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Botryocladia iridescens sp. nov. (Rhodymeniaceae, Rhodophyta) from Puerto Rico, Caribbean Sea

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Abstract – A new species, *Botryocladia iridescens* D.L.Ballant. *et* H.Ruiz is described from coral reef habitats in Puerto Rico. The new species possesses a brilliant and patterned iridescence and has been collected in mid- to deep-water cryptic reef habitats. *Botryocladia iridescens* is diminutive, to 7 mm high and with vesicles to 6 mm in diameter, and most commonly produces one or two primary vesicles on a short simple or once forked, solidly parenchymatous axis. Vesicle walls are composed of two medullary and two cortical layers. The vesicles are uniformly covered by a surface cortical layer and frequently anastomose laterally with contiguous vesicles of the same or adjacent plants. Spherical to pyriform gland cells are borne on inner medullary cells or medullary cells which project into the vesicle cavity. Regrowth of an axis from the base of old vesicles is common. Tetrasporangia are cruciately divided, measuring 14-20 µm in diameter and are scattered in the cortex. Gametophytes are monoecious, the partially protuberant cystocarps measuring to 500 µm in diameter. Spermatangia are located in discrete clusters and are cut off singly or in pairs from elongate spermatangial mother cells. The new species is also recorded from Grenada, West Indies.

***Botryocladia iridescens* sp. nov/ Caribbean Sea / coral reef algae / Puerto Rico / marine red algae / Rhodophyta / Rhodymeniaceae / taxonomy**

Résumé – Une espèce nouvelle, *Botryocladia iridescens* D.L.Ballant. *et* H.Ruiz, est décrite des récifs de corail à Porto Rico. La nouvelle espèce possède une iridescence brillante qui forme des motifs réguliers. Elle a été récoltée dans des récifs cryptiques moyennement ou très profonds. *Botryocladia iridescens* est une espèce de très petite taille (jusqu' à 7 mm de haut, vésicules jusqu'à 6 mm de diamètre) qui produit une ou deux vésicules primaires sur un axe parenchymateux court, simple ou fourchu. Les parois des vésicules sont composées de deux couches médullaires et deux couches corticales. Les vésicules sont couvertes uniformément par une couche corticale superficielle ; elles présentent souvent des anastomoses latérales avec des vésicules contiguës appartenant ou non à la même plante. Des cellules glandulaires sphériques ou piriformes sont portées par les cellules médullaires internes ou par les cellules médullaires qui se projettent dans la cavité des vésicules. La nouvelle croissance d'un axe à partir de la base d'une vieille vésicule est fréquente. Les tétrasporanges sont divisés de façon croisée ; ils sont dispersés dans le cortex et leur diamètre est de 14-20 mm. Les gamétophytes sont monoïques et la diamètre de la partie protubérante des cystocarpes mesure jusqu'à 500 mm de diamètre. Les spermatanges sont situés dans des groupes discrets ; ils sont séparés individuellement ou en paires de leurs cellules-mère allongées. La nouvelle espèce a aussi été enregistrée aux Antilles à Grenade.

Algues des récifs coralliens /algues marines rouges / *Botryocladia iridescens* sp. nov / Mer des Caraïbes / Porto Rico / Rhodophyta / Rhodymeniaceae / taxinomie

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INTRODUCTION

The genus *Botryocladia* (J.Agardh) Kylin (1931) is a member of the Rhodymeniaceae belonging to the *Botryocladia* group of Kylin (1931). Classically the genus has been defined on the basis of possessing a solid parenchymatous axis, which bears hollow vesicular branches (Kylin, 1931). It further differs from other hollow-vesicled Rhodymeniaceae on the basis of its sympodial branching (Kylin, 1956; Millar, 1990). These simple morphological criteria as applied to separation of *Chrysymenia* from *Botryocladia* are currently being questioned with the recognition that the genera are not monophyletic and represent heterogenous assemblages (Saunders *et al*., 1999; Afonso-Carrillo & Sobrino, 2003; Wilkes *et al*., 2006). In fact, systematic difficulties within the Rhodymeniaceae (Rhodymeniales) as a whole have been well and increasingly documented (Saunders & Kraft, 1994, 1996; Huisman, 1995, 1996; Saunders, 2004). Furthermore, Wilkes *et al.* (2006) indicated that there was no apparent correlation between some primarily-utilized morphological characters (degree of cortication and disposition of gland cells) with molecular data sets as these characters are represented within multiple *Botryocladia* clades.

