals. Carpogonial and auxiliary-cell filaments both simple, occurring singly, in pairs or in clusters. Diploidization of the auxiliary cells effected by direct fusion with a presumably fertilized carpogonium, or its derivative cell, in the same (procarpic) or a separate (nonprocarpic) branch system. Tetrasporophytes unknown.

Type genus: *Acrothesaurum* Kraft & G.W.Saunders, gen. nov.

Additional genus: *Peleophycus* I.A.Abbott 1984, 325-327 (discussed below).

Acrothesaurum Kraft & G.W.Saunders, **gen. nov.**

Description: Thalli flaccid, lubricous; central-axial cells each bearing nodal whorls of sub-/pseudo-dichotomous determinate laterals; mid and lower axes corticated between nodal whorls by branched, basipetally directed rhizoids. Thalli monoecious; spermatangia borne directly on terminal and subterminal cells of whorl branchlets; carpogonial and auxiliary-cell filaments both unbranched, occurring singly, in pairs or in clusters on periaxial and one or two distal cells of whorl branchlets, directed basipetally, the auxiliary cells terminal. Diploidization of auxiliary cells effected by direct fusion with presumably fertilized carpogonia, the diploidized auxiliary cell either

365 borne on the same supporting cell as the donor carpogonium (procarpic) or on one of
366 several adjacent supporting cells (nonprocarpic); gonimoblast initials single,
367 carposporophytes composed of up to three synchronously maturing gonimolobes
368 consisting entirely of carposporangia. Proximal portions of diploidized auxiliary cells
369 frequently emitting one or two stout, basally directed filaments that fuse apically to
370 central-axial cells and/or adjacent lower whorl-branchlet cells. Tetrasporophytes
371 unknown.

372 *Etymology*: from "acro", referring to objects at an extremity, and "thesaurum",
373 for a treasury or treasure chamber, in reference to the terminal auxiliary cells that
374 receive and house the "precious" zygote nucleus that initiates the embryonic
375 carposporophyte.

376 *Type and only species*: *Acrothesaurum gemellifilum* Kraft & G.W.Saunders, sp.
377 nov.

378

379 ***Acrothesaurum gemellifilum*** Kraft & G.W.Saunders, **sp. nov.** Figs 6-8

380 *Description*: portion of thallus on holotype slide (Fig. 6a) banded, 14 mm in
381 height, 17.8 mm in width, the whole specimen (Fig. 6b) lubricous, 60 mm in height, 73
382 mm in greatest breadth, erect from a holdfast pad of consolidated rhizoidal filaments;
383 axes terete (Fig. 6c, j), irregularly radially branched to four orders, indeterminate lateral
384 initials scattered (Fig. 6c), arising on epi-periaxial cells of determinate whorl-laterals
385 (Fig. 6d); proximal axes to 1600 µm in diameter, 550-650 µm in lower first-order
386 laterals, narrowing to 10-20 µm at gradually tapered tips (Fig. 6c). Cells of central-
387 axial filaments 60-90 µm long, 18-25 µm wide (Fig. 6d), each with (3-)4 periaxial cells

388 at distal poles, the periaxial cells subtending a determinate subdichotomous whorl
389 branchlet with domed or lacrimiform (Fig. 6c, d), sometimes hair-terminated (Fig. 6e),
390 apical cells, the nodal appearance of fronds accentuated by the regular spacing of
391 adjacent whorls (Fig. 6a, d). Rhizoidal filaments basipetal, 2-4 µm in diameter, initially
392 simple (Fig. 6f), later branched (Fig. 6g), mostly arising from periaxial cells, also
393 frequently from apical and subapical cells of apparently non-functioning carpogonial
394 and auxiliary-cell branches, in mature axes issuing adventitious filaments
395 perpendicularly to fully corticate the central-axial cells between adjacent whorl-branch
396 nodes (Fig. 6h). Spermatangia spherical, 2.0-2.5 µm in diameter, borne singly or
397 usually in pairs or threes (occasionally fours) mostly on terminal cells of whorl-
398 branchlets (Fig. 6i, j), less frequently also singly or in pairs on subterminal cells.
399 Carpogonial and auxiliary-cell branches basipetally directed (Figs 6f, i, 7a-c), borne
400 individually and intermixed on periaxial or epi-periaxial supporting cells (Fig. 7b), the
401 auxiliary-cell branches usually three-celled, predominant (Fig. 7a, b), the carpogonial
402 branches scarcer, normally four-celled (Fig. 7a-c), rarely five-celled (Fig. 7d), the
403 carpogonia campanulate and with straight (Fig. 7c, d) or sinuous (Fig. 7e) trichogynes,
404 the carpogonia usually borne on an inflated, subspherical to ovoid hypogynous cell c.
405 7.5-8 µm x 6 µm (Fig. 7b-e); carpogonia frequently non-functional, at various stages of
406 breaking down (Fig. 7b, e), carpogonial branches then resembling three-celled
407 auxiliary-cell branches because hypogynous cells are the size and shape of auxiliary
408 cells (Fig. 7b, e). Auxiliary cells terminal, usually ovoid (Fig. 7a-c), 6-15 x 6-9 µm in
409 diameter; each functional carpogonial branch invariably associated with an adjacent
410 auxiliary-cell branch on the same or an adjoining supporting cell (Fig. 7a-c).

411 Diploidization of auxiliary cells effected by direct fusion of the presumably fertilized
412 carpogonium (Fig. 7f, g), the auxiliary cell enlarging, becoming eccentrically swollen
413 (Fig. 8a, b) and cutting off a single terminal gonimoblast initial (Figs 7g, 8a).
414 Gonimolobes compact, the auxiliary cell elongating, thickening distally (Fig. 8b-d),
415 darkly staining (Fig. 8a-e), frequently initiating two stout single-celled arms of
416 undetermined function proximal to the carposporophyte (Figs 7h, 8d, e), the arms
417 ultimately fusing apically with central-axial cells (Fig. 8b, d). Carposporophytes
418 globular (Figs 7h, 8c, e), at maturity 250-450 µm in diameter and composed of three
419 compact synchronously maturing gonimolobes of carposporangia (Fig. 8f), the
420 gonimolobes consisting of tightly folded filaments of pit-connected subspherical to
421 angular carposporangia 25-50 µm in diameter.
422 *Etymology*: from "gemellus" (paired or twinned), and "filum" (filament), in
423 reference to the adjacency of carpogonial and auxiliary-cell filaments that connect after
424 fertilization either procarpically or nonprocarpically.
425 *Holotype*: GWS016355, slide A (Fig. 6a). The holotype slide and six duplicate
426 slides (GWS016355B-G) permanently housed at UNB. Habit of the entire type
427 specimen was photographed before it was dried in silica as a voucher (Fig. 6b).
428 *Type locality*: Wynyard, Tasmania (40^{o} 58' 48.7" S; 145^{o} 45' 04" E), -12 m on
429 shell at Sanctuary Reef (*G.W. Saunders & K.R. Dixon*, 28 Jan. 2010).
430 *Distribution*: known only from the single holotype specimen.
431
432 **Schimmelmanniaceae** G.W.Saunders & Kraft, **fam. nov.**

Description: Thalli uniaxial, apical cell division transverse, the central-axial cells each bearing nodal whorls of sub-/pseudodichotomous determinate laterals. Procarpic; carpogonial and auxiliary-cell branches in pairs on supporting cells. Diploidization of auxiliary cells effected by direct fusion with presumably fertilized carpogonia, typically following division of the latter. Tetrasporophytes crustose; tetrasporangial division cruciate.

Type genus: *Schimmelmannia* Schousboe ex Kützing, 1849: 722.

Additional genus: *Gloeophycus* I.K.Lee & S.A.Yoo 1979, p. 347 (discussed below).

