

Notes on the marine algae of the Bermudas. 16. Two new epiphytic species of *Champia* (Champiaceae, Rhodymeniales), *C. hasselbringii* and *C. insularis*

Craig W. SCHNEIDER^{a*}, Maura K. GRIFFITH^a, Christopher E. LANE^b
& Gary W. SAUNDERS^c

^aDepartment of Biology, Trinity College, Hartford, CT 06106, USA

^bDepartment of Biological Sciences, University of Rhode Island,
Kingston, RI 02881, USA

^cCentre for Environmental and Molecular Algal Research, Department of Biology,
University of New Brunswick, Fredericton, New Brunswick, Canada E3B 5A3

Abstract – Using molecular-assisted alpha taxonomy, we have uncovered two new pseudocryptic species of *Champia* in Bermuda, as well as have demonstrated that the European *C. parvula*, a name previously applied to historical Bermuda collections, and *C. farlowii* recently described from southern New England, USA, are not part of the island flora. We present COI-5P and *rbcL*, as well as morphological, evidence to describe two endemic species for the islands, *C. hasselbringii* sp. nov. and *C. insularis* sp. nov. They are similar species with compressed but variable axes and a wealth of overlapping anatomical characteristics. However, the two species are distinguished not only by their genetics, but also by reproductive features that are commonly found in the small prostrate, epiphytic species. It is possible that *C. hasselbringii* could be the correct name for species reported in warm waters of the western Atlantic as *C. compressa* or *C. vieillardii*, species with type localities in South Africa and New Caledonia, respectively.

Bermuda / *Champia* / Champiaceae / *C. hasselbringii* sp. nov. / *C. insularis* sp. nov. / COI-5P / phylogenetics / *rbcL* / Rhodymeniales / Rhodophyta

INTRODUCTION

A great deal has changed in the marine algal flora of Bermuda since an historical checklist was published 15 years ago (Schneider, 2003), much of change due to the introduction of molecular-assisted alpha taxonomy (MAAT) in the islands (Cianciola *et al.*, 2010). For the red algal genus *Champia*, only two species have historically been reported for Bermuda, *C. parvula* (C. Agardh) Harvey (Schneider, 2003) and *C. salicornioides* Harvey (Schneider & Lane, 2005). A recent report on *Champia* along the northeastern coast of the United States cast doubt on *C. parvula*, with its type locality in Spain, being present in the western Atlantic Ocean. Using

* Corresponding author: cschneid@trincoll.edu

two genetic markers and morphological characteristics, Griffith *et al.* (2017) demonstrated that *Champia* collections from southern New England, USA, could no longer be ascribed to *C. parvula* (Irvine & Guiry, 1983) and represented the new species, *C. farlowii* M.K. Griffith, C.W. Schneider & C.E. Lane. They suggested that all records previously called *C. parvula* in the Americas be looked at using molecular techniques to better understand the diversity in this complex of species still identified under the European name.

Champia parvula was first reported in Bermuda by Collins & Hervey (1917) as small (to 3 cm) epiphytic plants, and the authors distributed local examples under this binomial in the exsiccata *Phycotheca Boreali-Americana (P.B.-A.)* as specimen no. 1934 (Collins *et al.*, 1913). A year later, Howe (1918) considered this species as one of the “more common and conspicuous algae occurring in the islands” in his algal section of Britton’s *Flora of Bermuda*. Subsequently, Taylor & Bernatowicz (1969) described their Bermuda collections as *C. parvula* but “not as well developed as in more northern waters.” Presently, as the “northern” collections Taylor & Bernatowicz (1969) would have been referring to are now considered *C. farlowii* and no longer represent European *C. parvula* after molecular and morphological analysis (Griffith *et al.*, 2017), the assessment of specimens from Bermuda became a necessity. We have made many collections of *C. ‘parvula’* in Bermuda during the past decades that morphologically match the early 20th century collections of Collins and Hervey, and as part of our effort to barcode all of the marine algae of the islands (Cianciola *et al.*, 2010), subjected many of them to molecular analysis in order to determine whether they align with *C. parvula*, *C. farlowii* or another species in the growing cluster of species in the *C. parvula* complex (Griffith *et al.*, 2017). Some of our *Champia* collections from Bermuda were more distinctly compressed than others from the area, leading us to consider flattened species reported in the western Atlantic, *C. compressa* Harvey, *C. vieillardii* Kützinger and *C. taironensis* Bula-Meyer, as new to the islands. Given all of the uncertainty associated with these collections, we undertook a molecular-assisted alpha taxonomic study (MAAT) of the genus to identify species of *Champia* in the Bermuda flora to sort out relationships with congeners.

MATERIALS & METHODS

Sample collection, preservation and anatomy

Collection locations were marked with a Garmin™ eTrex H GPS (Olathe, Kansas, USA) or using Google Earth Pro, vers. 7.3.0.3832 © 2017. Small portions of specimens were dried on silica gel for DNA extraction and also preserved in 4-5% Formalin in seawater for anatomical study, the remainder being pressed onto rag paper as archival herbarium vouchers. Liquid preserved samples were sectioned using an 880 American Optical (San Diego, California, USA) freezing microtome, with sections and whole mounts mounted in 30% corn syrup with acidified 1% aniline blue in a ratio of 20:1 with a drop of Formalin added as a medium preservative. Live specimens chosen for DNA analysis were photographed using a Nikon Coolpix AW100 digital camera (Nikon Inc., Tokyo, Japan) and whole mounts were digitized on an Epson ET-2650 scanner (Seiko Epson Corp., Suwa, Nagano, Japan). Photomicrographs were taken using Zeiss Axioskop 40 microscope (Carl

Zeiss, Oberkochen, Germany) equipped with a model 11.2 Spot InSight 2 digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA). The digital images were composed in Adobe Photoshop™CS6 v. 13.0.1 (Adobe Systems, San Jose, California, USA). Herbarium abbreviations follow the online Index Herbariorum <<http://sweetgum.nybg.org/ih/>> and standard author initials were taken from Brummitt & Powell (1992). The *P.B.-A. exsiccata* cited here is part of CWS' personal herbarium.

Molecular sequencing and analysis

Sub-samples of specimens dried in silica gel for subsequent DNA analysis are listed in Table 1 along with sequences selected from GenBank for comparison. The dried samples were ground in liquid nitrogen and stored at -20°C . Samples processed at the University of New Brunswick (UNB) had their DNA extracted as in Saunders & McDevit (2012). The DNA of samples processed at the University of Rhode Island (URI) was extracted using the Macherey-Nagel (Bethlehem, Pennsylvania, USA) NucleoSpin Plant II kit. Mitochondrial COI-5P and *rbcL* were amplified and sequenced as outlined in Saunders & Moore (2013). PCR products generated at UNB and URI were sequenced at the Genome Quebec sequencing facility and the Rhode Island Genomics and Sequencing Center using the Applied Biosystems Inc. 3130xl Genetic Analyzer (Life Technologies, Grand Island, New York, USA).

