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INTEGRATING MOLECULAR AND TRADITIONAL
SYSTEMATIC TECHNIQUES TO REDEFINE
RED ALGAL (RHODOPHYTE) DIVERSITY IN THE
BERMUDA ISLANDS
BY
THEA RUTH POPOLIZIO

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2015

DOCTOR OF PHILOSOPHY DISSERTATION
OF
THEA RUTH POPOLIZIO

APPROVED:

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UNIVERSITY OF RHODE ISLAND
2015

ABSTRACT

Molecular-assisted alpha taxonomy (MAAT) is a groundbreaking methodology that combines molecular tools with traditional morphological investigations. From studies using these methods, researchers can determine whether specimens with different morphologies are actually one entity exhibiting high phenotypic plasticity or are multiple genetic species with convergent morphologies, an important breakthrough for phycologists since algae are notoriously difficult to identify on morphology alone. Molecular-assisted techniques have also significantly increased the rate of novel species discovery among the algae, especially rhodophytes. From our own biodiversity assessments, we have learned that numerous members of Bermuda's macroalgal flora have been misnamed, overlooked, or have not been identified as accepted species. Seaweed diversity in the islands overall, as well as the percentage of endemic species, is presumably underestimated. To explore this hypothesis, MAAT methods have been applied to extensive collections of Bermuda seaweeds accumulated since 2010 along with robust phylogenetic analyses incorporating comparative sequence data from around the world. This dissertation examines several results of these efforts. Four genera have been added to the Bermuda flora — *Hommersandiophycus*, *Trichogloeopsis*, *Yamadaella* and *Laurenciella*, and a number of species uncovered that are new reports for the islands – *Centroceras gasparrinii*, *C. hyalacanthum*, *C. microacanthum*, *Liagora mannarensis*, *Trichogloeopsis pedicellata*, *Laurencia dendroidea*, *L. catarinensis* and *Palisada flagellifera*. Eight species new to science have also been described — *Helminthocladia kempii*, *Liagora nesophila*, *Yamadaella grassyi*, *Chondrophycus planiparvus*, *Laurenciella namii*, *Crassitegula laciniata*,

Centroceras arcii and *C. illaqueans*. Over the course of this study, we have accumulated 1875 DNA vouchered specimens collected from 157 sites around the Bermuda platform, as well as 317 specimens from the Florida Keys and 236 from St. Croix in the Caribbean Antilles, all paramount for present and future work. What we have learned already from this small archipelago suggests a overwhelming underrepresentation of diversity in historical records of the islands macroalgal flora, and highlights the importance of generating an accurate baseline dataset for future monitoring efforts.

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This work would not have been possible without the support of my advisor, Chris Lane. Prior to graduate school at URI, I was an educator with few years of professional experience, some people skills, a limited scientific research background and a healthy dose of ambition. You saw something promising in my unconventional background, and gave me a shot. *Thank you.* I can say without hesitation that the past several years have been some of the best of my life, largely because of the opportunities you have provided and prioritized.

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URI and in Bermuda. Special thanks to Robbie for helping to make BAMZ a second home for me during my field year, for being my dive buddy even when the water was “cold,” and for introducing me to Southwest Breaker, one of the prettiest dive sites in Bermuda.

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am so fortunate to have your light in my life. I am sorry for all the soccer games and family dinners I have missed, and hope I can make up for it in the upcoming years.

John— there are few people who demonstrate the kind of patience and selflessness that seems to come so naturally to you. Your generous and optimistic spirit, constant encouragement, and steady love, care and support have contributed immeasurably to my success. You have shown me that it is possible (and worth it) to persevere in challenging circumstances for meaningful work. I'm so lucky you're my lobster.

This work is dedicated to my parents, who recognized early that the sea was good for my soul, and made many sacrifices to ensure it was always a significant part of our lives. Being encouraged with equal emphasis to read, make art, and play outside has played an important role in the person I have become, and the work that I do. Mom and Dad, with deep gratitude and bottomless love, thank you for being my greatest allies.

PREFACE

This dissertation was formatted in accordance with the manuscript format guidelines established by the Graduate School of the University of Rhode Island. Chapter 2, “Notes on the marine algae of the Bermudas. 13. *Helminthocladia kempii* sp. nov. (Nemaliales, Liagoraceae) based upon *H. calvadosii sensu auct.* from the western Atlantic” was published in *Cryptogamie, Algologie* on February 21, 2014. Chapter 3, “A molecular evaluation of the Liagoraceae *sensu lato* (Nemaliales, Rhodophyta) in Bermuda, including *Liagora nesophila* sp. nov. and *Yamadaella grassyi* sp. nov.” has been submitted to the *Journal of Phycology*. Chapter 4, “Molecular analysis resolves the taxonomy of the *Laurencia* complex (Rhodomelaceae, Ceramiales) in Bermuda and uncovers novel species in the western Atlantic Ocean” is in preparation for publication in *Phycologia*. For uniformity, all title pages and references are formatted according to the requirements of the *Journal of Phycology*, and all tables, figures and figure headings are labeled with Arabic numerals. All formatting for manuscripts in the Appendices remain as prescribed by the corresponding publication.

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*Humanity is exalted not because we are so far above other living creatures,
but because knowing them well elevates the very concept of life.*

-E.O. Wilson

CHAPTER ONE

GENERAL INTRODUCTION

Bermuda is an isolated archipelago at the interface of tropical and warm temperate biogeographic zones, making it an ideal location for biodiversity assessments. Situated just over 1000 km east of North Carolina in the Sargasso Sea, Bermuda's climate, water temperature and marine life are heavily influenced by the Gulf Stream (Locke et al. 2013). Because of the seasonal water temperature oscillations (ranging from 18° C in winter to 28°C in summer) the macroalgal assemblage of Bermuda is made up of warm-water tolerant species from the western mid-Atlantic that have persisted during the last ice age, cool-water tolerant Caribbean species carried northward by the Gulf Stream that recolonized the islands since the Pleistocene, and endemic species that have evolved there (Schneider and Searles 1998a). Despite its distant location from North America and tropical summer temperatures, Bermuda's small size presently supports only ca. 450 species of red, brown and green seaweeds, and endemism among these groups is reportedly less than 2% (Schneider and Searles 1998a).

Reports of benthic macroalgae from Bermuda began to appear in the literature in the last half of the 19th century based upon collections made on just a few voyages, including the “*Challenger* Expedition” to the islands (Kemp 1857; Rein 1873; Dickie 1874; Hemsley 1884; Murray 1888, 1889). In 1917, F.S. Collins and A.B. Hervey produced the first comprehensive marine flora of the islands and distributed the bulk of their specimens in five volumes entitled ‘The Algae of Bermuda’ as part of *Phycotheca Boreali-Americana* (P.B.-A., Collins et al. 1912-1917). In 1949, W.R. Taylor of the University of Michigan collected in Bermuda with his student, A.J. Bernatowicz (Taylor 1952). Taylor included their data along with that of previous

collectors in his comprehensive *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas* (1960). Later, Taylor and Bernatowicz (1969) produced an annotated list of the most common shallow water macroscopic seaweeds of the Bermudas.

Years later, using Scuba and surface-supplied air (SSA), R.B. Searles of Duke University and his former student, C.W. Schneider (1987) collected seaweeds from 12-50 m depths around the islands on the NOAA funded R/V *Seahawk* cruises of 1983 and 1985. Prior to this, the algae present in offshore reef communities around Bermuda had been largely neglected. From 1999 to the present, C.W. Schneider of Trinity College and C.E. Lane of the University of Rhode Island have conducted extensive annual collections from the intertidal to 36 m in Bermuda in an effort to better understand the islands' macroalgal flora. Their work has resulted in the addition of ~90 species to the flora, the exclusion of ~30 names historically applied to Bermuda taxa, and the descriptions of 17 novel species and three new genera (Popolizio et al. 2013 [Chapter 2]; Schneider 2000, 2004; Schneider and Lane 2005, 2007, 2008; Schneider et al. 2006, 2010, 2011a, 2011b, 2012, 2014a, 2014b; Schneider and Searles 1997a, 1997b, 1998a, 1998b; Saunders et al. 2006; Schneider and Wynne 2009; Wynne and Schneider 1996).

From biodiversity assessments like those referenced above, we have learned that a significant number of Bermuda's macroalgal species have either been misnamed, overlooked, or represent dark taxa— specimens that presently aren't identified as a known species. Seaweed diversity in the islands, and conceivably the percentage of endemic species, is likely underestimated. To explore this hypothesis,

we have applied molecular-assisted alpha taxonomy (hereafter referred to as MAAT) to extensive collections of Bermuda seaweeds accumulated in August of 2010 and from January to December of 2012. Collections were made from shore access points around Bermuda, as well as by boat, and are representative of a variety of habitats ranging from the intertidal zone to as deep as 36 meters (Fig. 1) Many of the 157 collecting sites indicated in Fig. 1 were visited repeatedly throughout 2012, to capture seasonal variation in macroalgal assemblages with changing sea surface temperatures across the reef platform.

For taxonomists, MAAT is a groundbreaking methodology that combines molecular tools with traditional morphological investigations (see Cianciola et al. 2010 [Appendix A] for a complete review of MAAT and its application to biodiversity studies). For most groups of eukaryotes, the 5' end of the mitochondrion-encoded cytochrome oxidase I gene (COI-5P) has been established as an ideal, nearly universal, marker that is sensitive enough to differentiate between even closely related species, while remaining conservative enough for comparisons at the taxonomic family-level (Saunders 2005). COI-5P barcode data analysis is an efficient screening tool for identifying genetic species groups in the red algae, and is useful for determining divergences within and among species (based on nucleotide differences in aligned sequences).

In the small representative sampling from Bermuda collections made during the course of this study, the number of genetic species estimated by the COI-5P barcode data is nearly twofold that of currently reported numbers (Wynne 2011) for these taxa in the islands, with 45 presently recognized species in this subsample

forming ~82 unique genetic entities in preliminary sequence analyses (Table 1). For example, in the taxon rich red algal order Ceramiales, the data has revealed unreported diversity in the genus *Centroceras* and among genera in the *Laurencia* complex. These are discussed in detail in Appendix C (Schneider et al. 2015) and in Chapter 4, respectively. *Wrangelia*, *Spyridia* and *Dasya*, three common western Atlantic genera also in the Ceramiales, are examples of future candidates for robust molecular and morphological analyses. In the genus *Wrangelia* alone, a single morphological species (*Wrangelia* cf. *penicillata*) is shown to be four genetic entities (Fig. 2). This is a classic illustration of diversity that would presumably go undetected without the relatively fast, powerful assessment methods employed in MAAT. The data produced thus far during this study also suggest that the red algal genera *Dichotomaria*, *Chondria*, *Ceramium*, *Chrysomenia*, *Gracilaria* and *Polysiphonia/Neosiphonia* in Bermuda are in need of further molecular and morphological examination.

For phylogenetic assignment of novel species or of taxa that appear to be misplaced based on the results of DNA barcoding, additional molecular markers and alpha taxonomy are employed to confirm the data, ensuring a robust classification. Standard molecular markers (*e.g.*, the large or small subunit [LSU, SSU] of the ribosomal cistron; the plastid-encoded RuBisCO [*rbcL*] operon) are often used, but the precise genes used to further investigate targeted taxa are generally chosen based on the taxonomic level the researcher is aiming to resolve, as well as the comparative data available in GenBank (<http://www.ncbi.nlm.nih.gov>). From these data, phylogenetic trees are constructed with the broadest relevant sampling possible, allowing us to differentiate between undescribed cryptic species and

unrecognized additions to the Bermuda flora described from other places in the world. In this way, we can also place Bermuda taxa into a larger biogeographical context.

Cryptic species, in the truest sense, cannot be distinguished using morphological characters, which may overlap partially or entirely across genetic species groups. In other words, regardless of the genetic data that indicate they are distinct entities, morphological examination in the field or lab could lead the researcher to either of two (or more) names that are tied to identical descriptions. For example, in a new study of the tribe Laurenceiae, or the ‘*Laurencia* complex’ (Ceramiales, Rhodomelaceae), the novel genus *Laurenciella* was ascertained through molecular sequence data as sister to the *Laurencia sensu stricto* clade. Though an *rbcL* sequence divergence of nearly 10% clearly indicated that the two clades differed enough to be separated into distinct genera, no morphological characters could be found to formally support the erection of a new genus (Cassano et al. 2012). Thus, *Laurenciella* gen. nov. was published with the support of molecular data alone, the first macroalgal genus where this was done.

Pseudo-cryptic species, however, appear superficially identical to congeners, but once molecular distinctness is established, morphological characters can be carefully observed and measured to establish unique identifiers (Maggs et al. 2007). The ‘*Centroceras clavulatum* complex’ (Ceramiales, Ceramiaceae) for example, was considered to be a cosmopolitan complex of cool temperate to tropical red algae, until molecular sequencing of a number of isolates worldwide greatly restricted its biogeography to the Pacific Ocean (Won et al. 2009). By combining morphological

analysis of 52 Bermuda collections previously identified as *C. clavulatum* (C. Agardh) Mont. with phylogenetic analysis of *rbcL* and SSU gene sequences for several isolates, we identified two novel species, *C. arcii* C. W. Schneid., Cianciola & Popolizio and *C. illaqueans* C. W. Schneid., Cianciola & Popolizio, and expanded the distributions of three taxa recently segregated from the ‘complex’ in the western Atlantic (*C. gasparrinii* (Menegh.) Kütz., *C. hyalacanthum* Kütz. and *C. micracanthum* Kütz.) to include Bermuda (Schneider et al. 2015 [Appendix C]). Prior to this study, *Helminthocladia kempii* Popolizio, C.W. Schneid. & C.E. Lane (Popolizio et al. 2013 [Chapt. 2]) had historically been reported from Bermuda as *H. calvadosii* J.V. Lamour., a species with an eastern Atlantic type locality, and *Liagora nesophila* Popolizio, C.W. Schneid. & C.E. Lane prop. sp. nov. (Popolizio et al. 2015 [Chapt. 3]) was previously identified as the pan-tropical species *L. ceranoides* J.V. Lamour. In all of these instances, similarities in gross morphology were deceiving to researchers, but molecular data revealed their distinctiveness, and the detection of informative morphological characters followed, as MAAT implies.

In some cases, revisions to the flora are due to over-classification, where taxa exhibit phenotypic plasticity due to environmental factors such as wave action or temperature and can be misconstrued as distinct species. For example, from data gathered after a 2010 collecting trip to Bermuda, it was determined that the alga described as *Platoma cyclocolpum* (Mont.) F. Schmitz in Bermuda was genetically distinct from specimens of this taxon collected from the type locality (Canary Islands). Remarkably, *P. cyclocolpum* was determined to be the winter (juvenile) “morph” of *P. gelatinosum* (M. Howe) C.W. Schneid., McDevit, G.W. Saunders & C.E. Lane comb.

nov. (formerly *Nemastoma gelatinosum* M. Howe) a taxon that appears remarkably different in gross morphology at different times of the year (Schneider et al. 2011b). This is merely one example that demonstrates the importance of molecular tools to the proper identification of morphologically plastic species.

The scenarios discussed above emphasize the utility of molecular tools for determining whether specimens with different morphologies are actually one entity exhibiting high phenotypic plasticity or are multiple genetic species with convergent morphologies. Molecular techniques are also significantly increasing the rate at which phycologists can identify novel specimens, and as such, an overwhelming number of studies indicate that biodiversity among the algae (rhodophytes in particular) is vastly underestimated (Maggs et al. 2007). For instance, recent analysis of the Sebdeniaceae in the Pacific presented seven undescribed or unknown specimens (Kraft and Saunders 2011). Initially thought to be a species-poor group, the family was originally monotypic with *Sebdenia*. Schneider et al. (2006) added a second genus to the family after molecular and morphological data recovered the novel taxon *Crassitegula walsinghamii* C.W. Schneid., C.E. Lane & G.W. Saunders from limestone sinkhole pools in Bermuda. After its publication, the biogeographical range of the monotypic genus *Crassitegula* was extended to the Pacific and a third genus was added to the family, *Lesleigha* (Kraft and Saunders 2011). Furthermore, our work has uncovered a second novel species of *Crassitegula* from Bermuda, *C. laciniata* C.W. Schneider, Popolizio & C.E. Lane (Schneider et al. 2014a) discussed in detail in Appendix B. Molecular sequencing has been paramount to identifying several other taxa new to science in Bermuda, e.g., the

novel genus and species *Archestenogramma profundum* C.W. Schneid., Chengsupanimit & G.W. Saunders (Schneider et al. 2011a) and the novel species *Halopeltis pellucida* C.W. Schneid. & G.W. Saunders (Schneider et al. 2012), *Meredithia crenata* C.W. Schneid., G.W. Saunders & C.E. Lane (Schneider et al. 2014b), *Ethelia umbricola* K.R. Dixon, G.W. Saunders & C.W. Schneid. prop. sp. nov. (Dixon et al. 2015), *Yamadaella grassyi* Popolizio, C.W. Schneid. & C.E. Lane prop. sp. nov. (Popolizio et al. 2015 [Chapt. 3]), *Chondrophycus planiparvus* Popolizio, C.W. Schneid. & C.E. Lane prop. sp. nov. and *Laurenciella namii* Popolizio, C.W. Schneid. & C.E. Lane (Chapt. 4).

Combining molecular data with alpha-taxonomic techniques has allowed us to corroborate historical reports of Bermuda taxa that previously were based on morphological data alone. We have verified reports of numerous rhodophyte species in the islands, including *Laurencia microcladia* Kütz., *L. intricata* J.V. Lamour., *Palisada perforata* (Bory) K.W. Nam and *Yuzurua poiteaui* (J.V. Lamour.) Martin-Lescanne (Chapt. 4), *Titanophycus validus* (Harv.) Huisman, *Ganonema farinosum* (J.V. Lamour.) K.C. Fan & Yung C. Wang, *Gloiocallis dendroidea* (P. Crouan & H. Crouan) S.-M. Lin, Huisman & D.L. Ballant. (Popolizio et al. 2015 [Chapt. 3]) and effectively excluded the species *Helminthocladia calvadosii* (Popolizio et al. 2013 [Chapt. 2]), *Liagora ceranoides* (Popolizio et al. 2015 [Chapt. 3]) and *Laurencia obtusa* (Huds.) J.V. Lamour. (Chapt. 4) from the Bermuda flora based on the results of molecular sequence analyses. We have also confirmed the presence of three members of the chlorophyte genus *Codium* that have been historically reported for Bermuda, C.

intertextum Collins & Herv., *C. taylorii* P.C. Silva and *C. decorticatum* (Woodw.) M. Howe (Schneider et al. 2014a [Appendix B]).

Molecular tools can also lead us to the discovery of taxa in the study region that have been overlooked in previous assessments. The Indo-Pacific species *Liagora mannarensis* V. Krishnamurthy & Sundararajan and Caribbean species *Trichogloeopsis pedicellata* (M. Howe) I.A. Abbott & Doty were uncovered in our investigation of the Liagoraceae in Bermuda, and are examined in Chapt. 3. Another Indo-Pacific species, *Palisada flagellifera* (J. Agardh) K.W. Nam was detected among our collections of species in the ‘*Laurencia* complex’ in Bermuda, and is presented in Chapt. 4. Specimens previously identified as *Gracilaria curtissiae* J. Agardh have been genetically and morphologically connected with *G. occidentalis* (Børgeson) M. Bodard from the Gulf of Mexico (Schneider et al. 2014a [Appendix B]) and the genus *Predaea*, not previously reported for the islands, tied to specimens from Mexico identified as *P. goffiana* D.L. Ballant., H. Ruiz & Aponte (Schneider et al. 2014a [Appendix B]). To determine whether our specimens truly represent *P. goffiana* or are novel, comparative material is needed from the type locality of Puerto Rico. Our data has also identified the green alga *Codium carolinianum* Searles in Bermuda after sequencing material of it from the type locality of North Carolina (Schneider et al. 2014a [Appendix B]).

When a researcher discovers a species that is new to science, she chooses from the collection a specimen to represent the holotype (or type specimen). All future reports of this taxon are compared to the type prior to applying its name. Linking a specimen to the type is the only unmistakable way to validate whether a specimen one

has collected is, or is not, the same entity (and in cases of genuine crypsis, comparing molecular sequence data from the specimens in question with the type). Type specimens are essential to phycological investigations because they anchor the classification system by representing the “true” or “standard” example of a described taxon (*i.e.* species, genus, etc.). The type locality is the location from which the holotype specimen was collected, and serves a similar purpose, especially in molecular phylogenetic studies when it is not possible or practical to gather molecular data from an archival type specimen. Logically, a morphologically comparable specimen identified from the type locality is more likely to be classified correctly than a species with the same name from a disjunct geographical location.

Many of the early phycologists who identified thousands of western Atlantic algal specimens were European, and their bias is evident in the names they applied to specimens collected outside of the eastern North Atlantic. Many species in the Caribbean Sea, for example, are designated by European binomials (see Popolizio et al. 2013 [Chapt. 2]). To determine whether these names were accurately applied in the past for Bermuda and the Caribbean, a comparison must be made between representatives from the collection site and from or near the type locality of the collected and field-identified specimen. For example, historically known specimens of *Laurencia obtusa* from Bermuda were compared with those from the British Isles type locality. As a result of such comparisons, several species with a type locality in Bermuda presently have molecular sequence data associated with them, and serve as valuable references for comparative studies when all are mounted in the online genetic database, GenBank. Some examples are *Helminthocladia kempii*, *Liagora nesophila*

prop. sp. nov., *Yamadaella grassyi* prop. sp. nov. and *Hommersandiophycus pectinatus* (Collins & Herv.) Popolizio, C.W. Schneid. & C.E. Lane prop. comb. nov. (Chapt. 3); *Chondrophycus planiparvus* prop. sp. nov. and *Laurenciella namii* prop. sp. nov (Chapt, 4); *Platoma gelatinosum*, *Asteromenia bermudensis* G.W. Saunders, C.E. Lane, C.W. Schneid. & Kraft, *Crouania elisiae* C.W. Schneid., *Griffithsia aestivana* C.W. Schneid. & C.E. Lane, *Chondria curvilineata* Collins & Herv., *Trichogloea herveyi* W.R. Taylor, *Dasya spinuligera* Collins & Herv., *Nitophyllum wilkinsoniae* Collins & Herv., *Seirospora purpurea* M. Howe, *Halymenia pseudofloresii* Collins & M. Howe, *Chondracanthus saundersii* C.W. Schneid. & C.E. Lane and the green alga *Cladophora longicellulata* C. Hoek.

It is important to note that together, the cases considered here and in the following chapters represent only a small fraction of the work that is yet to be done to establish a complete flora for the islands using molecular tools. Of the 1875 DNA vouchered red algal specimens collected in Bermuda since 2010, ~675 have been barcoded, and far less than those subjected to additional molecular analysis and alpha-taxonomic morphological examination. The prospective extent of macroalgal diversity that is yet to be discovered in this small archipelago is staggering.

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Example Taxon (Rhodophyta)	# currently recognized	# found with MAAT analysis
<i>Liagoraceae</i>	5	9
<i>Laurencia</i> complex	9	≥13
<i>Centroceras clavulatum</i>	1	5
<i>Crassitegula</i> spp.	1	2
<i>Spyridia</i> spp.	2	4
<i>Ceratodictyon</i> spp.	2	≥4
<i>Amphiroa</i> spp.	4	8
<i>Jania</i> spp.	2	7
<i>Dasya</i> spp.	10	8
<i>Wrangelia</i> spp.	3	6
<i>Peyssonnelia</i> spp.	3	7
<i>Cryptonemia</i> spp.	2	6
<i>Lobophora variegata</i> (Phaeophyta)	1	4
Total	45	≥82

Table 1. Changes in taxon number for multiple species and genera of Bermuda macroalgae based on molecular-assisted alpha taxonomic (MAAT) analysis.

Figure 1. Collection sites in Bermuda accessed for this study in 2010 and 2012, with depth (m) indicated by pin color. Offshore sites are displayed in the smaller locus map in the upper left corner. The red box represents the area shown in the larger map.

Figure 2. UPGMA tree of Ceramiales subgrouping based on COI-5P sequences (68 taxa, 557 sites)

CHAPTER TWO

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NOTES ON THE MARINE ALGAE OF THE BERMUDAS.

13. *HELMINTHOCLADIA KEMP* SP. NOV. (NEMALIALES, LIAGORACEAE)

BASED UPON *H. CALVADOSII SENSU AUCT.*

FROM THE WESTERN ATLANTIC¹

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Abstract —Since the initial western Atlantic collections in the Florida Keys and Bermuda during the mid-1800s, *Helminthocladia calvadosii sensu auct.* (type locality: Calvados, France) has also been identified from the Caribbean Sea and as far south as northern Brazil. Prior to this study, collections from the eastern and western Atlantic had not been compared using molecular-assisted alpha taxonomy. Recent winter-spring collections of *H. calvadosii* from Bermuda display an overall habit that is distinct from eastern Atlantic plants of the same species, appearing more similar to *H. reyesii* (type locality: Canary Islands). Utilizing markers for the mitochondrial COI-5P, we have elucidated the relationships between Bermudian isolates and *H. calvadosii* from near the type locality, verifying their generic placement within the Liagoraceae and demonstrating their distinctiveness. Using vegetative and reproductive characteristics, we conclude that specimens historically identified as *H. calvadosii* from Bermuda represent a novel species, and propose *Helminthocladia kempii* Papolizio, C.W. Schneid. et Chengsupanimit sp. nov. for them.

COI-5P / *Helminthocladia calvadosii* / *Helminthocladia kempii* sp. nov. / Liagoraceae / Nemaliales / Rhodophyta

Résumé — Notes sur les algues marines des Bermudes. 13...

INTRODUCTION

Despite the heavy influence of the Caribbean on the marine flora of Bermuda (Schneider & Searles, 1998), the taxonomic preconceptions of early workers had a major influence on seaweed floristics for the archipelago. These 19th century botanists often used familiar European binomials when cataloging or reporting on the Bermudian flora (Kemp, 1857; Rein, 1873; Dickie, 1874; Hemsley, 1884; Murray, 1888, 1889). Many of the names they applied have not changed since their 19th century identifications. While some European names are still used in the local flora, many western Atlantic specimens have not been tested genetically for sequence comparison with their eastern Atlantic namesakes. In some cases, these species exhibit some morphological disparity between eastern and western populations. Previous studies clearly demonstrate the need for molecular sequence analysis in determining whether eastern and western Atlantic entities are cryptic species or single species with amphi-Atlantic distributions. Schneider & Lane (2005) showed that collections attributed to *Chondracanthus acicularis* (Roth) Fredericq (type locality: Adriatic Sea) in Bermuda represented a distinct species, *C. saundersii* C.W. Schneid. et C.E. Lane. Similarly, *Halymenia floresii* (Clemente) C. Agardh (type locality: Spain) *sensu auct.* from Bermuda was determined to be a narrow morph of *Halymenia pseudofloresii* Collins et M. Howe by Schneider et al. (2010), who further suggested that all western Atlantic specimens attributed to *H. floresii* require molecular sequencing and comparison. Most recently, a study by Schneider et al. (2011) showed that what had been identified as *Platoma cyclocolpum* (Mont.) F. Schmitz (type locality: Canary

Islands) in the western Atlantic was phylogenetically distinct from specimens found in the eastern Atlantic. Parente, Saunders & McDevit (unpublished) have shown that a Bermudian specimen of *Scytosiphon* is a genetic match with European, Azorean and Canarian *S. lomentaria* (Lyngb.) Link, thus a species still truly amphi-Atlantic, as the early workers in the western Atlantic had it.

The present study aims to resolve another instance of an early application of a European binomial, *Helminthocladia calvadosii* (J.V. Lamour. ex Duby) Setch., for plants living in the western Atlantic. It remains as one of only two species reported for the genus in the region (Wynne, 2011). This species was first collected in the western Atlantic in the Florida Keys in the 1850s. Harvey (1853) initially identified this specimen as *Helminthora divaricata* (C. Agardh) J. Agardh, the binomial that was subsequently applied to specimens collected and reported from Bermuda by Kemp (1857). When later workers studied these American specimens, they found them to be better allied with a different European-based genus and species, *Helminthocladia calvadosii* (type locality: Calvados, France) [fide Collins et al., 1915, Phycotheca Boreali-Americana (P.B.-A.) no. 2035], agreeing with Harvey (1853) that the American plants might be distinct from European *Helminthora divaricata*, the name he had applied. *Helminthocladia calvadosii* remains in use for similar specimens collected in Bermuda (Schneider, 2003) and the western Atlantic (Wynne, 2011).

We have noticed that *Helminthocladia calvadosii* in Bermuda has obvious habit differences with specimens from the eastern Atlantic, and as such, we compared anatomical characteristics, as well as COI-5P gene sequences of Bermuda specimens, to measurements and sequences in GenBank from specimens collected in Europe. We

have also extended our investigations to congeners with overlapping characteristics to determine whether our specimens of *H. calvadosii sensu auct.* from Bermuda require taxonomic action.

MATERIAL AND METHODS

Standard Methods

Collections were made in shallow water (0-2 m) and individuals were pressed fresh onto herbarium paper as permanent vouchers. Small fragments were excised prior to pressing, part desiccated in silica gel and the remaining preserved in 4-5% Formalin-seawater for anatomical study. Site locations were taken using a Garmin™ eTrex H (Olathe, Kansas, USA). Field habit photographs were taken using a Canon PowerShot S90 camera (Tokyo, Japan), herbarium specimens were scanned on an HP Photosmart Premium scanner model C-309a (Hewlett-Packard Company, Palo Alto, California, USA), and photomicrographs were taken using Carl Zeiss Axioskop 40 microscope (Oberkochen, Germany) equipped with a model 4.2 Spot InSight QE digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA). A Zeiss camera lucida was used for the cellular line drawings. All digital images were composed in Adobe Photoshop™ CS5 Extended v. 12.0.2 (Adobe Systems, San Jose, California, USA). Voucher specimens are deposited in GALW, MICH, MSM, NY, US, the Bermuda Natural History Museum and Herbarium CWS. Herbarium abbreviations follow the online Index Herbariorum (<http://sweetgum.nybg.org/ih/>) and standard author initials are from Brummitt & Powell (1992). The *P.B.-A.* specimen used in this study is from the exsiccata in CWS' personal herbarium.

The extra-Bermuda collections were processed at the University of New Brunswick following established field and vouchersing protocols (Saunders & McDevit, 2012). Vouchers are deposited in UNB and all of the pertinent metadata are publicly available in the dataset HELMN01 on the BOLDSYSTEMS web site (www.barcodinglife.org) and summarized here (Table 1).

Molecular Methods

Silica dried samples for DNA analysis were ground in liquid nitrogen and stored at -20°C (Table 1). For Bermudian collections, DNA was extracted from 100 µl ground material using the DNA extraction buffer from Saunders (1993) followed by incubation at 23°C for 1 hr and then incubation on ice chips for 20 min. Samples were spun at 10,000xg for 10 minutes and the elute was transferred into the extraction column of the Sigma-Aldrich (St. Louis, MO) GenElute Plant Genomic Miniprep Kit. The remaining protocol was followed according to manufacturer's protocol. DNA extraction of non-Bermuda collections followed published protocols (Saunders & McDevit, 2012).

For Bermudian collections the COI-5P region was PCR amplified with the Takara Ex-Taq DNA polymerase kit (PanVera, Madison, WI, USA) in an Eppendorf AG Mastercycler epGradient thermal cycler (Hamburg, Germany). Amplified DNA was treated with Sigma-Aldrich GenElute PCR Clean-Up Kit following the manufacturer's protocol and the purified PCR product was sequenced at the Rhode Island Genomics and Sequencing Center using the ABI 3130xl genetic analyzer. For the non-Bermuda collections the COI-5P was amplified following published protocols

that do not require subsequent cleaning of the product (Saunders & McDevit, 2012) with the sequencing outsourced to Genome Québec (www.genomequebec.com). The actual primer pair used with each specimen is available in the dataset HELMN01 on the BOLDSYSTEMS web site and with each entry at GenBank (Table 1).

Fourteen COI-5P sequences from representative *Helminthocladia* spp., including those available through GenBank and newly determined here, were included in an alignment with 14 sequences (outgroup) from the closely allied species *Cumagloia andersonii* (Huisman et al., 2004). Sequences were first aligned in Geneious Pro on a MacPro (OS X version 10.6.8) and the appropriate model parameters estimated (AIC) in Modeltest (v. 3.06; Posada & Crandall, 1998) as implemented in PAUP. The selected model was used to complete maximum-likelihood analyses in PHYML 3.0 (Guindon & Gascuel, 2003) with BIONJ used to designate the starting tree, best of nearest-neighbor interchange (NNI) or subtree pruning and regrafting (SPR) branch-swapping options, and with the tree topology, branch lengths and substitution rates optimized. Data partitioning was not implemented. Branch support was estimated using 500 bootstrap replicates.

RESULTS AND DISCUSSION

Helminthocladia 'calvadosii' has been found from January to April in intertidal and shallow subtidal habitats on both the southern and northern shores of Bermuda Is., as well as the northwestern coast of St. George's Is., at rocky sites experiencing moderate to heavy wave action. Although previously recorded as

abundant from a number of Bermuda's bays (Taylor & Bernatowicz, 1969), at present we have found this species to be infrequent to rare except at the Spanish Point site where it remains abundant. The winter-spring collections were found when seawater temperatures in Bermuda average from 18-20°C, the cooler end of the warm temperate biogeographic range. Of the sixteen currently accepted species of *Helminthocladia* (Guiry & Guiry, 2012), most are found in warm temperate seas in the spring-summer months (O'Dwyer & Alfonso-Carrillo, 2001), and a few are found in tropical seas (Guiry & Guiry, 2012). Because biogeographical distribution patterns of species are primarily controlled by the physiological tolerance of seaweeds' life cycle stages to temperature (Bartsch et al., 2012), we can infer that Bermuda's *H. 'calvadosii'* is acclimated to the cooler winter water temperatures found there, distinguishing it from other species in the genus.

Helminthocladia from Bermuda has a distinctly subdichotomous branching pattern of axes that can clearly be observed near the apices of branches (Figs 2, 3). This developmental pattern is at odds with plants of *H. calvadosii* from Atlantic European and Mediterranean waters. European specimens of *H. calvadosii* (Kützinger, 1866; Hauck, 1885, as *H. purpurea* (Harv.) J. Agardh; Gayral, 1966; Dixon & Irvine, 1977; O'Dwyer & Alfonso-Carrillo, 2001) are shown to have prominent irregularly or pectinately branched main axes (see Kützinger, 1866, pl. 62c, as *Nemalion purpureum* (Harv.) Chauv.; Hauck, 1885, fig. 17a, as *H. purpurea*; O'Dwyer & Alfonso-Carrillo, 2001, fig. 1). Despite currently being placed in *Helminthocladia*, the specimens from Bermuda actually have a branching pattern more similar to *Helminthora divaricata*, the species with which they were initially allied in the western Atlantic. Other than its

macroscopic habit, no other single feature exclusively distinguishes Bermuda specimens of *Helminthocladia* 'calvadosii' from morphologically similar species in the genus. However, a collective analysis of a variety of vegetative and reproductive characters demonstrates its distinctiveness from the European type and similar congeners (Table 2).

Bermuda collections have longer cortical fascicles, to 230 μm , than European specimens of *Helminthocladia calvadosii* (<180 μm cortical fascicles) (Table 2; O'Dwyer & Afonso-Carrillo, 2001). Although measurements of anatomical and morphological features are often variable for algae in general, considerable differences in overall thallus size exist between European and Bermudian collections determined as *H. calvadosii*. While *H. calvadosii* produces individuals to 60 cm in Europe, specimens from Bermuda measure only to 19 cm in the later parts of growing season in the islands. In addition, the main axes in the largest Bermuda samples are about one-fourth the diameter of mature European specimens. Therefore, it appears that morphologically and anatomically, the European and Bermudian specimens attributed to *H. calvadosii* do not represent the same species (Table 2). In fact, the size and general habit of our Bermuda collections are more reminiscent of the smaller, more recently described, species *H. reyesii* O'Dwyer et Afonso-Carr. from the Canary Islands. However, despite their habit similarities, Bermudian *Helminthocladia* 'calvadosii' and *H. reyesii* have some differences in cellular dimensions. Both the apical and basal cells of cortical filaments are one half or less in *H. 'calvadosii'* than *H. reyesii* (Table 2). Bermudian *H. 'calvadosii'* generally has shorter cortical fascicles

(to 230 mm) than *H. reyesii* (to 400 mm), as well as a fewer number of cells making up the cortical filaments (3-6 cells vs. the 6-8 cells in *H. reyesii*).

While reproductive characters are less obvious and more difficult to locate in specimens, they provide strong evidence that Bermudian *Helminthocladia* '*calvadosii*' is distinct from its congener in the Canary Islands. O'Dwyer & Afonso-Carrillo (2001) note that the occurrence and abundance of post-fertilization sterile filaments in species of *Helminthocladia* can vary considerably. *Helminthocladia reyesii* produces numerous moniliform sterile filaments from the two supra-supporting cells (distal to the supporting cell), these partially or completely surrounding the carpogonial branch (O'Dwyer & Afonso-Carrillo, 2001). When fully developed, these sterile cells form a mass below the gonimoblast (O'Dwyer & Afonso-Carrillo, 2001). Bermudian *H. 'calvadosii'* produces sterile, elongated cells that wrap around post-fertilization carpogonial branches, however the sterile cells are merely a few, and develop primarily from the supporting cell of the carpogonial branch. Moreover, gonimoblast filament diameter is smaller in *H. 'calvadosii'* when compared with both European *H. calvadosii* and *H. reyesii* (Table 2).

Only three species of *Helminthocladia* other than *H. reyesii* are subdichotomously branched: *H. andersonii* Searles et S.M. Lewis (type locality = North Carolina, USA), *H. densa* (Harv.) F. Schmitz et Hauptfl. (t. l. = Tasmania), and *H. dotyi* Womersley (t. l. = Victoria, Australia). Among these, *H. andersonii* is the only other species currently recognized in the genus from the western Atlantic (Searles & Lewis, 1983). It differs from Bermudian *H. 'calvadosii'* by its small size (to 5 cm tall), overall habit and lax medulla, production of descendant rhizoids from sterile

cells, and a strongly developed involucre around the gonimoblasts (Searles & Lewis, 1983). The two Australian species, *H. densa* and *H. dotyi*, are repeatedly subdichotomous and thus more densely branched than the Bermuda specimens, the latter being a small species, 2-7 cm tall, with a massive base (Womersley, 1965; Huisman, 2006). *Helminthocladia densa* has barely enlarged terminal cells on cortical fascicles (Womersley, 1965), unlike the specimens collected in Bermuda. Both of these species lack the heavy investment of adventitious branches around their axes, and neither would be confused with the Bermuda collections.

Evaluating the COI-5P barcode sequences, Bermudian specimens form a distinct genetic species group that resolves deeply among the other included species of *Helminthocladia* being most closely allied (Fig. 1) to *H. rhizoidea* Doty et I.A. Abbott, a species with unique rhizoidal features (Doty & Abbott, 1961). This genetic species group did not align with *H. calvadosii* from near the type locality in France (Fig. 1), corroborating our morphological findings. Unfortunately, we were unable to obtain fresh material of *H. reyesii* for DNA sequencing and comparison from workers in the Canary Is. who describe the species as rarely and not predictably collected (Afonso-Carrillo, pers. comm.). It should also be noted that specimens attributed to *H. australis* resolved into two distinct genetic species indicating that future species-level taxonomic work remains for this genus (Fig. 1).

Therefore, given the morphological and molecular evidence, Bermudian collections of *Helminthocladia*, ascribed for the past hundred years as *H. calvadosii*, are proposed here as a new species:

***Helminthocladia kempii* Popolizio, C.W. Schneid. et Chengsupanimit sp. nov.**

(Figs 2-11).

