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A revision of the genus *Cryptonemia* (Halymeniaceae, Rhodophyta) in Bermuda, western Atlantic Ocean, including five new species and *C. bermudensis* (Collins *et* M. Howe) *comb. nov.*

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Left Header: C. SCHNEIDER ET AL.

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ABSTRACT

Cryptonemia specimens collected in Bermuda over the past two decades were analyzed using gene sequences encoding the large subunit of the nuclear ribosomal DNA and the large subunit of RuBisCO as genetic markers to elucidate their phylogenetic positions. They were additionally subjected to morphological assessment and compared with historical collections from the islands. Six species are presently found in the flora including *C. bermudensis* comb. nov., based on *Halymenia bermudensis*, and the following five new species: *C. abyssalis*, *C. antricola*, *C. atrocotalis*, *C. lacunicola* and *C. perparva*. Of the eight species known in the western Atlantic flora prior to this study, none is found in Bermuda. Specimens reported in the islands in the 1900s attributed to *C. crenulata* and *C. luxurians* are representative of the new species, *C. antricola* and *C. atrocotalis*, respectively.

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KEYWORDS Bermuda; *Cryptonemia*; *C. abyssalis* sp. nov.; *C. antricola* sp. nov.; *C. atrocotalis* sp. nov.; *C. bermudensis* comb. nov.; *C. lacunicola* sp. nov.; *C. perparva* sp. nov.; Halymeniaceae; LSU rDNA; *rbcL*; Rhodophyta; UPA

Introduction

At the time W.R. Taylor (1960) published his flora of the western Atlantic tropical and subtropical waters, two species in the red algal genus *Cryptonemia* (Halymeniaceae) had been reported for Bermuda, and only three for the entire region. The earliest record of *Cryptonemia* in Bermuda was Rein's (1873) report of *C. crenulata* (J. Agardh) J. Agardh, a species later listed in the flora by Collins & Hervey (1917) and Howe (1918). The other species reported in these early 20th century floras was *C. luxurians* (C. Agardh) J. Agardh, which had been discovered earlier in Bermuda and misidentified as the delesseriaceous *Botryoglossum platycarpum* (Turner) Kützing by Kemp (1857). Over the past two decades, we have made numerous collections of *Cryptonemia* in Bermuda, which form the basis of the present report. At present, there are 45

Cryptonemia species listed as “current” in AlgaeBase (Guiry & Guiry, 2017), only eight of which are found in the western Atlantic (Wynne, 2017). In the last 15 years, little molecular work has been done on the genus, consisting of a few sequences from around the world (Gavio & Fredericq, 2002; Kim *et al.*, 2012; Calderon *et al.*, 2014; D’Archino *et al.*, 2014; Yang & Kim, 2014). Using molecular barcoding and phylogenetics, we have identified six species in the genus for Bermuda, including five new to science, and have investigated the early records of *Cryptonemia* from the last century.

Materials and methods

Sample collection and processing

Deepwater collections were made on the 2016 cruise of the R/V *Baseline Explorer* by technical divers, and the other samples were collected by SCUBA diving or snorkeling. Voucher specimens are deposited in KIRI, MICH, NY, the Bermuda Natural History Museum, UNB, US and CWS’s personal herbarium. Herbarium abbreviations follow the online *Index Herbariorum* <<http://sweetgum.nybg.org/ih/>> and standard author initials of taxa follow Brummitt & Powell (1992). Site locations for all collections were taken using a Garmin™ eTrex H GPS (Olathe, Kansas, USA).

Specimens were pressed fresh on herbarium paper and many were photographed live. Fragments or entire individuals were dried in silica gel for DNA extraction and, when possible, additional fragments were preserved in 4-5% Formalin in filtered sea water for anatomical study. Thin sections were made with an American Optical freezing microtome model 880 (San Diego, California, USA). The sections were mounted in 30% corn syrup with acidified 1% aniline blue in a ratio of 20:1 with a few drops of Formalin as a medium preservative. Herbarium specimens were scanned on an HP 309a Photosmart Premium scanner (Hewlett-Packard Company, Palo Alto, California, USA), and photomicrographs were taken using Zeiss Axioskop 40 microscope

(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). Unless otherwise mentioned, the *Phycotheca Boreali-Americana* (*P.B.-A.*) exsiccata referred to represents the set belonging to the first author. Data for some species were collected from archived specimens and labels digitized on the online *Macroalgal Herbarium Portal* (<http://macroalgae.org/portal/index.php>).

Molecular methods

Specimens used in molecular analyses are recorded in Table 1. After collection, specimens were silica-dried and total DNA was extracted following Saunders & McDevit (2012). For the assignment of specimens to genetic groups, data for the *rbcL*-3P (3' end of the plastid ribulose-1,5-bisphosphate carboxylase large subunit) and in some cases the UPA [universal plastid amplicon: Sherwood & Presting (2007), as a secondary marker] were amplified as outlined in Saunders & Moore (2013). These data were aligned in Geneious v.10.1.3 (<http://www.geneious.com>; Kearse *et al.*, 2012) with the former subjected to barcode gap analyses by calculating the uncorrected distances between specimens [n =33 (including 3P region for specimens for which the longer *rbcL* fragment was determined; Table 1); 800 bp (Milton *et al.* 2013)]. For phylogenetic analyses, partial LSU (large subunit of the nuclear ribosomal cistron; owing to a conflict for LSU and *rbcL* for one specimen, additional LSU data were generated for other specimens of the *C. bermudensis*/*C. lacunicola* complex; Table 1) and a longer region of the *rbcL* was amplified for each of the genetic groups identified previously (Saunders & Moore, 2013). Sequence data were generated with the Big Dye Terminator Cycle Sequencing Ready Reactions BDA sequencing kit (Applied Biosystems, Foster City, California, USA) and bidirectional reads assembled (excluding the 5' and 3' primer regions) using Geneious

for all markers with additional data sourced from GenBank (Table 1). Individual LSU (n = 20; 2796 bp) and *rbcL* (n = 38; 1269 bp) alignments, as well as a concatenated (n = 13; 4064 bp) alignment, were generated for phylogenetic analyses.