DISCUSSION

The combination of more taxa, notably some key lineages previously poorly studied or newly discovered, additional sequence data and exploration of phylogenetic analyses that account for site saturation have resulted in increased resolution among the deep-diverging lineages of Rhodymeniophycidae. This problematic portion of the red algal tree of life (Withall and Saunders 2006) was considered solvable in the analyses of Verbruggen et al. (2010), which was consistent with the results here. Phylogenetic reconstruction can be difficult when sequences become saturated and when deep evolutionary events have occurred in relatively close succession such that the available signal is masked by noise in an alignment. Site stripping as performed here can enhance the signal to noise ratio improving phylogenetic inference (Verbruggen 2012). Logically, a node of interest in evolutionary time will be impacted such that resolution

of deeper nodes could see greater improvement with more site removal relative to more recent nodes. However, as more and more sites are removed signal will also be removed and support across the phylogeny will degrade. Although stochastic events can complicate matters, the previous patterns were generally observed in our analyses (Fig. 1, Table 1). As with many studies Bayesian posterior probabilities were typically higher in support of various relationships than were the corresponding ML bootstrap percentages (Table 1). Although the former values are typically considered to overestimate support (see Wróbel 2008), our analyses of progressively more conservative alignments showed enhanced ML bootstrap support for relationships with high posterior probability support in our original analyses of the full alignment (Table 1). For the current alignment and model at least, higher posterior probabilities in analyses of the full alignment appeared to be indicative of phylogenetic signal for the resolved relationships (e.g., Table 1 nodes E and H), i.e., Bayesian posterior probabilities may have been more indicative of evolutionary relationships when the full alignment was analyzed than were the ML bootstrap values. It should also be noted that the latter values are typically considered as conservative estimators of support (Wróbel 2008). Although the general applicability of site stripping for enhancing phylogenetic resolution awaits more study, the novel phylogenetic insights generated here have necessitated a suite of taxonomic changes at the familial and ordinal levels to represent the full diversity of the lineages under study and to adhere to the principle of monophyly.

Agardh (1876) originally described *Catenellopsis oligarthra* as a species of *Catenella*, at the time placed in the Solieriaceae. Kylin (1932) transferred it to

479 *Nemastoma* based on tetrasporangial anatomy, but Chapman (1979) later described the
480 new genus *Catenellopsis* (Gymnophloeaceae/'Nemastomataceae') because the
481 carposporangia were formed strictly in constricted regions of the saccate thalli. Later,
482 when erecting the monospecific family Catenellopsidaceae, Robins (1990) compared
483 the reproductive anatomy of *C. oligarthra* to several other families. Although he did
484 not observe carpogonia or early post-fertilization development, Robins (1990)
485 considered the post-fertilization anatomy of *C. oligarthra* to be so distinct that even its
486 ordinal position was uncertain. Nevertheless Robins (1990) provisionally retained
487 *Catenellopsis* and the Catenellopsidaceae in the Gigartinales. Our molecular data, the
488 first published for this species, resolved *Catenellopsis* as an isolated lineage sister to the
489 remainder of the Rhodymeniophycidae necessitating the recognition of this taxon at the
490 ordinal level.
491 Members of the Bonnemaisoniaceae (represented by *Asparagopsis* and *Delisea*)
492 and two members of the Naccariaceae (*Naccaria* and *Reticulocaulis*) group together
493 (although the Bonnemaisoniaceae is paraphyletic and further familial level study is
494 needed in this order), but *Atractophora*, previously assigned to the Naccariaceae, does
495 not join this clade (Fig. 1). The systematic position of *Atractophora* has long been
496 debated. In describing *A. hypnoides* Crouan and Crouan (1848) posited an alliance with
497 *Dudresnaya*. Agardh (1863) transferred this species to *Naccaria*, which he placed in his
498 family Wrangelieae (although given the rank of '*ordo*' Agardh's name was equivalent to
499 a family), while Zerlang (1889) again recognized *Atractophora* as a distinct genus.
500 Schmitz and Hauptfleisch (1897) allied *Atractophora*, *Naccaria* and *Wrangelia* in the
501 Wrangelieae of the family Gelidiaceae, with Oltmanns (1904) soon after recognizing

502 the family Wrangeliaceae for these three genera. Kylin (1928) erected the Naccariaceae
503 to include *Naccaria* and *Atractophora*. He discussed the relationships of the
504 Naccariaceae and suggested, based on post-fertilization development, that the family
505 was allied to the Bonnemaisoniaceae, which at that time was included in the Nemaliales
506 (as Nemalionales). Feldmann and Feldmann (1942) separated the Bonnemaisoniaceae
507 from the Nemaliales owing to the heteromorphic life cycle of *Asparagopsis* and
508 *Bonnemaisonia* and proposed the Bonnemaisoniales. Kylin (1956) did not recognize
509 the Bonnemaisoniales and included the Naccariaceae (including *Atractophora*,
510 *Naccaria* and *Neoardissonia*) and Bonnemaisoniaceae (including *Asparagopsis*,
511 *Bonnemaisonia*, *Delisea*, *Leptophyllis* and *Ptilonia*) at the end of his treatment of the
512 Nemaliales (as Nemalionales).
513 Fan (1961), discussing relationships of the Gelidiales, stressed a major
514 difference between *Atractophora* and *Naccaria* in that the nutritive filaments associated
515 with carpogonial branches were produced by the supporting cell versus the hypogynous
516 cell, respectively. Fan considered that both genera displayed direct development of the
517 gonimoblast from the carpogonium like the Gelidiales, and felt that the
518 Bonnemaisoniaceae should be recognized at the ordinal level, as proposed by Feldmann
519 and Feldmann (1942). However, Papenfuss (1966) and Dixon and Irvine (1977)
520 continued to place the Bonnemaisoniaceae in the Nemalionales.
521 Pueschel and Cole (1982) showed that both *Atractophora hypnoides* and
522 *Bonnemaisonia hamifera* Hariot have pit plugs characterized by a membrane only and
523 lacking plug caps, whereas *bona fide* Nemaliales have two-layered plug caps. They
524 considered this as strong evidence in support of the Bonnemaisoniales as distinct from

525 the Nemaliales and continued to include the Bonnemaisoniaceae and Naccariaceae in
526 the former order. However, it is important to note that this pit-plug type is shared by
527 virtually all Rhodymeniophycidae and provides no evidence on the relationships
528 between these two families and the many orders of this subclass (Saunders and
529 Hommersand 2004). Womersley (1996) speculated that the Naccariaceae may not be
530 related to the Bonnemaisoniaceae owing to significant differences in the reproductive
531 structures including the diffuse rather than compact gonimoblast and the complete
532 absence of a pericarp.

533 Separation of *Atractophora* from the rest of the Naccariaceae is not entirely
534 unexpected despite similarities of the uniaxial mucilaginous erect gametophytes and
535 mature carposporophytes composed of diploid tissue tightly surrounding the primary
536 axis, intermixed with sterile filaments, and lacking a consolidated pericarp. There are
537 several potentially significant vegetative differences, such as the transverse apical cell
538 division in *Atractophora* (Fig. 3c) compared to the oblique division in *Naccaria* (Kylin
539 1928, fig. 7A) and *Reticulocaulis* (Schils et al. 2003, fig. 23), the number of periaxial
540 cells cut off each axial cell (four in *Atractophora* versus two in members of the
541 Bonnemaisoniales *sensu stricto*), and the absence of secondary pit connections in
542 *Atractophora* (Figs 3d, 5c) versus their presence in *Naccaria* and *Reticulocaulis* (Schils
543 et al. 2003). There are many similarities in female development between *Atractophora*
544 and *Naccaria*, such as the presence of nutritive-cell clusters on the carpogonial branch
545 (Kylin 1928, Chihara and Yoshizaki 1972, Hommersand and Fredericq 1990), which
546 was the basis of their association with the Bonnemaisoniaceae. However, there are also
547 significant differences between the early post-fertilization development of *Atractophora*

548 and the other Naccariaceae, the most important of which is that whereas in
549 *Atractophora* the supporting cell (= auxiliary cell) fuses with the fertilized carpogonium
550 (Fig. 4h), in *Naccaria* and *Reticulocaulis* it remains discrete (Kylin 1928, Schils et al.
551 2003). As reported by Kylin (1928), in *Atractophora* the gonimoblast develops from
552 the carpogonial element of the fusion cell (Fig. 4g), not from the auxiliary cell. We
553 have also observed fusion between the carpogonium and hypogynous cell (Fig. 4g), and
554 among some of the lateral (nutritive) cells, which was not reported by Kylin (1928), but
555 resembles that in *Reticulocaulis* in which the fertilized carpogonium fuses directly with
556 the hypogynous cell via the expansion or breakdown of the pit connection (Schils et al.
557 2003).