Specimens of *Champia* from Bermuda were assigned to genetic groups using mitochondrial COI-5P barcode sequences (13 specimens; 664 sites; Table 1). To place the new Bermuda species into a phylogenetic context, individual COI-5P (19 specimens; 664 sites; Table 1) and *rbcL* (28 specimens; 1358 sites; Table 1) alignments, and a concatenated (28 specimens; 2022 sites; Table 1) alignment were subjected to phylogenetic analyses. Maximum likelihood (ML) analyses were completed with RaxML (Stamatakis, 2014) in Geneious 10.2.3 (Kearse *et al.*, 2012) with a GTR+I+G model, partitioned by gene and codon, with 1000 bootstrap replicates completed to assess robustness. New molecular sequences were uploaded to GenBank (<http://www.ncbi.nlm.nih.gov/>) and BOLD (<http://www.barcodinglife.org/>) (Table 1).

RESULTS & DISCUSSION

Mitochondrial COI-5P data revealed two distinct genetic entities of epiphytic *Champia* from Bermuda. The first had four specimens (*C. hasselbringii*; Table 1) that were only 1-2 bp (base pairs) or 0.3% divergent. The second group with nine collections (*C. insularis*; Table 1) only displayed 0-1 bp or 0.15% divergence. The two species were not closely related being >9% (62-64 bp) divergent in COI-5P. The distance between these two genetic groups was reiterated in phylogenetic analyses in which they were separated by a number of *Champia* spp., which nonetheless included a number of novel unnamed species as well as genetic groups to which the same morphospecies name was applied (e.g., *C. parvula*; Fig. 1). The two new epiphytic *Champia* spp. from Bermuda are described here.

Table 1. Collection details for isolates included in the molecular analyses of this study with newly generated GenBank accession numbers in bold type

<i>Species</i>	<i>Voucher nos.</i>	<i>Collectors/date</i>	<i>Collection site</i>	<i>COI-5P</i>	<i>rbcL</i>
<i>Champia bifida</i> Okamura	GWS018588	G. Saunders, H-G. Choi/20 May 2010	Rocky Reef at Lighthouse 'Point' Piyangdo Island, Jeju, South Korea	HQ544129	MH308225
<i>Champia compressa</i> Harvey		B. Gavio, B. Wysox/10 Aug. 2000	Florida Middle Ground, Florida, USA	—	AY294358
<i>Champia expansa</i> Yendo	GWS018695	G. Saunders, H-G. Choi/19 May 2010	Channel between Little & Big Munseom Islands, Jeju, South Korea	HQ544156	MH308221
<i>Champia farlowii</i> M.K.Griffith, C.W.Schneider <i>et</i> C.E.Lane	GWS017871	B. Clarkston, D. McDevit, M. Bruce, A. Savoie, C. Longtin/14 April 2010	Garbage Beach Breakwater, Woods Hole, Massachusetts, USA	HM915110	MH308220
<i>Champia hasselbringii</i> C.W.Schneider <i>et</i> G.W.Saunders sp. nov.	CWS/CEL 10-6-11 [BDA0061]	C. Schneider, C. Lane, T. Popolizio, D. McDevit/19 Aug. 2010	Tucker's Town Bay, Castle Harbour, Bermuda	HQ93262	MH308224
	CWS/CEL 10-24-20 [BDA0384]	C. Schneider, C. Lane, T. Popolizio, D. McDevit/23 Aug. 2010	Cathedral Rock, south shore, Bermuda	KY033969	—
	CWS 10-29-6 [BDA0439]	C. Schneider/24 Aug. 2010	Offshore of High Point, Bermuda I., Bermuda	MH378438	—
	CWS/CEL/TRP 12-8-15 [BDA0557] Holotype	C. Schneider, C. Lane, T. Popolizio/17 Jan. 2012	Brackish Pond Flats, north shore, Bermuda	MH378425	—
<i>Champia insularis</i> C.W.Schneider <i>et</i> G.W.Saunders	GWS001257 (CWS/CEL 01-16-10)	C. Schneider, C. Lane/14 Nov. 2001	John Smith's Bay, south shore Bermuda I., Bermuda	—	MH308215
	CWS/CEL 10-5-24 [BDA0033]	C. Schneider, C. Lane, T. Popolizio, D. McDevit/19 Aug. 2010	Frick's Beach, Tucker's Town, south shore Bermuda I., Bermuda	HQ93252	MH308226
	TRP/CWS 12-29-10 [BDA0797]	T. Popolizio/2 Feb. 2012	Gibbet I., Flatts Inlet, Bermuda	MH378426	—

Species	Toucher nos.	Collectors/date	Collection site	COI-5P	rbcl
	TRP/CWS 12-29-10 [BDA0798]	T. Popolizio/2 Feb. 2012	Gibbet I., Flatts Inlet, Bermuda	MH378427	—
	TRP/CWS 12-32-8 [BDA0844] Holotype	T. Popolizio/13 Feb. 2012	Walsingham Bay, Castle Harbor, Bermuda	MH378428	—
	TRP/CWS 12-32-8 [BDA0845]	T. Popolizio/13 Feb. 2012	Walsingham Bay, Castle Harbor, Bermuda	MH378437	—
	TRP/CWS 12-35-9 [BDA0878]	T. Popolizio/17 Feb. 2012	South of White Flats, north of Bermuda Is., Bermuda	MH378429	—
	TRP/CWS 12-83-5 [BDA1260]	T. Popolizio/24 July 2012	Natural Arches, south shore Bermuda I., Bermuda	MH378439	—
	TRP/CWS 12-149-14 [BDA1643]	T. Popolizio/6 Nov. 2012	North Rock, north shore Bermuda I., Bermuda	MH378436	—
	TRP/CWS 12-174-6 [BDA1836]	T. Popolizio/12 Dec. 2012	Spanish Point Park, north shore Bermuda I., Bermuda	MH378430	—
<i>Champia japonica</i> Okamura		M. Suzuki/16 May 2006	Shizuoka, Shimoda, Toji, Japan	—	AB383121
<i>Champia lubrica</i> Mas. Suzuki et Yoshizaki	TNS-AL 178606	M. Suzuki/4 Aug. 2008	Uranohama, Yamada, Shimohei County, Iwate Prefecture, Japan	—	AB693118
<i>Champia parvula</i> (C.Agardh) Harvey	CHA1102	P. Diaz-Tapia	San Pedro de Veigue, Sada, Spain	—	KF356059
	LLG 1603	L. Le Gall, J.M. Urge, F. Rousseau/15 July 2008	Les Haies de la Conchee, Saint-Malo, Brittany, France	KY033971	—
	CLT 173	H. Ruiz/17 July 2005	Seaward of Media Luna Reef, La Parguera, Puerto Rico	—	EU086464
<i>Champia 'parvula'</i>	CLT 198	H. Ruiz/5 May 2006	Culebra, Puerto Rico	—	EF613312
	CLT221	D.L. Ballantine, H. Ruiz/ 1 Oct. 2007	Turramote Reef, La Parguera, Puerto Rico	—	EU670596
<i>Champia vieillardii</i> Kützing	CLT286	H. Ruiz/24 Jul. 2008	La Parguera, Puerto Rico	—	FJ212299
<i>Champia</i> sp. 3Cocos	GWS037907	G. Saunders, K. Dixon/ 11 Dec. 2013	Aquarium Wall, south of Horsburg I., Cocos (Keeling) Is., Australia	MH308213	MH308227

Table 1. Collection details for isolates included in the molecular analyses of this study with newly generated GenBank accession numbers in bold type (*continued*)

