Cryptogamie, Algol., 2003, 24 (2): 93-106

Botryocladia caraibica (Rhodymeniales, Rhodophyta), a new species from the Caribbean

Brigitte GAVIO[*1] *& Suzanne FREDERICQ*

University of Louisiana at Lafayette, Department of Biology, Lafayette, LA 70504-2451, USA

[1] *Present address: CICESE, Depto de Ecologia, Apdo Postal 2732, Ensenada, Baja California, Mexico CP 22800*

(Received 28 October 2002, accepted 5 February 2003)

Abstract — A new species, *Botryocladia caraibica* sp. nov., is described from the Caribbean Sea on the basis of comparative morphology and *rbc*L sequence analysis. The new species is often confused with and reported in the literature as *B. pyriformis* (Børgesen) Kylin. In *B. pyriformis* a section of the solid stipe shows an abrupt transition between the cortex and medulla, and the vesicle wall is composed of two cell-layers with irregular and almost complete cortication; in *B. caraibica* the transition between cortex and medulla is more gradual, and the vesicle wall is 3-5 cell-layered with complete cortication. Reproductive structures in *B. caraibica* are unknown. Tetrasporangia of *B. pyriformis* are newly documented.

***Botryocladia* / Caribbean / phylogeny / *rbc*L / Rhodophyta / Rhodymeniales / taxonomy**

Résumé — ***Botryocladia caraibica* (Rhodymeniales, Rhodophyta), une nouvelle espèce de la Mer des Caraïbes**. Une nouvelle espèce, *Botryocladia caraibica* sp. nov., est décrite de la mer des Caraïbes, à partir de la morphologie et de l'analyse du séquençage du gène *rbc*L. La nouvelle espèce est souvent confondue avec le *B. pyriformis* (Børgesen) Kylin, caractérisée par un stipe solide marquant une transition nette entre le cortex et la zone médullaire, une cortication irrégulière et presque complète et une paroi vésiculaire composée de deux couches; la transition dans le *B. caraibica* est plus graduelle, la cortication est complète, et la paroi de la vésicule comporte 3-5 couches. Les structures reproductives du *B. caraibica* sont inconnues. Les tétrasporocystes du *B. pyriformis* sont décrits ici pour la première fois.

***Botryocladia* / Caraïbes / phylogénie / *rbc*L / Rhodophyta / Rhodymeniales / taxinomie**

INTRODUCTION

The genus *Botryocladia* was proposed by Kylin in 1931 to accommodate those species of *Chrysymenia* J. Agardh possessing partly solid axes. Feldmann (1945) further divided the genus into two sections: Sect. Microphyseae, including

* Correspondence and reprints: bgavio@yahoo.com

small-sized species with branches bearing only a small number of vesicles, and the Sect. Botryoideae, including larger species bearing many vesicles that resemble clusters of grapes and with axes that are frequently branched (Schneider & Lane, 2000; Ballantine & Aponte, 2002). With about 35 species known worldwide, *Botryocladia* is the second largest genus in the family Rhodymeniaceae (Rhodymeniales), and is widely distributed in tropical and subtropical waters (Schnetter, 1978; Aponte-Diaz, 1988; Ballantine & Aponte, 2002).

Characters used to distinguish the species include thallus size, size and shape of the vesicles, degree of branching of the axes, extent of cortication, number of cell layers comprising the vesicle wall, presence or absence and number and shape of gland cells, monoecious or dioecious gametophytes, shape and size of cystocarp, and size of tetrasporangia (Ballantine, 1985; Brodie & Guiry, 1988; Ballantine & Aponte, 2002).

In the western Atlantic, nine species of *Botryocladia* have been reported to date (Ballantine & Aponte, 2002). One of the most commonly reported species is *B. pyriformis* (Børgesen) Kylin, originally described from vegetative specimens dredged in 30-32 m depth north of St. John, off America Hill west of Thatch Island, in the Dutch West Indies as *Chrysymenia pyriformis* (Børgesen, 1910: 187, figs. 8, 9). Subsequently transferred to *Botryocladia* by Kylin (1931, p. 18), *B. pyriformis* has since been recorded from North Carolina to Brazil (Taylor, 1960; Ballantine, 1989; Schneider & Searles, 1991; Wynne, 1998; Littler & Littler, 2000). The taxonomic confusion surrounding this species along with reports of misidentification have already been noted (Ballantine, 1985).

Morphological and molecular analyses inferred from material erroneously identified as *B. pyriformis* from Martinique, French West Indies, and Caribbean Panamá have led to the identification of a new species that bears a superficial resemblance to *B. pyriformis*. We report the findings in the present paper and compare the new species with genuine *B. pyriformis*.

MATERIALS AND METHODS

Morphological analysis

Specimens of *B. pyriformis* were collected from offshore Louisiana, northwestern Gulf of Mexico, using dredging at a depth of 50-70 m (Tab. 1), while those of the new species were collected by scuba diving in Caribbean Panamá and Martinique, French West Indies (Tab. 1). Specimens were mounted on herbarium sheets or preserved in silica gel and 5 % Formalin/seawater, and deposited in the herbarium of the University of Louisiana at Lafayette (LAF).

Whole-mount slides and cross-sections were made by hand with a stainless razor blade and stained with aniline blue. Photographs of sections were taken on an Olympus BX60 Photomicroscope (Olympus, Melville, NY, USA) with a Polaroid DMC Ie digital camera (Polaroid Inc., Cambridge, MA, USA). Habits of specimens were scanned using a Microtek Scanmaker III (Microtek, Redonda beach, CA, USA). Digital images were edited and assembled on plates using Photoshop 5.0.

Molecular analyses

Samples used for molecular analyses were desiccated in the field in silica gel. Chloroplast-encoded *rbc*L sequences were produced for nine recently col-

lected samples of *Botryocladia*. Collection information listed in Tab. 1 includes specimen locality, date and collector's name, percentage of *rbc*L sequenced, and Genbank accession numbers. Two species of *Rhodymenia*, *R. pseudopalmata* and *R. corallina* (Rhodymeniaceae) were used as the outgroup.

