AUGOPHYLLUM, A NEW GENUS OF THE DELESSERIACEAE (RHODOPHYTA) BASED ON *rbc*L SEQUENCE ANALYSIS AND CYSTOCARP DEVELOPMENT[1]

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A new genus, *Augophyllum* Lin, Fredericq et Hommersand gen. nov. related to *Nitophyllum*, tribe Nitophylleae, subfam. Nitophylloideae of the Delesseriaceae, is established to contain the type species *Augophyllum wysorii* Lin, Fredericq et Hommersand sp. nov. from Caribbean Panama; *Augophyllum kentingii* Lin, Fredericq et Hommersand sp. nov. from Taiwan; *Augophyllum marginifructum* (R. E. Norris et Wynne) Lin, Fredericq et Hommersand comb. nov. (*Myriogramme marginifructa* R. E. Norris et Wynne 1987) from South Africa, Tanzania, and the Sultanate of Oman; and *Augophyllum delicatum* (Millar) Lin, Fredericq et Hommersand comb. nov. (*Nitophyllum delicatum* Millar 1990) from southeastern Australia. Like *Nitophyllum*, *Augophyllum* is characterized by a diffuse meristematic region, the absence of macro- and microscopic veins, procarps consisting of a supporting cell bearing a slightly curved four-celled carpogonial branch flanked laterally by a cover cell and a sterile cell, a branched multicellular sterile group after fertilization, absence of cell fusions between gonimoblast cells, and tetrasporangia transformed from multinucleate surface cells. *Augophyllum* differs from *Nitophyllum* by the blades becoming polystromatic inside the margins, often with a stipitate cylindrical base, the possession of aggregated discoid plastids neither linked by fine strands nor forming bead-like branched chains, spermatangia and procarps initiated at the margins of blades, not diffuse, and a cystocarp composed of densely branched gonimoblast filaments borne on a conspicuous persistent auxiliary cell with an enlarged nucleus. Analyses of the *rbc*L gene support the separation of *Augophyllum* from *Nitophyllum*. An investigation of species attributed to *Nitophyllum* around the world is expected to reveal other taxa referable to *Augophyllum*.

Lin et al. (2001a) emended the subfamily Nitophylloideae of the family Delesseriaceae to contain two tribes, Martensieae, with one genus, *Martensia*, and Nitophylleae, with eight genera: *Nitophyllum* Greville (1830), *Arachnophyllum* Zanardini (1843), *Polyneurella* Dawson (1944), *Polyneuropsis* Wynne, McBride et West (1973), *Calonitophyllum* Aregood (1975), *Valeriemaya* Millar et Wynne (1992), *Papenfussia* Kylin (1938), and *Radicilingua* Papenfuss (1956). The subfamily Nitophylloideae is well defined, characterized by procarps consisting of a four-celled carpogonial branch flanked laterally by cover cells and a single sterile cell. A massive fusion cell and fusions between gonimoblast cells are absent (Maggs and Hommersand 1993, Lin et al. 2001a,b, Lin and Fredericq 2003).

Three of the species described here were referred to in Lin et al. (2001a) as *Nitophyllum* sp. 1 from Caribbean Panama, *Nitophyllum* sp. 2 from southern Taiwan, and *Nitophyllum delicatum* (Millar 1990) from southeast Australia. Here we include *Myriogramme marginifructa* Norris et Wynne (1987) from South Africa. In molecular analyses these four species form a clade that is distinct from the clade containing the type species of *Nitophyllum*, *N. punctatum*. A new genus, *Augophyllum*, is proposed to include the type species, *Augophyllum wysorii*, and the three other species.

MATERIALS AND METHODS

Collections were made by SCUBA. Algal samples for the molecular study were desiccated in silica gel or preserved in 95% alcohol. Voucher specimens and materials used in the morphological study were fixed in 10% formalin/seawater and

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stored in 5% formalin/seawater or pressed as herbarium sheets and deposited in the Herbarium of the University of Louisiana at Lafayette (LAF), USA. Herbarium abbreviations follow Holmgren et al. (1990). Whole-mount material and hand sections were treated with Wittmann's aceto-iron-hematoxylin-chloral hydrate (Wittmann 1965) and mounted in 50% Hoyer's mounting medium (Lin et al. 2001c, Lin and Fredericq 2003). Microphotographs were taken on a BX51 microscope (Olympus, Tokyo, Japan) with a digital camera (Pixera Pro600ES, Tokyo, Japan). Because the new genus is a member of the tribe Nitophylleae and subfamily Nitophylloideae of the Delesseriaceae, the reproductive development of the type species of this tribe, *Nitophyllum punctatum*, was included for illustrative and comparative purposes.

DNA samples were prepared using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the instructions of the manufacturer. DNA sequencing procedures are as described in Lin et al. (2001a) and Lin and Fredericq (2003). New sequence data and those first generated in Lin et al. (2001a) and Lin and Fredericq (2003) were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis. Phylogenetic analyses were performed using the maximum parsimony (MP) and Bayesian methods available in the computer programs PAUP* v.4.0 beta 10 (Swofford 2003) and MrBayes v.3.0 beta 4 (Huelsenbeck and Ronquist 2001). New *rbc*L sequences were generated for two members of the new genus (see Table 1 for

the GenBank numbers). Outgroup species were selected following Lin et al. (2001a).

The *rbc*L alignment initially included 1467 sites, but because information was missing for the 5′ ends of many sequences, the first 60 sites were excluded from the analyses. A set of sequences from 31 representative taxa belonging to the subfamilies Phycodryoideae and Nitophylloideae were selected for the analyses, with two members of the tribe Delesserieae in the subfamily Delesserioideae serving as the outgroup (Table 1). Parsimony heuristic searches and calculation of bootstrap proportion values (BP) were made as described in Lin et al. (2001a), with 5000 bootstrap replicates completed for the MP analysis. Decay indices (Bremer 1988) representing the number of steps less parsimonious than minimal at which branches were no longer resolved were determined based on strict consensus analysis of cladograms found by relaxing parsimony sequencing one step at a time, up to five steps.

The model used in the Bayesian analysis was the general-time-reversible model of nucleotide substitution with invariable sites and gamma-distributed rates for the variable sites (GTR + I + Γ). This model was selected based on maximum likelihood ratio test implemented by the software Modeltest version 3.06 (Posada and Crandall 1998) with a significance level of 0.01. For the Bayesian analysis, we ran four chains of the Markov chain Monte Carlo (one hot and three cold), sampling 1 tree every 10 generations for 1,000,000 generations starting with a random tree. Stationarity was reached at

TABLE 1. List of species used in *rbc*L analysis and accession numbers in GenBank.

Species	Collection information/references	GenBank accession number[a]
"*Hemineura*" *cruenta* Harvey	Lin et al. 2001a	AF257453, 98%
Abroteia orbicularis J. Agardh	Lin et al. 2001a	AF254149, 98%
Acrosorium decumbens (J. Agardh) Kylin	Lin et al. 2001a	AF254151, 94%
Acrosorium venulosum (Zanardini) Kylin	Lin et al. 2001a	AF254156, 95%
Augophyllum delicatum (Millar) Lin, Fredericq et Hommersand comb. nov. [= *Nitophyllum delicatum* Millar]	Lin et al. 2001a	AF257400, 97%
Augophyllum kentingii sp. nov.	Sail Rock, Kenting National Park, Taiwan, coll. S. M. Lin, 14.iii.2002. (This study)	AY680694, 99%
Augophyllum marginifructa (R. E. Norris et Wynne) Lin, Fredericq et Hommersand comb. nov.	Sodwana Bay, KwaZulu-Natal, South Africa, coll. S. Fredericq & O. De Clerck, 10.ii.2001. (This study)	AY680695, 97%
Augophyllum wysorii sp. nov. (= *Nitophyllum* sp. 1)	Lin et al. 2001a	AF257402, 97%
Botryoglossum platycarpum (Turner) Kützing	Lin et al. 2001a	AF254151, 94%
Calonitophyllum medium (Hoyt) Aregood	Lin and Fredericq 2003	AF254166, 97%
Cladodonta lyallii (Hooker et Harvey) Skottsberg	Lin et al. 2001a	AF257169, 94%
Cryptopleura ramosa (Hudson) Kylin ex Newton	Lin et al. 2001a	AF254175, 96%
Delesseria decipiens J. Agardh	Lin et al. 2001a	AF254181, 94%
Delesseria sanguinea (Hudson) Lamouroux	Lin et al. 2001a	AF254182, 86%
Drachiella spectabilis Ernst et Feldmann	Lin et al. 2001a	AF254183, 93%
Hymenena venosa (Linnaeus) Krauss	Lin et al. 2001a	AF257365, 98%
Martensia australis Harvey	Lin et al. 2001b	AF257374, 94%
Martensia elegans Hering	Lin et al. 2001a	AF257375, 98%
Martensia formosana Lin, Hommersand et Fredericq	Lin et al. 2004	AY253663, 97%
Martensia lewisiae Lin, Hommersand et Fredericq	Lin et al. 2004	AY253661, 96%
Martensia martensii (Schmitz) Lin, Fredericq et Liao	Lin et al. 2001b	AF257407, 93%
Martensia pavonia (C. Agardh) J. Agardh	Lin et al. 2001a	AF257379, 91%
Myriogramme livida (Hooker et Harvey) Kylin	Lin et al. 2001a	AF257391, 95%
Myriogramme manginii (Gain) Skottsberg	Lin et al. 2001a	AF257392, 93%
Neuroglossum binderianum Kützing	Lin et al. 2001a	AF257394, 98%
Nienburgia andersoniana (J. Agardh) Kylin	Lin et al. 2001a	AF254396, 97%
Nitophyllum hommersandii Lin et Fredericq	Lin and Fredericq 2003	AY118270, 96%
Nitophyllum punctatum (Stackhouse) Greville	Lin et al. 2001a	AF257402, 97%
Phycodrys rubens (Linnaeus) Batters	Lin et al. 2001a	AF257429, 95%
Platyclinia taylorii Levring	Lin et al. 2001a	AF257432, 98%
Polyneuropsis stolonifera Wynne, McBride et West	Lin et al. 2001a	AF257439, 93%
Schizoseris condensata (Reinsch) Ricker	Lin et al. 2001a	AF257444, 94%