<i>Species</i>	<i>Voucher nos.</i>	<i>Collectors/date</i>	<i>Collection site</i>	<i>COL-5P</i>	<i>rbcL</i>
<i>Champia</i> sp. 4Cocos	GWS037757	G. Saunders, K. Dixon/ 5 Dec. 2013	Winter Wall, south of Horsburgh I., Cocos (Keeling) Is., Australia	MH308214	MH308228
<i>Champia</i> sp. 5Cocos	GWS037842	G. Saunders, K. Dixon/ 9 Dec. 2013	Broccoli Bommites, south of Direction I., Cocos (Keeling) Is., Australia	MH308210	MH308217
<i>Champia</i> sp. 1FL	CWS/CEL/TRP [KW316]	C. Schneider, C. Lane, T. Popolizio, D. McDevit/ 1 June 2013	Overseas Highway Trail, Key West, Florida, USA	MH37843	MH378424
<i>Champia</i> sp. 2FL	CWS/CEL/TRP [KW135]	C. Schneider, C. Lane, T. Popolizio, D. McDevit/ 29 May 2013	White St. Pier, Key West, Florida, USA	MH378434	MH378423
<i>Champia</i> sp. 1LH	GWS023006	G. Saunders, K. Dixon, R. Withall/22 Nov. 2010	Roach Wall, Lord Howe Is., New South Wales, Australia	KY033967	MH308216
<i>Champia</i> sp. 1NSW	GWS025270	G. Saunders/8 Dec. 2012	Korora Beach, Coffs Harbour, New South Wales, Australia	KY033973	MH308223
<i>Champia</i> sp. 2NSW	GWS032746	G. Saunders, K. Dixon/ 11 Dec. 2012	Mutton Bird Island (N), Coffs Harbour, NSW, Australia	MH308212	MH308222
<i>Champia</i> sp. 1STX	TRP/CEL/EDS [STX036]	T. Popolizio, C. Lane, E. Salomaki/19 Nov. 2013	Alien Nation Reef, off Frederiksted Pier, St. Croix, USVI	MH378433	MH378422
<i>Champia</i> sp. 2STX	TRP/CEL/EDS [STX0190]	T. Popolizio, C. Lane, E. Salomaki/23 Nov. 2013	Cables Reef, north of Frederiksted Pier, St. Croix, USVI	MH378432	MH378421
<i>Champia</i> sp. 3STX	TRP/CEL/EDS [STX228]	T. Popolizio, C. Lane, E. Salomaki/24 Nov. 2013	Fort Frederik Beach, Frederiksted Pier, St. Croix, USVI	MH378431	MH378420
<i>Champia</i> sp. 1TAS	GWS016176	G. W. Saunders, K. Dixon & L. Kraft/27 Jan. 2010	Boat Harbour Beach, Tasmania, Australia	HM918086	MH308219
<i>Champia</i> sp. 4WA	GWS025526	G. Belton/14 Nov. 2010	Blackwall Reach, Swan River, Western Australia, Australia	MH308211	MH308218

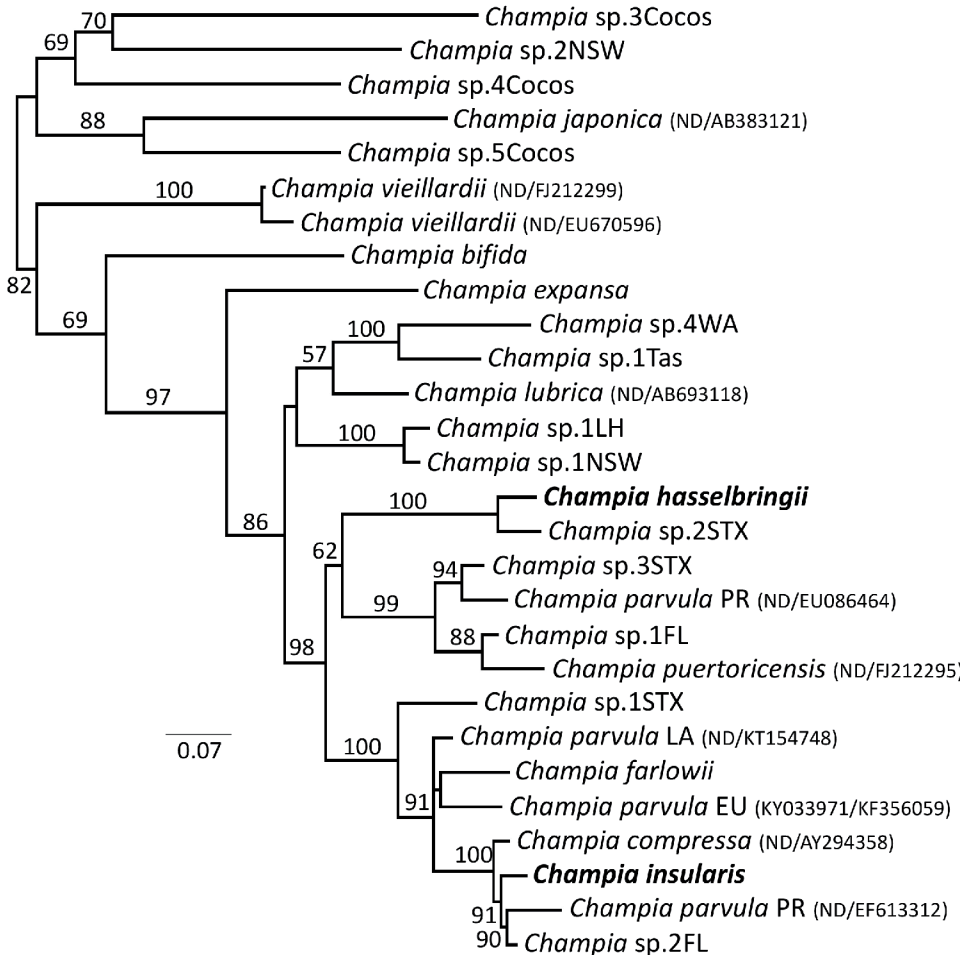


Fig. 1. RaxML phylogenetic tree generated from the concatenated alignment using the GTR+I+G model, 1000 replicates of bootstrap resampling. Values <50 are not shown. Data were either generated for this study or acquired from GenBank with accession numbers included in parentheses after the names (COI-5P/*rbcL*). Scale bar represents substitutions per site.

***Champia insularis* C.W. Schneider & G.W. Saunders, sp. nov.**

Figs 2-10

Thallus rosy-red, spreading and erect to 3 cm tall (Figs 2, 3), axes terete or slightly compressed, axes attached to other branches by 2° rhizoidal fascicles issued from cortical cells on the contact surface (Figs 4, 7), at times forming mats interconnected with other algae; axes divided into segments by single-layered septa; mature segments shallowly pinched at the septa or not at all in distal segments, at times more so in some lower mature axes; segments 250-850 µm diam. and 250-700 µm in length, decreasing distally; branches alternate to secund and irregular,

formed at or in between septa, at times with more than one branch arising from the same segment (Figs 4-7); septal cells lightly pigmented, forming a complete layer of irregularly polygonal cells, 25-50 μm diam. and 30-85 long; cortex 2-layered, the larger (inner) cells forming a complete layer of ellipsoidal to irregularly polygonal and ovoidal cells, 15-50 diam. and 24-175 μm long in mature segments, the long cells paralleling plant axes, cutting off an incomplete layer of smaller subspherical, reniform, pyriform to ovoid, cortical cells outwardly, 5-16 μm in longest dimension; inner cortical cells of distal segments issuing longitudinal, descending filaments 7-12 μm diam. that produce obovoid to spherical gland cells, 9-17 μm diam. in the interior cavity (Fig. 8); sporangia scattered under the cortex of internodal segments, tetrahedrally divided, obovoid to spherical, larger forms 80-110 μm diam. (Fig. 9); cystocarps urceolate and ostiolate at maturity, 520-600 diam., 640-760 μm tall, occasionally clustered (Fig. 10), carposporangia obpyriform to 100 μm long; spermatangia unknown.