Botryocladia, with some 42 species world-wide (Ballantine & Aponte, 2002a; Gavio & Fredericq, 2003; Wilkes *et al*. 2006; Guiry & Guiry, 2008), is (along with *Rhodymenia*) the largest genus by far of both the family Rhodymeniaceae and the order Rhodymeniales. Three *Botryocladia* species [*B. bahamense* D.L.Ballant. *et* Aponte (2002a), *B. caraibica* Gavio *et* Fredericq (2003) and *B. ballantinei* Gavio *et* Fredericq (2005)] have been described from the Caribbean region within the last six years, and the genus is now represented by 11 species in the tropical and subtropical western Atlantic Ocean (Gavio & Fredericq, 2003, 2005; Wynne, 2005), five of which are now known from Puerto Rico (Ballantine & Aponte, 2002b).

Recent collections from coral reef habitats at 6-23 m depths offshore from La Parguera, Puerto Rico, have yielded specimens of a diminutive *Botryocladia* which possesses a combination of characters that clearly separate it from other members of the genus.

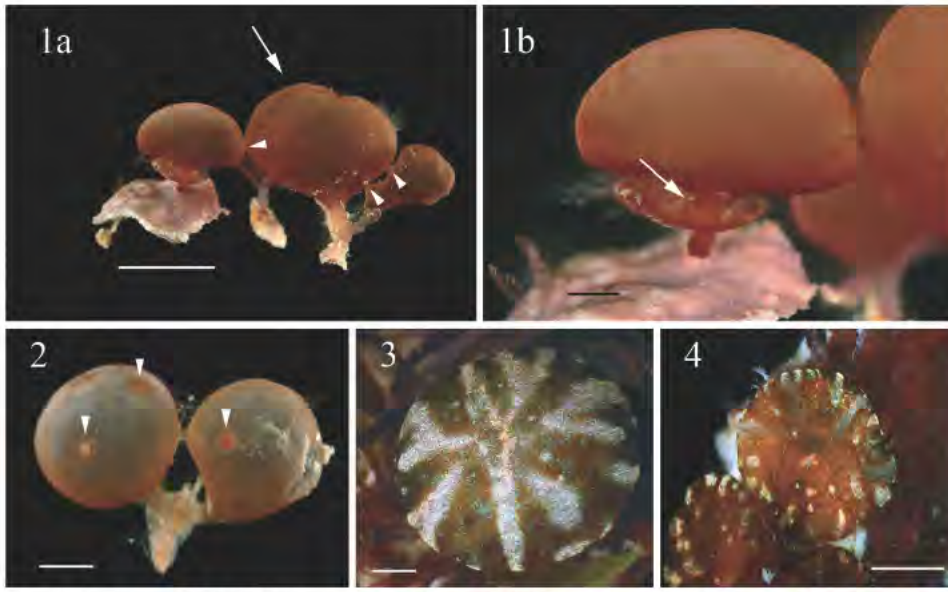
MATERIALS AND METHODS

Specimens were collected by SCUBA diving and were preserved in 10% formalin/seawater. Transections (30 µm thick) were made using an American Optical Cryo-Cut freezing microtome. Microscopic preparations were stained in acidified 1% aniline blue and mounted in 60% Karo® corn syrup on microscope slides. Photomicrographs were taken with a SPOT RE digital camera through an Olympus BMAX light microscope. The plates were assembled from digital photographs utilizing Adobe Photoshop CS2. The holotype is deposited in US and paratypes in US, MICH, and MSM. Herbarium abbreviations follow Holmgren *et al*. (1990) and authority designations are according to Brummitt & Powell (1992).