[a]The number after the accession number is the percentage of the gene sequenced.

generation 8220. Thus, trees saved until generation 8220 were the "burn-in" of the chain, and inferences about the phylogeny were based on those trees sampled after generation 8220. A 50% consensus tree (majority rule as implemented by PAUP*) was computed from the $99,178 + 1$ trees saved after the burn-in point.

RESULTS

Augophyllum Lin, Fredericq et Hommersand, gen. nov.

Type: Augophyllum wysorii Lin, Fredericq et Hommersand sp. nov.

Etymology: "*Augophyllum*"from the Greek "auge": luster, shine, and phyllo: leaf, referring to its light-reflecting blades.

Thalli constantes ex laminis crispatis palmatisque, exorientes stipite unico recto vel ramoso; plerumque polystromatici praeter margines, laminae maculis nitentibus iridoaureis et/vel circularibus marmoratis super paginas, membranaceae venis microscopicis macroscopicisque aut costis absentibus; plasti discoidei, compacti sub pagina cellulae, non concatenati per tenuia fila vel formantes catenis ramosis ad instar pilularum; cystocarpia restricta ad margines vel partes supernas laminarum fertilium; procarpia constantia ex cellula sustinenti ferenti lateralem cellulam sterilem singularem et filum carpogoniale leviter curvatum 4–cellulatum ambientem ad cellulam obtectam lateralem, cellula sterilis dividens et ramificans duplo vel triplo post fecundationem; cellula auxiliari abscissa apicaliter e cellula sustinenti, non producenti cellulam pedem sed amplificanti remanentique distincta basi carposporophyti; primarium initium gonimoblasti abscissum terminaliter e cellula auxiliari, crescens terminaliter per aliquot segmenta et ramificans bilateraliter; secundaria initia gonimoblasti abscissa lateraliter e cellula auxiliari; synapses inter cellulas gonimoblasti et inter cellulam auxiliarem et cellulam sustinentem dilatantes nucleis gonimoblasti accrescentibus; synapses inter cellulis gonimoblasti dilatantes sed cellulae non conjungentes; fila gonimoblasti evoluta valde ramosaque maxime vice in Nitophyllum*; sori tetrasporangiorum irregulariter circulares, formati prope margines laminae, dispersi raro super laminis fertilibus; initium tetrasporangii multinucleatum cum omnibus nucleis praeter unum degenerantibus, nucleo remanenti subienti meiosis; matura tetrasporangia tetraedrica divisa.*

Thalli consisting of ruffled, palmate blades, arising from a single, straight, or branched stipe; mostly polystromatic except the margins, blades with brilliant golden iridescent and/or circled mottled spots on surfaces, texture membranous with microscopic and macroscopic veins or midribs absent; plastids discoid, compact beneath cell surface, not linked by fine strands or forming bead-like, branched chains; cystocarps restricted to margins or upper parts of fertile blades, procarps consisting of a supporting cell bearing a single sterile cell and a slightly curved four-celled carpogonial branch flanked by a lateral cover cell, the sterile cell dividing and branching two to three times after fertilization; auxiliary cell cut off apically from supporting cell, not cutting off a foot cell but enlarging and remaining distinct at the base of the carposporophyte; primary gonimoblast initial cut off terminally from the auxiliary cell, growing terminally for a few segments and branching bilaterally; secondary gonimoblast initials cut off laterally from auxiliary cell; pit connections between gonimoblast cells and between the auxiliary cell and supporting cell broadening with gonimoblast nuclei enlarging; pit plugs between gonimoblast cells broadening but the cells not fusing; gonimoblast filaments strongly developed and highly branched compared with those of *Nitophyllum*; tetrasporangial sori irregularly circular, initiated near the blade margins, rarely scattered over fertile blades; tetrasporangial initials multinucleate with all but one nucleus degenerating, the remaining nucleus undergoing meiosis; mature tetrasporangia tetrahedrally divided.

Augophyllum wysorii Lin, Fredericq et Hommersand, sp. nov.

Thalli constantes ex 1–3 laminis crispatis palmatisque, 3–5 cm lati, 1–2 cm longi, exorientes stipite unico vel ramoso, 2–3 cm alto; laminae maculosae nitentibus iridoaureis circularibus marmoratis super paginas, membranaceae et polystromaticae omnino praeter ad margines; plasti discoidei, aggregati simul infra paginam cellulae; thalli masculi ignoti; procarpia et cystocarpia restricta ad margines laminarum fertilium, procarpia non fecundata degenerascentia mox post fecundationem; explicatio cystocarpii ut in generis; cystocarpia ostiolis rostratis, crescentia utroque laminarum et restricta ad margines laminae; carposporangia teminalia, maturantia in seriebus sequentibus; sori tetrasporangiorum circulares utroque laminarum fertilium, 0.4–3 mm diam., pro parte maxima restricti ad margines laminae.

Thalli consisting of 1–3 ruffled, palmate blades, 3–5 cm broad by 1–2 cm high, arising from a single or branched stipe, 2–3 cm tall; blades mottled with brilliant golden iridescent circular spots on surface, membranous and polystromatic throughout except at the margins; plastids discoid, aggregated together beneath the cell surface; males unknown; procarps and cystocarps restricted to margins of fertile blades, unfertilized procarps degenerating soon after fertilization; cystocarp development as for the genus; cystocarps with beaked ostioles, developing on both sides of blades and restricted to blade margins, carposporangia terminal, maturing sequentially; tetrasporangial sori on both sides of fertile blades, circular, 0.4–3 mm in diameter, mostly restricted to blade margins.

Etymology: "*wysorii*," in honor of our phycological colleague Brian Wysor, who collected the specimens and who is a specialist of Panamanian marine algae.

Holotype: In LAF, no. BW01006-2, tetrasporophyte (Fig. 1a). Isotype in LAF, no. BW01006-1, cystocarpic specimen (Fig. 1b).

Type locality: Galeta (STRI-Research Station), Colon, Caribbean Sea, Panama (9°24.4′ N, 79°52.0′ W)

Distribution: Known only from the type locality.

