

Notes on the marine algae of the Bermudas. 9. The genus *Botryocladia* (Rhodophyta, Rhodymeniaceae), including *B. bermudana*, *B. exquisita* and *B. flookii* spp. nov.

CRAIG W. SCHNEIDER¹* AND CHRISTOPHER E. LANE²†

¹Department of Biology, Trinity College, Hartford, CT 06106-3100, USA

²Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS B3H 1X5, Canada

C.W. SCHNEIDER AND C.E. LANE. 2008. Notes on the marine algae of the Bermudas. 9. The genus *Botryocladia* (Rhodophyta, Rhodymeniaceae), including *B. bermudana*, *B. exquisita* and *B. flookii* spp. nov. *Phycologia* 47: 614–629. DOI: 10.2216/08-44.1

Using morphological and molecular analyses (*rbcL* gene sequences), three new species, *Botryocladia bermudana*, *B. exquisita* and *B. flookii*, are described from the coastal waters and national aquarium of Bermuda. The new taxa are joined by two others in the island's flora, *B. occidentalis* and *B. wynnei*, the last representing a new distributional record. The cryptic species, *B. bermudana*, is the most common of all *Botryocladia* species in the islands and was known from Bermuda in the past as *B. pyriformis* (Børgesen) Kylin. Analysis of *rbcL* sequences shows that *B. bermudana* is most closely related to *B. caraibica* from the Caribbean. These two species share many characteristics in addition to their phylogenetic positioning. *Botryocladia exquisita* is anatomically and molecularly most similar to the much smaller *B. monoica* from the Caribbean and Gulf of Mexico, and *B. flookii* bears a striking superficial resemblance to *B. macaronesica* from the Canary Islands. Several anatomical and reproductive characteristics differentiate the two.

KEY WORDS: Bermuda, *Botryocladia*, *B. bermudana* sp. nov., *B. exquisita* sp. nov., *B. flookii* sp. nov., Rhodymeniaceae, Rhodophyta

INTRODUCTION

The genus *Botryocladia* (J. Agardh) Kylin (1931) presently contains 40 species (Guiry & Guiry 2008) and includes some of the most recognizable and beautiful red algae. As presently constituted, the genus is considered by Saunders *et al.* (1999) to be a heterogenous grouping of species 'united only by the possession of hollow vesicles formed in various ways on a solid stipe'. Several recent treatments of *Botryocladia* have provided extensive tabular comparisons of the features delineating species (Brodie & Guiry 1988; Schneider & Lane 2000; Afonso-Carrillo & Sobrino 2003; Gavio & Fredericq 2003; Afonso-Carrillo *et al.* 2006; Wilkes *et al.* 2006), and various workers have recently segregated species assigned to the genus into *Gloiosaccion* Harvey and *Irvinea* Guiry based on distinctive morphology (Womersley 1996) or molecular sequences (Saunders *et al.* 1999; Wilkes *et al.* 2006). At present, only *Irvinea* is considered, along with *Botryocladia*, as plants with hollow, mucilage-filled vesicles atop solid short to extended stipes, with *Gloiosaccion* being similar but having the vesicles arising singly or in clusters directly from a holdfast. Over time, various morphological concepts within *Botryocladia* and these two closely related genera have been distinguished by a wide array of characters, from the length of terete stipes, shape and size of the mucous-filled vesicles, the pattern of outer cortication of vesicles, number of secretory

cells, type of secretory cell bearing cells, and immersion or protrusion of the cystocarps. As pointed out by Afonso-Carrillo *et al.* (2006), DNA sequences from more species need to be added to those referenced in public databases in order to discover the relationships between them and to define generic boundaries, because 'presently the genus *Irvinea* exists only at the molecular level'.

When Taylor (1960) published his comprehensive flora of the tropical and subtropical western Atlantic seaweeds, he listed only two species in the genus from this part of the world, *Botryocladia occidentalis* (Børgesen) Kylin and *B. pyriformis* (Børgesen) Kylin, both listed with ranges including Bermuda. Since then, several taxa of *Botryocladia* have been found and described in the greater Caribbean, so that presently 11 species are found from North Carolina to Brazil inclusive of all of the offshore islands in the western Atlantic (Wynne 2005). Since we began collecting marine macroalgae in Bermuda more than two decades ago, we have amassed scores of *Botryocladia* specimens that we report on here, adding to, and clarifying, the list of two species recorded here since early in the 20th century [Collins & Hervey 1917; Howe 1918; both as *Chrysymenia uvaria* (L.) J. Agardh and *C. pyriformis* Børgesen].

MATERIAL AND METHODS

Standard methods

Underwater collections were made by snorkeling or scuba, and specimens were pressed fresh onto herbarium paper with

* Corresponding author (craig.schneider.1@trincoll.edu).

† Present address: Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, USA.

fragments preserved in 4–5% formalin–sea water. Site locations were taken using a Garmin™ GPS III Plus (Olathe, Kansas, USA). For microscopic examination, specimens were sectioned using a American Optical (San Diego, California, USA) freezing microtome and sections were stained and mounted on glass slides in 30% corn syrup and 1% aniline blue in a ratio of 20:1 with a few drops of formalin as a medium preservative. Habit photographs were taken with a Nikon Coolpix 8800VR (Tokyo, Japan) and photomicrographs using a Carl Zeiss Axioskop 40 microscope (Oberkochen, Germany) equipped with a model 11.2 Spot InSight 2 digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA). Herbarium specimens were scanned on an HP 2175 scanner (Hewlett-Packard Company, Palo Alto, California, USA). The digital images were composed in Photoshop™ 7.0 (Adobe Systems, San Jose, California, USA). Voucher specimens are deposited in the first author's personal herbarium, with duplicates of most sent to MICH, MSM, NY and the Bermuda Natural History Museum, and some to C, DUKE, GALW and US. Holotypes of the new species were deposited in US. Unless otherwise noted, the *Phycotheca Boreali-Americana* (*P.B.-A.*) *exsiccata* referred to represents the set originally purchased by Wellesley College (Massachusetts) now belonging to the first author. Herbarium abbreviations follow Holmgren *et al.* (1990) and standard author initials are from Brummitt & Powell (1992).

Molecular methods

Specimens were cleaned of epiphytes and dried on silica gel immediately after bringing the field collections into the lab. The dried samples were ground under liquid nitrogen and stored at –20°C. DNA extraction, amplification of the large subunit of the Rubisco operon (*rbcL*) and DNA sequencing were performed as previously detailed in Schneider & Lane (2005).

Complementary, overlapping sequences were edited and aligned using Sequencher v4.5 (Gene Codes Corp., Ann Arbor, Michigan, USA) and consensus sequences from multiple species were aligned in MacClade v4.08 (Madison & Madison 2003). Maximum Likelihood (ML) analyses were performed using PhyML v2.4.4 (Guindon & Gascuel 2003) using the GTR model, four substitution rate categories and invariable sites with all model parameters estimated during the analysis. Bayesian analyses were done using MrBayes v 3.0 (Huelsenbeck & Ronquist 2003), which was run three times independently for 1,000,000 generations using four chains and the GTR + I + Γ model. Trees were sampled every 100 generations and the likelihood values stabilized around 3000 trees, which were discarded as 'burn-in'. The remaining 7000 trees were used to calculate the consensus tree.

OBSERVATIONS AND DISCUSSION

Botryocladia bermudana C.W. Schneider & C.E. Lane, *sp. nov.*

Figs 1–12

Plantae atrorubrae roseae ad 65 mm elatas per parva discoidea aptera affixae stipites unam ad multas vesiculas ferentes

producentes; vesiculae obpyriformes vel obovoideae et saepe elongescentes ad 5–8 (–11) mm diametro et 10–15 (–20) mm longitudine; stipites teretes et ramosae, ad 1 mm diametro, solidi; parietes vesiculae 3 (–4) laminarum constatae, cellulae intimae medullae 90–120 μ m crassitudine et 60–150 μ m longitudine in sectione, polygoniae in aspectu paginae cum cellulis magnis et parvis intermixtis; cellulae transmutatae et nontransmutatae secretoriferae medulla interior prodientes et 1–10 (–15) pyriformes ad clavatas cellulas secretorias ferentes, ad 22 μ m diametro et 26 μ m longitudine; cortex vesiculis 1–2 stratorum, completus; tetrasporangia in soris dispersis discretis corticis exterioris, sphaerica ad subsphaerica, cruciata divisa, ad 22–34 μ m a 26–48 μ m; plantae dioeciae, spermatangia in expansis soris in exterioribus corticalibus cellulis fasciculata, 1 μ m diametro; cystocarpia in superis partibus vesicularum evoluta, protrudentia ambo intrinsecus et extrinsecus pariete vesiculae, 750–1350 μ m diametro; carposporangia rectangularia ad irregulariter angulata 12–16 μ m diametro et 19–24 μ m longitudine.

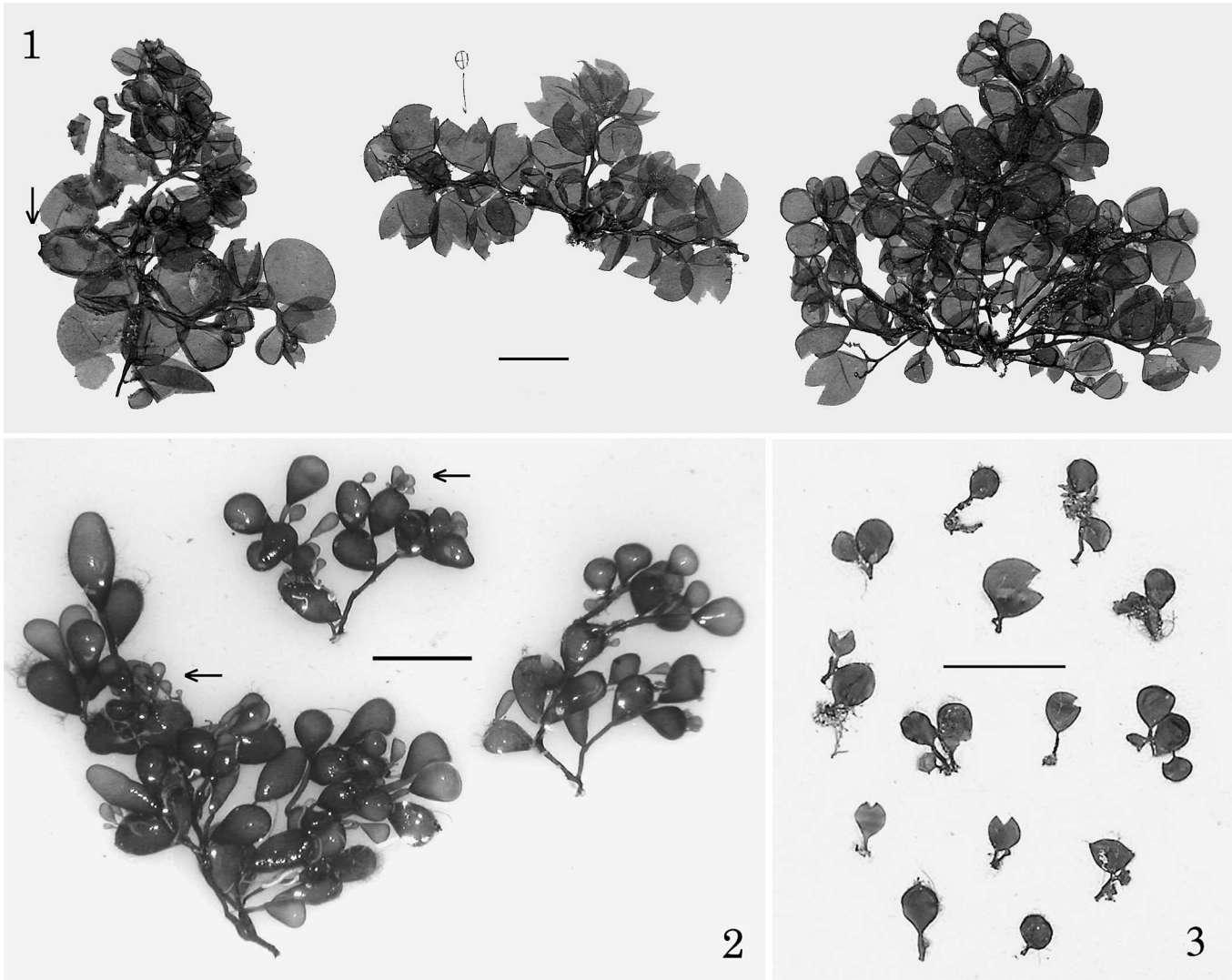
DESCRIPTION: Plants dark rosy-red, to 65 mm tall, attached by small discoid holdfasts, giving rise to stipes bearing one to many vesicles (Figs 1–3); vesicles when young small and spherical (Fig. 3), becoming obpyriform or obovoid and elongating to 5–8 (to 11) mm diam. and 10–15 (to 20) mm long (Fig. 2); stipes terete and branched, to 1 mm diam., solid (Fig. 8); vesicle walls consisting of three (to four) layers (Figs 4, 5), innermost cells of the medulla 90–120 μ m thick, 60–150 μ m long in section, polygonal in surface view with large and small cells intermingled (Fig. 7); specialized and nonspecialized secretory cell bearing cells produced from the inner medulla bearing 1–10 (to 15) pyriform to clavate secretory cells, to 22 μ m diam. and 26 μ m long (Figs 9, 10); vesicle cortex of 1–2 layers, complete (Figs 4–6); tetrasporangia in scattered discrete sori in the outer cortex, spherical to ellipsoidal, cruciately divided, 22–34 μ m by 26–48 μ m; dioecious, spermatangia in spreading sori on outer cortical cells (Fig. 12), 1 μ m diam.; cystocarps forming in upper portions of vesicles, protruding both inwardly and outwardly from the vesicle wall (Fig. 11), 750–1350 μ m diam.; carposporangia rectangular to irregularly angular, 12–16 μ m diam. and 19–24 μ m long.