Description — Plants reddish-brown in color, erect, arising from a simple discoidal holdfast, to 19 cm tall; axes mucilaginous, smooth and slippery but firm, and subdichotomously branched (Figs 2, 3); main axes 0.5-2.5 mm in diameter, typically overwhelmed by short, irregularly disposed adventitious lateral branches (Figs 2, 3), some of these remaining undeveloped and dichotomous, whereas others become additional leading axes; cortical assimilatory filaments branched into fan-like clusters, 3-6 cells from base to apex, and to 230 μm in length (Fig. 4); basal cortical cells 7-10 μm in diameter and 18-35 μm long with shorter subterminal cells 12-15 μm diameter and 15-22 μm long, and apical cells 10-20 μm in diameter and 20-35 μm long; medullary axes composed of filaments with cells 11-27 μm in diameter and 70-175 μm long; gametophytes dioecious, spermatangial clusters paniculate with globose spermatangia 2-5 μm in diameter (Fig. 5); carpogonial branches consisting of 3-4 cells (Fig. 6), 7.5-10.0 μm in diameter and 30-38 μm long; first division of the carpogonium transverse or obliquely transverse after fertilization and following excision of the trichogyne (Figs 7, 9); gonimoblast filaments issued from divided carpogonium cells (Figs 10, 11), comprised of cells 3.5-4.5 μm in diameter and 7.0-9.0 μm long, producing carposporangia 3.0-5.0 μm in diameter and 7.0-9.0 μm long; few sterile post-fertilization cells present, arising from the supra- and infra-supporting cells adjacent to the carpogonial branch, and wrapping around the carpogonial branches (Figs 10, 11); a weak involucre of elongate sterile filaments forming around

the carposporophytes produced from adjacent cortical cells of the same assimilatory fascicle (Figs 8, 11).

Etymology: Named for the Rev. Alexander Ferrie Kemp (1822-1884), a Scottish-born Canadian Presbyterian minister posted to Bermuda during the 1850s when he collected and published the first report on the algae of the islands (Kemp, 1857), including the first collection of the red alga that now bears his name.

Misapplied names for Bermuda: *Helminthora divaricata* (C. Agardh) J. Agardh *sensu auct.*; *Helminthocladia calvadosii* Mont. *sensu* W.R. Taylor, 1960, p. 432, pl. 80, fig. 2.

Holotype: C.W. Schneider 12-10-14, 18.i.2012, cove immediately east of Spanish Point Park, Bermuda Is., Bermuda, western Atlantic, 32°18'25.5"N, 64°48'48.6"W, 0-1 m on rock [MICH] (BOLD, BERMR312-12; GenBank, KC250437) (Fig. 2);
isotypes KIRI, NY, PC, US, UNB and Herb. CWS (BOLD, BERMR313-12; GenBank, KC250436) (Fig. 3).

Paratypes — Bermuda: F.S. Collins, P.B.-A. no. 2035 [Collins et al., 1915, as *H. calvadosii*], 27.iv.1912, Long Bird Is.; C.W. Schneider (CWS)/C.E. Lane (CEL) 02-5-35, 13.iv.2002, Whalebone Bay, St George's Is., 32°21'49.00"N, 64°42'45.77"W, depth 1 m; CWS/CEL 03-19-3, 1.iv.2003, Battery Park Beach, St. George's Is., 32°22'48.32"N, 64°39'54.68"W, tide pool; CWS/CEL 03-21-4, 1.iv.2003, West

Whale Bay, Southampton, Bermuda Is., 32°15.3'N, 64°48.6'W, intertidal rock;
 CWS/CEL 03-31-18, 4.iv.2003, Tobacco Bay, St George's Is., 32°23'20.1"N,
 64°40'44.1"W, depth 2 m; CWS/CEL 08-31-2, 16.ii.2008, cove immediately east of
 Spanish Point Park, Bermuda Is., loc. cit.; CWS/CEL 08-43-7, 20.ii.2008, West Whale
 Bay, loc. cit.; T.R. Popolizio (TRP) /CWS 12-30-6, Grape Bay Beach, Bermuda Is.,
 32°17'03.6"N, 64°45'52.6"W, intertidal rock; TRP/CWS 12-33-6, 15.ii.2012, Bowen
 Pt., Shelly Bay, Bermuda Is., 32°20'45.2"N, 64°42'30.4"W, depth 1-2 m.; TRP/CWS
 12-51-1, 11.iv.2012, Whalebone Bay, loc. cit. **Florida:** I.M. Lamb no. A-34,
 8.iii.1958, Haulover Pier, N. Miami, intertidal pilings [MICH, as *H. calvadosii*].

Geographic distribution: In the western Atlantic, *Helminthocladia kempii* was first reported by Harvey as *Helminthora divaricata* in his *Nereis Boreali-Americana* (1853) based on a single Key West, Florida specimen. Of this specimen, Harvey thought that in branching pattern it may possibly be distinct from European specimens of *H. divaricata* he had seen, but in many anatomical characteristics, the eastern and western Atlantic specimens were quite the same. Following Harvey, early workers called Bermuda specimens *H. divaricata* (Kemp, 1857; Rein, 1873; Hemsley, 1884; Murray, 1889). Setchell (in Collins et al., 1915) alerted Collins & Hervey (1917) that the Bermuda specimens were actually *Helminthocladia calvadosii*, the name presented to all western Atlantic specimens until now. After examining *P.B.-A.* 2035 from Bermuda, Feldmann (1939) suspected that the American specimens of *H. calvadosii* were not only different from the European type but possibly a new species of Nemalion. Taylor (1960, pl. 43, fig. 5, as *H. calvadosii*) illustrated a typical western

Atlantic form of *H. kempii*, probably using a Bermuda specimen as the model, as he had numerous specimens from the islands and only one from Florida in MICH at that time. Since Taylor (1960), *H. calvadosii* has been reported for the western Atlantic from Dominica (Taylor, 1969), Venezuela (Díaz-Piferrer, 1970) and Brazil (Guimarães et al., 1990). The only of these reports with morphological and anatomical data is that from Brazil (Guimarães et al., 1990), and it shows that these South American plants likely do not represent *H. kempii*. The Brazilian plants differ in height and branching pattern, and are monoecious, all features similar to European *H. calvadosii*, despite sharing some anatomical dimensions with *H. kempii* (Table 2; Guimarães et al., 1990). Further work, including genetic analyses, is necessary to determine the specific placement of these South American and Caribbean plants relative to European *H. calvadosii* and North American *H. kempii*.

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Table 1. List of the isolates used in study with collection details and BOLD/GenBank accession numbers (**bolded** if newly sequenced in this study).

Name	Voucher	BOLD	GenBank	Collector(s)	Collection site
<i>Cumagloia andersonii</i> (Farl.) Setch. & N.L. Gardner	GWS001399	ABMMC5750-09	HM916454	G.W. Saunders	Blowhole at Bradys Beach, Bamfield, BC, Canada
	GWS002881	ABMMC653-06	HQ603209	G.W. Saunders	‘Sparlingia Pt.’ Bradys Beach, Bamfield, BC, Canada
	GWS002882	ABMMC654-06	HQ603208	G.W. Saunders	‘Sparlingia Pt.’, Bradys Beach, Bamfield, BC, Canada
	GWS003158	ABMMC835-06	HQ603210	G.W. Saunders	Pachena Beach, Bamfield, BC, Canada
	GWS003219	ABMMC1078-06	HQ603211	G.W. Saunders	Bradys Beach, Bamfield, BC, Canada
	GWS004303	ABMMC1096-06	HQ603217	G.W. Saunders, B. Clarkston & D. McDevit	Pachena Beach, Bamfield, BC, Canada
	GWS004304	ABMMC1309-07	HQ603216	G.W. Saunders, B. Clarkston & D. McDevit	Pachena Beach, Bamfield BC, Canada
	GWS004305	ABMMC1310-07	HQ603215	G.W. Saunders, B. Clarkston & D. McDevit	Pachena Beach, Bamfield BC, Canada
	GWS004393	ABMMC1312-07	HQ603213	G.W. Saunders, B. Clarkston & D. McDevit	Botanical Beach, Port Renfrew, Vancouver I., BC, Canada
	GWS004593	ABMMC1104-06	HQ603214	G.W. Saunders, B. Clarkston & D. McDevit	Pachena Beach, Bamfield, BC, Canada
	GWS009153	ABMMC2198-08	HQ603212	G.W. Saunders & B. Clarkston	Waypoint #45 from Land’s End to Pachena Beach, Bamfield, BC, Canada
	GWS021723	ABMMC11691-10	HQ603206	B. Clarkston, K. Hind & S. Toews	Soberanes Point, California, USA
	GWS021922	ABMMC12110-10	HQ603207	B. Clarkston, K. Hind & S. Toews	Santa Cruz (Four Mile), California, USA

	JAW4767	ABMMC14744-11	KC250433	J. West	Otter Point, Oregon, USA
<i>Helminthocladia australis</i> Harv.	GWS014805	ABMMC7082-10	HM917412	G.T. Kraft & G.W. Saunders	Point Lonsdale Lighthouse Reef, Victoria, Australia
	GWS016628	ABMMC8367-10	HM918280	G.W. Saunders, L. Kraft & K. Dixon	The Springs, Point Lonsdale, Victoria, Australia
	GWS022786	OZSEA195-10	KC250429	R. Withall	Lord Howe I., Neds Beach, NSW, Australia
	GWS022787	OZSEA196-10	KC250434	R. Withall	Lord Howe I., Neds Beach, NSW, Australia
	GWS022788	OZSEA197-10	KC250430	R. Withall	Lord Howe I., Neds Beach, NSW, Australia
	GWS022789	OZSEA198-10	KC250438	R. Withall	Lord Howe I., Neds Beach, NSW, Australia
<i>Helminthocladia calvadosii</i> (Duby) Setch.	LLG3069	CNEM002-10	HQ603218	Y. Galdu, L. Le Gall & A. Besnier	Beniguet, Finistère, Brittany, France
<i>Helminthocladia dotyi</i> Womersley	GWS002626	ABMMC8944-10	KC355084	G.W. Saunders	Remarkable Cave near Port Arthur, Tasmania, Australia
<i>Helminthocladia hudsonii</i> J. Agardh	LLG3000	CNEM003-10	HQ603219	L. Le Gall, J.M. Utge & Y. Turpin	Lampaul île Ségat, Finistère, Brittany, France
<i>Helminthocladia kempii</i> sp. nov.	CWS12-10-14 (BDA591)	BERMR312-12	KC250437	C.W. Schneider	Cove east of Spanish Point, Bermuda I., Bermuda
	CWS12-10-14 (BDA592)	BERMR313-12	KC250436	C.W. Schneider	Cove east of Spanish Point, Bermuda I., Bermuda
	TRP12-30-6 (BDA813)	BERMR314-12	KC250432	T.R. Popolizio	Grape Bay, Bermuda I., Bermuda
	TRP12-33-6 (BDA856)	BERMR316-12	KC250435	T.R. Popolizio	Bowen Pt., Shelly Bay, Bermuda I., Bermuda
	TRP12-51-1 (BDA1013)	BERMR317-12	KC250431	T.R. Popolizio	Whalebone Bay, St. George's I., Bermuda
<i>Helminthocladia rhizoidea</i> Doty & I.A. Abbott	HADB03858	—	HQ423009	A. Kurihara	Malaekahana Beach Park, Oahu, Hawaii

Table 2. Comparison of *Helminthocladia* from Bermuda and other morphologically related Atlantic species.

	<i>Helminthocladia calvadosii</i>	<i>Helminthocladia</i> ' <i>calvadosii</i> '	<i>Helminthocladia kempii</i> sp. nov.	<i>Helminthocladia reyesii</i>
Geographic distribution	Atl. Europe, Mediterranean	Brazil	Bermuda, Florida	Canary Islands
Thallus				
Height (cm)	to 60	20-60	to 19	to 26
Main axis diam. (mm)	0.4-15.0	5-15	0.5-2.5	to 10
Branching pattern	radially to irregularly branched; lateral adventitious branches few to many, long, simple or little branched	alternately lateral to irregularly branched, numerous simple adventitious branches covering axes	subdichotomously branched, axes obscured by numerous short adventitious laterals, mostly simple, few branched	main axes initially subdichotomous to 5 orders of branching; adventitious laterals perpendicular to the main axes, usually short and simple or branched
Medullary filaments				
Size 2 mm fr. apex (diam. x length μ m)	3-6 x 80-150	nd	3-12 x 35-70	5-22 x 60-200
Size 15 mm fr. apex (diam. x length μ m)	to 12 x to 160	nd	11-27 x 70-175	20-50 x 200-350
Cortical fascicles				
Number of cells, base to apex	4-6	3-5 ^a	3-6	6-8
Length of cortical fascicles (μ m)	up to 180	up to 250 ^a	up to 230	up to 400
Rhizoidal connections of cortical filaments	absent	absent	absent	absent

Basal cell (diam. x length in μm)	7.5-9.0 x 20-30	7.5-10.0 x 25-35 ^a	7-10 x 18-35	7.5-20.0 x 52-100
Subterminal cell (diam. x length μm)	13.0-17.5 x 15-20	9.5-12.0 x 15-25 ^a	12-15 x 15-22	6-19 x 11-28 ^b
Apical cell (diam. x length μm)	(15-)20-26(-32) x 27-36(-50)	15-23 x 25-35(-52) ^a	10-20 x 20-35	20-35 x 36-60
Reproduction				
Gametophyte	monoecious	monoecious	dioecious	monoecious or dioecious
Spermatangium diam. (μm)	3-5	2.5-5.0 ^a	2-5	2-3
Spermatangial clusters	paniculate	paniculate	paniculate	paniculate
Carpogonial branch (diam. x length μm)	8-10 x 21-23	7.5-11 x 20-22 ^a	7.5-10.0 x 30-38	10-13 x 24-43
Number carpo gonial branch cells	(2-)3(-4)	3-4 ^a	3-4	3(-4)
First carpo gonial division	oblique or transverse	oblique	transverse or oblique	transversely oblique
Post-fertilization fusion of carpo gonial branch cells	present	absent	absent, the pit-connections often greatly enlarged post-fertilization	present
Sterile post-fertilization filaments	absent to common, arising from the supra- and infra-supporting cells, occasionally from adjacent cortical cells	nd	present but few, arising from the supra- and infra-supporting cells	few or forming a compact mass of moniliform cells below the gonimoblast
Gonimoblast filament (diam. x length μm)	4-8 x 12-15	2-4 x 9-15 ^a	3.5-4.5 x 7-9	3-5 x 10-16
Mature carposporophyte diam. (μm)	100-270	85-130	50-80	140-250

Carposporangia (diam. x length μm)	7-9 x 15-20	3.5-6.5 x 7.0-12.5 ^a	3-5 x 7-9	5-7 x 11-20
Seasonal appearance	May-Oct	Apr-Sept	Jan-Apr	(Mar-) Apr-Jul (-Aug)
References	Dixon & Irvine (1977) O'Dwyer & Afonso-Carrillo (2001)	Guimarães <i>et al.</i> (1990)	Present study	O'Dwyer & Afonso- Carrillo (2001)

^a calculated from

Guimarães *et al.* (1990)

^b calculated from O'Dwyer & Afonso-Carrillo (2001)

nd = no data

Figure 1. Maximum likelihood phylogeny generated from the COI-5P alignment resolving the Bermudian collections assigned here to *H. kempii* sp. nov. as sister to *H. rhizoidea* and only distantly related to *H. calvadosii*. Bootstrap support (% of 500 replicates) is indicated along the branches. Refer to Table 1 for collection data.

Figures 2-7. *Helminthocladia kempii* sp. nov. **2.** Holotype specimen, *CWS* 12-10-14 (DNA0591). Scale bar = 2 cm. **3.** Isotype specimens. Scale bar = 2 cm. **4.** Cortical fascicle, *CWS* 12-10-14. Scale bar = 25 μ m. **5.** Spermatangial cluster on cortical fascicle, *CWS* 12-10-14. Scale bar = 25 μ m. **6.** Three- and four-celled carpogonial branches with extended trichogynes, *CWS/CEL* 03-31-18. Scale bar = 25 μ m. **7.** Post-fertilization carpogonia with early gonimoblast initials (arrows) and withering trichogynes, *CWS/CEL* 03-31-18. Scale bar = 25 μ m. **8.** Carposporophyte with weakly investing sterile involucreal filaments, *CWS/CEL* 03-31-18. Scale bar = 25 μ m.

Figures 9-11. *Helminthocladia kempii* sp. nov. Scale bar = 25 μ m. **9.** Four-celled carpogonial branch with divided carpogonium and previously cut-off trichogyne. **10.** Early gonimoblast initial divisions on carpogonial branch with associated sterile cell (trichogyne already excised and missing). **11.** Young carposporophyte showing enlargement of pit connections between carpogonial branch cells, gonimoblast initials cut off upper portion of carpogonial branch, sterile cell pit-connected to a supra-supporting cell and formation of sterile filaments. **Abbreviations:** cs, carposporophyte; dc, divided carpogonium; gbi, gonimoblast initials; isc, infra-supporting cell; sf, sterile filament; sc, supporting cell; ssc, supra-supporting cell; stc, sterile cell; t, trichogyne.

CHAPTER THREE

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A MOLECULAR EVALUATION OF THE LIAGORACEAE *SENSU LATO*
(NEMALIALES, RHODOPHYTA) IN BERMUDA, INCLUDING
LIAGORA NESOPHILA SP. NOV. AND *YAMADAELLA GRASSYI* SP. NOV.

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We have undertaken a comprehensive, molecular-assisted alpha-taxonomic (MAAT) examination of the rhodophyte family Liagoraceae *sensu lato*, a group that has not previously been targeted for molecular studies in the western Atlantic. Sequence data from three molecular markers indicate that in Bermuda alone there are 10 species in nine different genera. These include the addition of three genera to the flora — *Hommersandiophycus*, *Trichogloeopsis* and *Yamadaella*. *Liagora pectinata*, a species with a type locality in Bermuda, is phylogenetically allied with Indo-Pacific species of *Hommersandiophycus*, and the species historically reported as *L. ceranoides* for the islands is morphologically and genetically distinct from that taxon, and is herein described as *L. nesophila* sp. nov. Molecular sequence data has also uncovered the Indo-Pacific *L. mannarensis* in Bermuda, a long-distance new western Atlantic record. DNA sequences of *Trichogloeopsis pedicellata* from the type locality (Bahamas) match with local specimens demonstrating its presence in Bermuda. We describe *Yamadaella grassyi* sp. nov. from Bermuda, a species phylogenetically and morphologically distinct from the generitype, *Y. caenomyce* of the Indo-Pacific. Our data also indicate a single species each of *Ganonema*, *Gloiocallis*, *Helminthocladia*, *Titanophycus* and *Trichogloea* in the flora.

Key index words: Bermuda, COI-5P, *Liagora*, *L. nesophila* sp. nov., Liagoraceae *sensu lato*, LSU, *Ganonema*, *Gloiocallis*, *Helminthocladia*, *Hommersandiophycus*, *H. pectinatus* comb. nov., *rbcL*, *Titanophycus*, *Trichogloea*, *Trichogloeopsis*, *Yamadaella*, *Y. grassyi* sp. nov.

Abbreviations: COI-5P, 5' region of the cytochrome oxidase subunit 1 gene; LSU, nuclear large subunit ribosomal DNA; *rbcL*, large subunit ribulose-1,5-bisphosphate carboxylase/oxygenase gene

The first molecular phylogenetic investigation of the red algal order Nemaliales (Schmitz 1892) was conducted by Huisman et al. (2004) who used nuclear large subunit ribosomal DNA (LSU) sequences to firmly establish three familial lineages within the order: the Liagoraceae (Kützinger 1843), Galaxauraceae (Parkinson 1983) and Scinaiceae (Huisman et al. 2004). Le Gall and Saunders (2010) used DNA barcoding to categorize the Nemaliales for the coasts of Canada and France, discovering unreported diversity within the genus *Nemalion* and confirming the familial distinctions of Huisman and coworkers. The Liagoraceae *sensu lato* is by far the most diverse of the families in the order, with 26 genera and 153 species currently accepted worldwide (Guiry and Guiry 2014). Its members are widely distributed in tropical and temperate seas, but exhibit the greatest diversity in warm waters (Huisman 2002, 2006). Lin et al. (2015) have recently recognized six families in the order, separating out three families from the Liagoraceae by resurrecting the Nemaliaceae and creating two monogeneric new families, the Liagoropsidaceae and the Yamadaellaceae.

The distinctive anatomical and reproductive characteristics of the Liagoraceae *sensu lato* include the presence of multiaxial thalli with a subdichotomously or trichotomously branched filamentous cortex, and post-fertilization development where the gonimoblast initiates directly from one or both cells of the divided carpogonium

(Huisman 2002, 2006). Traditionally, genera and species have predominantly been recognized based on distinguishing reproductive characteristics such as the size, shape and origin of the carpogonial branch, the nature of the gonimoblast (compact vs. diffuse), the presence (or lack thereof) and arrangement of the sterile filaments associated with the carposporophyte, as well as spermatangial development (Kraft 1989, Huisman 2002, Lin et al. 2015). In addition, vegetative characters such as branching patterns, degree of calcification, and cortical cell shape and size are common identifiers, albeit generally less salient. Given that a great number of species in the Liagoraceae have remarkably similar morphologies, the group has suffered a long history of taxonomic ambiguity (Lin et al. 2013). To remedy this, modern diagnostics of generic (and specific) placement integrate DNA sequence analysis with the examination of carposporophyte development and other characters, resulting in a stronger taxonomic organization and a more accurate representation of biodiversity.

Though members of the Liagoraceae *sensu lato* were significantly consolidated following several morphological studies in the 20th century (e.g., Abbott 1990a, 1990b), the increasing use of molecular sequence data in systematic research over the past decade has resulted in the descriptions of several new genera and species (Huisman et al. 2004). For example, the novel species *Helminthocladia kempii* Popolizio, C.W.Schneid. et Chengsupanimit was recently described from Bermuda in the western Atlantic (Popolizio *et al.* 2013), the genus and species *Yoshizakia indopacifica* S.-M. Lin, Huisman & C. Payri was newly erected from the Indo-Pacific (Lin et al. 2013), and the genera *Hommersandiophycus* and *Gloiocallis* were segregated from *Ganonema* (Lin et al. 2014). Continuing from our investigation of

Helminthocladia in Bermuda, the present study represents the first comprehensive analysis of the Liagoraceae and Yamadaellaceae using molecular tools for specimens from a region of the western Atlantic.

MATERIALS AND METHODS

Standard methods

Collections were made in shallow water (0-3 m) or via scuba (0-23 m) and site locations were taken using a Garmin™ eTrex H (Olathe, Kansas, USA). A portion of each specimen used for DNA analysis was then dried on silica gel and the remainder of the thallus was pressed onto herbarium paper as a permanent voucher. Selected fragments were preserved in 4-5% Formalin in seawater for anatomical study. Specimens for squash mounts were briefly soaked in an 8% solution of HCL to decalcify. Squashes 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. Live specimens chosen for DNA analysis were photographed using a Canon Powershot s90 digital camera (Canon Inc., Tokyo, Japan) and dried herbarium specimens were scanned on an HP 309a Photosmart Premium scanner (Hewlett-Packard Company, Palo Alto, California, USA). 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). Voucher specimens of some numbers are deposited in KIRI, MICH, NY, the

Bermuda Natural History Museum (BAMZ) and CWS's personal herbarium.

Herbarium abbreviations follow the online Index Herbariorum

<<http://sweetgum.nybg.org/ih/>> and standard author initials are from Brummitt and Powell (1992).

Molecular methods

Specimens used in molecular analyses are recorded in Table 1. Silica dried samples for DNA analysis were ground in liquid nitrogen and stored at -20°C . DNA was extracted from 0.1-0.5 μl ground material using the Sigma-Aldrich (St. Louis, Missouri, USA) GenElute Plant Genomic Miniprep Kit according to manufacturer protocol, with 500 μl of modified lysis solution (50 μl 10% TWEEN 20 and 5 μl of 20 mg/ml ProK), as well as 1 hr of incubation at 23°C followed by 20 min on ice (Saunders and Druehl 1993).

DNA was amplified via polymerase chain reaction (PCR) with the Takara Ex-Taq DNA polymerase kit (PanVera, Madison, WI, USA) in an Eppendorf AG Mastercycler epGradient thermal cycler (Eppendorf, Hamburg, Germany). To assign all specimens to species groups, two oligonucleotide primers were used for both sequencing and amplification of the COI-5P mitochondrial marker, GWSFn (Le Gall and Saunders 2010) and GWSRx (Saunders and McDevit 2012). A denaturation cycle of 94°C for 4 min was followed by 38-42 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 7 min. Several specimens were selected for additional sequencing of the plastid-encoded RuBisCO (*rbcL*) and nuclear large subunit rDNA (LSU) operons. Amplification and/or sequencing reactions for

rbcL were conducted using the primers and thermal profile demonstrated by Freshwater and Rueness (1994). In some instances, the 3' portion of the *rbcL* was alternatively amplified and sequenced using the primers (TLF4 with *rbcLrevNEW*) and amplification profile presented by Saunders and Moore (2013). The LSU marker was amplified and prepared for sequencing using the primers and protocols outlined by Harper and Saunders (2001) and Saunders and Moore (2013). All amplified DNA was treated with the Qiagen (Redwood City, California, USA) QIAquick PCR Purification Kit following the manufacturer's protocol, and the purified PCR product was sequenced at the Rhode Island Genomics and Sequencing Center using the Applied Biosystems Inc. 3130xl Genetic Analyzer (Life Technologies, Grand Island, New York, USA).

COI-5P barcode sequences from representative liagoracean spp., including those available through GenBank and newly determined here, were aligned using the MUSCLE (multiple sequence comparison by log-expectation) alignment program in Geneious (v. 6.1.8 available from <http://www.geneious.com>). To visually characterize genetic variability among specimens, the UPGMA clustering algorithm was applied to the COI-5P alignment (105 specimens, 541 sites) with Tamura-nei-corrected distances (default setting). The resulting tree was used to demarcate genetic species groups. Based on these groups, and with comparative data available from GenBank, one specimen from each species and/or geographic location was selected for phylogenetic analysis using *rbcL* and LSU sequences. The most variable portion of the 3' "Z" fragment (Saunders and Moore 2013) of the LSU marker was missing from several GenBank specimens (~215 bp). The remaining sequence data in this fragment were

highly conserved, between 97.1% and 99.8% identical across all genera in the family. Thus, the alignment was trimmed of the “Z” fragment and the more variable region representing the “X” and “Y” fragments (Saunders and Moore 2013) retained for analysis. The best models of evolution for the individual gene regions *rbcL* (50 taxa, 1270 sites) and LSU (33 taxa, 1586 sites) were determined in jModelTest 2 (version 2.1.5; Darriba et al. 2012). The selected phylogenetic model (GTR+I+G in both instances) was used to complete both maximum likelihood (ML) and Bayesian analyses for each gene. Both the *rbcL* and LSU maximum likelihood phylogenies were estimated using the RAxML graphical user interface (Silvestro and Michalak 2012) with branch support calculated using 1000 bootstrap replicates. Bayesian analysis of *rbcL* was conducted in MrBayes v.3.2.2 (Ronquist and Huelsenbeck 2003) and run with four parallel chains (three heated + one cold) with branch lengths optimized during the run for one million generations. The initial 2500 trees were discarded as the burn-in, and posterior probabilities were estimated based on the remaining trees. The LSU analysis was conducted with the same parameters as *rbcL*, but with two million generations. Stationarity was attained after the first 500,000 generations (burnin = 5000 trees). Both the *rbcL* and LSU gene trees include members of the closely related Scinaiceae and Galaxauraceae families as outgroups. All trees (Figs 1, 2 and S1) were manipulated for presentation using FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

RESULTS AND DISCUSSION

Molecular observations

Our DNA barcode analyses included 105 sequences from members of the Liagoraceae and Yamadaellaceae; 78 of these were from Bermuda collections. The final COI-5P barcode alignment consisted of 541 base pairs, of which 40.5% were informative characters. This tree (Fig. S1) was used to initially align genetic species groupings (Saunders and McDevit 2012). The 78 Bermuda specimens resolved as ten genetic species groups (Fig. S1) and allied with six established genera for which comparative sequence data were available (*Ganonema*, *Helminthocladia*, *Hommersandiophycus*, *Liagora*, *Titanophycus* and *Yamadaella*). Specimens identified as *Trichogloea herveyi* W.R. Taylor matched published barcode data (Le Gall and Saunders 2010) for this species collected from the type locality (= Bermuda). Two species groups formed distinct clades that were not clustered with the other genera, and *rbcL* data later revealed these to be *Gloiocallis dendroidea* (P. Crouan et H. Crouan) S.-M. Lin, Huisman et D.L. Ballantine (syn. *Ganonema dendroideum* (P. Crouan et H. Crouan) D.L. Ballantine et Aponte, previously reported from Bermuda) and *Trichogloeopsis pedicellata* (M. Howe) I.A. Abbott et Doty (unreported from Bermuda). The COI-5P distance values of 11.7-23.2% indicated that this gene is highly variable among liagoracean genera.

To place them into a phylogenetic context, a representative of each of the previously determined genetic species groups was then included in single gene (LSU, *rbcL*) phylogenetic analyses along with a diverse representation of liagoracean taxa

(Table 1) mined from GenBank. Tree topologies from maximum likelihood and Bayesian analyses were largely consistent, thus a single tree is presented for each gene (Figs 1 and 2). The *rbcL* phylogeny is illustrated by the Bayesian tree, with posterior probabilities and ML bootstraps both indicated at the nodes (Fig. 1). Our results confirmed the presence of nine genera and ten species in Bermuda. Each genus is represented by a single species in Bermuda, with the exception of *Liagora*, which includes two species. Phylogenetic relationships conformed predominantly to those published by Lin et al. (2014) but with moderate to weak support for the position of the *Ganonema farinosum* (J.V. Lamouroux) K.C. Fan et Yung C. Wang clade, which at the moment appears ambiguous when comparing the two analyses. We did not include *Dotyophycus abbottiae* Kraft in our *rbcL* analysis because we were not able to obtain a correct sequence. This may have altered our results as *G. farinosum* resolves as a sister clade to *D. abbottiae* in the Lin et al. (2014) analysis. Intergeneric distances of 8.7-17.5% denote high levels of *rbcL* genetic variation among the Liagoraceae at the generic level, similar to the COI-5P result. For genera present in Bermuda, the greatest interspecific variation existed for *Liagora* (5.6-11.0%) and *Hommersandiophycus* (4.6-10.8%), with the least occurring between western Atlantic *Titanophycus validus* (Harvey) Huisman, G.W. Saunders et A.R. Sherwood and Indo-Pacific *T. setchelliae* (Yamada) S.-M. Lin, S.-Y. Yang et Huisman (2.0-2.3%) and western Atlantic *Trichogloeopsis pedicellata* and Pacific *T. mucosissima* (Yamada) I.A. Abbott et Doty (3.5%). The *rbcL* data revealed several species that occupied unique branches on the tree, including a distinct species, basal in the *Liagora* clade, which LSU data later confirmed as the first Atlantic report of *L. mannarensis* V.

Krishnamurthy et Sundararajan. Also in the *Liagora*, specimens initially identified as *L. ceranoides* J.V. Lamouroux grouped as a distinct sister clade, with 6.8-7.0% genetic distance in *rbcL* sequences from specimens of *L. ceranoides* from around the world, including near the type locality. *Liagora pectinata* (type locality = Bermuda) did not group with other species of *Liagora* in the *rbcL* tree, instead, it appeared as a basal lineage of the newly described *Hommersandiophycus* (Lin et al. 2014). This is the first evidence of this genus in the Atlantic Ocean. Though *Trichogloeopsis pedicellata* had not previously been recovered in Bermuda, our sequence data exposed a 100% identity match to a sequence of the species from its Bahamas type locality. The genus *Yamadaella* was not included in the former study by Lin et al. (2014), however our data show it as the most basal lineage among the genera of the Liagoraceae *sensu lato* included in our *rbcL* tree (Fig. 1) with nearly full support in the Bayesian analysis. In a recent study using *psaA* and *rbcL* sequences, Lin et al. (2015) found *Yamadaella* forming a distinct clade basal to an emended Liagoraceae *sensu stricto* and created the Yamadaellaceae to contain it. COI-5P and LSU sequence data indicate that our samples represent a unique *Yamadaella* species in this formerly monospecific genus, expanding its range to include Bermuda.

For the LSU analysis, the ML result is shown, with bootstraps and Bayesian posterior probabilities respectively at the nodes (Fig. 2). Many of these support values are noticeably problematic, despite the generic placement of specimens across the tree being mainly congruent with that of *rbcL*. The LSU sequence alignment indicated that intergeneric distances of 0.8-7.5% were remarkably smaller than in COI-5P and *rbcL* due to the highly conserved nature of this gene, potentially resulting in weak support

values for the topology recovered. Some comparative sequence data from GenBank were not as reliable for this gene compared with *rbcL*, therefore the absence of data needed to resolve the relationships among liagoracean taxa may also contribute to the lack of support, especially toward the backbone of the tree. However, we chose to present the LSU analysis in spite of the difficulty resolving a well-supported tree, because the data confirm several important findings. Most notably, the LSU data were essential for identifying an unknown specimen grouping with the genus *Liagora* in the previously sequenced genes. The LSU sequence was identical to a GenBank sequence of *L. mannarensis* from western Australia. Our COI-5P data showed that we collected a number of specimens most closely related to *Yamadaella caenomyce* (9.5% distance) from Hawaii. and LSU sequence data confirmed with full support that Bermuda specimens belonged in the same genus, but were distinct from the Indo-Pacific generitype. Also, although its position within the *Liagora* clade is uncertain in the LSU analysis, the data provide further evidence that *Liagora ceranoides*, the species known in Bermuda prior to this publication, does not appear to be present in Bermuda waters. Instead, the collections represent a novel taxon that is closely related to *L. ceranoides* from other parts of the world. Finally, the placement of *Liagora pectinata* as a basal lineage in the *Hommersandiophycus* clade was well supported in the Bayesian LSU analysis. Both of the latter two examples corroborate the relationships shown in the more cogent *rbcL* tree.

Future molecular studies of the Liagoraceae family would benefit from greater sampling and sequencing effort, especially for regions such as the Caribbean Sea where little comparative molecular data has been made available.

Systematic treatment

***Liagora* J.V. Lamouroux, 1812**

***Liagora mannarensis* V. Krishnamurthy et Sundararajan, 1985, p. 59 (Figs 3-7)**

Description: Thalli to 8 cm high, pale pink, with moderate calcification.

Branching irregularly alternate, axes 2.0 mm near base, tapering to 0.5 mm distally.

Medullary filaments 6 -15 μm diam. Cortical fascicles 180-350 μm in overall length, composed of dichotomously branched filaments; lower cells of the cortical filaments elongate to cylindrical, 6-15 μm diam., 25-41 μm long; upper cortical cells elongate to obovate, 4-12 μm diam., 9-23 μm long, some ultimate cortical cells nearly spherical with single long terminal hairs. Gametophytes monoecious. Carpogonial branches 2-4-celled, very small and inconspicuous, 7.5-10.5 μm diam. and 19-27 μm long, borne on proximal regions of cortical fascicles; fusion cells formed after presumed fertilization. Carposporophytes 60-103 μm high and 108-127 μm wide; involucrel filaments sparsely developed, 2.5-9.5 μm diam., 48-192 μm long and tapering to their apices, not mingling with the gonimoblast, but rather spread out horizontally away from the carposporophyte; carposporangia terminal on gonimoblast filaments, irregularly elongate to obovoid, 6.0-7.5 μm diam. and 9-15 μm long. Two spermatangial mother cells on terminal cortical cells, each bearing a pair of spermatia, 3 μm diam.

Tetrasporangia unknown.

Type locality: Pudumadam, Tamil Nadu, India, Indian Ocean.

Bermuda collections: C.W. Schneider (CWS)/Christopher E. Lane (CEL) 09-34-21, fertile, 20 Mar. 2009, Middle buoy, Eastern Blue Cut channel, north of Daniel's

Head, Somerset Is., 32°23'20.6"N, 64°53'19.9"W, 15-16 m; *T.R. Popolizio (TRP)* 12-42-3 (Fig. 3; data in Table 1).

Distribution: India, Western Australia, Bermuda.

Remarks: Only two known representatives of this rare species have been collected, each at the same location in 2009 and 2012, several miles offshore at a depth of 15-16 m, attached to coral rubble. The collections were initially field-identified as *Liagora ceranoides*, but our COI-5P and *rbcL* sequence data later showed that the 2012 specimen was genetically distinct from all other *Liagora* specimens collected and sequenced from the islands. Moreover, this specimen did not group with any other liagoracean species from ours or the comparative data, instead resolving on its own branch, basal to the *Liagora* clade in both analyses. A publicly available LSU sequence of *L. mannarensis* from Western Australia was identical to LSU data from our Bermuda specimen (Fig. 2). An examination of the morphology and reproductive characters of our plant agreed with the description of collections from Western Australia (Huisman 2002), as well as the type from India (Krishnamurthy and Sundararajan 1985). Clearly, *L. mannarensis* is cryptic with what has been called *L. ceranoides* in Bermuda and the only way one can reliably distinguish them (other than using molecular markers) is to observe the production of gonimoblast initials from both the proximal and distal cells of the transversely divided carpogonium. According to Huisman (2002), all other known species of *Liagora* generate gonimoblast filaments exclusively from the distal cell. Carpogonial branches in our Bermuda specimens are remarkably small (Fig. 4), making observation of the reproductive characters important for characterizing the species difficult, however we were able to

locate a fertilized carpogonium where longitudinal division of both initial daughter cells was evident (Fig.5). The Bermuda specimens appeared to have more weakly developed involucreal filaments (Fig. 7) compared with Western Australian specimens (Huisman 2002). Interestingly, Krishnamurthy and Sundararajan (1985) note their Indian specimens are present only in fall and winter, disappearing abruptly from the flora in spring; we collected both of our well-developed Bermuda plants in March, suggesting the species may have a similar growing season in the western Atlantic. This study represents the first report of *L. mannarensis* in the Atlantic Ocean, and is one example of several Indo-Pacific genera or species in the Liagoraceae and Yamadellaceae discovered in this region.

***Liagora nesophila* Popolizio, C.W. Schneider et C.E. Lane sp. nov. (Figs 8-14)**

Description: Thalli to 14 cm, whitish to brownish rose, usually with dark red tips, lightly to moderately calcified. Branching dichotomous to many orders, with obtusely angled dichotomies at the branch apices, and some with adventitious branches present on main axes that at times appear pinnate. Axes 0.2-1.5 mm diam. Medullary filaments 17-19 μm diam. Cortical fascicles 170-320 μm in overall length, composed of dichotomously branched filaments. Lower cells of the cortical filaments cylindrical to ellipsoidal, 7-12 μm diam. and 33-48 μm long; upper cells obovoid to nearly spherical, 9-12 μm diam. and 9-32 μm long; terminal cortical cells nearly spherical, 4-5 μm diam. Gametophytes monoecious or dioecious. Carpogonial branches 4-celled, curved, at times sharply, very small and inconspicuous, to 9 μm diam. and borne distally on mid- to proximal regions of the cortical fascicles.

Carpogonial branch cells unfused early in development, in some instances with widening of pit connections; fusion cells present in mature carposporophytes. Gomimoblasts compact, 96-190 μm wide and 48-110 μm high. Carposporangia terminal on gonimoblast filaments, obovoid, 5-9 μm diam. and 8-19 μm long. Sterile involucre filaments unbranched, 45-100 μm long, the cells ovoid to cylindrical, 6 μm diam. at base, tapering distally to 2.5 μm , and subtending the gonimoblast in an unusual, extensive arrangement. Spermatangia formed on outer cortical cells, 1.5-3.0 μm diam. Easily identified by comparison to the type *rbcL* sequence (GenBank: KR005369).

Holotype (designated here): CWS/CEL 8-5-9, 12 Feb. 2008, junction of Horseshoe and Higgs Is. at Town Cut, St. George's Harbour, 32° 22.570'N, 64° 39.847'W, 1 m (KIRI, GenBank KR005369 [Fig. 8]; Isotypes GALW, MICH, MSM, NY, US, the Bermuda Natural History Museum [BAMZ] and CWS's herbarium).

Additional collections (Paratypes): Specimens listed in Table 1 as CWS/CEL/TRP 12-10-10, TRP 12-28-3, TRP 12-32-9, TRP 12-37-7, TRP 12-51-3 and TRP 12-62-4.

Misapplied name for Bermuda: *Liagora ceranoides* J.V. Lamouroux *sensu auct.*

Etymology: *nesophila* (L, f.), for "island-loving," thus far known only from the Bermuda archipelago.

Distribution: At present, endemic to Bermuda, western Atlantic Ocean.