RESULTS AND OBSERVATIONS

***Botryocladia iridescens* D.L.Ballant. *et* H.Ruíz, sp. nov.** **Figs 1-12**

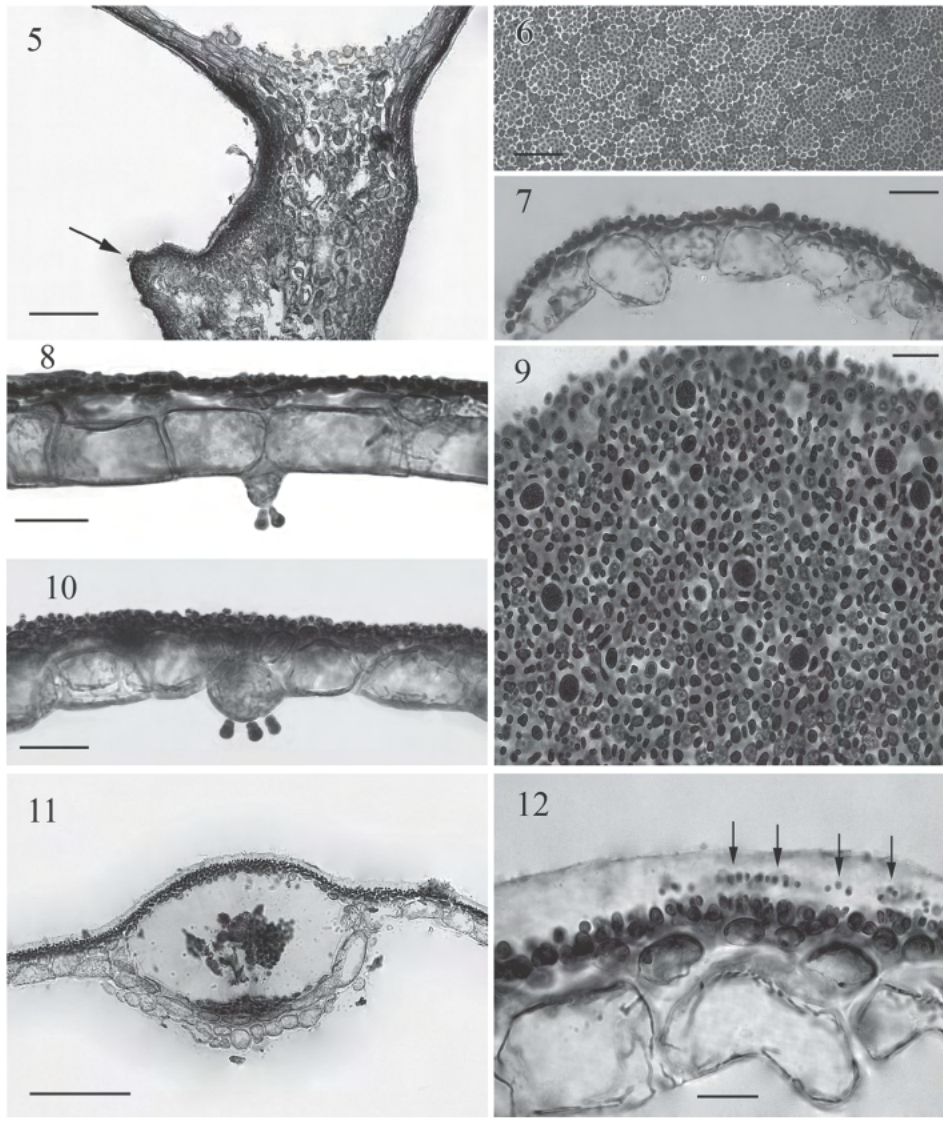
Diagnosis: *Algae usque ad 9 mm altae; axes usque ad 750 µm diametro et usque ad 4 mm longi, vesiculas 1-2 pyriformes usque ad sphaericas usque ad 6 mm diametro typice facientes, coniunctionibus lateralibus frequentibusque; paries vesicularum e*



Figs 1-4: *Botryocladia iridescens* D.L.Ballant. *et* Ruiz, sp. nov. **1a.** Wet-habit of several plants including the holotype (arrow) showing anastomosed adjacent vesicles (arrowheads) (*D.L.B. 6342*). Scale bar = 5 mm. **1b.** Enlargement of plant at left showing regrowth of the axis from the base of a disintigrating vesicle (arrow) (*D.L.B. 6342*) Scale bar = 1 mm. **2.** Habit of female gametophytic plant with cystocarps (arrowheads) (*D.L.B. 6301*). Scale bar = 2 mm. **3.** Vesicle viewed from above showing a stellate pattern of iridescence (*D.L.B. 6307*). Scale bar = 1 mm. **4.** Vesicle viewed from above showing a spotted pattern of iridescence (*D.L.B. 6376*). Scale bar = 2 mm.