DIAGNOSIS: Differing from other members of the '*Botryocladia pyriformis*-complex' by a suite of characters including vesicle medulla and wall thickness, and number of secretory cells per supporting cell (Table 1).

HOLOTYPE: *W.R. Taylor (WRT)/A.J. Bernatowicz (AJB)* 49-1724 (two cards, one split, all in a single packet), cystocarpic, \oplus , 11 May 1949, on shaded intertidal rocks, Hunt Island, Port Royal Bay (Little Sound), Bermuda, western Atlantic (housed in MICH; Isotype MICH) (Fig. 1).

ETYMOLOGY: Named for the beautiful islands where this species is commonly collected from intertidal rock to deep offshore waters and is presently endemic.

SELECTED COLLECTIONS: **Bermuda**—*F.S. Collins (FSC)* 7076, April 1912, Tucker's Town [specimen referred to as *Chrysymenia pyriformis* Borgesen by Collins & Hervey 1917, p. 114 (NY)]; *WRT/AJB* 49-957A, 25 April 1949, fishpond, Tucker's Town Bay, Bermuda Island (MICH); *AJB* 51-832, 1 February 1951, Nonsuch Island, Castle Harbour, 0–1 m (MICH); *J.J. Frederick (JFF)* 60-484, 19 April 1960, Challenger Bank off Bermuda, 32°02'26"N, 65°04'51"W, 72.7 m (MICH); *JFF* 60-568, 15 July 1960, off Gurnet Rock, 32°19'00"N, 64°39'00"W, 50.8 m (MICH); *WRT* 61-227, 6 April 1961, Five Fathom Hole, off St George's Island, 19 m; *C.W. Schneider (CWS)/R.B. Searles (RBS)* 85-10-17, 8 June 1985, Northeast Breakers, 32°30.5'N, 64°39.4'W, from 29 m; *CWS/C.E. Lane (CEL)* 99-24-3, 25 July 1999, Warwick Long Bay, south shore, Bermuda Island, 32°15.3'N, 64°48.6'W, depth 1–3 m; *CWS/CEL* 01-10-6, 11 November 2001, Battery Point Park, St George's Island, 32°22.6'N, 64°39.8'W, 0–1 m; *CWS/CEL* 01-13-17, 12 November 2001, Elbow Beach, Bermuda Island, 32°16.3'N, 64°46.7'W, on boilers from 1–2 m; *CWS/CEL* 01-15-8, 13 November 2001,



Figs 1–3. *Botryocladia bermudana* sp. nov. All scale bars = 1 cm.
Fig. 1. Portion of holotype (*WRT/AJB* 49-1724 [MICH]), cystocarps on left specimen (arrow).
Fig. 2. Bermuda Aquarium specimen (*CWS/CEL* 02-11-1) with secondary vesicles (arrows).
Fig. 3. Wild, grazed examples, West Whale Bay (*CWS/CEL* 01-15-8).

West Whale Bay, Southampton, Bermuda Island, 32°15.3'N, 64°48.6'W, on intertidal rock (Fig. 3); *CWS/CEL* 02-11-1, 16 April 2002, Bermuda Aquarium reef tank #20, Flatts, Harrington Sound (Fig. 2); *CWS/CEL* 03-21-5, 1 April 2003, West Whale Bay *loc. cit.*, on intertidal rock (GenBank accession no. EU 977501); *CWS/CEL* 03-36-9, 3 October 2003, West Whale Bay *loc. cit.*, on boilers 1–3 m; *CWS/CEL* 03-48-1, cystocarpic, ⊕, 7 October 2003, Bermuda Aquarium reef tank #20, *loc. cit.* (GenBank accession no. EU 977500); *CWS/CEL* 03-51-23, male, 7 October 2003, Gibbet Island, 32°19'56.1"N, 64°44'32.2"W, 1–2 m on rock and *Codium intertextum* Collins & Herv.; *CWS/CEL* 05-10-21, 20 July 2005, North Rock, north of Bailey Bay Flatts, 32°28.3'N, 64°46.1'W, depth 18 m; *CWS/CEL* 05-19-13, 22 July 2005, John Smith's Bay, Canton Point, south shore, Bermuda Island, 32°19.6'N, 64°46.6'W, depth 10 m; *CWS/CEL* 06-5-3, 19

June 2006, Tobacco Bay, St George's Island, 32°23.333'N, 64°40.733'W, depth 2–3 m; *CWS/CEL* 06-8-18, 20 June 2006, west of Eastern Blue Cut channel marker, 32°23'22.1"N, 64°53'23.0"W, depth 12 m (GenBank accession no. EU 977495); *CWS/CEL* 06-14-15, ⊕, 21 June 2006, outer reef off Frick's Beach, Bermuda Island, 32°19.9'N, 64°40.3'W, depth 10 m; *CWS/CEL* 08-22-9, 15 February 2008, off Gurnet Rock, mouth of Castle Harbour, 32°20.162'N, 64°39.731'W, depth 17.4 m.

Remarks

This new species is by far the most common *Botryocladia* in the waters surrounding Bermuda, and is found from intertidal rock to at least 73 m on offshore reefs throughout

Figs 4–12. *Botryocladia bermudana* sp. nov. **Figs 4, 5.** Cross-sections of vesicle walls (*CWS/CEL* 05-20-1). Scale bar = 25 μm.
Fig. 6. Outer cortex of vesicle wall in surface view (*CWS/CEL* 05-20-1). Scale bar = 25 μm.
Fig. 7. Inner medulla of vesicle wall, surface view (*CWS/CEL* 05-20-1). Scale bar = 100 μm.

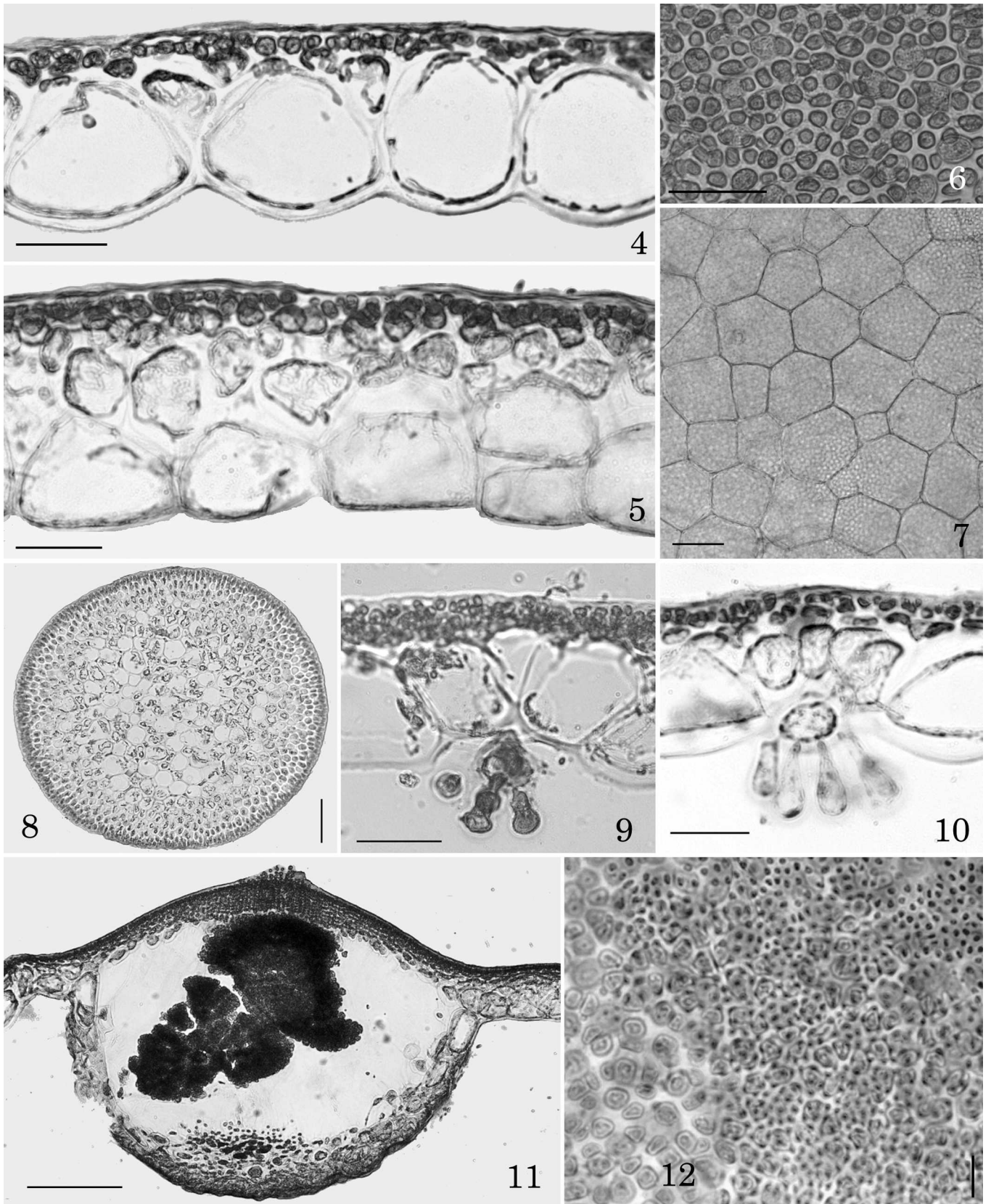


Fig. 8. Cross-section of stipe (*CWS/CEL* 03-36-9). Scale bar = 100 μ m.

Figs 9, 10. Secretory cell bearing cells off inner medulla (*CWS/CEL* 05-20-1, 06-8-18). Scale bars = 25 μ m.

Fig. 11. Cross-section through a cystocarp (*CWS/CEL* 03-48-1). Scale bar = 200 μ m.

Fig. 12. Surface view, spermatangial sorus (*CWS/CEL* 03-51-23). Scale bar = 10 μ m.

the year. Typically, *B. bermudana* is found as small plants (less than 1 cm tall) with one or two spherical to subspherical vesicles atop short stipes (Fig. 3) protected in rock crevices or overtopped by larger algae on the coral 'boiler' reefs of the south shore, in these cases being afforded some protection from the constant grazing pressure of herbivores and omnivores, particularly parrot fishes (*Scarus* spp. and *Sparisoma* spp.). Normally, this species is found as scattered, cryptic individuals. The most extensive populations of these few-vesicled plants are found at the western end of the islands on the 'roofs' of wave and biologically eroded intertidal carbonate rock, areas where the herbivores are allowed little time at high tide to mow down the significant populations.