Remarks: Kemp (1857, as *Liagora pulverulenta* C. Agardh) first reported *L. ceranoides* from Bermuda, and abundant collections over the past several decades

have been recognized as such. *Liagora ceranoides* (type locality = St. Thomas, Virgin Is., Caribbean Sea) is reputedly the most widely distributed species of the genus (Guiry and Guiry 2014), known from throughout the northern and eastern regions of the Atlantic Ocean and parts of the Indo-Pacific. Publicly available sequence data for specimens identified as *L. ceranoides* exists from throughout its range and we were also able to generate DNA sequences for a specimen collected in St. Kitts, a Caribbean island in close proximity to the type locality. Our phylogenetic analysis demonstrates that *L. ceranoides* is indeed a pantropical species, however, the Bermuda specimens are not equivalent to those sequenced from elsewhere, including those from St. Kitts (Fig. 1). Based on these data, along with a thorough, comprehensive assessment of all existing species of *Liagora*, we consider the pantropical clade in our analysis to be *L. ceranoides sensu stricto*, and our Bermuda species a novel taxon. To ensure that prior names now subsumed under *L. ceranoides* were taken into consideration, we eliminated all taxonomic synonyms of it reported from the western Atlantic based on a combination of morphological character descriptions and geographic distributions. None of the synonyms of *L. ceranoides* were described from Bermuda or warm temperate regions of the Atlantic; thus, tying an older epithet to a cryptic species from this region would be problematic at best.

Liagora nesophila shares several features with *Liagora ceranoides* and other analogous species in the ‘ceranoides’ group as outlined by Huisman (2002) but with some plausible distinctions. For example, *L. nesophila* does not display the broader medullary filaments exhibited by *L. ceranoides*, *L. izziae* Huisman (type locality = Western Australia) and *L. wilsoniana* Zeh (type locality = Victoria, Australia) or the

longer and sometimes narrower assimilatory filaments associated with *L. izziae* and *L. ceranoides*. *Liagora nesophila* has only been observed with 4-celled carpogonial branches, whereas *L. izziae* can have up to six cells in female branches. *Liagora izziae* also possesses considerably smaller carposporangia than *L. nesophila* and other closely related species. The morphological and reproductive characters of *L. wilsoniana* overlap almost entirely with *L. nesophila*, with the exception of the involucre which is composed of broad, branched filaments in the former species, compared with slender, unbranched, tapering filaments subtending the gonimoblast in the new species (Fig. 13). *Liagora maderensis* (type locality = Madeira Is.) also has a remarkably similar morphology to *L. nesophila*, but does not form a distinct post-fertilization fusion cell like the new species (Fig. 12). . Because *Liagora nesophila* is somewhat morphologically cryptic, no single characteristic can differentiate this new species from all congeners. However, by recognizing a combination of morphological and anatomical characters as demonstrated above, it may be possible to distinguish *L. nesophila* from cryptic relatives. Gross habit features include light thallus calcification and branch apices that commonly bifurcate at wide angles. The markedly small and strongly curved carpogonial branch that remains unfused early in gonimoblast development (Fig. 11) as well as the relatively small carposporophyte diameter and elaborate arrangement of sterile filaments subtending the gonimoblast are notable diagnostic characters (Figs 12 and 13). In the end, however, DNA sequence data are most reliable in separating the two western Atlantic species. Since our molecular data thus far have not revealed *L. ceranoides* from Bermuda in our extensive collections, it is reasonable to assume that non-sequenced specimens from the islands identified as

such by earlier workers (see Schneider 2003) are all *L. nesophila*, effectively removing *L. ceranoides* from the flora, and providing a geographic distinction for these two species.

***Trichogloea* Kützinger, 1847**

***Trichogloea herveyi* W.R. Taylor, 1951, p. 119**

Type locality: Cooper's Island (presently the southeastern portion of St. David's Is.), Bermuda, western Atlantic Ocean.

Collections: Specimens listed in Table 1.

Distribution: Bermuda, Gulf of Mexico, eastern Caribbean, Venezuela.

Remarks: Several Bermuda collections of *Trichogloea herveyi* were processed for DNA analysis (Table 1). The sequence data verified earlier morphological investigations that established *T. herveyi* as the only species of the genus in Bermuda.

***Helminthocladia* J. Agardh, 1851**

***Helminthocladia kempii* Popolizio, C.W. Schneider et Chengsupanimit in Popolizio et al. 2013. p. 239**

Type locality: Spanish Point, Bermuda, western Atlantic Ocean.

Collections: Specimens listed in Table 1.

Distribution: Bermuda, Florida.

Remarks: After both molecular and morphological evidence indicated that Bermuda specimens were distinct from *Helminthocladia calvadosii*, we established the novel species *H. kempii* (Popolizio et al. 2013).

***Trichogloeopsis* I.A. Abbott et Doty, 1960**

***Trichogloeopsis pedicellata* (M. Howe) I.A. Abbott et Doty, 1960, p. 638 (Figs 15-19)**

Basionym: *Liagora pedicellata* M. Howe 1920, p. 556.

Description: Thalli to 24 cm high, brownish-white to rose-red, with light calcification. Branching irregularly alternate, in some cases with opposite branching in distal areas, and occasionally secund, axes to 2 mm diam. Cortical fascicles consisting of di- or tri-chotomously branched filaments, overall fascicle length 200-600 μm . Upper cells of the cortical filaments obovate, some nearly spherical, 9.5-21.5 μm in diam. and 12-36 μm long, proximal cells elongate, 7-17 μm in diam. and 24-84 μm long. Gametophytes monoecious, carpogonial branches 4-celled, straight, conspicuous and abundant, 9-23 μm diam. and 37.5-67.5 μm long produced on extended pedicels, usually with two carpogonial branches per supporting cell. Fusion cells not produced after fertilization. Involucres not generated around the carposporophyte, but sterile rhizoidal filaments subtend developing gonimoblasts. Carposporophytes 60-180 μm in diam. with obpyriform, or less commonly, obovoid or ellipsoid carposporangia, 6-18 μm diam. and 15-32 μm long. Spermatangia apical or subapical, inconspicuous; tufts 10-24 μm diam.; spermatangia 3-7 μm long and 3-4.5 μm diam.

Type locality: Cockburn Harbor, South Caicos Is., Bahamas, western Atlantic Ocean.

Collections: Specimens listed in Table 1.

Distribution: Bahamas, Puerto Rico, Florida, Bermuda.

Remarks: This represents the first report of *Trichogloeopsis pedicellata* from Bermuda. Based upon the carpogonial branch shape and the unique sterile filament development associated with the carposporophyte, Abbott and Doty (1960) transferred *Liagora pedicellata* to their new genus *Trichogloeopsis*. In habit, Bermuda collections are similar to *Gloiocallis dendroidea* from Bermuda and other western Atlantic sites. These two species are situated in the same clade in our phylogenetic analyses (Fig. 1) with a genetic distance of 10.8% for *rbcL*. Molecular sequence data from our specimens plainly illustrate that *T. pedicellata* from Bermuda corresponds to sequences from the Bahamas Island chain, the type locality of this species (Fig. 1).

Trichogloeopsis is characterized by the presence of short sterile filaments that issue from below the gonimoblast during carposporophyte development, these referred to as descending ‘gonimorhizoids’ (Abbott and Doty 1960) or ‘sterile rhizoids’ (Vélez-Villarmil et al. 2000). All species of *Trichogloeopsis* display this trait, but only *T. pedicellata* possesses pedicels, the particularly long bearing or stalk cells at the base of the carpogonial branches (the namesake of the western Atlantic species). Our collections from Bermuda demonstrate both of these distinctive characters (Figs 16 and 17). Surprisingly, Bermuda specimens commonly exhibit multiple carpogonial branches originating from a single basal supporting cell (Fig. 19), a feature Huisman and Kraft (1994) attribute to *Ganonema*. Others who have discussed *T. pedicellata* from the Caribbean Sea have not mentioned this conspicuous trait.

***Yamadaella* I.A. Abbott, 1970**

***Yamadaella grassyi* Popolizio, C.W. Schneider et C.E. Lane, sp. nov. (Figs 20-25)**

Description: Plants typically appearing as small, hemispherical, brownish-rose clumps across areas of intertidal rock, usually in areas of considerable surf, to 5.0 cm high and 7.5 cm wide. Axes mostly uniform, to 1 mm diam. with moderate to heavy calcification. Branching dichotomous with annular constrictions obvious in the upper portions of the branches. Cortical fascicles composed of 3-5-times dichotomously branched filaments, overall to 240 µm in length. Apical cortical cells swollen, obpyriform to clavate, 15-21 µm long and 9-12 µm diam., some with apical hairs. Gametophytes dioecious, spermatangia obovate, 2-3 µm diam., formed from stalk cells on the subapical cells of the cortical filaments, either singly or in pairs. Carpogonial branches 3-celled, 65-85 µm long, borne distally on supporting cortical cells. Carpogonial branch cells elongate to ellipsoidal, 7.5-16.5 µm diam. with basal cells only slightly longer than central cells. Post-fertilization fusion present. Carpogonia narrowly conical, first divisions lateral; fertilized carpogonia issuing opposite gonimoblast initials at distal ends. Involucral filaments not produced. Single, terminal, undivided, elongate-ellipsoid to obovoid carposporangia, 6-14 µm diam. and 19-26 µm long.

Holotype (designated here): BDA 1184 (=T.R. Popolizio 12-74-1), fertile, June 18, 2012, Doe Bay Cove, Paget, south shore Bermuda I., Bermuda, western Atlantic Ocean, 32°17'21.7" N, 64°45'30.4" W, on intertidal rock (MICH, GenBank KR005300 [Fig. 20]; isotypes KIRI, NY, US and CWS's herbarium; GenBank KR005291, KR005298).

Additional collections (Paratypes): Specimens listed in Table 1 as TRP 12-27-6, TRP 12-30-4, TRP 12-75-1, TRP 12-76-1, TRP 12-77-1, TRP 12-140-1, TRP 12-172-2 and TRP 13-22-3.

Etymology: Named for Roger “Grassy” Simmons whose local expertise, support and friendship were invaluable during the first author’s field collection year in Bermuda, and who introduced her to the site where this species was first discovered.

Distribution: Thus far, only known from the type locality and nearby intertidal sites on the southeastern shore of Bermuda Is., from Grape Bay to Devonshire Bay.

Remarks: The genus *Yamadaella* was described by Abbott (1970) for a single species, *Yamadaella caenomyce* (Decaisne) I.A. Abbott (type locality = Philippines). This Indo-Pacific species was recently characterized using MAAT techniques by Lin et al. (2015). The new species from the intertidal zone along the south shore of Bermuda Island is distinguished from the generitype by its longer carpogonial branches (65-85 μm vs. 40-60 μm) and narrower carpogonial branch cells (7.5-16.5 μm vs. 12.5-25.0 μm). Basal cells of the carpogonial branches of *Y. grassyi* are elongate but only slightly longer than other elongate cells of the fertile branches (Fig. 22), while those in *Y. caenomyce* are 1.5 times longer than other fertile branch cells, the middle cell often being nearly cuboidal (Abbott 1970, fig. 5). The carpogonia of *Y. grassyi* are narrowly conical (Fig. 22) while those in the generitype are flask-shaped (broadest basally) (Abbott 1970, figs 3-5). Finally, we have observed only dioecious gametophytes in the many Bermuda specimens rather than strictly monoecious gametophytes for Indo-Pacific populations (Abbott 1970, Lin et al. 2015). *Yamadaella grassyi* exhibits inflated, wedge-shaped terminal cortical cells that are typical for the

genus (Fig. 21) and allow it to be easily distinguished from the other genera reported here.

Originally thought to be restricted to the Indo-Pacific region, *Yamadaella caenomyce* was later reported by Wynne and Huisman (1998) from the Caribbean Sea following a morphological investigation of specimens from the Dominican Republic (Greater Antilles). Phylogenetic sequence analysis has clarified the evolutionary relationship between Bermuda collections and *Yamadaella* from the Indo-Pacific (Figs 2 and S1), but unfortunately, we were not able to obtain sequence data from the Caribbean specimens. While it is logical to suggest the Caribbean specimens are more likely allied to *Y. grassyi* from Bermuda than to *Y. caenomyce* from the Indo-Pacific, we have noted some anatomical distinctions between Bermuda and Caribbean material. Vegetative characters are comparable, but terminal cortical cells are often broader in Bermuda material (8-15 μm vs. 8-10 μm in Caribbean material). Carpogonial branches are shorter and sometimes broader in Bermuda specimens, and both carposporangia and spermatangia are smaller than reported for Caribbean collections. Also, Caribbean specimens are reported to be monoecious as is true of the generitype (Lin et al. 2015), while only dioecious specimens have been collected in Bermuda. Acknowledging that morphological and reproductive characteristics within species can vary temporally and spatially, we feel that molecular data is imperative for verifying the relationship of *Yamadaella* specimens collected in the western Atlantic.

All representatives of *Yamadaella grassyi* were collected on intertidal rock of adjacent beaches of Bermuda's rocky, turbulent southeastern shore between October and June. Despite returning to these same collection sites throughout the year, the new

species was not present during the summer months of July-September, when sea surface temperatures in Bermuda are highest.

***Ganonema* K.C. Fan et Yung C. Wang, 1974**

***Ganonema farinosum* (J.V. Lamouroux) K.C. Fan et Yung C. Wang, 1974, p. 492**

Basionym: *Liagora farinosa* J.V. Lamouroux 1816, p. 240.

Type locality: Suez, Egypt, Red Sea.

Collections: Specimens listed in Table 1.

Distribution: Pantropical.

Remarks: *Ganonema farinosum* was first reported from Bermuda by J. Agardh (1896) as *Liagora corymbosa* J. Agardh, the islands being a syntype locality for his new species. Later, Howe (1920) subsumed *L. corymbosa* in *Liagora farinosa*. When Fan and Wang (1974) described their new genus *Ganonema* based on carpogonial branch position, they selected *L. farinosa* as the generitype. As presently understood, *G. farinosum* is found in tropical to warm-temperate seas worldwide. Our molecular comparison of *rbcL* confirms that specimens identified as *G. farinosum* from Bermuda are conspecific with those from the Indo-Pacific region (Western Australia, Taiwan, Philippines) with only 0.6% genetic distance between Bermuda and Western Australian specimens. Additionally, collections identified as *G. farinosum* from the Florida Keys are likely the same species, with only 1.7% variation in COI-5P compared with Bermuda sequences. However, specimens sequenced from Hawaii are distinct from western Atlantic collections in both our COI-5P and LSU analysis (6.1% and 1.5% distance, respectively) indicating that *G. farinosum* may represent a larger

species complex. Material from the type locality is not available for molecular comparison to place species from other parts of the world into the correct taxonomic context. Since *G. farinosum* is thought to be pantropical, and because Indo-Pacific specimens group with our western Atlantic specimens, we will continue to identify our collections from Bermuda as they are currently reported.

***Titanophycus* Huisman, G.W. Saunders et A.R. Sherwood, 2006**

***Titanophycus validus* (Harvey) Huisman, G.W. Saunders et A.R. Sherwood, 2006, p. 119.**

Basionym: *Liagora valida* Harv. 1853, p. 138.

Type locality: Sand Key, Florida, USA, western Atlantic Ocean.

Collections: Specimens listed in Table 1.

Distribution: Reported as pantropical, but not corroborated by molecular data.

Remarks: The first report of *Liagora valida* from Bermuda was made by Kemp (1857) and this was followed by numerous reports, as it is common during summer months. In the last decade, this species was moved to a new genus, *Titanophycus* (Huisman et al. 2006), based on the generic character of a distinctive cup-like involucre subtending the gonimoblast (Lin et al. 2011). *Titanophycus* was monotypic until Lin and co-workers (2011) described *T. setchellii* (Yamada) S.-M. Lin, S.-Y. Yang et Huisman from Japan. Outside its wide distribution in the western Atlantic, *T. validus* has also been reported from Australia, Taiwan, Japan, and Hawaii, indicating that as presently circumscribed, it is a pan-tropical species. However, additional molecular data is needed for specimens collected in the Indo-Pacific region to validate

this. Lin et al. (2011) remark that the specimens they collected in Taiwan do not correspond molecularly, despite reports of *T. validus* from the region. They also observed that sequences from Hawaii grouped with their *T. setchellii*, suggesting possible crypsis. Our phylogenetic analyses agree with these findings, illustrating that “*T. validus*” from Hawaii is distinct from Bermuda material and collections from near the type locality, with 6.9-7.1% genetic distance for COI-5P (Fig. S1). Moreover, *T. validus* from Western Australia exhibits 0.5% distance from Bermuda specimens in the portion of the highly conserved LSU gene sequenced in this study (Fig. 2). Further molecular analysis of specimens from Indo-Pacific regions may support the restriction of *T. validus* to warm waters of the western Atlantic Ocean.

***Gloiocallis* S.-M. Lin, Huisman et D.L. Ballantine, 2014**

***Gloiocallis dendroidea* (P. Crouan et H. Crouan) S.-M. Lin, Huisman et D.L. Ballantine, 2014, p. 49**

Basionym: *Helminthora dendroidea* P.L. Crouan et H.M. Crouan in Mazé & Schramm 1878: 178

Type locality: Pointe-a-Pitre, Ilet a Cochons, Guadeloupe, Caribbean Sea.

Collections: Specimens listed in Table 1.

Distribution: Bermuda, Florida, Bahamas, Caribbean, Brazil, Mariana Is.

Remarks: Taylor (1960) was the first to report *Liagora mucosa* M. Howe from Bermuda, and later, this species was reduced to synonymy of *L. dendroidea* (P. Crouan et H. Crouan) I.A. Abbott (Abbott 1990b). Subsequently, Ballantine and Aponte (2002) transferred *L. dendroidea* to *Ganonema* based on reproductive characters associated with the carpogonial branch and spermatangial arrangement.

When a recent phylogenetic analysis rendered *Ganonema* polyphyletic, a new genus, *Gloiocallis*, was established for *G. dendroideum* (Lin et al. 2014). At present, *Gloiocallis* is monotypic and is sister to *Trichogloeopsis*. It is characterized by lacking the descending gonimorhizoids associated with *Trichogloeopsis*, and by possessing carpogonial branches that are produced terminally or laterally from the cortical filaments (compared with *Ganonema* in which they originate in a special accessory branch system). Our Bermuda specimens' morphologies conform to those described for *G. dendroidea* by Huisman (2002), Ballantine and Abbott (2006) and Lin et al. (2014). Abbott (1990a) remarked on the similarity between *G. dendroidea* and *T. pedicellata*, stating they were “equally gelatinous and pinnately-paniculately branched.” She also noted that *T. pedicellata* was more common in the Caribbean waters she had examined them from than the former species was. Conversely, in Bermuda *G. dendroidea* is far more common than *T. pedicellata*.

When Ballantine and Aponte (2002) transferred *Liagora dendroidea* to *Ganonema*, they mentioned that polycarpogonial branches were occasionally produced in this species. As evidenced in this study, this is yet another feature shared by *Gloiocallis* and the closely related genus *Trichogloeopsis*. At present, the only conspicuous morphological character used to distinguish the two is the lack of descending gonimorhizoids in the latter species. It is worth noting that the intergeneric distance between *T. pedicellata* and *G. dendroidea* (10.8% for *rbcL*) is similar to values found for interspecific relationships among the Liagoraceae. For example, the genetic distance between *H. pectinatus* and other members of *Hommersandiphycus* (9.2-10.8%) or between *Liagora viscida* (Forsskål) C. Agardh (generitype) from Spain

and *L. ceranoides* (10.7-11.0%), *L. nesophila* (9.4%), *L. albicans* J.V. Lamouroux (9.2%) and *L. mannarensis* (10.2%).

***Hommersandiophycus* S.-M. Lin et Huisman in Lin et al., 2014**

***Hommersandiophycus pectinatus* (Collins et Hervey) Popolizio, C.W. Schneider et C.E. Lane, comb. nov. (Figs 26-29)**

Basionym: *Liagora pectinata* Collins et Hervey 1917, p. 100

Collections: Specimens listed in Table 1.

Description: Thalli to 20 cm high, brownish-rose to dark red, with light to moderate calcification. Branching alternate to irregularly alternate, sometimes opposite above, axes 1.0-3.0 mm diam. Ultimate branchlets often pectinate or secund. Medullary filaments 30-50 µm diam. Cortical fascicles 3-4 times irregularly dichotomous, occasionally with secund branching. Overall filament length 360-440 µm. Proximal cells of the cortical filaments 6-19 µm diam. and 23-52 µm long, upper cells 6.0-14.5 µm diam. and 10.5-33.0 µm long, apical cells 7-12 µm diam. and 11-24 µm long. Gametophytes dioecious, carpogonial branches straight to slightly curved, 15.0-22.5 µm diam. and 45-64 µm long. Sterile filaments form from the supporting cell of the carpogonial branch, the involucre being weakly developed (or not a true involucre as the filaments do not surround the gonimoblast but rather, spread horizontally as in a “nest” or “collar”). Carposporophytes 120-200 µm diam.; carposporangia 24 µm long and 10 µm diam. Spermatangia occur in dense compound sori, borne of spermatangial supporting cells cut off laterally from upper regions (usually the uppermost three cells) of cortical filaments. Tetrasporangia unknown.

Type locality: Cooper's Is. (presently the southeastern portion of St. David's Is.), Bermuda, western Atlantic Ocean.

Collections: CWS/R.B. Searles 85-1-7, South of Gibbs Hill Light and Sinky Bay, Bermuda I., Bermuda western Atlantic Ocean, 32° 13.0'N, 64° 50.5'W, collected at 29 m; additional collections listed in Table 1.

Distribution: Bermuda, Bahamas, Martinique.

Remarks: The genus *Hommersandiophycus* was recently segregated from *Ganonema* in Lin et al. (2014) for a phylogenetically distinct clade of species that produce compact gonimoblasts, unfused carpogonial branches and compound clusters of spermatangia. The movement of *Liagora pectinata* to *Hommersandiophycus*, based upon their position in our phylogenetic trees, represents the first report of the new genus in the western Atlantic (Figs 2, 3 and S1). Prior to this study, *Hommersandiophycus* was known only from the Indo-Pacific region, and encompassed three species — *H. borowitzkae* (Huisman) S.-M. Lin et Huisman, *H. clavatus* (Yamada) S.-M. Lin et Huisman and *H. samaensis* (C.K. Tseng) S.-M. Lin et Huisman. Evidence from the COI-5P and LSU results indicate that *Ganonema yoshizakii* Huisman, I.A. Abbott & A.R. Sherwood from Hawaii may also be a member of this genus (Figs 2 and S1). An important diagnostic character of this genus is the nature of the loosely developed sterile filaments around carposporophytes (Fig.28), distinguishing *Hommersandiophycus* from its liagoracean sibling *Ganonema*. Lin et al. (2014) describe spermatangia initiating in complex cluster from lateral, adventitious parental cells in upper parts of assimilatory filaments to be a generic character of *Hommersandiophycus*. Our specimens conform to this unifying feature,

having dense bunches of spermatangia originating on apical and subapical cortical cells (Fig. 29). In addition to its restricted geography, the pectinately arranged branchlets often found on well-developed specimens of *H. pectinatus* (Fig. 26) differentiate the western Atlantic species from its Indo-Pacific congeners.

Concluding remarks

The Indo-Pacific region is the most speciose region for the Liagoraceae (Lin et al. 2013), with 25 genera and 152 species presently reported (Guiry and Guiry 2014). With records of 13 genera and only 24 species, the warm temperate to tropical western Atlantic region is comparatively depauperate (Guiry and Guiry 2014). However, we have demonstrated that the diversity of this group in the western Atlantic, and Bermuda in particular, has been undervalued. The number of liagoracean genera in Bermuda have nearly doubled from the five reported prior to our investigation (Schneider 2003); we have determined that at least nine genera are present. Furthermore, we have extended the range of the Caribbean species *Trichogloeopsis pedicellata* to its northernmost outpost in Bermuda, and confirmed that specimens previously described as *Ganonema dendroidea* in Bermuda are equivalent to *Gloiocallis dendroidea* from Brazil. We transferred *Liagora pectinata* with a type locality in Bermuda to the recently segregated *Hommersandiophycus*, thus expanding the range of an Indo-Pacific genus to include the Atlantic Ocean. *Liagora mannarensis*, previously known only from the Indian Ocean, is now recognized for the first time in the Bermuda flora and the Atlantic Ocean. Moreover, we have described two novel species, *Liagora nesophila* and *Yamadaella grassyi*. The latter is the first report of the

genus *Yamadaella* in Bermuda. Convincing molecular evidence from this study suggests *Liagora ceranoides* is not present in the waters surrounding Bermuda.

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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</i>	<i>Collectors/Date</i>	<i>Site</i>	<i>COI-5P</i>	<i>rbcL</i>	<i>LSU</i>
Liagoraceae						
<i>Akalaphycus setchelliae</i> (Yamada) Huisman, I.A. Abbott et Sherwood	—	S.-M. Lin/ 28 Apr. 2003	Jian Shan, Penghu Archipelago, W. Taiwan		GU357697	—
	IA 28755	M. LeGrande/ 5 May 2002	Mokuleia, Oahu, Hawaiian Islands, USA	—	—	AY523825
	ARS00070	J. Huisman/ 11 Mar. 2003	Mali Point, Oahu, Hawaiian Islands, USA	HQ422870	—	—
<i>Cumagloia andersonii</i> (Farl.) Setch. et N.L. Gardner	GWS021723	B. Clarkston, K. Hind, S. Toews/ 17 May 2010	Soberanes Point, California, USA	HQ603206	—	—
	NTOU-18vii2011-CA G0156	S.-M. Lin/ 18 July 2011 G.W. Saunders/ 8 Aug. 1993	Seal Rock, Oregon, USA Brady's Beach, Bamfield, British Columbia, Canada	—	JX878365	—
<i>Dermonema pulvinatum</i> (Grunow) Fan	ARS03552	A. Kurihara/ 1 Mar. 2008	Poipu Beach Park, Kauai, Hawaiian Islands, USA	HQ422985	—	—
<i>Dermonema virens</i> (J. Agardh) Pedroche et Ávila Ortiz	—	S.-M. Lin/ 9 Mar. 2008	Lung Keng, Pingtung Co., southern Taiwan	—	HQ901787	—

<i>Dotyophycus abbottiae</i> Kraft	G0404	G.T. Kraft & G.W. Saunders/ 10 Nov. 1995	Anemone Mound, Easter Group, Abrolhos I., Western Australia	—	—	AY570370
<i>Dotyophycus yamadae</i> (Ohmi et Itono) Abbott et Yoshizaki	ARS00991	E. Zablackis, K. DeDermid/ 7 Apr. 1984	Maili Point, Oahu, Hawaiian Islands, USA	HQ422733	—	—
<i>Ganonema farinosum</i> (J.V. Lamour.) K.C. Fan et Yung C. Wang	—	S.-L. Liu/ 29 Aug. 2002	Horse Bridge, Green Island, Taitung Co., E. Taiwan	—	GU357699	—
	—	S.-L. Liu/ 12 Feb. 2003	Bulusan, Luzon Island, Philippines	—	GU357700	—
	19.10.11.1.11	J. Huisman/ 16 Oct. 2011	Kimberley, Western Australia	—	KF667111	—
	IA28656	J. Huisman/ 2 Apr. 2002	Makapuu tidepool, Oahu, Hawaiian Islands, USA	—	—	AY424880
	ARS00037	J. Huisman/ 2 Apr. 2002	Makapuu, Oahu, Hawaiian Islands, USA	HQ422850	—	—
<i>CWS/CEL/TR</i> <i>P</i> 12-19-3 [BDA0659]		C.W. Schneider, C.E. Lane, T.R. Popolizio/ 19 Jan. 2012	Annie's Bay, St David's I., Bermuda (2-3 m)	KR005322	KR005373	—
<i>TRP</i> 12-37-2 [BDA0888]		T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005313	—	—
<i>TRP</i> 12-37-2 [BDA0889]		T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005312	—	—
<i>TRP</i> 12-37-3 [BDA0890]		T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005319	—	—
<i>TRP</i> 12-47- 13[BDA0986]		T.R. Popolizio/ 26 Mar. 2012	Shell Point, Tucker's Town, Castle Harbour, Bermuda I. (0-2 m)	KR005314	—	—

	<i>TRP</i> 12-48-3 [BDA0995]	T.R. Popolizio/ 29 Mar. 2012	Cahow Islets, Turtle Beach, St. Davids I., Bermuda (0-2 m)	KR005318	—	—
	<i>TRP</i> 12-53-2 [BDA1022]	T.R. Popolizio/ 5 May 2012	Building Bay, St George's I., Bermuda (0-1 m)	KR005315	—	KR005376
	<i>TRP</i> 12-56-2 [BDA1044]	T.R. Popolizio/ 8 May 2012	Achilles Bay, St. Georges I.,	KR005321	—	—
	<i>TRP</i> 12-59-7 [BDA1081]	T.R. Popolizio/15 May 2012	Cove opposite Barry Lane, St. George's I., Bermuda	KR005317	—	—
	<i>TRP</i> 12-63-9 [BDA1123]	T.R. Popolizio/ 26 May 2012	Church Bay, south shore Bermuda I., Bermuda (0-3 m)	KR005323	—	—
	<i>TRP</i> 12-78-4 [BDA1206]	T.R. Popolizio/ 20 June 2012	The wreck of the <i>Pelinaion</i> , south shore Bermuda I., Bermuda (10-23 m)	KR005311	—	—
	<i>TRP</i> 12-81-3 [BDA1242]	T.R. Popolizio/ 26 June 2012	Fort St. Catherine's, St. George's I., Bermuda (0-3 m)	KR005316		
	<i>TRP</i> 12-81-5 [BDA1244]	T.R. Popolizio/ 26 June 2012	Fort St. Catherine's, St. George's I., Bermuda (0-3 m)	KR005320	—	—
	<i>CWS/CEL/TR</i> <i>P</i> 13-7-3 [KW063]	C.W. Schneider, C.E. Lane, T.R. Popolizio/ 28 May 2013	Ft. Zachary Taylor, Key West, Florida, USA (3-4 m)	KR005310	KR014259	KR005375
<i>Ganonema yoshizakii</i> Huisman, I.A. Abbott et Sherwood	IA28822	R. Okano/ 25 May 2002	Pepeekeo, Hawaii Island, Hawaii, USA	—	—	AY424876
	ARS00054	R. Okano/ 25 May 2002 M.H.	Peepeekeo, Hawaiian Islands, USA	HQ422853	—	—

<i>Gloiocallis dendroidea</i> (P. Crouan et H. Crouan) S.-M. Lin, Huisman et D.L. Ballant.	FLUSA-1997- Lm	Hommersand/ 10 Mar. 1997	West Summerland, Key West, Florida, USA	—	KF667101	—
	<i>CWS/CEL/TR</i> <i>P</i> 12-8-6 [BDA0550]	C.W. Schneider, C.E. Lane, T.R. Popolizio/ 17 Jan. 2012	Brackish Pond Flats, north shore, Bermuda (3-4 m)	KR005356	—	—
	<i>CWS/CEL/TR</i> <i>P</i> 12-8-6 [BDA0551]	C.W. Schneider, C.E. Lane, T.R. Popolizio/ 17 Jan. 2012	Brackish Pond Flats, north shore, Bermuda (3-4 m)	KR005355	KR005371	—
	<i>CWS/CEL/TR</i> <i>P</i> 12-19-10 [BDA0667]	C.W. Schneider, C.E. Lane, T.R. Popolizio/ 19 Jan. 2012	Annie's Bay, St. David's I., Bermuda (2-3 m)	KR005357	—	—
	<i>CWS/CEL/TR</i> <i>P</i> 12-19-10 [BDA0668]	C.W. Schneider, C.E. Lane, T.R. Popolizio/ 19 Jan. 2012	Annie's Bay, St. David's I., Bermuda (2-3 m)	KR005358	—	KR005380
	<i>TRP</i> 12-37-5 [BDA0892]	T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005361	—	—
	<i>TRP</i> 12-37-5 [BDA0893]	T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005359	—	—
	<i>TRP</i> 12-47-6 [BDA0978]	T.R. Popolizio/ 26 Mar. 2012	Shell Point, Tucker's Town, Castle Harbour, Bermuda I. (0-2 m)	KR005354	—	—
	<i>TRP</i> 12-47-6 [BDA0979]	T.R. Popolizio/ 26 Mar. 2012	Shell Point, Tucker's Town, Castle Harbour, Bermuda I. (0-2 m)	KR005362	—	—

	TRP 12-156-1 [BDA1701]	T.R. Popolizio/ 26 Nov. 2012	Officer's Beach, St. David's I., Bermuda (0-1 m)	KR005360	—	—
<i>Helminthocladia australis</i> Harv.	GWS02278	R. Withall/ 20 Nov. 2010	Ned's Beach, Lord Howe Island, NSW Australia	KC250430	—	—
<i>Helminthocladia calvadosii</i> (J.V. Lamour. ex Duby) Setchell	LLG3069	Y. Gladu, A. Besnier L. Le Gall	Beniguët, Finistère, Brittany, France	HQ603218	—	—
<i>Helminthocladia hudsonii</i> (C. Agardh) J. Agardh	LLG3000	L. Le Gall, J.M. Utge, Y. Turpin	Lampaul île Ségat, Finistère, Brittany, France	HQ603219	—	—
<i>Helminthocladia kempii</i> Popolizio, C.W. Schneid. et Chengsupanimit	[BDA0591]	C.W. Schneider/ 18 Jan. 2012	Cove east of Spanish Point, Bermuda I., Bermuda	KC250437	—	—
	[BDA0592]	C.W. Schneider/ 18 Jan. 2012	Cove east of Spanish Point, Bermuda I., Bermuda	KC250436	—	—
	[BDA0813]	T.R. Popolizio/ 7 Feb. 2012	Grape Bay Beach, Bermuda I., Bermuda	KC250432	—	—
	[BDA0856]	T.R. Popolizio/ 15 Feb. 2012	Bowen Pt., Shelly Bay, Bermuda I., Bermuda	KC250435	—	—
	TRP 12-51-1 [BDA1013]	T.R. Popolizio/ 11 Apr. 2012	Whalebone Bay, St. George's I., Bermuda	KC250431	KR005366	—
<i>Helminthocladia rhizoidea</i> (Doty et I.A. Abbott)	ARS03858	A. Kurihara/ 15 Mar. 2008	Malaekahana Beach Park, Oahu, Hawaiian Islands, USA	HQ423009	—	—
<i>Helminthora australis</i> J. Agardh ex Levring	MURU JB 629	J. Huisman/ 29 Oct. 2000	South of Cervantes, Western Australia, Australia	—	—	AY570373
<i>Helminthora divaricata</i> (C. Agardh) J. Agardh	LLG3071	L. Le Gall, J.M. Utge, Y. Turpin/Date not available	Beniguët, Finistère, Brittany, France	HQ603220	—	FR715032

<i>Hommersandiophycus borowitzkae</i> (Huisman) S.-M. Lin et Huisman	GWS000148	J. Huisman/ 10 Feb. 1997	Surf Pt., Barrow I., Western Australia, Australia.	—	—	AY570371
	NTOU- Phil2003-izz	S.-L. Liu/ 17 Feb. 2003	Bulusan, Philippines	—	KF667097	—
	NTOU-HK20 09-L-18a	S.-M. Lin/ 12 Feb. 2009	Haikou, Pintung Co., S. Taiwan	—	KF667093	—
<i>Hommersandiophycus clavatus</i> (Yamada) S.-M. Lin et Huisman	NTOU- DBS26iv2002 Lo2	S.-M. Lin/ 26 Apr. 2002	Dabaisha, Green Island, Taitung Co., E. Taiwan	—	KF667100	—
<i>Hommersandiophycus pectinatus</i> (Collins et Herv.) Popolizio, C.W. Schneid. et C.E. Lane	CWS 06-14-5	C.W. Schneider, C.E. Lane/ 21 June 2006	Due south of Frick's Beach, Tucker's Town, Bermuda (5-10 m)	HQ603226	—	—
	TRP 12-79-4 [BDA 1221]	T.R. Popolizio/ 20 June 2012	Cathedral Rock, off south shore Bermuda I., Bermuda (15-18 m)	KR005324	KR005374	KR005384
<i>Hommersandiophycus samaensis</i> (C.K.Tseng) S.-M. Lin et Huisman	IA28824	J. Huisman/ 24 May 2002	Leleiwi, Hawaii, Hawaiian Islands, USA	—	—	AY424879
	NTOU- HK2009-L-e ARS00068	S.-M. Lin/ 12 Feb. 2009 J. Huisman/ 24 May 2002	Haikou, Pintung Co., S. Taiwan Leleiwi Beach Park, Hawaiian Islands, USA	— HQ422868	KF667102 —	— —
<i>Izziella formanosa</i> (Yamada) S.-M. Lin, S.-Y. Yang et J.M. Huisman	—	S.-L. Liu/ 15 Jan. 2006	Chuan Fan Shi, Kenting National Park, Pingtung Co., S. Taiwan	—	GU357688	—

<i>Izziella kuroshioensis</i> S.-M. Lin, S.-Y. Yang et J.M. Huisman	—	S.-M. Lin/ 12 Feb. 2009	Hai Kuo, Pingtung Co., S. Taiwan	—	GU357685	—
<i>Izziella orientalis</i> (J. Agardh) Huisman et Schils	ARS02577	D. O'Doherty, A. Kurihara/ 18 May 2007	Kawailoa, Oahu, Hawaiian Islands, USA	HQ422602	—	—
<i>Liagora albicans</i> J.V. Lamour.	IA28764	J. Huisman/ 14 May 2002	Pupukea, Oahu, Hawaiian Islands, USA	—	—	AY424872
	ARS00056	J. Huisman/ 14 May 2002	Pupukea, Oahu, Hawaiian Islands, USA	HQ422860	—	—
	—	S.-M. Lin/ 27 Apr. 2006	Feng Chui Sha, Pingtung Co., southern Taiwan	—	HQ901786	—
<i>Liagora boergesenii</i> Yamada	—	S.-M. Lin/ 1 Nov. 2003	Wan Li Tong, Kenting National Park, Pingtung Co., S. Taiwan	—	GU357679	—
<i>Liagora ceranoides</i> J.V. Lamour.	MJW10405	M.J. Wynne/ 22 Nov. 1995	Major's Bay, St. Kitts, Lesser Antilles, Caribbean	—	KR014260	—
	—	M.H. Hommersand/ 12 Mar. 1997	Florida, USA	—	GU357682	—
	KZN-b 2294	O. De Clerck, T. Schils, H. Verbruggen, E. Demeulenare/ 7 Dec. 2003	Sodwana, South Africa	—	—	DQ873277
	GWS002065	G.T. Kraft/ 1 Feb. 2004	Leward side of Balls Pyramid, Lord Howe Is., Australia	—	—	DQ873278
	—	S.-L. Liu/ 17 Apr. 2005	Nan Wan, Kenting National Park, Pingtung Co., S. E. Taiwan	—	GU357681	—

	UNB JH05A2- 59/K10-H LLG2851	J. Huisman/ 25 October 2010	Cassini Is., Western Australia	—	JX878367	—
<i>Liagora distenta</i> (Mertens ex Roth) J.V. Lamour.		L. Le Gall/ 10 June 2009	Port-Cros, la Gabiniere, French Riviera, France	HQ603222	—	—
<i>Liagora julieae</i> I.A. Abbott et Huisman	IA28798	L.M. Hodgson/ 5 June 2002	Maili, Oahu, Hawaiian Islands, USA	—	—	AY424873
	ARS00050	L.M. Hodgson/ 5 June 2002	Maili Point, Oahu, Hawaiian Islands, USA	HQ422852	—	—
<i>Liagora mannarensis</i> V. Krishnam. et Sundararajan	GWS000147	J. Huisman/ 2 Feb. 1997	Anemone Lump, Abrolhos I., Western Australia, Australia	—	—	AY570374
	TRP 12-42-3 [BDA0944]	T.R. Papolizio/ 14 Mar. 2012	Middle buoy, Eastern Blue Cut channel, north of Daniel's Head, off Somerset I., Bermuda (16 m)	KR005353	KR005368	KR005382
<i>Liagora nesophila</i> Papolizio, C.W. Schneid. et C.E. Lane	CWS/CEL 08- 5-9	C.W. Schneider, C.E. Lane/ 12 Feb. 2008	Jct. Horseshoe/Higgs Is., Town Cut, St. George's Harbour, Bermuda (1 m)	—	KR005369	—
	CWS/CEL/TR P 12-10-10 [BDA0585]	C.W. Schneider, C.E. Lane, T.R. Papolizio/ 18 Jan. 2012	Spanish Point Park, north shore Bermuda I., Bermuda (0-1 m)	KR005349	—	—
	CWS/CEL/TR P 12-10-10 [BDA0586]	C.W. Schneider, C.E. Lane, T.R. Papolizio/ 18 Jan. 2012	Spanish Point Park, north shore Bermuda I., Bermuda (0-1 m)	KR005350	—	—
	TRP 12-28-3 [BDA779]	T.R. Papolizio/ 1 Feb. 2012	Old Ferry Crossing, St. George's I., Bermuda (0-1 m)	KR005348	—	—