Habitat and seasonality: The collections were all made in September. Presence of stipes suggests that thalli may be perennial. Plants were attached on rock overlying a sand/mud plain at 12–15 m depth.
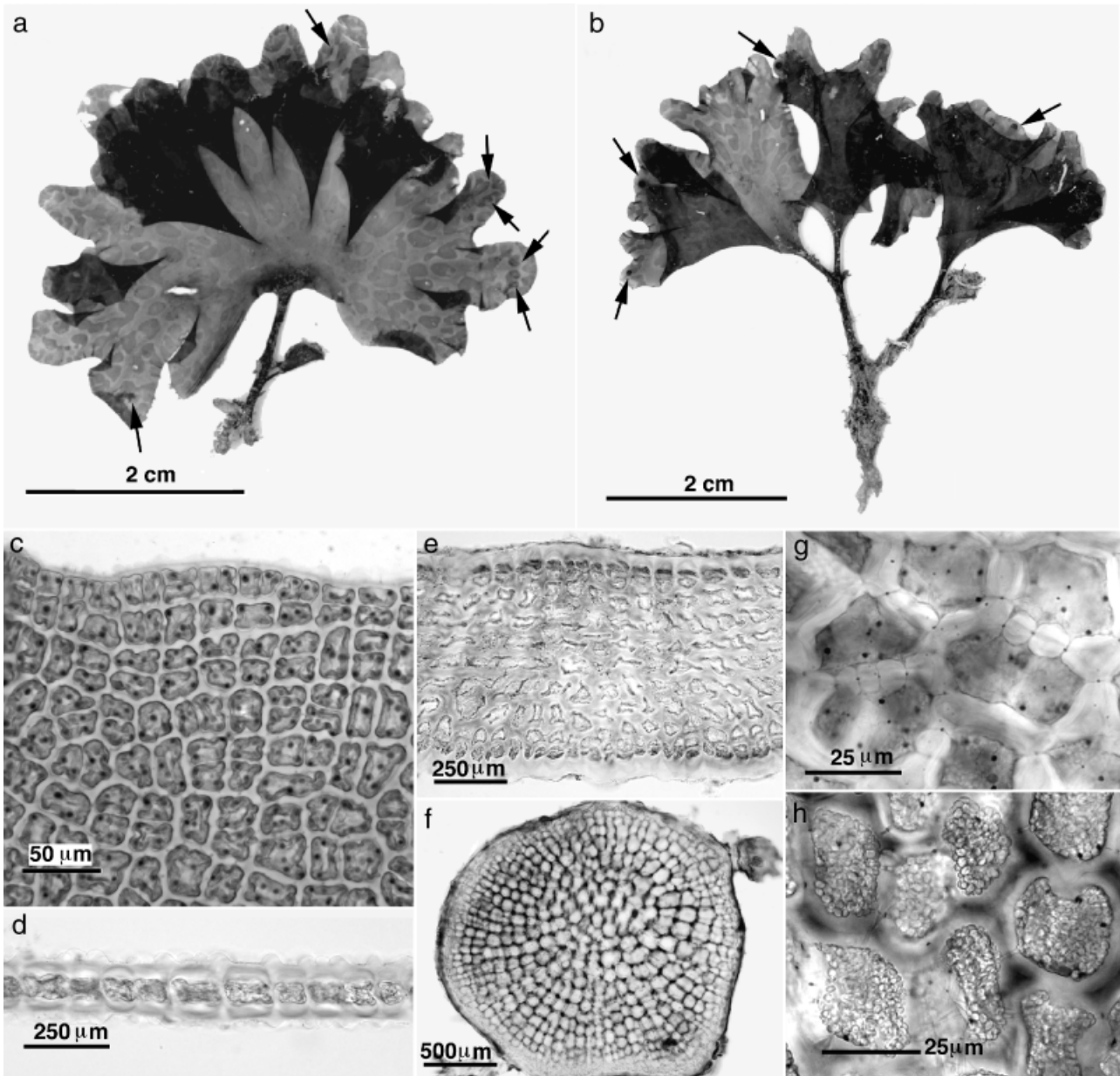
Fig. 1. *Augophyllum wysorii* (Caribbean Panama). Habit and vegetative morphology. (a) Holotype, tetrasporic plant showing tetrasporangial sori (arrows). (b) Isotype, cystocarpic plant with marginal cystocarps (arrows). (c) Young blade margin showing marginal and intercalary meristematic regions with multinucleate cells. (d) Cross-section through monostromatic thallus at margin. (e) Cross-section through polystromatic interior thallus. (f) Cross-section through stipe. (g) Surface view showing multiple pit connections between contiguous cells. (h) Surface view of discoid plastids.

Specimens examined: On rock over sand/mud plain, 12–15 m depth, Galeta (STRI-Research Station), Colon, Caribbean Sea, Panama, coll. W. Kooistra & B. Wysor, 21.ix.1999, tetrasporic (no. BW01006-1), female (no. BW01006-2).

Habit and vegetative structure: Thalli are 4–5.5 cm high, composed of one to three erect palmate blades (Fig. 1, a and b), 3–5 cm in width by 1–2 cm in height, arising from a single or branched stipe, 2–3 cm tall by 1.2–1.8 mm in diameter (Fig. 1f). The blades are membranous and bright red with brilliant golden iridescent circular mottled spots on surfaces (Fig. 1a).

Margins are entire and ruffled with irregularly marginal lobes, 4–9 mm wide (Fig. 1, a and c). The blades are polystromatic throughout (Fig. 1e), 55–530 μm in thickness, except the young monostromatic blade margins (Fig. 1d). Micro- and macroscopic veins are absent. Growth is diffuse by the meristematic activity of multinucleate marginal and intercalary cells (Fig. 1c), and secondary pit connections are abundant between contiguous multinucleate cells (Fig. 1g). Numerous parietal discoid plastids are densely aggregated beneath cell surfaces (Fig. 1h).
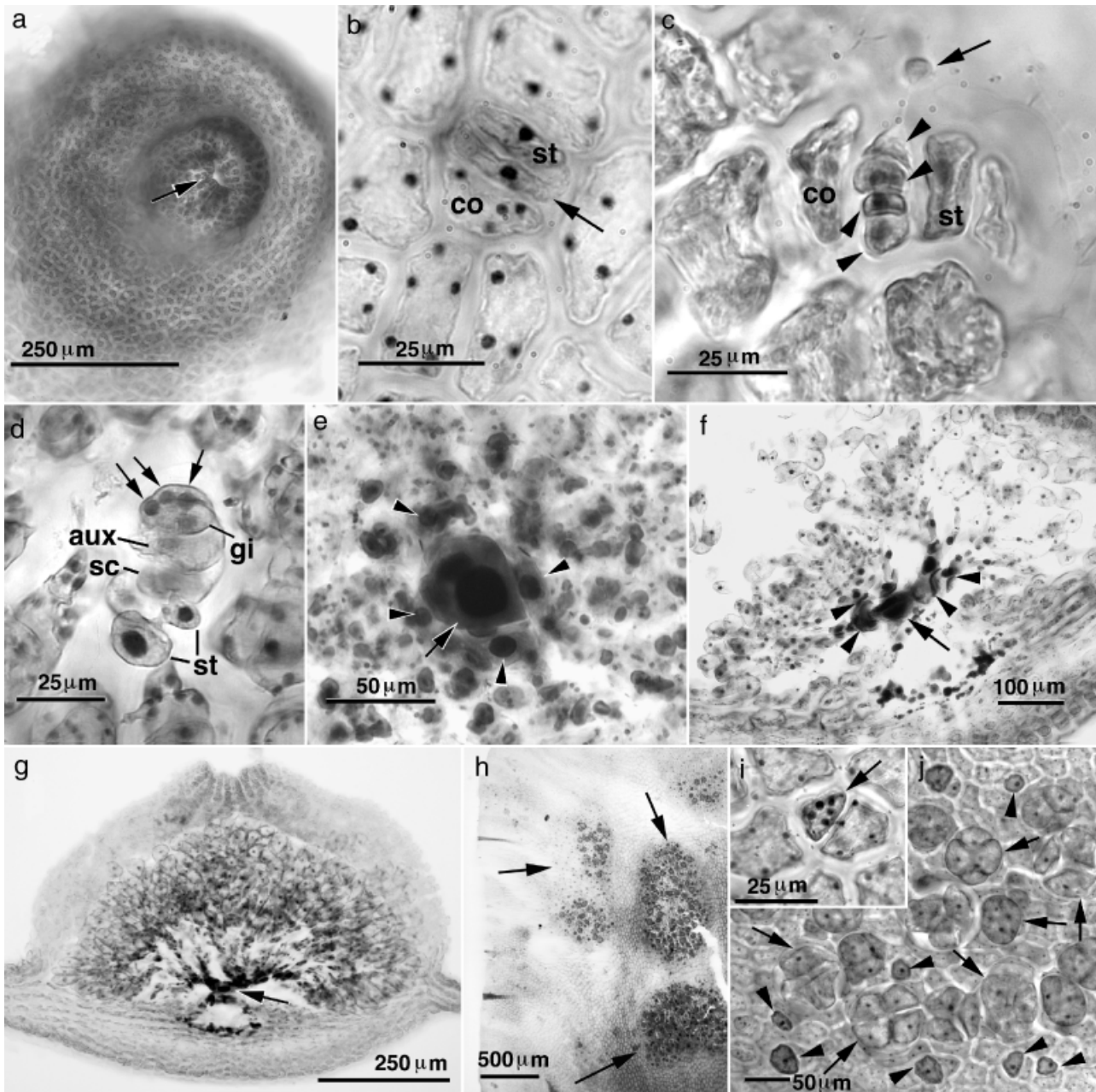
FIG. 2. *Augophyllum wysorii* (Caribbean Panama). Reproductive morphology. (a) Close-up of cystocarp with a beaked ostiole (arrow). (b) Early stage in procarp development showing an undivided cover cell (co) and supporting cell (arrow) bearing a lateral sterile cell (st). (c) Mature procarp showing supporting cell flanked by a cover cell (co) and bearing a four-celled carpogonial branch (arrowheads) with trichogyne (arrow) and a sterile cell (st). The supporting cell (not shown) is directly beneath the carpogonial branch. (d) Early stage of gonimoblast development showing the supporting cell (sc), the divided sterile group (st) with darkly staining nuclei, the auxiliary cell (aux), and a gonimoblast initial (gi) cutting off three young gonimoblast cells (arrows) acropetally. (e) Close-up of young cystocarp seen in surface view showing the auxiliary cell (arrow) with an enlarged darkly staining nucleus. Note that the gonimoblast cells (arrowheads) are not fused with the auxiliary cell. (f) Cross-section of an immature cystocarp showing the enlarged pit connections (arrowheads) between the auxiliary cell (arrow) and the inner gonimoblast cells. (g) Cross-section of a mature cystocarp showing the enlarged basal auxiliary cell (arrow) bearing densely branched gonimoblast filaments. (h) Surface view of some tetrasporangial sori (arrows). (i) Close-up of a multinucleate tetrasporocyte (arrow). (j) Close-up of tetrasporangial sorus showing uninucleate maturing tetrasporocytes (arrowheads) and mature tetrasporangia with tetrahedrally arranged tetraspores (arrows).