Botryocladia bermudana has also been a continuous inhabitant for over a decade in reef tank #20 at the Bermuda Aquarium in Flatts. In this artificial environment lacking herbivores and fed by waters of Harrington Sound, the plants are the largest we have collected in decades (Fig. 2). At first, individuals are short-stalked with perfectly spherical vesicles similar to those we find throughout "wild" Bermuda environments (Fig. 3). But without the vesicles and stalks being eaten away, the axis continues on past the original vesicle and produces additional vesicles ultimately demonstrating truly robust individuals to 6.5 cm with branched axes and numerous large obpyriform vesicles to 20 mm long and 11 mm diam. (Fig. 1). Such large-vesicled plants were collected both inshore and offshore regularly by the first workers to collect this species in Bermuda (Collins & Hervey 1917; Howe 1918) who identified their specimens as *Chrysomenia pyriformis* Borgesen [= *Botryocladia pyriformis* (Børgesen) Kylin]. Taylor and his students also collected large plants offshore in the 1940s–1960s, but most of their inshore populations were small and few-vesicled, typical of how the species is presently found. It seems apparent that the natural conditions affecting this species have changed over the past century, perhaps being as simple to explain as having a greater density of herbivores.

A few other species in the western Atlantic that were in the past relegated to *Botryocladia pyriformis* or have overlapping characteristics with it have recently been described as new based upon molecular analysis and/or morphological features, *B. bahamense* D.L. Ballantine & Aponte (Ballantine & Aponte 2002), *B. caraibica* Gavio & Fredericq and *B. ballantinei* Gavio & Fredericq (Gavio & Fredericq 2003, 2005). Along with *B. bermudana*, each of these could be considered members of the greater Caribbean '*B. pyriformis*-complex'. In the eastern Atlantic, specimens from the Canary Islands attributed to *B. pyriformis* prior to 2003 were all found to be representative of *B. canariensis* Afonso-Carrillo & Sobrino (Afonso-Carrillo & Sobrino 2003), until later collections affirmed *B. pyriformis* as well for the islands (Afonso-Carrillo & Sobrino 2004). Unfortunately, there are no obvious features that are wholly unique to *B. bermudana*, but a combination of differences in a suite of characters separates it from all other species in the genus (Table 1). Although *B. bermudana* is indeed superficially similar to *B. pyriformis*, the name it has historically been called in Bermuda, the latter has two to eight pyriform secretory cells that are formed on

medullary cells that are smaller than nonbearing cells, and these secretory cell bearing cells are not specialized or projected into the vesicle cavity (Børgesen 1910). Furthermore, unlike *B. bermudana* (Figs 4–6), the outer cortex in *B. pyriformis* initially forms as an incomplete layer over the transverse walls of the medulla in rosettes, then later fills the gaps and forms a near complete outer cortex on the vesicle (Børgesen 1910).

In fact, after comparing *rbcL* sequences (Fig. 13) and morphologies (Table 1), *B. bermudana* is most similar to the smaller-sized *B. caraibica* from Atlantic Panama (Gavio & Fredericq 2003) and Florida (Dawes & Mathieson 2008). Overall, the vesicle walls and inner medullary cell dimensions of *B. bermudana* are twice the size of those in *B. caraibica* (Gavio & Fredericq 2003). Both species produce specialized and unspecialized secretory cell bearing cells and some of the secretory cells are club-shaped in the new species (Fig. 10) unlike those in *B. caraibica* (Table 1). Like *B. caraibica* and unlike *B. pyriformis* (Gavio & Fredericq 2003), the vesicle wall of *B. bermudana* often does not adhere well to herbarium paper. Stipes of *B. caraibica* show a gradual transition of cell sizes from the center of the stipe outwards, with no cells being greatly inflated, while those of *B. pyriformis* have larger cells in the center that abruptly transition to smaller outer cortical cells (Gavio & Fredericq 2003). The stipes of *B. bermudana* are more similar to the former, with large central cells grading to the outer cortex. In the Bermuda species, however, small cells are cut off the larger central cells and fill in between them (Fig. 8) giving a mixed appearance similar to that of *B. ballantinei* (Gavio & Fredericq 2005) and that depicted for *B. pyriformis* in the Canaries (Afonso-Carrillo & Sobrino 2004). Clearly, *B. bermudana* is best described as a 'cryptic species' due to its similar habit to *B. caraibica* despite being identified as *B. pyriformis* beginning early in the 20th century. The *rbcL* sequences confirm that *B. bermudana* is unique from *B. caraibica* (Fig. 13) despite the lack of clear morphological markers to distinguish them.

In the Bermuda Aquarium reef tank, vesicles of *Botryocladia bermudana* are often found with other vesicles growing directly from them (Fig. 2). These are interpreted as secondary vesicles on stalks that arise directly from the vesicle wall, rather than epiphytic *B. bermudana* plants. This phenomenon could be an asexual development similar to that described for *B. pyriformis* by Ballantine (1989) who demonstrated proliferation of cortical cells into new plants. None of the 'wild' populations of *B. bermudana* have exhibited secondary vesicles so they are considered a culturing phenomenon.

It is worthy of mention that the Taylor/Bernatowitz 49-1724 reproductive '*Botryocladia pyriformis*' Hunt Island specimens cited in the literature (Ballantine 1989; Gavio & Fredericq 2003) were selected as the type collection for *B. bermudana* (Fig. 1). Therefore, subsequent descriptions of *B. pyriformis* may in fact be tainted by these reports of specimens that are now assigned to *B. bermudana*. Furthermore, none of the many Bermuda specimens of '*B. pyriformis*' in F, MICH and NY could be identified as the Børgesen species, all now representing *B. bermudana*, effectively removing the former from the island's flora.

Table 1. Comparison of similar small-sized *Botryocladia* species with few to many vesicles from the Atlantic Ocean.

Characteristics	<i>B. ballantinei</i> ¹	<i>B. bahamense</i> ²	<i>B. bermudana</i> ³	<i>B. canariensis</i> ⁴	<i>B. carabibica</i> ⁵	<i>B. pyriformis</i> ⁶	<i>B. shanksi</i> ⁷
Maximum plant height (mm)	15	25	65	18	40	45 (to 100)	70 (to 140)
Vesicle shape	spherical/obovoid	obovoid	obpyriform	obovoid/obpyriform	obpyriform	obovoid/obpyriform	pyriform
Vesicle size (mm)	8–12 × 9–15	2.5–4.0 × 3.5–7.0	2–11 × 2–14	4–5 × 5–7 (to 9)	1.5–10.0 × 2–12	5 (to 8) × 8 (to 12)	2.5–4.0 long
Thickness of vesicle wall (µm)	80–140	66–75	80–200	53–120	68–93	90	90–130
No. layers in vesicle wall	2–3	2–3	3–4	3	3–5	2–3	3–5
Cortication of vesicle wall	complete	initially incomplete	complete	incomplete	complete	nearly complete	complete
No. layers cortex	1	1	1–2	1	1–2	1	1–2
Medullary cell size (µm) (height × length in cross section [x.s.])	30 (to 80) × 36 (to 90)	to 42 × 100	90–120 × 60–150	53–180 diam.	35–45 × 75–85	80–220 diam.	90–205 diam.
No. layers medulla	1	2	1–2	1	2–3	1	2–3
Secretory cell bearing cells (SCBC)	none seen	specialized and unspecialized	specialized and unspecialized	specialized and unspecialized	specialized and unspecialized	specialized	specialized (and unspecialized)
No. secretory cells on SCBC	0	(2–) 4–6 (to 8)	1–15	3–5 (to 7)	2–5	2–8	1–5
Shape of secretory cells	—	obovoid	pyriform/clavate	obovoid/pyriform	obovoid/obovoid	obovoid/pyriform	spherical/pyriform
Secretory cell size (µm)	—	to 23 × 48	22–34 × 26–48	10–34 × 15–48	16–32 × 32–40	90–55 × 30–77	18–30 × 25–54
Tetrasporangia	ovoid	ovoid	spherical/ovoid	spherical/ellipsoidal	—	?	subpherical/ovoid
Size of tetrasporangia	16–25 × 25–30	to 25 × 56	24–39 × 25–48	22–34 × 26–48	—	?	20–30 × 25–50
Tetrasporangia with cover cells	?no	no	yes	yes	—	yes	yes
Cystocarp development	swelling in/out	swelling in/out	swelling in/out	—	—	swelling in	—
Cystocarp diam. (µm)	1000	750	750–1350	—	—	1000	—
Gametophytes	?dioecious	monoecious	dioecious	?dioecious	—	dioecious	—
Type locality	Louisiana, USA	Bahamas	Bermuda	Canary Islands	Atlantic Panama	Virgin Islands	Atl. Costa Rica

¹ Data from Gavio & Fredericq (2005).

² Data from Ballantine & Aponte (2002).

³ Present study.

⁴ Data from Afonso-Carrillo & Sobrino (2003).

⁵ Data from Gavio & Fredericq (2003).

⁶ Data from Bergesen (1910, 1920).

⁷ Data from Dawson (1962), Afonso-Carrillo & Sobrino (2004).

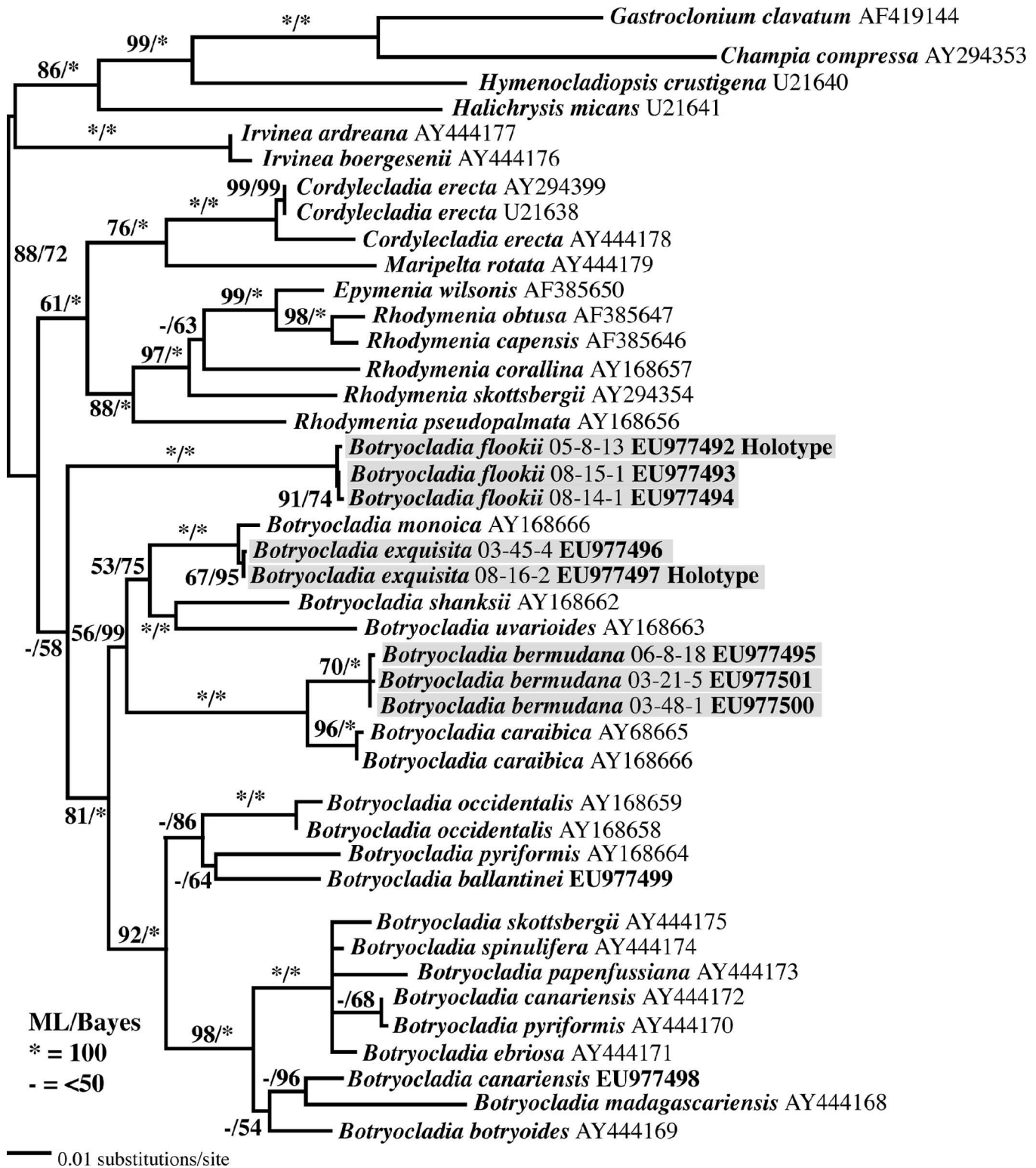


Fig. 13. Unrooted Bayesian tree based on *rbcL* sequence from select members of the Rhodymeniales. Bermudian *Botryocladia* samples are highlighted and GenBank accession numbers for sequences deposited here are in bold. All three species from Bermuda have high intraspecific similarity, but are divergent from their nearest relative. Support values for maximum likelihood and Bayesian analyses are given, respectively, and values of 100 are signified by '*', whereas values less than 50 are denoted with '-'. Maximum likelihood analyses produced a tree with identical topology.