For phylogenetic analyses, the best models for the individual gene regions LSU rDNA, and *rbcL* were first estimated (AIC) in Modeltest (v 3.06; Posada & Crandall, 1998) as implemented in PAUP* (Swofford, 2003) through Geneious. Bayesian Inference analysis was performed using MrBayes v3.2.2 (Ronquist *et al.* 2012). Two Metropolis-coupled Markov chain Monte Carlo (MCMCMC) runs consisting of one cold chain and three hot chains were performed with each run being sampled every 100 generations for 1,010,000 generations. After confirming that the average standard deviation of split frequencies between runs was below 0.01, the trees were merged. The resulting tree and posterior probabilities were calculated from the 20,202 trees generated. Maximum likelihood phylogenetic analysis was conducted under the GTR+I+G model using the raxmlGUI (Silvestro and Michalak 2011). Node support was calculated using 1000 replicates of bootstrap resampling.

Results

Our *rbcL*-3P gap analyses have revealed seven genetic groups for *Cryptonemia* in Bermuda (Table 2). These are assigned in the taxonomic section below to *C. abyssalis*, *C. antricola*, *C. atrocotalis*, *C. bermudensis*, *C. lacunicola*, *C. perparva* and *C. sp. 1_Bda*. There was almost no within-group genetic variation, with only *C. antricola* having a single variable site (Table 2). In contrast, between-group genetic variation was typically high with the exception of *C. bermudensis* and *C. lacunicola* with only four fixed differences (Table 2), but which nonetheless displayed a clear barcode gap (Table 2). Data generated for UPA added specimens to some of these previous groups, and were consistent with *rbcL*-3P when data were available for both markers, but no additional genetic species were uncovered.

Full *rbcL* (Fig. 1A) and LSU (Fig. 1B) analyses were largely congruent in their relative placement of novel *Cryptonemia* species. The nuclear LSU and plastid *rbcL* analyses for the initial specimens surveyed from the *Cryptonemia bermudensis*/*C. lacunicola* complex (CL020801; Fig. 2) were non-concordant. Consequently, LSU was sequenced for additional specimens for comparison with the plastid data (Fig. 1B; Table 1). Ultimately, only the initial specimen for which both of these markers was generated showed signs of introgression or incomplete lineage sorting, although the lack of LSU data for five additional collections of putative *C. lacunicola* leaves leaves uncertainty as to the true identity for these specimens (BDA0265, BDA0541, BDA0936, BDA1369, GWS011906; Table 1). Using samples that did not show signs of introgression, a concatenated tree of LSU and *rbcL* data improved support values within *Cryptonemia*, although the placement of *C. abyssalis* and *C. sp.1_Bda* within the genus was not resolved with certainty.

As discussed below, one of the *Cryptonemia* genetic groups is represented by a well-known species previously assigned to *Halymenia* with a type locality in the islands. Five other taxa are newly discovered species and some of these were formerly misassigned to other species of *Cryptonemia*. These are herein described as new species. One genetic group, *C. sp.1_Bda*, had insufficient material to be described as a new species at this time.

***Cryptonemia abyssalis* C.W. Schneider et Popolizio, sp. nov. (Figs 4-7)**

DESCRIPTION: Plants rosy-red and erect to 11 cm wide and 6 cm tall, composed of a successive series of reniform blades marginally producing multiple orders of blades in an opuntoid pattern (Fig. 4), arising from small discoidal holdfasts on short stipes (4 mm), adhering to paper when dried; oldest blades (pad-like) to 2 cm broad and 1.5 cm tall, inner portions of them 90-100 μm thick, slightly swollen at the margins (Fig. 5), the margins producing triangular pegs many of

which eventually develop into stipe-like extensions bearing new proliferous pads, margins at the site of pegs swollen to 200-220 μm thick (Fig. 6), the ultimate (developing) order of blades smaller than those produced earlier; medulla comprised of a compact cavity with dense intertwining, elongate filaments, 2.4-5.0 μm diam. interconnected with slightly enlarged ganglial cells; medulla giving rise to a 3-layered cortex on both surfaces; cortical cells subglobose to elongate, innermost layer of cells 4.5-7.5 μm diam., outer cortical cells 3-6 μm diam., irregularly rounded in surface view; monoecious, auxiliary cells found on plants with spermatangia; spermatangia globose to irregularly ovate and elongate, 2.5-4.5 μm in longest dimension, formed in irregular patches (Fig. 7) on both surfaces of blades; carpogonial branches, cystocarps and tetrasporangia not seen.

ETYMOLOGY: From a Latinization of the Gr. noun translated as *abyss* meaning “deep”, for the very deep subtidal habitat where it was collected offshore of Bermuda.

HOLOTYPE (designated here): *Craig W. Schneider (CWS)/Thea R. Popolizio (TRP)* 16-13-5 [BDA 1998], 31 Jul. 2016, ledge north northeast of St. George’s I., Bermuda, western Atlantic Ocean, 32°28’58.05”N, 64°35’04.31”W, depth 90 m [deposited in MICH; GenBank MF685028 (LSU), MF782465 (*rbcL*)] (Fig. 4); Isotype – Herb. CWS.

DISTRIBUTION: At present, endemic to the deep offshore reefs of Bermuda.

REMARKS: This new species truly defines the term “opuntoid” (Fig. 4) as it mimics a similar marginal production of cactus pads in the prickly-pear *Opuntia*, and its spectacular habit alone separates it from all of the 45 other currently accepted species in the genus *Cryptonemia*. Because the most appropriate epithet was already chosen for a species from the Gulf of

California, *C. opuntioides* E.Y. Dawson (1966), we selected the epithet for our new species from its habitat at great depth offshore of Bermuda. *C. opuntioides* is endemic to the northern Gulf of Mexico and “poorly known” (Norris, 2014). The type specimen is a small, eroded blade with two larger blades produced from its “eroded or grazed margins” (Dawson, 1966, fig. 6F; Norris, 2014, fig. 204C), which have no further orders of branching nor even the marginal pegs developed in *C. abyssalis*.