unico strato medulloso, cellulae medullosae 45-105 µm longae et 35-65 µm altae, cellulae medullosae et triangulares et mediocres magnitudine inter cellulas medullosas confertae, 25-40 µm diametro; stratum corticale crassum cellulas 2, stratum interius corticale cellulis sphaericis ad ovatas, usque ad 15 µm diametro, producentes cellulas corticales parvas et sphaericas, 5-7.5 diametro; glandicellulae hemisphaericae ad pyriformes, ad 12.5 µm diametro, in cellulis interioribus medullosis in cavitatem vesicularem projectis portatae; glandicellulae 1-3 per cellulas sustinentes productae; tetrasporangia paene sphaerica, in cortice dispersa, cruciatim divisa, 14-20 µm diametro; algae monoeciae; cystocarpia partim protrudentia, plerumque introrsum projecta, cystocarpia 440-495 µm diametro.

Algae to 9 mm in height with axes to 750 µm in diameter and to 4 mm long, axes typically producing 1 or 2 pyriform to spherical vesicles, to 6 mm in diameter with frequent lateral fusions; vesicle walls of a single medullary layer, medullary cells measure 45-105 µm in length and 35-65 µm in height with intermediate-sized triangular medullary cells, 25-40 µm in diameter, wedged between; cortical layer two cells in thickness, an inner cortical layer with cells spherical to ovate measure to 15 µm in diameter, these giving rise to small spherical cortical cells, 5-7.5 µm in diameter; gland cells spherical to pyriform, to 12.5 µm in diameter, borne on inner medullary cells or medullary cells which project into the vesicle cavity; gland cells are produced 1-3 per supporting cell; tetrasporangia nearly spherical, scattered in cortex, cruciately divided, 14-20 µm in diameter; algae monoecious, cystocarps partially protruding, mostly projecting inwardly; cystocarps 440-495 µm in diameter.



Figs 5-12. *Botryocladia iridescens* D.L.Ballant. *et* Ruiz, sp. nov. **5.** Longitudinal section through stipe and base of vesicle wall. Regrowth of the axis from a disintegrated vesicle is evident from scar on stipe (arrow) (*D.L.B. 5863*). Scale bar = 250 µm. **6.** Surface view of outer cortical wall of a vesicle showing complete cortical covering (*D.L.B. 6342*). Scale bar = 25 µm. **7.** Transection through vesicle wall showing medullary and cortical layers (*D.L.B. 6342*). Scale bar = 50 µm. **8.** Transection through vesicle wall showing two gland cells produced from small secondary medullary cell (*D.L.B. 5629*). Scale bar = 50 µm. **9.** Surface view of outer cortical wall of a vesicle showing complete cortical covering with scattered tetrasporangia (*D.L.B. 6342*). Scale bar = 25 µm. **10.** Transection through vesicle wall showing three gland cells produced from a protuberant medullary cell (*D.L.B. 5629*). Scale bar = 50 µm. **11.** Transection through cystocarp (*D.L.B. 5747*). Scale bar = 250 µm. **12.** Transection through vesicle wall showing spermatangia (arrows) (*D.L.B. 5747*). Scale bar = 25 µm.