558 *Atractophora hypnoides* resolved distant from the included Bonnemaisoniales
559 and as sister to the Peyssonneliales (Fig. 1). The sister relationship observed between
560 *Atractophora* and the Peyssonneliales was unexpected and intriguing, particularly
561 considering their contrasting morphologies and the anatomical similarities that
562 *Atractophora* shares with members of the Naccariaceae (Bonnemaisoniales), to which it
563 was previously attributed. However, there are some significant morphological links
564 between *Atractophora* and the Peyssonneliales. The tetrasporophyte of *Atractophora*
565 *hypnoides*, described by Crouan and Crouan (1859) as *Rhododiscus pulcherrimus*, was
566 placed provisionally in the Squamariaceae (= Peyssonneliaceae) by Denizot (1968).
567 The *Rhododiscus* phase of *Atractophora* consists of a compact disc with a
568 heterotrichous construction, in which prostrate filaments growing from a multiaxial
569 margin give rise to erect filaments (Fig. 5a-f). Tetrasporangia are formed terminally on
570 the erect filaments, large, and regularly cruciately divided (Fig. 5d-f), resembling those

571 of *Peyssonnelia* species (Maggs and Irvine 1983). Furthermore, during development of
572 male reproductive structures, members of the Peyssonneliaceae often exhibit a uniaxial
573 filamentous construction with whorls of periaxial filaments around each "axial" cell
574 (Kylin 1956, fig. 118B, Maggs and Irvine 1983, fig. 30). Post-fertilization development
575 in *Peyssonnelia* species reportedly involves the fusion of the carpogonium with the
576 hypogynous and subhypogynous cells of the carpogonial branch, but the auxiliary cell is
577 in a separate filament, i.e. nonprocarpic, and gonimoblasts arise either from a
578 diploidized auxiliary cell or directly from connecting filaments that form expansive
579 networks (Maggs and Irvine 1983, Dixon and Saunders 2013).
580 The question of whether the similarities between *Atractophora* and the
581 Peyssonneliales are sufficient to justify expanding the Peyssonneliales to include
582 *Atractophora* is a difficult one. Clearly, there are marked contrasts between
583 *Atractophora* and the Peyssonneliales, such as procarpic versus nonprocarpic
584 reproduction. The Peyssonneliales exhibits a consistent vegetative and reproductive
585 architecture such that we find it impossible to reconcile the assignment of *Atractophora*
586 to this order. We therefore propose placing the genus *Atractophora* in the
587 Atractophoraceae fam. nov., Atractophorales ord. nov.
588 Schils et al. (2003) noted similarities between the Naccariaceae and the
589 monotypic genus *Liagorothamnion*, described as an atypical member of the
590 Ceramiaceae (Huisman et al. 2000). These similarities include the formation of sterile
591 cell groups on the supporting cell and carpogonial branch cells. However, many key
592 features of its vegetative and post-fertilization development are closer to those of
593 *Atractophora* than to the rest of the Naccariaceae. In particular, the vegetative axis

consists of a narrow axial filament lacking the expanded “jacket” cells observed in the Naccariaceae (Huisman et al. 2000, Schils et al. 2003). The formation in *Liagorothamnion* of gonimoblasts from the fertilized carpogonium following fusion with the supporting cell also contrasts with *Naccaria* and the rest of the Naccariaceae, in which the carpogonium first fuses with the hypogynous cell (Kylin 1928, Hommersand and Fredericq 1990). Based on this anatomical evidence we propose that *Liagorothamnion* (for *Liagorothamnion mucoides* Huisman, D.L.Ballantine & M.J.Wynne) be placed in the Atractophoraceae.

Lindstrom (1987) erected the family Acrosymphytaceae based mainly on the terminal rather than intercalary position of the auxiliary cell for species of *Acrosymphyton* versus the Dumontiaceae *sensu stricto*. Lindstrom (1987) commented on similarities to the Calosiphoniaceae or Naccariaceae, but argued for a separate family because members of the Calosiphoniaceae have intercalary auxiliary cells while those of the Naccariaceae were considered procarpic in contrast to the terminal auxiliary cells and nonprocarpy of the Acrosymphytaceae.

Tai et al. (2001) provided molecular evidence in support of the Acrosymphytaceae as distinct from the Dumontiaceae and further suggested that this family might not even be a member of the Gigartinales. Saunders et al. (2004) expanded on that study and uncovered a strong association of the genus *Schimmelmannia*, at that time assigned to the Gloiosiphoniaceae (Gigartinales), with the Acrosymphytaceae. This was an interesting discovery because species of *Schimmelmannia*, despite being procarpic, produce a terminal auxiliary cell as in the Acrosymphytaceae and unlike the generitype of the Gloiosiphoniaceae (Kylin 1930).

617 Saunders et al. (2004) transferred *Schimmelmannia* to the Acrosymphytaceae despite
618 the respective procarpic versus nonprocarpic post-fertilization patterns, placing
619 taxonomic significance on the terminal auxiliary cells. The Acrosymphytaceae
620 (including *Schimmelmannia*) weakly resolved in a larger clade including the Ceramiales
621 (members of which also produce terminal auxiliary cells) and the Calosiphoniaceae.
622 Despite the previous molecular indications, Saunders et al. (2004) retained the
623 Acrosymphytaceae and Calosiphoniaceae in the Gigartinales arguing that formal
624 taxonomic proposals were premature. Subsequent molecular analyses by Withall and
625 Saunders (2006) were sufficiently robust to recognize a new order for *Acrosymphyton*
626 and *Schimmelmannia*, Acrosymphytales, solidly resolved as sister to the Ceramiales
627 with the Calosiphoniaceae as sister to the previous two orders. The Calosiphoniaceae
628 were considered *incertae sedis* pending study of the generitype *Calosiphonia* (Withall
629 and Saunders 2006). The Calosiphoniaceae remain a distinct lineage in our analyses
630 (Fig. 1); however, taxonomic proposals remain premature as we lack molecular data for
631 both the type of *Schmitzia* and, more importantly, *Calosiphonia*.
632 The resolution of our new Tasmanian species *Acrothesaurum gemellifilum*
633 within the Acrosymphytales prompted us to consider family-level taxonomy.
634 *Acrosymphyton* is highly distinctive from *Acrothesaurum* and *Schimmelmannia* as it is
635 characterized by carpogonial branches bearing pinnate laterals, production of primary
636 connecting filaments on presumed fertilization that first fuse with cells of the
637 carpogonial-branch laterals, which in turn issue lengthy septate secondary connecting
638 filaments that seek out distant (i.e. nonprocarpic) auxiliary cells terminating short,
639 unbranched “adventitious” filaments, diploidize them, and then continue on to effect

large numbers of further diploidizations (Sjöstedt 1926, Kraft 1981, fig. 1.1, Millar and Kraft 1984, figs 7-9, 15). *Schimmelmannia*, on the other hand, is procarpic with the supporting cell bearing both the carpogonial and auxiliary-cell branches, the former simple and not pinnate as in *Acrosymphyton* (Kylin 1930). Following fertilization in *Schimmelmannia* the carpogonium typically undergoes one or two divisions (with one exception; Ballantine et al. 2003), with one of the resulting cells fusing directly to the auxiliary cell (Kylin 1930), again in stark contrast to *Acrosymphyton*. Our new Tasmanian genus, *Acrothesaurum*, differs from both *Acrosymphyton* and *Schimmelmannia* in that following fertilization the carpogonium fuses directly with an auxiliary cell without intervening connecting filaments or connecting cells, respectively. It is further unusual in blurring the lines between procarpy and nonprocarpy in that auxiliary cells are diploidized both in the same and in separate branch systems by post-fertilization carpogonia. To avoid paraphyly (Fig. 1, Table 1), and in consideration of the significant anatomical differences for *Acrosymphyton* relative to *Acrothesaurum* and *Schimmelmannia*, we have recognized the latter two at the family level in the Acrosymphytales.