Secondary and higher order blades are commonly produced from branched stipes or from the margins of blades in *Cryptonemia* (Chiang, 1970). The generitype, *C. lomation* (Bertolini) J. Agardh, produces similar small reniform blades like those in *C. abyssalis* but these are formed from branched stalks, not the margins of blades (Bertolini, 1818, Pl. X, fig. 3, as *Fucus lomation* Bertolini). Another species with some morphological similarity is the eastern Atlantic/Mediterranean *C. tuniformis* (Bertolini) Zanardini which produces orders of blades on marginal stipe-like projections, but the blades are elongate and not nearly as regularly produced as the reniform blades of the new species (Kützing, 1868, fig. 94). Other species of *Cryptonemia* produce marginal blades, but none ever appear “opuntoid” as they are large strap-shaped blades with small bladelets (at times only in proximal or distal portions of the first-order blades), e.g. *C. atrocotalis* sp. nov. (see Schneider & Searles, 1991, fig. 323 as *C. luxurians*; account of the species below) and *C. heteronema* (M. Howe) Acleto & Zúñiga (2011, fig. 19). Other species in the genus produce small blades at the margins but most of these bladelets continue to develop into full-sized blades of 2nd or 3rd orders, e.g. *C. angustata* (Setchell & N.L. Gardner) E.Y. Dawson, *C. chiangii* Acleto, *C. hibernica* Guity & L.M. Irvine, *C. obovata* J. Agardh and *C. papenfussii* Y.-M. Chiang.

***Cryptonemia antricola* C.W. Schneider, C.E. Lane et G.W. Saunders, sp. nov.** (Figs 8-10)

DESCRIPTION: Plants dark rosy-red and erect to 2.5 cm tall, composed of narrowly ligulate, irregularly branched, cartilaginous blades (Fig. 8), arising from small discoidal holdfasts on short subterete stipes, often more than one upright from the same holdfast, not adhering well to paper when dried; blades to 2 mm wide and 57-90 μm thick, the margins entire (Fig. 9), proliferous blades re-growing terminally after being grazed by herbivores; medulla comprised of a compact cavity with few to dense intertwining, elongate filaments (Fig. 10), 2.5-3.5 μm diam. and occasional dark staining, swollen ganglial cells; medulla giving rise to a 2-(3-) layered cortex on both surfaces, subsurface cells subglobose to transversely elongate reaching 17 μm diam., outer cortical cells 3-10 μm diam., irregular to transversely elongate in transverse section, irregularly angular to rounded in surface view; gametangia and tetrasporangia unknown.

ETYMOLOGY: From the noun *antrum* (L., n.), grotto, for the subtidal cave where it persists at the type locality, and *-cola* (L. comp., indeclinable) for dweller or inhabitant, thus literally “grotto-dweller.”

HOLOTYPE (designated here): *CWS/Christopher E. Lane (CEL)* 10-14-33 [BDA0193], 21 Aug. 2010, rock floor of grotto entrance, Tobacco Bay, St. George’s I., Bermuda, western Atlantic Ocean, 32°23’20.1”N, 64°40’44.1”W, depth 2 m [deposited in MICH; GenBank MF685029 (LSU), MF782466 (*rbcL*)] (Fig. 8); Isotypes – UNB [BDA192], Herb. CWS.

SELECTED COLLECTIONS: Refer to Supplementary text file S1.

MISAPPLIED NAME FOR BERMUDA: *Cryptonemia crenulata*, *P.B.-A.* no. 2100 *sensu* Collins *et al.* (1916).

DISTRIBUTION: Thus far, known only from shaded near shore sites and offshore reefs in Bermuda, western Atlantic Ocean.

REMARKS: We first noticed a sizable matted population of this attractive species on the floor of a grotto opening in Tobacco Bay, where water surges with great energy. This type locality population of *Cryptonemia antricola* persists throughout the year (Supplementary text file, S1). The habitat that gives the new species its name, as living on the floors of grottos or caves, is where it was collected in Gravelly Bay by A.B. Hervey in 1913, the specimens later being distributed as part of *P.B.-A. exsiccata* identified as *C. crenulata* (Collins *et al.*, 1916; Collins & Hervey, 1917). These historical specimens are clearly not *C. crenulata*, being dramatically smaller in all dimensions and bearing smooth, non-crenulate margins. We have not seen specimens of what Rein (1873), followed by subsequent 19th century workers, earlier had called *C. crenulata* from Bermuda, but the Hervey *P.B.-A.* Gravelly Bay specimens clearly represent the morphology of *C. antricola* as described here. Collins & Hervey (1917) and Howe (1918) gave accounts of the same *P.B.-A.* no. 2100 describing the specimens as reduced, or smaller, forms than normally found for *C. crenulata*. Neither account mentioned marginal dentations (as typical of *C. crenulata*) for the Bermuda specimens, confirming the smooth margins of our own collections, and observations of theirs.

There are no species in the genus that compare to the overall habit of *Cryptonemia antricola*, therefore it represents a species with a unique branching pattern among congeners. It is larger in size than *C. perparva* (described below), but its blades are narrowly ligulate and much branched off long to short terete stipes and blade margins. Interestingly, the molecular data show that *C. antricola* is more closely related to sequences of *C. asiatica* M.Y. Yang & M.S. Kim and *C. luxurians* from Japan (Fig. 1A), rather than its congeners from Bermuda.