Holotype: *D.L. Ballantine (D.L.B.) 6342*, Edge insular shelf, La Parguera, Puerto Rico (17º53.423 N, 66º 59.320 W), 23m deep, coll. Héctor Ruiz (H.R.), 15.x.2004 (Alg. Coll. # US-209106). Isotype in MSM.
Paratypes: Puerto Rico: Edge insular shelf, La Parguera: *D.L.B. 5971,* 20 m, coll. H.R., 7.x.2003 (Deposited in MSM); *D.L.B. 6307*, *ibid*, 13.ix.2004 (MSM); *D.L.B. 6352*, 23 m, *ibid.*, 10.viii.2003 (MSM). Media Luna Reef, La Parguera, (17°56.093N, 67°02.931W): *D.L.B. 5620*, 12 m, coll. H.R., 2.xi.2001 (MSM); *D.L.B. 5629b*, 12 m, coll. D.L.B., H.R., 7.xi.2001 (MSM); *D.L.B. 5685*, 11 m, coll. H.R., 19.xii.2001 (MSM); *D.L.B. 5783*, *ibid*., 22.xi.2002 (MSM); *D.L.B. 6285*, 12 m, coll. H.R., 25.viii.2004 (MSM); *D.L.B. 6301*, 6 m, coll. H.R., 2.ix.2004 (MSM); *D.L.B. 6584,* 9 m, coll. H.R., 28.vi.2005 (MICH and US). Turrumote Reef, La Parguera (17°56.097N, 67°01.130W): *D.L.B. 5747*, 18 m, coll. H.R., 30.x.2002 (MSM); *D.L.B. 5776*, 12 m, *ibid*, 11.xi.2002 (MSM); *D.L.B. 5896*, 14 m, 9.vi.2003 (MSM); *D.L.B 6313*, 14 m, *ibid*, 28.ix.2004 (MSM). Enrique Reef: *D.L.B. 5863*, 7 m, coll. H.R., 4.iii.2003 (MSM). **Grenada:** *D.L.B. 5663*, Boss Reef, 12 m, coll. H.R., 16.xi.2001 (MSM); *D.L.B. 5671*, north coast, 11 m, *ibid*, 14.xi.2001.
Etymology: The new species is named for its brilliant iridescence.

Botryocladia iridescens is highly inconspicuous, having been observed exclusively in cryptic habitats in coral reef environments, i.e. in narrow crevices and under overhangs where it has been collected at depths of 6-23 m. *Botryocladia iridescens* reaches a maximum height of only 9 mm (Figs 1, 2) and when living displays a striking and stellate- or spot-patterned iridescence (Figs 3, 4). Plants have simple solid pseudoparenchymatous axes, which occasionally are once forked and measure to 4 mm long and to 750 µm in diameter. The axes typically bear a single pyriform to spherical terminal vesicle to 6 (– 7) mm in diameter. Occasionally a second vesicle is cut off laterally in a position immediately proximal to the first vesicle. Vesicle walls consist of three or four layers (Figs 7,8), the innermost composed of large colorless medullary cells 45-105 µm in length × 35-65 µm in height intermixed with intermediate-sized medullary cells 25-40 µm in diameter (Fig. 7). The cortex is complete (Fig. 6) and typically two-layered (Fig. 12), rarely three-layered. The innermost layer is composed of ovate to spherical cells (to 15 µm in diameter) and the outermost of one or two layers consisting of small spherical cells 5-7.5 µm in diameter. Secretory cells are spherical to obpyriform in shape, 12-13 µm in maximum diameter, and are generally cut off in pairs (Fig. 8), although one or three (Fig. 10) secretory cells have also been observed. In all cases the secretory cells are either cut off from protuberant medullary cells of otherwise normal size (Fig. 10) or from smaller adventitious cells cut off toward the center of the vesicle from ordinary medullary cells (Fig. 8).

Spherical to ovate cruciately divided tetrasporangia are terminal on subsurface cortical cells and are scattered throughout the otherwise unmodified cortex (Fig. 9), and measure 14-20 µm in diameter. Gametophytes are monoecious. Broadly domed cystocarps (Figs 2, 11) project equally into the vesicle cavity and outwardly, measure 440-500 µm in diameter. The gonimoblast measures 300-385 µm in diameter and carposporangia are to 18 µm in largest dimension. Spermatangia are located in discrete clusters (Fig. 12). They are cut off (singly or in pairs) from elongate spermatangial mother cells which measure to 3.5 µm in length.

Contiguous vesicles on the same or adjacent plants frequently anastomose laterally by one or two stout connections (Figs 1a, 2). An unusual feature of *B. iridescens* is the common occurrence of continued growth of the axis from the attachment point of a disintegrating vesicle (Fig. 1b). Evidence of the regrowth can be seen as scars which frequently are observed on axes (Fig. 5).