Reproductive structures: Male plants are unknown. Cystocarps and tetrasporangial sori are restricted to blade margins on both sides of fertile blades (Fig. 1, a and b). Procarps are initiated from a fertile central cell (Figs. 2b and 5a) and are abundant along both sides of the young blade margins (Figs. 2c and 5, b and c). A fertile central cell cuts off two pericentral cells, one of which functions as the cover cell initial

and the other as the supporting cell that produces a lateral sterile cell and a straight to slightly curved four-celled carpogonial branch. Very few carpogonia appear to have functional trichogynes, and unfertilized procarps soon degenerate with the result that few cystocarps develop on each fertilized blade. Cystocarps measure 0.8–1.3 mm in diameter at maturity with a slightly beaked ostiole (Fig. 2a). Fertilization and transfer of a fertilized nucleus to the auxiliary cell were not seen. After presumed fertilization, the sterile cell divides several times to produce a sterile group composed of a branched chain of five to six cells that remain attached to the supporting cell during early stages of cystocarp development (Figs. 2d and 5d). The gonimoblast initial is cut off transversely from the auxiliary cell and divides transversely to produce a short row of gonimoblast cells (Figs. 2d and 5d). Growth of the gonimoblasts is initially apical but becomes bilateral early in development (Figs. 2, e and f, and 5d). The auxiliary cell nucleus enlarges and becomes darkly staining, and the auxiliary cell serves as the basal cell of the carposporophyte throughout the development of the cystocarp (Fig. 2, e–g). As gonimoblast development progresses, the gonimoblast nuclei enlarge (Fig. 2e, arrowheads) and the primary pit connections between the gonimoblast cells and the auxiliary cell broaden (Fig. 2f, arrowheads). Fusion between gonimoblast cells is absent, and a large fusion cell is not formed (Fig. 2, f and g). The carposporangia are pyriform, 55–80 μm long by 28–40 μm wide, and are produced sequentially, appearing terminal.

Tetrasporangial sori are irregularly circular in shape (Fig. 2h), 0.4–3 mm in diameter, solitary or slightly aggregated and are scattered over the margin on both sides of fertile blades. Tetrasporocytes are transformed from multinucleate surface cells in the margins of young blades (Fig. 2i). Each tetrasporocyte enlarges and becomes darkly staining. Ultimately, all the nuclei but one degenerate (Fig. 2j, arrowheads). Mature tetrasporangia (Fig. 2j, arrows) are tetrahedrally divided, 80–110 μm in diameter.

Augophyllum kentingii Lin, Fredericq et Hommersand sp. nov.

Thalli constantes ex laminis prostrates decumbentibusque, 1–4 lobatis ad rotundis, 1–2 cm longis, 1–2.5 cm latis, exorientes stipite unico vel ramoso, 5–8 mm altis, laminae novae exorientes laminis prostrates veteribus coalescentesque rhizoideis marginalibus filamentosis vel hapteris discoideis infra laminas; laminae membranaceae polystromaticae omnino praeter ad margines, vivide ruberae maculis nitentibus iridoaureis, roseis vel cyaneoiridescentibus circularibus marmoratis super paginas; plasti discoidei, aggregati simul infra paginam cellulae; thalli masculi ignoti; procarpia et cystocarpia restricta ad margines laminarum fertilium vel dispersa super partes supernis laminarum fertilium; structura explicatioque procarpii ut in generis; procarpia non fecundata degenerascentia mox post fecundationem; cystocarpia crescentia utroque margines laminarum; cystocarpia ostiolis rostratis fila gonimoblasti multum ramosa et ferentia carposporangia teminalia,

tetrasporangia circulares aggregata in soris irregularibus, 0.5–0.8 mm x 0.7–1.5 mm diametro, vel genita singulariter dispersa super partibus superis laminarum fertilium.

Thalli composed of prostrate and overlapping decumbent, 1–4 lobed to rounded blades, 1–2 cm long by 1–2.5 cm wide, arising from a single or branched stipe, 5–8 mm high, new blades arising from old prostrate blades and becoming coalescent by marginal filamentous rhizoids or discoid haptera beneath the blades; blades membranous and polystromatic throughout except at the margins, bright red in color with brilliant golden, pink, or blue iridescent circular mottled spots on surface; plastids discoid, aggregated beneath cell surfaces; males unknown; procarps and cystocarps restricted to margins of fertile blades or scattered over upper parts of fertile blades; procarp structure and development as for the genus, unfertilized procarps degenerating soon after fertilization; cystocarps forming on both sides of blades, with beaked ostioles; gonimoblasts much branched and bearing terminal carposporangia; tetrasporangia aggregated in irregular sori, 0.5–0.8 mm by 0.7–1.5 mm in diameter, mostly restricted to blade margins, or borne singly scattered over upper parts of fertile blades.

Etymology: "*kentingii*" refers to the place, Kenting National Park, in which this new species was found.

Holotype: In Department of Natural Science Education, National Taitung University (NTTU), November 1, 1993, no. SML WLD-11-01-1993-1 (Fig. 3a).

Type locality: Wonglitung, Kenting National Park, Taiwan (21°55′ N, 120°49′ E).

Distribution: Sparsely distributed in the Kenting National Park, southern Taiwan.

Habitat and seasonality: The collections were made in March, April, July, November, and December. Presence of stipes indicates that this taxon may be a perennial. Plants were attached on reef rocks at 1–6 m depth.

Specimens examined: Kenting National Park, Taiwan: 1) Wanlitung, attached on reef rocks, 5–6 m deep, female, coll. S. M. Lin and M. L. Qiu, 01.xi.1993; attached on reef rocks, 2–6 m deep, female and tetrasporic, coll. S. M. Lin and M. L. Qiu, 06.vii. 1994; attached on reef rocks, 1–5 m deep female, coll. S. M. Lin and M. L. Qiu, 23.xii.1996; 2) Big Bay, attached on reef rocks, 2–6 m deep, tetrasporic, coll. S. M. Lin, 25.iii.2003; 3) Sail Rock, attached on reef rocks, 1–2 m deep, sterile, coll. S. M. Lin, 14.iii.2002.