One narrow species described with a question as to its generic placement, *Cryptonemia subdichotoma* Womersley & A. Bailey (1970), is only known from the Solomon Islands and there have been no other accounts of it since the protologue (Guiry & Guiry, 2015). This species is regularly pseudodichotomous, to 12 cm tall and with axes 1.5-3.0 mm broad and 100-130 μm thick (Womersley & Bailey, 1970), so a larger and thicker-bladed species than *C. antricola*.

***Cryptonemia atrocotalis* C.W. Schneider, C.E. Lane et G.W. Saunders, sp. nov.** (Figs 11-15)

DESCRIPTION: Plants with new growth membranous and pinkish-red, older portions firm, leathery, and dark rosy-red, to 25 cm tall, attached by small to large discoid holdfasts, giving rise to one or more simple to branched erect axes (Figs 11, 12); axes broadly ligulate above brief to long stipes which extend into the attenuate blade bases as midribs reaching 1/2 the total distance of the expanded blades or beyond, often several blades issued from extended older, terete stipes (Fig. 12) and holdfasts; blades pseudodichotomously to alternately branched 0.6-2.5 cm wide, with few to many marginal obovate to subcircular bladelets, these at times concentrated on the truncate to rounded apices; margins undulate (Fig. 11) and entire to erose; blades 75-300 μm thick in vegetative portions, swollen at the margins (Fig. 13), medulla comprising much of the blade width, composed of densely arranged, narrow filaments 2-5 μm diam., surrounded by swollen ganglial cells with bodies 7-23 μm diam., giving rise to a few-layered cortex (Fig. 13), progressively smaller from medulla outwards, surface cells 3-5 μm diam., irregularly angular to rounded in surface view; midribs more greatly thickened on the ventral surface by anticlinal tiers of cortical cells (Fig. 14); tetrasporangia globose and obovoid to ellipsoid, cruciately divided, 7-10 μm diam., 9-15 μm long, loosely to densely formed in marginal bladelets near the apices, displacing cortical cells at maturity (Fig. 15); dioecious, spermatangia in patches on marginal bladelets, short ellipsoid, 1.5-3.0 μm diam.; carposporophytes immersed in marginal bladelets or

blade tips, globose, 100-150 μm diam., with basal placentas, involucre filaments, and small cortical ostioles; carposporangia subquadrate to irregularly angled, 7.5-10.0 μm diam.

ETYMOLOGY: From *atro-* (L., comp.) for dark, and *costalis* (L., f.) for midrib.

HOLOTYPE (designated here): *TRP/CWS* 12-131-3 (BDA1543), 2 Oct. 2012, off Great Head Park, St. David's I., Bermuda, western Atlantic Ocean, 32°22'06.9"N, 64°38'30.1"W, depth 18 m on rock [deposited in MICH; GenBank MF685031 (LSU), MF782469 (*rbcL*)] (Fig. 11).

SELECTED COLLECTIONS: Refer to Supplementary text file S1.

DISTRIBUTION: From Bermuda and North Carolina, as currently known, but probably more widespread.

REMARKS: Originally collected in Bermuda during the mid-19th century and misidentified as the delesseriaceous *Botryoglossum platycarpum* (Kemp 1857), Collins & Hervey (1917) and Howe (1918) later recognized that the specimens belonged in the Cryptonemiales (= Halymeniales) and identified them as *Cryptonemia luxurians* (C. Agardh) J. Agardh (type locality: Brazil). This taxonomy was followed by subsequent floristic workers until *C. luxurians* was synonymized with *C. seminervis* (C. Agardh) J. Agardh [type locality: Cádiz, Spain] by Price *et al.* (1986). Recently however, Yang & Kim (2014) firmly re-established the two as distinct species using *rbcL* sequences from the type localities of *C. luxurians* and *C. seminervis* that showed a 2.7% sequence divergence between them. During that study, Yang & Kim (2014) described *C. asiatica* for what had until that time been identified as *C. luxurians* in Japan. When costate Bermuda specimens were subjected to *rbcL* analysis (Fig. 1A), they showed a difference of 10 base pairs

(bp), or 0.8% divergence, from Brazilian sequences (GenBank AF488813) revealing that these two are likewise unique species. There are morphological similarities between the two western Atlantic species with distinct midribs, *C. atrocotalis* and *C. luxurians*, both being comprised of ligulate blades with undulate margins and with abundant marginal proliferations on mature blades (Taylor, 1960, pl. 58, fig. 3; Yoneshigue-Braga, 1972; Schneider & Searles, 1991, fig. 323), thus representing cryptic sibling species (Figs 1, 3). Tetrasporangia of both western Atlantic species are found in these marginal proliferations (Taylor, 1960; Schneider & Searles, 1991), while in *C. seminervis* they are scattered in the cortex of blades (Irvine, 1983). *Cryptonemia seminervis* has broadly expanded fan-like blades and short, basal midribs, features differing from the ligulate blades of *C. atrocotalis* and *C. luxurians*, with midribs generally reaching half their lengths or more (Fig. 11; Kützing, 1869; Schneider & Searles, 1991).

Specimens of *Cryptonemia atrocotalis* from North Carolina (Schneider & Searles, 1973, 1991) are considerably larger (to 2.5 cm wide and 25 cm tall) and more robust than the ones we have collected in Bermuda from 13-18 m over the last decade, perhaps due to the grazing pressure on Bermuda reefs, a phenomenon we have observed for several “reduced forms” of macroalgal species in the islands (Schneider & Lane, 2008; Schneider & Flook, 2017). Howe (1918) reported island specimens that were approximately 10 cm tall, a bit larger than those than we have collected, the largest blades being 8 cm tall. Challenger Bank specimens collected from 52-64 m in 1960 off Bermuda are to 2 cm broad and 16 cm long with, small marginal proliferations like those in North Carolina. Despite the fact that our recent Bermuda specimens are considerably smaller than those we have collected in North Carolina (to 2.5 cm wide and 25 cm tall; Schneider & Searles, 1991), *rbcL* sequences show a near perfect match for specimens from the two locations. Bermuda blade widths (to 1 cm wide) are more similar to those reported as *C. seminervis* from Florida, 0.5-1.5 cm (Dawes & Mathieson, 2008), but it remains to be determined whether they are correctly identified using molecular sequencing. Yoneshigue-Braga

(1972) described Brazilian specimens of *C. luxurians* to 7 cm in height with blades 115-125 μm thick. Blades of *C. atrocotalis* can reach nearly three times that height and more than twice the thickness. Furthermore, Yoneshigue-Braga (1972, fig. 5) noted midribs often reaching from the base to the apex of many blades, the remainder covering 2/3 of the length of blades, all of these exceeding the costae of *C. atrocotalis*. Due to the great similarity of these two western Atlantic species with midribs, it is best to use molecular data to identify specimens, especially those in the Caribbean Sea and Gulf of Mexico.