DISCUSSION

Saunders *et al*. (1999) provided examples of genera within the Rhodymeniaceae that have a mixture of characters which cloud suprageneric assignment. They also listed a number of species considered problematic including species of *Chrysymenia* and *Botryocladia*. Difficulties have undoubtedly been exacerbated by the inclusion of species that contain conflicting characters within genera. For example, Saunders *et al.* (1999) found that *B. ardreana* J.Brodie *et* Guiry (1988) did not clade with other *Botryocladia* species according to their molecular data set. On this basis, they erected the genus *Irvinea* Guiry *in* Saunders *et al*. (1999). Nevertheless, the two genera as presently circumscribed still possess a number of shared morphological characters. In their transfer of *B. boergesenii* Feldmann (1935) and *B. hancockii* E.Y.Dawson (1944) to *Irvinea*, Wilkes *et al*. (2006) established the importance of branched (*Irvinea*) vs. unbranched (*Botryocladia*) vesicles as a distinguishing character. Nevertheless, vesicles are bifurcate in *B. adhaerens* E.Y.Dawson. Until further characters are identified to characterize *Irvinea* from *Botryocladia*, the former is definitively identifiable only on molecular bases.

On the basis of its possession of a solid axis, which bears hollow, non-divided vesicles, and when more than a single vesicle is present, they are produced sympodially, the new species is assigned to *Botryocladia*. By nature of is small size and limited axial development, *B. iridescens* belongs to the section Microphysae of Feldmann (1945). While, the Feldmann (*op. cit*.) divisions are almost certainly phylogenetically artificial, it does serve to divide the genus among morphological types bearing superficial resemblance. Those species in the Microphysae which, like *B. iridescens*, possess a complete cortical surface layer (Table 1) are: *B. adhaerens* E.Y. Dawson (1963), *B. ballantinei* Gavio *et* Fredericq (2005), *B. caraibica* Gavio *et* Fredericq (2003), *B. ganesanii* Aponte-Diaz (1988), *B. guineensis* D.M. John (1972), *B. kuckuckii* (Weber Bosse) Yamada *et* Tak. Tanaka (1938), *B. macaronesica* Afonso-Carr *et al*. (2006), *B. paucivesicaria* Stegenga, J.J. Bolton *et* R.J.Anderson (1997) and *B. skottsbergii* (Børgesen) Levring (1941), *B. spinulifera* W.R. Taylor *et* I.A. Abbott (1973) as well as *B. bahamense* which may be incompletely or completely corticated. Comparison of the features of these species shows that all differ in some significant way from the newly proposed species (Table 1). Cortication in *Botryocladia canariensis* Afonso-Carr *et* Sobrino (2003) is reported to be nearly complete (2003).

Wilkes *et al*. (2006) pointed out that among all *Botryocladia* species, only *B. spinulifera* has a single character that is unique to the genus (i.e. spine-like projections in the cortical matrix). Nevertheless, *B. iridescens* possesses several unusual features, the latter of which may be unique. The first of these is the common anastomosing between adjacent vesicles of the same or closely positioned plants. While uncommon in *Botryocladia*, this character is known in the Rhodymeniaceae genera *Halichrysis* (Ballantine *et al*., 2007) and *Chamaebotrys* (Huisman, 1996). The additional character of continuation of growth of the axis from the base of a vesicle after vesicle senescence is to our knowledge unique to the genus. However, the character of regrowth of an axis following apical structure senescence is also known in other Rhodymeniaceae genera such as *Maripelta* (Eiseman & Moe, 1981).

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Table 1. Comparative features of Botryocladia Section Microphysae with continuous cortication (Unk. = unknown)

Species	*Plant height (cm)*	*V sicle dimension (length × width) (mm)*	*Vesicle shape*	*Number medullary layers*	*Number cortical layers*	*Gland cells: number per cluster*	*Gland cells: specialized supporting cell*	*Gland cell dimensions (length × width) (µm)*	*Tetrasporangia dimensions (length × width) (µm)*	*Monoecious (Mon.)/ dioecious*	*Cystocarp breadth (µm)*	*Unusual features*	*References **
B. adhaerens E.Y. Dawson	0.6-0.8	6 × 2.2	Long-elliptical	2	1	3 or more	+	Unk.	Unk.	Unk.	Unk.	Primary vesicle frequently bifurcate, lateral fusions	1
B. bahamense D.L. Ballant. *et* Aponte	1-2.5	3.5-7 × 2.5-4	Ovate - pyriform	1-2	Incomplete – 1	(2)4-6(8)	+/–	To 48 × to 23	To 56 × 23	Mon.	– 750	Vesicle portions with complete and incomplete cortication	2
B. ballantinei Gavio *et* Fredericq	1.0-1.5	9-15 × 8-12	Spherical-obovate	1-2	1	0	Unk.	Unk.	30-35 × 16-25	Unk.	1000	No gland cells	3
B. caraibica Gavio *et* Fredericq	3-4	2-12 × 1.5-10	Ovoid-pyriform	3	1-2	2-5	+/–	32-40 × 16-32	Unk.	Unk.	Unk.		4
B. ganesanii Aponte-Diaz	3-3.5	20-25 × 10-18	Spherical - ovate	1-2	2	6-12(15)	–	30-45(-50) × 20-35	24-28 × 20	Mon.	700-900		5
B. guineensis D.M. John	2-5	15 (– 35) × 11(– 21)	Spherical - elongate-pyriform	2-3	1-2	4-8(12)	–	11-33 × 10-25	22-23 × 16-22	Unk.	600		6