		<i>TRP</i> 12-32-9 [BDA846]	T.R. Popolizio/ 13 Feb. 2012	Walsingham Bay, Castle Harbour, Bermuda I., Bermuda (0-1 m)	KR005345	KR014263	KR005383
		<i>TRP</i> 12-32-9 [BDA847]	T.R. Popolizio/ 13 Feb. 2013	Walsingham Bay, Castle Harbour, Bermuda I., Bermuda (0-1 m)	KR005346	—	—
		<i>TRP</i> 12-37-7 [BDA894]	T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005351	—	—
		<i>TRP</i> 12-37-7 [BDA895]	T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005352	—	—
		<i>TRP</i> 12-51-3 [BDA1015]	T.R. Popolizio/ 11 Apr. 2012	Whalebone Bay, St. George's I., Bermuda (0-1 m)	KR005344	—	—
		<i>TRP</i> 12-62-4 [BDA1109]	T.R. Popolizio/ 25 May 2012	Bowen Point, Shelly Bay, Bermuda I., Bermuda (0-2 m)	KR005347	—	—
	<i>Liagora viscida</i> (Forssk.) C. Agardh	GWS002481/ ODC 928	O. De Clerck/ 16 July 2003	Presqu' Ile de Giens, France	—	—	DQ873274
		—	Rodriguez- Prieto/ 12 Aug. 2005	El Golfet, Calella de Palafrugell, Spain	—	GU357678	—
		LLG2593	L. Le Gall/ 7 June 2009	Port-Cros, Montrémian, French Riviera, France	HQ603228	—	—
	<i>Liagora</i> sp. 1	ARS03538	A. Kurihara/ 17 Mar. 2007	Anahola Beach Park, Kauai, Hawaiian Islands, USA	HQ422956	—	—
	<i>Liagora</i> sp. 2	ARS03536	A. Kurihara/ 7 Sept. 2007	Oceanic Institute Reef, Oahu, Hawaiian Islands, USA	HQ422954	—	—
	<i>Liagora</i> sp. 3	ARS03335	A. Kurihara/ 17 Mar. 2007	Anini Reef, Kauai, Hawaiian Islands, USA	HQ422780	—	—
	<i>Macrocarpus perennis</i> (I.A. Abbott) S.-M. Lin, S.-Y. Yang et Huisman	IA28535a	N. Ogden/ 27 July 2001	Mali Point, Oahu, Hawaiian Islands, USA	—	—	AY424870

	NTOU23xii07 WLD125	S.-M. Lin/ 23 Dec. 2007	Wan Li Tong, Pingtung Co., Taiwan	—	HQ901783	—
<i>Macrocarpus</i> sp.	HEC8208a	E. Coppejeans	Nyali Beach, Mombasa, Kenya	—	KR014261	—
<i>Nemalion lubricum</i> Duby	LLG0143	L. Le Gall/ 18 May 2005	Platga els Castell, Palamos, Catalunya, Spain	HQ603231	—	FR715028
<i>Nemalion multifidum</i> (F. Weber et D. Mohr) Chauv.	GWS001817	G.W. Saunders	Mullaghmore Head, Connacht, Ireland	—	—	FR715027
<i>Neoizziella asiatica</i> S.-M. Lin, S.-Y. Yang et Huisman	—	S.-L. Liu/ 7 Nov. 2002	Cebu, the Philippines	—	HQ901788	—
	—	S.-M. Lin/ 23 Dec. 2007	Won Li Tong, Pingtung Co., southern Taiwan	—	HQ901778	—
	—	S.-M. Lin/ 24 July 2008	Serangan I., Bali, Indonesia	—	HQ901789	—
<i>Neoizziella divaricata</i> (C.K. Tseng) S.-M. Lin, S.-Y. Yang et Huisman	—	S.-L. Liu/ 16 Mar. 2006	Feng Gang, Pingtung Co., southern Taiwan	—	HQ901781	—
	ARS02620	D. Gebhart/ 5 Mar. 2007	Hunakai, Oahu, Hawaiian Islands, USA	HQ423117	—	—
<i>Stenopeltis gracilis</i> (Yamada et Tak. Tanaka) Itono et M. Yoshizaki	—	S.-M. Lin/ 26 Apr. 2002	Lung-Meng Port, Orchid Island, Taitung Co., S. E. Taiwan	—	GU357696	—
	ARS03722	30 Nov. 1999	Midway Atoll, Hawaiian Islands, USA	HQ422952	—	—

<i>Titanophycus setchellii</i> (Yamada) S.-M. Lin, S.-Y. Yang et Huisman	—	S.-M. Lin/ 26 Apr. 2002	Da Bai Sha, Green Island, E. Taiwan	—	GU357694	—
	HEC12207	O. Dargent	Makunduchi, Zanzibar	—	KR014262	—
<i>Titanophycus validus</i> (Harv.) Huisman, G.W. Saunders et Sherwood	GWS000152	J. Huisman/ 3 Oct. 1995	Abrolhos I., Western Australia, Australia	—	—	AY570375
	—	M.Hommersand/ 26 Mar. 2006	French Bay, San Salvador, Bahamas Islands	—	GU357693	—
	ARS00035	J. Huisman/ 2 Apr. 2002	Makapuu, Oahu, Hawaiian Islands, USA	HQ422845	—	—
	TRP 12-30-3 [BDA0809]	T.R. Popolizio/ 7 Feb. 2012	Grape Bay Beach, south shore Bermuda I., Bermuda (intertidal rock)	KR005343	—	KR005379
	TRP 12-37-4 [BDA0891]	T.R. Popolizio/ 24 Feb. 2012	Fort St. Catherine's, St. George's I., Bermuda (0-2 m)	KR005334	KR005370	—
	TRP 12-49-4 [BDA1004]	T.R. Popolizio/ 31 Mar. 2012	Lobster hole, off north shore Bermuda I., Bermuda (0-3 m)	KR005337	—	—
	TRP 12-63-3 [BDA1117]	T.R. Popolizio/ 26 May 2012	Church Bay, south shore Bermuda I., Bermuda (0-3 m)	KR005336	—	—
	TRP 12-65-1 [BDA1129]	T.R. Popolizio/ 27 May 2012	Fort Hill Bay, Cooper's Island, St. David's I., Bermuda (0-2 m)	KR005342	—	—
	TRP 12-78-9 [BDA1212]	T.R. Popolizio/ 20 June 2012	The wreck of the Pelinaion, south shore Bermuda I., Bermuda (10-23 m)	KR005341	—	—
	TRP 12-81-6 [BDA1245]	T.R. Popolizio/ 26 June 2012	Fort St. Catherine's, St. George's I., Bermuda (0-3 m)	KR005333	—	—

<i>Trichogloea herveyi</i> W.R Taylor	<i>TRP</i> 12-82-3 [BDA1248]	T.R. Popolizio/ 26 June 2012	Building Bay, St George's I., Bermuda (0-2 m)	KR005339	—	—
	<i>TRP</i> 12-82-3 [BDA1249]	T.R. Popolizio/ 26 June 2012	Building Bay, St George's I., Bermuda (0-2 m)	KR005340	—	—
	<i>TRP</i> 12-82-6 [BDA 1252]	T.R. Popolizio/ 26 June 2012	Building Bay, St George's I., Bermuda (0-2 m)	KR005338	—	—
	<i>TRP</i> 12-86-2 [BDA1284]	T.R. Popolizio/ 6 Aug. 2012	Spanish Point Park, north shore Bermuda I., Bermuda (0-3 m)	KR005335	—	—
	<i>CWS/CEL</i> 06-14-4	C.W. Schneider, C.E. Lane/ 21 Jun. 2006	Due south of Frick's Beach, Tucker's Town, Bermuda	HQ603260	—	—
	<i>CWS/CEL</i> 10-5-27 [BDA0036]	C.W. Schneider, C.E. Lane/ 19 Aug. 2010	Off Frick's Beach, Tucker's Town, Bermuda I., Bermuda (10-12 m)	KR005332	—	—
	<i>TRP</i> 12-60-2 [BDA1083]	T.R. Popolizio/ 15 May 2012	Tobacco Bay, St. George's I., Bermuda (0-2 m)	KR005325	—	—
	<i>TRP</i> 12-60-2 [BDA1084]	T.R. Popolizio/ 15 May 2012	Tobacco Bay, St. George's I., Bermuda (0-2 m)	KR005330	KR005367	KR005381
	<i>TRP</i> 12-60-3 [BDA1085]	T.R. Popolizio/ 15 May 2012	Tobacco Bay, St. George's I., Bermuda (0-2 m)	KR005328	—	—
	<i>TRP</i> 12-61-3 [BDA1091]	T.R. Popolizio/ 16 May 2012	North Rock, off north shore Bermuda I., Bermuda (10 m)	KR005326	—	—
	<i>TRP</i> 12-61-3 [BDA1092]	T.R. Popolizio/ 16 May 2012	North Rock, off north shore Bermuda I., Bermuda (10 m)	KR005327	—	—
	<i>TRP</i> 12-79-2 [BDA1218]	T.R. Popolizio/ 20 June 2012	Cathedral Rock, off south shore Bermuda I., Bermuda (15-18 m)	KR005329	—	—

<i>Trichogloeopsis mucosissima</i> (Yamada) I.A. Abbott et Doty	TRP 12-81-4 [BDA1243]	T.R. Popolizio/ 26 June 2012	Fort St. Catherine's, St. George's I., Bermuda (0-3 m)	KR005331	—	—
	NTOU-OkJP2009-Tm	M. Suzuki/ 26 Mar. 2009	Nashiro Beach, Itoman, Okinawa Prefecture, Japan,	—	KF667107	—
	FrenchBay-L.4	Don Ott/ 16 Mar. 2006	French Bay, southern end, San Salvador Island, Bahamas	—	KF667108	—
	TRP 12-58-10 [BDA1069]	T.R. Popolizio/ 9 May 2012	Crescent Reef, north shore Bermuda I., Bermuda (8 m)	KR005363	—	KR005378
	TRP 12-58-12 [BDA1071]	T.R. Popolizio/ 9 May 2012	Crescent Reef, north shore Bermuda I., Bermuda (8 m)	—	KR005372	—
<i>Yoshizakia indopacifica</i> S.-M. Lin, J. Huisman & Payri	NTOU-Lia1-3-8ii2010	S.-M. Lin/ 8 Feb. 2010	Nan Wan, Kenting National Park, Pintung County, S. Taiwan	—	JX878376	—
Galaxauraceae						
<i>Actinotrichia fragilis</i> (Forsskål) Børgesen	—	S. L. Liu and L. M. Liao/ 7 Nov. 2002	Sulpa Island, Cebu, Philippines	—	AY688010	—
	GWS000151	J. Huisman/ 17 Aug. 1995	Ningaloo Reef, Western Australia	—	—	AY570363
<i>Dichotomaria marginata</i>	GWS001893	H. Ruiz/ 20 Oct. 2003	Lajas, Turrumote Reef, La Parguera, Puerto Rico	—	KC134339	—
<i>Galaxaura rugosa</i> (Ellis et Solander) Lamouroux	SAP 101116	A. Kurihara & S. Shimada	Rottneest Island, Western Australia	—	AB258448	—

	GWS000143	J. Huisman/ 28 Nov 1995	Barrow Island, Western Australia	—	—	AY570368
<i>Tricleocarpa cylindrica</i> (Ellis et Solander) Huisman et Borowitzka	—	S. Fredericq/ 29 Mar. 1995	Guadeloupe, West Indies	—	AY688012	—
Scinaeaceae						
<i>Nothogenia fastigiata</i> (Bory) P.G. Parkinson	GWS001463	G.W. Saunders/ 25 Nov. 2002	Bicheno, Tasmania, Australia	—	KC134356	—
<i>Scinaia complanata</i> (Collins) Cotton	TRP 12-40-7 [BDA0928]	T.R. Popolizio/ 13 Mar. 2012	Gurnet Rock, mouth of Castle Harbour, s. shore, Bermuda (13 m)	—	KR005364	—
<i>Whidbeyella cartilaginea</i> Setch. et N.L. Gardner	GWS019662	G. Saunders & K. Dixon	Bamfield, Scotts Bay, British Columbia, Canada	—	—	FR717917
Yamadaellaceae						
<i>Yamadaella caenomyce</i> (Decne.) I.A. Abbott	GWS000152	J. Huisman/ 26 Nov. 1995	Eagles Nest, Barrow I., Western Australia, Australia	—	—	AY570378
	ARS02576	A. Kurihara/ 6 May 2007	Diamond Head, Oahu, Hawaiian Islands, USA	HQ422593	—	—
<i>Yamadaella grassyi</i> Popolizio, C.W. Schneid. et C.E. Lane	TRP 12-27-6 [BDA0766]	T.R. Popolizio/ 31 Jan. 2012	Ariel Sands, Cox's Bay, south shore Bermuda I. (intertidal)	KR005303	—	—

<i>TRP</i> 12-30-4 [BDA0810]	T.R. Popolizio/ Feb. 2012	7 Grape Bay Beach, south shore Bermuda I., Bermuda (intertidal)	KR005307	—	—
<i>TRP</i> 12-30-4 [BDA0811]	T.R. Popolizio/ Feb. 2012	7 Grape Bay Beach, south shore Bermuda I., Bermuda (intertidal)	KR005309	—	—
<i>TRP</i> 12-74-1 [BDA1184]	T.R. Popolizio/ 18 June 2012	Doe Bay, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005300	—	—
<i>TRP</i> 12-74-1 [BDA1185]	T.R. Popolizio/ 18 June 2012	Doe Bay, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005298	—	—
<i>TRP</i> 12-74-1 [BDA1186]	T.R. Popolizio/ 18 June 2012	Doe Bay, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005291	—	—
<i>TRP</i> 12-75-1 [BDA1187]	T.R. Popolizio/ 18 June 2012	Rocky Bay, Devonshire, south shore Bermuda I., Bermuda (intertidal)	KR005301	—	—
<i>TRP</i> 12-75-1 [BDA1188]	T.R. Popolizio/ 18 June 2012	Rocky Bay, Devonshire, south shore Bermuda I., Bermuda (intertidal)	KR005292	—	—
<i>TRP</i> 12-75-1 [BDA1189]	T.R. Popolizio/ 18 June 2012	Rocky Bay, Devonshire, south shore Bermuda I., Bermuda (intertidal)	KR005304	—	—
<i>TRP</i> 12-76-1 [BDA1190]	T.R. Popolizio/ 18 June 2012	Cox's Bay, Devonshire, south shore Bermuda I., Bermuda (intertidal)	KR005293	—	—
<i>TRP</i> 12-76-1 [BDA1191]	T.R. Popolizio/ 18 June 2012	Cox's Bay, Devonshire, south shore Bermuda I., Bermuda (intertidal)	KR005302	KR005365	—

<i>TRP</i> 12-76-1 [BDA1192]	T.R. Popolizio/ 18 June 2012	Cox's Bay, Devonshire, south shore Bermuda I., Bermuda (intertidal)	KR005297	—	—
<i>TRP</i> 12-77-1 [BDA1195]	T.R. Popolizio/ 18 June 2012	Southwest Grape Bay, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005305	—	—
<i>TRP</i> 12-77-1 [BDA1196]	T.R. Popolizio/ 18 June 2012	Southwest Grape Bay, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005306	—	—
<i>TRP</i> 12-77-1 [BDA1197]	T.R. Popolizio/ 18 June 2012	Southwest Grape Bay, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005299	—	—
<i>TRP</i> 12-140-1 [BDA1574]	T.R. Popolizio/ 13 Oct. 2012	East end of Grape Bay Beach, Paget, south shore Bermuda I., Bermuda (intertidal rock)	KR005294	—	KR005377
<i>TRP</i> 12-172-2 [BDA1813]	T.R. Popolizio/ 10 Dec. 2012	East end of Grape Bay Beach, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005296	—	—
<i>TRP</i> 12-172-2 [BDA1814]	T.R. Popolizio/ 10 Dec. 2012	East end of Grape Bay Beach, Paget, south shore Bermuda I., Bermuda (intertidal)	KR005295	—	—
<i>TRP</i> 13-22-3 [BDA1882]	T.R. Popolizio/ 18 Sep. 2013	Grape Bay Beach, south shore Bermuda I., Bermuda (intertidal)	KR005308	—	—

Figure 1. *rbcL* phylogeny: Bayesian analysis of sequence data from the Liagoraceae family with representative members of the Galaxauraceae and Scinaiceae included as outgroups. Posterior probabilities followed by maximum likelihood (ML) bootstrap values are shown at the nodes. An asterisk (*) denotes full support in both analyses, a dash (-) indicates less than 50% support.

Figure 2. Maximum likelihood analysis of LSU sequence data from the Liagoraceae family with representative members of the Galaxauraceae and Scinaiceae included as outgroups. ML bootstraps followed by Bayesian posterior probabilities are shown at the nodes. An asterisk (*) denotes full support in both analyses, a dash (-) indicates less than 50% support

Figures 3-7. *Liagora mannarensis* [TRP 12-42-3] **3.** Habit of live specimen, scale bar = 2 cm. **4.** Carpogonial branch (arrow). Scale bar 20 μm . **5.** Division of the carpogonium from both the proximal and distal cells of the first transverse division (arrows indicate distally divided cells). Scale bar = 10 μm . **6.** Fusion cell (arrow). Scale bar = 20 μm . **7.** Carposporophyte with sterile filaments. Scale bar = 40 μm .

Figures 8-14. *Liagora nesophila* sp.nov. **8.** Habit of holotype specimen [CWS/CEL 8-5-9]. Scale bar = 2 cm. **9.** Habit of live specimen [TRP 12-28-3]. Scale bar = 1 cm. **10.** Branch tip showing cortical fascicles [CWS/CEL 6-22-4]. Scale bar = 100 μm . **11.** Early developing distal gonimoblasts with unfused carpogonial branches (arrowheads) and developing sterile filaments [holotype, CWS/CEL 8-5-9]. Scale bar = 20 μm . **12.** Gonimoblasts emerging from distal end of fusion cell (arrow) and developing sterile filaments [holotype, CWS/CEL 8-5-9]. Scale bar = 20 μm . **13.** Carposporophyte showing extensive sterile filaments [TRP 12-47-2]. Scale bar = 100 μm . **14.** Spermatangia (arrowheads) [TRP 12-28-3]. Scale bar = 30 μm .

Figures 15-19. *Trichogloeopsis pedicellata* **15.** Habit [TRP 12-61-1]. Scale bar = 5 cm. **16.** Carposporophyte with carpogonial branch showing pedicel [TRP 12-61-1]. Scale bar = 40 μ m. **17.** Gonimoblast with descending gonimorhizoids (arrows) [TRP 12-58-10]. Scale bar = 40 μ m. **18.** Spermatangia (arrowheads) [TRP 12-58-12]. Scale bar = 20 μ m. **19.** Multiple carpogonial branches developing from one basal cell (arrows) [TRP 12-58-12]. Scale bar = 20 μ m.

Figures 20-25. *Yamadaella grassyi* sp. nov. **20.** Habit [holotype, TRP 12-74-1]. Scale bar = 2 cm. **21.** Cortical fascicles displaying obpyriform terminal cells [TRP 12-77-1]. Scale bar 40 μ m. **22.** Three-celled carpogonial branch [TRP 12-74-1]. Scale bar = 20 μ m. **23.** Fused carpogonial branch with gonimoblast initials (arrows) [TRP 12-76-1]. Scale bar = 20 μ m. **24.** Spermatangia (arrowheads) [TRP 12-27-6]. Scale bar = 10 μ m. **25.** Gonimoblast with terminal carposporangia (arrowheads) [TRP 12-76-1]. Scale bar = 20 μ m.

Figures 26-29. *Hommersandiophycus pectinatus* comb. nov. **26.** Habit, with branches bearing secund branchlets (arrow) [*CWS* 85-1-7]. Scale bar = 2 cm. **27.** Carpogonial branch with dividing carpogonium and trichogyne [*CWS* 83-7-5]. Scale bar = 20 μ m. **28.** Carposporophyte with loosely arranged sterile filaments [*CWS* 83-7-5]. Scale bar = 100 μ m. **29.** Spermatangia (arrowheads) [*TRP* 12-79-4]. Scale bar = 20 μ m.

Figure S1. Liagoracean species groups determined with UPGMA clustering of the COI-5P genetic barcode. Specimens with data generated for this study appear in bolded text. Where more than one specimen is represented for a single species group, n=number of specimens.

CHAPTER FOUR

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MOLECULAR ANALYSIS RESOLVES THE TAXONOMY
OF THE *LAURENCIA* COMPLEX (RHODOMELACEAE, CERAMIALES)
IN BERMUDA AND UNCOVERS NOVEL SPECIES
IN THE WESTERN ATLANTIC OCEAN

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ABSTRACT

In the last decade, molecular tools have revealed a significant number of previously unrecognized taxa in Bermuda's marine flora, especially among the red algae (Rhodophyta). A number of species have been persistently misidentified based on morphological similarities to species described from other localities. Some have been assigned to existing taxa not previously reported for the islands, and many have been determined to be novel species. Of late, researchers have substantially modified the 'Laurencia complex' in several regions around the globe. In Bermuda, we have confirmed the presence of 5 of the 6 genera described in this complex—*Laurencia*, *Chondrophycus*, *Palisada*, *Yuzurua* and *Laurenciella*. Ribulose biphosphate carboxylase (*rbcL*) chloroplast sequences and cytochrome oxidase I (COI-5P) mitochondrial sequences both support the recognition of at least two species for what has historically been labeled *L. obtusa*, grouping with *L. dendroidea* and *L. catarinensis* in phylogenetic analyses. One novel species in the genus *Chondrophycus* has been identified, and represents the first report of the genus in the western Atlantic that has been verified with molecular data. We describe a new species within the *Laurenciella* clade, genetically distinct from both the monotypic *L. marilzae* and an undescribed species from Brazil. A full description of *L. microcladia* based on recent collections from Bermuda and the Caribbean Sea is also included in this study.

KEY WORDS: Bermuda, *Chondrophycus*, COI-5P, *C. planiparvus* sp. nov., Laurencieae, *Laurencia*, *Laurenciella*, *L. namii* sp. nov., *Palisada*, *rbcL*, *Yuzurua*

INTRODUCTION

The ‘*Laurencia* complex’ is a diverse sub-grouping of red algae in the Rhodomelaceae family (tribe = Laurencieae Schmitz 1889) found in tropical and temperate seas worldwide, and contains 208 currently accepted species (Guiry & Guiry 2015). The group has received substantial attention from phycologists in the past several decades due to its convoluted taxonomy. Within the complex (and in addition to *Laurencia sensu stricto*) the genus *Osmundea* has been resurrected (Nam et al. 1994), *Chondrophycus* has been elevated to generic status (Garbary and Harper 1998), and three new genera have been named— *Palisada*, *Yuzurua*, and most recently, *Laurenciella* (Nam 2007; Martin-Lescanne et al. 2010; Cassano et al. 2009). Members of the ‘*Laurencia* complex’ are known to exhibit considerable morphological plasticity, which can make them challenging to diagnose from a morphological point of view. However, each of the genera has been shown to be well-supported monophyletic clades in multiple publications, except for the taxon-rich genus *Laurencia* (145 spp.). This group is in need of extensive molecular and morphological analysis and, likely, reorganization.

As a genus, *Laurencia* has already been significantly modified by molecular-assisted alpha taxonomy (MAAT). Erected by Lamouroux (1813: 131-132) for just eight species, *L. obtusa* (Hudson) J.V. Lamour. was later lectotypified as the generitype (Schmitz 1889). In his 1931 monograph of *Laurencia*, Yamada separated the genus into four sections based on morphological and anatomical features. Saito (1967) segregated *Laurencia sensu lato* into two subgenera, designating these either *Chondrophycus* or *Laurencia* based on differences in tetrasporangial arrangement and

the presence (or absence) of secondary pit connections. Decades later, the genus *Osmundea* (Stackhouse 1809) was resurrected by Nam (1994) within the complex, and separated from *Laurencia* by the reproductive characters of tetrasporangial and spermatangial branch origin. By this time, it was also established that *Laurencia* possessed four pericentral cells while the other genera had only two. Using morphological cladistics, Garbary and Harper (1998) elevated the subgenus *Chondrophycus* to generic rank as sister to *Osmundea* based primarily on the presence of two (rather than four) pericentral cells in vegetative axes and trichoblast-type spermatangial development. *Osmundea* exhibits filament-type spermatangial development. Many of the species nested within the *Chondrophycus* clade resolved by Garbary and Harper have since been transferred to *Palisada*, a genus proposed and later validated by Nam (2007) based on Yamada's (1931) section *Palisadae* for *Laurencia*, with tetrasporangial development unique from that of *Chondrophycus*. Martin-Lescanne et al. (2010) segregated *Yuzurua* from *Palisada sensu stricto* on molecular evidence, adding a fifth genus to the complex. The authors determined the morphological traits of *Yuzurua* to mainly overlap with those of *Palisada*, but with secondary pit connections between cortical cells in the former, and lacking the characteristic palisade-like cells of the latter genus. A sixth genus, *Laurenciella*, has recently been added to the complex for a molecularly distinct clade that is entirely morphologically cryptic with sister genus *Laurencia sensu stricto* (Cassano et al. 2012b).

Members of the tribe Laurencieae are distinguished from the closely related genus *Chondria* by both vegetative and reproductive characters. *Chondria* displays

five pericentral cells per axial cell (mostly obvious only at the apices); genera in the *Laurencia* complex produce either four or two (Womersley 2003). Spermatangial plates form in *Chondria*; alternatively, development is from trichoblasts located within apical pits in Laurencieae (Nam 1999).

Several workers in the past two decades have provided a phylogenetic basis for reorganization of the *Laurencia* complex within a global context (Cassano et al. 2009, Gil-Rodriguez et al. 2009, Martin-Lescanne et al. 2010). Comprehensive surveys of the group using molecular tools are less common for discrete localities like Bermuda, but the results are particularly interesting when compared to historical accounts of the islands' flora. Reports of *Laurencia* in Bermuda begin to appear in the literature in the mid 19th century. The earliest reports of the Reverend Alexander Ferrie Kemp (1857) included *L. obtusa* (Huds.) J.V. Lamour. and *L. papillosa* C. Agardh (now *Palisada perforata* (Bory) K.W. Nam). *Laurencia obtusa* (type locality = England) was subsequently recorded from the islands by workers over the next century (Rein 1873, Dickie 1874, Hemsley 1884, Murray 1888, Collins et al. 1916 [as *P.B.-A.* 42:2092], Collins and Hervey 1917, Howe 1918, Tandy 1936, Bernatowicz 1952) followed by additional accounts in the 20th century. *Laurencia obtusa* var. *crucifera* Kütz. was reported by Dickie (1874), and var. *gracilis* (C. Agardh) Zanardini by Collins and Hervey (1917). Collins and Hervey (1917) reported *L. paniculata* J. Agardh from Bermuda and this species is presently regarded as a junior synonym of *L. obtusa*. Rein (1873) provided the first account of *Yuzurua poiteaui* (J.V. Lamour.) Martin-Lescanne (as *L. gemmifera* Harv.). Howe (1918) included *L. intricata* J.V. Lamour., *L. microcladia* Kütz., and *P. corallopsis* (Mont.) Senties, M.T. Fujii & Díaz-Larr. (as *L.*

corallopsis M. Howe) in his survey of the islands' flora. Almost a century later, Schneider and Lane (2005, 2007, 2010) added *L. decumbens* Kütz., *Yuzurua iridescens* (M.J. Wynne & D.L. Ballant.) Senties, M.J. Wynne, Cassano, Gil-Rodriguez & M.T. Fujii [as *Chondrophycus iridescens* (M.J. Wynne & D.L. Ballant.) Garbary & J.T. Harper] and *L. caraibica* P.C. Silva to the Bermuda flora.

The present study provides the molecular and morphological evidence needed to establish *Laurencia dendroidea* J. Agardh, *L. catarinensis* Cord.-Mar., & M.T. Fujii, *P. flagellifera* (J. Agardh) K.W. Nam, and novel species of *Laurenciella* and *Chondrophycus* as constituents of the *Laurencia* complex in the Bermuda flora, and to verify previous reports of the following members of this tribe: *L. intricata* J.V. Lamour., *L. microcladia* Kütz., *P. perforata* (Bory) K.W. Nam, *P. corallopsis* (Mont.) Senties, M.T. Fujii & Díaz-Larr., *Y. poiteaui* (J.V. Lamour.) Martin-Lescanne and *Y. iridescens* [as *P. iridescens* (M.J. Wynne & D.L. Ballant.) K.W. Nam]. Unfortunately, our attempts to obtain sequence data from archival material of *L. caraibica* and *L. decumbens* collected in Bermuda in the past have been unsuccessful.

MATERIALS AND METHODS

Standard methods

Collections were made in shallow water (0-3 m) or via scuba (0-23 m), and site locations were taken using a Garmin™ eTrex H (Olathe, Kansas, USA). A portion of each specimen used for DNA analysis was dried on silica gel and the remainder of the thallus was pressed onto herbarium paper as a permanent voucher. Selected fragments were preserved in 4-5% Formalin in seawater for anatomical study. 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. Live specimens chosen for DNA analysis were photographed using a Canon Powershot s90 digital camera (Canon Inc., Tokyo, Japan) and dried herbarium specimens were scanned on an HP 309a Photosmart Premium scanner (Hewlett-Packard Company, Palo Alto, California, USA). 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). Voucher specimens of some numbers are deposited in KIRI, MICH, NY, the Bermuda Natural History Museum (BAMZ) and Herbarium C.W. Schneider. Herbarium abbreviations follow the online Index Herbariorum <<http://sweetgum.nybg.org/ih/>> and standard author abbreviations are from Brummitt and Powell (1992).

Molecular methods

Specimens used in molecular analyses are recorded in Table 1. Silica dried samples for DNA analysis were ground in liquid nitrogen and stored at –20°C. DNA was extracted from 0.1-0.5 µl ground material using a GenElute Plant Genomic Miniprep Kit according to manufacturer protocol (Sigma-Aldrich, St. Louis, Missouri, USA) with 500 µl of modified lysis solution (50 µl 10% TWEEN 20, and 5 µl of 20 mg/ml ProK, as well as 1 hr 23°C incubation followed by a 20 min incubation on ice (Saunders and Druehl 1993).

DNA was amplified via polymerase chain reaction (PCR) with the Takara Ex-Taq DNA polymerase kit (PanVera, Madison, WI, USA) in an Eppendorf AG Mastercycler epGradient thermal cycler (Eppendorf, Hamburg, Germany). To assign all specimens to species groups, two oligonucleotide primers were used for both sequencing and amplification of the COI-5P mitochondrial marker, GWSFn (Le Gall and Saunders 2010) and GWSRx (Saunders and McDevit 2012). A denaturation cycle of 94°C for 4 min was followed by 38-42 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 1 min and a final extension of 72°C for 7 min. Several specimens were selected for additional sequencing of the plastid-encoded RuBisCO (*rbcL*) operon. Four oligonucleotide primers were used for both amplification and sequencing of two overlapping fragments (forward- RR1 5' ATGTCTAACTCTGTAGAAG 3' and reverse RR4 5' TTCAGCTCTTTCATACAT 3') and (forward- RrIf 5' TCTCAGCCTTTTATGCGTTG 3' and reverse Rrr 5' ATCTCACTATTCTATACTCC 3'). A denaturation cycle of 94°C for 4 min was followed by 35 cycles of 94°C for 1 min, 47°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 7 min. Amplified DNA was treated with the QIAquick PCR Purification Kit following the manufacturer's protocol (Qiagen Redwood City, California, USA), and the purified PCR product was sequenced at the Rhode Island Genomics and Sequencing Center using the Applied Biosystems Inc. 3130xl Genetic Analyzer (Life Technologies, Grand Island, New York, USA).

COI-5P sequences from representative species within the *Laurencia* complex, including those available through GenBank and newly determined here, were included in an alignment using the MUSCLE (multiple sequence comparison by log-

expectation) alignment program in Geneious (v. 6.1.8 available from <http://www.geneious.com>). To visually characterize genetic variability among specimens, the UPGMA clustering algorithm was applied to the COI-5P alignment (180 specimens, 458 sites) with Tamura-nei-corrected distances (default setting). The resulting tree was used to demarcate genetic species groups. Based on these groups, and with comparative data available from GenBank, one specimen from each species and/or geographic location was selected for phylogenetic analysis using *rbcL* sequences. The best models of evolution for the individual gene region *rbcL* (85 taxa, 1217 sites) were determined in jModelTest 2 (version 2.1.5; Darriba et al. 2012). The selected phylogenetic model (GTR + I + G in both instances) was used to complete both maximum likelihood (ML) and Bayesian analyses for each gene. The *rbcL* maximum likelihood phylogeny was estimated using the RAxML graphical user interface (Silvestro and Michalak 2012) with branch support calculated using 1000 bootstrap replicates. Bayesian analysis of *rbcL* was conducted in MrBayes v.3.2.2 (Ronquist and Huelsenbeck 2003) and run with four parallel chains (three heated + one cold) with branch lengths optimized during the run for one million generations. The initial 3650 trees were discarded as the burn-in, and posterior probabilities were estimated based on the remaining trees. The *rbcL* gene tree includes members of the genera *Chondria* and *Bostrychia* as outgroups. All trees (Figs 1, 2) were manipulated for presentation using FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

RESULTS AND DISCUSSION

Our DNA barcode (COI-5P) analysis included 180 sequences from members of the *Laurencia* complex; 170 of these were generated for this study from specimens collected in Bermuda, the Florida Keys, and St. Croix, USVI. Ten additional sequences were downloaded from GenBank, and represented several specimens collected in the Pacific (mainly Hawaii) and two species of *Osmundea* from France. The final COI-5P alignment consisted of 458 base pairs, of which 35.2% were informative characters. Intergeneric distance values for the five genera represented in our collections (all members of the ‘*Laurencia* complex’) were 8.3-12.9%. The barcode analysis was primarily used to organize our large dataset into 13 distinct genetic species groups (Fig. 1). For further generic and specific identification, the *rbcL* gene was far more informative, especially given the extent of molecular studies using this gene in the past decade.

To place them into a phylogenetic context, a representative of each of the previously determined genetic groups was included in a single gene (*rbcL*) analysis along with a diverse representation of taxa designated to the complex mined from GenBank (Table 1). Tree topologies from maximum likelihood (ML) and Bayesian analyses of *rbcL* were consistent; the phylogeny is illustrated by the ML tree, with bootstraps and posterior probabilities indicated at the nodes (Fig. 2). Phylogenetic associations among genera in the tribe conformed largely to those published by Cassano et al. (2012b). Our analysis similarly depicts the sister relationship of *Laurencia* and *Laurenciella*, with *Yuzurua* resolving basally to this subgroup, but as in the aforementioned papers’ results, only the Bayesian posterior probabilities show

strong support for these two relationships. Whereas the phylogeny in Cassano et al. suggests *Palisada* is the basal genus in the complex, ours instead indicates a well-supported basal grouping of the *Osmundea-Chondrophycus* clade.

Intergeneric *rbcL* distances in the complex were 8.7-14.5%, similar to the COI-5P distance results. For genera present in our collections, the greatest interspecific variation occurs among *Chondrophycus* (2.1-8.9%) and *Laurencia* (2.5-9.0%). That the higher range of these values overlap with intergeneric distances in the complex is indicative of the need for greater taxon sampling (including type specimens or specimens from type localities) and robust molecular analyses in these groups, and it is not unreasonable to expect that future studies will propose additional generic diversification within the *Laurencia* complex.

Both COI-5P and *rbcL* sequence data support the recognition of at least two species for collections long called *L. 'obtusum'* in Bermuda, both of which are only distantly related to *L. obtusum* from Europe. These species represent examples of western Atlantic entities with a misidentified European binomial placed upon them by phycologists of the 19th and early 20th centuries (see Popolizio et al. 2013). Both *Laurencia dendroidea* and *L. catarinensis* were recently described from Brazil (Cassano et al. 2012a; Machín-Sánchez 2012) and represent new reports for Bermuda. Our collections of *L. dendroidea* and *L. catarinensis* have all been attributed to *L. obtusum* in the past, but molecular data from this study suggest *L. obtusum* does not exist in the Bermuda Islands, and may not be present in the tropical western Atlantic. Both ML and Bayesian analyses present strong support for the conspecificity of Bermuda specimens with the respective species from Brazil and the Canary Islands. These data

also show that both species are present in the Florida Keys and St. Croix in the US Virgin Islands. Notably, specimens we have identified as having “red” habits in our collections group only within the *L. dendroidea* clade, along with green and purple-green morphs of this species. All specimens with thalli we have described as ‘green with pink tips’ in our field notes all fall within the *L. catarinensis* clade. It is likely that Howe (1918) was examining collections of *L. catarinensis* when describing ‘*L. obtusa*’ from the islands as “subglobose tufts” that are “often greenish with red tips,” features that appear to characterize the habit of this species based on our observations and genetic sequences.

The presence of *Laurencia intricata* in Bermuda has been verified with molecular data. Sequence data shows that this species, first reported for the islands by Howe (1918), is indeed conspecific with specimens from the Caribbean Antilles, the type locality of this species. We have discovered a specimen of *L. intricata* among Hervey’s archived material collected at Heron Bay, Bermuda (*P.B.-A* no. 1937, as *Laurencia tuberculosa* J. Agardh). This exsiccate has specimens of this number representing at least two heterotypic members of the ‘*Laurencia*-complex’. Early workers moved all *P.B.-A*. no. 1937 material to *Laurencia poiteaui*, yet one representative specimen (MICH 6605) is *L. intricata*, while the other (MICH 622078) is likely *Y. poiteaui*.

The first account of *Laurencia microcladia* in the literature is by Kützing (1865: p. 22, pl. 60, figs b, c) who illustrates material from the West Indies in a way that distinctly highlights the virgate habit of this species. Børgeson (1915) later reported *L. obtusa* var. *gelatinosa* (Desf.) J. Agardh from the region, a name that has

since been placed in synonymy with *L. microcladia*. Collins and Hervey (1917) describe their collections (reported as *L. obtusa* var. *gelatinosa*) as a “low and slender form of exposed rocky shores,” archetypal characteristics of this species. Howe (1918) also notes that intertidal collections of *L. microcladia* from Bermuda are included in “*Phyc. Bor.-Am.* 1888, as *L. obtusa* var. *gelatinosa*.” In his account, Børgesen highlights the pyramidal shape of the thallus, nature of verticillate branching with swollen, clavate branchlets, and the tendency of this species to exist in particularly exposed habitats. His illustration of this species, which he reports from the US Virgin Islands, bears a remarkable resemblance to our specimens of *L. microcladia* from St. Croix, USVI (Fig. 3). Taylor (1960) provides the first comprehensive description of *L. microcladia* for the western Atlantic region, which fits observations of our specimens from both Bermuda and St. Croix with morphological overlap in all characters for which data have been reported (Table 2).