***Cryptonemia bermudensis* (Collins et M. Howe) C.W. Schneider, C.E. Lane et G.W.**

Saunders, comb. nov. (Figs 16, 17)

BASIONYM: *Halymenia bermudensis* Collins et M. Howe 1916, *Proc. Amer. Acad. Arts & Sci.*, vol. 53, p. 180.

TYPE LOCALITY: Castle Harbour, near Tucker's Town, Bermuda I., Bermuda, western Atlantic Ocean (**Holotype**: *Halymenia bermudensis*, leg. F.S. Collins no. 7074, 25 Apr. 1912 [NY]; isotypes distributed as *Phycotheca Boreali-Americana* [1915], Fasc. XLI, no. 2050).

SELECTED COLLECTIONS: Refer to Supplementary text file S1.

DISTRIBUTION: Bermuda, Florida, and historically more widespread from the Caribbean and Gulf of Mexico to Brazil, but genetic comparisons from these regions are required.

MISAPPLIED NAME FOR BERMUDA: *Halymenia floridana* J. Agardh (Guimarães & Fujii 1998, Schneider 2003; see remarks below).

REMARKS: The most common, widespread and broadest of the *Cryptonemia* species in Bermuda (4-30 cm; Collins & Howe, 1916), this taxon was originally designated *Halymenia bermudensis* by Collins & Howe (1916), and as many as 80 isotypes were distributed as *P.B.-A.* no. 2050 (Collins *et al.*, 1915) to herbaria throughout the world. This species is found in a variety of inshore and offshore subtidal locations, and much of the year is particularly abundant in Walsingham Pond and Harrington Sound. The mature form of *Cryptonemia bermudensis* is comprised of multiple broad blades that are invariably lobate, these arising from distinct branched stipes that reach 1 cm in length (Collins & Howe, 1916). The terete stipes occasionally extend into the blade as short basal midribs. The youngest specimens are originally obovate to suborbicular in shape, but once they reach a few cm high, they form deep lobes or branches, these extending to 2-3 orders.

In a morphological study of the foliose species of *Halymenia* in Brazil, *H. bermudensis* was moved by Guimarães & Fujii (1998) into synonymy with *H. floridana* J. Agardh (type locality = Florida, USA). It is clear from our molecular work that the highly variable Bermuda specimens from throughout the islands, including the type locality, fall in a well-supported clade (Figs 1, 3) along with other species of *Cryptonemia*. Thus, this taxon needs reassignment to the genus as we propose above. Based upon observations of type material, the species to which *H. bermudensis* had been reassigned, *H. floridana*, has larger and less dissected blades (Guimarães & Fujii, 1998, fig. 9) than the highly branched species from Bermuda (Fig. 15). Although Collins & Howe (1916) mentioned that *Halymenia bermudensis* had some similarities with *H. floridana*, they distinguished the two species using a suite of characters. In our *rbcL* analysis (Fig. 1A), three specimens from Brazil identified as *H. floridana* are shown to represent a genetic species in *Cryptonemia*, unique from a sister clade with *C. bermudensis* from the type locality and *C. lacunicola* (see account below). At this time, given the taxonomic assignment of *H. floridana* to

Halymenia, we suggest that prior to moving this species to *Cryptonemia* that specimens from its type locality in Florida need to be sequenced and analyzed to demonstrate their genetic relationship with Brazilian isolates identified as the same species. It is possible, especially at a time when geographically cryptic species are being discovered locally in Bermuda and elsewhere around the globe (e.g., Saunders *et al.*, 2006; Schneider & Lane, 2008; Schneider *et al.*, 2010), that *H. floridana* and the Brazilian isolates are distinct species possibly in different genera.

Cryptonemia bermudensis is most closely related to another species in Bermuda, *C. lacunicola* (Figs 1, 3), a taxon with which it is found growing sympatrically in Walsingham Pond. In early stages of growth, these two species are impossible to distinguish morphologically. We also collected several small, branched specimens from the Dry Rocks Reef off Key West, Florida, that were a perfect genetic match to *C. bermudensis* from its type locality, and we suspect that it may be shown to be more widespread in warm western Atlantic waters with further molecular studies.

***Cryptonemia lacunicola* C.W. Schneider, C.E. Lane et G.W. Saunders, sp. nov. (Figs 18-23)**

DESCRIPTION: Plants light to dark rosy-red and erect to 15 cm tall, composed of broadly ligulate, pseudodichotomously to irregularly branched blades; single, or occasionally a few, proximally tapering blades on simple to branched stipes to 1 cm long, arising from small discoidal holdfasts or eroded axes, the stipes at times extending into proximal portions of the blades as short midribs; blades increasing in breadth distally, to 3 cm wide and 80-150 μm thick below branching nodes in upper portions, the margins entire, often undulate (Figs 18-20), but appearing crenate distally due to an abundance of incipient branching; medulla comprised of a loosely woven, mucilaginous matrix with fine intertwining, elongate filaments (Figs 21, 22), 1.5-3.5 μm diam., some running from one cortex to another, all interspersed with coarse, darkly staining

filaments generally 4-8 μm diam. directly connected to large densely staining, stellate ganglial cells (Fig. 23); medulla giving rise to a 2-(3-) layered cortex composed of cells 4.5-9.0 μm diam., transversely elongate in transverse section, irregularly rounded angular in surface view; gametangia and tetrasporangia unknown.