Habit and vegetative structure: Thalli are composed of 1–4 lobed, flabellate to rounded blades, 1–2 cm long by 1–2.5 cm wide, arising from a single or sometimes branched stipe, 5–8 mm high and 0.6–1.2 mm in diameter (Fig. 3, a–c and e). When old, flabellate blades are decumbent and several overlapping blades (Fig. 3b) may coalesce by marginal filiform filaments (Fig. 3f) or discoid haptera. Blades are membranous and bright red and mottled with blue or brilliant golden iridescent circular spots and with entire slightly curved or irregularly lobed margins 5–16 mm wide (Fig. 3b). New blades arise from lower parts of stipes
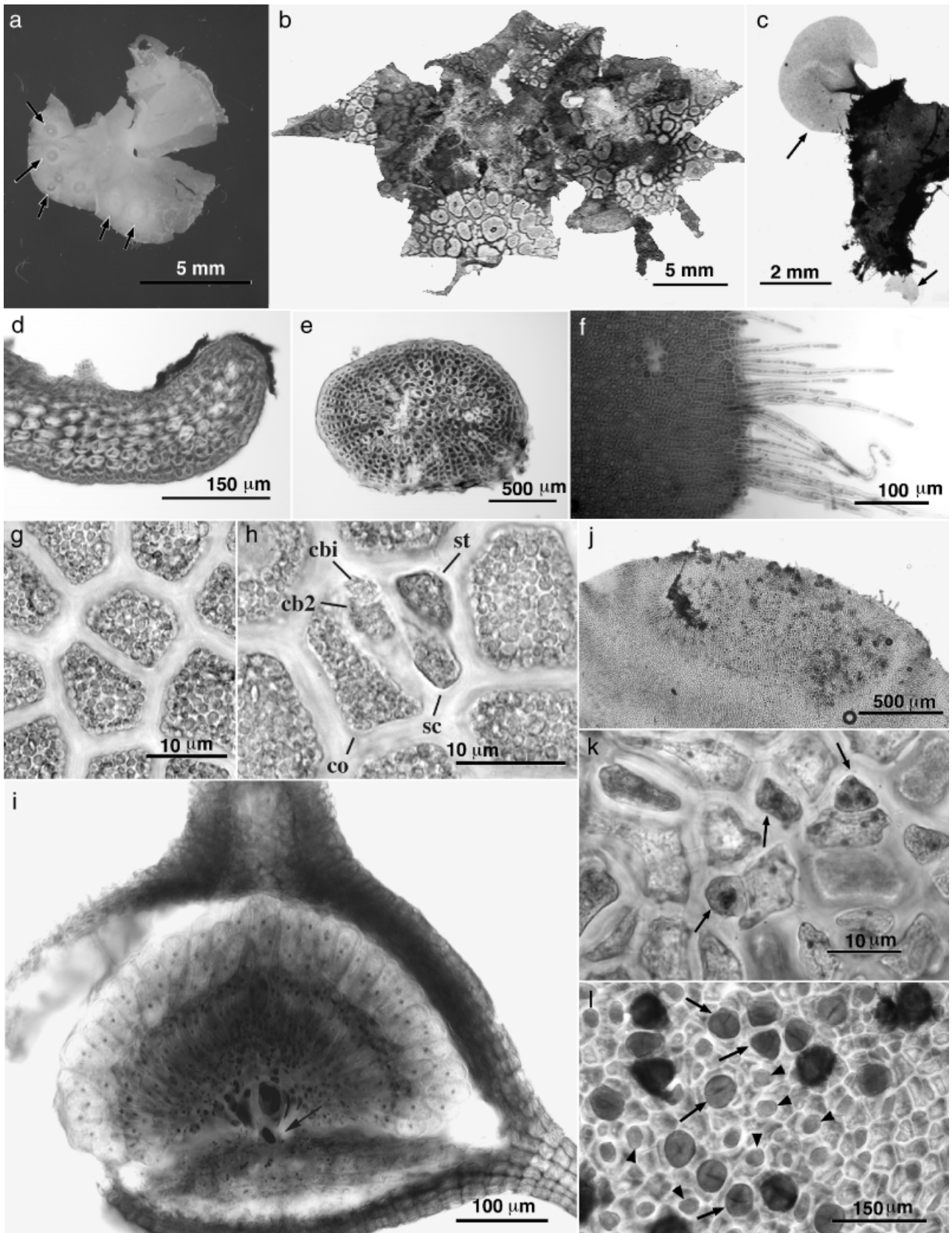
or from margins of prostrate blades (Fig. 3c). The blades are polystromatic throughout, three- to eight-celled layers (75–165 µm) thick (Fig. 3d), except at the margins of young blades. Microscopic and macroscopic veins are absent. Growth is diffuse and takes place as in the genus by meristematic activity of multinucleate marginal and intercalary cells. Secondary pit connections are abundant between contiguous multinucleate cells. Plastids are parietal, discoid, and numerous beneath the surface layers of cells (Fig. 3g).

Reproductive structures: Male plants are unknown. Cystocarps are scattered over both sides of the upper parts of fertile blades (Fig. 3a). Few functional procarps are found near young blade margins (Fig. 3h), and unfertilized procarps soon degenerate after fertilization. The few cystocarps that developed on fertile blades are 1–1.3 mm in diameter at maturity with beaked ostioles (Fig. 3i). Fertilization and transfer of a derivative of the fertilization nucleus to the auxiliary cell was not seen. Fusion does not occur between gonimoblast cells, and a large fusion cell is absent. A modified auxiliary cell with an enlarged nucleus functions as the basal cell of the carposporophyte, as in the type species *Augophyllum wysorii*. The carposporangia are borne terminally and are pyriform, 50–82 µm long by 25–37 µm wide. Tetrasporangia are formed in irregularly circular sori, 0.7–1.5 mm in diameter, or borne singly or in small aggregations and scattered over both sides of upper parts of blades (Fig. 3j). Tetrasporocytes are transformed from enlarged, darkly staining, multinucleate surface cells (Fig. 3k). Mature tetrasporangia are 60–80 µm in diameter and tetrahedrally divided (Fig. 3l).

Augophyllum marginifructum (R. E. Norris et Wynne) Lin, Fredericq et Hommersand comb. nov.

Basionym: Myriogramme marginifructa: R. E. Norris et Wynne (1987, pp. 381–5, figs. 1–16).

Type locality: Sodwana Bay, KwaZulu-Natal, South Africa.

Additional distribution: Tanzania (Coppejans et al. 2000), Sultanate of Oman (Wynne 2001b).

FIG. 3. *Augophyllum kentingii* (Kenting National Park, southern Taiwan). Vegetative and reproductive morphology. (a) Holotype, cystocarpic plant (arrows indicate the position of cystocarps inside margins). (b) Freshly collected sample showing several coalescent plants with circular mottled spots on surfaces. (c) Young blades (arrows) issuing from old prostrate blade. (d) Cross-section through polystromatic blade. (e) Cross-section of stipe. (f) Marginal filiform rhizoids. (g) Surface view of discoid plastids. (h) Early stage in procarp development showing an undivided cover cell (co) and supporting cell (sc) bearing a lateral sterile cell (st) and a young carpogonial branch with the second cell (cb2) and the carpogonial branch initial (cbi) in focus. (i) Cross-section of a mature cystocarp showing the enlarged basal auxiliary cell (arrow) bearing densely branched gonimoblast filaments. (j) Surface view of a tetrasporangial sorus (arrows). (k) Close-up of multinucleate tetrasporocytes (arrows). (l) Close-up of tetrasporangial sorus showing maturing tetrasporocytes (arrowheads) and mature tetrasporangia (arrows).

Specimens examined: Sodwana Bay, KwaZulu-Natal, South Africa; tetrasporic and female, coll. S. Fredericq & O. De Clerck, 10.ii.2001, and stored at LAF (SF-2-10-01-1-3).

Morphological observations. Thalli consist of one to two strongly ruffled blades, up to 15 cm tall (Fig. 4a), arising from a single or branched stipe, 3–4 cm in length. Blades are membranous and polystromatic throughout except at the margins. Plastids are discoid, aggregated beneath the cell surface. Spermatangia are borne in elliptical sori at the blade margins (Norris and Wynne 1987). Procarps and cystocarps are restricted to the margins of fertile blades, developing on both sides of the blades, with unfertilized procarps degenerating soon after fertilization. The architecture of the procarp (Fig. 4, b and c) and development of the cystocarp are as seen in the type species, *Augophyllum wysorii*. An enlarged auxiliary cell serves as the basal cell of the gonimoblasts (Fig. 4d). Carposporangia are terminal and pyriform, 55–70 µm long by 35–45 µm wide, and evidently produced sequentially. Tetrasporangial sori are irregularly circular in shape, 0.3–1.2 mm in diameter, solitary or aggregated and scattered over the margin on both sides of fertile blades (Fig. 4e). Tetrasporocytes are transformed from multinucleate surface cells in the margin of young blades (Fig. 4f). Mature tetrasporangia are 50–70 µm in diameter and tetrahedrally divided (Fig. 4g).