Protocols for DNA extraction, gene amplification, cycle sequencing, and alignment are as reported in Gavio & Fredericq (2002). We were not able to sequence the first 618 base pairs (bp) of the 1467 bp *rbc*L (58 % sequenced) in both samples of the new species despite using different combination pairs of primers.

The generated sequence data were compiled and aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and exported for phylogenetic analysis in PAUP and MacClade (Maddison & Maddison, 2000). Phylogenetic analyses were performed using the Maximum Parsimony, Neighbor Joining and Maximum Likelihood algorithms available in the computer program PAUP (v. 4.0b10, Swofford, 2002). For Maximum Likelihood the aligned sequences were first analyzed with the software Modeltest v. 3.0 (Posada & Crandall, 1998) which compared different models of DNA substitutions in a hierarchical hypothesis-testing framework to select a base substitution model that best fit the sequence data. The optimal model found was a TrN +G evolutionary model (Tamura-Nei model + Gamma distribution). The parameters were as follows: assumed nucleotide frequencies A = 0.3020; C = 0.1681; G = 0.2172; T = 0.3127; substitution rate matrix with A-C substitutions = 1.0000, A-G = 7.1886, A-T = 1.0000, C-G = 1.0000, C-T = 21.7360, G-T = 1.0000; proportion of sites assumed to be invariable = 0; rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.1173. These values were imported into a maximum likelihood analysis using heuristic search (PAUP).

Support for nodes was determined by calculating bootstrap proportion values (Felsenstein, 1985) using Neighbor Joining (5000 bootstrap resamplings), Maximum Parsimony (5000 bootstrap resamplings) and Maximum Likelihood methods (100 bootstrap resamplings).

RESULTS

Morphological observations

Botryocladia caraibica sp. nov., Figs 1-5.

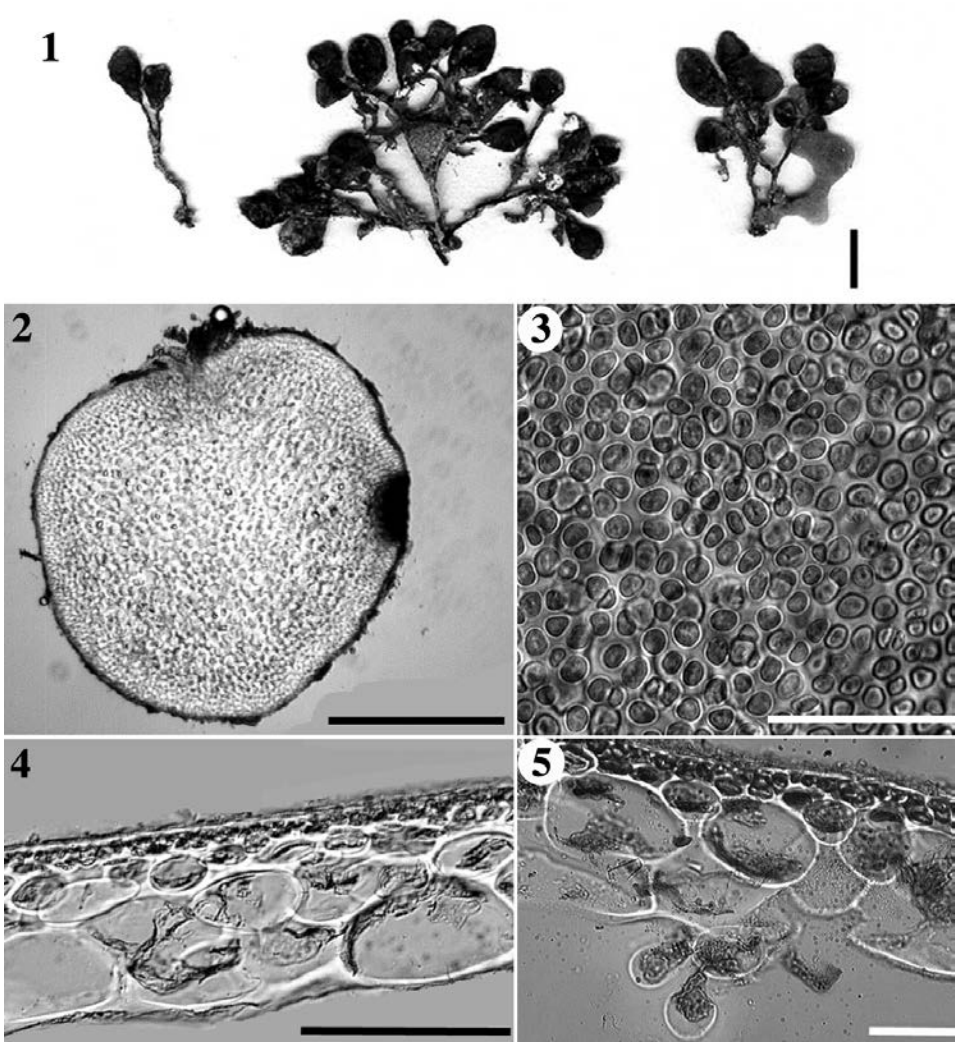
Missapplied name: *Botryocladia pyriformis sensu* Taylor 1960 (pl. 64, fig. 2) non (Børgesen) Kylin.

Type locality and holotype. Long Bay Point, Isla Colón, Bocas del Toro, Republic of Panamá, 9°24.00'N, 82°13.39'W; on rock, 12-15 m, 19 October 1999. *Brian Wysor* (BW# 1279), #Algol. Coll. US#204327 (Fig. 1).

Isotypes. LAF.

Distribution. Caribbean Panamá; Martinique.

Additional specimens studied. Panamá: Long Bay Point, Isla Colón, Bocas del Toro, Republic of Panamá, 9°24.00'N, 82°13.39'W; on hard substratum, 8 m, 19 October 1999, BW#1229; Martinique: Diamant's Rock, 14º26.94'N, 61º02.41'W, 14 June 1995, *S. Fredericq, M. Littler, D. Littler & B. Brooks*, DML#30935, All specimens are deposited at UL Lafayette (LAF).