The blurring of the procarpic and nonprocarpic conditions in this family may represent a transitional state from the procarpic Schimmelmanniaceae to the elaborate nonprocarpy characteristic of the Acrosymphytaceae (Fig. 1). The sister relationship of the Acrosymphytales to the procarpic Ceramiales, combined with the early divergence of the procarpic Schimmelmanniaceae, render it parsimonious to conclude that procarpy is ancestral to nonprocarpy in the Acrosymphytales. For this lineage at least, this

662 reverses the long-standing paradigm that nonprocarpy is an ancestral condition to
663 procarpy and that the Ceramiales are the apogee of red algal evolution (Kylin 1956).
664 *Acrothesaurum gemellifilum* is another novel addition to our knowledge of
665 Tasmanian algal biodiversity as it displays vegetative characters seemingly typical of
666 the Gloiosiphoniaceae (Gigartinales), a family heretofore unknown in Australia
667 (Womersley 1994). Molecular analyses, however, revealed an unexpected alliance with
668 the Acrosymphytales, a small order including species that classical morphologists
669 would not have been likely to classify correctly. Recognition of the Acrosymphytales
670 as presented here emphasizes the importance of the terminal auxiliary cell as a
671 diacritical marker among "gloiosiphonioid" taxa (Yeh and Yeh 2008, p. 337). The
672 affinities of the genera *Gloeophycus* and *Peleophycus* need consideration as both are
673 atypical members of the Gloiosiphoniaceae in being characterized by terminal auxiliary
674 cells.
675 *Gloeophycus* is lubricous in habit and lacks the pinnate carpogonial branch of
676 the Acrosymphytaceae (Lee and Yoo 1979). It is procarpic and in this regard more
677 reminiscent of the Acrothesauraceae and Schimmelmanniaceae, although more akin to
678 the latter (Kaneko et al. 1980). Pending much needed insights of molecular data this
679 genus is provisionally placed in the Schimmelmanniaceae, Acrosymphytales.
680 *Peleophycus* is a Hawaiian endemic monotypic genus (Abbott 1984). Limited
681 LSU data (664 bp) in GenBank (HQ421875) for *Peleophycus multiprocarpius*
682 I.A.Abbott solidly ally this genus to the Acrothesauraceae (not shown). Like
683 *Acrothesaurum*, *P. multiprocarpius* is lubricous, has similarly structured laterals
684 (Abbott 1984, figs 2, 7, 8), spermatangia (Abbott 1984, fig. 9), and

carpogonial/auxiliary-cell branches (Abbott 1984, figs 5, 6). It differs in the relative simplicity of its rhizoidal cortication (Abbott 1984, fig. 4), which apparently does not produce perpendicular corticating filaments, the division of presumably fertilized carpogonia and the diploidization of auxiliary cells by a connecting cell produced by a derivative cell of the divided carpogonium, and an apparent lack of the stout tubular gonimoblasts that arise from proximal auxiliary-cell surfaces to connect to subtending central-axial cells. Whereas *Peleophycus* was reported as strictly procarpic (Abbott 1984, p. 330), the possibility that carpogonia can diploidize auxiliary cells in separate branch systems as noted here for *Acrothesaurum* should be explored.

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Figure legends

Fig. 1. Bayesian phylogeny for multigene alignment analyzed with full partitioning. Letters at nodes refer to respective support values in Table 1. New taxa in bold type. The outgroup Corallinophycidae have been cropped from the figure to facilitate presentation. Scale indicates substitutions per site.

Fig. 2. Specimen CO00288 from the brothers Crouan herbarium housed at the Muséum National d'Histoire Naturelle - Marinarium de Concarneau (CO) is here designated as the lectotype of *Atractophora hypnoides*.

887

888 Fig. 3a-m. Vegetative and reproductive structures of *Atractophora hypnoides*
889 gametophytes in culture. The culture was isolated from tetraspores of field-collected
890 *Rhododiscus pulcherrimus*. [Abbreviations: ax, axial cell; b, first (basal) carpogonial
891 branch cell; c, carpogonium; cs, carposporangium; f, fusion cell; g, gonimoblast cell; gi,
892 gonimoblast initial; h/hy, hypogynous cell; l1-l4, lateral cells of carpogonial branch; s,
893 spermatangium; sm, spermatangial mother cell; sp, spermatium; su, supporting cell; tr,
894 trichogyne.]
895 a. Uniaxial erect axis of *A. hypnoides* 18 d after inoculation of tetraspores, showing
896 distichous branching pattern.
897 b. Erect axes after 29 d in culture, developing cruciate arrangement of whorl branchlets.
898 c. Apex of thallus after 46 d, showing transverse apical cell division, repositioning of
899 whorls around axial cells by elongation of axial cells, and terminal hair (arrow).
900 d. Cortication of axis (at 90 d) by downgrowing rhizoidal filaments produced by basal
901 cells of whorl branchlets (shaded).
902 e. Tufts of spermatangial mother cells bearing spermatangia after 46 d.
903 f-i. Stages of development of carpogonial branch.
904 j. Diagram of structure of mature carpogonial branch showing tufts of small "nutritive"
905 cells on the hypogynous cell, first carpogonial branch cell, and fourth lateral cell.
906 k. Carpogonial branch after fertilization, with spermatium attached to trichogyne.
907 Carpogonium and hypogynous cell appear to have fused; protuberance has developed
908 from supporting cell, to which lateral cell (l) is attached.

l. First carpogonial branch cell has apparently been included in fusion structure formed by carpogonium, hypogynous cell and supporting cell, which has produced branching gonimoblast filaments.

m. Gonimoblast filaments of mature carposporophyte, bearing terminal carposporangia, after 95 d in culture.

Scale bars represent: a, b, 50 µm; c, 20 µm; d, 20 µm; e-h, j-m, 25 µm.

Fig. 4a-i. Reproductive structures of *A. hypnoides* gametophytes in culture.

[Abbreviations as for Fig. 7.]

a. Thallus apex bearing tufts of spermatangial branches (arrows).

b. Spermatangial mother cells giving rise to spermatangia.

c. Thallus with spermatangial tufts and mature carpogonial branch (arrow) with long trichogyne and numerous attached and unattached spherical spermatia.

d. Young carpogonial branch with developing trichogyne.

e. Mature carpogonial branch showing 8-celled structure.

f, g. Post-fertilization development, with interpretation of complex structure drawn in multiple focal planes. Carpogonium has fused with supporting cell, still attached to axial cell (large arrow) to form fusion cell (shaded); gonimoblast initial and gonimoblast filaments produced from carpogonial end of fusion cell (shown in f). Second fusion cell consisting of hypogynous cell and first carpogonial branch cell (lies over carpogonium); third fusion cell formed by lateral cells 2 and 4; three groups of small nutritive cells (nut; small arrow) attached to these fusion cells.