Interestingly, *Laurencia microcladia*, one of the most pervasive species in Bermuda and the Caribbean Antilles (Taylor 1960), has not been included in any molecular studies of the *Laurencia* complex that have been conducted over the past several years in Mexico or Brazil (e.g. Martin-Lescanne 2010; Fujii et al. 2011; Senties et al. 2011; Cassano et al. 2012a; Cassano et al. 2012b; Machín-Sánchez et al. 2012; Machín-Sánchez et al. 2014; Mateo-Cid 2014). Fujii and Senties (2005) document previous reports of *L. microcladia* in Brazil (Oliveira Filho 1977, Cordeiro-Marino 1978, Pinheiro-Joventino et al. 1998, Figueiredo et al. 2004) but do not include the species in their detailed monograph of the complex in Brazil. The authors do, however, include *L. venusta*, a species from Japan reported for the first time in the

Atlantic by Senties et al. (2001) from the Mexican Caribbean, and described to be “entirely in concordance” with Japanese material examined by Saito (1964) and without “significant differences” from Yamada’s (1931) type specimen. The authors briefly mention the similarities between Mexican *L. venusta* and *L. chondriodes* Børgesen from the West Indies, but do not discuss similarities with other western Atlantic species of the genus, including *L. microcladia*, with which it shares a significant number of morphological characteristics. The features of *L. microcladia* from the western Atlantic, including Bermuda, conform to all of the vegetative and reproductive characters described for *L. venusta* from this region, except that in *L. venusta*, sparse, verticillate branching is emphasized. *Laurencia microcladia* is densely branched and possesses verrucose branchlets that are clustered in a manner that creates a whorled appearance (Fig 4), however, branching overall is more irregular than verticillate (Fig 7). Additionally, though the observed diameters for tetrasporangia and cystocarps in the two species overlap, the upper limits of these tend to be larger in *L. microcladia* than in *L. venusta*, especially compared with Caribbean specimens of the latter species (Table 2).

Our *rbcL* sequences from specimens of *Laurencia microcladia* are almost identical to those from *L. venusta* collected in Caribbean Mexico, with only 0.2-0.4% divergences between the sequences. Specifically, 2 of 1217 base pair were different between our St. Croix specimen and the GenBank data for *L. venusta* from Caribbean Mexico; 5 differences were present between both Caribbean specimens and Bermuda specimens. It is quite possible that the entities shown in the previous analyses are genetic variants of *L. microcladia*, a name that would be precedent over *L. venusta* by

more than half a century. Alternatively, the two may represent cryptic species from different ocean basins, but given the near genetic equivalence of these specimens as clearly demonstrated by our data, we think this is unlikely. Molecular sequence data for specimens of *L. venusta* from Japan will be required to bring clarity to this puzzling matter.

Our specimens of *Laurencia microcladia* are also closely related in *rbcL* sequence analysis to the recently described *L. laurahuertana* Mateo-Cid, Mendoza-Gonzalez, Senties & Díaz-Larr. (Mateo-Cid et al. 2014) with 0.5-0.7% interspecific variation. As such, the vegetative and reproductive characteristics observed in this species are also presented for comparative purposes in Table 2. *Laurencia laurahuertana* is distinguished from *L. microcladia* by its unusually diminutive size and exclusively being an epiphyte of seagrasses. *Laurencia microcladia* specimens collected in Bermuda are strictly intertidal with the exception of two shallow subtidal specimens from Spanish Point and Shelly Bay, but tidal exposure at these locations is possible during the lunar cycle. The presence of fertile specimens for *L. laurahuertana* suggests that these are not simply immature stages in the life cycle of a larger species. Other notable differences in *L. laurahuertana* include cortical cell projections (absent in *L. microcladia*), markedly smaller tetraporangial, cystocarpic and carposporangial diameters, and the absence of a sterile apical cell in spermatangial stichidia.

The molecular sequence data we have produced verifies that *Palisada perforata* in Bermuda is conspecific with specimens from the type locality in the Canary Islands, Spain. The species is the most common member of the genus *Palisada* in our collections, and is predominantly intertidal with a few exceptions from shallow

subtidal sites in the Florida Keys and a single subtidal collection from a shallow (3-4 m) site off Bermuda's north shore. Originally known from the Indian Ocean, *Palisada flagellifera* has been shown to be pan-tropical with reports from Cuba (Areces et al. 2003), Brazil (Fujii et al. 2006) and the Canary Islands (Gil-Rodriguez et al. 2010), and is newly established here for Bermuda based on molecular data.

Accounts of *Palisada corallopsis* (as *Laurencia corallopsis*) in Bermuda (type locality = Cuba) have appeared in the literature since the early 20th century [as *Laurencia corallopsis* (Mont.) M. Howe by Howe 1918, Frederick 1963; as *L. cervicornis* Harv. by Collins et al. (1917, as *P.B.-A.* 44: 2187), Collins & Hervey 1917; as *Chondrophycus corallopsis* (Mont.) K.W. Nam by Schneider 2003]. Harvey's (1853) *L. cervicornis* (type locality = Key West) had long been merged with *P. corallopsis* (Howe 1918) until Littler and Littler argued that it was much smaller in habit and had smaller surface cells than *P. corallopsis* (Dawes & Mathieson 2008). The Littlers were the first in modern times to resume using the name *L. cervicornis* for Caribbean material (Littler and Littler 2000).

Specimens field-identified as *Palisada corallopsis* from Bermuda and the Florida Keys are genetically variable, as shown by both the COI-5P and *rbcL* analyses (Figs 1, 2). For the latter gene, one genetic entity from Bermuda is closely related (0.6% divergence) and likely conspecific with specimens from Caribbean Mexico also identified as *P. corallopsis*. The other is sister to specimens we collected in the Florida Keys (2.4% divergence). Further study is needed to determine whether either of our Bermuda variants of *P. corallopsis* fits the protolog of *L. cervicornis* from Key West. Moreover, we must determine whether our collections from the Florida Keys, which

also display genetic variation when compared with Caribbean specimens' sequence data, can be morphologically tied to this long-synonymized name. Our COI-5P barcode investigation indicated a group within the *Palisada* clade that was distinct from reported Bermuda species. The *rbcL* phylogeny allies this grouping with *P. flagellifera* from Brazil and the Canary Islands, Spain, but comparative molecular data is not available for specimens from the Indian Ocean type locality of this species. The present report represents the first record of *P. flagellifera* in the Bermuda Islands.

Reports of the species currently known as *Yuzurua poiteaui* appear in the earliest literature for Bermuda as *Laurencia gemmifera* Harv. (Rein 1873, Dickie 1874, Hemsley 1884). This species was archived *pro parte* as *P.B.-A* 1937 (as *L. tuberculosa*; Collins et al. 1913), and as mentioned above, represents *Y. poiteaui* in a heterotypic collection (a specimen of the same *P.B.-A.* number in another fascicle can be attributed to *L. intricata*). Collins & Hervey (1917) first reported the species in Bermuda as *Laurencia poiteaui* (J.V. Lamour.) M. Howe; this name was later transferred to *Chondrophycus* (Nam 1999) and *Palisada* (Nam 2006, 2007) based on phylogenetic and morphological evidence. Using the *rbcL* sequences of *Palisada poiteaui* (J.V. Lamour.) K.W. Nam (as *Chondrophycus poiteaui*) and *C. gemmiferus* (Harvey) Garbary & Harper, Díaz-Larrea et al. (2007) concluded that the latter species, different only in minor anatomical characters, should be reduced to a synonym of *P. poiteaui* (Senties and Diaz-Larrea 2008). Molecular data has also prompted the elevation of *Yuzurua*, initially a subgenus of *Chondrophycus* recognized by Nam (1999), to the generic level (Martin-Lescanne et al. 2010) with *Y. poiteaui* as the generitype. This species appears to be relatively rare in Bermuda presently, with

only one collection in 2012 confirmed with molecular data. *Yuzurua poiteaui* specimens were far more abundant in our Florida Keys collections.

Recently, sequence data has shown that *Palisada iridescens* is closely aligned to *Yuzurua*, and a new combination, *Y. iridescens* is proposed in a forthcoming publication (Senties et al. in press). Our data agree with this transfer, as previous records of this species in Bermuda (Schneider and Lane 2007, as *C. iridescens*) group with the *Yuzurua* clade in our COI-5P barcode analysis (Fig. 1). Our analysis has also resolved a unique sequence from a Bermuda specimen in this clade sister to *Y. poiteaui* (4.3% divergence). Thus far, we are only able to confirm a single specimen with sequence data representing this entity, and will require additional collections with matching sequences before we verify that these represent a unique, third species in the genus. For the time being this taxon will be regarded as *Yuzurua* sp. 2 Bermuda (Figs 1, 2).

Our COI-5P barcode analysis and *rbcL* phylogeny have both uncovered a unique taxon in the genus *Chondrophycus* in Bermuda, herein described as the novel species *C. planiparvus* (Figs 1, 2). Sequences from *Chondrophycus* cf. *undulatus* (Yamada) Garbary & Harper, *C. dotyi* (Y. Saito) K.W. Nam, *C. succisus* (A.B. Cribb) K.W. Nam and one undescribed species of *Chondrophycus*, all collected in the Hawaiian Islands, group with our Bermuda species in COI-5P distance analysis. The *rbcL* data shows that the new species is closely related (0.8% divergence) to an undescribed specimen collected from Flower Garden Banks in the Gulf of Mexico (Fujii et al. 2006). These are arranged sister to the rest of the *Chondrophycus* clade,

which consists *C. cf. undulatus* and several undescribed species from New Caledonia, and *C. tranoi* from the Philippines.

Saito (1967) split the genus *Laurencia* into two subgenera, *Laurencia* and *Chondrophycus*, on the basis of tetrasporangial development relative to the axis (parallel or right-angle) and presence or absence of secondary pit connections between cortical cells. He later determined these features were unreliable since some species possessed features of both subgenera, namely a parallel arrangement of tetrasporangia and no secondary pit-connections (Saito 1982). Species exhibiting this combination of characters would later be attributed to *Osmundea* (Nam 1994). Ultimately, though, Garbary and Harper (1998) elevated the subgenus *Chondrophycus* to generic rank based on morphological cladistics, including the absence of secondary pit connections. The genus was shown to be polyphyletic in a molecular analysis by Abe et al. (2006) with disjunct clades sister to either *Osmundea* or *Laurencia*. The species in the latter clade were transferred to *Palisada* (Nam 2007), a genus defined by palisade-shaped epidermal cells, and distinguished from *Chondrophycus* by differences in tetrasporangial development.

Nam (1999) proposed an infrageneric classification scheme for *Chondrophycus* including four subgenera— *Chondrophycus*, *Kangjaewonia*, *Palisada* and *Yuzurua*. Members of the subgenus *Chondrophycus* (containing *C. cartilagineus* (Yamada) Garbary & J.T. Harper as the type, as well as several others) exhibit right-angle tetrasporangia, but lack secondary pit connections. For species displaying both right-angle arrangement of tetrasporangia and secondary pit connections, Nam (1999) circumscribed the subgenus *Yuzurua*, which has since been elevated to generic status

and is irrefutably distinct in phylogenetic analyses from *Chondrophycus* (Martin-Lescanne 2010). Furthermore, Nam (1999) defined the section Parvipapillatae for members of subgenus *Yuzurua* demonstrating epidermal cell projections at branchlet apices in transverse section, and designated *C. parvipapillatus* (C.K.Tseng) Garbary & J.T.Harper as the type species of this section. Several years later, Nam (2006) proposed the genus *Palisada* following a morphological cladistics analysis that resolved two paraphyletic clades of *Chondrophycus* species. *Chondrophycus parvipapillatus* fell into the clade that did not include *C. cartilagineus*, the generitype, and thus was transferred to *Palisada*. Interestingly, our specimens from Bermuda exhibit the three traits Nam (2006) used to separate *C. parvipapillatus* within the genus *Chondrophycus*: right-angle arrangement of tetrasporangia, possession of secondary pit connections and apical cortical cell projections. Secondary pit connections are noted to be sporadic in *P. parvipapillata*, but are frequent and conspicuous in the *Chondrophycus* from Bermuda, and in the former species, cortical cell projections are present throughout the thallus, whereas these are only occasionally seen in the latter, strictly at branch apices. In our analyses, COI-5P sequence data from a Hawaiian specimen of *P. parvipapillata* nests the species within the *Palisada* clade (Fig. 1), providing molecular evidence for its correct placement in the genus and dismissing it as a possible range extension from Bermuda species, despite some morphological similarities.

Of the species of *Chondrophycus* exhibiting compressed axes, only *C. kangjaewonii* lacks sequence data available from GenBank. But, this species is easily distinguished from Bermuda specimens by the parallel arrangement of its

tetrasporangia, which are also three or four times larger in diameter than tetrasporangia in Bermuda collections. The comparisons of *Chondrophycus* species with compressed axes are shown in Table 3. Our novel Bermuda species is distinguished from all of these in having upright axes with a length of 2 cm or smaller (Figs 8-11) and by the conspicuous secondary pit connections present between cortical cells (Figs 14 and 15), a character that historically has been used to segregate morphological subclades of taxa. The novel species of *Chondrophycus* from Bermuda represents the only current species of the genus that we know of to possess secondary pit connections. However, this trait is not synapomorphic in other genera in the *Laurencia* complex, including in the sister genus *Osmundea*. McIvor et al. (2002) suggest that secondary pit connections may be an ancestral state that was subsequently lost in some lineages or species in the complex, a concept that is not easily rationalized given our poor knowledge of pit connection functionality on the whole. Nevertheless, the presence of this character in the Bermuda specimens provides an interesting contrast to other members of the genus, especially given that it is the only member of the genus presently known in the Atlantic Ocean.

Branching patterns exhibited by *Chondrophycus* in Bermuda vary from irregular to alternate and sometimes opposite; members of the compressed Pacific species show each of these morphologies — *C. undulatus* with opposite branching, *C. kangjaewonii* (K.W. Nam & C.H. Sohn) Garbary & J.T. Harper with alternate, *C. succisus* displaying both of these patterns and *C. dotyi* bearing irregular branches similar to some specimens from Bermuda. Our specimens occasionally exhibit cortical

cell projections at branch apices, a trait shared by *C. dotyi* and *C. kangjaewonii*, but lacking in *C. undulatus* and *C. succisus*.

Notably, all of the compressed species of the genus from the Pacific Ocean are found in intertidal habitats (or shallow subtidal, as in *C. kangjaewonii*). Our Bermuda specimens have been collected exclusively in subtidal waters off the south shore of the islands, from depths between 13 and 23 m during the winter from December to March, when water temperatures in Bermuda are at their lowest. These findings suggest that this novel species is adapted to cooler water temperatures and lower light levels than its most similar congeners.

The genus *Laurenciella* was segregated from *Laurencia* (Cassano et al. 2012b) on the basis of molecular sequence data, which showed it to be a distinct clade despite its generic features being indistinguishable from those of *Laurencia*. The type of the genus, *Laurenciella marilzae* (Gil-Rodriguez, Senties, Diaz-Larrea, Cassano & M.T. Fujii) Gil-Rodriguez, Senties, Diaz-Larrea, Cassano & M.T. Fujii was originally described as *Laurencia marilzae* Gil-Rodriguez, Senties, Díaz-Larr., Cassano & M.T. Fujii from the Canary Islands (Gil-Rodriguez et al. 2009) based on *rbcL* sequences, and characterized morphologically by the distinctive yellow-orange color of its habit, cortical cells that project markedly in cross-section, and the presence of ‘*corps en cerise*’ in all cells of the thallus. Corroborating evidence from both molecular and morphological analyses of specimens collected in the Mexican Caribbean showed that this species was also present in the tropical western Atlantic Ocean (Senties et al. 2011).

Our molecular findings show a clear relationship between *Laurenciella marilzae* and specimens collected in Bermuda and the Florida Keys. Both genetic variation (3.8% divergence) and differences in overall habit morphology suggest that these latter collections are a distinct second species of the genus, described herein as a novel species of *Laurenciella*. Not excepting the shared features of *Laurenciella* and *Laurencia sensu stricto* (i.e., four pericentral cells in axial segments; presence of ‘*corps en cerise*’; presence of secondary pit connections), the microscopic vegetative characters of *L. marilzae* and the proposed new species predominantly overlap. In particular, the size and shape of outer cortical cells in surface view (Figs 19 and 22), the size and shape of medullary cells (Fig. 21), the conspicuous projecting of cortical cells in cross-sectional view (Fig. 18) and the presence of trichoblasts with three to four orders of subdichotomous branching are all shared characteristics. In addition, Bermuda and the Florida Keys plants are distinctly yellow-orange in color (Fig. 20), an identifying feature that is noted for specimens of *L. marilzae* from the type locality and from the Caribbean Sea. However, this does not appear to be a characteristic of the genus as a whole, since deep-sea Brazilian specimens, as well as our undescribed genetic isolate (*Laurenciella* sp. 2 Bermuda in Figs 1, 2) are markedly red in color.

Though the vegetative morphology appears to be largely cryptic between *Laurenciella marilzae* and the proposed new species, the two species are quite distinct in overall habit. The Bermuda and the Florida Keys plants reach twice the height of *L. marilzae* and have a thallus texture that is fleshy rather than cartilaginous. The main axes of the proposed new species are slender and are distinctly lax (Fig. 20), never displaying the characteristic “turf” form possessed by *L. marilzae* in the Caribbean Sea

(Sentíes et al. 2011). Branching patterns in Bermuda and the Florida Keys plants are more irregular than alternate (Fig. 17), with upper portions of the thallus tending to be profusely branched while lower portions are often denuded at maturity (Fig. 20). The habitats of both Caribbean and Canary Islands specimens of *L. marilzae* are documented as exposed sites in the low intertidal zone (Gil-Rodríguez et al. 2009, Sentíes et al. 2011). Our collections in Bermuda and the Florida Keys exclusively come from the subtidal, and the species is especially common in shallow, relatively protected habitats in both locations.

After a thorough assessment of species in *Laurencia* reported for the region, we determined that our newly collected western Atlantic specimens are unique and cannot be attributed to an existing epithet in the ‘*Laurencia*-complex’ for which molecular data is unavailable. We did consider the historical name *Laurencia obtusa* var. *gracilis* (C. Agardh) Kütz. based on the illustrations and Latin description of Kützting (1865), which we interpreted as “delicate branches spreading widely,” and of Collins and Hervey (1917) who describe it as a “delicate, soft and slender form.” A request was made to the Lund Herbarium for scanned images of the original *Chondria obtusa* var. *gracilis* that C. Agardh reported from the West Indies, but unfortunately we have been unable to obtain this historical material to date. Thus, we believe linking this variety (which we did not observe in our collections from St. Croix in the West Indies) to our specimens would be unwise at present.

A second genetically distinct species of *Laurenciella* from Bermuda has been exposed in this study, resolving as sister to *L. marilzae* (1.4% divergence) with moderate support. This entity, listed as ‘*Laurenciella* sp. 2 Bermuda’ (Figs 1, 2) for

the present, groups closely with an undescribed species of *Laurenciella* (Cassano et al. 2012b) from Brazil (0.9% divergence). As with *Yuzurua* sp. 2 Bermuda, we have thus far confirmed a single collection representing this taxon, and have conservatively refrained from describing it as a third species in the genus until additional material is collected from the region.

Taxonomic treatment

Laurencia microcladia Kütz. (1865: 22, pl. 60: figs b, c) Figs 4-7

DESCRIPTION: Plants to 6.5 cm tall, fleshy, green to deep purplish-red, often with distinct purple-red branchlet tips, forming clumps with several associated upright axes; individual axes narrowly pyramidal or sometimes with secondary branches not varying much in length from base to tip of main axes; main axes to 0.5 mm diam. with discoidal holdfasts; densely irregularly branched in all directions, some branches clustered creating a whorled appearance; branchlets clavate to narrowly turbinate, to 2 mm long; densely clustered ultimate branches imparting a verrucose appearance.

Vegetative axes with 4 pericentral cells; secondary pit connections present; in surface view cortical cells 15-50 µm diam., irregularly rounded to rounded rectangular in upper branch regions, elongate-angular to elongate-ovoid below; corps en cerise present; outer cortical cell projections absent. In transverse section, outer cortical cells appearing subquadrate or campanulate to ovoid, 12-36 µm diam.; medullary cells 45-120 µm diam. Branch tips with deep apical pits, trichoblasts emerging from the pits, dichotomously branched to 6 or more orders, and expanding in diam. distally.

Tetrasporangia arranged parallel to axis, spherical to slightly ovoid, 60-100 µm diam.; cystocarps urn-shaped, situated near branchlet tips, 600-820 µm diam., carposporangia

obpyriform, 30-60 μm diam. and 80-125 μm long. Spermatia formed on dense fascicles issued from lower portions of trichoblasts, spherical to obovoid, 2-3 μm diam.

TYPE LOCALITY: West Indies, Caribbean Sea, western Atlantic Ocean.

DISTRIBUTION: Adriatic, Mediterranean, Azores, Canary Islands, Cape Verde Islands, Bermuda, Florida, Central America, Caribbean, Brazil, N. Africa, Arabian Gulf, Singapore, Vietnam, Polynesia, Samoa (Guiry and Guiry 2015).

Chondrophycus planiparvus Popolizio, C.W. Schneid. & C.E Lane *sp. nov.* Figs 8-16

DESCRIPTION: Plants to 2 cm high, distinctly compressed, rosy-red, cartilaginous; main axes to 2 mm diam. with discoidal holdfasts; branching irregular to opposite or alternate, with branch apices sometimes appearing trifurcated; branchlets to 2 mm in diameter. Vegetative axes with 2 pericentral cells; secondary pit connections abundant; cortical cells arranged longitudinally in surface view, cells ovoid in upper branch regions, irregularly angular and elongated below, 9-55 μm diam. and 20-100 μm long; outer cortical cells non-palisade, obtriangulate to obovate in transverse section, sometimes with constrictions at the base appearing hastate, 12-35 μm diam. and 9-27 μm long, some acute cortical cell projections present at branch apices; lenticular thickenings absent in the walls of medullary cells. Extensive trichoblasts present in shallow apical pits, dichotomously branched, to 1000 μm long.

Tetrasporangia slightly ovoid, arranged at right angles to the axis, 43-68 μm diam. and 48-96 μm long. Gametangia unknown.

HOLOTYPE (designated here): BDA 0934 (=TRP 12-40-13), March 13, 2012,
Gurnet Rock, mouth of Castle Harbour, 32°20'22.7"N, 64°39'44.8"W, 13 m.

Holotype DNA barcode: GenBank XXXXXX, COI-5P; GenBank XXXXXX, *rbcL*

PARATYPES: Specimens listed in Table 1 as *CWS/CEL 9-30-9*, *TRP 12-40-13*, *TRP 12-148-6* and *TRP 12-160-8*.

ETYMOLOGY: From the Latin, describing the typical habit of this flattened (*'planus'*) and relatively diminutive (*'parvus'*) species.

DISTRIBUTION: Thus far only known from the Bermuda Islands.

Laurenciella namii Popolizio, C.W. Schneid. & C.E. Lane *sp. nov.* Figs 17-22

DESCRIPTION: Plants to 15 cm tall, yellow with deep red branchlets, fleshy; main axes lax, slender, to 1 mm diam. with discoidal holdfasts; axes becoming denuded in lower portions at maturity; upper portions demonstrating profuse irregular branching; ultimate branchlets cylindrical to clavate, to 2 mm long. Vegetative axes with 4 pericentral cells; secondary pit connections present; in surface view cortical cells 20-45 µm diam., irregularly rounded-polygonal to ovoid, becoming more elongated in lower portions of branches, outer cortical cells often appearing obpyriform at branch apices; corps en cerise present; outer cortical cells acutely projecting, the surface appearing crenate in longitudinal section. In transverse section, two layers of pigmented cortical cells with three inner layers of colorless irregularly globose

medullary cells 30-115 μm diam., decreasing in size radially toward the cortex. Outer cortical cells rounded-rectangular to ovoid or campanulate, 25-40 μm diam. and 20-35 μm long. Deep apical pits at branch apices bearing dense trichoblast systems to 220 μm long with 3-4 orders of subdichotomous branching. Reproductive structures unknown. Distinct from other members of the *Laurencia* complex by the COI-5P and *rbcL* molecular sequences.

HOLOTYPE (designated here): BDA 0597 (=CWS/CEL/TRP 12-11-2), Jan. 18, 2012, Spanish Point Park, north shore Bermuda I., western Atlantic Ocean, 32°18'26.4"N, 64°48'56.6"W, 0-2 m.

Holotype DNA barcode: GenBank XXXXXX, COI-5P

PARATYPES: Specimens listed in Table 1 as CWS/CEL/TRP 10-16-12, CWS/CEL/TRP 10-33-13, CWS/CEL/TRP 10-33-15, CWS/CEL/TRP 12-8-1, TRP 12-143-4, CWS/CEL/TRP/DCM 13-9-3, CWS/CEL/TRP/DCM 13-9-17, CWS/CEL/TRP/DCM 13-9-25, CWS/CEL/TRP/DCM 13-14-7 and TRP 13-20-4

ETYMOLOGY: An honorific name for Professor Ki Wan Nam of Pukyong National University, Korea, who has played a significant role in the taxonomic diversification of the *Laurencia* complex using both morphological and phylogenetic data.

DISTRIBUTION: Bermuda and the Florida Keys, USA as presently known.

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Table 1. Collection details and GenBank accession numbers for isolates included in barcode (one representative per species and/or geographic region) and in phylogenetic analyses (all specimens).

Taxa	Voucher/ Collection data/ Reference	COI-5P Accession numbers	<i>rbcL</i> Accession numbers
<i>Bostrychia radicans</i> (Montagne)	USA, Mississippi, St. Louis Bay, 11 Feb. 1998, C.F.D. Gurgel	–	AF259497
Montagne in Orbigny	Brazil, Rio de Janeiro, Armacao dos Buzios, Praia Rasa, 13 Jan.	–	AF259497
<i>Chondria collinsiana</i> M. Howe	2005, V. Cassano and J.C. De-Paula	–	AF259497
<i>C. dasyphylla</i> (Woodw.) C. Agardh	USA, North Carolina, New Hanover Co., Wrightsville Beach	–	U04021
<i>Chondrophycus dotyi</i> (Saito) K.W. Nam	USA, Hawaiian Is., Oahu, Waikiki Natatorium, 4 Apr. 2001, K. Shultz	HQ423050	–
<i>C. planiparvus</i> Popolizio, C.W. Schneid. & C.E. Lane prop. sp. nov.	Bermuda, Gurnet Rock, mouth of Castle Harbour (13 m), 13 Mar. 2012, T. Popolizio; Holotype: BDA 0934 [TRP 12-40-13]	xxxxxxx	xxxxxxx
<i>C. succisus</i> (Cribb) K.W. Nam	USA, Hawaiian Is., Molokai, Honolui Maloo Bay Beach, 11 Feb. 2007, A. Kurihara	GU223884	–
<i>C. tranoi</i> (Ganzon-Fortes) K.W. Nam	Philippines, A.O. Lluisma	–	AF489864
<i>C. cf. undulatus</i>	New Caledonia, Loyalty Island, Mare, 22 Mar. 2005, C. Payri	–	FJ785307
<i>C. cf. undulatus</i>	USA, Hawaiian Is., Maui, 12 Dec. 2007	GU223886	–
<i>Chondrophycus</i> sp.	USA, Texas, Flower Garden Banks Sanctuary, Feb. 2000, S. Fredericq	–	AF465817
<i>Chondrophycus</i> sp. 1	New Caledonia, Loyalty Island, Lifou, 26 Mar. 2005, C. Payri	–	FJ85309
<i>Chondrophycus</i> sp. 2	New Caledonia, Loyalty Island, Mare, 21 Mar. 2005, C. Payri	–	FJ85310
<i>Chondrophycus</i> sp. 3	New Caledonia, Loyalty Island, Beautemps/Beaupre, 6 Apr. 2005, C. Payri	–	FJ85311

<i>Laurencia</i> cf. <i>brongniartii</i>	Australia, Tarcoala Beach, S. Fredericq, 1993	—	EF061654
<i>L. cf. brongniartii</i>	Taiwan, Makang Harbour, S. Fredericq, 11 July, 1993	—	AF465814
<i>L. caraibica</i> P.C. Silva	Mexico, Quintana Roo, Cancun, Isla Mujeres, 23 Feb. 2006, A. Senties	—	EF658642
<i>L. catarinensis</i> Cordeiro-Marino & M.T. Fujii	Brazil, Rio Grande do Norte, Maracajau, 24 June 2006, M.T. Fujii and I.B. Silva [voucher SP400112]	—	—
<i>L. catarinensis</i>	Spain, Canary Islands, Tenerife, El Pris, 4 May 2008, M.C. Gil-Rodríguez, M.T. Fujii, V. Cassano and J. Díaz-Larrea	—	KF492776
<i>L. catarinensis</i>	Bermuda, St. David's I., Fort Hill Bay, Cooper's Island (0-2 m), 27 May 2012, T. Papolizio; BDA 1130 [TRP 12-65-2]	xxxxxxxx	xxxxxxxx
<i>L. catarinensis</i>	USA, Florida, Key West, East Dry Rocks Reef (7 m), 30 May 2013, C.W. Schneider, C.E. Lane and T. Papolizio; KW 208 [CWS/CEL/TRP 13-13-16]	xxxxxxxx	xxxxxxxx
<i>L. catarinensis</i>	US Virgin Islands, St. Croix, Fredericksted Pier (0-1 m), 24 Nov. 2013, T. Papolizio and E. Salomaki; STX 222 [TRP 13-40-5]	xxxxxxxx	xxxxxxxx
<i>L. dendroidea</i> J. Agardh	Brazil, Sao Paulo, Ubatuba, Praia do Felix, 31 Aug. 2000, M.T. Fujii	—	AF465810
<i>L. dendroidea</i>	Spain, Canary Islands, Tenerife, Puerto de la Cruz, 13 July 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Senties	—	EF686000
<i>L. dendroidea</i>	Bermuda, Middle buoy, Eastern Blue Cut channel, north of Daniel's Head, Somerset Is. (16 m), 20 Aug. 2010, C.W. Schneider, C.E. Lane and T. Papolizio; BDA 0137 [CWS/CEL 10-12-38]	xxxxxxxx	xxxxxxxx
<i>L. dendroidea</i>	USA, Florida, Key West, Fort Zachary Taylor (3-4 m), 28 May 2013, C.W. Schneider, C.E. Lane, D. McDevit and T. Papolizio; KW 064 [CWS/CEL/TRP 13-7-2]	xxxxxxxx	xxxxxxxx

<i>L. dendroidea</i>	US Virgin Islands, St. Croix, Salt River Bay (0-1 m), 21 Nov. 2013, C.E. Lane, T. Popolizio and E. Salomaki; STX 111 [CEL/TRP 13-30-2]	xxxxxxxx	xxxxxxxx
<i>L. flexuosa</i> Kütz.	South Africa, S. KwaZulu-Natal, Palm Beach, 7 Feb. 2001, S. Fredericq	—	AF465815
<i>L. intricata</i> J.V. Lamour.	Mexico, Campeche, Campeche Bay, 14 Feb. 1999, C.F.D. Gurgel	—	AF465809
<i>L. intricata</i>	Cuba, Ciego de Avila, Cayo Coco, 25 Sep. 2005, M.T. Fujii	—	GU330238
<i>L. intricata</i>	Bermuda, Bermuda Is., Fairyland Creek (0-1 m), 25 Aug. 2010, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0483 [CWS/CEL 10-32-5]	xxxxxxxx	xxxxxxxx
<i>L. intricata</i>	USA, Florida, Key West, White Street Pier (0-2 m), 29 May 2013, C.W. Schneider, C.E. Lane, D. McDevit and T. Popolizio; KW 131 [CWS/CEL/TRP 13-9-23]	xxxxxxxx	xxxxxxxx
<i>L. intricata</i>	US Virgin Islands, St. Croix, Deep Wrecks (20 m), 19 Nov. 2013, C.E. Lane, T. Popolizio and E. Salomaki; STX 010 [CEL/TRP 13-23-9]	xxxxxxxx	xxxxxxxx
<i>L. cf. keutzingii</i>	New Caledonia, Loyalty Is., Ouvea, 31 Mar. 2005, C. Payri	—	FJ785322
<i>L. laurahuertana</i> Mateo-Cid, Mendoza-Gonzalez, Senties & Díaz-Larrea	Mexico, Quintana Roo, Punta Herrero, 2012, A.C. Mendoza-Gonzalez and L.E. Mateo-Cid	—	KF279401
<i>L. cf. majuscula</i>	New Caledonia, Ile des Pins, 2 Dec. 2005, C. Payri	—	FJ785312
<i>L. majuscula</i>	USA, Hawaiian Is., Molokai, 10 Feb. 2007	GU223887	—
<i>L. cf. mariannensis</i>	New Caledonia, Lagon Sud-Ouest, Ilot Laregnere, 11 July 2003, C. Payri	—	FJ785313
<i>L. cf. mcdermidiae</i>	New Caledonia, Ile des Pins, 29 Nov 2005, C. Payri	—	FJ785314
<i>L. mcdermidiae</i>	USA, Hawaiian Is., Oahu, 8 Apr. 2007	GU223878	—

<i>L. microcladia</i>	Bermuda, south shore Bermuda I., Capt. Williams' Bay (intertidal rocks/pools), 15 Jan. 2012, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0523 [<i>CWS/CEL/TRP</i> 12-2-2]	xxxxxxx	xxxxxxx
<i>L. microcladia</i>	US Virgin Islands, St. Croix, Turtle Deli Beach (intertidal rocks/pools), 20 Nov. 2013, C.E. Lane, T. Popolizio and E. Salomaki; STX 079 [<i>CEL/TRP</i> 13-26-2]	xxxxxxx	xxxxxxx
<i>L. natalensis</i> Kylin	South Africa, S. KwaZulu-Natal, Palm Beach, 7 Feb. 2001, S. Fredericq	—	AF465816
<i>L. cf. nidifica</i>	New Caledonia, Ile des Pins, 30 Nov. 2005, C. Payri	—	FJ785315
<i>L. nidifica</i> J. Agardh	USA, Hawaiian Is., Oahu, 20 May 2007	GU223888	—
<i>L. nipponica</i> Yamada	Japan, Hokkaido, Muroran, 5 June 2003	GU223875	—
<i>L. obtusa</i> (Hudson) J.V. Lamour.	Ireland, County Donegal, Fanad Head, 6 July 1998, C.A. Maggs	—	AF281881
<i>L. oliveirana</i> Yoneshigue	Brazil, Rio de Janeiro, Arraial do Cabo, Ponta da Cabeça, 7 July 2008, V. Cassano and J.C. De-Paula	—	JF810352
<i>L. pyramidalis</i> Bory ex Kütz.	France, Brittany, Roscoff, 5 Dec. 2002, F. Rousseau	—	FJ785316
<i>L. venusta</i> Yamada	Mexico, Quintana Roo, Puerto Morelos, Punta Brava, 18 Apr. 2004, J.D. Larrea and A. Senties	—	EF061655
<i>L. viridis</i> Gil-Rodríguez & Haroun	Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 6 Oct. 2005, M.C. Gil-Rodríguez	—	EF685999
<i>Laurenciella marilzae</i> (Gil-Rodríguez, Senties, Díaz-Larrea, Cassano & M.T. Fujii) Gil-Rodríguez, Senties, Díaz-Larrea, Cassano & M.T. Fujii	Spain, Canary Islands, Tenerife, Punta del Hidalgo, 12 July 2006, M.C. Gil-Rodríguez	—	EF686002
<i>L. marilzae</i>	Mexico, Isla Mujeres, Quintana Roo, 2008, A. Senties and M.T. Fujii	—	HQ115065

<i>L. marilzae</i>	Brazil, Sao Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25 Mar. 2007, R. Rocha-Jorge	—	GU938189
<i>L. namii</i> Popolizio, C.W. Schneid. & C.E. Lane prop. sp. nov.	Bermuda, Spanish Point Park, north shore Bermuda I., (0-2 m), 25 Aug. 2010, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0502 [<i>CWS/CEL</i> 10-33-13]	xxxxxxxx	xxxxxxxx
<i>L. namii</i>	Bermuda, Spanish Point Park, north shore Bermuda I., (0-2 m), 18 Jan. 2012, C.W. Schneider, C.E. Lane and T. Popolizio; Holotype: BDA 0597 [<i>CWS/CEL</i> 12-11-2]	xxxxxxxx	—
<i>L. namii</i>	USA, Florida, Key West, White Street Pier (0-2 m), 29 May 2013, C.W. Schneider, C.E. Lane, D. McDevit and T. Popolizio; KW 109 [<i>CWS/CEL/TRP</i> 13-9-5]	xxxxxxxx	xxxxxxxx
<i>Laurenciella</i> sp. 2 Brazil	Brazil, Espirito Santo, Anchieta, Ilhote de Ubu, 30 June 2007, E. Stein [voucher SP399.938]	—	—
<i>Laurenciella</i> sp. 3 Brazil	Brazil, Sao Paulo, Ilha Vitoria, 19 May 2008, M.T. Fujii [voucher SP399.939]	—	—
<i>Laurenciella</i> sp. 2 Bermuda	Bermuda, Gibbet I., mouth of Flatts Inlet (1-2 m), 2 Feb. 2012, T. Popolizio; BDA 0806 [<i>TRP</i> 12-29-16]	xxxxxxxx	xxxxxxxx
<i>Osmundea blinksii</i> (Hollenberg & Abbott) K.W. Nam	USA, California, San Mateo County, Ano Nuevo, Greyhound Rock, 17 July 1996, M.H. Hommersand	—	AY172575
<i>O. oederi</i> (Gunnerus) G. Furnari	Ireland, County Donegal, St John's Point, 12 Oct. 1999, C.A. Maggs	—	AF281880
<i>O. osmunda</i> (S.G. Gmelin) K.W. Nam	Ireland, County Donegal, St John's Point, 12 Oct. 1999, C.A. Maggs	—	AF281877
<i>O. osmunda</i>	France, Brittany, Le Loup, 19 May 2011, L. Couceiro and M. Robuchon	KJ960875	—

<i>O. pinnatifida</i> (Hudson) Stackhouse	Spain, Canary Is., La Palma, La Fajana de Barlovento, 24 Jan. 2008, M.C. Gil-Rodríguez	—	EF686005
<i>O. pinnatifida</i>	Ireland, Galway, Black Head, H.-G. Choi and M.D. Guiry	—	JX828140
<i>O. pinnatifida</i>	France, Brittany, Le Loup, 8 Mar. 2012, L. Couceiro and M. Robuchon	KJ960886	—
<i>O. sanctarum</i> M.T. Fujii & R. Rocha-Jorge	Brazil, Sao Paulo, Parcel do Sul, Laje de Santos Marine State Park, 19 Apr. 2012, R. Rocha-Jorge, M.B. Barros-Barreto, I.B. Silva & M.T. Fujii	—	KC012600
<i>O. sinicola</i> (Setchell & Gardner) K.W. Nam	USA, California, Orange County, Crescent Beach, 28 May 2002, S. Murray	—	AY588407
<i>O. spectabilis</i> (Postels & Ruprecht) K.W. Nam	Mexico, Baja California, Punta Santo Thomas, 2 July 1996, M.H. Hommersand	—	AY172574
<i>O. splendens</i> (Hollenberg) K.W. Nam	Mexico, Baja California, Bahia Colnett, Drift, 2 July 1996, M.H. Hommersand	—	AY172576
<i>O. truncata</i> (Kutz) K.W. Nam & Maggs	Ireland, Lough Hyne, County Cork, 11 Nov. 1999, C.A. Maggs	—	AF281879
<i>O. truncata</i>	Spain, Canary Is., Tenerife, La Barranquera, 5 May 2008, M.C. Gil-Rodríguez, M.T. Fujii, V. Cassano and J. Díaz-Larrea	—	JF781523
<i>Palisada corallopsis</i> (Montagne) Senties, M.T. Fujii & Díaz-Larrea	Mexico, Quintana Roo, Cancun, Chaac-Mol Beach, 21 Aug. 2005, J. Díaz-Larrea and A. Senties	—	EF061646
<i>P. cf. corallopsis</i>	Bermuda, Brackish Pond Flats, inner reef, north shore (3-4 m), 17 Jan. 2012, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0559 [CWS/CEL/TRP 12-8-16]	xxxxxxx	xxxxxxx
<i>P. cf. corallopsis</i>	Bermuda, Bailey's Reef, north shore Bermuda I. (7 m), 17 Aug. 2012, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 1336 [CWS/CEL/TRP 12-94-2]	xxxxxxx	xxxxxxx

<i>P. cf. corallopsis</i>	USA, Florida, Cudjoe Key, Summerland Bridge (1-3 m), C.W. Schneider, C.E. Lane, D. McDevit and T. Popolizio; KW 234 [CWS/CEL/TRP 13-14-17]	xxxxxxx	xxxxxxx
<i>P. flagellifera</i> (J. Agardh) K.W. Nam	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras, 3 Aug. 2005, V. Cassano and M.B. Barros-Barreto	—	GU330221
<i>P. flagellifera</i>	Spain, Canary Is., Tenerife, Playa Paraiso, 12 July 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Senties	—	EF685998
<i>P. flagellifera</i>	Bermuda, SW of North Rock BAMZ “pink sand” collecting site (12 m), 16 Nov. 2012, T. Popolizio; BDA 1665 [TRP 12-151-8]	xxxxxxx	xxxxxxx
<i>P. parvipapillata</i> (C.K. Tseng) K.W. Nam	USA, Hawaiian Is., Oahu, Hauula Beach Park, Sep. 18 2007, A. Kurihara	GU223895	—
<i>P. patentiramea</i> (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii	Philippines, A.O. Lluisma	—	AF489862
<i>P. perforata</i> (Bory) K.W. Nam	Brazil, Rio de Janeiro, Parati, Praia Vermelha, 30 Dec. 2005, V. Cassano	—	EU256331
<i>P. perforata</i>	Spain, Canary Is., Tenerife, Puerto de La Cruz, San Telmo, 14 July 2006, M.C. Gil-Rodríguez, M.T. Fujii and A. Senties	—	EU256329
<i>P. perforata</i>	Mexico, Quintana Roo, Cancun, Isla Mujeres, 2 Mar. 2007, A. Senties and M.C. Gil-Rodríguez	—	EF658541
<i>P. perforata</i>	Bermuda, Gravelly Bay, Bermuda I., (intertidal rock/pools), 22 Aug. 2012, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0314 [CWS/CEL 10-21-4]	xxxxxxx	xxxxxxx
<i>P. perforata</i>	USA, Florida, Key West, Low Key Channel (1-3 m) 27 May 2013, C.W. Schneider, C.E. Lane, D. McDevit and T. Popolizio; KW 016 [CWS/CEL/TRP 13-6-15]	xxxxxxx	xxxxxxx
<i>P. cf. robusta</i>	New Caledonia, Lifu, 23 Mar. 2005, C. Payri	—	FJ785321

<i>P. yamadana</i> (Howe) K.W. Nam [as <i>Laurencia yamadana</i> M.A. Howe]	USA, Hawaiian Is., Maui, Kihei, 5 Apr. 2006	—	GQ252550
<i>Palisada</i> sp.	Philippines, A.O. Lluisma	—	AF489865
<i>Yuzurua iridescens</i> (M.J. Wynne & D.L. Ballant.) Senties, M.J. Wynne, Cassano, Gil-Rodríguez & M.T. Fujii	Bermuda, Southhampton, Bermuda I., West Whale Bay boiler reefs (2-3 m), 20 Jan. 2012, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0707 [<i>CWS/CEL/TRP</i> 12-21-14]	xxxxxxxx	xxxxxxxx
<i>Y. poiteaui</i> (J.V. Lamour.) Martin- Lescanne	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, 16 Apr. 2004, J. Díaz-Larrea and A. Senties	—	EF061648
<i>Y. poiteaui</i>	Cuba, La Habana, Rincon de Guanabo, 29 July 2005, J. Díaz- Larrea and A.A. Mallea	—	EF061650
<i>Y. poiteaui</i>	Bermuda, St. George's I., Ferry Reach, BIOS dock (0-1 m), 19 Aug. 2010, C.W. Schneider, C.E. Lane and T. Popolizio; BDA 0083 [<i>CWS/CEL</i> 10-11-3]	xxxxxxxx	xxxxxxxx
<i>Y. poiteaui</i>	USA, Florida, Key West, White Street Pier (0-2 m), 29 May 2013, C.W. Schneider, C.E. Lane, D. McDevit and T. Popolizio; KW 141 [<i>CWS/CEL/TRP</i> 13-9-33]	xxxxxxxx	xxxxxxxx
<i>Yuzurua</i> sp. 2	Bermuda, Southhampton, Bermuda I., West Whale Bay boiler reefs (0-3 m), 30 May 2012, T. Popolizio; BDA 1153 [<i>TRP</i> 12- 69-3]	xxxxxxxx	xxxxxxxx

Table 2. Morphological character comparisons of *Laurencia microcladia* from the type locality and from Bermuda, *L. venusta* from the type locality and the Caribbean and *L. laurahuertana* from the type locality.