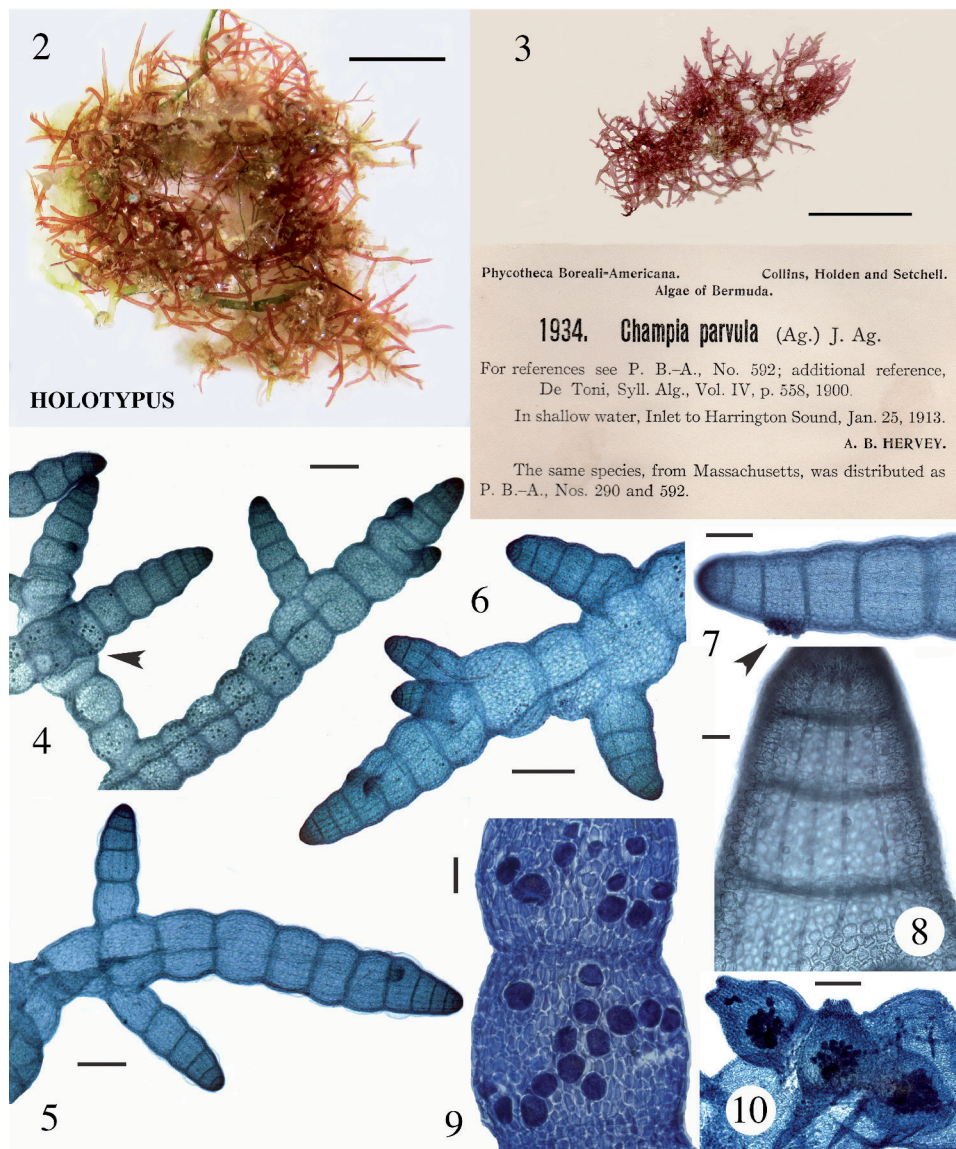
Holotype (designated here): *Thea R. Popolizio (TRP)/Craig W. Schneider (CWS)* 12-32-8 (BDA0844), \oplus , 13 Feb. 2012, Walsingham Bay, Castle Harbour, Bermuda I., 32°19'25.0"N, 64°44'11.5"W, depth 0-1 m, Bermuda, western Atlantic Ocean [MICH] (Fig. 2); *isotype*, (BDA0845) Herb. CWS.

Selected collections: BERMUDA—*A.B. Hervey, P.B.-A.* no. 1934 (Collins *et al.*, 1913, as *C. parvula*), \oplus , 25 Jan. 1913, inlet to Harrington Sound, Flatts, Bermuda I. (Fig. 3); *CWS/Christopher E. Lane (CEL)* 01-16-10 (GWS001257), 14 Nov. 2001, John Smith's Bay, Bermuda I., 32°19.0'N, 64°42.8'W, from 7-9 m; *CWS/CEL* 10-5-24 (BDA0033), 19 Aug. 2010, off Frick's Beach, Tucker's Town, Bermuda I., 32°19'56.0"N, 64°40'20.7"W, from 10-12 m; *TRP/CWS* 12-29-10 (BDA0797), \oplus , 2 Feb. 2012, Gibbet I., mouth of Flatts Inlet, 32°19'20.1"N, 64°44'35.7"W, from 1-2 m; *TRP/CWS* 12-35-9 (BDA0878), 17 Feb. 2012, south of White Flats, north shore Bermuda I., 32°20'01.1"N, 64°44'23.3"W, from 0-4 m; *TRP/CWS* 12-83-5 (BDA1260), 24 Jul. 2012, Natural Arches, south shore Bermuda I., 32°19'48.0"N, 64°41'06.8"W, from 7 m; *TRP/CWS* 12-149-14 (BDA1643), cystocarpic, 6 Nov. 2012, North Rock, north shore Bermuda I., 32°28'26.2"N, 64°46'28.5"W, from 9 m; *TRP/CWS* 12-174-6 (BDA1836), \oplus , 12 Dec. 2012, Spanish Point Park, Bermuda I., 32°18'26.4"N, 64°48'56.6"W, from 0-2 m.

Distribution: Presently known only from Bermuda, western Atlantic.

Etymology: Named *insularis* (L., f.) for the new species presence throughout the islands of Bermuda.