ETYMOLOGY: From *lacuna* (L., f.), pool, for the sink-hole pools where it persists throughout the year in Walsingham Park, and *-cola* (L. comp., indecl.) for dweller or inhabitant, thus literally “pool-dweller.”

HOLOTYPE (designated here): *Craig W. Schneider/Christopher E. Lane* 05-9-15, 19 Jul. 2005, Cliff Pool, Walsingham Park, Bermuda I., Bermuda, western Atlantic Ocean, 32°20'50.4"N, 64°42'41.3"W, depth 0-2 m on rock [deposited in MICH; GenBank MG191639 (UPA), MG191579F782469 (LSU)] (Fig. 18); Isotypes — NY, Herb. CWS.

SELECTED COLLECTIONS: Refer to Supplementary text file S1.

DISTRIBUTION: Thus far, known only from Bermuda.

REMARKS: Our numerous collections of the new species from shaded inland sinkholes and cave mouths in Walsingham Park have some features in common with its congener and genetic sibling, *Cryptonemia bermudensis*. When we first collected it, it was considered a “stretched out” shade morphology of the more broadly bladed *C. bermudensis*. Our genetic sequences, however, show them to be distinct species. *Cryptonemia lacunicola* has narrower blades (generally 2 cm or less in width as compared to the significantly wider blades of *C. bermudensis*, to 12 cm), appearing strap-like and more open in habit (Figs 18-20), somewhat similar in gross morphology

to congeneric species known in the Caribbean and Brazil (Guiry & Guiry, 2015), *C. bengryi* W.R. Taylor (type locality = Jamaica) and *C. limensis* (Kütz.) J.A. Lewis (type locality = Peru). Genetic material of these species collected in the Caribbean and Brazil is unavailable at present, but there are morphological differences. *Cryptonemia bengryi* has blades borne on long, terete stipes and is deeply cleft to palmately lobed (Taylor, 1960; Díaz-Piferrer, 1965). *Cryptonemia limensis* has palmately organized blades lacking stipes with broadly ovate lobes and retuse apices (Acleto, 1973, as *C. chiangii*ACLETO and *C. peruviana*ACLETO; Lewis, 1990), features not seen in the new species. One Pacific species presently considered a synonym of *C. limensis*, *C. guaymasensis* (E.Y. Dawson) E.Y. Dawson from Baja California, has narrower lobes than the more typical plants of the species from South America, but it too has retuse apices causing Lewis (1980) to synonymize them.

Cryptonemia lacunicola has pseudodichotomous branching pattern that sets it apart from *C. bermudensis*, branching to 4-5 orders of magnitude at maturity, the blades increasing in width from the base to the apex (Fig. 20). *Cryptonemia bermudensis* produces broadly ovate to cuneate blades significantly broader (to 12 cm) than the new species (to 3 cm) and remain tightly branched to just a few orders (Fig. 16). Anatomically, the two species cells sizes and arrangements are mostly indistinguishable. Both have coarse, darkly staining medullary filaments networking between substellate ganglia as well as dimorphic finer medullary filaments traversing the central core of the axes. Collins & Howe (1916) reported the coarser medullary filaments from 6-20 μm diam. for *C. bermudensis*, whereas those in *C. lacunicola* range from 4-8 μm diam., but these hardly represent a character warranting species level discussions. In short, the genetic differences and overall habit are the only way to differentiate these sympatric species in Bermuda, particularly as they appear able to occasionally hybridize (Fig. 2).

Cryptonemia lacunicola is found in a robust population throughout the entire year at the type locality and other nearby sinkholes in Walsingham Park on Bermuda I. Many of these

inland areas lack the large herbivorous fish that would reduce individuals to a smaller cropped habit on coastal reefs, a pattern seen contrasting many other species in sinkholes and nearshore boiler reefs (e.g., *Botryocladia* spp., see Schneider & Lane, 2008). Interestingly, hundreds of collected specimens from throughout the entire year have not turned up reproductive characteristics. Collins & Howe (1916, p. 172) were equally frustrated by the lack of reproduction in their hundreds of specimens of *C. bermudensis*, and we too have discovered none in our collections.

Cryptonemia perparva C.W. Schneider, C.E. Lane et G.W. Saunders sp. nov. (Figs 24-31)

DESCRIPTION: Single, or occasionally a few, blades arising from often long stipes on small discoidal holdfasts (Fig. 24), blades not adhering well to paper; rosy-red blades simple, to 1.3 cm tall, obovate, elliptical to subspherical in outline, margins entire; blades 40-100 μm thick in vegetative portions, medulla comprised of a compact cavity with few to many intertwining, elongate filaments (Fig. 26), 1.5-4.5 μm diam. and large, oddly lobate to stellate swollen ganglial cells (Fig. 25); medulla giving rise to a 2-(3-) layered cortex, subsurface cells small, subglobose to flattened reaching 10 μm diam., outer cortical cells 3-6 μm diam., irregularly globose to elongate in transverse section, irregularly angular to rounded in surface view; dioecious, subglobose auxiliary cells in ampullae, 10-17 μm diam. (Fig. 26), produced from medullary filaments; carposporophytes immersed in blades, globose, 100-250 μm in diam., with stretched involucrel filaments, dramatically swelling both blade surfaces to 270 μm thick (Figs 27, 28); carposporangia subglobose to irregularly elongate, 9.5-14.5 μm diam.; spermatangia formed in extensive sori on both blade surface from elongated spermatangial mother cells produced directly or on one-celled pedicels produced from outer cortical cells, spermatia ovoid to ellipsoidal, 1 μm diam. (Fig. 29); tetrasporangia ellipsoid (Fig. 31), cruciately divided, 10-12 μm diam., 9-18 μm

long, clustered over the surface of both blade surfaces and compressing adjacent cortical cells (Fig. 30).