***Botryocladia exquisita* C.W. Schneider & C.E. Lane, sp. nov.**

Figs 14–23

Plantae saturatae roseae ad 30 mm elatas per parva discoidea haptera affixae parvos simplices stipites unam ad multas vesiculas ferentes producentes; vesiculae obovoideae, aliquantum elongatae maturitate 6 mm diametro et 12 mm longitudine; stipites tenues, teretes, solidi et ramosi, ad 0.5 mm diametro; parietes vesiculae 33–36 µm crassitudine et duorum laminarum constatae, medullae cellularum magnarum ad 24 µm crassitudine et 39 µm longitudine in sectione compositae, circularium et ellipticarum ad irregularibus in aspectu paginae, elongatarum maturitate; cellulae transmutatae et nontransmutatae secretoriferae medulla interior prodientes et 2–3 pyriformes cellulas secretorias ferentes, 22–26 µm diametro; cortex incompletus, reticuli cellularum super parietes cellularum medullarum formatarum compositus; tetrasporangia ignota; plantae verosimiliter dioeciae, spermatangia ignota; cystocarpia super vesiculas dispersa, ostiolis, protrudentia ad exteriorum et interiori vesiculae, ad 0.5 mm diametro; carposporangia subglobosa ad irregulariter angulata 10–20 µm diametro in dimensione magno.

DESCRIPTION: Plants intensely pink, to 30 mm tall, attached by small discoid holdfasts, giving rise to stipes bearing one to many vesicles (Figs 14, 15); vesicles obovoid, somewhat elongating at maturity to 6 mm diam. and 12 mm long; stipes delicate, terete, solid and branched, to 0.5 mm diam.; vesicle walls 33–36 µm thick and consisting of two layers (Fig. 18), medulla composed of large cells to 24 µm thick and 39 µm long in section, circular and elliptical to irregular in surface view (Fig. 19), elongating at maturity; specialized and nonspecialized secretory cell bearing cells produced from the inner medulla bearing two to three pyriform secretory cells, 22–26 µm diam. (Figs 20, 21); cortex incomplete, composed of a network of small angular cells forming above the junctions of medullary cells (Fig. 19); tetrasporangia unknown; most likely dioecious, spermatangia unknown; cystocarps scattered over vesicles, ostiolate, protruding to the exterior and interior of the vesicle (Fig. 23), to 0.5 mm diam.; carposporangia subglobose to irregularly angular, 10–20 µm diam. in greatest dimension.

DIAGNOSIS: Differing from other *Botryocladia* species with small, nonelongate, spherical to pyriform vesicles and incomplete cortices by its number of cell layers in the vesicle walls and its production of specialized and unspecialized secretory cell bearing cells (Table 2).

HOLOTYPE: CWS/CEL 08-16-2, cystocarpic, 13.ii.2008, Bermuda Aquarium reef tank #3, Harrington Sound, Flatts, Bermuda, western Atlantic, 32°20.7'N, 64°42.8'W (GenBank accession no. EU 977497) (deposited in US; Isotypes MICH, NY, Herb. CWS) (Figs 14, 15).

ETYMOLOGY: From the Latin *exquisita*, for delicate, fine, exquisita.

SELECTED COLLECTIONS: **Bermuda**—CWS/CEL 99-22-26, 24 July 1999, Gibbet Island, Gibbons Bay, north shore, Bermuda Island, 32°19.4'N, 64°44.6'W, depth 1 m; CWS/CEL 03-45-4, cystocarpic, 6 October 2003, John Smith's Bay, Canton Point, south shore, Bermuda Island, 32°19.0'N, 64°42.8'W, depth 5–8 m (GenBank accession no. EU 977496); CWS/CEL 08-22-19, 08-22-36, cystocarpic, 15 February 2008, off Gurnet Rock, mouth of Castle Harbour, 32°20.162'N, 64°39.731'W, depth 17.4 m (Figs 16, 17).

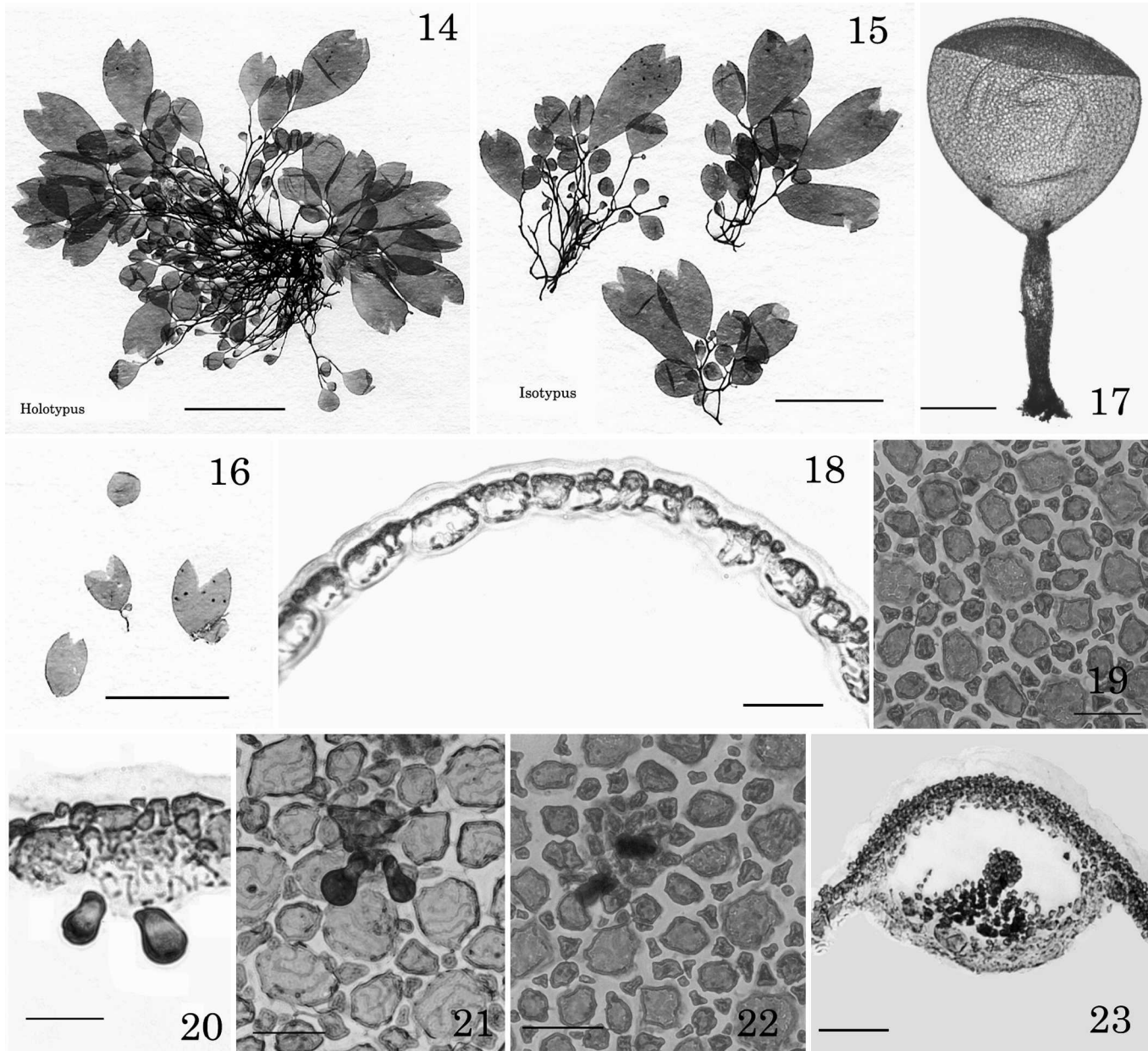
Remarks

This beautiful and delicate new species with vesicle walls to only 36 µm thick was first found as a dense cluster growing at the source pipe of inflowing seawater in a reef tank at the Bermuda Aquarium. A subsequent fertile collection with short stalks and 7 mm long vesicles two days later

confirmed its presence offshore (Fig. 16). This species perhaps suffers the same fate as *Botryocladia bermudana* in the wild, appearing as small, short-stalked specimens due to grazing pressure, but growing extensive branched axes to at least 3 cm tall if not eaten away as is the case for the Aquarium specimens (Figs 14, 15). These latter plants were selected as the type collection due to their more elaborate size and features despite growing in an artificial habitat. The Bermuda Aquarium pipes in water from the adjacent Harrington Sound and utilizes natural light above the tanks. Furthermore, the Aquarium has a policy of never importing exotic species of any kind for their displays, relying solely on the biota naturally occurring in island waters. Therefore, algae that 'appear' in the tanks probably arrive either on rocks collected from the island reefs or as spores from Harrington Sound, a huge 'inland' sound with an inlet to the north shore of Bermuda Island.

Botryocladia exquisita has similarities to a number of species, especially those with small, spherical to pyriform vesicles and incomplete cortices at maturity (Table 2). In the *rbcL* tree (Fig. 13), *B. exquisita* clusters closely with Gulf of Mexico specimens of *B. monoica* Schnetter, a species with a type locality in Caribbean Colombia (Schnetter 1978). If simply comparing the organization of vesicle walls, these two species are indeed similar, but a suite of characteristics shows distinct differences (Table 2). *Botryocladia monoica* is a minute species usually with only one spherical vesicle 2 mm or less in diameter on a short stalk (Schnetter 1978), markedly different from the much larger-sized and -vesicled new taxon. Both the Atlantic *B. monoica* and Pacific *B. tenuissima* W.R. Taylor have similar incomplete cortices to that in *B. exquisita*, but both occasionally produce a third layer as the cortical cells covering the junctions of medullary cells divide anticlinally to produce outer cortical cells. This has not been seen in *B. exquisita*. For species with a similar incomplete cortex, only two are greater than 15 mm, *B. exquisita* and *B. leptopoda* (J. Agardh) Kylin. This latter species, however, is twice the overall size, has vesicles half the size, and has three layers in its vesicle walls relative to the new species from Bermuda.