Remarks: Our COI-5P barcoding results showed that *Champia insularis* formed a single genetic group (BDA0033, BDA0797, BDA0798, BDA0844, BDA0845, BDA0878, BDA1260, BDA1643, BDA1836: $n = 9$) divergent at only 0-1 bp or 0.15%. Previously, this new species from Bermuda was identified as the European *C. parvula* (Collins *et al.*, 1913; Collins & Hervey, 1917; Howe, 1918; Schneider, 2003), yet using molecular sequencing *C. parvula* has not been shown to occur in the western Atlantic (Griffith *et al.*, 2017). Collins & Hervey (1917) mentioned that their specimens were epiphytic, mostly presenting tetraspores, and were seldom over 3 cm tall. Their 1913 specimens were distributed in the *P.B.-A. exsiccata* as no. 1934 (Collins *et al.*, 1913, as *C. parvula*; Fig. 3), and we were able to link these collections to recently sequenced specimens by tetrasporangial size. Of note, Howe (1918) reported 10 cm tall specimens of *C. parvula* in his treatment of the Bermuda flora, but no island specimens available to him (NY), or elsewhere that we can discover, approach that size [dried specimens and labels digitized on the (US) National Science Foundation supported online *Macroalgal Herbarium Portal* (<http://macroalgae.org/portal/index.php>), including hundreds of collections made by Collins, Hervey and Howe among others from the early 1900s]. As he seems to have done for other species, Howe (1918) must have used the broad concept of the species



Figs 2-10. *Champia insularis* C.W. Schneider & G.W. Saunders, *sp. nov.* 2. Holotype as a living mat prior to dry-pressing [TRP/CWS 12-32-8]. 3. *P.B.-A.* no. 1934, as *C. parvula*, from Harrington Sound, Bermuda (Collins *et al.*, 1913). 4-6. Branching habits with an anastomosed pair (arrowhead) [TRP/CWS 12-174-6]. 7. Apex of a branch with descending rhizoidal holdfast (arrowhead) [TRP/CWS 12-174-6]. 8. Apex of branch showing descending medullary filaments and associated gland cells [TRP/CWS 12-174-6]. 9. Axial segments of holotype with tetrasporangia [TRP/CWS 12-32-8]. 10. Clustered cystocarps [TRP/CWS 12-149-14]. Scale bars: 2 = 1 cm, 3 = 2 cm, 4-6 = 500 μ m, 7 = 200 μ m, 8 = 50 μ m, 9 = 100 μ m, 10 = 300 μ m.

Table 2. Morphological data for selected *Champia* species discussed in the paper from protologues and reports of specimens near the type locality only

	<i>Champia compressa</i>	<i>Champia parvula</i>	<i>Champia insularis</i> <i>sp. nov.</i>	<i>Champia hasselbringii</i> <i>sp. nov.</i>	<i>Champia puertoricensis</i>	<i>Champia taironensis</i>	<i>Champia vieillardii</i>
Plant height	procumbent with erect, 2-5 (-15) cm	to 10 cm	prostrate and spreading, uprights to 3 cm tall	prostrate	1.0-4.5 cm	to 6 cm	semi-prostrate to 4.5 cm tall
Axis morphology	strongly compressed, subterete below	terete or slightly compressed	terete to slightly compressed	strongly compressed above, subterete below	terete	markedly compressed	flattened
Axis diam.	2-3 mm	1-2 mm	250-850 µm	440 µm to 1.1 mm	1.0-1.3 mm	1.0-2.5 mm	2-4 mm
Segment height	shorter than broad, less than 0.25 times diam. in older portions	shorter than broad in younger portions; 1-2 times diam. in older portions	mostly shorter than broad throughout; 0.75-1. times diam. in mature portions	shorter than broad distally; as long as broad in mature portions	height equals diam.	shorter than broad in all portions, more pronounced in younger portions; 0.33 times diam. in older portions	0.3-0.5 times diam.
Branching pattern	alternate to subopposite and opposite, pinnate and bipinnate; branches tapering to bases	variable, usually alternate	alternate and opposite	alternate and opposite	opposite	opposite, some alternate	alternate, occasionally bipinnate and tripinnate
Branch origin		at or slightly above septa	at septa or between nodes	at septa or slightly above	intermodal	at and above septa	intermodal

	<i>Champia compressa</i>	<i>Champia parvula</i>	<i>Champia insularis</i> <i>sp. nov.</i>	<i>Champia</i> <i>hasselbringii</i> <i>sp.</i> <i>nov.</i>	<i>Champia</i> <i>puertoricensis</i>	<i>Champia</i> <i>taironensis</i>	<i>Champia</i> <i>vieillardii</i>
Branch anastomosing	present	present	present	absent	not reported	not reported	present
Septum constriction	slight but obvious	slight	slight, occasionally marked	marked	slight	marked	none
Septal cell dimensions (surface)	not reported	10-15 µm diam., 500-700 µm long	25-50 µm in diam., 30-85 µm long	39-72 µm long, 69-81 µm long	not reported	35-57 µm in diam., 41-67 µm long	not reported
Cortical cells (surface)	complete layer of larger ovoid to irregular cells, 32-50 µm × 45-55 µm, with an incomplete layer of smaller cells at interstices, 12-20 µm diam.	complete layer of larger axially elongated cells, covered by an incomplete layer of smaller cells, 10-30 µm	complete layer of ellipsoidal and irregularly polygonal to ovoid cells, 15-50 × 24-175 µm, cutting off an incomplete layer of smaller subspherical, reniform, pyriform to ovoid cells, 5-16 µm in diam.	complete layer of irregularly polygonal to ovoid cells, 30-55 µm diam., 42-125 µm long, cutting off an incomplete layer of smaller subspherical to ovoid cells, 5-15 µm in diam.	complete layer of irregularly shaped cells, 20-25 µm diam, 45-75 µm long, cutting off an incomplete layer of smaller subspherical and reniform to ovoid cells	complete layer of larger irregularly polygonal to ovoid cells, 25-75 µm long, 27-76 × 17-47 µm, covered by an incomplete layer of smaller subspherical to ovoid cells, 6-11 µm	complete layer of irregularly polygonal to ovoid cells, 25-75 µm long, cutting off an incomplete layer of smaller subspherical to ovoid cells, 15-30 µm long
Medullary filament diam.	ca. 10 µm	10-15 µm	7-12 µm	4.0-10.5 µm	not reported	13 µm	15-20 µm
Gland cell diam.	ca. 15 µm	15 µm	9-17 µm	8.5-11.0 µm	10.0-12.5 µm	20-26 µm	10-12 µm
Spermatangia diam.		2-3 µm	unknown	unknown	2.5 µm	unknown	not reported

Table 2. Morphological data for selected *Champia* species discussed in the paper from protologues and reports of specimens near the type locality only (*continued*)

	<i>Champia compressa</i>	<i>Champia parvula</i>	<i>Champia insularis</i> <i>sp. nov.</i>	<i>Champia hasselbringii</i> <i>sp. nov.</i>	<i>Champia puertoricensis</i>	<i>Champia taironensis</i>	<i>Champia vieillardii</i>
Cystocarp shape	conical to urceolate	subspherical and ostiolate	urceolate, ostiolate	broadly ovoid, ostiolate	urceolate	broadly ovoid to urceolate and ostiolate	urceolate
Cystocarp dimensions	2 mm long × 1.6 mm diam.	to 1 mm diam.	520-600 µm in diam., 640-760 µm long	740 µm diam., 620 µm long	490-670 µm diam., 500-700 µm long	579-730 µm diam., 660-792 µm long	to 600 µm diam.
Carposporangia shape	conical to obpyriform	conical	obpyriform	obpyriform	irregular	pyriform	not reported
Carposporangia length		50-120 µm	to 100 µm	to 93 µm long	55 µm long	80-115 µm	not reported
Tetrasporangial shape	spherical	spherical	obovoid, appearing spherical in surface view	obovoid, appearing spherical in surface view	spherical	not reported	spherical
Tetrasporangial diam.	to 100 µm	55-120 µm	80-110 µm	55-75 µm	40-60 µm	77-83 µm	80-100 µm
Type locality	Cape Province, South Africa	Cádiz, Spain	Bermuda	Bermuda	Puerto Rico	Parque Natural Nacional Tairona, Caribbean Colombia	New Caledonia
References	Harvey, 1849; Millar, 1990; Stegenga <i>et al.</i> , 1997; De Clerck <i>et al.</i> , 2005	Irvine & Guiry, 1983	Present study	Present study	Lozada-Troche & Ballantine, 2010	Bula-Meyer, 1997	Kutzing, 1866; Dawson, 1954; Lawson & John, 1987; Millar, 1990; Masuda <i>et al.</i> , 2001