931 h. Fusion cell (large arrow) with group of small nutritive cells (small arrow indicates
932 one cell).
933 i. Branching gonimoblast filaments with terminal cells developing into carposporangia.
934 Scale bars represent: a, 100 µm; b, d, 5 µm; c, 20 µm; e-i, 10 µm.
935
936 Fig. 5a-f. Vegetative and reproductive structures of *Atractophora hypnoides*
937 tetrasporophytes in culture.
938 a. Poorly attached spore (left) produced long filament that by lateral branching formed a
939 disc after 31 d.
940 b. Radial vertical section of crust (at 102 d), showing origin of erect filaments from
941 basal layer cells, and elongated apical cells.
942 c. Stained crust from below. Polyflabellate pattern formed by cessation of growth of
943 most filaments soon after branching.
944 d. Radial vertical sections of crust showing origin of two erect filaments from long basal
945 layer cells, and pigmented apical cells (shaded) that develop into tetrasporangia (t)
946 following transfer to short daylengths (8 h).
947 e. Surface view of crust with mature tetrasporangia; patchy appearance results from
948 discharge of spores.
949 f. Vertical section through field-collected tetrasporophyte (as *Rhododiscus*
950 *pulcherrimus*) with developing and mature tetrasporangia.
951 Scale bars represent: a, 50 µm; b, d, 20 µm; c, e, f, 25 µm.
952

953 Fig. 6a-j. *Acrothesaurum gemellifilum* vegetative and spermatangial features of
954 holotype (GWS016355).
955 a. Slide-mounted fragment serving as holotype.
956 b. Habit of entire specimen from which holotype slide was prepared.
957 c. Gradually tapering tips of indeterminate laterals, with higher-order laterals (arrows)
958 arising from epi-periaxial cells of whorl branchlets.
959 d. Whorl laterals borne on periaxial cells ringing distal poles of central-axial cells;
960 higher-order lateral (arrowhead) growing from epi-periaxial cell (arrow).
961 e. Apical cells of whorl-laterals that project into vegetative hairs (arrows).
962 f. Rhizoids initiated basipetally from periaxial cells that also bear carpogonial (arrow)
963 and auxiliary-cell (arrowhead) branches.
964 g. Basipetally growing rhizoids (arrowheads) producing lateral branches.
965 h. Complete internodal cover of central-axial cells by perpendicular determinate laterals
966 borne on rhizoidal filaments.
967 i. Whorl lateral of monoecious gametophyte with spermatangia borne singly
968 (arrowheads) or in multiples of two or three (double arrowheads) formed terminally or
969 subterminally, with an auxiliary-cell branch (arrow) directed basipetally from a
970 periaxial cell.
971 j. Cross-section of a gametophyte axis with terminal spermatangia on whorl laterals
972 borne on the four periaxial cells (arrowheads) surrounding the central-axial filament.
973
974 Fig. 7a-h. *Acrothesaurum gemellifilum* features of pre- and presumably post-
975 fertilization events (GWS016355). (Designations “a”, “b”, “ac” are basal, epibasal and

auxiliary cells, respectively; designations “1”, “2”, “3”, “cp” are basal, epibasal, hypogynous cells and carpogonia, respectively. “sc” = supporting cell of carpogonial and/or auxiliary-cell filaments.)

a. Periaxial and epi-periaxial (double arrowheads) cells bearing numerous auxiliary-cell branches (arrowheads) and an immature carpogonial branch with rudimentary trichogyne (arrow).

b. Periaxial and epi-periaxial cells bearing auxiliary-cell (arrowheads) and carpogonial branches, trichogyne of one (arrow) apparently breaking down and leaving hypogynous cell the size and position of an auxiliary cell.

c. Terminal auxiliary cells (ac) and three carpogonial branches with early trichogynes (arrows).

d. An anomalous five-celled carpogonial branch, carpogonium bearing a long sinuous trichogyne (arrow).

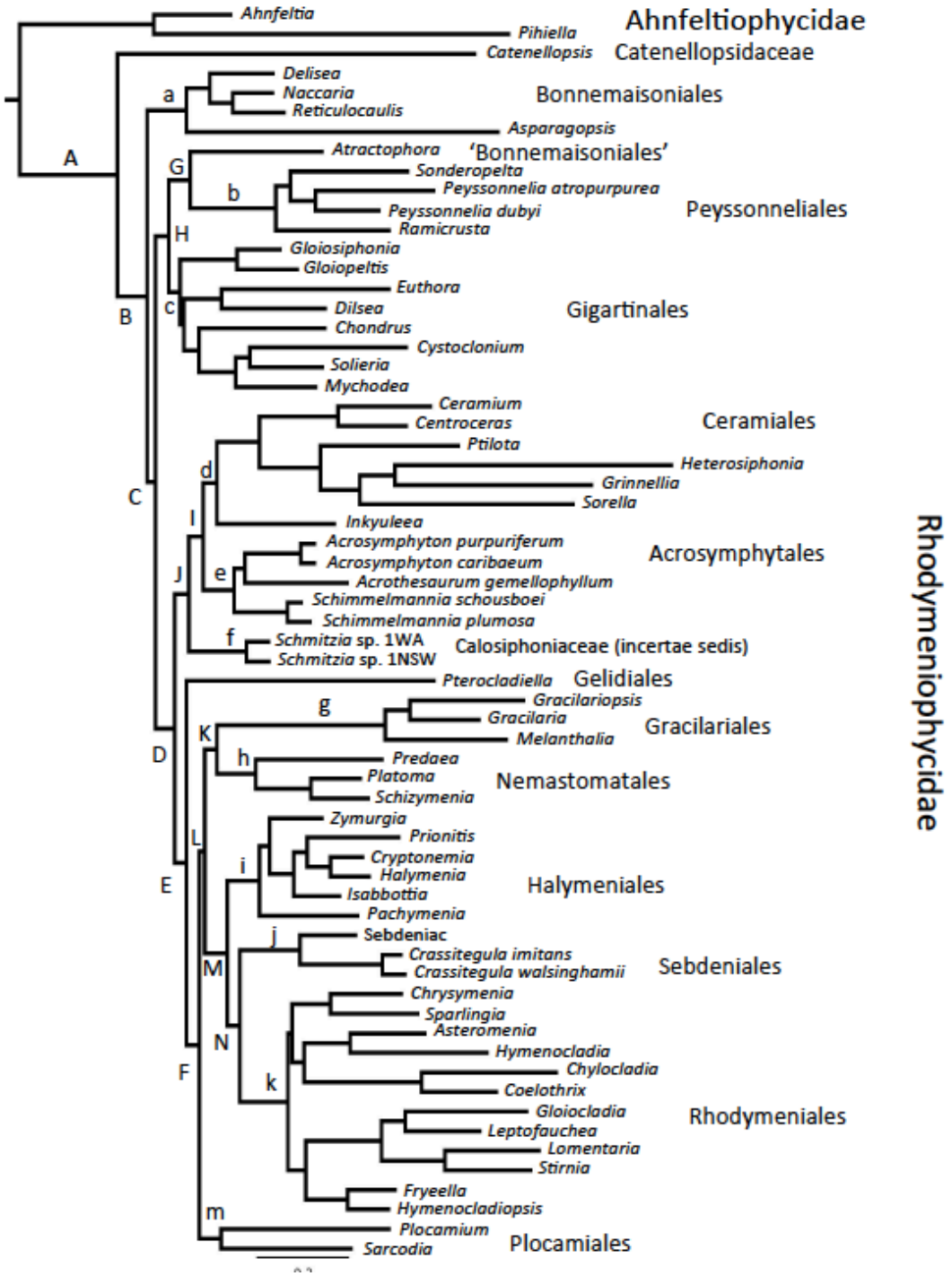
e. Carpogonial branch with adjacent auxiliary cells (ac), trichogyne (arrow) extending to whorl surface and apparently bearing attached spermatium (arrowhead).

f. Attachment of carpogonium to auxiliary cell (arrowhead) of sibling filament on supporting cell (sc) and cutting off of single gonimoblast initial (1’gbl).

g. Three-celled stage of early gonimoblast (gbl) following procarpic fusion of carpogonium and auxiliary cell (arrow).

h. As first gonimolobe matures, two basally directed arms of unknown function grow from extended auxiliary cell (ac).