Figs 1-5. *Botryocladia caraibica* sp. nov. Fig. 1. Habit of holotype specimens, Long Bay Point, Isla Colón, Bocas del Toro, Republic of Panamá; scale bar = 1 cm. Fig. 2. Cross-section through solid axis; scale bar = 0.5 mm. Fig. 3. Surface view of outer cortical vesicle wall showing complete cortication; scale bar = 40 µm. Fig. 4. Cross-section through vesicle wall showing 4-5 cell layers; scale bar = 100 µm. Fig. 5. Gland cells produced from supporting cell; scale bar = 40 µm.

Florida : Station 7, Loggerhead Key, Dry Tortugas, 5-8 m, 12 July 1924, *W.R. Taylor*, MICH#9132. Loggerhead Key, Dry Tortugas, 12 November 1924, *W.R. Taylor*, US#33702

Habitat. Subtidal at 8-15 m depths, attached to rock or dead coral.

Etymology. The species epithet refers to the present distributional range of the new species.

Description. *Plantae 3-4 cm altae, erectus axis cylindricus 1-3 cm longus, 1.2 mm diam., ramificationibus irregularibus, 2-30 vesiculis. Axis constitus e cellulis subsphaericis 23-50 µm diam. in centro. Vesiculae 2-12 mm longae, 1.5-10 mm latae. Paries vesicularum 68-93 µm crassa, constitua e 3-5 stratis. Cortex e 1-2 stratis cellularum sphaericarum 2-4 × 3-9 µm. medulla e 2-3 stratis magnarum cellularum, internum stratum e cellulis 35-45 × 75-85 µm, externum stratum e cellulis 8-18 × 16-32 µm. Glandi-cellulae ovoideae ad obovatae, 16-32 × 32-40 µm, 2-5 aggregatae, e cellulis medullaris normalibus aut sustinantibus cellulis 32-42 µm procreatae. Gametophyta et tetrasporangia ignota.*

Plants are erect, to 3-4 cm in height (Fig. 1). A basal disc bears a single axis, 1-3 cm long and up to 1.2 mm thick, which branches irregularly once to multiple times (Fig. 1). Each thallus possesses 2-30 vesicles; single-vesicled plants were not observed. The solid axes are composed of subspherical medullary cells, reaching 23-50 µm in diameter in the central region (Fig. 2) and decreasing in size towards the periphery. The cortex is composed of 1-2 layers of cells, 6-8 µm in diameter. Vesicles measure 2-12 mm in length and 1.5-10 mm in width (Fig. 1) and are filled with viscous, whitish mucilage. The vesicle wall is 68-93 µm thick. Cortication of the vesicle wall is complete (Fig. 3), with 1-2 cortical layers of deeply pigmented, roundish to elliptical cells (in cross section) 2-4 × 3-9 µm. The outermost cortical cells are roundish-ovate, 2-5 × 4-8 µm in surface view. The medulla is composed of 2-3 layers of large, colorless, elongate cells (in cross section) (Figs 4, 5). Cell size increases toward the center of the vesicle, in the outermost layer cells measure 8-18 × 16-32 µm, grading to 35-45 × 75-85 µm in the innermost layer (Fig. 4).

Gland cells are present, borne in clusters of 2-5 either on a modified "supporting" cell or on an ordinary medullary cell (Fig. 5). Gland supporting cells measure 32-42 µm. Gland cells are ovoid to obovate, 16-32 × 32-40 µm. Reproductive plants unknown.

Molecular analyses

Eleven samples, representing six species of *Botryocladia* and two outgroup species of Rhodymenia (Tab. 1), were newly sequenced for inclusion in a phylogenetic tree (Fig. 6).

Intraspecific *rbc*L sequence divergence within species of *Botryocladia* was less than 1 % (Tab. 2). For example, *Botryocladia occidentalis* from three localities in the northern Gulf of Mexico showed 3-12 base pair differences (99.3-99.9 % sequence identity), whereas *B. caraibica* from Martinique and Panamá showed 1 bp difference (99.9 % sequence identity). In contrast, interspecific *rbc*L sequence divergence among *Botryocladia* species varied from 5.5-9.7 % (Tab. 2).

Botryocladia caraibica and *B. pyriformis* showed 8.9 % sequence divergence. The two species are placed in separate clades in the phylogenetic tree (Fig. 6). *Botryocladia pyriformis* is the sister taxon of *B. occidentalis* with moderate to strong (68-90 %) bootstrap support; *B. caraibica* clusters weakly with *B. monoica* from the Northwestern Gulf of Mexico. This is the first record of *B. monoica* in the Gulf of Mexico.

DISCUSSION

On morphological grounds, *B. caraibica* can be placed in the Section Botryoideae. It superficially resembles *B. pyriformis* in size and branching, but the texture of the new species is more leathery, its color darker red, and the overall