Species	<i>L. microcladia</i>	<i>L. microcladia</i>	<i>L. venusta</i>	<i>L. venusta</i>	<i>L. laurahuertana</i>
Collection Location	West Indies (type locality)	Bermuda	Japan (type locality)	Mexico	Mexico (type locality)
Thallus color	gray-green to purple-green	green to deep purplish-red, often with distinct purple-red branchlet tips	brown, deep brown or purplish brown with pink branch tips	pale green	pale green
Length of upright axes (cm)	to 10	to 6.5	to 8	to 7	to 0.7
Branching pattern	densely branched; alternate to irregular, dense above	densely irregularly branched in all directions; some branches clustered creating a whorled appearance	irregularly alternate, subopposite or subverticillate; occasionally curved branches with secund ultimate branchlets	sparse, verticillate, 2-4 branches per verticille, less commonly opposite, alternate or irregular	sparse, dichotomous below, irregularly alternate above
Outer cortex surface cell diameter (μm)	30-60	15-50	not reported	32-52	not reported
Outer cortex cross-sectional cell projection	not reported	absent	absent or slight	not reported	present (undulate)
Tetrasporangial arrangement	not reported	parallel	parallel	parallel	not reported

Tetrasporangia diameter (μm)	65-100; tetrahedrally divided; spherical to oval	spherical to slightly ovoid; 60-100 diam.	75-86	40-80	60-65
Cystocarp shape	spherical to urn-shaped; near branchlet tips	urn-shaped, situated near branchlet tips	urn-shaped to conical or ovoid without a protuberant ostiole	prominent; laterally positioned to bearing branchlet; urn-shaped to conical	present; urn-shaped
Cystocarp diameter (μm)	500-700	600-820	600-700	400-600	450-500
Carposporangia	not reported	obpyriform, 80-125 μm long x 30-60 μm diam.	not reported	100-150 μm long x 30-50 μm diam.	pyriform; 90-100 μm long x 22-25 μm diam.
Spermatangia	not reported	spherical to ovoid; 4.5-10 μm diam.; apical sterile cells present	ovoid; 10-12 x 5-7 μm ; apical sterile cells present	ovoid; 6-11 x 4-7 μm ; apical sterile cells present	ovoid, 11-13 x 3-4 μm ; apical sterile cells absent
References	Taylor 1960; Howe 1918; Littler & Littler 2000; Dawes & Mathieson 2008	this study	Nam et al. 2000; Mateo-Cid et al. 2012	Senties et al. 2001	Mateo-Cid et al. 2014

Table 3. Morphological character and habitat comparisons for species of *Chondrophycus* with compressed axes.

Species	<i>C. planiparvus</i> sp. nov.	<i>C. undulatus</i>	<i>C. succisus</i>	<i>C. dotyi</i>	<i>C. kangjaewonii</i>
Type locality	Bermuda	Japan	Australia	Hawaii	Korea
Length of upright axes (cm)	to 2 cm	to 6 cm	to 8 cm	to 5 cm	to 10 cm
Branching pattern	distichous; irregular to alternate or opposite	distichous; opposite (pinnate)	distichous; opposite (pinnate) or alternate	distichous; alternate, opposite or irregular	distichous; alternate
Secondary pit-connections	present (abundant, conspicuous)	absent	absent	absent	absent
Outer cortex surface cell view	ovoid in upper regions of branches, irregularly angular and elongated below	quadrangular	rounded polygonal to longitudinally elongate	nearly isodiametric	elongated ovoid
Outer cortex cross-sectional cell projection	occasionally acute cortical cell projections present at branch apices	absent	absent	domed projections present in ultimate branchlets	absent or occasionally slightly projecting at branch apices
Tetrasporangial arrangement	right-angle	right-angle	right-angle	right-angle	parallel
Tetrasporangia diameter (µm)	48-96 long x 43-68 diam.; slightly ovoid	not reported	not reported	not reported	180-230 diam.
Habitat	from 13-23 m on coral; never intertidal	low intertidal, more commonly on basalt rock	intertidal; on eroded coral and basalt pools	intertidal; on eroded coral and basalt platforms	low intertidal on rock or coral or subtidal to 3 m
References	this study	Yamada 1931; Nam 1990; Abbott	Cribb 1958; Saito 1969; Abbott	Saito 1969; Abbott	Nam & Sohn 1994

Figure 1. Species groups among members of the *Laurencia* complex determined with UPGMA clustering of the COI-5P genetic barcode. Specimens with data generated for this study appear in bolded text. Where more than one specimen is represented for a single species group, n=number of specimens.

Figure 2. *rbcL* phylogeny: Maximum likelihood analysis of sequence data from the *Laurencia* complex with *Bostrychia* and *Chondria* included as outgroups. Maximum likelihood (ML) bootstrap values followed by Bayesian posterior probabilities are shown at the nodes. An asterisk (*) denotes full support in both analyses; a dash (-) indicates less than 50% support. Specimens with data generated for this study appear in bolded text.

In Børgeson (1915):
Fig. 238. *Laurencia*
obtusa (Huds.) Lamour.
var. *gelatinosa* (Desf.)
J. Ag. Part of a male
plant (About 3:1)

Laurencia microcladia from St. Croix,
USVI [voucher STX 079]

Figure 3. A comparison of the illustration by Børgeson (1915) of *L. microcladia* (as *Laurencia obtusa* var. *gelatinosa*) from the US Virgin Islands, and an herbarium specimen of *L. microcladia* from this study collected in St. Croix, USVI, Caribbean Antilles. Scale bar = 2 mm.

Figures 4-7. *Laurencia microcladia* **4.** Habit with numerous characteristic narrowly pyramidal axes [TRP 12-30-11/BDA 0818]. Scale bar = 2 cm. **5.** Longitudinal section of branch tip showing tetrasporangia [TRP 12-172-3]. Scale bar = 250 μ m. **6.** Whole mount of cystocarpic female specimen, illustrating urn-shaped cystocarps and obpyriform carposporangia [CWS 3-21-1]. Scale bar = 500 μ m. **7.** Whole mount of tetrasporic specimen, displaying irregular branching in all planes and parallel arrangement of tetrasporangia [CWS 3-21-1]. Scale bar = 250 μ m.

Figures 8-16. *Chondrophycus planiparvus* sp. nov. **8.** Habit of live specimen [*TRP* 12-40-13/BDA 934] (holotype). Scale bar = 5 mm. **9.** Herbarium specimen [*CWS/CEL* 9-30-9]. Scale bar = 1 cm. **10.** Habit of live specimen, *TRP* [12-160-8/BDA 1741]. Scale bar = 5 mm. **11.** Herbarium specimen [*TRP* 12-148-6/BDA 1625]. Scale bar = 2 cm. **12.** Longitudinal section showing irregularly angular to ovoid surface cells [*TRP* 12-148-6/BDA 1626]. Scale bar = 50 μ m. **13.** Branch tip with extensive trichoblasts [*CWS/CEL* 9-30-9]. Scale bar = 200 μ m. **14.** Secondary pit connections between adjacent cortical cells [*TRP* 12-40-13/BDA 0934]. Scale bar = 40 μ m. **15.** Longitudinal section of cortex showing irregularly angular surface cells and secondary pit connections [*CWS/CEL* 9-30-9]. Scale bar = 50 μ m. **16.** Longitudinal section of branch tip showing right-angle arrangement of tetrasporangia [*TRP* 12-160-8/BDA 1741]. Scale bar = 100 μ m.

Figures 17-22. *Laurenciella namii* sp. nov. **Fig. 17.** Whole mount displaying irregular branching [*CWS/CEL/TRP/DCM* 13-9-17/KW 125]. Scale bar = 500 μ m. **Fig. 18.** Transverse section of branch showing outer cortical cells with slight projections creating a crenate margin [*CWS/CEL/TRP/DCM* 13-9-17/KW 125]. Scale bar = 50 μ m. **Fig. 19.** Longitudinal section of branch tip illustrating apical pit, trichoblasts, and crenate outer margins [*CWS/CEL/TRP/DCM* 13-9-5/KW 109]. Scale bar = 50 μ m. **Fig. 20.** Habit of live holotype specimen [*CWS/CEL/TRP* 12-11-2/BDA 0597]. Scale bar = 2 cm. **Fig. 21.** Transverse section of branch showing two outer layers of corticated cells and three inner layers of colorless, irregularly globose medullary cell layers [*CWS/CEL/TRP/DCM* 13-9-17/KW 125]. Scale bar = 100 μ m. **Fig. 22.** Longitudinal section of branch tip; orientation shows depth of apical pit and developing trichoblasts [*CWS/CEL/TRP/DCM* 13-9-17/KW 125]. Scale bar = 50 μ m.

CHAPTER FIVE

GENERAL CONCLUSION

Over the course of this study, we have accumulated 1875 DNA vouchers specimens collected from 157 sites around the Bermuda platform. For comparative purposes, we have also collected 317 DNA vouchers specimens from the Florida Keys and 236 from St. Croix in the Caribbean Antilles.

Five different species of the red algal genus *Centroceras* are known from Bermuda after 59 specimens were analyzed for one species currently reported, *C. clavulatum*. Using MAAT techniques, this formerly reported member of the Bermuda flora has been determined to be restricted to the Pacific Ocean. Three of the five species of *Centroceras* now known in Bermuda are also found in the Caribbean Sea (*C. gasparrinii*, *C. micracanthum* and *C. hyalacanthum*); the remaining two are newly described, *C. arcii* and *C. illaqueans*.

Two genetic species that are both morphologically identified as the red alga *Laurencia obtusa* are not genetically allied to specimens of that taxon collected near the type locality (England). These have been determined to be *L. dendroidea* and *L. catarinensis*, both with type localities in Brazil. Thus, *L. obtusa* has been removed from the Bermuda flora. We have presented the first report of the Indo-Pacific species *Palisada flagellifera* in the islands. We have also described two novel species in the ‘*Laurencia* complex’—*Laurenciella namii*, a second species in the newly erected monospecific genus *Laurenciella*, and *Chondrophycus planiparvus*, which also represents the first report of this genus in Bermuda and in the Atlantic Ocean to be verified with molecular sequence data.

In the red algal family Liagoraceae, we have proposed the addition of three genera to the Bermuda flora—*Hommersandiphycus*, *Trichogloeopsis* and

Yamadaella. *Hommersandiophycus* and *Yamadaella* are also first reports for the Atlantic Ocean. Our Bermuda *Y. grassyi* represents a novel species, and only the second reported species of this genus worldwide. Two additional novel species, *Helminthocladia kempii* and *L. nesophila*, have been described and are genetically and morphologically distinct from the *H. calvadosii* and *L. ceranoides*, the respective morphological species historically identified for those collections in the past. We have no evidence that the two latter species are part of the Bermuda flora either presently or in the past. The Indo-Pacific species *Liagora mannarensis* was uncovered during this work, and is the first report of this species in Bermuda and in the Atlantic Ocean.

We have also described *Crassitegula laciniata*, a second new species in the genus in Bermuda. Thus far, *Crassitegula* is known only from Bermuda and from Lord Howe Island, off the eastern coast of Australia. These taxa are examples of a fascinating phenomenon materializing with the support of MAAT methods, where sibling endemic species are discovered from half a world away (see Appendix B). This is certainly a trend that warrants further exploration.

What little we have learned from this small chain of islands in Bermuda could have broad implications for the Caribbean flora to the south, and also suggests greater levels of endemism in Bermuda than previously thought. While many Caribbean algal taxa are considered to be pantropical, molecular data may reveal certain groups that appear to be more limited in scope than currently accepted, warranting future biodiversity studies in this region. Additionally, because we suspect that such a large number of marine plants are yet to be described in the western Atlantic, it is not unreasonable to predict that a significant number of novel species will continue to be

exposed both in Bermuda and the tropical seas of the Americas. Our results from examining the Liagoraceae in Bermuda, as well as preliminary data suggesting additional cryptic diversity in the Caribbean, demonstrates the need for extensive collections in the order Nematiales throughout the western Atlantic. In the '*Laurencia* complex', more work has been done by groups in Mexico and Brazil, but the entire Antillean chain needs to be examined further to identify the northern and southern distributional limits of the Caribbean flora. Extensive sampling of the Greater and Lesser Antilles, areas poorly studied for molecular systematics, would be instructive in piecing together the biogeographical implications of this work for the warm temperate western Atlantic.

Though practice of MAAT and the type method should be routine for taxonomists, it is unfortunately not always so simple. The ability to make these important comparisons depends on either (1) genetic sequence data for organisms from the type locality being available in a public database, such as GenBank, or (2) having connections with a network of colleagues around the globe who have the ability to access and collect material from type localities so that data can be produced if it is not currently available in databases. Often these can be problematic, because (1) the open-source databases are missing a considerable number of the known species, as well as inconsistency in the molecular markers used to make comparisons between lineages, and (2) phycologists may not have colleagues in proximity to a particular type locality, or if they do, the specimens may be rare or difficult to access, or the person may not have the expertise necessary to identify and collect the proper material. Hopefully, these challenges will become more conquerable as the practice of

analyzing and publishing molecular sequence data in phycological studies becomes the rule rather than the exception. Accordingly, we should look toward improved procedures for making data available and relatively standardized, to facilitate meaningful use by the phycological community. The establishment of digital museums where the morphology, geography, ecology and genetic information associated with biological collections can be accessed, analyzed and synthesized by researchers is a compelling prospect with potentially far-reaching outcomes.

The precision of biotic and genetic inventories may be especially important in the face of climate change, since inaccurate baseline surveys will mask species loss. The global climate has increased in temperature approximately $0.74 \pm 0.18^{\circ}\text{C}$ over the past century, with further increase of 2.0 to 4.5°C predicted in the next century (IPCC 2007). Numerous studies indicate that rising sea temperature is affecting the geographic ranges of various marine animals (Hendriks et al. 2006, Parmesan 2006). Many of these studies target large, migratory species such as turtles and fish, which may be directly responding to temperature, or to a variety of indirect factors like oceanographic currents or availability of prey. To get a better understanding of the potential impact of rising sea temperature in coastal habitats, we should study the diversity and distribution of resident, immobile organisms. Sessile organisms like seaweeds are pertinent organismal markers of environmental change, because once individuals are established, they cannot flee the environment. Thus, changes in the distribution of sessile species over longer (*i.e.*, inter-generational) time scales will be a function of survival under ambient conditions. In a recent analysis of herbarium records from macroalgae collected at the tropical-temperate transition on both coasts

of Australia, Wernberg et al. (2011) discovered that over a relatively short period of ~70 years, communities in the southern (temperate) region progressively came to resemble communities in the equatorial (tropical) region. A study conducted along the rocky coast of Portugal documented a northward extension of warm-temperate seaweeds (Lima et al. 2007). Despite growing evidence that range-shifts are occurring among macroalgal communities, seaweeds remain an understudied group in climate change research.

An accurate and complete understanding of marine biodiversity in places like Bermuda, which also sits at a climatic boundary, would serve as a reliable indicator of compounding problems that may result from ocean warming. Despite Bermuda's warm temperate latitude (32°N), the Gulf Stream carries warm water from the Florida and Antilles currents northward to the islands. Current average monthly temperatures (18-30°C) allow for some tropical flora to thrive for much of the year, but mid-winter temperatures are presently too cool to support invasions of all of the Caribbean tropical marine flora and fauna. Bermuda maintains small, relatively rare populations of macroalgae that could represent Pleistocene relicts. However, if water temperatures and sea levels rise due to global warming over the coming decades, these Pleistocene relicts may disappear from Bermuda, and the islands could become a northern outpost for additional Caribbean plants and animals [*e.g.*, the coral *Acropora palmata* Lamarck and the non-invasive strain of the alga *Caulerpa taxifolia* (M.Vahl) C. Agardh]. Furthermore, warm water species that are presently scarce in Bermuda may become more competitive, and as sea temperatures increase in the next several

decades, could become dominant members of the flora with unpredictable consequences.

The creation of a baseline dataset to compare against future algal invasions or losses due to changing water temperatures may become an essential reference tool for the future monitoring of marine biodiversity in Bermuda, as well as southeastern North America and the Caribbean. Studies that aim to discover new organisms and understand the characteristics and patterns of genetic and/or morphological variation in related organisms, will ultimately allow researchers to measure the condition of individual species and ecosystems over time, informing conservation management and policy when necessary. Emergent scientific research and monitoring in this realm are critical on both local and global scales, as new technologies improve our ability to sustainably manage biodiversity.

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APPENDIX A

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Using Molecular-Assisted Alpha Taxonomy to Better Understand Red Algal Biodiversity in Bermuda

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Abstract: Molecular-assisted alpha taxonomy has recently become an effective practice in reassessing biodiversity and floristics for a variety of different organisms. This paper presents a series of examples that have been drawn from biodiversity work being carried out for the marine red algae of Bermuda. Molecular sequencing of DNA from Bermuda samples has already begun to greatly alter the makeup of the flora as it was known just decades ago, and will help set a new database for future comparison as climate change affects species compositions in the islands.

Keywords: algae; Bermuda; convergence; cryptic species; molecular-assisted alpha taxonomy (MAAT), Rhodophyta

1. Introduction

Molecular-assisted alpha taxonomy (MAAT) has recently emerged as an effective technique due to its ability to conquer the challenges of classifying many organisms prone to simple or convergent morphologies. Here we review its use in marine macroalgae, particularly the red algae (Rhodophyta), a group whose individuals are infamously difficult to identify due to characteristic obstacles such as heteromorphic life cycles, evolutionary convergence, and the influence of environmental factors on phenotype expression. The incorporation of molecular data to the understanding of red algal classification has fundamentally altered the way in which we understand this group. Prior to the advent of gene sequencing, taxonomic placement among red algae often could not be definitive without reproductive structures. Individuals discovered with only vegetative characteristics were often classified by their relatedness to species only at the generic level, an inexact science at best. Likewise, convergent evolution and recent speciation often produce cryptic species — impossible to differentiate at the gross morphological level, but in fact genetically distinct. Molecular analyses can now be used to reveal discrete algal ancestries obscured by seemingly identical appearances as a result of converging on a similar morphology. At times, however, the reverse is true. Many species exhibit great morphological diversity based upon the environmental conditions under which a population of marine algae grows (protected vs. exposed environments, shallow subtidal vs. deep-water habitats) and this diversity is often misinterpreted as multiple species. Thus, two fundamental questions guide molecular-assisted alpha taxonomy: Will the alleged conspecifics hold up under molecular scrutiny (Fig. 1a), or are distinct

entities present (Fig. 1b)? Or, conversely, will the data confirm or reject apparent differences between uniquely classified individuals (Fig. 1c)?

Although alpha taxonomy is generally performed in similar ways across algal groups, the molecular element of MAAT studies can vary depending on the taxa in question. In some cases, little is known about the species being examined. This is particularly true in instances where reproductive features are unknown, and in such cases, more ‘traditional’ molecular phylogenetic methods are often used. These include the use of full, or nearly full, gene or ribosomal RNA (rRNA) sequences and in-depth likelihood and Bayesian analysis. In cases where the goal is examining potentially closely-related species, the approach might be to use an established “DNA barcode”, such as the 5’ portion of the cytochrome oxidase I gene (COI-5’) encoded in the mitochondrion, the internal transcribed spacer (ITS) of the nuclear rRNA or a portion of the plastid 23S ribosomal subunit (UPA). These DNA regions may be used individually, or in combination, to determine whether there is an obvious distinction between intra- and interspecies genetic variation, but this method can be subject to sampling biases [e.g., 1]. Accordingly, the sampling strategy for barcoding studies is geared towards obtaining multiple samples of each possible species, in order to better assess species boundaries at the morphological and molecular level. Whereas none of the above methods will give an absolute identity to an alga in every instance, the molecular results can form a framework in which it is possible to re-assess recognized “taxonomically informative” characters or establish novel ones. In combination, each technique can inform the other to establish a more robust classification scheme.

Over the past decade, two of the authors (CWS, CEL) have been applying MAAT to the marine flora of Bermuda in order to better understand the algal biodiversity of this isolated and small island grouping. The Bermudas are among the northernmost islands in the world to support a tropical marine biota [2, 3], including the highest latitude coral reefs in the western Atlantic [4]. Eighty-seven percent of the species living in Bermuda are known from the Caribbean Sea, and despite their small size and distance from the tropics proper, the islands of Bermuda support approximately 30% of the 1442 species known in the tropical and subtropical western Atlantic [5], the bulk of them residing in the Caribbean proper. The “lifeline” of the biota of Bermuda is the swift-flowing Gulf Stream, which brings tropical waters from the Gulf of Mexico and Caribbean via the Florida and Antilles Currents, these originating with the Northern and Southern Equatorial Currents off northern South America [2, 4, 6]. Because of seasonal water temperature oscillations, the macroalgal assemblage of Bermuda is made up of warm-water tolerant species from the western mid-Atlantic that have persisted since re-colonization during the last ice age [7], cool-water tolerant Caribbean species carried by the current northward and an additional 3% that are endemic to this “postage stamp” in the Atlantic Ocean.

Bermuda is a particularly good example of how the use of MAAT can redefine a marine flora because of its location, the history of taxonomic work on the islands and the manageable number of taxa. As a small and isolated archipelago, dispersal to and from Bermuda is relatively rare, leading to an environment amenable to the evolution of new species through genetic isolation. Additionally, despite the heavy influence of the Caribbean on the marine flora of Bermuda, a major obstacle to

understanding seaweed biodiversity surrounding the islands has been the North Atlantic biases of early visiting botanists. All of the early descriptions of algal species from Bermuda were reported by individuals from either New England or Europe [8] and many species were misidentified based on a superficial likeness to North Atlantic algae. Many of Bermuda's algal species, therefore, have had to be painstakingly decoupled from the names of their northern relatives. Investigations based on morphology alone, for reasons outlined above, are often inadequate to convincingly reclassify species that cannot be found reproductive, thus making use of MAAT in this process a critical step towards understanding Bermuda's marine biodiversity.

The first compiled list of species in the marine flora of Bermuda was "*The Algae of Bermuda*" in 1917 by F.S. Collins and A.B. Hervey [9]. This report included 342 species of marine algae, many with names of recognized eastern North Atlantic species. This report [9] was the last complete flora strictly dedicated to the algae of Bermuda. A year later, M.A. Howe [10] contributed the algal section to N.L. Britton's 1918 *Flora of Bermuda*, but he only included the more common and more conspicuous algae occurring in the islands and again exhibited a northern bias. In the 1940s, W.R. Taylor first visited Bermuda with his student, A.J. Bernatowicz [11], and he included their data along with previous collectors in his 1960 comprehensive *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas* [12]. Nearly a decade later, Taylor and Bernatowicz [13] produced an annotated list of the most common shallow water macroscopic seaweeds of the Bermudas. At that time, 50 years after the Collins and Hervey report, 40 new species of algae were added to the Bermuda flora and, within the context of the Caribbean, many Bermudian seaweed

names were changed. These works brought the total marine red, green and brown species in the islands to 380.

From the time of Taylor's work until late in the 20th century, additions to the Bermuda marine flora have been published only sporadically. However, beginning in 1997, more than 30 new species and new records to the islands were added to the flora culminating in a checklist of taxa [8]. Since then, nearly 50 more have been added [14-21], bringing the total flora to 449 species. Of this number, 258 are red algae. Several additional questionable records of European taxa reported in the first half of the 20th century have yet to be confirmed and are not included in the total. It is important to note that 65 species in the flora have their type localities (origin of the specimen used to define a species) in Bermuda, several of which are presently synonymized with other species described from distant geographic locations. Below we review the significant impacts MAAT has recently had on the understanding of Bermudian and western Atlantic seaweed diversity.

2. Convergence, Hidden Diversity and Sorting It Out

Classification and taxonomy in red algae has traditionally been based on reproductive structures. In particular, the development of female gametangia, or "carpogonia", both before and after fertilization, and the development of tetrasporangia, when found, are extremely valuable taxonomically. The sporophytic stage in the well-known triphasic red algal life history develop sporangia that meiotically produce four tetraspores. These sporangia divide in one of three distinct division patterns typical for genera, and at times, higher taxonomic units. Characters derived from these stages of the life history are critical in differentiating the majority

of red algal species, but many collections have either only male reproductive features or none at all, making morphological convergence of two species at the gross anatomy level a vexing problem.

2.1 Convergence

A red algal species recently discovered in Bermuda, *Chondracanthus saundersii* C.W. Schneid. et C.E. Lane (Gigartinales, Gigartinaceae), was discovered devoid of female gametangia or tetrasporangia [15]. Although male spermatangia were present in some collections, these were not helpful for generic assignment. As a genus, the taxonomy of *Chondracanthus* Kütz. was first revised morphologically [22], then recently using the large subunit of the chloroplast-encoded RuBisCo gene (*rbcL*) [23]. After *rbcL* sequencing of Bermuda specimens from Walsingham Pond and Harrington Sound collected at various times of the year, these collections grouped within, but were not identical to, other *Chondracanthus* sequences [15]. The collections of *C. saundersii* were found to be morphologically identical to specimens historically collected in Bermuda, and throughout much of the Caribbean, known as *C. acicularis* (Roth) Fredericq. However, at the molecular level the western Atlantic specimens are quite distinct from sequences produced from isolates collected in Europe, the area from which *C. acicularis* was originally described. Based on the morphological re-examination of material from both sides of the Atlantic, character differences were discovered. The primary morphological distinction between *C. acicularis* and *C. saundersii* is the narrower, flattened lubricous axes and less dense medulla of *C.*

saundersii, but these characters alone would not have convinced all taxonomists of the distinction between the two species using strictly traditional practices.

Chondracanthus saundersii is also smaller and less copiously branched than another European *Chondracanthus* once also thought to occur in Bermuda, *C. teedei* (Mert. ex Roth) Kütz. [15]. *Chondracanthus teedei* was likewise removed from the flora of Bermuda after morphological observations of historical specimens from the islands also showed them to belong to the newly described *C. saundersii*. Thus, molecular work in Bermuda became the platform upon which European *C. acicularis* and *C. teedei* were questioned or removed as members of the western Atlantic flora. Collections of *C. acicularis* from Haiti, Cuba, and Brazil were also annotated as *C. saundersii*, thus affecting their floras. The Bermuda focused study [15] did report two western Atlantic records (North Carolina, Florida) that for the present remain as *C. acicularis*, but even these locations should be sampled and their genetics compared against European sequences of this species in order to confirm that this species remains as a true member of both the eastern and western Atlantic floras.

2.2 Hidden Diversity

In a similar manner, *Botryocladia pyriformis* (Børgesen) Kylin is an example of a Caribbean red algal species name being applied to a wide range of morphologies in the Atlantic Ocean. *Botryocladia bermudana* C.W. Schneid. et C.E. Lane was one of the species previously known as *B. pyriformis* in Bermuda [17] prior to the advent of MAAT. Collections of *B. pyriformis* from the Canary Islands, Gulf of Mexico and the Caribbean, but not from its type locality in the West Indies, nor Bermuda, had previously been examined using MAAT. Molecular analyses had already carved out

four species from specimens previously attributed to *B. pyriformis*: *B. bahamense* D.L. Ballant. et Aponte, *B. canariensis* Afonso-Carrillo et Sobrino, *B. ballantinei* Gavio et Fredericq and *B. caraibica* Gavio et Fredericq [24-27]. *Botryocladia bermudana*, presently an endemic to Bermuda, recently became the sixth member of the “*B. pyriformis* complex,” again using MAAT [17]. This species is by far the most common of the three *Botryocladia* species in the islands [17], and is found at depths ranging from the intertidal to 73 m. But, since its first report in Bermuda by Collins and Hervey [9] and Howe [10], this taxon had been a cryptic species under the name of *B. pyriformis*. In reality, it clusters closely with newly described and sequenced *B. caraibica* from Atlantic Panama and Florida in a molecular phylogeny, not *B. pyriformis*. Morphologically, these sister species are differentiated by the greater thickness of *B. bermudana* vesicle medullary cells and walls, and occasional club-shaped secretory cells, although no single defining, non-overlapping feature stands alone to separate *B. bermudana* from *B. caraibica* [17]. This molecular sequencing allowed the discovery of this new *Botryocladia* with minimal anatomical differences from the species it had previously been assigned to in Bermuda [17].

To make matters worse for traditional taxonomists, herbivores in the natural environment of *Botryocladia bermudana* beget mostly populations of algal individuals under 1 cm tall, creating the illusion of a second species when compared with 6.5 cm, highly branched individuals residing in a protected environments, such as the reef tank in the Bermuda National Aquarium. The Bermuda Aquarium has only ever housed anything in its tanks that has not been collected locally, making it an occasional sanctuary for native marine algae that end up in tanks lacking herbivores. It appears

that the parrot fish which graze *B. bermudana* in “wild” habitats have been an important recent force shaping the scattered *B. bermudana* populations, as Collins and Hervey [9] never collected its smaller “cropped” form, instead collecting luxurious large specimens in inshore environments where today they cannot be found [17]. Stunted or cropped growth has also been observed among offshore *Botryocladia exquisita* C.W. Schneid. et C.E. Lane. Despite over a decade of collecting Bermudian algae in all habitats, large, developed plants have only ever been collected in the reef tanks of the Bermuda Aquarium [17]. The small number of cell layers in the *B. exquisita* vesicle walls, which are less than 36 μm thick, and the production of both specialized and non-specialized secretory cell bearing cells in *B. exquisita*, distinguish it from a similar Caribbean and Gulf of Mexico *Botryocladia* species, *B. monoica* Schnetter [17]. The cropped small offshore specimens of *B. exquisita* could easily have been misidentified as *B. monoica* had molecular analysis not been performed.

Despite its relative rarity in Bermuda, a third species of *Botryocladia*, *B. flookii* C.W. Schneid. et C.E. Lane, has been described from the intertidal to 30 m. Although the gross morphology of *B. flookii* is virtually identical to that of *B. macaronesica* Afonso-Carrillo, Sobrino, Tittley et Neto of the Canary Islands, it differs from the eastern Atlantic species in the lateral attachment of its vesicles, its large secretory bearing cells and the development of the medulla into the vesicle cavity below carposporophytes [17]. Unfortunately, no molecular data exist for *B. macaronesica* nor was any available for DNA extraction at the time of the study [17]. While there is no single outstanding morphological feature which separates *B. flookii* from all other *Botryocladia* species, the genus *Irvinea* was split from *Botryocladia* on the basis of

molecular genetics alone [28]. Since this split, confidence in the use of morphology to separate *Botryocladia* species has seriously diminished.

Another red alga that contained cryptic species within its binomial in a variety of locations worldwide, including Bermuda, was *Asteromenia peltata* (W.R. Taylor) Huisman *et* A. Millar [28]. This species was first described from Venezuela based solely on vegetative material [30, as *Fauchea peltata* W.R. Taylor]. Since that original report from the Caribbean, *A. peltata* has been reported as a cosmopolitan tropical species [29]. Recent molecular work [21], however, has carved up '*A. peltata*' into five regional *Asteromenia* species aligned with the Hymenocladaceae rather than the Rhodymeniaceae [31]. During this recent study [21], two species of *Asteromenia* were reported in Bermuda, *A. peltata* and *A. bermudensis* G.W. Saunders, C.E. Lane, C.W. Schneid. *et* Kraft where previously only the former was known. New samples of '*Asteromenia peltata*' from Hawaii, Fiji, French Polynesia and other locations where it has been reported from and not sequenced in this study are now in need of molecular analysis to discover which species they actually represent, as *A. peltata* has only been verified using gene sequencing in the western Atlantic Ocean [21]. Once again, molecular work created a domino effect of species in need of additional clarification.

Centroceras clavulatum (C. Agardh) Mont. (Ceramiales, Ceramiaceae) has been a reported cosmopolitan alga in the biogeographical literature [3, 32]. This species has been recorded from the Pacific, Atlantic and Indian Oceans and places such as Pacific South America, California, North Carolina and Bermuda to Brazil, southeast Asia, Australia, the South Seas and all of the coasts of Africa, to name a few [33]. Thus, it ranges from cool temperate to tropical waters [3]. This was the case

until, once again, MAAT greatly restricted the biogeography of *C. clavulatum*, in this case to the Pacific Ocean [32]. Molecular phylogenetic trees illustrating the relationships between various *Centroceras* specimens were produced using small subunit (SSU) and large subunit (LSU) nuclear encoded genes, and ribulose biphosphate carboxylase (*rbcL*) chloroplast DNA. Three significant changes were made in the taxonomy of this species described in the 19th century [34, as *Ceramium clavulatum* C. Agardh]. First, true *Centroceras clavulatum* was found to be restricted to the Pacific coasts of the Americas from southern California to Chile, southern Australia and New Zealand. Because *C. clavulatum* had previously been reported from Bermuda, these collections now required analysis both morphologically and molecularly in order to determine their true identity. Next, this study [32] resurrected three western Atlantic species from the ‘*C. clavulatum* complex’ which had previously been retired as synonyms of *C. clavulatum*: *C. gasparinii* (Menegh.) Kütz., *C. hyalacanthum* Kütz. and *C. micracanthum* Kütz. [35] . Lastly, Won et al. [32] also added two new species to the genus, one from South Africa and the other from southern Chile.

2.3 Morphological Plasticity

Halymenia pseudofloresii Collins et M. Howe is a noteworthy Bermudian red algal species because of its distinctive morphological plasticity. Prior to molecular analysis, a similar species, *H. floresii* (Clemente) C. Agardh, was also a member of the Bermuda flora [8]. Nuclear LSU ribosomal DNA and a protein-coding elongation factor were sequenced and compared with sequences from other Halymeniales using bayesian and likelihood molecular phylogenetic methods. The cytochrome oxidase

subunit I (COI-5P) from the mitochondria and universal plastid amplicon (UPA) were likewise sequenced from five Bermuda isolates including *H. pseudofloresii* and *H. floresii* morphs in order to investigate the possibility of intraspecific divergence [19]. The type locality of *H. pseudofloresii* is not precise, being described as from “a grotto near Walsingham” [36], clearly within the national park of that name in Bermuda. There are a number of ponds and pools (or grottos) in the Walsingham area, all of which are connected by a system of underground saltwater caves. Several specimens from this tidally fed system were collected for molecular and morphological analysis. Molecular analysis showed no more than a single base pair difference between the COI-5P of any two of the isolates, which included distinct morphologies such as narrowly pinnate (*H. floresii* morph), intermediate, and typical broad fronds (*H. pseudofloresii* morph). Likewise, no differences were observed in the UPA of any of the Bermuda specimens tested [19]. Thus despite the range of appearance, molecular data from several common markers clearly indicates that they all represent a single, phenotypically plastic species in the islands [19]. Additionally, all of the Bermuda plants were distinct from a sequence obtained from freshly collected material of *H. floresii* from near its type locality in Spain, thus bringing into question other reports of this European species from the western Atlantic. Further comparisons with sequences from other members of the Halymeniales in this study [19] suggest that *Halymenia* is a polyphyletic genus as it currently stands, opening up another avenue for molecular work. Clearly, other species found in Bermuda and elsewhere which are observed as morphological continua between distinct morphologies could benefit from the refined techniques of MAAT [19]. Some may, in fact, represent distinct species with

overlapping morphologies, while others such as *H. pseudofloresii* outlined here represent species with highly variable morphologies.

2.4 Remaining Barriers to Sorting it All Out

Clarifying taxonomic issues often requires more than just obtaining samples of the species in question. It is often necessary to examine type specimens and then critical to collect and process DNA samples from the type locality and from potentially related species. For that reason, many species remain in limbo while comparative collections are being made. This process can take years, because some species have only been collected once or exist in remote locations. Additionally, habitats have changed since the time early 20th century collections were made and locating comparative material can be impossible. This is especially a problem because early vegetative algal collections, in particular, had their broader taxonomic placements misjudged as often as they were erroneously classified at the genus or species level. For example, when Taylor [37] described the genus and species *Rhododictyon bermudensis* W.R. Taylor from Bermuda, he tentatively placed it in the family Dasyaceae (Ceramiales). This new genus and species was later moved to the Ceramiaceae prompted by the discovery of tetrasporangia in offshore collections from North Carolina [38]. The tetrasporangia of *R. bermudensis* form at the ends of filaments protruding from the lower, older cells of the blade. Such an arrangement is similar to that of *Compsothamnion* Nägeli and was used to propose an alliance with the tribe Compsothamnieae [38]. Because taxonomically critical female gametangia have yet to be discovered for this genus, molecular analysis is required to firmly establish the taxonomic position of this monotypic genus in its family and tribe [38].