ETYMOLOGY: From *perparva* (L., adj., f.) = very small, for the overall size of the species.

HOLOTYPE (designated here): CWS/CEL 05-19-12, 22 July 2005, outer reef, John Smith's Bay, off Canton Pt., south shore of Bermuda I., Bermuda, western Atlantic Ocean, 32°19'09.6"N, 64°42'46.6"W, depth 10 m on coral [deposited in MICH; GenBank MG191585 (LSU)] (Fig. 24);
Isotypes – UNB, Herb. CWS.

SELECTED COLLECTIONS: Refer to Supplementary text file S1.

DISTRIBUTION: Endemic to Bermuda, western Atlantic Ocean, as presently known.

REMARKS: *Cryptonemia perparva* is a common summer species on the south shore boiler reefs off Bermuda Island. They are found along with *Botryocladia bermudana* C.W. Schneider et C.E. Lane as understory plants somewhat protected from grazing fish by a number of larger macroalgae in this high-energy environment including species of *Dictyota*, *Padina*, *Laurencia* and *Dasya spinuligera* Collins et Hervey. The small blades of *C. perparva* could have been overlooked as germlings of a number of other *Cryptonemia* species, but their reproductive state in blades less than 1 cm tall demonstrates that they are mature despite their small stature. Furthermore, *rbcL* and LSU sequences confirmed that they are distinct from the other species in the genus in Bermuda (Figs 1, 3).

Although carpogonial branch ampullae have not been found in our specimens, female plants of *Cryptonemia perparva* produce great quantities of small auxiliary cell ampullae (Fig.

25) typical of the genus (Kylin, 1925; Sjoestedt, 1926; Chiang, 1970), often in positions adjacent to others such that after presumed fertilization the cross-sections look like well-stuffed sausages (Fig. 28). Auxiliary cells are produced from ampullar filaments on short stalk cells, and are large and subglobose surrounded by ampullar filaments following the pattern described for other *Cryptonemia* species (Kylin, 1925; Sjoestedt, 1926; Chiang, 1970). Small ellipsoidal spermatia are produced in dense, slightly raised sori on both surfaces of male blades from elongate spermatangial mother cells (Fig. 29). Tetrasporangia are formed within the thallus at the interface of the filamentous medulla and the inner row of cortical cells (Fig. 31). When they expand in size and divide cruciately, the sporangia compress the surrounding cortical cells into arcing elongate cells, leaving themselves uncovered by outer cortical cells at the surface (Fig. 30).

The new species bears comparison with other small and diminutive *Cryptonemia* species worldwide. Although the epithet for the western Atlantic *C. delicatula* Joly *et* Cordeiro (type locality = São Paulo, Brazil) suggests a small and delicate species, in reality it extends to 9 cm tall when proliferous, and 6 cm tall when not (Joly, 1965; Joly *et al.*, 1966), whereas *C. perparva* barely exceeds 1 cm tall even at reproductive maturity. A subspecies from South America, *C. delicatula* ssp. *venezuelensis* Ganesan (type locality = Venezuela) is smaller than the nominate variety (1.2-3.0 cm tall) and differs from *C. perparva* due to the presence of its long terete stalks giving rise to the broadening simple blades above (Ganesan, 1975). *Cryptonemia taylorii* I.A. Abbott (type locality = Socorro I., Revillagigedo Archipelago, Mexico) is 4-8 cm tall and 4-6 cm wide, dimensions that dwarf the new species. This eastern Pacific species is very thin, 45-60 μm , accounting for its torn and fragmented habit at maturity (Abbott, 1967). *Cryptonemia veleroae* (E.Y. Dawson) E.Y. Dawson (type locality = Gulf of California, Mexico) is as tall as 4 cm at maturity (Dawson, 1944, as *Callymenia veleroae* E.Y. Dawson; Norris, 2014), but has several blades arising from a common holdfast and the blades are split by lacerations as they increase in

height. Another small species, *C. papenfussii* Chiang (type locality = Natal, South Africa), reaches only 3.5 cm high and produces multiple blades from short filiform stalks (Chiang, 1970) unlike the new species. One *Cryptonemia* species that is truly diminutive, *C. parva* Zhang et B.M. Xia (type locality = Xisha I., China), reaches only 1.4 cm in height, but this species becomes subdichotomously lobed or irregularly palmate at maturity and produces tetrasporangia in nemathecial sori (Zhang & Xia, 1983; Xia, 2004), whereas the similar-sized *C. perparva* mostly remains as simple blades at maturity and bears scattered, non-nemathecial tetrasporangia.

Discussion

Prior to this study, only eight species of *Cryptonemia* were known from the tropical western Atlantic Ocean (Wynne, 2017). The description of five new species from Bermuda represents a significant addition for the region and suggests a biodiversity hotspot for the genus in this area. With the limitations of collecting in deep water well beyond the limits of SCUBA, we only turned up a single spectacular specimen of *C. abyssalis* in two weeks of mesophotic collecting. All of the other species found in Bermuda are abundant and seen throughout fairly long growing seasons, some representing misidentifications made by earlier workers in the islands. With molecular sequencing available as a tool, many such problems can now be eliminated, and the result is a more accurate assessment of the flora of these Atlantic islands.

Key to Bermuda species of *Cryptonemia*

- 1a. Plants with midribs extending to one half the length of the blade..... *C. atrocotalis*
- 1b. Plants lacking midribs or with only basal midribs entering the blades..... 2
- 2a. Plants less than 3 cm tall at maturity..... 3
- 2b. Plants greater than 3 cm tall at maturity..... 4
- 3a. Simple obovate to subspherical blades, occasionally a few rising from terete stipes.....