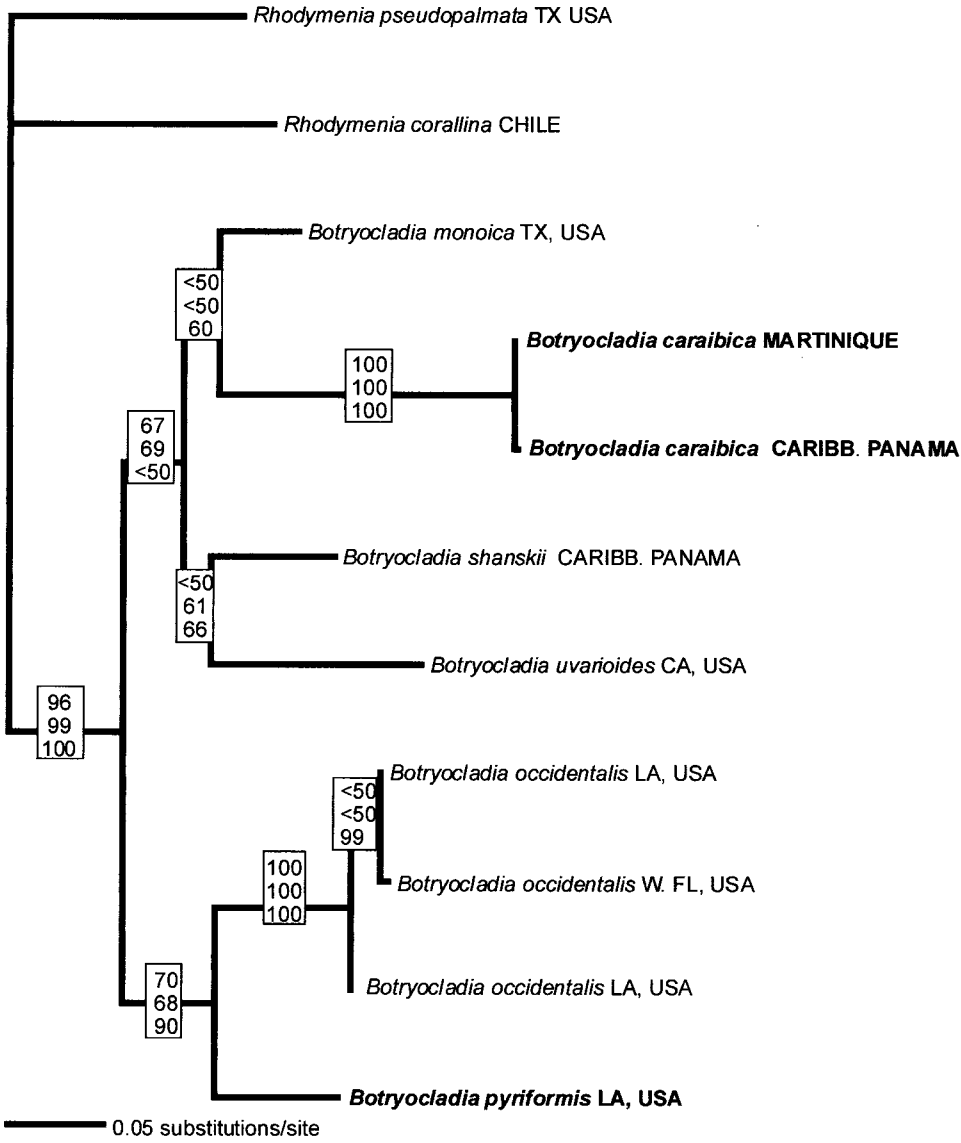


Fig. 6. *Rbc*L Maximum Likelihood tree. Bootstrap proportion values are shown at the nodes for Maximum Likelihood (top; 100 replicates), Maximum Parsimony (center: 5 000 replicates) & Neighbor Joining analyses (bottom; 5 000 replicates).

thallus shape different (compare Fig. 1 with Figs 7-8). In *B. pyriformis* the solid stipe shows an abrupt transition between cortex and medulla (Fig. 9) whereas in *B. caraibica* the transition is more gradual (Fig. 2). The cortication of *B. pyriformis* is irregular and almost complete (Fig. 10) whereas in the new species it is complete (Fig. 3); the vesicle wall of *B. pyriformis* is two cell-layered (Fig. 11), whereas it is 3-5 cell-layered in *B. caraibica* (Fig. 4). The shape of the gland cells is also dif-

Table 1. List of species used in *rbc* analysis with GenBank accession number.

Species	*Location*	*Collector, collection date*	*Portion of* rbcL *sequenced*	*GenBank accession number*
Botryocladia caraibica Gavio et Fredericq sp. nov.	Rocher du Diamant, Martinique,~20 m 14˚ 26.94'N, 61˚ 02.40'W	S. Fredericq, D.L. & M.M. Littler & B. Brooks, 14/VI/95	619-1467 (58%)	AY168665
*Botryocladia caraibic*a Gavio et Fredericq sp. nov.	Long Bay Point, Isla Colón, Panama On rocks, 8 m	B. Wysor, 19/X/99	618-1467 (58%)	AY168666
Botryocladia monoica Schnetter	E. Flower Garden Bank, Flower Garden Banks National Marine Sanctuary, TX, USA, ~30 m	B. Gavio & B. Wysor, 29/II/00	32-1467 (98%)	AY168658
Botryocladia occidentalis (Børgesen) Kylin	Dredged, 66 m, offshore LA, USA, 28˚03.451'N 92˚27.345'W	S. Fredericq, 25/V/00	43-866 917-1467 (94%)	AY168659
Botryocladia occidentalis (Børgesen) Kylin	Dredged, 50-55 m, offshore LA, USA 28˚ 06.470'N 90˚ 55.359'W	S. Fredericq, 30/VI/01	43-1467 (97%)	AY168660a
Botryocladia occidentalis (Børgesen) Kylin	Florida Middle Grounds, FL, USA 28˚ 33.064'N 89˚16.468'W, ~25 m	B. Gavio & B. Wysor, 12/VIII/00	43-747 1020-1467 (79%)	AY168661
Botryocladia pyriformis (Børgesen) Kylin	Dredged, 58 m, offshore LA USA 28˚ 06.480'N 90˚55.361'W	S. Fredericq, 27/V/00	606-1381 (53%)	AY168664
Botryocladia shanskii Dawson	Long Bay Point, Isla Colon, Panama, at 12-15 m, on rocks	B. Wysor, 19/X/99	9-1467 (99%)	AY168662
Botryocladia uvarioides Dawson	Drift, Ocean Beach, San Diego, CA, USA	B. Gavio & B. Wysor, 15/VII/00	33-1467 (98%)	AY168663
Rhodymenia corallina (Bory) Greville	Intertidal, Playa Choya, Coquimbo, Chile	S. Fredericq & M.E. Ramírez, 19/I/95	32-1467 (98%)	AY168657
Rhodymenia pseudopalmata (Lamouroux) Silva	Intertidal, Port Aransas jetty, TX, USA	C.F. Gurgel, 17/V/98	9-1467 (99%)	AY168656

Table 2. Uncorrected p distances (percentage) in *rbc*L sequences among the species of *Rhodymenia* and *Botryocladia* used in this study.