Yet this species is only found at great depth and in small numbers when found, an example of a microscopic taxon difficult to find enough material for both DNA and morphological study.

Flahaultia tegetiformans W.R. Taylor (Gigartinales, Solieriaceae) provides an example of a red alga that may not have been placed in the correct genus. Despite numerous collections from a variety of locations in Jamaica none were ever collected with gametangia [39]. Taylor described this species as a prominent member of the Jamaican flora from three subtidal sites. Again, Taylor's pressed herbarium collections were made prior to sample preservation for DNA extraction, and no recent workers have collected fresh material for genetic sequencing. The Jamaican vegetative specimens sat upon Taylor's lab bench for years awaiting fertile collections that were never found; eventually, he assigned the new species to the genus *Flahaultia* Bornet on the basis of its resemblance to the eastern Atlantic *F. appendiculata* Bornet. Although a recent report of *F. tegetiformans* from the Greater and Lesser Antilles has been made [40], no specimens have been sequenced from either the type locality nor these recently collected sites. Such an analysis is necessary to ensure that the Antillean specimens are indeed the same as those collections from the type locality in Jamaica, and also whether the species is properly assigned to *Flahaultia*. Thus, the taxonomic placement of *F. tegetiformans* remains uncertain.

Although *Crassitegula walsinghamii* C.W. Schneid., C.E. Lane et G.W. Saunders appears in habit and vegetative morphology very similar to *Flahaultia tegetiformans*, sequences of its SSU nuclear DNA from Bermuda were used to firmly place it in the Sebdeniaceae (Sebdeniales), rather than the Solieriaceae. This

classification was affirmed by the remarkable similarity in female sexual structures and post-fertilization stages observed between *C. walsinghamii* and *Sebdenia* (J. Agardh) Berthold when female material was found in a collection discovered after molecular sequencing was completed [18].

Another recent discovery in Bermuda was the endemic *Griffithsia aestivana* C.W. Schneid. et C.E. Lane [16]. It was identified without the benefit of gametophytes or gene sequencing, but was placed in *Griffithsia* C. Agardh owing to its obvious morphological resemblance to *G. capitata* Børgesen from the Canary Islands and other like members of the genus. The new species was based upon the formation of tetrasporangia located on whorled stalk cells at the distal ends of axial cells, among other things. Whorls occur around the two penultimate cells at the tips in the similar *G. capitata*; whorls occur on long cylindrical cells from mid-axis to a few cells below an apex in *G. aestivana*. Although *G. aestivana* seems to have unique characteristics, molecular analysis of *G. aestivana* is still necessary to ensure that it is a unique species, and the taxonomy will not change until that is completed [16].

Despite the fact that MAAT is becoming a standard procedure in floristics and phylogenetics, not all new species are being preserved for DNA extraction. Some are simply so small and of unknown identification in the field, that they remain to be determined in the lab long after the collections are made. *Woelkerlingia sterreri* C.W. Schneid. et M.J. Wynne was recently described from Bermuda [20]. This species was collected at 10 m, extremely well established on the unusual habitat created by a free-moving discarded linen tablecloth. The presence of distinctive 2-celled fertile female filaments and other characters of the procarp were critical in placing the specimens in

the genus *Woelkerlingia* Alongi, Comaci *et* G. Furnari. However, no molecular work has been done within this genus to corroborate its taxonomic assignment; therefore, it is questionable whether or not all the genera of small fuzzy reds in the Wrangeliaceae are supported genetically, including *Woelkerlingia* [20].

3. Conclusions

The barriers to understanding algal biodiversity are problematic on a global scale for reasons covered here. Even relatively heavily studied genera in marine floras that were thought to be well understood using morphology and anatomy, have been revised based on recent MAAT studies [41-43], suggesting that substantial taxonomic revisions are likely over the next few decades. Although the MAAT method will almost certainly be superseded by novel techniques as methodologies advance, the combination of molecular data and microscopical observations has proven to be a robust approach to solving many long-standing taxonomic controversies among algal groups, particularly within red algae. Ultimately, the extent to which MAAT studies expand will likely depend on the stabilization of markers for various groups of the Tree of Life and continued training of students in alpha taxonomy – a skill that is rapidly declining in prevalence as descriptive morphological taxonomic work has fallen out of favor. The strengths of MAAT studies are, like any type of research, entirely dependent on the input data. DNA barcoding has been lauded as a major step forward in species identification, but several studies [1, 44] have shown that sampling biases, either geographic or taxonomic, can result in incorrect species delimitations. What is acceptable in one lineage of organisms, however, may not work in another.

Ironically, the very diversity of Eukaryotes confounds our attempts to apply a universal marker to understand that diversity.

However, the short-term importance of understanding global biodiversity in an era when it is being depleted means that methods to do so must be identified, even if we have to adapt to each lineage independently. The examples we present here, as well as many others, have shown that the ability to definitively place vegetative algal samples in a taxonomic framework is an enormous asset to biodiversity studies in places where some species are only ever found as vegetative thalli. The accuracy of biotic inventories may be exceedingly important in the face of global climate change, as inaccurate baseline surveys will mask species loss. Numerous recent publications (including a special section of the August 11 issue of *Science* in 2006) suggest that rising sea temperature is already affecting the ranges of various marine animals, from turtles to fish. However, migrating animals may be responding directly to temperature or to any number of indirect factors, such as currents or prey items. In order to get a better understanding of the potential effect of rising sea temperature on the coastline, attached, rather than swimming, organisms would seem to be obvious environmental indicator species. Thus, an accurate and complete understanding of marine biodiversity in places like Bermuda, which sit at a climatic boundary, may be the first solid indicators of larger problems.

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Figure 1. Possible scenarios resulting from the application of molecular-assisted alpha taxonomy. Species of algae, classified based on morphology, are represented diagrammatically. In many cases, the traditional morphological classification reflects the molecular data **(a)**. However, convergent evolution **(b)** can produce distantly related species that are morphologically similar. Molecular data has been instrumental in clarifying this type of situation, as well as instances where morphologically dissimilar specimens represent the same species **(c)**. This occurs most commonly either when the complete life history of an organism with heteromorphic alternation of generations is unknown, or in some species that exhibit a large degree of environmentally-influenced morphological plasticity.

APPENDIX B

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Notes on the marine algae of the Bermudas. 14. Five additions to the benthic flora, including a distinctive second new species of *Crassitegula* (Rhodophyta, Sebdeniales) from the western Atlantic Ocean

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ABSTRACT

A new red algal species, *Crassitegula laciniata* (Sebdeniaceae, Sebdeniales), is described from recent collections made on offshore Bermuda reef habitats, and is distinguished morphologically and molecularly (COI-5P, LSU gene sequences) from congeners in Bermuda and Australia. In addition, four other species are reported as new members of the growing Bermuda macroalgal flora, the chlorophytes *Anadyomene lacerata* and *Codium carolinianum*, and the rhodophytes *Gracilaria occidentalis* and *Predaea goffiana*.

KEY WORDS: *Anadyomene*, Bermuda, COI-5P, *Codium*, *Crassitegula*, *C. laciniata* sp. nov., *Gracilaria*, LSU, morphology, *Predaea*, *rbcL*, Rhodophyta, Sebdeniaceae, taxonomy.

INTRODUCTION

The isolated western Atlantic islands of Bermuda (32°18'N, 64°46'W) have proved to be not only rich in macroalgal biodiversity, but also host to an invaluable reserve of genetic information for taxonomists. More than 70 species of benthic marine algae, as well as 15 subspecific taxa, have their type localities in Bermuda, these representing nearly 20% of the local flora and 5% of the approximate 1400 tropical and subtropical marine algae in the western Atlantic (Wynne 2011). The power of molecular sequencing for distinguishing between morphologically ambiguous taxa and for inferring evolutionary relationships at all taxonomic levels has directed a great deal of recent attention to regions of rich biodiversity such as Bermuda as well as to the type localities of the species that presently are found in

Bermuda. Specimens from type localities, if not the archival types themselves, have become even more critical for accurate species determinations (Schneider *et al.* 2010, 2011).

Shortly after the genus *Crassitegula* was proposed for a single species from Bermuda (Schneider *et al.* 2006), a second species was described from almost half a world away at Lord Howe Island, eastern Australia (Kraft & Saunders 2011). Now, relying on molecular as well as classical methodologies, a third *Crassitegula* has come to light, again in Bermuda. This entity is described, and, in addition, four new records of Caribbean macroalgae for Bermuda are documented as a result of our recent explorations.

MATERIAL AND METHODS

Collections were made using scuba, and the specimens were pressed fresh onto herbarium paper with fragments preserved in 4-5% Formalin-seawater as well as dried in silica gel for molecular analysis. Site locations were determined using a Garmin™ GPS III Plus (Olathe, Kansas, USA). Samples selected for DNA analysis were photographed live using a Canon Powershot s90 camera (Canon Inc., Tokyo, Japan) prior to desiccation. Herbarium specimens were scanned on an HP Photosmart Premium scanner model C-309a (Hewlett-Packard Company, Palo Alto, California, USA) and photomicrographs were taken using a Zeiss Axioskop 40 microscope (Oberkochen, Germany) equipped with a model 4.2 Spot InSight QE digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA) interfaced with Spot

Software v. 5.1.3. The digital images were composed in Adobe Photoshop™7.0 (Adobe Systems, San Jose, California, USA). Voucher specimens are deposited in the first author's personal herbarium (CWS), with some duplicates sent to KIRI, MICH, NY, UNB, US and the Bermuda Natural History Museum (BAMZ). The *Phycotheca Boreali-Americana* (*P.B.-A.*) *exsiccata* referred to represents the set originally purchased by Wellesley College (Massachusetts, USA) that now belongs to the first author. When listed, herbarium abbreviations follow the online *Index Herbariorum* (<http://sweetgum.nybg.org/ih/>) and standard author initials/abbreviations follow Brummitt & Powell (1992).

Specimens used in molecular analyses and their assigned GenBank numbers are listed in Table S1. Some species reported here were sequenced for COI-5P to assign specimens to genetic species groups (Saunders & McDevit 2012). For phylogenetic analyses, 28S rDNA (LSU) or *rbcL* sequences were amplified following Saunders & Moore (2013). Portions of permanent vouchers placed in silica gel were ground in liquid nitrogen and stored at -20°C. DNA was extracted from 0.5 µl ground material using the Sigma-Aldrich (St. Louis, MO) GenElute Plant Genomic Miniprep Kit according to manufacturer protocol, with 500 µl of modified lysis solution (50 µl 10% TWEEN 20, and 5 µl of 20 mg/ml ProK), as well as 1 hr 23°C incubation followed by a 20 min incubation on ice (Saunders & Druehl 1993). DNA was amplified via polymerase chain reaction (PCR) with the Takara Ex-Taq DNA polymerase kit (PanVera, Madison, Wisconsin, USA) in an Eppendorf AG Mastercycler epGradient thermal cycler (Eppendorf, Hamburg, Germany) with the COI-5P bidirectional primer pair and thermal profile outlined by Saunders & McDevit

(2012). Amplified DNA was treated with Sigma-Aldrich GenElute PCR Clean-Up Kit per manufacturer protocol (Sigma, St. Louis, Missouri, USA). Purified PCR product was sequenced at the Rhode Island Genomics and Sequencing Center using the ABI 3130xl genetic analyzer (Life Technologies, Grand Island, New York, USA).

Sequences were assembled with Geneious software (v. 6.1.5 available from <http://www.geneious.com>). Alignments were generated with additional COI-5P and LSU data from GenBank using the BLASTx and BLASTn algorithm (Altschul *et al.* 1990). The following regions of ambiguous alignment were removed from the LSU analysis: 486-508, 628-655, 662-670, 1285-1295, 1959-1992 and 1997-2047. Base pair numbers are from the alignment. The best model of evolution was determined for the gene regions COI-5P (39 taxa, 676 sites) and LSU (12 taxa, 2747 sites) using jModel Test (Posada 2008). Maximum Likelihood (ML) was performed with PhyML software in Geneious, using model HKY85+I+G (COI-5P) and GTR+I+G (LSU) with 500 bootstrap replicates. Bayesian analyses were conducted for both COI-5P and LSU using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003) and run with four parallel chains including one heated chain, with branch lengths optimized during the run for two million generations. The first 100,000 generations were discarded as the ‘burn-in’. Posterior probabilities were estimated based on the remaining trees. The consensus tree was manipulated for presentation using FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>). *RbcL* chloroplast gene sequences of Bermuda and North Carolina *Codium* specimens (Table 1) were generated and supplied to us from DNA extracted by H. Verbruggen according to the methods of Verbruggen *et al.* (2007). For *Gracilaria occidentalis*, we compared our sequences

with GenBank *rbcL* sequence AY049322, and for *Predaea goffiana*, *rbcL* sequences FJ868814-FJ868819.

TAXONOMIC NOTES

Chlorophyta, Ulvophyceae, Cladophorales, Anadyomenaceae

***Anadyomene lacerata* D.S. Littler & Littler 1991, p. 105, figs 6-14.**

Figs 1-3

TYPE LOCALITY: Isla Monito, Puerto Rico, Caribbean Sea, western Atlantic.

COLLECTION: Bermuda—*C.W. Schneider (CWS)/C.E. Lane (CEL)* 10-12-6, 20 August 2010, vic. Middle Buoy, Eastern Blue Cut channel off Daniel's Head, Somerset Is., 32°23'20.6"N, 64°53'19.9"W, depth 9-10 m.

A single collection of this species was made in a species-rich habitat off the north shore of Bermuda. It conformed in all macroscopic and anatomical characteristics to *Anadyomene lacerata* (Littler & Littler 1991). Its distinctive lacerate margins (Figs 1, 2) bordered by elongate “vein” cells (Fig. 2) separated it from the locally common *A. stellata* (Wulfen) C. Agardh. One other *Anadyomene* species from the archipelago, *A. howei* D.S. Littler & Littler (Schneider & Lane 2007), also has elongate “vein” cells along the outer margins (Littler & Littler 1991). This latter species, however, has a pattern of randomly organized, oval-shaped interstitial cells between the veins (Littler & Littler 1991), in contrast to those in *A. lacerata* which are elongated and parallel, basically perpendicular to the vein cells, and separated by rows of shorter interstitial cells (Figs 2, 3) (Littler & Littler 1991).

This collection from Bermuda represents a new northernmost range extension for this offshore subtidal species, previously known from 15-60 m depths in the Gulf of Mexico, the Caribbean Sea, and off the coasts of Brazil and Venezuela (Littler & Littler 1991).

Bryopsidales, Codiaceae

***Codium carolinianum* Searles 1972, p. 19, figs 1a, 2.**

Fig. 4

TYPE LOCALITY: Offshore reefs, Onslow Bay, North Carolina, western Atlantic Ocean.

COLLECTION: Bermuda—*CWS/CEL* 9-34-10 [BDA0087], 20 March 2009, vic. Middle Buoy, Eastern Blue Cut channel off Daniel's Head, Somerset Is., 32°23'20.6"N, 64°53'19.9"W, depth 15-16 m; *CWS/CEL* 10-12-2, 20 August 2010, *loc. cit.*, depth 9-10 m.

Not previously known from Bermuda, recent collections from a seemingly persistent offshore mat of this prostrate species are a good morphological match to specimens from Onslow Bay, North Carolina, USA (the type locality). We obtained and sequenced the *rbcL* portion of the chloroplast genome from two offshore North Carolina specimens for comparison (Table S1, Fig. 7). Sequence H.1009 (Bermuda) is a 100% match to the NC collections, and H.1015 (Bermuda) differs by one base pair (out of 705 bp).

Previously, this species has been reported in the western Atlantic from the Carolinas (Schneider & Searles 1991), the Gulf of Mexico-Florida (Dawes &

Mathieson 2008), and Trinidad in the Caribbean (Duncan & Lee Lum 2006). Haroun *et al.* (2002) reported *Codium carolinianum* from the Canary Islands in the eastern Atlantic, specimens that should be sequenced for confirmation. As it is highly unlikely that this densely compact benthic species could have been dispersed laterally across the Gulf Stream from the Carolinas, or survived in the abyssal depths between Bermuda and mainland America; however, it seems probable that *C. carolinianum* found a warm water refuge in the Caribbean during the Pleistocene and was distributed north to the Carolinas and Bermuda after the final glaciers retreated 10,000 to 12,000 BP, when subtropical water temperatures recovered in the more northerly habitats where it flourishes today (Schneider & Searles 1998).

Rhodophyta, Florideophyceae, Gracilariales, Gracilariaceae

***Gracilaria occidentalis* (Børgesen) M. Bodard 1965, p. 874, figs 1a-1e.**

Fig. 6

BASIONYM: *Rhodymenia occidentalis* Børgesen 1920, p. 387, figs 371, 372.

SYNTYPE LOCALITIES: St. John and St. Thomas, Virgin Islands, Caribbean Sea, western Atlantic Ocean.

COLLECTION: Bermuda—*CWS/CEL* 10-12-24 [BDA0114-116], cystocarpic/male, 20 August 2010, vic. Middle Buoy, Eastern Blue Cut channel off Daniel's Head, Somerset Is., 32°23'20.6"N, 64°53'19.9"W, depth 9-10 m.

A morphological analysis of recently collected specimens from Bermuda (Fig. 6) has shown them to represent *Gracilaria occidentalis*, both in habit and anatomy. They compared favorably with an isosyntype of *Rhodymenia occidentalis* Børgesen in

MICH (Fig. 5; from 18-21 m between St. Thomas and St. John, Virgin Islands [Børgesen 1920]). They also matched specimens collected at depths to 59 m off Bermuda (Challenger Bank) in August 1960 by J.J. Frederick, specimens determined by W.R. Taylor as *G. curtissiae* J. Agardh. These Challenger Bank specimens alone were the basis of the report of *G. curtissiae* from Bermuda (Frederick 1963; Schneider 2003). The lectotype of *G. curtissiae* [L] and other specimens from Florida (*P.B.-A.* no. 432 [Collins *et al.* 1898]) show this species to be significantly broader, thicker and more irregularly branched than *G. occidentalis*. *RbcL* sequences of the 2010 Bermuda collections were a 100% match to a sequence of *G. occidentalis* from offshore Louisiana, USA, as identified by Gurgel & Fredericq (2004) and Gurgel *et al.* (2004), and posted to GenBank as AY049322. This report effectively removes *G. curtissiae* from the flora of Bermuda and represents a new northern limit of distribution for *G. occidentalis* in the western Atlantic.

Nemastomatales, Nemastomataceae

***Predaea goffiana* D.L. Ballantine, H. Ruiz & Aponte 2002, p. 385, figs 1-11.**

Figs 8-10

TYPE LOCALITY: Laurel Reef, off La Parguera, Puerto Rico, Caribbean Sea, western Atlantic.

COLLECTIONS: Bermuda—*CWS/CEL* 06-14-8, 21 June 2006, offshore of Frick's Beach, Tucker's Town, 32°19'56.7"N, 64°40'21.0"W, depth 10 m; *CWS* 10-24-23 [BDA0388-391], 23 August 2010, Cathedral Rock, off the south shore of Bermuda Is., 32°20'31.1"N, 64°39'24.2"W, on a shaded horizontal rock at 17 m;

CWS/CEL/T.R. *Popolizio* (TRP) 12-22-4, 21 January 2012, Cathedral Rock, *loc. cit.*, 14-15 m; TRP 12-79-5, 20 June 2012, Cathedral Rock, *loc. cit.*, 15-18 m; TRP 12-175-1, 16 December 2012, Cathedral Rock, *loc. cit.*, 15 m.

During the last decade, we have collected in offshore waters on the south shore of Bermuda Is. an elegant, monoecious *Predaea* (Fig. 8), the first of this genus reported from the archipelago. Sequences of *rbcL* from this robust, rubbery, compactly branched *Predaea* are shown to be a 99.8% match (three changes among 1345 bp) to sequences of the *P. goffiana* in GenBank cited by Gabriel *et al.* (2009) from Campeche Banks, Mexico. In that paper, Gabriel and co-workers analyzed five sequences from material collected and determined by S. Fredericq and colleagues from deep offshore waters. Interestingly, our distinctive plants from Bermuda (Fig. 8) exhibit a different overall habit to thalli described in the protologue of *P. goffiana* from the type locality off Puerto Rico (Ballantine *et al.* 2002), despite having a similar internal anatomy that includes distinctively tapering filaments in the cortical fascicles (Figs 9, 10). Because the *P. goffiana* sequences uploaded to GenBank are from the western Caribbean, and the type is from the eastern Caribbean, there has to be some doubt that they indeed are representative of *P. goffiana*. As we have been unable to locate specimens from Puerto Rico for genetic analysis, and because the type collection was preserved in formalin/seawater (David Ballantine, pers. comm.), it remains uncertain if they are a genetic match to the specimens in GenBank. Nevertheless, because our collections from Bermuda represent the same taxon as those from Mexico deposited in GenBank, we tentatively have accepted the Gabriel *et al.*

(2009) identification of *P. goffiana* until topotype material of this species is located and sequenced.

Sebdeniales, Sebdeniaceae

Crassitegula laciniata* C.W. Schneider, Popolizio & C.E. Lane, *sp. nov.

Figs 13-17

DESCRIPTION: Plants saxicolous, arising from small primary holdfasts and short erect stipes, the dorsiventral blades developing perpendicularly from the stipe, therefore prostrate and spreading, with branch tip, margin and laminal ventral secondary holdfasts attaching to rocky substrata or other thalli to form an imbricating mat; blades firm, smoothly textured, brownish-red in color, with occasional marginal dentations that are the precursors to adventitious branches; blades initially transversely ovate to angular, 0.6-1.6 cm wide, the primary blade then proliferating finger-like branches from distal margins; blades 270-500 μm thick, the medulla densely filled with long, thin filaments from 2-5 μm in diam. with occasional connecting stellate ganglia; cortex 3-4 layered with irregular to subglobose large inner thick-walled cortical cells 28-36 μm diam., grading to smaller-celled outer cortical cell layers, the heavily pigmented surface cells irregularly rounded to spherical and small, 3.5-5.0 μm in diam., arranged in a mostly continuous cortex of arching rows in surface view, the chloroplasts at times reticulate; gametophytes presumably dioecious, spermatia spherical to elongate, 1.5-3.0 μm diam., produced from outer cortical cells in small rounded to irregular and slightly raised, pigmented sori on dorsal surfaces; female characteristics, carposporophytes and tetrasporangia unknown.

HOLOTYPE (deposited in UNB and designated here): *T.R. Popolizio* 12-91-1, 11 August 2012, [Fig. 14; BDA1305, GenBank KF561832].

ISOTYPES: KIRI, MICH, Herb. CWS [Figs 13, 15; BDA1306, GenBank KF561833].

TYPE LOCALITY: Hog Breaker off the north shore of Bermuda Island, Bermuda, western Atlantic, 32°27'47.7"N, 64°49'48.9"W, depth 12 m.

PARATYPES (all Bermuda): *CWS/CEL* 10-12-37 [BDA0134-0135], male, 20 August 2010, vic. Middle Buoy, Eastern Blue Cut channel off Daniel's Head, Somerset Is., 32°23'20.6"N, 64°53'19.9"W, depth 9-10 m; *TRP* 12-66-1 [BDA1131-35], 28 May 2012, Hog Reef, off north shore Bermuda Is., 32°27'26.3"N, 64°50'06.5"W, depth 12 m; *TRP* 12-117-1 [BDA1486-88], 20 September 2012, Hog Breaker, *loc. cit.*; *TRP* 12-125-1 [BDA1514], 26 September 2012, off Sinky Bay, south shore Bermuda Is., 32°14'35.9"N, 64°50'10.2"W, depth 7 m.

ETYMOLOGY: *laciniatus* (adj., L.), "slashed into narrow divisions with taper-pointed incisions" (Stearn 1966).

Based on amplified COI-5P barcode regions of the mitochondrial genome that are widely used to assign species to genetic species groups (Fig. 11; Saunders & McDevit 2012), and LSU rDNA sequences to infer phylogenetic relationships (Fig. 12), *Crassitegula laciniata* nested with the two known species in the genus, *C. walsinghamii* C.W. Schneider, C.E. Lane & G.W. Saunders and *C. imitans* G.W. Saunders & Kraft within the Sebdeniaceae (Gigartinales). They represent the second endemic species of *Crassitegula* from Bermuda (Schneider *et al.* 2006) and only the third described in the genus (Kraft & Saunders 2011). Unlike *C. walsinghamii* which

occurs from shallow inshore and inland habitats of Bermuda to as deep as 18 m offshore, the new species has thus far been found only on reefs from 7-12 m depths off both the north and south shores. Individual blades of *C. laciniata* develop perpendicularly to their short erect stipes and produce secondary attachments terminally from acute tips, margins or even ventral positions on blade surfaces, the blades then spreading across rocks as in the other species of the genus.

Although the anatomy was typical for the genus (Schneider *et al.* 2006), *Crassitegula laciniata* was easily distinguished morphologically as it was more branched and dissected than both the generitype and its remarkably similar southern hemisphere congener, *C. imitans* (Kraft & Saunders 2011). The latter two species have suborbicular proliferations at the margins, this being the form taken as they spread over rock surfaces whether the surface is vertical or horizontal. Conversely, *C. laciniata* in early development was seen as small transversely ovate to angular blades that produce occasional minute marginal dentations, most of these distally, that develop long, narrow finger-like branches (Figs 13, 15). As the plant matures, these narrow, acute-tipped branches broaden to become strap-shaped and often dichotomous (Figs 14, 15). Thus, mature thalli totally differed in habit from the juvenile blades that initiated them (Fig. 14).

We made several collections of this new species in spring and summer, mostly off the north shore of Bermuda, and although each consisted of numerous individuals, no tetrasporophytes or gametophytes have been encountered such as have been detailed for the other two *Crassitegula* species by Schneider *et al.* (2006) and Kraft & Saunders (2011). Therefore, molecular data have been necessary to characterize the

generic placement of the third species, which, as already stated, conformed to the genus *Crassitegula* in its anatomical features (Figs 16, 17).

As pointed out in Kraft & Saunders (2011), the Sebdeniaceae is now much richer in genera and species than it was when Kylin (1932) created the family for the single genus *Sebdenia*. Its monogeneric status persisted until Schneider *et al.* (2006) added the genus *Crassitegula*, which was soon followed by the addition of *Lesleigha* by Kraft & Saunders (2011). Significant diversity in the family has been discovered from the Indo-Pacific region, from Australia to Hawaii, where *Lesleigha* and its three species were recently described (Kraft & Saunders 2011). In that same paper, *C. imitans* was described from Lord Howe Is., off the eastern coast of mainland Australia, adding *Crassitegula* to an emerging list of genera shown to have sibling, endemic species there and in Bermuda - *Asteromenia*, *Halopeltis* and *Meredithia* (Saunders *et al.* 2006; Saunders & McDonald 2010; Schneider *et al.* 2012, 2014).

In our phylogenetic analyses of *Crassitegula*, we included samples from Bermuda of the related species, *Sebdenia flabellata* (J. Agardh) P.G. Parkinson, along with those that are genetically distinct from Lord Howe Is. but are retained under the same name, the field identification assigned by Gary Saunders and Gerry Kraft (pers. comm.). It is clear that the two island groupings from different hemispheres harbor unique, yet cryptic, species in the genus. As the basionym of *S. flabellata* was based on collections from syntype localities (Key West, Florida, USA; Guadeloupe, West Indies) in the western Atlantic (Schneider & Wynne 1991), it is logical that the Bermuda specimens are more likely allied to the Caribbean type than they are to those from tropical/subtropical Australia. Sequences from collections of *S. polydactyla*

(Børgesen) M.S. Balakrishnan (an entity presently considered a synonym of *S. flabellata*) from the type locality or nearby in India should be used to see if they are genetically the same as the Lord Howe specimens. Whether or not the populations from Australia (including those from Western Australia and the southern Great Barrier Reef – Huisman, Saunders & Kraft, pers. comm.) are equivalent to a reinstated *S. polydactyla* or represent one or more novel cryptic species remains for the moment uncertain.

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

Species	Voucher	Collector	Site	COI-5P	<i>rbcL</i>	LSU
Bryopsidaeeae						
<i>Bryopsis plumosa</i> (Huds.) C. Agardh	BA47428, Museo Bernadino Rivadavia	M. Ciancea	Mara del Plata, Buenos Aires, Argentina	—	JQ755422	—
Codiaceae						
<i>Codium adhaerens</i> C. Agardh	SMG05-35 (GENT)	D. Gabriel	Azores	—	EF107959	—
<i>Codium arabicum</i> Kütz.	JH9 (GENT)	J.M. Huisman	Australia	—	EF107969	—
	SD0509370 (L)	S. Draisma	Indonesia	—	EF107964	—
	Isolate 121	—	Ishigaki I., Okinawa, Japan	—	AB102984	—
	DML40360 (US)	D.S. & M.M. Littler	Fiji	—	EF107965	—
<i>Codium arenicola</i> P.C. Silva & M.E. Chacana	G.046 (GENT)	C.E. Payri	New Caledonia	—	JQ706332	—
<i>Codium carolinianum</i> Searles	CWS/CEL09-34-10 (H.1009)	C.W. Schneider & C.E. Lane	Middle Buoy, Eastern Blue Cut Channel, Bermuda	—	KF761642	—
	CWS/CEL10-12-2 (BDA0087; H.1015)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	—	KF761643	—
	NC weed 352 (LPT0068)	D.W. Freshwater & R. Muñoz	Onslow Bay, North Carolina, USA	—	KF761646	—

	NC weed 370 (LPT0069)	D.W. Freshwater & R. Muñoz	Onslow Bay, North Carolina, USA	—	KF761647	—
<i>Codium cylindricum</i> Holmes	Isolate 125	—		—		—
<i>Codium</i> <i>decorticans</i> (Woodw.) M. Howe	<i>CWS/CEL</i> 10-6-5 (BDA0054; H.1019)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Tateyama, Chiba, Japan Tucker's Town Bay chasm, Bermuda	—	KF761637	—
	<i>CWS/CEL</i> 10-26-2 (BDA0413; H.1020)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Shark Hole, Harrington Sound, Bermuda	—	KF761638	—
	CDNC07 HV2742 (GENT)	— H. Verbruggen	North Carolina, USA Buzios (Forno Beach), Rio de Janeiro, Brazil	— —	EF107980 JQ950543	— —
<i>Codium duthieae</i> P.C. Silva	HEC15348 (GENT)	E. Coppejans	South Africa	—	EF107982	—
<i>Codium fragile</i> (Suringar) Har.	Isolate 31	—	Tateyama, Chiba, Japan	—	AB103021	—
<i>Codium intertextum</i> Collins & Herv.	<i>CWS/CEL</i> 08-30-1 (H.1022)	C.W. Schneider & C.E. Lane	Cove east of Spanish Pt. Park, Bermuda	—	KF761636	—
	<i>CWS/CEL</i> 10-16-6 (BDA0219; H.1017)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Whalebone Bay, St. George's, Bermuda	—	KF761634	—
	<i>CWS/CEL</i> 10-33-8 (BDA0497; H.1018)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Spanish Point Park, Bermuda	—	KF761635	—
	HV2767 (GENT)	H. Verbruggen	Buzios (Forno Beach), Rio de Janeiro, Brazil	—	JQ950539	—
	HV2741 (GENT)	H. Verbruggen	Buzios (Forno Beach), Rio de Janeiro, Brazil	—	JQ950544	—
	HV343 (GENT)	H. Verbruggen	Jamaica	—	EF108023	—

<i>Codium isthmocladum</i> Vickers	DML59073 (US)	D.S. & M.M. Littler	Florida, USA	—	EF108036	—
	HV917 (GENT)	H. Verbruggen	Jamaica	—	EF108032	—
	DML30307 (US)	D.S. & M.M. Littler	Puerto Rico	—	EF108026	—
<i>Codium recurvatum</i> Verbruggen	TZ0023 = HV2012 (GENT)	H. Verbruggen	Mbudya I., Tanzania	—	JQ706336	—
<i>Codium taylorii</i> P.C. Silva	CWS/CEL10-26-1 (BDA0411; H.1011)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Shark Hole, Harrington Sound, Bermuda	—	KF761644	—
	HV1062 (GENT)	H. Verbruggen	Florida, USA	—	EF108084	—
	DML59088 (US)	D.S. & M.M. Littler	Florida, USA	—	EF108082	—
	HV906 (GENT)	H. Verbruggen	Jamaica	—	EF108083	—
	DML55046 (US)	D.S. & M.M. Littler	Belize	—	EF108080	—
	HV2750 (GENT)	H. Verbruggen	Buzios (Forno Beach), Rio de Janeiro, Brazil	—	JQ950542	—
	KZN2K4-27 (GENT)	E. Tronchin	South Africa	—	EF108088	—
	DHO2-178 (GENT)	T. Schils	Oman	—	EF108086	—
	SOCANC3 (GENT)	—	Socotra, Yemen	—	EF108089	—
Ostreobiaceae						
<i>Ostreobium quekettii</i> Bornet & Flahault	SAG 6.99	J.A. West	Philippines	—	FJ715720	—
Gracilariaceae						
<i>Gracilaria occidentalis</i> (Børgesen) M. Bodard	CWS/CEL10-12-24 (BDA0114)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF761651	KF761639	—

	<i>CWS/CEL</i> 10-12-24 (BDA0115)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF761652	KF761640	—
	<i>CWS/CEL</i> 10-12-24 (BDA0116)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF761653	KF761641	—
	FG#153	C.F.D. Gurgel & S. Fredericq	Offshore Louisiana, USA	—	AY049322	—
Nemastomataceae						
<i>Predaea goffiana</i> D.L. Ballant., H. Ruiz & Aponte	<i>CWS/CEL</i> 10-24-23 (BDA0388)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Cathedral Rock, south shore, Bermuda	—	KF761645	—
	NSF-II-(17-1, 78- 13.79-10, 95-12, 97- 1, 100-14)	S. Fredericq, D. Gabriel, C.F.D. Gurgel & T.O. Cho	Campeche Banks, Mexico	—	FJ868814- 19	
Sebdeniaceae						
<i>Crassitegula imitans</i> G.W. Saunders & Kraft	GWS001076	G.W. Saunders & G.T. Kraft	Noddy I., Admiralty Group, Lord Howe I., Australia	JN641180	—	DQ343700
	GWS002026	R. Withall	Yellow Rock, Lord Howe, I., Australia	HM915970	—	—
	GWS002071	G.W. Saunders	Ned's Beach, Lord Howe I., Australia	JN641181	—	—
	GWS002089	G.W. Saunders	Malabar Reef, Lord Howe I., Australia	JN641182	—	—
	GWS023521	G.W. Saunders, K. Dixon & R. Withall	Algae Hole North, Lord Howe I., Australia	JN641792	—	—
<i>Crassitegula laciniata</i> C.W. Schneid., Popolizio & C.E. Lane sp. nov.	<i>CWS/CEL</i> 10-12-37 (BDA0134)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF561827	—	—

<i>Crassitegula walsinghamii</i> C.W. Schneid., C.E. Lane & G.W. Saunders	<i>CWS/CEL</i> 10-12-37 (BDA0135)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF561828	—	—
	<i>TRP</i> 12-66-1 (BDA1132)	T.R. Popolizio	Hog Reef, north shore, Bermuda	KF561829	—	—
	<i>TRP</i> 12-66-1 (BDA1133)	T.R. Popolizio	Hog Reef, north shore, Bermuda	KF561830	—	—
	<i>TRP</i> 12-66-1 (BDA1134)	T.R. Popolizio	Hog Reef, north shore, Bermuda	KF561831	—	—
	<i>TRP</i> 12-91-1 (BDA1305)	T.R. Popolizio	Hog Breaker, north shore, Bermuda	KF561832	—	—
	<i>TRP</i> 12-91-1 (BDA1306) Isotype	T.R. Popolizio	Hog Breaker, north shore, Bermuda	KF561833	—	KF761654
	<i>TRP</i> 12-117-1 (BDA1487)	T.R. Popolizio	Hog Breaker, north shore, Bermuda	KF561834	—	—
	<i>TRP</i> 12-117-1 (BDA1488)	T.R. Popolizio	Hog Breaker, north shore, Bermuda	KF561835	—	—
	<i>TRP</i> 12-125-1 (BDA1514)	T.R. Popolizio	Sinky Bay, south shore, Bermuda	KF561836	—	—
	GWS001245 Isotype	G.W. Saunders	Walsingham Pond, Bermuda	JN641185	—	EF033623
	GWS001260	G.W. Saunders	Walsingham Pond, Bermuda	JN641184	—	—
	<i>CWS/CEL</i> 10-5-30 (BDA0038)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Off Frick's Beach, Tucker's Town, Bermuda	KF772612	—	—

	CWS/CEL10-12-34 (BDA0130)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Middle Buoy, Eastern Blue Cut Channel, Bermuda	KF761648	—	—
<i>Crassitegula</i> sp.1WA	GWS025666	K. Dixon, R. Dixon & G. Belton	The Basin, Rottneest I., Australia	JN641183	—	JN602192
<i>Lesleighta</i> <i>hawaiiensis</i> Kraft & G.W. Saunders	G0436	G.T. Kraft	Maunalua Bay, Oahu, Hawaii, USA	JN641187	—	DQ343699
<i>Lesleighta howensis</i> Kraft & G.W. Saunders	GWS002087	G.W. Saunders	Malabar Reef, Lord Howe I., Australia	HM915995	—	—
	GWS002088	G.W. Saunders	Malabar Reef, Lord Howe I., Australia	JN641188	—	JN602198
	GWS022878	G.W. Saunders, K. Dixon & R. Withall	Malabar Reef, Lord Howe I., Australia	JN641190	—	—
	GWS022923	G.W. Saunders, K. Dixon & R. Withall	Malabar Reef, Lord Howe I., Australia	JN641189	—	—
<i>Lesleighta yamadae</i> (Okamura & Segawa) G.W. Saunders & Kraft	GWS018503	G.W. Saunders & H.-G. Choi	Big Munseom I., Jeju, South Korea	JN641192	—	JN602195
	GWS018683	G.W. Saunders & H.-G. Choi	Big Munseom I., Jeju, South Korea	HQ544145	—	—
	GWS018686	G.W. Saunders & H.-G. Choi	Big Munseom I., Jeju, South Korea	HQ544148	—	—
<i>Lesleighta</i> sp. 1Phil	GWS025841	K. Dixon	South Balatasan, Oriental Mindoro, Philippines	JN641191	—	JN602193
<i>Halymenia floresii</i> (Clemente) C. Agardh	GWS011961	E. Bercibar	Armacao de Pere, Portugal	GQ862071	—	GQ471912

<i>Halymenia pseudofloresii</i> Collins & Herv. <i>Sebdenia flabellata</i> (J. Agardh) P.G. Parkinson	<i>CWS/CEL</i> 05-8-7 (CL033701)	C.W. Schneider & C.E. Lane	Walsingham Pond, Bermuda	GQ862075	—	GQ471915
	<i>CWS/CEL</i> 03-27-2 (CL031201)	C.W. Schneider & C.E. Lane	Green Bay, Harrington Sound, Bermuda	JN641197	—	JN602196
	<i>CWS/CEL</i> 05-9-4 (GWS011903)	C.W. Schneider & C.E. Lane	Cliff Pool, Walsingham Park, Bermuda	JN641196	—	—
	<i>CWS/CEL</i> 10-6-1 (BDA0051)	C.W. Schneider, C.E. Lane, D.C. McDevit & T.R. Popolizio	Tucker's Town Bay, Castle Harbour, Bermuda	KF761649	—	—
	<i>TRP</i> 12-36-4 (BDA0884)	T.R. Popolizio	Cathedral Rock, south shore, Bermuda	KF761655	—	—
	<i>TRP</i> 12-91-4 (BDA1310)	T.R. Popolizio	Hog Breaker, north shore, Bermuda	KF761650	—	—
	GWS002027	R. Withall	Yellow Rock, Lord Howe I., Australia	HM915971	—	—
	GSW023452	G.W. Saunders, K. Dixon & R. Withall	Algae Hole North, Lord Howe I., Australia	JN641198	—	JN602194
	GSW023509	G.W. Saunders, K. Dixon & R. Withall	Algae Hole North, Lord Howe I., Australia	JN641199	—	—

Figs 1-6. Bermuda marine algae.