998 Fig. 8a-f. *Acrothesarum gemellifilum* gonimoblast and carposporophyte features
999 (GWS016355). (Photo annotations as in Fig. 7.)
1000 a. Fused carpogonia (arrow) and auxiliary cell (ac), latter viewed end-on and stoutly
1001 connected to gonimoblast initial (gi), which subtends early first gonimolobe.
1002 b, c. Diploidized auxiliary cells seen side-on (ac), cells eccentrically swollen and
1003 bearing early (5b) and mid (5c) primary gonimolobes.
1004 d. Terminal fusion of two auxiliary-cell arms (arrows) to adjacent central-axial cells
1005 (arrowheads).
1006 e. Mature primary gonimolobe on auxiliary cell (arrow) that has issued two inwardly
1007 growing arms (arrowheads) that are yet to fuse with central-axial cells.
1008 f. Mature carposporophyte consisting of three gonimolobes (gl-1, -2, -3) of successively
1009 maturing crops of carposporangia.
1010
1011 Supplementary Material
1012 Fig. S1. An alternative topology generated by neighbor-joining with the HKY model to
1013 assess the effect of the starting tree on our SiteStripper analyses.
1014 Table S1. A list of the taxa used in this study with the corresponding GenBank
1015 accessions for the five genes used in phylogenetic analyses.
1016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Asparagopsis
Atractophora
'Bonnemaisoniales'
Sonderopelta
Peyssonnelia atropurpurea
Peyssonnelia dubyi
Peyssonneliales
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Gloiosiphonia
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Dilsea
Gigartinales
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Cystoclonium
Solieria
Mychodea
Ceramium
Centroceras
Ceramiales
Ptilota
Heterosiphonia
Grinnellia
Sorella
Inkyuleea
Acrosymphyton purpuriferum
Acrosymphyton caribaeum
Acrosymphytales
Acrothesaurum gemellophyllum
Schimmelmannia schousboei
Schimmelmannia plumosa
Schmitzia sp. 1WA
Schmitzia sp. 1NSW
Calosiphoniaceae (incertae sedis)
Pterocladiella
Gelidiales
Gracilariopsis
Gracilaria
Gracilariales
Melanthalia
Predaea
Platoma
Nemastomatales
Schizymenia
Zymurgia
Prionitis
Cryptonemia
Halymenia
Halymeniales
Isabbottia
Pachymenia
Sebdeniac
Crassitegula imitans
Crassitegula walsinghamii
Sebdeniales
Chrysymenia
Sparlingia
Asteromenia
Hymenocladia
Chylocladia
Coelothrix
Rhodymeniales
Gloiocladia
Leptofauchea
Lomentaria
Stirnia
Fryeella
Hymenocladiopsis
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Sarcodia
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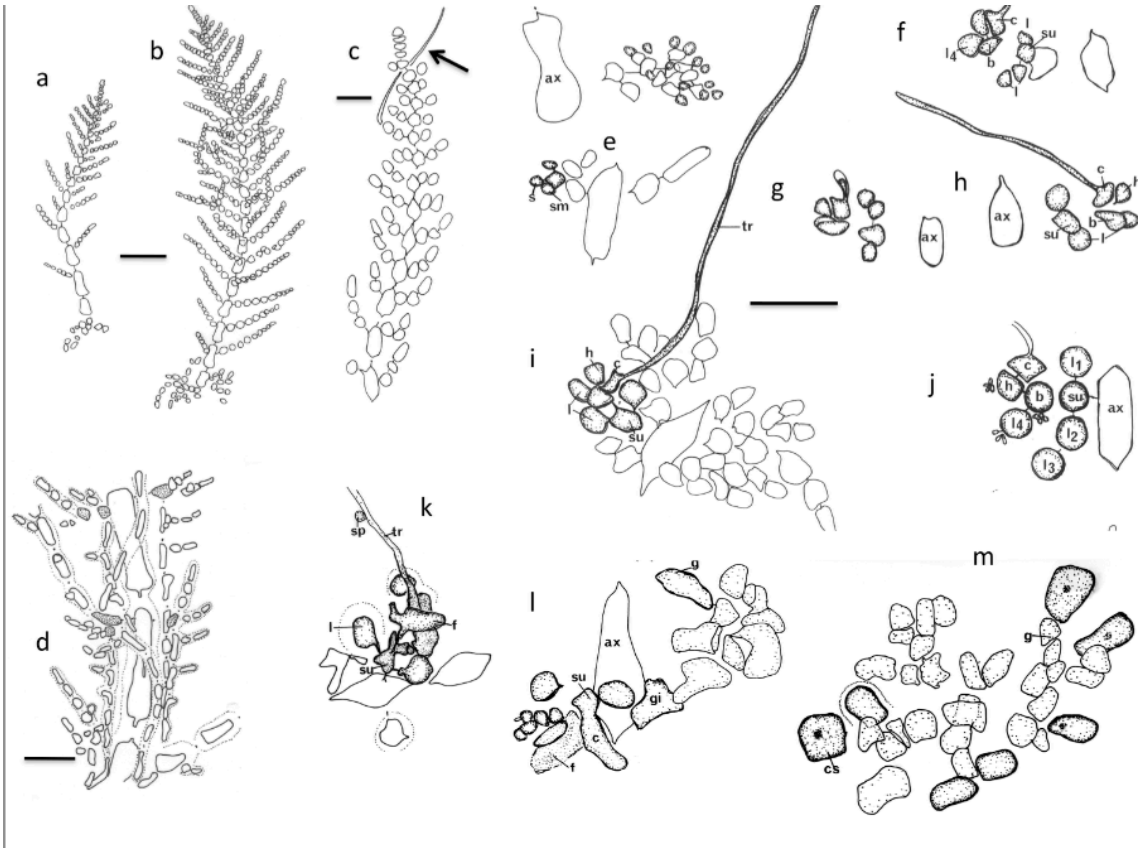

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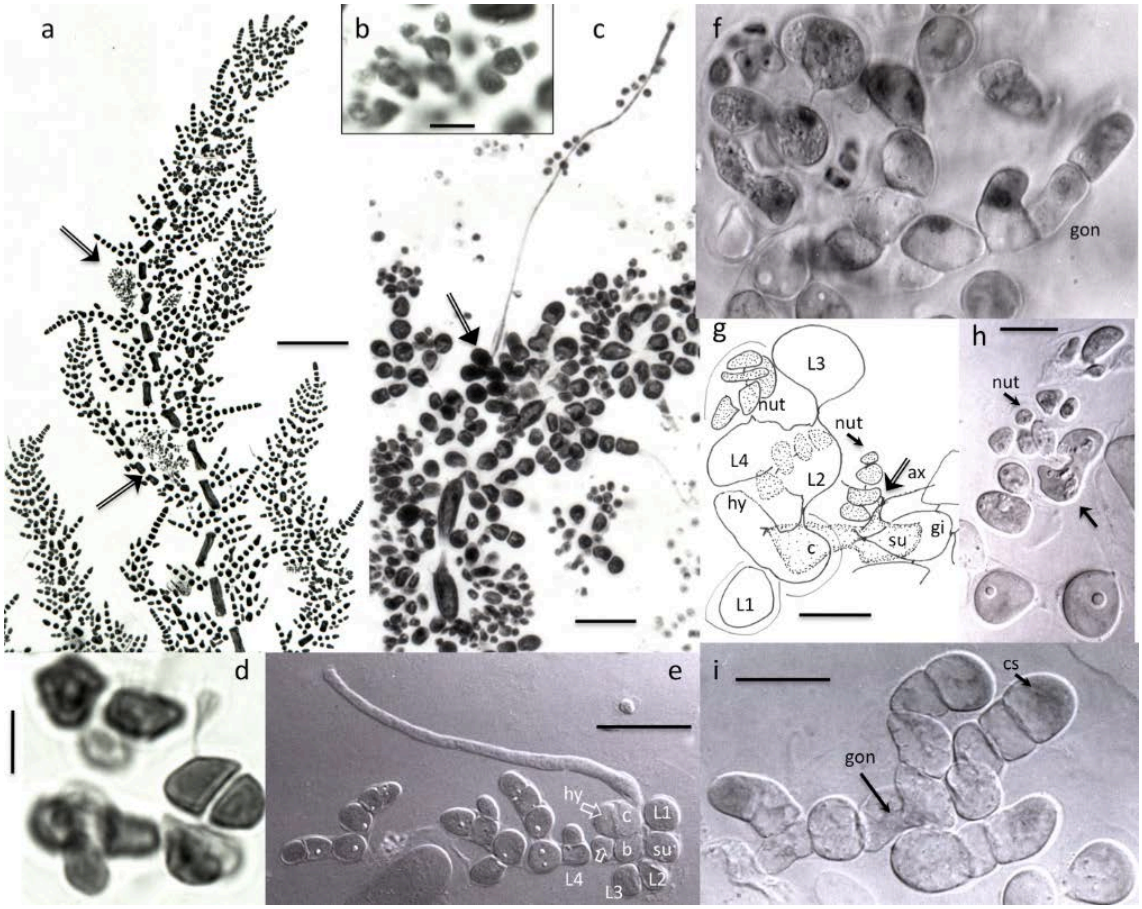