		1	2	3	4	5	6	7	8	9	10	11
1	*Rhodymenia pseudopalmata*	–										
2	*R. corallina*	0.07862	–									
3	*Botryocladia monoica*	0.07998	0.09053	–								
4	*B. occidentalis* (LA) sample 1	0.09438	0.09352	0.08208	–							
5	*B. occidentalis* (LA) sample 2	0.087	0.08491	0.07423	0.00873	–						
6	*B. occidentalis* (FL)	0.09148	0.08834	0.0778	0.00257	0.0026	–					
7	*B. shanskii*	0.08225	0.09396	0.05631	0.08054	0.07498	0.07534	–				
8	*B. uvarioides*	0.09678	0.09476	0.06761	0.09743	0.08894	0.09737	0.06822	–			
9	*B. pyriformis*	0.0998	0.10485	0.07161	0.06473	0.05569	0.06669	0.08015	0.09027	–		
10	*B. caraibica* (Martinique)	0.10948	0.11056	0.07114	0.08948	0.07448	0.09235	0.08693	0.09773	0.08955	–	
11	*B. caraibica* (Panama)	0.11052	0.11162	0.07333	0.09069	0.07557	0.09385	0.08803	0.09652	0.08809	0.00118	–

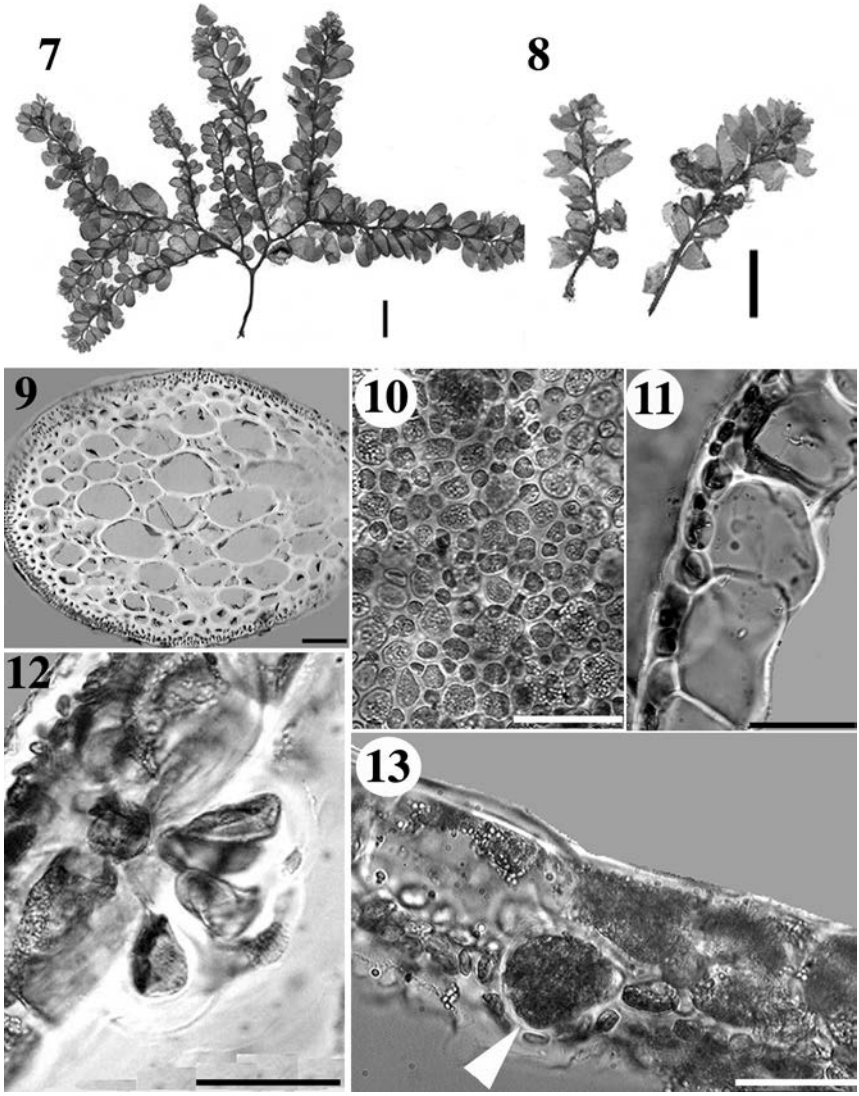
ferent (see Figs 5 & 12). As only sterile specimens were available for study, the reproductive features of our new taxon remain unknown. Our collections did include, however, tetrasporophytic plants of *B. pyriformis* (Fig. 13), which are newly recorded for the species.

Including the present finding, there are currently ten species of *Botryocladia* reported for the Western Atlantic. Distinguishing features of the species are given in Table 3. *Botryocladia caraibica* can be distinguished from *B. bahamense* Ballantine & Aponte, *B. monoica* Schnetter, *B. papenfussiana* Ganesan & Lemus, and *B. wynnei* Ballantine by its size, and by the fact that it has complete cortication. *Botryocladia spinulifera* Taylor & Abbott is peculiar in having spiny outgrowths on the outer cortical cell layer. *Botryocladia shanskii* Dawson has a fewer number of vesicles that are much smaller, whereas *B. ganesanii* Aponte-Diaz has much larger vesicles with a three cell-layered vesicle wall. *Botryocladia occidentalis* (Børgesen) Kylin can be distinguished by its abundant, spherical, mostly single gland cells.

The *rbc*L tree topology indicates that among the species under study, *B. pyriformis* is closely related to *B. occidentalis*, whereas *B. caraibica* is distant from the *B. occidentalis-B. pyriformis* clade.

Børgesen (1920) reported *B. pyriformis* as growing in deepwater only, at depths greater than 30 m. However, the species has subsequently been repeatedly reported from shallower depths from several regions of the Caribbean and Western Atlantic, from North Carolina to Brazil.