in the early part of the 20th century from throughout its range, and did not use measurements of specimens strictly from Bermuda. Southern New England specimens, to which Collins *et al.* (1913) linked their specimens with those from Bermuda, and which Howe would have been locally familiar with in New York, were known at the time as *C. parvula*. These specimens were recently described as *C. farlowii* (Griffith *et al.*, 2017), and our analyses find that the Bermuda isolates are, along with *C. parvula*, not genetically matched to this cold-water species (Fig. 1). *Champia insularis* is much smaller (to 3 cm tall) than *C. farlowii* which has upright axes to 8 cm, and its axes are strikingly more delicate (to 850 μm vs. 1-2 mm in diam.; Griffith *et al.*, 2017). The New England species is represented by erect, widely branched individuals, while *C. insularis* when fully developed is matted and spreading, the axes often anastomosing with each other on contact.

With the addition of *Champia insularis* along with *C. farlowii* and *C. puertoricensis* Lozada-Troche & D.L. Ballantine, the *C. parvula*-complex presently includes three species in the western Atlantic Ocean and seven in seas worldwide (Griffith *et al.*, 2017). Although there are some anatomical similarities (Table 2), *C. insularis* is much smaller and more delicately branched than *C. puertoricensis*, a species with opposite branching initiated in between septa (Lozada-Troche & Ballantine, 2010).

***Champia hasselbringii* C.W. Schneider & G.W. Saunders, sp. nov. Figs 11-18**

Thallus rosy-red (Fig. 12), prostrate and spreading, attached to macroalgae and hard substrata by a fibrous holdfast and 2° rhizoidal fascicles issued from cortical cells on the contact surface (Fig. 16); axes terete below, compressed distally, divided into segments by single-layered septa; mature segments distinctly barrel-shaped and markedly pinched at the septa (Figs 11-13), 440-1100 μm diam., shorter than broad distally, about as long as broad in lowermost segments, increasing in breadth towards the apex of axes but abruptly decreasing in length and width in the final few distal segments (Figs 13, 17); branches alternate and opposite, formed at or just above septa (Fig. 11); septal cells colorless, polygonal, reticulate, 39-72 μm broad and 69-81 μm long; cortex 2-layered, the larger (inner) cells forming a complete layer of rounded ellipsoidal to polygonal cells, 30-55 diam. and 42-125 μm long in mature segments, cutting off an incomplete layer of smaller subspherical to ovoid, cortical cells outwardly, 5-15 μm in longest dimension; hairs deciduous, when seen found distally, originating from small, outer cortical cells (Fig. 15); inner cortical cells of distal segments issuing longitudinal, descending filaments, 4.0-10.5 μm diam. that produce obovoid to spherical gland cells 8.5-11.0 μm diam. in the interior cavity (Figs 13, 14); sporangia scattered under the cortex of internodal segments, tetrahedrally divided, spherical, larger forms 55-70 μm diam. (Fig. 17); cystocarps broadly ovoid and ostiolate at maturity, 740 diam. and 620 μm tall (Fig. 18), carposporangia obpyriform to 93 μm long; spermatangia unknown.

Holotype (designated here): *Craig W. Schneider/Christopher E. Lane/Thea R. Popolizio* 12-8-15 (BDA0557), cystocarpic, 17 Jan. 2012, Brackish Pond Flats, off north shore of Bermuda I., 32°21'07.0"N, 64°48'02.5"W, depth 3-4 m, Bermuda, western Atlantic Ocean [MICH] (Fig. 11); isotype, Herb. CWS.

Selected collections: BERMUDA-CWS/*Richard B. Searles (RBS)* 85-23-14, ⊕, 19 June 1985, The Spit, northeast of Little Head, St. David's Is., 32° 22.4'N, 64° 38.5'W, depth 1-12 m; CWS 96-4-9a, ⊕, 2 Jul. 1996, Coot Pond, Achilles Bay, St. Georges I., 32°23.2'N, 64°40.66'W, from 1 m; CWS 96-5-11, ⊕, 3 July 1996, Bailey's Bay, Bermuda I., 32° 20.8'N, 64° 43.4'W, depth 3-4 m; CWS/CEL 05-19-18, ⊕, 22 Jul. 2005, John Smith's Bay, Canton Point, south shore, Bermuda I., 32°19.6'N, 64°46.6'W, from 10 m; CWS/CEL

09-3-7, ⊕, 15 Mar. 2009, Fairyland Creek, Bermuda I., 32°17'41.9"N, 64°48'05.2"W, from 1 m; *CWS/CEL* 10-6-11 (BDA0061), 19 Aug. 2010, Tucker's Town Bay chasm, Castle Harbour, Bermuda I., 32°21'01.8"N, 64°41'31.0"W, from 1 m; *CWS/CEL* 10-24-20 (BDA0384), 23 Aug. 2010, Cathedral Rock, off Castle Harbour and south shore of Bermuda I., 32°20'31.1"N, 64°39'24.2"W, from 15-17 m; *CWS* 10-29-6 [BDA0439], 24 Aug. 2010, offshore west of High Point, Bermuda I., Bermuda, 32°15'18.72"N, 65°02'11.76"W, from 35-36 m.