Prior to the collections of large specimens mentioned above, a few pink, tiny (less than 1 mm diam.) plants of *B. exquisita* from Bermuda (Fig. 17) were at first thought to represent *B. monoica*. But genetic analysis showed them to have the exact *rbcL* sequences as much larger specimens of *B. exquisita* (Fig. 13). They had pink vesicles, short, occasionally branched stipes, protruding cystocarps, incomplete and nonconnected outer cortication of angular cells, and specialized stellate secretory cell bearing cells with (1-)2(-3) secretory cells. These characteristics matched the protologue of *B. monoica* (Schnetter 1978), however several of these Bermuda individuals were female plants with abundant cystocarps on the vesicles but lacked spermatangia, a character at odds with *B. monoica*. When we compared the *rbcL* sequences of *B. exquisita* to *B. monoica* from Texas (30 m, Gulf of Mexico, leg. Gaudio & Wylor, 29 February 2000, GenBank AY168658), they resolve as sister taxa but show enough divergence to indicate that they are distinct species (Fig. 13). It would be helpful to sequence *B. monoica* from Colombia, the type locality, to be certain it matches those sequences from the Texas material, speci-



Figs 14-23. *Botryocladia exquisita* sp. nov.
Fig. 14. Habit of holotype, cystocarpic (CWS/CEL 08-16-2 [US]). Scale bar = 1 cm.
Fig. 15. Habit of an isotype, cystocarpic (CWS/CEL 08-16-2). Scale bar = 1 cm.
Fig. 16. Habit of offshore specimen, cystocarpic (CWS/CEL 08-22-19). Scale bar = 1 cm.
Fig. 17. Habit of a tiny, single-vesicled plant reminiscent of *B. monoica* (CWS/CEL 08-22-36). Scale bar = 0.5 mm.
Fig. 18. Cross-section of vesicle wall (CWS/CEL 10-16-2). Scale bar = 25 μ m.
Fig. 19. Outer incomplete cortex of vesicle wall in surface view (CWS/CEL 08-16-2). Scale bar = 25 μ m.
Fig. 20. Cross-section of vesicle wall showing non-modified medullary cell bearing two secretory cells (CWS/CEL 08-16-2). Scale bar = 25 μ m.
Fig. 21. Inner vesicle surface view, modified secretory cell bearing cell with two secretory cells (CWS/CEL 08-16-2). Scale bar = 25 μ m.
Fig. 22. Surface view of cortex covering a secretory cell bearing cell with two secretory cells (CWS/CEL 08-16-2). Scale bar = 25 μ m.
Fig. 23. Cross-section through a cystocarp (CWS/CEL 08-16-2). Scale bar = 100 μ m.

mens that lacked secretory cells (Gavio & Fredericq 2005), a feature disagreeing with the protolog. Given that our minute fertile specimens are genetically the same as our large, full-grown plants, it should be obvious that other minute *Botryocladia* species might indeed be early vesicles on plants that if allowed to develop fully might look significantly different macroscopically.

Secretory cell bearing cells are either nonmodified medullary cells or specially modified smaller cells that project into the vesicle cavity (Figs 20, 21). This duality is known for a number of *Botryocladia* species (Table 1), but none of the species most similar in other features to *B. exquisita* (Table 2). The incomplete cortex of the vesicle eventually fills and completely covers the medullary cells below (Fig. 22).

Table 2. Comparison of *Botryocladia* species with small, spherical to pyriform vesicles and incomplete cortexes at maturity.

Characteristics	<i>B. exquisita</i> ¹	<i>B. leptopoda</i> ²	<i>B. microphysa</i> ³	<i>B. monoica</i> ⁴	<i>B. tenuissima</i> ⁵
Maximum plant height (mm)	30	100	9	3.6	<10
Vesicles per individual	numerous	numerous	1 (to few)	1 (to 3)	1
Vesicle shape	obovoid	obovoid	obovoid/obpyriform	spherical	obovoid
Vesicle maximum height (mm)	12	3	6	1.9	<9
Vesicle maximum diam. (mm)	6	3	6.5	2.0	2.25
Thickness of vesicle wall (µm)	33–36	60	60	?	?
No. layers in vesicle wall	2	3	3	2 (to 3)	2 (to 3)
No. layers cortex	1	2	1	1 (to 2)	1 (to 2)
No. layers medulla	1	1	1	1	1
Secretory cell bearing cells (SCBC)	specialized and unspecialized	unspecialized	—	specialized	unspecialized
No. secretory cells on SCBC	2–3	1 (to 2)	0	1–3	?
Shape of secretory cells	pyriform	pyriform	—	pyriform-spherical	—
Size of tetrasporangia (µm)	—	—	22–28 diam × 30–40 long	to 28 diam × 38 long	to 24 long
Gametophytes	?dioecious	?dioecious	dioecious	monoecious	?
Cystocarp strongly protuberant	+	+	+	+	?
Cystocarp maximum diameter (µm)	500	250	?	350	?
Known geographical distribution	Bermuda	Indo-Pacific	Mediterranean, IndoPacific	Caribbean, Gulf of Mexico	Galapagos, Am. Samoa, ?Hawaii

¹ Present study.² Data from Kylin (1931), Børgesen (1934), Norris (1989).³ Data from Hauck (1885), Kuckuck (1912), Cormaci *et al.* (1976).⁴ Data from Schnetter (1978).⁵ Data from Taylor (1945), Abbott (1999).***Botryocladia flookii* C.W. Schneider & C.E. Lane, sp. nov.**

Figs 24–34

Plantae brunneolae vel rubrae roseae ad 42 mm elatas per parva discoidea haptera affixae parvos simplices stipites unam ad multas vesiculas ferentes producentes; vesiculae obovoideae et saepe elongescentes (3–) 10–19 mm diametro et (10–) 18–38 mm longitudine, locos conjunctionum ad vesiculas propinquas producentes; stipites parvi, teretes ad 0.5 mm diametro, solidi; parietes vesiculae 3 (–4) laminorum constatae, cellulae intimae medullae 45–130 µm diametro et 55–135 µm longitudine et polygoniae in aspectu paginae cum cellulis magnis et parvis intermixtis et cum cellulis medullosis accrescentibus in cavitatem internam vesiculae projectis et 1–5 pyriformes ad obovoideas cellulas secretorias producentibus, 14–34 µm diametro; cortex exterior incompletus in initio, fere ubi maturus completens; cortex inferior cellularum intermediarum super parietes cellularum medullosarum formatus; tetrasporangia in soris dispersis discretis corticis exterioris, sphaerica ad subsphaerica, cruciata divisa, 15–23 µm in diametro; plantae monoeciae, spermatangia in exterioribus corticalibus cellulis fasciculata, 1 µm diametro; cystocarpia super vesiculas dispersa, leviter protrudentia ad exterium vesiculae, ad 775 µm diametro, ostiolis; carposporangia subglobosa, obovoidea ad irregulariter angulata 10–15 µm diametro et 12–25 µm longitudine.

DESCRIPTION: Plants brownish- to rosy-red, to 45 mm tall, attached by small discoid holdfasts, giving rise to short simple stipes bearing one to many vesicles (Figs 24–26); vesicles obovoid and often elongating, (3–) 10–19 mm diam. and (10–) 18–38 mm long, producing lateral attachments to adjacent vesicles (Fig. 26); stipes short, terete, to 0.5 mm diam., solid; vesicle walls consisting of three (to four) layers, innermost cells of the medulla 45–130 µm diam., 55–135 µm long and polygonal in surface view with large and small cells intermingled and with enlarged medullary cells projecting into the inner vesicle cavity and producing one to five pyriform to obovoid secretory cells, 14–34 µm diam. (Figs 31, 32); outer cortex initially incomplete (Fig. 28), becoming nearly complete when mature (Fig. 30); inner cortex of intermediate-sized cells formed above the walls of the medullary cells; tetrasporangia in scattered discrete sori in the outer cortex (Fig. 33), spherical to subspherical, cruciately divided, 15–23 µm in diameter; monoecious, spermatangia scattered on outer cortical

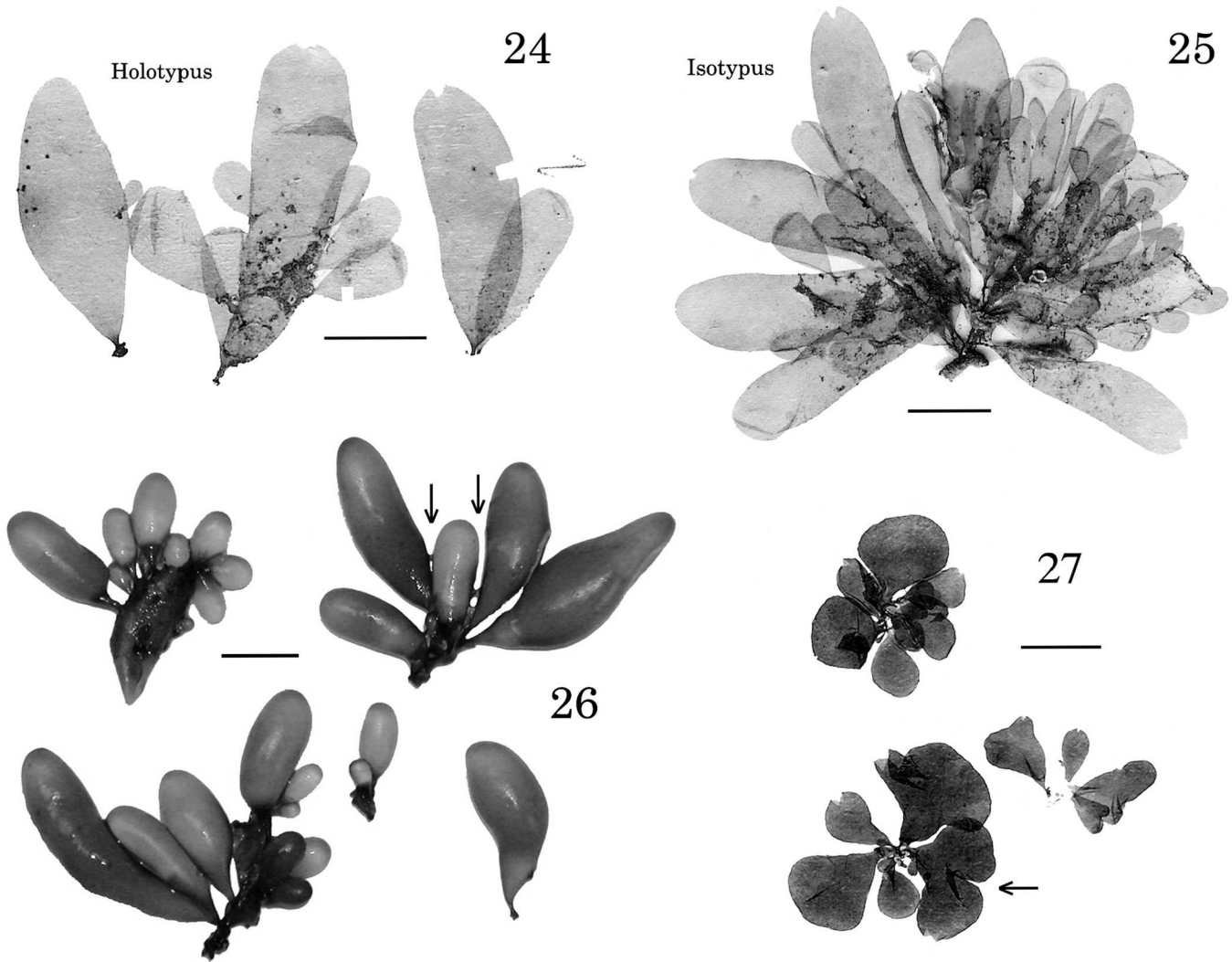
cells, 1 µm diam.; cystocarps scattered over vesicles, slightly protruding to the exterior of the vesicle (Fig. 34), to 775 µm diam., ostiolate; carposporangia subglobose, obovoid to irregularly angular, 10–15 µm diam. and 20–25 µm long.

DIAGNOSIS: Differing from the similar *Botryocladia macaronesica* Afonso-Carrillo, Sobrino, Tittley & Neto of the eastern Atlantic by the lateral attachments on vesicles, enlarged and projecting secretory bearing cells, and development of the medulla below carposporophytes and into the vesicle cavity (Table 2).

HOLOTYPE: CWS/CEL 05-8-13, cystocarpic-male, ⊕, 19 July 2005, Walsingham Pond, Hamilton Parish, Bermuda Island, Bermuda, western Atlantic, 32°20.7'N, 64°42.8'W, depth 6 m on the north ledge (GenBank accession no. EU 977492) (deposited in US; Isotypes MICH, Herb. CWS) (Figs 24, 25).