Ballantine (pers. comm.) observed that *Botryocladia pyriformis* is often misidentified, and Littler & Littler (2000, p. 232) remarked that an unknown species of *Botryocladia* in the Caribbean is often misidentified as *B. pyriformis*. Børgesen's photograph and drawings of type material (Figs 14-19) are consistent with the deepwater taxon we collected and sequenced for *rbc*L (Figs 7-13). As Børgesen (1920, p. 400) pointed out, this plant has a "rather soft and slimy consistency, and adheres strongly to the paper". Our deepwater material of *B. pyri-*



Figs 7-13. *Botryocladia pyriformis*. Figs 7-8. Habit of specimens collected offshore Louisiana, USA; scale bars = 1 cm. Fig. 9. Cross-section through solid axis; scale bar = 100 µm. Fig. 10. Surface view of outer cortical vesicle wall showing almost complete cortication; scale bar = 40 µm. Fig. 11. Cross-section through two-layered vesicle wall; scale bar: 40 µm. Fig. 12. Gland cells produced from medullary cell; scale bar = 50 µm. Fig. 13. Cross-section through vesicle wall showing tetrasporangium (arrowhead); scale bar = 40 µm.

formis is indeed very slimy and soft, and light pink in color. In contrast, *B. caraibica* is more leathery in texture, dark red, is heavily epiphytized (contrary to the situation in *B. pyriformis*) and it does not easily adhere to herbarium paper.

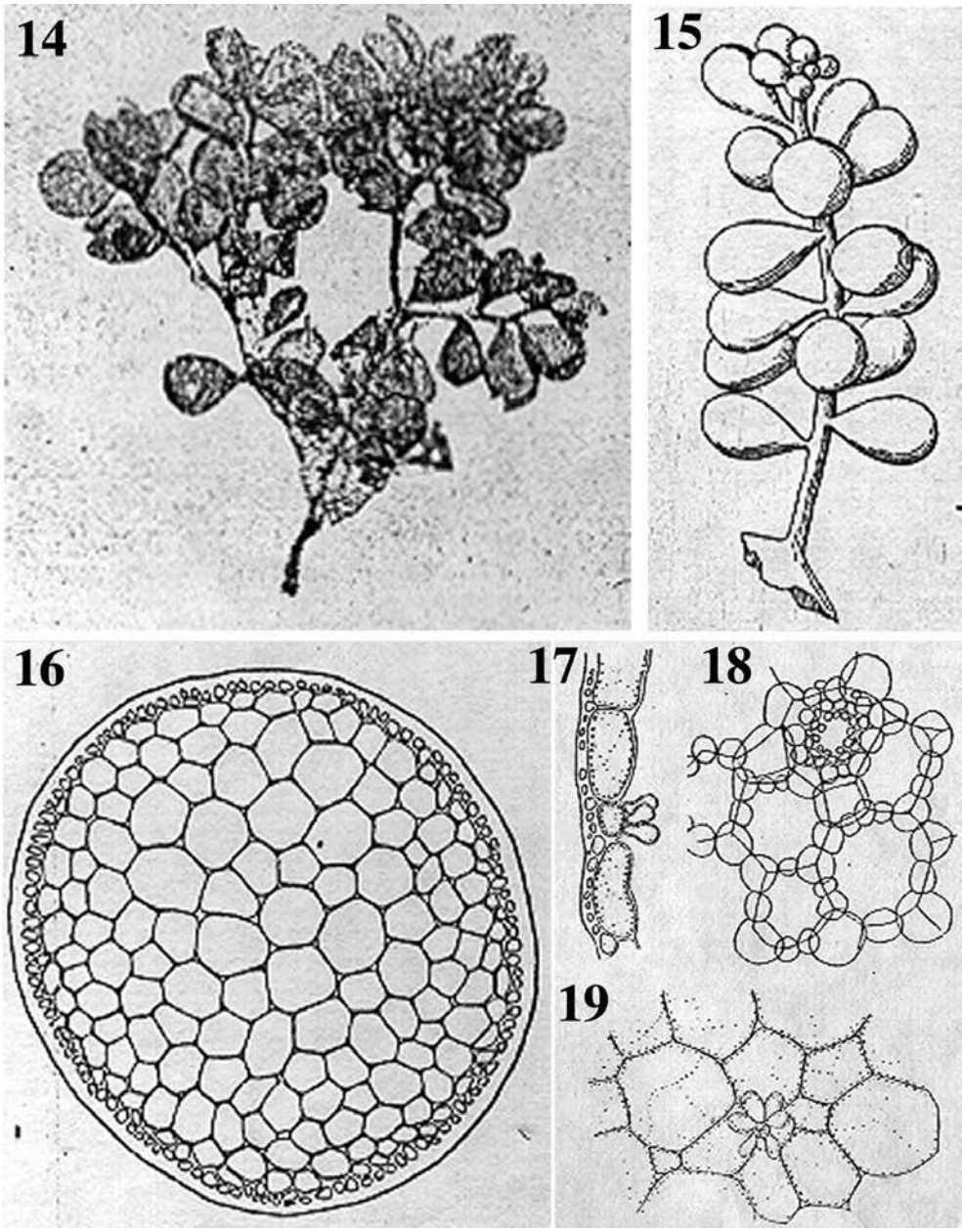
Table 3. Vegetative and reproductive characteristics distinguishing the Caribbean species of *Botryocladia*. Most of the information has been adopted from the original description.