.....	<i>C. perparva</i>
3b. Branched ligulate blades to 2 mm wide.....	<i>C. antricola</i>
4a. Plants with blades growing from marginal projections of other blades in an opuntoid pattern.....	<i>C. abyssalis</i>
4b. Plants not opuntoid.....	5
5a. Plants pseudodichotomously branched, composed of strap-like blades generally 2 cm or less wide.....	<i>C. lacunicola</i>
5b. Plants with broad obovate lobed blades arising from terete branched axes, blades well over 2 cm at maturity.....	<i>C. bermudensis</i>

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No potential conflict of interest was disclosed by the authors.

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Author contributions

All of the authors conceived the project and wrote/edited the manuscript. The morphological work was carried out by C.W. Schneider and the genetic analyses were conducted in the laboratories of G.W. Saunders and C.E. Lane.

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Figure Captions

Fig. 1. Maximum likelihood phylogenetic trees of (A) *rbcL* and (B) LSU data from *Cryptonemia* and related taxa. Support values represent bootstrap proportion / and Bayesian posterior probability. Bootstrap values below 50 and posterior probabilities below 0.60 are not shown.

Fig. 2. Specimens of *Cryptonemia bermudensis* and *C. lancunicola* for which nuclear (LSU) and plastid (*rbcL* and or UPA) data were available. At least one specimen (CL020801) shows clear evidence of introgression or incomplete lineage sorting, while five others (BDA0265, BDA0541, BDA0936, BDA1369, GWS011906) have only plastid data and may be bona fide *C. lancunicola* or *C. bermudensis* introgressed with the plastid of the former.

Fig. 3. Maximum likelihood phylogenetic tree of concatenated LSU and *rbcL* sequences. Support values represent bootstrap proportion / and Bayesian posterior probability. Bootstrap values below 50 and posterior probabilities below 0.60 are not shown.

Figs 4-10. Bermuda *Cryptonemia* species. **Figs 4-7.** *Cryptonemia abyssalis* sp. nov., CWS/TRP 16-13-5 [BDA1998]. **Fig. 4.** Holotype. **Fig. 5.** Transverse section through blade at swollen margin. **Fig. 6.** Transverse section through blade at swollen margin at proliferous peg. **Fig. 7.** Collapsed section of a male sorus showing surface spermatia. **Figs 8-10.** *Cryptonemia antricola* sp. nov. **Fig. 8.** Holotype, CWS/CEL 10-14-33 [BDA0193]. **Fig. 9.** Habit, CWS/CEL 03-52-9. **Fig. 10.** Transverse section through blade, CWS/CEL/TRP 12-18-18. Scale bars: Fig. 4, 2 cm; Figs 5, 6, 100 μ m; Fig. 7, 40 μ m; Figs 8, 9, 1 cm; Fig. 10, 50 μ m.

Figs 11-15. *Cryptonemia atrocotalis* sp. nov. **Fig. 11.** Holotype, TRP/CWS 12-131-3 [BDA1543]. **Fig. 12.** Habit, TRP/CWS 12-79-7. **Fig. 13.** Transverse section through blade at

swollen margin, *TRP/CWS* 12-131-3. **Fig. 14.** Transverse section through blade midrib, *TRP/CWS* 12-131-3. **Fig. 15.** Surface layer displacement by subcortical tetrasporangia, *CWS* 799. Scale bars: Figs 11, 12, 2 cm; Figs 13, 14, 100 μm ; Fig. 15, 10 μm .

Figs 16-23. Bermuda *Cryptonemia* species. **Figs 16, 17.** *Cryptonemia bermudensis*, *CWS/CEL* 09-33-2. **Fig. 16.** Collection of specimens from the type locality, Tucker's Town Bay. **Fig. 17.** Transverse section through blade. **Figs 18-23.** *Cryptonemia lacunicola* sp. nov. **Fig. 18.** Holotype, *CWS/CEL* 05-9-15. **Fig. 19.** Specimen from the type locality, Cliff Pool, Walsingham Park. *CWS/CEL* 08-39-4. **Fig. 20.** Specimen from the type locality, *CWS/CEL* 06-16-5. **Fig. 21.** Transverse section through blade, *CWS/CEL* 05-4-4. **Fig. 22.** Transverse section through blade of isotype demonstrating fine and coarse medullary filaments, *CWS/CEL* 05-9-14. **Fig. 23.** Surface view of cortex showing medullary ganglion, *CWS/CEL* 01-13-28. Scale bars: Fig. 16, 18-20, 2 cm; Figs 17, 21, 100 μm ; Fig. 22, 50 μm ; Fig. 23, 20 μm .

Figs 24-31. *Cryptonemia perparva* sp. nov. **Fig. 24.** Holotype, *CWS/CEL* 05-19-12. **Fig. 25.** Transverse section near margin showing enlarged ganglial cells in medulla, *CWS/CEL* 03-4-30. **Fig. 26.** Ampullar filaments surrounding the auxiliary cell prior to fertilization, *CWS/CEL* 03-4-30. **Fig. 27.** Cystocarp in transverse section with mature carposporangia, *CWS/CEL* 03-4-30. **Fig. 28.** Contiguous cystocarps swelling the thallus, *CWS/CEL* 03-4-30. **Fig. 29.** Elongate spermatangial mother cells in a dense sorus produced from outer cortical cells, *CWS/CEL* 06-4-13. **Fig. 30.** Tetrasporangia in section and surface view through cortex demonstrating cortical cell displacement between swollen sporangia, *CWS/CEL* 01-13-28. **Fig. 31.** Cruciate tetrasporangia formed at the interface of the inner cortex and medulla, *CWS/CEL/TRP* 12-20-7. Scale bars: Fig. 24, 1 cm; Figs 25, 26, 29-31, 25 μm ; Figs 27, 50 μm ; Fig. 28, 150 μm .

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A revision of the genus *Cryptonemia*
(Halymeniaceae, Rhodophyta) in
Bermuda, western Atlantic Ocean,
including five new species and *C.*
bermudensis (Collins & M. Howe) comb. nov.

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Taylor and Francis

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