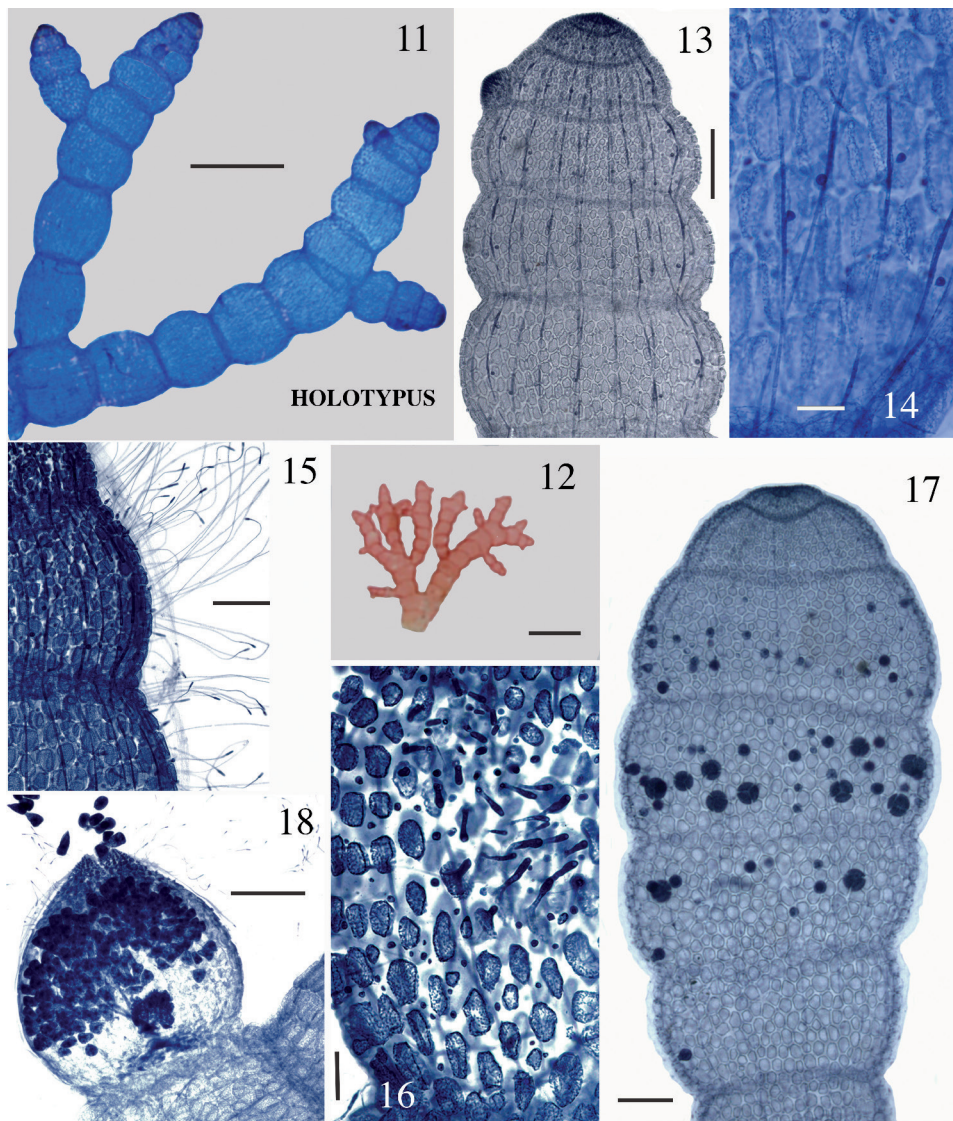
Distribution: Presently known only from Bermuda, western Atlantic.

Etymology: The epithet *hasselbringii* is an honorific named for Timothy L. Hasselbring (1974–2012), beloved conservationist and educator in Bermuda who assisted our offshore collecting as Captain of the *Endurance* for the Bermuda Zoological Society.

Remarks: This new epiphytic species (BDA0061, BDA0384, BDA0439, BDA0557: n = 4) only showed differences at 1-2 bp (0.3%) using COI-5P barcoding. Although we have made several collections of *Champia hasselbringii* on larger algae in Bermuda, it is not as common or obvious as *C. insularis*. Its flattened axes and short, swollen segments would have made it improbable that it would have been identified as *C. parvula* in the past in the islands. Rather, *C. hasselbringii* is more similar to three compressed-axis species reported from the Caribbean Sea, *C. compressa*, *C. taironensis* and *C. vieillardii*. In the western Atlantic, *Champia compressa* had been reported from Brazil and Colombia, but these plants are now attributed to either *C. vieillardii* or *C. taironensis* (Bula-Meyer, 1997; Wynne, 1998, p. 90). A recent collection from deep water off the Gulf Coast of Florida (Gavio & Fredericq, 2005) was assigned to *C. compressa* after following the distinctions between the two outlined by Millar (1990).

Unlike *Champia hasselbringii*, *C. taironensis* (type locality, Caribbean Colombia; Bula-Meyer, 1997) has compressed axes that arise from a discoid holdfast forming an erect habit to 6 cm tall, and has much broader axes (to 2.5 mm) than the new species. Despite these dramatic size differences, both *C. hasselbringii* and *C. taironensis* have very similar looking distal segments and branching patterns (Bula-Meyer, 1997). These distal segments also recall *C. compressa* (type locality, South Africa; Harvey, 1838, 1849; Stegenga *et al.*, 1997) and *C. vieillardii* (type locality, New Caledonia; Kützing, 1866), species that have been repeatedly reported in regional or island floras of the Caribbean Sea; in fact, the two species names have been used separately as well as interchangeably in many reports. When Taylor (1960) compiled his western Atlantic warm water flora, *C. compressa* was relegated as an “uncertain record.” Since then, this species has once again gained acceptance as a member of the warm western Atlantic flora (Wynne, 2017) after clarifications of the distinguishing features that separate it from *C. vieillardii* (Dawson, 1954; Millar, 1990; Masuda *et al.*, 2001).

Champia hasselbringii contains plants that are markedly compressed to flattened above, but that have some lower decumbent axes that are terete to only slightly compressed. Individuals attach to larger macroalgae by rhizoidal clusters issued from cortical cells of the prostrate axes (Fig. 16). Unlike the flattened *C. vieillardii* (Kützing, 1866; Dawson, 1954; Millar, 1990; Masuda *et al.*, 2001), *C. hasselbringii* has obvious constrictions at the septa or “nodes” (Figs 11, 13, 17). A character to distinguish *C. vieillardii* from *C. compressa* noted by Dawson (1954) for Vietnamese plants and corroborated by Masuda *et al.* (2001) for their specimens from Malaysia, was that the former produces some slender, subterete ultimate branches whereas *C. compressa* does not. *Champia hasselbringii* in Bermuda has lower axes that are terete and therefore narrower than the flattened axes above. From *C. insularis*, *C. hasselbringii* is vegetatively distinguished by its much smaller size,



Figs 11-18. *Champia hasselbringii* C.W. Schneider & G.W. Saunders, *sp. nov.* **11.** Portion of holotype specimen mounted on a glass slide [CWS/CEL/TRP 12-8-15]. **12.** Habit of specimen [CWS/CEL 10-6-11]. **13.** Apex of branch showing descending medullary filaments [CWS/CEL 05-19-18]. **14.** Detail of medullary filaments with associated gland cells [CWS/CEL/TRP 12-8-15]. **15.** Extended hairs produced by incomplete layer of small outer cortical cells [CWS/CEL/TRP 12-8-15]. **16.** Early development of 2° rhizoidal attachment being issued from cortical cells [CWS/CEL/TRP 12-8-15]. **17.** Axial segments near apex with tetrasporangia [CWS/CEL 05-19-18]. **18.** Cystocarp releasing carposporangia on holotype [CWS/CEL/TRP 12-8-15]. Scale bars: 11 = 1 mm, 12 = 2 mm, 13, 18 = 200 μm, 14, 16 = 50 μm, 15 = 100 μm, 17 = 150 μm.

more compressed axes and the obvious constrictions of segments at all of the nodes (Table 2). Importantly, however, both the size of its tetrasporangia and the shape of its cystocarps also distinguish it from *C. insularis* (Table 2).

The two specimens listed as *Champia parvula* var. *prostrata* L.G. Williams (type locality, North Carolina, USA) for Bermuda (Schneider & Searles, 1997) are representative of *C. hasselbringii*, effectively removing this taxon from the flora. Neither of the taxa identified as *C. parvula* and *C. parvula* var. *prostrata* from the southeastern United States has been genetically studied at this time.

CONCLUSIONS

Champia insularis and *C. hasselbringii* are added to the macroalgal flora of Bermuda, the former now representing the early reports of *C. parvula* in the flora. *Champia insularis* is the newest species in the *C. parvula*-complex (Griffith *et al.*, 2017) bringing the total to seven now separated out of the pantropical to cold temperate distribution of *C. parvula sensu lato* after molecular treatments in portions of the attributed range. The two new species are pseudocryptic but can be distinguished by non-overlapping reproductive features (Table 2).