	Thallus height (mm)	*Vesicle shape*	*Vesicle length (mm)*	*Vesicle cortication*	*No. of layers in wall*	*No. of gland cells*	*Gland cell shape*	*Gland cell length (μm)*	*Gland cell position*	*Monoecious dioecious*	*Cystocarp diameter (μm)*	*Tetrasporangial dimensions (μm)*
Botryocladia bahamense Ballantine & Aponte 2002	10-25	ovoid	3.5-7	incomplete	2	2-8	obovoid	48	sessile/ on supporting cell	monoecious	to 750	to 25 × 56
Botryocladia caraibica sp. nov.	*30-40*	ovoid-pyriform	*2-12*	*complete*	*3-5*	*2-5*	obovoid	30-40	sessile/ on supporting cell	unknown	unknown	unknown
Botryocladia ganesanii Aponte-Diaz 1988	30-35	spherical-ovoid	20-25	complete	3	6-15	ovoid-pyriform	25-50	sessile	monoecious	700-900	20 × 24-28
Botryocladia monoica Schnetter 1978	3.6	spherical	1.5-1.9	incomplete	2-3	1-3	ovoid-pyriform	20-55	on supporting cell	monoecious	350	28 × 38
Botryocladia occidentalis (Børgesen) Kylin 1931	200 -	ovoid-pyriform to subspherical	4-5	complete	2-3	1-2	spherical	11-15‡	sessile	unknown	unknown	unknown
Botryocladia papenfussiana Ganesan & Lemus 1972	10-40	obovoid	8-37	incomplete	2-3	1-8	spherical-ovoid	17-45	sessile	dioecious	500-660	20-33
Botryocladia pyriformis (Børgesen) Kylin 1931	45	obovoid-pyriform	4-12	nearly complete	2	4-8	pyriform	30-35‡	sessile	dioecious*	unknown	30 × 36‡
Botryocladia shanskii Dawson 1962	70	pyriform	2.5-4	complete	2-3	1-several	obovoid‡	8-10‡	on supporting cell	unknown	unknown	unknown
Botryocladia spinulifera Taylor and Abbott 1973	10-20	subspherical, ellipsoidal-compressed	2.2-2.6	complete	2-3	1 (scarce)	?	?	unknown	unknown	unknown	15-18 × 28
Botryocladia wynnei Ballantine 1985	6-15	obovoid	6-14	incomplete	3	1-17	ovoid-pyriform	17-26	sessile/ supporting cell	monoecious	440-670	18-24

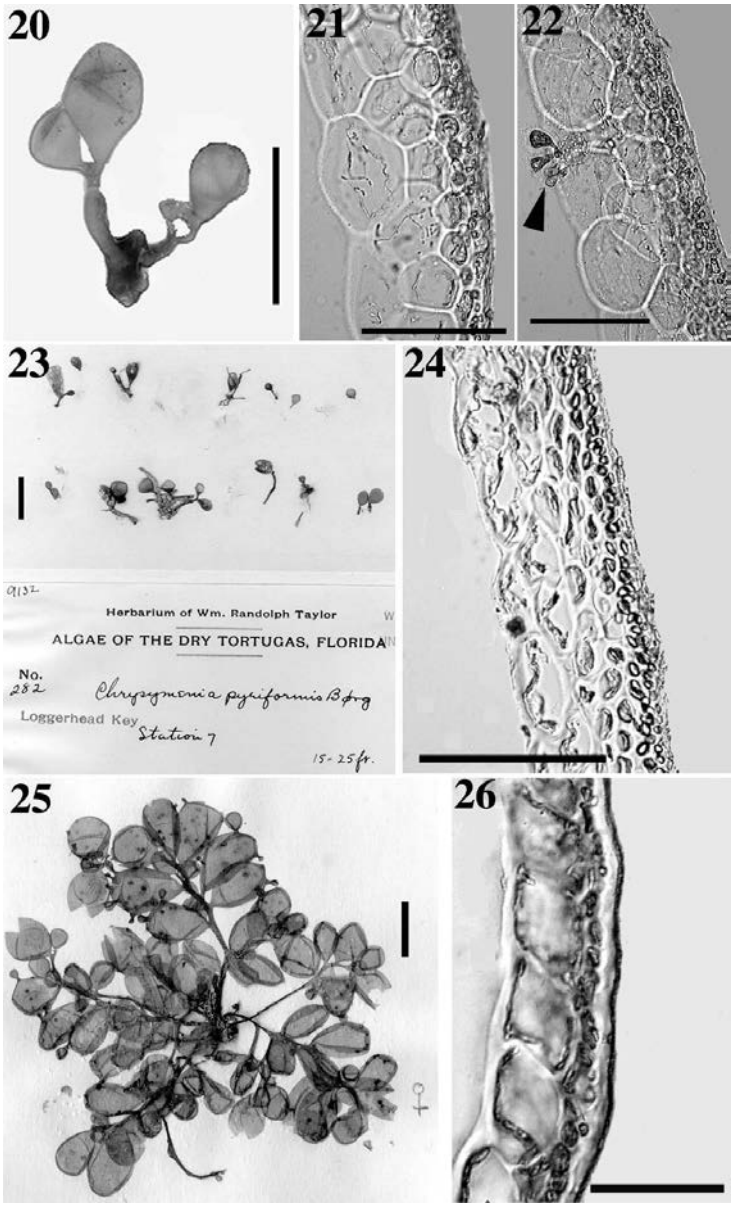
‡based on personal observations

* see Ballantine 1989

Note added in proofs: Several authors have described the gland cells in *Botryocladia* as “ovoid” but “obovoid” (egg shaped and attached at the narrow end) is more appropriate.



Figs 14-19. *Botryocladia pyriformis* reproduction of original drawings from Børgesen 1920. Fig. 14. Habit (fig. 384, p. 400). Fig. 15. Drawing of specimen with unbranched axis (fig. 385, p. 401). Fig. 16. Drawing of cross-section through solid axis (fig. 387, p. 402). Fig. 17. Drawing of cross-section through two-layered vesicle wall, with a group of gland cells borne on medullary cell (fig. 386a, p. 401). Fig. 18. Drawing of surface view of outer cortical vesicle wall (fig. 386b, p. 401). Fig. 19. Drawing of medullary cells, including one with gland cells (fig. 386c, p. 401).



Figs 20-24. *Botryocladia caraibica*, identified by W.R. Taylor as *B. pyriformis*. Fig. 20. Habit of specimen US#33702 from Loggerhead Key, Dry Tortugas, Florida; scale bar = 1 cm. Fig. 21. Cross-section through vesicle wall showing several cell layers; scale bar = 100 µm. Fig. 22. Cross-section through vesicle wall with gland cells (arrowhead); scale bar = 100 µm. Fig. 23. Habit of specimen MICH#9132 from Loggerhead Key, Dry Tortugas, Florida; scale bar = 1 cm. Fig. 24. Cross-section through vesicle wall showing several cell layers; scale bar = 100 µm.
Figs. 25-26. *Botryocladia pyriformis*, collected and identified by W.R. Taylor. Fig. 25. Habit of specimen US#72689 from Hunt Island, Port Royal Bay, Bermuda; scale bar = 1 cm. Fig. 26. Cross-section through vesicle wall showing two cell layers; scale bar = 50 µm.