ETYMOLOGY: Named for Christopher Flook of Marine Operations at the Bermuda Aquarium, an exceptional diver, collector and naturalist whose knowledge of the Bermuda reefs is second to none and whose expertise put us on top of many exciting new algal finds in Bermuda, including this species named for him.

SELECTED OTHER COLLECTIONS [PARATYPES]: **Bermuda**—CWS/RBS 85-2-47, cystocarpic, 6 June 1985, southwest of Chub Heads, 32°17.6'N, 65°01.0'W, depth 19 m; CWS/RBS 85-22-11, ⊕, 18 June 1985, off Elbow Bay, 32°15.0'N, 64°45.5'W, depth 21–30 m; A.R. Cavaliere 432, cystocarpic-male, 10 June 1993, Shark Hole, eastern Harrington Sound, Bermuda Island, 32°20.252'N, 64°42.260'W; CWS/CEL 02-5-33, cystocarpic-male, 13 April 2002, eastern point of Whalebone Bay, St Georges Island, 32°21.911'N, 64°42.782'W, depth 1 m; CWS/CEL 03-16-9, cystocarpic-male, ⊕, 31 March 2003, Walsingham Pond, *loc. cit.*, depth 5 m (Fig. 26); CWS/CEL 03-49-1, cystocarpic-male, 7 October 2003, Bermuda Aquarium reef tank #10, Flatts, Harrington Sound; CWS/CEL 06-12-4, ⊕, 21 June 2006, north side of Castle Island, Castle Harbor, 32°20'28.4"N, 64°40'21.3"W, intertidal caves; CWS/CEL 06-7-8, ⊕, 19 June 2006, Whalebone Bay, *loc. cit.*, 0–1 m; CWS/CEL 06-17-9, ⊕, 22 June 2006, Walsingham Pond, *loc. cit.*, depth 5 m; CWS/CEL 08-13-15, 13 February 2008, Walsingham Pond, *loc. cit.*, depth 4 m; CWS/CEL 08-14-1, ⊕, 13 February 2008, Bermuda



Figs 24–27. *Botryocladia flookii* sp. nov.
Fig. 24. Habit of holotype (*CWS/CEL* 05-8-13 [US]). Scale bar = 1 cm.
Fig. 25. Habit of an isotype (*CWS/CEL* 05-8-13). Scale bar = 1 cm.
Fig. 26. Habit of living specimens (*CWS/CEL* 03-16-9), with lateral attachments between adjacent vesicle (arrows). Scale bar = 1 cm.
Fig. 27. Habit of plants from Bermuda Aquarium reef tank (*CWS/CEL* 08-14-1), with flattened vesicles, some with bifurcated tips (arrow). Scale bar = 1 cm.

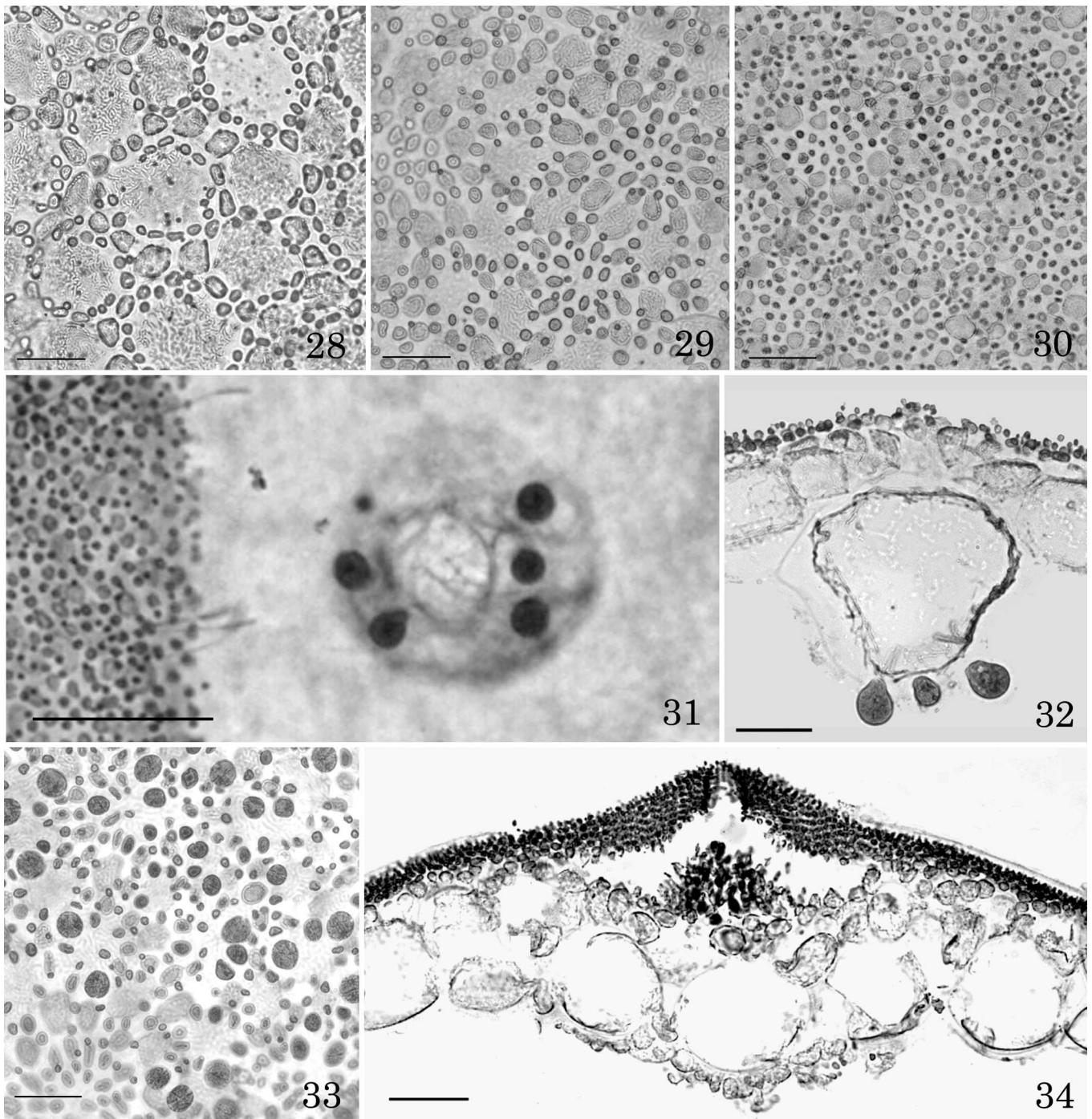
Aquarium, reef tank #20, *loc. cit.* (GenBank accession no. EU 977494) (Fig. 27); *CWS/CEL* 08-15-1, 13 February 2008, Bermuda Aquarium, reef tank #13, *loc. cit.* (GenBank accession no. EU 977493); *CWS/CEL* 08-17-1, cystocarpic-male, 13 February 2008, Bermuda Aquarium reef tank #23, *loc. cit.*; *CWS/CEL* 08-22-15, 15 February 2008, off Gurnet Rock, mouth of Castle Harbour, 32°20.162'N, 64°39.731'W, depth 17.4 m.

Remarks

This new large-vesicled *Botryocladia* is uncommon in the islands, but when found is an obvious member of the flora from the shallow subtidal to 30 m in depth. It joins a select subset of this large genus with few and large vesicles, short stipes and complete or near-complete surface cortication including: *B. adhaerens* E.Y. Dawson from the eastern Pacific (Dawson 1963), *B. chiajeana* (Meneghini) Kylin and *B. macaronesica* from the eastern Atlantic and Mediterranean (Afonso-Carrillo *et al.* 2006), and *B. paucivesicaria*

Stegenga, J.J. Bolton & R.J. Anderson from South Africa (Stegenga *et al.* 1997). Of these four, *B. flookii* is most similar to *B. macaronesica* in habit with its large, elongated vesicles, but it has a number of differences, including lateral vesicle fusions and specialized secretory cell bearing cells, that separate the two sister taxa (Table 3). *Botryocladia adhaerens* is a smaller species firmly affixed to other macroalgae or solid substratum

A distinctive feature of the new species is its ability to generate secondary vesicles from the primary vesicles issued from the short solid stipes (Figs 24–26). This feature is found in only two other *Botryocladia* species, *B. macaronesica* from the eastern Atlantic (Afonso-Carrillo *et al.* 2006) and *B. hancockii* E.Y. Dawson from the Gulf of California (Dawson 1944). The latter species was recently transferred to *Irvinea* because the branched vesicles were interpreted as having indeterminate growth, a delineating feature of *Irvinea ardreana* (J. Brodie & Guiry) Guiry



Figs 28–34. *Botryocladia flookii* sp. nov.

Fig. 28. Incomplete cortex of a young vesicle wall in surface view (*CWS/CEL* 05-8-13). Scale bar = 25 μ m.

Fig. 29. Intermediate cortex beginning to cover medullary cells of vesicle wall in surface view (*CWS/CEL* 03-16-9). Scale bar = 25 μ m.

Fig. 30. Near-complete cortex of mature vesicle wall in surface view (*CWS/CEL* 03-16-9). Scale bar = 25 μ m.

Fig. 31. Inner vesicle surface view of specialized secretory cell bearing cells with six secretory cells (*CWS/CEL* 03-16-9). Scale bar = 100 μ m.

Fig. 32. Cross-section of vesicle wall showing expanded medullary cell bearing three secretory cells (*CWS/CEL* 03-19-9). Scale bar = 100 μ m.

Fig. 33. Portion of a tetrasporangial sorus in the outer cortex of mature vesicle in surface view (*CWS/CEL* 03-16-9). Scale bar = 25 μ m.

Fig. 34. Cross-section through a developing cystocarp (*CWS/CEL* 02-5-33). Scale bar = 100 μ m.

(Wilkes *et al.* 2006). However, the secondary vesicles formed as laterals from a primary vesicle exhibited by *B. hancockii* have determinate growth similar to *B. flookii* and *B. macaronesica* and consequently are very different to the dichotomously branched indeterminate vesicles of

I. ardreana. In that the *rbcL* sequences show great distance between *I. ardreana* and *B. flookii* (Fig. 13), it is reasonable to retain *B. hancockii* in *Botryocladia* until sequenced and compared with known *Botryocladia* and *Irvinea* sequences.

Table 3. Morphological and geographical comparison of *Botryocladia flookii* sp. nov. and *B. macaronesica* (data from present study and Afonso-Carrillo *et al.* 2006).