* References: 1) Dawson, 1963; 2) Ballantine & Aponte, 2002a; 3) Gavio & Fredericq, 2005; 4 Gavio & Fredericq, 2003; 5) Aponte Diaz, 1988; 6) John, 1972; 7) Weber van-Bosse, 1928; 8) Littler & Littler, 2003; 9) Børgesen, 1929; 10) Afonso-Carillo, *et al*., 2006; 11) Stegenga *et al*., 1997; 12) Børgesen, 1924; 13) Taylor & Abbott. 1973; 14) This paper.

Table 1. Comparative features of Botryocladia Section Microphysae with continuous cortication (Unk. = unknown) *(continued)*

Species	*Plant height (cm)*	*V sicle dimension (length × width) (mm)*	*Vesicle shape*	*Number medullary layers*	*Number cortical layers*	*Gland cells: number per cluster*	*Gland cells: specialized supporting cell*	*Gland cell dimensions (length × width) (µm)*	*Tetrasporangia dimensions (length × width) (µm)*	*Monoecious (Mon.)/ dioecious*	*Cystocarp breadth (µm)*	*Unusual features*	*References **
B. kuckuckii (Weber Bosse) Yamada et Tak.Tanaka	1-2.5	8-15 × 6-7	Spherical - ovate	3	2-3	8-10	Unk.	Unk.	Unk.	Unk.	Unk.		7, 8
B. macaronesica Afonso-Carrillo *et al*.	1.5	4-30 × 1.5-4	Ellipsoidal - oblong	1?	1?	(1)2-4	-	To 28 diam.	Unk.	Unk.	Unk.	Primary vesicle frequently lobed	9, 10
B. paucivesicaria Stegenga, J.J. Bolton *et* R.J. Anderson	To 5.5	To 5 × – 17	Elongate-clavate	3	2	– 10	+/–(?)	To 36 × to 25	To 45 × 22	Unk.	Unk.	Carpospor-ophyte completely immersed	11
B. skottsbergii (Børgesen) Levring	1-2	5-6 × 4-5	Spherical - pyriform	1-2	1-2	10-20 or more	–	40 × 20	Unk.	Unk.	Unk.	Gland cells rarely divided into two cells	12
B. spinulifera W.R. Taylor *et* I.A. Abbott	Creeping, to 0.3	2.2-3.5 × 1.4-1.7	Pyriform - ovate - obovate	1	1	1	–	7.5 diam.	To 28 × 15-18	Unk.	Unk.	Creeping axis, vesicle wall with spine-like projections	13, 14
B. iridescens D.L.Ballant. *et* Ruiz	0.9	6.0	Spherical - pyriform	2	2	1-3	+/–	12.5 diam.	14-19	Mon.	440-495	Regrowth from old vesicles	14

* References: 1) Dawson, 1963; 2) Ballantine & Aponte, 2002a; 3) Gavio & Fredericq, 2005; 4 Gavio & Fredericq, 2003; 5) Aponte Diaz, 1988; 6) John, 1972; 7) Weber van-Bosse, 1928; 8) Littler & Littler, 2003; 9) Børgesen, 1929; 10) Afonso-Carillo, *et al*., 2006; 11) Stegenga *et al*., 1997; 12) Børgesen, 1924; 13) Taylor & Abbott. 1973; 14) This paper.

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