Taylor (1928, 1960) reported *Botryocladia pyriformis* occasionally from the intertidal, and he noted that the deep-water plants are morphologically different, with larger bladders. We examined specimens collected by Taylor from Loggerhead Key, Dry Tortugas, Florida (US#33702; MICH#9132) and these specimens correspond to *B. caraibica* (Figs 20-24). However, a specimen collected by Taylor from the same locality (MICH#11230) as well as a specimen collected from Hunt Island, Port Royal Bay, Bermuda (US#72689) (Figs 25-26) fit Børgesen's description of *B. pyriformis*.

We therefore propose a new species, *B. caraibica*, for the taxon that is morphologically and genetically distinct from *B. pyriformis sensu* Børgesen. We postulate that most of the records in the Western Atlantic referred to as *B. pyriformis* may represent *B. caraibica*, especially if these plants were found at moderate depths. We speculate that the real *B. pyriformis* is a deepwater species only, and probably restricted to the Caribbean. In our dredging trips in the NW Gulf of Mexico we have never found specimens with subsolitary large-sized bladders as mentioned by Taylor (1960) and Schneider & Searles (1991); detailed morphological and, perhaps, molecular analyses should reveal whether this entity is *B. pyriformis* or a different species.

Acknowledgements. We wish to thank B. Wysor for collecting the material from Panamá, J. Norris for providing critical literature, and D. Ballantine for helpful discussion on the genus *Botryocladia*. SF thanks M. and D. Littler for participation in the 1995 cruise to the Lesser Antilles on board the *R/V Sea Diver*. We thank E. Hickerson, G.P. Schmahl, D.L. Felder and S.A. Earle for the invitation to participate in collecting trips, and the crew of the *M/V Rinn* boats, the *R/V Pelican* (LUMCON), and the *R/V Gunther* (NOAA) for logistical support. This project was funded in part by a J. Bennett Johnston Foundation grant and UL Graduate Student Organization awards to BG, and by DOE grant DEFG02-97ER12220, NSF grant DEB9903900 and NURC-NOAA grant NA96RU-0260 to SF. We thank J.N. Norris (Smithsonian Institution) and M.J. Wynne (Michigan University herbarium) for sending herbarium specimens determined by W.R. Taylor as *B. pyriformis*, and C. Schneider & D. Ballantine for constructive comments on the manuscript.

REFERENCES

APONTE-DIAZ M., 1988 — *Botryocladia ganesanii* sp. nov. (Rhodophyta, Rhodymeniales) from the Caribbean coast of Venezuela. *Cryptogamie, Algologie* 9: 43-52.

BALLANTINE D., 1985 — *Botryocladia wynnei* sp. nov. and *B. spinulifera* (Rhodymeniales, Rhodophyta) Taylor & Abbott from Puerto Rico. *Phycologia* 24: 199-204.

BALLANTINE D., 1989 — Reproduction in Caribbean plants of *Botryocladia pyriformis* and *B. wynnei* (Rhodymeniales, Rhodophyta). *Phycologia* 28: 237-242.

BALLANTINE D. & APONTE N.E., 2002 — *Botryocladia bahamense* sp. nov. (Rhodymeniaceae, Rhodophyta) from the Bahamas, western Atlantic. *Cryptogamie, Algologie* 23: 123-130.

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. III. Rhodophyceae. *Dansk Botanisk Arkiv* 3: 369-504.

BRODIE J. & GUIRY M.D., 1988 — Life history and reproduction of *Botryocladia ardreana* sp. nov. (Rhodophyta, Rhodymeniales) from Portugal. *Phycologia* 27: 109-130.

FELDMANN G., 1945 — Révision du genre *Botryocladia* Kylin (Rhodophycées, Rhodyméniacées). *Bulletin de la Société d'Histoire Naturelle de l'Afrique du Nord* 35: 49-61.

FELSENSTEIN J., 1985 — Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

GAVIO B. & FREDERICQ S., 2002 — *Grateloupia turuturu* (Halymeniceae, Rhodophyta) is the correct identity for the non-native species in the Atlantic known as *G. doryphora. European Journal of Phycology* 37: 349-360.

KYLIN H., 1931 — Florideenordnung Rhodymeniales. *Lunds Universitets Årksskrift, Ny Följd, Andra Avdelningen* 27(11), 48 pp.

LITTLER D.S. & LITTLER M.M., 2000 — *Caribbean Reef Plants*. Offshore Graphics, Inc., 542 pp.

MADDISON D. R. & MADDISON W. P., 2000 — *MacClade 4: Analysis of Phylogeny and Character Evolution.* Version 4.0. Sinauer Associates, Sunderland, Massachusetts.

POSADA D. & CRANDALL K.A., 1998 — Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

SCHNEIDER C.G. & SEARLES R.B., 1991 — *Seaweeds of the Southeastern United States: Cape Hatteras to Cape Canaveral*. Duke University Press, 553 pp.

SCHNEIDER C.W. & LANE C.E., 2000 — A new species of *Botryocladia* (Rhodymeniales, Rhodophyta) from the Galapagos Islands. *Cryptogamie, Algologie* 21: 167-175.

SCHNETTER R., 1978 — *Botryocladia monoica* (Rhodymeniales, Rhodophyta), a new species from the Caribbean coast of Colombia. *Phycologia* 17: 13-15.

SWOFFORD D.L., 2002 — *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods)*, version 4.0b10. Sinauer Associates, Sunderland, MA.

TAYLOR W.R., 1960 — *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*. Ann Arbor, Michigan, University of Michigan Press, 870 pp.

WYNNE M.J., 1998 — A checklist of benthic marine algae of the tropical and subtropical western Atlantic: first revision. *Nova Hedwigia* 116: 1-155.