Characteristics	<i>B. flookii</i>	<i>B. macaronesica</i>
Primary vesicle width (mm)	9–19	to 7
Primary vesicle length (mm)	15–38	to 45
Secondary attachment of vesicles	present	absent
Secondary vesicle formation	present, radial	present, radial
Cell layers in vesicle	3 (to 4)	3 (to 4)
Vesicle wall thickness (µm)	55–180	70–115
Cortication of vesicle walls	incomplete to near complete	near complete
Outer cortex cell diam. (µm)	3.5–7.5	5–12
Medullary cells surface view (µm)	55–135 long, 45–130 broad	84–175 long, 67–115 broad
Secretory cell bearing cells (SCBC)	projecting into cavity	unmodified
No. secretory cells/SCBC	1–5	(1 to) 2–4 (to 8)
Secretory cells (µm diam.)	pyriform (14–34)	spherical (15–18)
Secretory cells in stipe	absent	present
Tetrasporangial sori	discrete scattered sori	in slightly raised nemathecium
Tetrasporangia (µm diam.)	(sub)spherical (15–23)	subspherical (17–30)
Gametophytes	monoecious	monoecious
Position of cystocarps (µm diam.)	immersed (330–640)	immersed (825–1050)
Medulla division under cystocarp	present	absent
Position of spermatangia	scattered	scattered in middle of vesicles
Geographical distribution	western Atlantic: Bermuda	eastern Atlantic: Azores, Madeira, Canaries, Cape Verdes

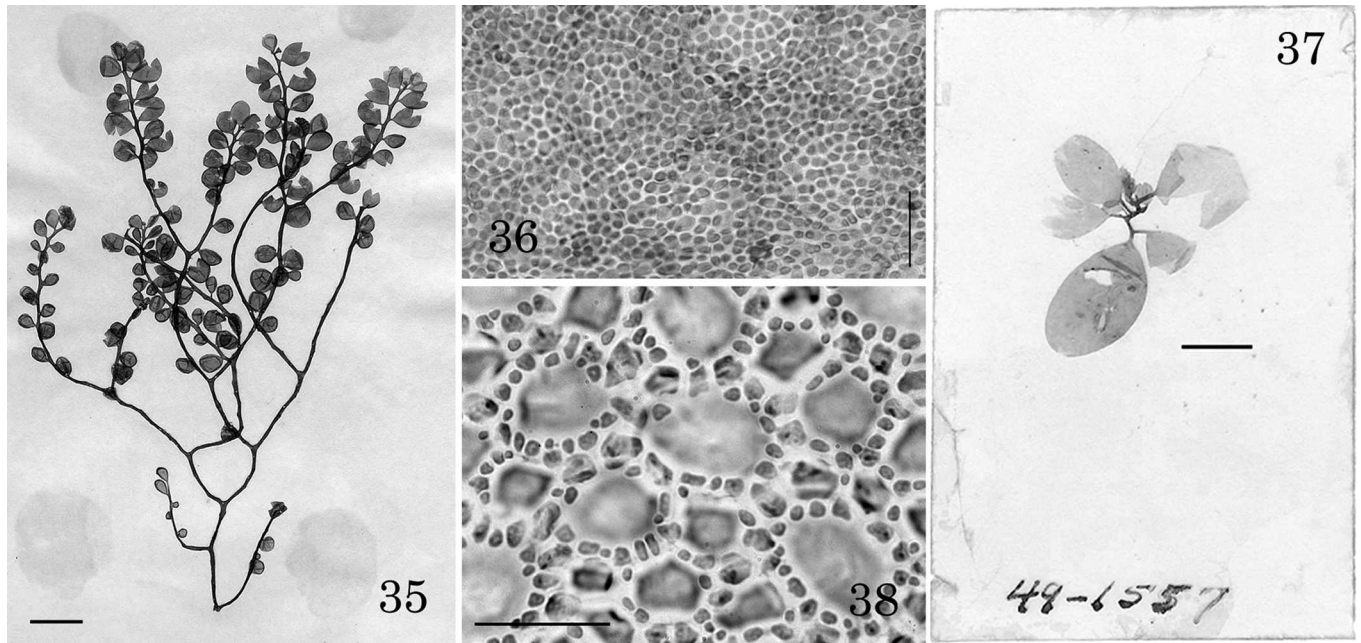
In one gametophytic *B. flookii* specimen, a new vesicle appeared to be germinating from a mature cystocarp, but all of the truly secondary vesicles on other specimens appeared on vegetative or tetrasporic plants. Another obvious feature of *B. flookii* is apparent in most field collected material – as the primary vesicle elongates, it bends in the middle, appearing somewhat kidney-shaped (Fig. 26).

The cystocarps of *B. flookii* are unusual among this small group of *Botryocladia* species with which it is being compared due to shared features. The carposporophytes have the usual pericarp development around the upper portions of the developing gonimoblasts, but they also have a proliferous medulla below the carposporophyte swelling into the mucous-filled cavity of the fertile vesicles (Fig. 34). In many species of *Botryocladia* that develop smaller cells from the larger-celled medulla, e.g. *B. ballantinei* Gavio & Fredericq (Gavio & Fredericq 2005), *B. botryoides* (Wulfen) Feldmann [Kuckuck 1912, as *Chrysomenia uvaria* (Wulfen) J. Agardh], *B. kuckuckii* (Weber-van Bosse) Yamada & Tak. Tanaka (Yamada & Tanaka 1938), *B. madagascariensis* G. Feldmann (Norris 1989) and *B. pianaarii* R.E. Norris (Norris 1989), none are shown to have the large-celled original medulla intact below the cystocarp as it was prior to cystocarp formation. One species however, *B. pseudodichotoma* (Farlow) Kylin, is illustrated with its large medullary layer intact while producing a few smaller cells into the cavity under a mature cystocarp (Bliding 1928, as *Chrysomenia pseudodichotoma* Farlow).

Of the 40 species currently recognized in *Botryocladia* (Guiry & Guiry 2008), only two other than *B. flookii* have vesicles with lateral attachments affixing them together or secondarily to the substratum, *B. adhaerens* (Dawson 1963) and *B. connexa* J.F. Zhang & B.M. Xia (Zhang & Xia 1978). Additionally, Littler & Littler (1997, 2000) illustrate *B. shanksii* E.Y. Dawson with lateral attachments, but such a feature of the vesicles was not noted in the protologue of *B. shanksii* (Dawson 1962) nor in subsequent reports of the species (e.g. Ganesan *et al.* 1985; Afonso-Carrillo &

Sobrinho 2004). Given Dawson's (1962) description in the protologue of 'stiffly bushy' plants with 'several to many...irregularly dichotomous solid axes' to 7 cm high, it seems unlikely that the solitary plants illustrated by Littler & Littler (1997, 2000) are *B. shanksii*. In any case, the Littlers' plants attributed to *B. shanksii* have vesicles that are much smaller than those described as *B. flookii*, the vesicles are not reported as generating secondary vesicles, and among several anatomical differences are reported to have a thick cortex consisting of three to four layers (Littler & Littler 1997, 2000). These specimens should nevertheless be compared with *B. flookii* to see if they represent a new range extension of the new species. Both *B. adhaerens* and *B. connexa* affix vesicles to other macroalgae or hard substratum, each species appearing attached and spreading, not extended upright as seen in *B. flookii*. Additionally, *B. adhaerens* and *B. connexa* are much smaller plants than *B. flookii*, the latter having more layers in vesicle cell wall and a cortex that is complete throughout development (Zhang & Xia 1978). *Botryocladia adhaerens* has similar cortex development to the new species, but all cell dimensions are, like the habit, smaller than those in the new species (Dawson 1963).

In one regard, *Botryocladia flookii* is similar to *B. bahamense* from the Bahamas, in that both species initially produce small bladders with an incomplete pattern in their outer cortices forming first over the transverse walls of the large, nearly colorless cells of the medulla (Fig. 28). As the vesicles enlarge and mature, the cortication eventually fills in between making the outer cortex a nearly complete layer (Figs 29, 30). Despite this similarity, *B. bahamense* has much smaller (to 7 mm long and 4 mm wide) and more frequent vesicles on its longer stipes, ovate tetrasporangia to twice the size of those in *B. flookii*, and no development of the medulla below the cystocarp into the central vesicle cavity (Ballantine & Aponte 2002). Furthermore, *B. bahamense* has secretory cells that are either borne directly on unmodified medullary cells or on other special secretory



Figs 35–38. *Botryocladia* species.

Fig. 35. *B. occidentalis*, habit (*Phycotheca Boreali-Americana* no. 1933, as *Chrysymenia uvaria*). Scale bar = 1 cm.

Fig. 36. *B. occidentalis*, complete cortex of vesicle wall in surface view (*P.B.-A.* no. 1933). Scale bar = 25 μ m.

Fig. 37. *B. wynnei*, mica whole mount (*AJB* 49-1557 [MICH]). Scale bar = 1 cm.

Fig. 38. *B. wynnei*, incomplete cortex of vesicle wall in surface view (*AJB* 49-1557 [MICH]). Scale bar = 50 μ m.

cell bearing cells that project into the central cavity. In *B. flookii*, all secretory cells are borne on greatly enlarged and specialized medullary cells that project into the vesicle cavity (Figs 31, 32). These specialized swollen medullary cells that initiate secretory cells are, in fact, most similar to those that develop in the Pacific *B. connexa* (Zhang & Xia 1978, figs 2–5, 7).

In February 2008, *B. flookii* was found in large numbers in four different display tanks at the Bermuda Aquarium. Unlike the other two new Bermuda species that also grow in Aquarium tanks, this species develops a different macroscopic habit than when found in the wild. Small plants of *B. flookii* are obpyriform, but the larger mature ones become flattened terminally, appearing turbinate (Fig. 27). Some of these vesicles become furrowed apically as if ready to branch, similar to cultured forms of *Irvinea ardreana* (Brodie & Guiry 1988, as *B. ardreana* J. Brodie & Guiry). These characteristics have not been found in wild populations. Unlike *B. flookii*, among other things *I. ardreana* is dioecious, has three layers in vesicle walls and exhibits no fusions between vesicles (Brodie & Guiry 1999).

Based on our analyses of *rbcL* sequence data, *B. flookii* does not cluster within the other *Botryocladia* sequences, but rather as a sister taxon with only weak support (Fig. 13). It may be shown at a later date that *B. flookii* should be separated into a new genus, but the number of *Botryocladia* species available for molecular analysis is far from complete. In addition, there are no obvious morphological features of *B. flookii* that set it apart from other members of *Botryocladia*. *Irvinea* however, was separated from *Botryocladia* based on molecular sequence data alone, indicating that morphology may be misleading within the Rhodymeniaceae. Further taxonomic changes

will have to await a broader sampling of *Botryocladia* and related taxa.

Botryocladia occidentalis (Børgesen) Kylin 1931, p. 18.

Figs 35, 36

BASIONYM: *Chrysymenia uvaria* var. *occidentalis* Børgesen 1920, p. 403, fig. 388.

SYNTYPE LOCALITIES: In the sound between St John and St Thomas, and the sea north of St Thomas, Virgin Islands, Caribbean Sea, western Atlantic.

SELECTED SPECIMENS EXAMINED: **Bermuda**—*W.G. Farlow*, 1888, Walsingham, as *Chrysymenia uvaria* (C. Agardh) J. Agardh, in Farlow *et al.* (1881), *Algae Exsic. Am. Bor.* no. 150 (FH, MICH); *FSC*, April 1912, Harrington Sound, as *C. uvaria* (FH, NY); *FSC* 7169, 1 May 1912, Walsingham, as *C. uvaria* (NY); *A.B. Hervey*, 18 January 1913, Castle Harbor, near Walsingham House, Bermuda Island, as *C. uvaria* in Collins *et al.* (1913), *Phycotheca Boreali-Americana* no. 1933 (CWS, NY) (Fig. 35); *M.M. Brooks*, 1924, Walsingham Pond ('Tom Moore's Lake') (MICH); *AJB* 49-1926, 30 May 1949, Abbot's Cliff, Harrington Sound, 0–1 m (MICH); *AJB* 49-2222, 6 September 1949, Tucker's Town Bay, Bermuda Island, 0–1 m (MICH); *AJB* 51-662, ⊕, 15 January 1951, Red Hole drift, St David's Island (MICH); *JJF* 60-166, 19 April 1960, Challenger Bank off Bermuda, 32°03'50"N, 65°05'15"W, 52.9 m (MICH); *WRT* 61-286, 7 April 1961, Challenger Bank, 65 m (MICH).

Remarks

This ampho-Atlantic species (Afonso-Carrillo & Sobrino 2003) was the first *Botryocladia* reported from Bermuda (Kemp 1857, as *Chrysymenia uvaria*). It is distinctive in Bermuda with its long axes (to 21 cm) and vesicles with single secretory cells on unmodified medullary cells. Like *B.*

bermudana, *B. occidentalis* has a complete outer cortex on vesicles (Fig. 36).

Interestingly, we have not collected *B. occidentalis* in our extensive *Botryocladia* collections over the past two decades. In the late 1800s and early 1900s, collections by Collins, Hervey, Farlow and Howe were commonly collected from shallow inshore habitats that no longer appear to develop plants of this species during any time during the year. The most recent collections were taken from deep offshore habitats, perhaps the present day refuge for *B. occidentalis* in Bermuda.

***Botryocladia wynnei* D.L. Ballantine 1985, p. 199, figs 1–5.**

Figs 37, 38

TYPE LOCALITY: Offshore of La Parguera, Puerto Rico, Caribbean Sea, western Atlantic.

COLLECTION: **Bermuda**—*AJB* 49-1557 (as *B. pyriformis*), 2 May 1949, shallow subtidal, Cripple-gate Bay, Harrington Sound, Bermuda Island (MICH) (Fig. 37).