Augophyllum delicatum (A. J. K. Millar) Lin, Fredericq et Hommersand, comb. nov.

Basionym: Nitophyllum delicatum A. J. K. Millar 1990, pp. 420–2, fig. 54, A–F.

Holotype: MELU, AM202, Muttonbird Island, Coffs Harbour, New South Wales, Australia, coll. A. J. K. Millar, 8.ii.1980.

Specimens examined: SCUBA 13 m, Kallymenia flats, off Hole-in-the-wall, Jervis Bay, New South Wales, Australia, coll. A. J. K. Millar and D. Hardin, 24.x.1995 (tetrasporic); SCUBA, 10 m, North Side, Muttonbird Island, Coffs Harbour, New South Wales, Australia, coll. P. W. Gabrielson and A. J. K. Millar 13.viii.1982 (tetrasporic), 16.viii.1982. (tetrasporic, cystocarpic), stored at NCU.

The description and illustrations in Millar (1990) are in agreement with our more limited observations and are in accordance with the diagnostic characters of *Augophyllum*.

Molecular analyses. Thirty-one delesseriacean taxa belonging to the subfamilies Phycodryoideae and Nitophylloideae were selected for the *rbc*L sequence analysis with two members of the Delesserioideae serving as the outgroup. The final *rbc*L data matrix was restricted to 1407 total sites. In both parsimony (Fig. 6) and Bayesian analyses (Fig. 7) the topology of the trees was congruent. Parsimony analysis revealed two most-parsimonious trees (tree length = 2096, Consistency Index (CI) = 0.403, Retention Index (RI) = 0.575, and 470 informative characters for the entire *rbc*L data set). Tree lengths of 100,000
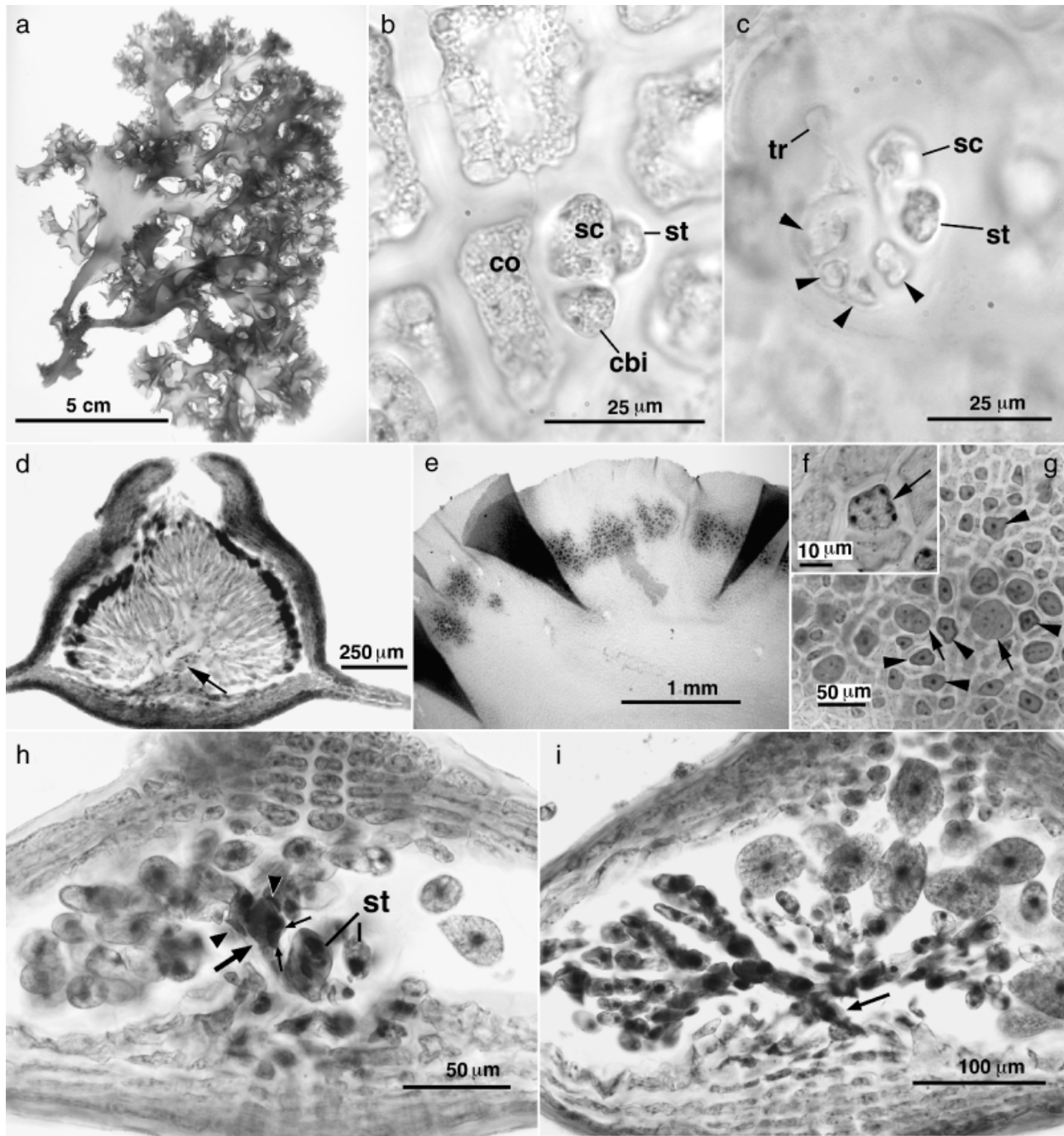
FIG. 4. (a–g) *Augophyllum marginifrucum* (Sodwana Bay, Natal, South Africa). (a) Habit of tetrasporangial plant with ruffled margins. (b) Prefertilization procarp showing an undivided cover cell (co), a supporting cell (sc) that has cut off a lateral sterile cell (st), and a carpogonial branch initial (cbi). (c) Mature procarp showing supporting cell (sc) bearing a carpogonial branch (arrowheads) with trichogyne (tr) and a lateral sterile cell (st). (d) Cross-section of mature cystocarp showing the enlarged basal auxiliary cell (arrow) bearing densely branched gonimoblast filaments and terminal carposporangia. (e) Close-up of marginal portion of tetrasporic plant bearing tetrasporangial sori. (f) Multinucleate tetrasporocyte (arrow). (g) Close-up of tetrasporangial sorus showing uninucleate maturing tetrasporocytes (arrowheads) and mature tetrasporangia with tetrahedrally arranged tetraspores (arrows). (h and i) *Nitophyllum punctatum* (St. Denis, Île d'Orelon, France). (h) Young carposporophyte showing the multicellular sterile group (st) and fusion cell (large arrow) containing two nuclei (small arrows). Note the broaden pit connections (arrowheads) between the fusion cell and inner cells of gonimoblast filaments. (i) Immature carposporophyte with small basal fusion cell (arrow) bearing relatively uncongested gonimoblast filaments and terminal carposporangia.

randomly generated trees had a skewed distribution ($g_1 = -0.68026$, $P < 0.01$), indicating the presence of nonrandom structure (Hillis and Huelsenbeck 1992, Hillis et al. 1993).

With the Delesserieae in the subfamily Delesserioideae used as the outgroup, the *Augophyllum* clade is fully supported (BP = 100, Bayesian posterior possibilities [PP] = 100) in the Nitophylloideae, but the sister relationship of *Augophyllum* is not resolved and remains equivocal (Figs. 6 and 7). The Nitophylleae is shown to be paraphyletic in the MP analysis, forming a grade leading to the Martensieae (Fig. 6, MP), and is unresolved in its position vis-à-vis the Martensieae (Fig. 7, MrBayes). The Martensieae is a terminal monophyletic clade with full support (BP = 100, PP = 83). There are two strongly supported groups within the Nitophylleae, one consisting of the type of *Nitophyllum*, *N. punctatum*, along with *Nitophyllum hommersandii* from Taiwan and "*Hemineura*" *cruenta* Harvey (BP = 96, PP = 100), and the other consisting of *Augophyllum wysorii* gen. et sp. nov. from Caribbean Panama, *Augophyllum kentingii* sp. nov. from Taiwan, *Augophyllum marginifructum* from South Africa, and *Augophyllum delicatum* from S. E. Australia (BP and PP = 100). Interspecific *rbc*L sequence divergence varied between 8.35% and 9.26% among species of the *Nitopyllum punctatum* group and between 2.78% and 4.99% within the *Augophyllum* clade. *Calonitophyllum medium* from Atlantic USA clusters weakly with the Martensieae in MP (Fig. 6) and with higher support using MrBayes (Fig. 7). The position of *Polyneuropsis stolonifera* from California is weakly resolved in the Nitophylloideae using both analyses.