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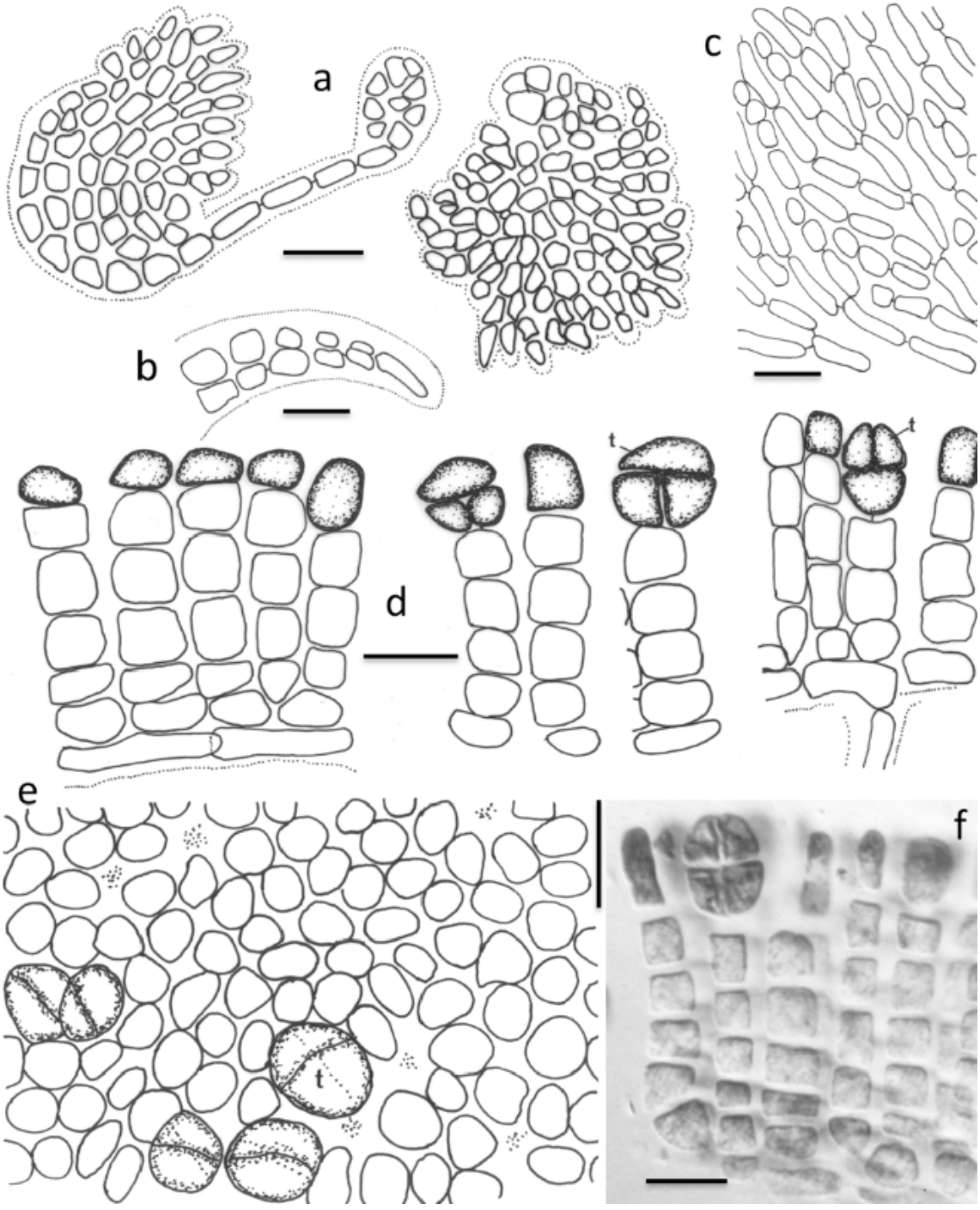

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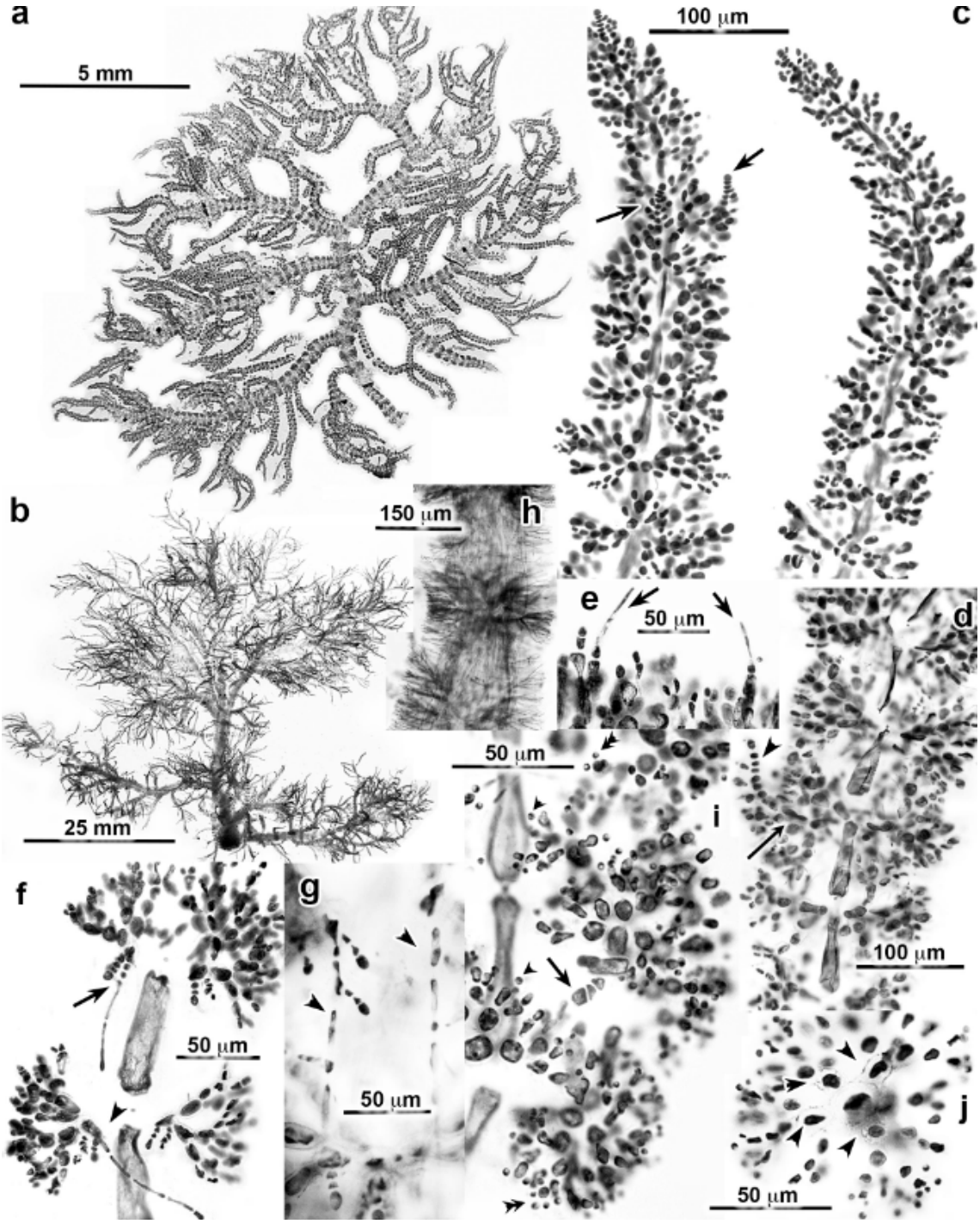