Remarks

A large specimen with an incomplete cortex (Fig. 38) discovered in a survey of the MICH *Botryocladia* species from Bermuda revealed a nice example of *B. wynnei* (identified by the collector as *B. pyriformis*) and represents a first report for the islands. Since its original report from Puerto Rico (Ballantine 1985), *B. wynnei* has been found in the southeastern United States (Schneider & Searles 1991), the Bahamas (Ballantine & Aponte 2005), the Canary Islands (Haroun *et al.* 1993) and the Arabian Sea (Wynne 2001). Throughout its range, *B. wynnei* has been found in deep-water collections, so this report from the shallow subtidal is anomalous. The largest vesicle on the Cripple-gate specimen is 1.5 cm long and 1 cm in diameter.

ACKNOWLEDGEMENTS

Travel support was provided by a Charles A. Dana Professorship. We thank the curators at FH, MICH and NY for specimen loans. Chris Flook, LeeAnne Hinton and Patrick Talbot (Bermuda Aquarium) provided logistical support while in Bermuda, and Gary Saunders and Tanya Moore (University of New Brunswick) provided some of the DNA sequences. We thank John Huisman and an anonymous reviewer for helpful comments on the manuscript. This is contribution no. 154 to the Bermuda Biodiversity Project (BBP) of the Bermuda Natural History Museum.

REFERENCES

ABBOTT I.A. 1999. *Marine red algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu. 477 pp.
 AFONSO-CARRILLO J., RODRIGUEZ-PRIETO C., BOISSET F., SOBRINO C., TITTLE I. & NETO A.I. 2006. *Botryocladia chiajeana* and *Botryocladia macaronensis* sp. nov. (Rhodymeniaceae, Rhodophyta) from the Mediterranean and the eastern Atlantic, with a discussion on the closely related genus *Irvinea*. *Phycologia* 45: 277–292.

AFONSO-CARRILLO J. & SOBRINO C. 2003. Vegetative and reproductive morphology of *Botryocladia botryoides*, *B. occidentalis* and *B. canariensis* sp. nov. (Rhodymeniaceae, Rhodophyta) from the Canary Islands. *Phycologia* 42: 138–150.
 AFONSO-CARRILLO J. & SOBRINO C. 2004. Two amphi-Atlantic species of *Botryocladia* (Rhodymeniales, Rhodophyta) in the Canary Islands (Eastern Atlantic). *Cryptogamie, Algologie* 25: 147–159.
 BALLANTINE D.L. 1985. *Botryocladia wynnei* sp. nov. and *B. spinulifera* (Rhodymeniales, Rhodophyta) Taylor & Abbott from Puerto Rico. *Phycologia* 24: 199–204.
 BALLANTINE D.L. 1989. Reproduction in Caribbean plants of *Botryocladia pyriformis* and *B. wynnei* (Rhodymeniales, Rhodophyta). *Phycologia* 28: 237–242.
 BALLANTINE D.L. & APONTE N.E. 2002. *Botryocladia bahamense* sp. nov. (Rhodymeniaceae, Rhodophyta) from the Bahamas, western Atlantic. *Cryptogamie, Algologie* 23: 123–130.
 BALLANTINE D.L. & APONTE N.E. 2005. An annotated checklist of deep-reef benthic marine algae from Lee Stocking Island, Bahamas (western Atlantic) II. Rhodophyta. *Nova Hedvigia* 80: 147–171.
 BLIDING C. 1928. Studien über die Florideenordnung Rhodymeniales. *Lunds Universitets Årsskrift, Ny Följd* 24(3): 1–74.
 BØRGESEN F. 1910. Some new or little known West Indian Florideae. II. *Botanisk Tidsskrift* 30: 177–207.
 BØRGESEN F. 1920. The marine algae of the Danish West Indies, Part 3: Rhodophyceae (6), with addenda to the Chlorophyceae, Phaeophyceae, and Rhodophyceae. *Dansk Botanisk Arkiv* 3: 369–498.
 BØRGESEN F. 1934. Some marine algae from the northern part of the Arabian Sea with remarks on their geographical distribution. *Kongelige Danske Videnskabernes Selskab, Biologiske Meddelelser* 11(6): 1–72, pls 2.
 BRODIE J. & GUIRY M.D. 1988. Life history and reproduction of *Botryocladia ardreana* sp. nov. (Rhodymeniales, Rhodophyta) from Portugal. *Phycologia* 27: 109–130.
 BRUMMITT R.K. & POWELL C.E. 1992. *Authors of plant names*. Royal Botanic Gardens, Kew. 732 pp.
 COLLINS F.S. & HERVEY A.B. 1917. The algae of Bermuda. *Proceedings of the American Academy of Arts & Sciences* 53: 1–195.
 COLLINS F.S., HOLDEN I. & SETCHELL W.A. 1913. *Phycotheca Boreali-Americana (Exsiccata), algae of North America*. Fascicle XXXIX. Algae of Bermuda. Nos. 1901–1950, Malden, Massachusetts.
 CORMACI M., FURNARI G. & SCAMMACCA B. 1976. Osservazioni su alcune specie della flora algale dell'isola Linosa. *Bollettino delle Sedute dell'Accademia Gioenia di Scienze Naturali in Catania, Ser. IV* 12: 109–113.
 DAWES C.J. & MATHIESON A.C. 2008. *The seaweeds of Florida*. University Press of Florida, Gainesville. 592 pp.
 DAWSON E.Y. 1944. The marine algae of the Gulf of California. *Allan Hancock Pacific Expeditions* 3: 189–453.
 DAWSON E.Y. 1962. Additions to the marine flora of Costa Rica and Nicaragua. *Pacific Naturalist* 3: 375–395.
 DAWSON E.Y. 1963. Marine red algae of Pacific Mexico. Part 6. Rhodymeniales. *Nova Hedvigia* 5: 437–476, pls 19.
 FARLOW W.G., ANDERSON C.L. & EATON D.C. 1881. *Algae exsiccatae Americae borealis*. Fascicle IV, nos 131–180. Boston, Massachusetts.
 GANESAN E.K., ALFONZO O. DE, APONTE M. & GONZÁLEZ A. 1985. Studies on the marine algal flora of Venezuela VIII. 4 new additions. *Boletín del Instituto Oceanográfico, Universidad de Oriente* 24: 237–246.
 GAVIO B. & FREDERICQ S. 2003. *Botryocladia caraibica* (Rhodymeniales, Rhodophyta), a new species from the Caribbean. *Cryptogamie, Algologie* 24: 93–106.
 GAVIO B. & FREDERICQ S. 2005. New species and new records of offshore members of the Rhodymeniales (Rhodophyta) in the northern Gulf of Mexico. *Gulf of Mexico Science* 2005: 58–83.

- GUINDON S. & GASCUEL O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.
- GUIRY M.D. & GUIRY G.M. 2008. AlgaeBase, version 4.2. World-wide electronic publication, National University of Ireland, Galway. Available at: <http://www.algaebase.org> (27 May 2008).
- HAROUN R.J., PRUD'HOMME VAN REINE W.F., MÜLLER D.G., SERRAO E. & HERRERA R. 1993. Deep-water macroalgae from the Canary Islands: new records and biogeographical relationships. *Helgolander Meeresuntersuchungen* 47: 125–143.
- HAUCK F. 1885. *Die meeresalgen Deutschlands und Oesterreichs*. Rabenhorst, Kryptogamen-Flora, Bd 2, Leipzig, Germany. 575 pp.
- HOLMGREN P.K., HOLMGREN N.H. & BARNETT L.C. 1990. *Index herbariorum, I. The herbaria of the world*, 8th ed. New York Botanical Garden, New York. [Regnum Vegetabile, vol. 120]. 693 pp.
- HOWE M.A. 1918. Algae. In: *Flora of Bermuda* (Ed. by N.L. Britton), pp. 489–540. Charles Scribner's Sons, New York.
- HUELSENBECK J. & RONQUIST F. 2003. MrBayes, version 3.0. Evolutionary Biology Centre, Uppsala University.
- KEMP A.F. 1857. Notes on the Bermudas and their natural history, with special reference to their marine algae. *Canadian Naturalist and Geologist* 2: 145–156.
- KUCKUCK P. 1912. Untersuchungen über *Chrysymenia*. *Wissenschaftliche Meeresuntersuchungen, Neue Folge* 5: 209–227.
- KYLIN H. 1931. Die florideenordnung Rhodymeniales. *Lunds Universitets Årsskrift, Ny Följd, Andra Afdelningen* 27(11): 1–48, pls 1–20.
- LITTLER D.S. & LITTLER M.M. 1997. An illustrated marine flora of the Pelican Cays, Belize. *Bulletin of the Biological Society of Washington* 9: 1–149.
- LITTLER D.S. & LITTLER M.M. 2000. *Caribbean reef plants*. Offshore Graphics, Inc., Washington, DC. 542 pp.
- MADISON W. & MADISON D. 2003. MacClade, version 4.08. Sinauer Associates, Sunderland, Massachusetts.
- NORRIS R.E. 1989. Natalian *Botryocladia* (Rhodymeniales, Rhodophyceae), including description of a new long axis-forming species. *Botanica Marina* 32: 131–148.
- SAUNDERS G.W., STRACHAN I.M. & KRAFT G.T. 1999. The families of the order Rhodymeniales (Rhodophyta): a molecular-systematic investigation with a description of Faucheaceae fam. nov. *Phycologia* 38: 23–40.
- SCHNEIDER C.W. & LANE C.E. 2000. A new species of *Botryocladia* (Rhodymeniales, Rhodophyta) from the Galápagos Islands. *Cryptogamie, Algologie* 21: 167–175.
- SCHNEIDER C.W. & LANE C.E. 2005. Notes on the marine algae of the Bermudas. 7. Additions to the flora including *Chondracanthus saundersii* sp. nov. (Rhodophyta, Gigartinales) based on *rbcl* sequence analysis. *Phycologia* 44: 72–83.
- SCHNEIDER C.W. & SEARLES R.B. 1991. *Seaweeds of the southeastern United States. Cape Hatteras to Cape Canaveral*. Duke University Press, Durham. 554 pp.
- SCHNETTER R. 1978. *Botryocladia monoica* (Rhodymeniales, Rhodophyceae), a new species from the Caribbean coast of Colombia. *Phycologia* 17: 13–15.
- STEGENGA H., BOLTON J.J. & ANDERSON R.J. 1997. *Seaweeds of the South African west coast*. Bolus Herbarium, University of Cape Town, Cape Town. 655 pp.
- TAYLOR W.R. 1945. Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands. *Allan Hancock Pacific Expeditions* 12: 1–528.
- TAYLOR W.R. 1960. *Marine algae of the eastern tropical and subtropical coasts of the Americas*. University of Michigan Press, Ann Arbor. 879 pp.
- WILKES R.J., MCLIVOR L. & GUIRY M.D. 2006. Vegetative morphology and *rbcl* phylogeny of some members of the genera *Botryocladia* and *Irvinea* (Rhodymeniaceae, Rhodophyta). *Phycologia* 45: 481–494.
- WOMERSLEY H.B.S. 1996. *The marine benthic flora of Southern Australia. Rhodophyta – Part IIIB*. Flora of Australia Supplementary Series Number 5. Australia Biological Resources Study, Canberra. 392 pp.
- WYNNE M.J. 2001. New records of benthic marine algae from the Sultanate of Oman, northern Arabian Sea. II. *Nova Hedwigia* 72: 347–374.
- WYNNE M.J. 2005. A checklist of benthic marine algae of the tropical and subtropical western Atlantic: second revision. *Nova Hedwigia, Beiheft* 129: 1–152.
- YAMADA Y. & TANAKA T. 1938. The marine algae from the island of Yonakumi. *Scientific Papers of the Institute of Algological Research, Faculty of Science Hokkaido University* 2: 53–86.
- ZHANG J. & XIA B. 1978. Studies on some marine red algae of the Xisha Islands, Guangdong Province, China. I. *Studia Marina Sinica* 12: 27–39, pl 1.

Received 28 May 2008; accepted 6 August 2008
Associate editor: Alan Millar