<center>DISCUSSION</center>

Nitophyllum Greville (1830, *nom cons.*), lectotype species *N. punctatum* (Stackhouse) Greville (see Silva 1952), was established to include thin membranous delesserioid plants that lack a percurrent midrib and possess hemispherical cystocarps immersed in the thallus membrane and tetrasporangia borne in distinct scattered sori. Numerous species from around the world were added to *Nitophyllum* in the years that followed. An attempt by J. Agardh (1898) to divide the genus into subgenera and tribes (sections) was largely unsuccessful. Agardh's studies and his worldwide collections, nonetheless, provided a foundation for a revision of the Delesseriaceae by Kylin (1924) in which he divided the family into 11 "Gruppen" (groups) that form the basis of our present classification of the family. The Nitophyllum group with one genus, *Nitophyllum*, and a small number of species was 1 of the 11. Additional species that could not be assigned elsewhere were treated by Kylin as "species inquirendae."
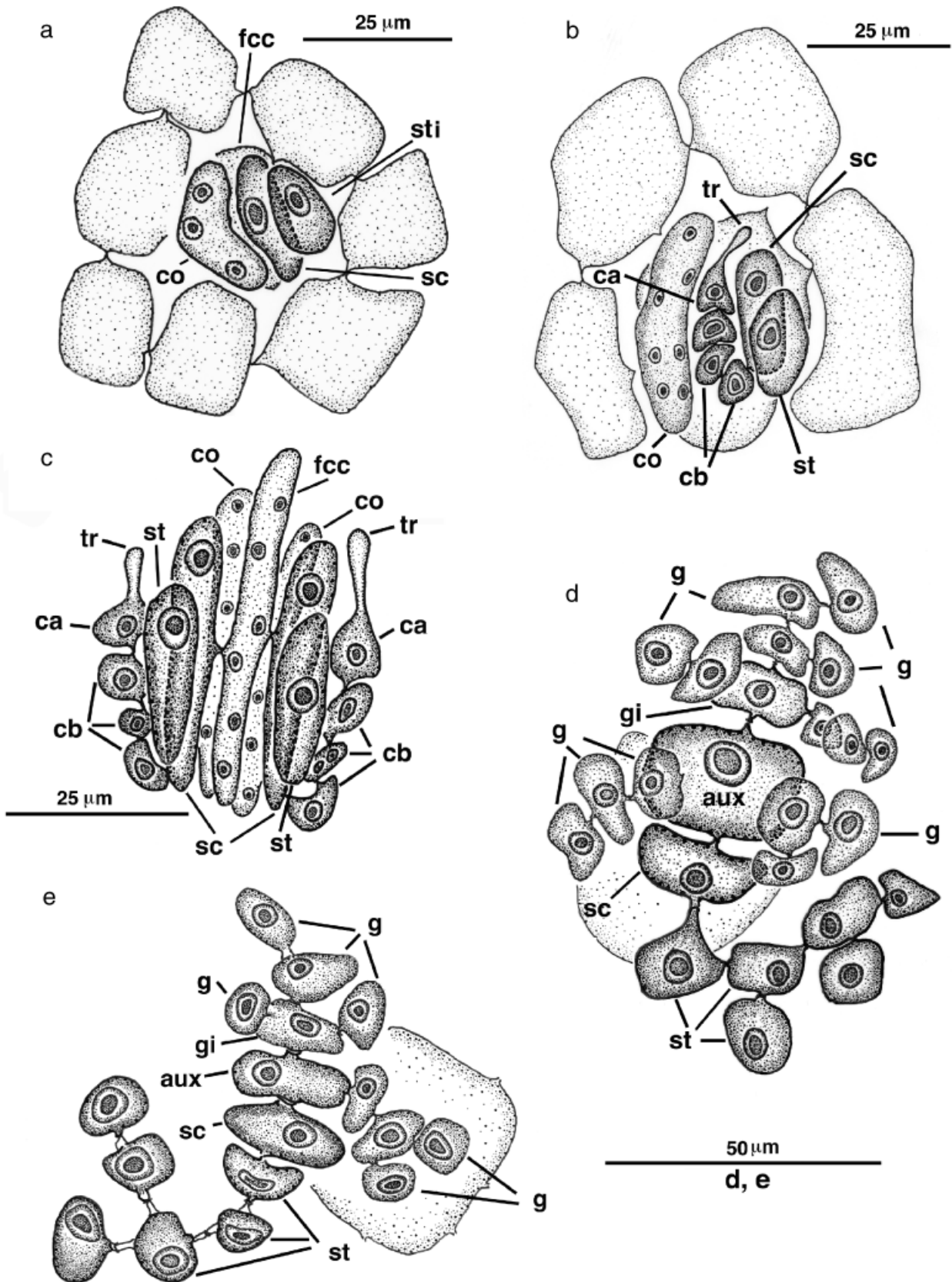
Kylin (1924, 1956) defined the Nitophyllum group to include membranous plants without transversely dividing apical cells that grow by marginal meristems into a monostromatic thallus that becomes polystromatic below and lacks midribs and macroscopic or microscopic veins. Cystocarps were said to bear terminal carposporangia, and cystocarps and tetrasporangial sori are scattered over the thallus surface. The precise development of the procarp before fertilization provided the defining character of the Nitophyllum group. A fertile central cell cuts off two cells, usually on both sides of the thallus, a cover cell initial and the supporting cell of the carpogonial branch. The supporting cell cuts off a single sterile cell laterally and a straight to slightly curved four-celled carpogonial branch situated between the cover cell group and the sterile cell. This character was unique to *Nitophyllum* in Kylin's treatment.

Wynne (2001a) formally recognized Kylin's groups as tribes along with all other groups published since Kylin, 23 in all. Three of these, the Nitophylleae Schmitz (1892), the Valeriemayeae Wynne et Millar in Wynne (2001), and the Martensieae Wynne (2001), possess *Nitophyllum*-type procarps. A fourth tribe, the Papenfussieae Wynne (2001), is similar except that cover cells are absent and there is a space above the area where they might have formed (Kylin 1938). In the same year, Lin et al. (2001a) identified a clade that included representatives of all these tribes and proposed that genera having a *Nitophyllum*-type procarp should be placed in an emended subfamily Nitophylloideae separate from a new subfamily, the Phycodryoideae Lin, Fredericq et Hommersand. The *rbc*L tree in Lin et al. (2001a) and the one shown here in Figure 6 identify a monophyletic terminal clade Martensieae and a paraphyletic tribe Nitophylleae in the Nitophylloideae that includes representatives of the Valeriemayeae.

The Valeriemaya group was originally separated by Millar and Wynne (1992) to include species having procarps that resembled those of *Nitophyllum* or *Papenfussia* but with thallus branches that grow by means of a single transversely dividing apical cell. The procarps of *Valeriemaya* Millar et Wynne (1992), *Calonitophyllum* Aregood (1975), *Radicilingua* Papenfuss (1956; cf. Maggs and Hommersand 1993), and *Martensia* (Lin et al. 2004) are all of the *Nitophyllum* type. Those of *Polyneuropsis* Wynne, McBride et West (1973) are of the *Papenfussia* type in that they lack cover cells; however, the genus is distinct in having procarps that bear a multicellular sterile group before fertilization. Procarp development is unknown in *Arachnophyllum* Zanardini (1843) and *Polyneurella* Dawson (1944). *Valeriemaya* is represented in Figure 6 by "*Hemineura*" *cruenta*, a New Zealand species that stands close to *Valeriemaya* but has not been transferred to it. Recognition of the monotypic genera *Polyneuropsis* and *Calonitophyllum* is justified based on morphological evidence, although their position is unsupported in *rbc*L trees.

Two groups are strongly supported in our molecular analyses within the Nitophylleae, one consisting of "*Hemineura*" *cruenta*, *Nitophyllum hommersandii*, and *Nitophyllum punctatum* (BP = 96, PP = 100) and the other comprising the genus *Augophyllum*, containing *A. wysorii*, *A. kentingii*, *A. marginifructum*, and *A. delicatum* with full support. *Nitophyllum hommersandii* Lin and

FIG. 6. MP tree for *rbc*L sequences showing the inter- and intrageneric relationships for some members of the subfamilies Nitophylloideae and Phycodryoideae using two species in the Delesserieae as outgroup taxa. Bootstrap proportion values (>50%) for MP (5000 replicates) are shown above nodes; decay indices are shown below nodes. Branch lengths are proportional to the amount of sequence change.