Our molecular analysis also shows a broad range of unnamed genetic species, as well as a number of genetic groups assigned to known morphospecies. For example, the *C. parvula*-complex, which had members variously associated with the two new species described herein, had four genetic groups including Australian, Caribbean, and Floridian specimens (Fig. 1, Table 1). A comprehensive systematic treatment is needed to sort out species beyond the scope of this report and much work remains for species assigned to the genus *Champia*.

Acknowledgements. This work was funded by NSF DEB grants 1120688 and 1120652 to CWS and CEL respectively, and by the Charles A. Dana Foundation to CWS. This research is based in part upon work conducted using the Rhode Island Genomics and Sequencing Center supported by the National Science Foundation (MRI Grant No. DBI-0215393 and EPSCoR Grant No. 0554548), the US Department of Agriculture (Grant Nos. 2002-34438-12688, 2003-34438-13111 and 2008-34438-19246), and URI. The work at UNB was supported by Discovery and Accelerator grants to GWS from the Natural Sciences and Engineering Research Council of Canada, as well as funding from the Canada Foundation for Innovation and the New Brunswick Innovation Foundation. We would like to thank Dr. Thea Popolizio for her help in collecting many of the specimens. This is contribution no. 265 to the Bermuda Biodiversity Project (BBP) of the Bermuda Aquarium, Natural History Museum and Zoo (BAMZ), Department of Environment and Natural Resources.

REFERENCES

- BULA-MEYER G., 1997 — Las especies de *Champia* (Rhodophyta: Champiaceae) de talo aplanado y una nueva del Caribe Colombiano. *Caldasia* 19: 83-90.
- BRUMMITT R.K. & POWELL C.E., 1992 — *Authors of plant names*. Kew, Royal Botanic Gardens. [iv] + 732 p.
- CIANCIOLA E.N., POPOLIZIO T.R., SCHNEIDER C.W. & LANE C.E., 2010 — Using molecular-assisted alpha taxonomy to better understand red algal biodiversity in Bermuda. *Diversity* 2010: 946-958. < <http://www.mdpi.com/1424-2818/2/6/946/>>
- 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.

- COLLINS F.S. & HERVEY A.B., 1917 — The algae of Bermuda. *Proceedings of the American academy of arts & sciences* 53: 1-195.
- DAWSON E.Y., 1954 — Marine plants in the vicinity of the Institut Océanographique de Nha Trang, Viêt Nam. *Pacific science* 8: 372-469.
- DE CLERCK O., BOLTON J.J., ANDERSON R.J. & COPPEJANS E., 2005 — *Guide to the seaweeds of KwaZulu-Natal*. Scripta Botanica Belgica Vol. 33. 296 p.
- 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.
- GRIFFITH M.K., SCHNEIDER C.W., WOLF D.I., SAUNDERS G.W. & LANE C.E., 2017 — Genetic barcoding resolves the historically known red alga *Champia parvula* from southern New England, USA, as *C. farlowii* sp. nov. (Champiaceae, Rhodymeniales). *Phytotaxa* 302 (1): 77-89.
- HARVEY W.H., 1838 — *The genera of South African plants, arranged according to the natural system*. Cape Town, A.S. Robertson. lxxvi + 429 p.
- HARVEY W.H., 1849 — *Nereis australis, or algae of the southern ocean...* (Part 2). London, Reeve Brothers, pp. 65-124, pls XXVI-L.
- HOWE M.A., 1918 — Algae. In: Britton N.L. (ed.), *Flora of Bermuda*, New York, Charles Scribner's Sons, pp. 489-540.
- IRVINE L.M. & GUIRY M.D., 1983 — Rhodymeniales. In: Irvine, L.M. *Seaweeds of the British Isles. Vol. 1. Rhodophyta, Part 2A. Cryptonemiales (sensu stricto), Palmariales, Rhodymeniales*. London, British Museum (Natural History), pp. 77-98.
- KEARSE M., MOIR R., WILSON A., STONES-HAVAS S., CHEUNG M., STURROCK S., BUXTON S., COOPER A., MARKOWITZ S., DURAN C., THIERER T., ASHTON B., MEINTJES P. & DRUMMOND A., 2012 — Geneious Basic: an integrated and extendable desktop soft-ware platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647-1649. doi:10.1093/bioinformatics/bts199. PMID:22543367.
- KÜTZING F.T., 1866 — *Tabulae phycologicae...* Vol. 16. Nordhausen, [iii +] 35 pp., 100 pls.
- LAWSON G.W. & JOHN D.M., 1987 — The marine algae and coastal environment of tropical West Africa (second edition). *Nova hedwigia, beiheft* 93: vi + 1-415.
- LOZADA-TROCHE C. & BALLANTINE D.L., 2010 — *Champia puertoricensis* sp. nov. (Rhodophyta: Champiaceae) from Puerto Rico, Caribbean Sea. *Botanica marina* 53: 131-141.
- MASUDA M., KOGAME K., KAWAGUCHI S. & PHANG S.M., 2001 — Taxonomic notes on marine algae from Malaysia. V. Five species of Rhodymeniales. *Botanica marina* 44: 81-88.
- MILLAR A.J.K., 1990 — Marine red algae of the Coff's Harbour region, northern New South Wales. *Australian systematic botany* 3: 293-593.
- SAUNDERS G.W. & MCDEVIT D.C., 2012 — Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. *Methods in molecular biology* 858: 207-222.
- SAUNDERS G.W. & MOORE T.E., 2013 — Refinements for the amplification and sequencing of red algal DNA barcode and RedToL phylogenetic markers: a summary of current primers, profiles and strategies. *Algae* 28: 31-43.
- SCHNEIDER C.W., 2003 — An annotated checklist and bibliography of the marine macroalgae of the Bermuda islands. *Nova hedwigia* 76: 275-361.
- 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., 1997 — Notes on the marine algae of the Bermudas. 2. Some Rhodophyta, including *Polysiphonia tongatensis* and a discussion of the *Herposiphonia secunda/tenella* complex. *Cryptogamie, algologie* 18: 187-210.
- STAMATAKIS A., 2014 — RAxML version 8: a tool for phylogenetic analyses and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312-1313. doi.org/10.1093/bioinformatics/btu033.
- STEGENGA H., BOLTON J.J. & ANDERSON R.J., 1997 — *Seaweeds of the South African west coast*. Cape Town, Bolus Herbarium, University of Cape Town. [ii] + 655 p.
- TAYLOR W.R., 1960 — *Marine algae of the eastern tropical and subtropical coasts of the Americas*. Ann Arbor, University of Michigan Press. xi + 879 p.
- TAYLOR W.R. & BERNATOWICZ A.J., 1969 — Distribution of marine algae about Bermuda. *Bermuda Biological Station for Research special publication* 1: 1-42.
- WYNNE M.J., 1998 — A checklist of benthic marine algae of the tropical and subtropical western Atlantic: first revision. *Nova hedwigia, beiheft* 116: iii + 1-155.
- WYNNE M.J., 2017 — A checklist of benthic marine algae of the tropical and subtropical western Atlantic: fourth revision. *Nova hedwigia, beiheft* 145: 1-202.