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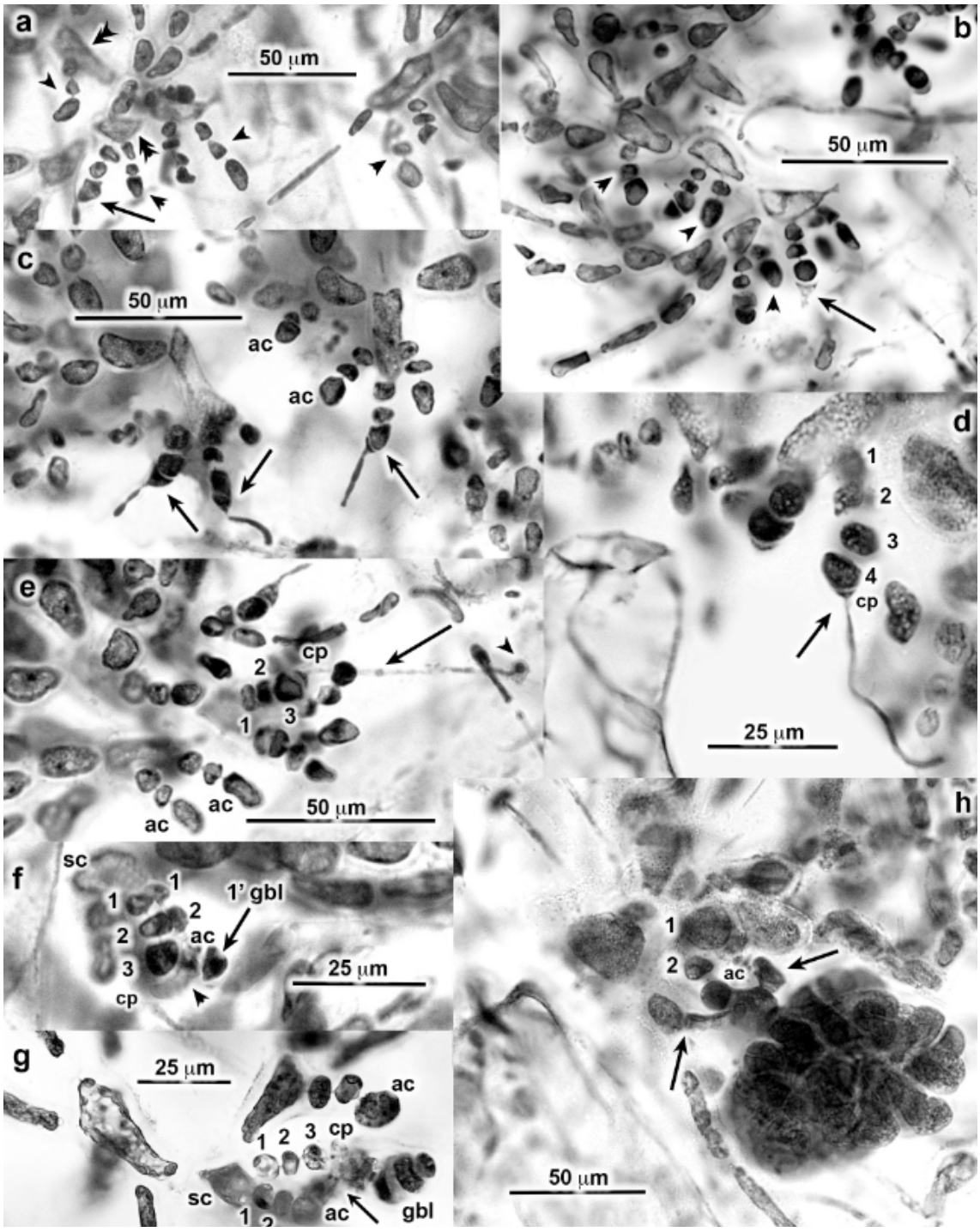
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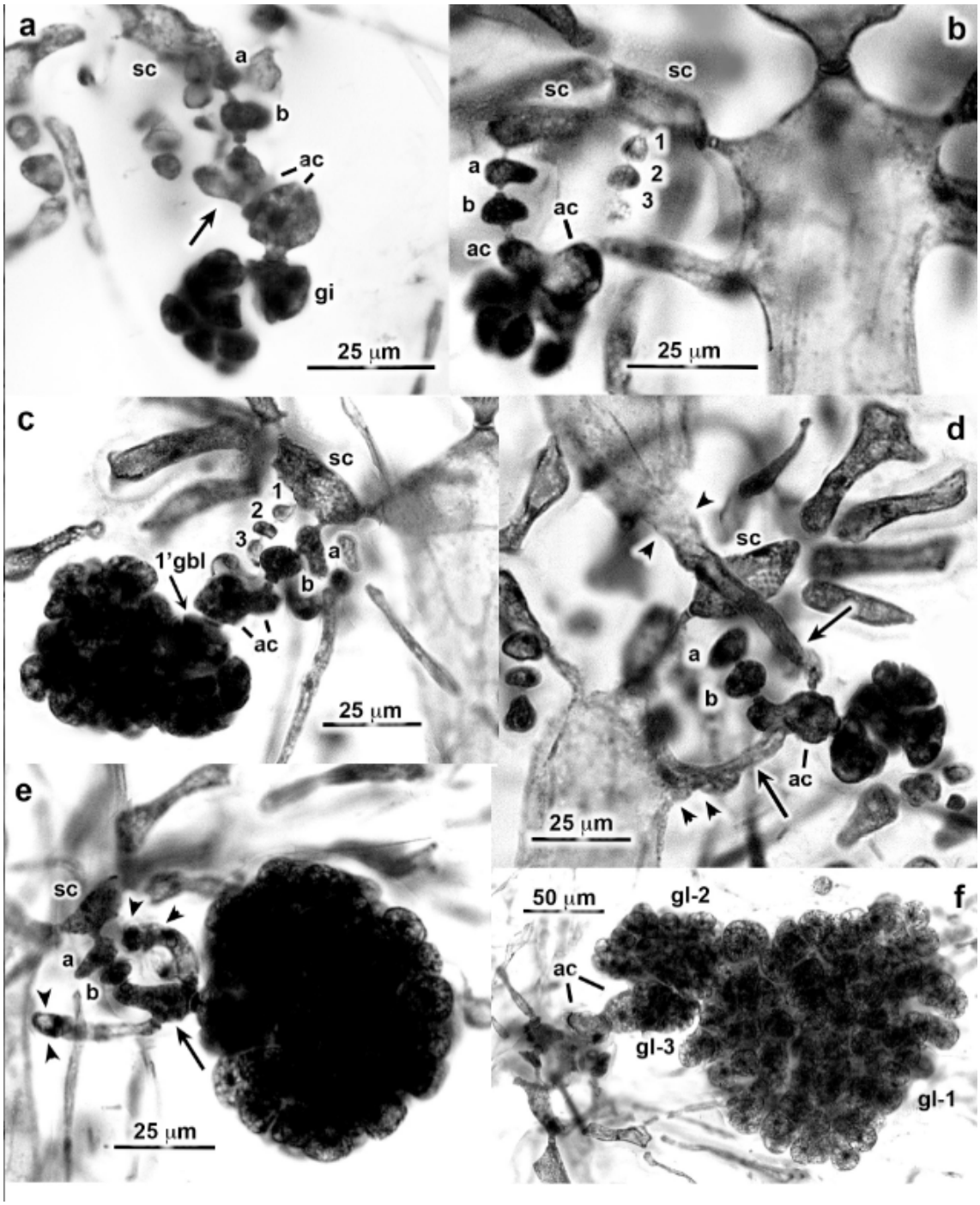
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