FIG. 5. (a–d) *Augophyllum wysorii*. Reproductive features. (a) Young procarp showing a fertile central cell (fcc) cutting off a cover cell (co) and a supporting cell (sc) bearing a lateral sterile cell initial (sti). (b) Mature unfertilized procarp showing the supporting cell (sc) flanked by a cover cell (co) and bearing a four-celled carpogonial branch (cb) and a sterile cell (st). A trichogyne (tr) has developed from the carpogonium (ca). (c) Cross-section of a fertile blade showing two procarps formed from the fertile central cell (fcc) directly opposite each other. Labeling as in b. (d) Early stage of gonimoblast development showing the supporting cell (sc), the branched sterile group (st), the enlarged auxiliary cell (aux), the primary gonimoblast initial (gi), and terminal and lateral gonimoblast filaments (g). (e) *Nitophyllum punctatum* (St. Denis, Île d'Orelon, France). An early stage of gonimoblast development similar to d of *A. wysorii* showing the supporting cell (sc), the branched sterile group (st), the relatively small auxiliary cell (aux), the primary gonimoblast initial (gi), and terminal and lateral gonimoblast filaments (g).
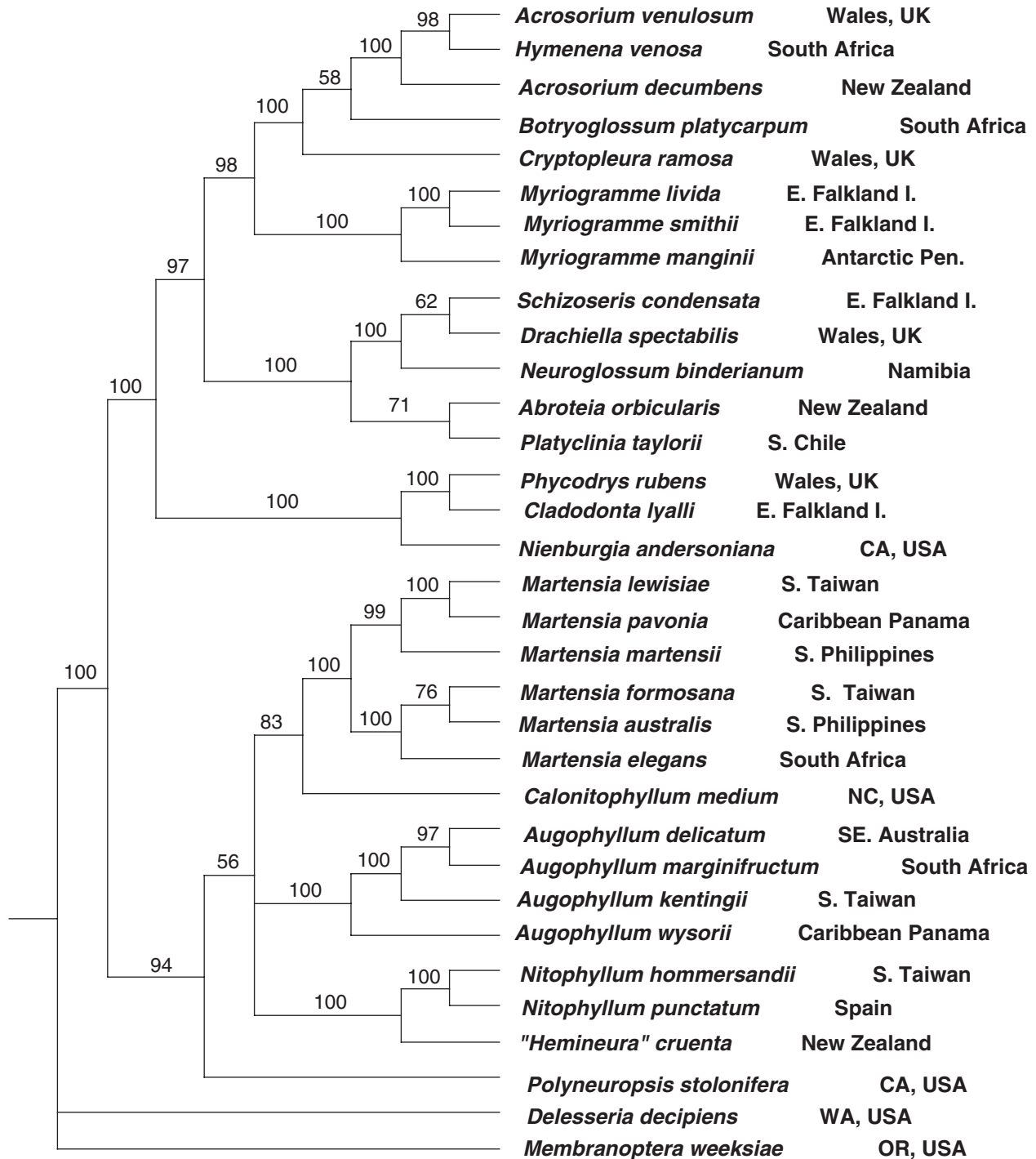
FIG. 7. Bayesian tree showing the phylogenetic position of *Augophyllum*. Fifty percent majority-rule consensus of 99,178 + 1 trees saved after the "burn-in" point trees. The evolutionary model used in the Bayesian analysis was the general-time-reversible model of nucleotide substitution with invariable sites and gamma-distributed rates for the variable sites (GTR + I + Γ) selected by a maximum likelihood ratio test.

Fredericq (2003) is a well-supported sister to the type species of *Nitophyllum*, *N. punctatum* (BP = 99, PP = 100). A comparison of *Nitophyllum hommersandii* with *N. punctatum* confirms that the plastids are parietal, composed of discoid platelets linked by fine strands or forming bead-like branched chains (Maggs and Hommersand 1993, Lin and Fredericq 2003, Fig. 7). In *Nitophyllum punctatum* the supporting cell fuses with the auxiliary cell to produce a small fusion cell (Fig. 4h in this study) that may incorporate a few inner gonimoblast

cells to form the basal cell of the carposporophyte (Kylin 1924; Fig. 4, h and i, in this study). Something similar may also happen in *N. hommersandii*, although the results are unclear (Lin and Fredericq 2003).

In contrast to *Nitophyllum*, the new genus *Augophyllum* is characterized by 1) plastids aggregating beneath the cell surface without being linked by fine strands or forming bead-like branched chains; 2) procarps restricted to the margins of fertile blades; 3) the auxiliary cell persistent, remaining distinct with an enlarged nucleus and functioning as the basal cell of the gonimoblasts throughout carposporophyte development; and 4) the carposporophyte highly branched with cell fusions between gonimoblast cells absent and with pit plugs broadening between gonimoblast cells. The key distinction between *Augophyllum* and *Nitophyllum* lies in the features of cystocarp development. After cutting off gonimoblast initials, the auxiliary cell enlarges and remains distinct at the base of the carposporophyte throughout the maturation of the cystocarp in *Augophyllum*, whereas in *Nitophyllum* the auxiliary cell fuses with the supporting cell that becomes binucleate and remains inconspicuous during the development of carposporophyte. It should be noted that the fusion cell was shown to be multinucleate by Kylin (1924, p. 78, Fig. 57C). The gonimoblast filaments are strongly developed and relatively highly branched in *Augophyllum* compared with those of *Nitophyllum* (compare Figs. 2g, 3i, and 4d with Fig. 4i).

The four species of *Augophyllum* recognized in this study are easily separated by their external morphology. *Augophyllum marginifructum* is large, up to 15 cm high, whereas *A. wysorii* is smaller, only 4–5.5 cm in height, and is identifiable by its palmate ruffled blades arising from a single or branched stipe with the blades a brilliant golden iridescent with circular mottled spots on the surface. Although *A. delicatum* is similar in size to *A. wysorii*, it differs in that the blades are deeply lobed and not mottled by golden iridescent spots. Moreover, the cystocarps and tetrasporangial sori are scattered over all but the lower one third of the fertile blades in *A. delicatum* (Millar 1990, p. 569, Fig. 54, A and C), not restricted to the margins of fertile blades. *Augophyllum kentingii* is the smallest species in the genus, being less than 2 cm high, and is characterized by a thallus consisting of prostrate and decumbent, flabellate, or rounded blades with brilliant blue to golden iridescent and circled mottled spots on the surface.

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