Fig. 1. *Anadyomene lacerata* (CWS/CEL 10-26-6), habit. Scale bar = 1 cm.

Fig. 2. *A. lacerata* (CWS/CEL 10-26-6) venation at margins of blade. Scale bar = 500 μ m.

Fig. 3. *A. lacerata* (CWS/CEL 10-26-6) venation pattern of blade. Scale bar = 500 μ m.

Fig. 4. *Codium carolinianum* (CWS/CEL 09-34-10), habit. Scale bar = 2 cm.

Fig. 5. *Rhodymenia occidentalis*, isosyntype (F. Børgesen no. 2073, Cruz Bay, Great St. James I., Virgin Is., 23 Mar 1906 [MICH]). Scale bar = 2 cm.

Fig. 6. *Gracilaria occidentalis* (CWS/CEL 10-12-24), habit. Scale bar = 2 cm.

Fig. 7. Bayesian analysis based upon an alignment of *Codium rbcL* sequences. Branch labels are posterior probabilities and maximum likelihood bootstraps, respectively. The branch label asterisk (*) represents a posterior probability of 1, or in the case of only one asterisk, both a posterior probability of 1 and 100% bootstrap support. The branch label dash (-) indicates a support value of less than 55%.

Figs 8-10. *Predaea goffiana* (CWS/CEL 10-24-23), habit.

Fig. 8. Thallus habits. Scale bar = 2 cm.

Fig. 9. Cortical fascicle demonstrating branching pattern and distally attenuating filaments. Scale bar = 20 μ m.

Fig. 10. Cortical fascicle with acute apical cells. Scale bar = 25 μ m.

Fig. 11. Bayesian inference based upon an alignment of mitochondrial COI-5P sequences of members of the Sebdeniales (outgroup *Halymenia*). Branch labels are posterior probabilities and maximum likelihood bootstraps, respectively. The branch label asterisk (*) represents a posterior probability of 1 and 100% bootstrap support. The branch label dash (-) indicates a support value of less than 60%.

0.2

Fig. 12. Bayesian inference based upon an alignment of ribosomal 28S (LSU) sequences of members of the Sebdeniales (outgroup *Halymenia*). Branch labels are posterior probabilities and maximum likelihood bootstraps, respectively. The branch label asterisk (*) represents a posterior probability of 1 and 100% bootstrap support.

Figs 13-17. *Crassitegula laciniata* sp. nov., type collection (TRP 12-99-1).

Fig. 13. Isotype (BDA1306), dorsal view of immature thallus with narrow finger-like branches. Scale bar = 1 cm.

Fig. 14. Holotype (BDA1305), dorsal view of mature habit with acute-tipped broadened axes and dichotomous branching. Scale bar = 1 cm.

Fig. 15. Photograph of isotypes, grouping of habits from the type collection. Scale bar = 1 cm.

Fig. 16. Cross-section of isotype material, demonstrating multilayered cortex and filamentous medulla. Scale bar = 200 μ m.

Fig. 17. Dorsal view of outer cortex of isotype material showing arching arrangement of cortical cells. Scale bar = 20 μ m.

APPENDIX C

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A molecular-assisted alpha taxonomic study of the genus *Centroceras* (Ceramiaceae, Rhodophyta) in Bermuda reveals two novel species

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When the generitype *Centroceras clavulatum*, a presumed cosmopolitan warm temperate to tropical red alga, was discovered to have a biogeographic distribution limited to the Pacific Ocean using molecular and morphological evidence, the taxonomy in the genus *Centroceras* was thrown into chaos worldwide. An analysis of what species was, or were, previously identified as *C. clavulatum* in Bermuda is the focus of the present molecular (COI-5P, *rbcL*) and morphological study. Two novel species are proposed, *C. arcii* sp. nov. and *C. illaqueans* sp. nov., and the distributions of three taxa recently segregated in the ‘*C. clavulatum* complex’ of the western Atlantic, *C. gasparrinii*, *C. hyalacanthum* and *C. micracanthum*, have been expanded to include Bermuda. *Centroceras arcii* is shown to be morphologically cryptic with *C. micracanthum*, and remains best distinguished by its COI-5P barcode sequence.

Key Words: Bermuda, *Centroceras* spp., *C. arcii* sp. nov., *C. illaqueans* sp. nov., Ceramiaceae, COI-5P, *rbcL*, Rhodophyta.

INTRODUCTION

In the early 20th century when the first modern compilation of algal species for Bermuda was completed by Collins and Hervey (1917), only a single *Centroceras* species was recorded, at that time regarded as *Ceramium clavulatum* C. Agardh. This species was thought to be the “commonest and most variable Bermudian representative of the Ceramiaceae” (Howe 1918). Howe (1918) recognized that *Centroceras clavulatum* (C. Agardh) Mont. was distinguished from *Ceramium* by its nodal spines, and that their “length, shape, and abundance” varied greatly among individuals found in the many different habitats of the islands. Despite this, at the time Taylor (1960) completed his comprehensive flora of the warm waters of the western

Atlantic, only a single species of *Centroceras* was again reported. Since then and prior to the advent of molecular sequencing, only two other species had been added to the western Atlantic flora, *C. internitens* Gallagher et Humm (Gallagher and Humm 1983) and *C. minutum* Yamada (Littler et al. 2008), both small epiphytes that could not be confused with the larger *C. clavulatum*.

Until recently, *Centroceras clavulatum* was considered a poster child for cosmopolitan marine algae (Lüning 1990, van den Hoek and Breeman 1990). It had been recorded from the Atlantic, Pacific and Indian Oceans (Guiry and Guiry 2014), and from warm temperate to tropical waters (Lüning 1990). Barros-Barreta et al. (2006) produced an *rbcL* ML tree that first demonstrated there were at least two genetic species within *C. clavulatum* in the Atlantic, one from Brazil a second from the Canary Islands. A molecular-assisted alpha taxonomic (MAAT) study by Won and coworkers (2009) restricted the biogeographic range of *C. clavulatum* to the Pacific Ocean (type locality = Peru). The Won et al. (2009) study segregated out five species from the Gulf of Mexico and Caribbean, southern Chile and South Africa from a limited set of sequenced samples. They also reported that three western Atlantic species were resurrected from the '*C. clavulatum* complex;' *C. gasparrinii* (Menegh.) Kütz., *C. hyalacanthum* Kütz. and *C. micracanthum* Kütz., all listed as synonyms of *C. clavulatum* since the middle of the 19th century (Agardh 1851). Because *C. clavulatum* was until now one of just two species reported from Bermuda, island collections were in need of both molecular and morphological assessment to determine their taxonomic identities, the focus of the present study.

MATERIALS & METHODS

Specimens were collected by scuba or snorkeling at the depths reported in Table 1 and locations recorded using a Garmin TM GPS III Plus (Garmin Ltd., Olathe, KS, USA). All of the species were found in similar lower intertidal to shallow subtidal habitats on coral reefs or as epiphytes on other macroalgae. Only one species, *Centroceras micracanthum*, was found extending into deep water habitats. Portions of each specimen were dried on silica gel for DNA extraction, fixed in 4-5% Formalin in seawater, and the remaining pressed as herbarium vouchers. Prior to drying, all DNA vouchers were photographed using a Canon Powershot s90 camera (Canon Inc., Tokyo, Japan). Axes with apical regions were whole-mounted onto glass slides, and liquid-preserved specimens were sectioned at 25 μm using an American Optical freezing microtome (American Optical, Buffalo, NY, USA). All of the mounts were affixed permanently in a 20:1 solution of 30% Karo™ corn syrup and 1% aniline blue. Herbarium specimens were scanned on an HP Photosmart Premium (Hewlett-Packard Company, Palo Alto, CA, USA), and 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, MI, USA). The digital images were assembled using Adobe Photoshop™ CS6 v.13.0.1 (Adobe Systems, San Jose, CA, USA). Voucher specimens are deposited in the first author's personal herbarium (CWS), with some duplicates sent to KIRI, MICH, NY and the Bermuda Natural History Museum (BAMZ). The referenced *Phycotheca Boreali-Americana* (P.B.-A.) exsiccata represents the set belonging to the first author, herbarium abbreviations follow the online *Index Herbariorum*

(<http://sweetgum.nybg.org/ih/>), and standard author initials/abbreviations follow Brummitt and Powell (1992).

Silica-dried samples were ground in liquid nitrogen and stored at -20°C. Sample DNA was extracted from 0.5 µL ground material using the Sigma-Aldrich GenElute Plant Genomic Miniprep Kit (Sigma-Aldrich Corp., St. Louis, MO, USA) according to manufacturer protocol, with a modified lysis solution (50 µL of 10% TWEEN 20 and 5 µL of 20 mg/mL ProK), as well as 1 hr at 23°C incubation followed by a 20 min. incubation on ice (Saunders and Druehl 1993). DNA was amplified via polymerase chain reaction (PCR) with the Takara Ex-Taq DNA polymerase kit (PanVera, Madison, WI, USA) in an Eppendorf AG Mastercycler epGradient thermal cycler (Eppendorf, Hamburg, Germany). To assign all specimens to species groups, two oligonucleotide primers were used for both sequencing and amplification of the COI-5P mitochondrial marker, GWSFn (Le Gall and Saunders, 2010) and GWSRx (Saunders and McDevit, 2012). Several specimens were selected for additional sequencing of the plastid-encoded RuBisCO operon. Four oligonucleotide primers were used for both amplification and sequencing of two overlapping fragments (forward- RR1 5' ATGTCTAACTCTGTAGAAG 3' and reverse RR4 5' TTCAGCTCTTTCATACAT 3') and (forward- RrIf 5' TCTCAGCCTTTTATGCGTTG 3' and reverse Rrr 5' ATCTCACTATTCTATACTCC 3'). A denaturation cycle of 94°C for 4 min was followed by 38 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and a final extension of 72°C for 7 min. Samples were stored at 4°C until removed from the system. Amplified DNA was treated with Sigma-Aldrich GenElute PCR Clean-Up Kit

per manufacturer protocol, and the purified PCR product was sequenced at the Rhode Island Genomics and Sequencing Center using the Applied Biosystems Inc. 3130xl Genetic Analyzer (Life Technologies, Grand Island, New York, USA).

Sequence data were aligned using the MUSCLE (multiple sequence comparison by log-expectation) alignment program in Geneious (v. 6.1.8 available from <http://www.geneious.com>) followed by neighbor-joining (NJ) analysis of the COI-5P barcode alignment (59 specimens, 567 sites) with Tamura-nei-corrected distances (default setting). This tree was used to identify genetic species groups.

The best models of evolution for the individual gene regions COI-5P (10 taxa, 567 sites) and *rbcL* (30 taxa, 1289 sites), after removing identical sequence replicates as identified in the previous analysis, were first estimated in jModelTest volume 2.1.5 (Guindon and Gascuel 2003, Darriba et al. 2012). The model GTR+I+G model was implemented for both COI-5P and *rbcL*. Maximum-likelihood analyses for the COI-5P data were conducted using the PHYML 3.0 (Guindon et al. 2010) plugin for Geneious, with BIONJ used to designate the starting tree, best of nearest-neighbor interchange (NNI) or subtree pruning and regrafting (SPR) branch-swapping options, and with the tree topology, branch lengths and substitution rates optimized. Branch support was estimated using 1000 bootstrap replicates. Bayesian analyses were conducted using MrBayes v.3.2.2 (Ronquist and Huelsenbeck 2003) and run with four parallel chains including three heated chains, with branch lengths optimized during the run for one million generations. The initial 2500 trees were discarded as the burn-in. Posterior probabilities were estimated based on the remaining trees. The *rbcL* maximum-likelihood phylogeny was estimated using the RAxML graphical user

interface (Silvestro and Michalak 2012) with 1000 bootstrap replicates. Bayesian posterior probabilities were generated in MrBayes, with the same parameters as in the COI-5P analysis. Stationarity was attained after the first 250,000 generations (burnin=2500 trees). The COI-5P (Fig. 1) and *rbcL* (Fig. 2) consensus trees were both manipulated for presentation using FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

TAXONOMIC RESULTS AND DISCUSSION

Based on our many Bermuda DNA samples and available sequences from GenBank (Table 1), COI-5P barcode analyses (Fig. 1) and *rbcL* phylogenies were generated (Fig. 2). As shown in these trees, the three species Won et al. (2009) resurrected for the western Atlantic Ocean by aligning type specimens with their recent genetic material, *Centroceras gasparrinii*, *C. hyalacanthum* and *C. micracanthum*, are found in the Bermuda flora. In addition, two novel species were delineated in our molecular analyses. During the 20th century, all of these five species would have been identified as *C. clavulatum* in the islands (Schneider 2003, p. 301). Along with the new genetic information, we follow Won et al. (2009, fig. 14) in using nodal cortical units and the origin, number and shape of cortical cells, glands and spines produced from cortical initials as distinguishing features among the species. The descriptions presented for each species below reflect genetically determined specimens from Bermuda, Florida and the U.S. Virgin Islands.

***Centroceras arcii* C. W. Schneid., Cianciola et Popolizio, *sp. nov.* (Figs 3, 8A)**

Description. Plants brownish- to rosy-red, with prostrate and erect axes 160-260 (-300) μm in diam. and to 5 cm tall (Fig. 3A & B); overall branching pattern pseudodichotomous with a branch or pair of branches usually occurring in the notches of main branches at apices (Fig. 3C); branches forming at intervals of 7-14 axial cells on the main axes, the branches divaricate and arched reflexively with straight or forcipate apices; adventitious branches developing from periaxial cells in lower portions (Fig. 3C); uniseriate, multicellular rhizoids issued from prostrate axes and lower portions of erect axes; 5-12 straight, 2-celled spines whorled at axial cell nodes, one always found in notches of dichotomous branches, 24-40 μm diam. and 80-130 μm long (Fig. 3D); distal ends of axial cells cutting off 14-16 periaxial cells, these remaining at the nodes during axial cell elongation (Fig. 3E); periaxial cells cutting off two cortical initials acropetally and one basipetally (Fig. 8A); the first cortical initial cutting off one spine and one elongate cortical cell, one gland cell and one elongate cortical cell, or two elongate cortical cells, the second cortical initial cutting off one elongate acropetal cortical cell and one basipetal cortical filament, the third cortical initial cutting off one basipetal filament; basipetal filaments made of mostly staggered cortical cell files of 8-27 cells, the cells rectangular in surface view, the smallest being 3-10 μm diam. and 9-43 μm long, the largest being 6-17 μm diam. and 8-54 μm long, these files completely corticating axial cells from node to node (Fig. 3F); tetrasporangia formed in whorls at the nodes (Fig. 3D), one produced from each periaxial cell, spherical to subspherical, 26-55 μm diam. and 38-75 μm long, subtended by 0-3 involucral filaments; gametangia not seen.

Diagnosis. This species is morphologically cryptic with *Centroceras micracanthum*, but differs genetically in its COI-5P barcode sequences (refer to Table 1 for GenBank numbers).

Etymology. Named for A. Ralph Cavaliere (ARC), Professor Emeritus at Gettysburg College, who introduced the first author to the beauty of the algae, to commemorate his retirement as the beloved Charles H. Graff Professor of Biology. ARC spent many summers in Bermuda studying and teaching the local marine flora and publishing a number of works on seaweeds including his local field guide with Wolfgang Sterrer, *Bermuda's Seashore Plants and Seaweeds* (1998). The epithet also is suggestive of *arcus* (L.), a bow-like curved line forming reflexively in distal portions of this species.

Holotype. *Craig W. Schneider (CWS)/Christopher E. Lane (CEL)* 09-13-6, 16 Mar. 2009, Horseshoe Bay grotto, south shore Bermuda Is., 32°15'01.1"N, 64°49'23.9"W, Bermuda, western Atlantic Ocean [MICH] (Fig. 3A), GenBank no. KP222800; Isotypes—KIRI, NY, UNB, US, Herb. CWS.

Paratypes: Bermuda—*CWS/CEL* 08-9-2 (⊕), *CWS/CEL* 09-1-3, *CWS/CEL* 09-25-6, *CWS/CEL/TRP* 12-21-10 [BDA0701], *TRP* 12-27-13 [BDA0773], *TRP* 12-34-4 [BDA0870], *TRP* 12-50-6 [BDA1012], *TRP* 12-53-3 [BDA1023], *TRP* 12-63-5 [BDA1119], *TRP* 12-69-2 [BDA1152], *TRP* 12-70-4 [BDA1058], *TRP* 12-82-4 [BDA1250]. St. John, Virgin Is.—*TRP* 10-3-4 (for collection details see Table 1).

Distribution. Bermuda, Virgin Islands.

Remarks. *Centroceras arcii* is the most common member of the genus in Bermuda, and is sister to the morphologically cryptic and sympatric *C. micracanthum*

in a separate clade according to COI-5P and *rbcL* analyses. Sequence divergence between the new species and *C. micracanthum* is ~1% for *rbcL* and ~9% for COI-5P, the latter clearly demonstrating genetic separation.

The most obvious morphological feature of *Centroceras arcii* is its divaricate, reflexive branching (Fig. 3B) giving the plants an arching overall habit, a feature found in all mature collections of the species thus far. Unfortunately, *C. micracanthum* demonstrates at least some reflexive branching in ca. 75% of our collections and when this is the case, this species is difficult to distinguish from *C. arcii*. Won et al. (2009, fig. 7b) demonstrated reflexive branching in *C. micracanthum*. Thus, *C. arcii* and *C. micracanthum* are cryptic in their anatomies (Table 2). At the extremes of ranges for certain characters, e.g., spine length, number of cells in descending cortical files and median sizes of tier cells, there are differences between the two species, but for the most part, they demonstrate overlapping ranges for cell dimensions and numbers (Table 2). At the extreme, cells composing basipetal cortical files are more numerous in *C. arcii* than those in *C. micracanthum*. Invariably, *C. arcii* has one or two branches formed in the notches of distal dichotomies, and such branches are uncommon in *C. micracanthum*. In the end, the two species are nearly indistinguishable and, as noted above, require gene sequencing to differentiate them with certainty.

As is also true for *Centroceras micracanthum*, in all but the first periaxial cell cut off in *Centroceras arcii*, three cortical initials form, two acropetally and one basipetally (Fig. 8A). The first periaxial cell cuts off three cortical initials acropetally and one cortical initial basipetally for a total of four cortical initials. In all cortical initial groups, the first cortical initial cuts off one spine and a single elongate cortical

cell, one gland cell and a single elongate cortical cell, or two elongate cortical cells.

The production of cortical cells from acropetal cortical initials in *C. arcii* is the same as that found in *C. micracanthum* and *C. illaqueans*, all producing only two cells from their first cortical initials. In *C. arcii*, the largest spines at nodes are longer than those of any other members of the '*C. clavulatum* complex' in Bermuda.

***Centroceras gasparrinii* (Meneghini) Kützing 1849, p. 689 (Figs 4, 8B)**

Basionym. *Ceramium gasparrinii* Meneghini 1844, p. 186

Description. Plants dark rosy-red with prostrate and erect axes 120-220 μm in diam. and to 7 cm tall; branching pattern pseudodichotomous with a branch or pairs of branches usually occurring in the notches of main branches at apices, branches forming at intervals of 10-12 axial cells on the main axes, the branches fastigate with slightly forcipate apices (Fig. 4A); adventitious branches developing from periaxial cells in lower portions; uniseriate, multicellular rhizoids issued from prostrate axes and lower portions of erect axes; 5-8 straight, 2-celled spines whorled at axial cell nodes, one almost always found in notches of dichotomous branches, 14-25 μm diam. and 33-75 μm long (Fig. 4B & D); distal ends of axial cells cutting off 12-16 periaxial cells, these remaining at the nodes during axial cell elongation (Fig. 4C); periaxial cells cutting off two cortical initials acropetally and one basipetally (Fig. 8B); the first cortical initial cutting off one spine and one ovoid cortical cell, one gland cell and one ovoid cortical cell, or two ovoid cortical cells, the second cortical initial cutting off one acropetal cortical cell and one basipetal cortical filament, the third cortical initial cutting off one basipetal filament; basipetal filaments made up of mostly staggered

cortical cell files of 12-35 cells, the cells rectangular in surface view, the smallest 4-10 μm diam. and 11-15 μm long, the largest 5-11 μm diam. and 12-18 μm long, these completely corticating axial cells from node to node (Fig. 4D); gland cells ovoid; gametangia and tetrasporangia not seen in Bermuda collections.

Type locality. Palermo, Sicily, Mediterranean Sea.

Distribution. Widely distributed in warm temperate to tropical zones in the Atlantic, Pacific and Indian Oceans.

Selected collections. Bermuda—*CWS/CEL* 09-5-11, *CWS/CEL* 10-23-2 [BDA0355], *CWS/CEL* 10-27-2 [BDA0429], *CWS/CEL* 10-31-1 [BDA0470], *CWS/CEL/TRP* 12-14-1 [BDA0627], *CWS/CEL/TRP* 12-16-2 [BDA0631], *CWS/CEL/TRP* 12-20-13 [BDA0685], *TRP* 12-27-1 [BDA0760], *TRP* 12-32-5 [BDA0841] (for collection details see Table 1).

Remarks. Except for first periaxial cell cut off at apices of *Centroceras gasparrinii*, each periaxial cells produce two cortical initials acropetally and one basipetally for a total of three initials (Fig. 8B). The first periaxial cell produces three cortical initials acropetally and one basipetally for a total of four initials. Each of the cortical initials after the first produces a descending uniseriate file of cortical cells, and together these completely cover internodal regions. *Centroceras gasparrinii* is distinguished from all other congeners in Bermuda by its ovoid terminal acropetal cells (Fig. 4B), which are elongated in all of the other species, the small size of its spines, and its small rectangular cells in descending cortical filaments. Won and coworkers report whorled 3-celled spines at the nodes in *C. gasparrinii* but illustrate 2-celled spines (Won et al. 2009, fig. 14b) similar to those found in the Bermuda

specimens. Later, Won (2010) explained that he included the cortical initial as one of the cells of the spines. All of the other characteristics that Won et al. (2009) report for *C. gasparrinii* are in conformity with those found in Bermuda specimens.

It is not surprising that *Centroceras gasparrinii* occurs in Bermuda, as it is presently the most geographically widespread of all of the 15 currently accepted species in the genus (Guiry and Guiry 2014). Using *rbcL* sequences, Won et al. (2009) identified this species from a number of western Atlantic locations from Brazil to Mexico and Florida, but report a range that also includes the Mediterranean Sea, the Indian Ocean, Asia, South Africa, Hawaii, Pacific Central America and California. It seems likely that *C. gasparrinii* represents many of the specimens identified in the past as *C. clavulatum* giving it its former worldwide distribution. Bermuda specimens in the Collins et al. (1913) exsiccata, *Phycotheca Boreali-Americana*, distributed as no. 1948, *Ceramium clavulatum* C. Agardh, are *C. gasparrinii*. An earlier *P.B.-A.* collection distributed as *Centroceras clavulatum* from Key West, Florida (Collins et al. 1895, no. 148a) likewise represents *C. gasparrinii*.

***Centroceras hyalacanthum* Kützinger 1842, p. 742 (Figs 5, 8C)**

Description. Plants beige-pink with prostrate and erect axes 110-170 μm in diam. and to 6 cm tall; branching pattern pseudodichotomous often with a branch occurring in the notches of main branches at apices, branches forming at intervals of 8-15 axial cells on the main axes, the branches erect with forcipate apices (Fig. 5A); adventitious branches developing from periaxial cells in lower portions; uniseriate, multicellular rhizoids issued from prostrate axes and lower portions of erect axes; 6-10 straight, 2-

celled spines whorled at axial cell nodes, one almost always found in notches of dichotomous branches, 15-18 μm diam. and 40-68 μm long (Fig. 5B); distal ends of axial cells cutting off 10-13 periaxial cells, these remaining at the nodes during axial cell elongation (Fig. 5C); periaxial cells cutting off two cortical initials acropetally and one basipetally (Fig. 8C); the first cortical initial cutting off one spine and two elongate cortical cells, one gland cell and two elongate cortical cells, or three elongate cortical cells, the second cortical initial cutting off one elongate acropetal cortical cell and one basipetal cortical filament, the third cortical initial cutting off one basipetal filament; basipetal filaments made up of mostly staggered cortical cell files of 7-11 cells, the cells elongate rectangular in surface view, the smallest 2-5 μm diam. and 21-28 μm long, the largest 5-10 μm diam. and 19-33 μm long, these completely corticating axial cells from node to node (Fig. 5D); gland cells ovoid; tetrasporangia formed in whorls at the nodes, one produced from each periaxial cell, subspherical, 35-43 μm diam. and 38-55 μm long, subtended by 0-2 involucre filaments; gametangia not seen.

Type locality. Antilles (French West Indies), Caribbean Sea

Distribution. Bermuda, Florida, Caribbean Sea

Selected collections. Bermuda—*CWS/CEL* 09-5-9, *CWS/CEL* 10-27-2 [BDA0428], *CWS/CEL* 10-30-12b [BDA0462], *CWS/CEL/TRP* 12-10-13 [BDA0590], *CWS/CEL/TRP* 12-18-15 [BDA0653], *TRP* 12-171-3 [BDA1810]. Florida, USA—*CWS/CEL/TRP* 13-8-17 [KW090], *CWS/CEL/TRP* 13-16-5 [KW265] (for collection details see Table 1).

Remarks. Multiple Bermuda isolates clustered with Florida, USA and Caribbean sequences of *Centroceras hyalacanthum* in our COI-5P and *rbcL* trees (Figs 1, 2). In *Centroceras hyalacanthum*, the first periaxial cell cuts off three cortical initials acropetally and one cortical initial basipetally for a total of four cortical initials. On the rest of the periaxial cells, only three cortical initials form, two acropetally and one basipetally (Fig. 8C). In all cortical initial groups, the first cortical initial cuts off one spine and two elongate cortical cells, one gland cell and two elongate cortical cells, or three elongate cortical cells. This production of cortical cells from acropetal cortical initials is unique among all of the species thus far isolated from Bermuda (Figs 5B, 8C), the rest producing only two cells from their first cortical initials. In addition, descending cortical cell files are made of elongate rectangular cells (Fig. 5D). This developmental pattern of the cortex initiated at the nodes is in perfect accord with that demonstrated by Won et al. (2009) for the species including their observations on the lectotype specimen they designated. Amongst all of the species in the genus found in Bermuda, *C. hyalacanthum* has the narrowest axes, 110-170 μm in diam. (Table 2), the most delicate, from Florida and the Caribbean, being even narrower than those reported by Won et al. (2009), at 140-165 μm in diam.

In the same paper in which Kützing (1842) wrote the protologue for *Centroceras hyalacanthum*, he also described an additional Caribbean species, *C. oxyacanthum* Kütz. (type locality = Cuba), and later described *C. brachyacanthum* Kütz. (type locality = Antilles, West Indies) (Kützing, 1863). After an examination of types of all three species, Won et al. (2009) considered the latter two species to be heterotypic synonyms of *C. hyalacanthum*.

***Centroceras illaqueans* C. W. Schneid., Cianciola et Popolizio, *sp. nov.* (Figs 6, 8D)**

Description. Plants light rosy-red, with prostrate and erect axes 140-210 μm in diam. and to 2 cm tall (Fig. 6A); branching pattern pseudodichotomous, rarely with a branch forming in the notches of main branches at apices (Fig. 6B); branches forming at intervals of 11-12 axial cells on the main axes, branches erect with straight to slightly forcipate apices (Fig. 6B); adventitious branches occasionally developing from periaxial cells in lower portions; uniseriate, multicellular rhizoids issued in profusion from prostrate axes and lower portions of erect axes; 0-3 straight, 2-celled spines formed at axial cell nodes and in the notches of branches, one almost always found in notches of dichotomous branches, 12-14 μm diam. and 33-39 μm long (Fig. 6D); distal ends of axial cells cutting off 10-12 periaxial cells, these remaining at the nodes during axial cell elongation (Fig. 6C); except for the first abaxial periaxial cell, periaxial cells cutting off two cortical initials acropetally and one basipetally (Fig. 8D); the first cortical initial cutting off one spine and one elongate cortical cell, one gland cell and one elongate cortical cell, or two elongate cortical cells; the second cortical initial cutting off one elongate acropetal cortical cell and one basipetal cortical filament; the third cortical initial cutting off one basipetal filament; basipetal filaments made up of 8-9 cortical cells in tiered files, the cells isodiametric to short rectangular in surface view, the smallest 7-8 μm diam. and 10-13 μm long, the largest 5-14 μm diam. and 20-25 μm long, these completely corticating axial cells from node to node (Fig. 6D & E); gland cells ovoid; gametangia and tetrasporangia not seen.

Diagnosis. Differing from other species in the genus by its unique COI-5P barcode, and from the morphologically similar *Centroceras hommersandii* Won, T.O. Cho et Fredericq (2010) by producing more than one elongated acropetal cell from the cortical cell units.

Etymology. *illaqueans* (L., part., n.) = entrapping, for the habit of the new species sifting and affixing sand among its erect filaments in intertidal pools.

Holotype. CWS/CEL 09-2-9, 15 Mar. 2009, tidal pool, rocky point south of beach, Capt. Williams' Bay, Bermuda Is., 32°18'09.3"N, 64°44'22.4"W, Bermuda, western Atlantic Ocean [MICH] (Fig. 6A), GenBank nos KP222781, KP222802; Isotypes—KIRI, NY, US, Herb. CWS.

Distribution. Endemic to Bermuda and currently known only from the type locality.

Remarks. *Centroceras illaqueans* is genetically distinct from all the other sequences in our analyses, and is grouped with *C. hyalacanthum* in our trees (Figs 1, 2). The new species is distinguished from other *Centroceras* species in Bermuda by the length of its terminal elongated acropetal cells which are mostly equal to or longer than spines at the nodes (Fig. 6D & E, Table 2), and the alignment of its basipetal filaments which typically line up with other descending files in tiers of short isodiametric cells (Fig. 6D & E). In addition, the new species differs from *C. hyalacanthum* by its fewer number of spines at the nodes, spine length and acropetal cell length (Table 2). A recently described species from Natal, South Africa, *C. hommersandii*, shares its small size with *C. illaqueans*, but has axes that are narrower in diameter than the Bermuda species and produces only a single elongated acropetal

cortical cell in each cortical cell unit, being produced from the second cortical initial (Won et al. 2010). The first cortical initial of *C. hommersandii* produces a spine or gland cell and an ovoid acropetal cortical cell (Won et al. 2010), unlike the elongated cell produced by *C. illaqueans*.

Despite the numerous collections of *Centroceras* sequenced from Bermuda, *C. illaqueans* was found only once. In this extensive population, the tips of erect axes emerged approximately one half centimeter out of sandy sediment on the bottom of a large, shallow tidal pool on the south shore. Repeated visits to the type locality have not uncovered this species again.

***Centroceras micracanthum* Kützing 1842, p. 741 (Figs 7, 8E)**

Description. Plants pink to dark rosy-red, with prostrate and erect axes 130-240 μm in diam. and to 2-4 cm tall; branching pattern pseudodichotomous, branches forming at intervals of 9-12 axial cells on the main axes, the branches divaricate with forcipate apices, some arched reflexive (Fig. 7A); adventitious branches developing from periaxial cells in the lower portions; uniseriate, multicellular rhizoids issued from prostrate axes and lower portions of erect axes; 2-8 straight, 2-celled spines whorled at axial cell nodes, one almost always found in notches of dichotomous branches, 15-40 μm diam. and 48-118 μm long (Fig. 7B-D); distal ends of axial cells cutting off 14-16 periaxial cells, these remaining at the nodes during axial cell elongation (Fig. 7C); periaxial cells cutting off two cortical initials acropetally and one basipetally (Fig. 8E); the first cortical initial cutting off one spine and one elongate cortical cell, one gland cell and one elongate cortical cell, or two elongate cortical cells, the second cortical

initial cutting off one elongate acropetal cortical cell and one basipetal cortical filament, the third cortical initial cutting off one basipetal filament; basipetal filaments made up of mostly staggered cortical cell files of 5-17 cells, the cells elongate rectangular in surface view, highly variable in size, from 2-12 μm diam. and 14-116 μm long, these completely corticating axial cells from node to node (Fig. 7D), at times these cells lined up in tiers; gland cells ovoid; gametangia and tetrasporangia not seen in Bermuda collections. This species is best distinguished from *C. arcii* by its COI-5P barcode sequence (see Table 1 for GenBank numbers).

Type locality. Rio de Janeiro, Brazil, western Atlantic Ocean.

Distribution. Brazil, Caribbean, Bermuda, Cape Verde, Mediterranean, Polynesia.

Selected collections. Bermuda—*CWS/CEL* 09-16-5, *CWS/CEL* 10-11-4a [BDA0084], *CWS/CEL* 10-14-24 [BDA0179], *CWS/CEL* 10-30-12a [BDA0460], *CWS* 12-10-9 [BDA0583], *CWS/CEL/TRP* 12-11-12 [BDA0611], *CWS/CEL/TRP* 12-18-14 [BDA0652], *CWS/CEL/TRP* 12-19-2 [BDA0658], *TRP* 12-28-2 [BDA0778], *TRP* 12-46-3 [BDA0971], *TRP* 12-59-3 [BDA1075], *TRP* 12-134-4 [BDA1561], *TRP* 12-153-6 [BDA1682], *TRP* 12-169-1 [BDA1795], *TRP* 12-173-9 [BDA1825]. Florida, USA—*CWS/CEL/TRP* 13-6-29 [KW039], *CWS/CEL/TRP* 13-16-25 [KW288] (for collection details see Table 1).

Remarks. In our *rbcL* analysis, sequences isolated from Bermuda specimens grouped with *Centroceras micracanthum* from Florida and the Caribbean (Fig. 2), and our morphological characters matched those described from the same areas (Won et al. 2009). In the same work with the protologue of *C. micracanthum*, Kützinger (1842) also described *C. leptacanthum* Kütz. (type locality = Genoa, Italy), *C. cryptacanthum*

Kütz. (type locality = Antilles, West Indies) and *C. macracanthum* Kütz. (type locality = Brazil), all of which are now considered heterotypic synonyms of *C. micracanthum* after an examination of type material (Won et al. 2009). Given its widespread distribution in the western Atlantic (Guiry and Guiry 2014), it is not surprising that *C. micracanthum* is found in Bermuda with many other macroalgal species also known in the Caribbean. Based upon morphological characteristics, Florida specimens of *C. clavulatum* distributed as no. 1347 in the Collins et al. (1906) exsiccatae, *Phycotheca Boreali-Americana*, are better identified as *C. micracanthum*. The label noted that these Key West specimens correspond to *C. leptacanthum*, a species that Won et al. (2009) maintained under *C. micracanthum* as noted above.

As discussed above, *Centroceras micracanthum* is most difficult to distinguish morphologically from *C. arcii* in Bermuda. Its habit of reflexive branching in about 75% of specimens and overlap of anatomical characteristics make it truly cryptic with *C. arcii*. Therefore, this species is best identified by comparing genetic sequences with those available in the public domain.

CONCLUSION

The use of molecular-assisted alpha taxonomy has allowed for the resolution of five species in the ‘*Centroceras clavulatum* complex’ in Bermuda. Won et al. (2009, fig. 14) used stylized diagrams of nodal cortical units for each of the species they covered in their seminal study of this complex. They depicted the origin, number and shape of cortical cells, glands and spines produced from cortical initials as distinguishing features among the species, and we have done the same for nodal cortical initials

bearing spines (Fig. 8), and the developmental patterns of our two new species can be compared in this way. Three of the five Bermudian species are recorded in their most northerly locations in the western Atlantic, and the remaining two, *C. arcii* and *C. illaqueans*, are newly described from the islands. Studies in other parts of the world's warm temperate to tropical seas will undoubtedly extend or shrink the distributional ranges of some *Centroceras* species, and thousands of herbarium specimens in the genus worldwide will need further study and annotation. Only then will we have a more accurate picture of the biogeography of the many species in the genus.

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

Species	Voucher	Collectors/Date	Site	COI-5P	<i>rbcL</i>
Ceramiaceae					
<i>Centroceras arcii</i> C.W. Schneid., Popolizio et C.E. Lane	<i>CWS/CEL</i> 09-13-6 (Holotype)	C.W. Schneider, C.E. Lane/ 16 Mar. 2009	Horseshoe Bay, Bermuda Is., Bermuda (0-1 m)	—	KP222800
	<i>CWS/CEL</i> 09-25-6	C.W. Schneider, C.E. Lane/ 18 Mar. 2009	Jct. Higgs/Horseshoe Is., St. George's Harbour, Bermuda (1 m)	KP222763	
	<i>TRP</i> 10-3-4	T.R. Popolizio/16 Mar. 2010	Salt Pond Bay, Virgin Islands National Park, SE St. John, USVI	—	KP222801
	<i>CWS/CEL/TRP</i> 12-21-5 [BDA0692]	C.W. Schneider, C.E. Lane, T.R. Popolizio/20 Jan. 2012	West Whale Bay, Bermuda Is., Bermuda (2-3 m)	KP222752	
	<i>CWS/CEL/TRP</i> 12-21-10 [BDA0701]	C.W. Schneider, C.E. Lane, T.R. Popolizio/20 Jan. 2012	West Whale Bay, Bermuda Is., Bermuda (2-3 m)	KP222753	—
	<i>TRP</i> 12-27-12 [BDA0772]	T.R. Popolizio/31 Jan. 2012	Cox's Bay, Bermuda Is., Bermuda (tide pool)	KP222759	
	<i>TRP</i> 12-27-13 [BDA0773]	T.R. Popolizio/31 Jan. 2012	Cox's Bay, Bermuda Is., Bermuda (tide pool)	KP222764	—
	<i>TRP</i> 12-34-4 [BDA0870]	T.R. Popolizio/16 Feb. 2012	Devonshire Bay, Bermuda Is., Bermuda (tide pool)	KP222761	—
	<i>TRP</i> 12-50-6 [BDA1012]	T.R. Popolizio/10 Apr. 2012	Stonehill Bay, Bermudas Is., Bermuda (0-3 m)	KP222757	—
	<i>TRP</i> 12-53-3 [BDA1023]	T.R. Popolizio/5 May 2012	Jct. Barry Rd./Salt Spray Ln., St George's Is., Bermuda (tide pool)	KP222762	—
	<i>TRP</i> 12-54-3 [BDA1027]	T.R. Popolizio/6 May 2012	Wreck of the <i>Cristobal Colon</i> , Bermuda Is., Bermuda (10 m)	KP222754	—

<i>Centroceras clavulatum</i> (C. Agardh) Mont.	TRP 12-63-5 [BDA1119]	T.R. Popolizio/26 May 2012	Church Bay, Bermuda Is., Bermuda (0-3 m)	KP222756	—
	TRP 12-69-2 [BDA1152]	T.R. Popolizio/30 May 2012	West Whale Bay, Bermuda Is., Bermuda (0-3 m)	KP222755	—
	TRP 12-70-4 [BDA1158]	T.R. Popolizio/30 May 2012	Grape Bay, Bermuda Is., Bermuda (intertidal)	KP222758	—
	TRP 12-82-4 [BDA1250]	T.R. Popolizio/26 June 2012	Jct. Barry Rd./Salt Spray Ln., St George's Is., Bermuda (0-2 m)	KP222760	—
	TC2900	M. H. Hommersand/13 Nov. 2004	Ahipara Bay, New Zealand	—	DQ374328
	LAF-6-viii-95-1-1 [TC2209]	M.H. Hommersand, F. Hommersand/6 Aug. 1995	Werribee, Servage Form, S. Australia	—	DQ374330
	C76	7 Jul. 1999	Pohang, Guryongpo, S. Korea	EU194971	—
	LAF-10-xii-99-1-1 [TC293]	T.O. Cho/10 Dec. 1999	Lovers Point, Pacific Grove, Monterey Co., California, USA	—	DQ374325
	LAF-30-viii-03-1-1 [B9]	N. Arakaki/30 Aug. 2003	Punta La Cruz, Ancon, Lima, Peru	—	DQ374331
	LAF-22-i-04-1-1 [B4]	E.F. García/22 Jan. 2004	Antofagasta, northern Chile	—	DQ374332
<i>Centroceras gasparrinii</i> (Menegh.) Kütz.	LAF-19-I-97-1-1 [B107]	E. Coppejans/19 Jan. 1997	Sri Lanka, west coast, Indian Ocean	—	DQ374291
	LAF-19-iv-99-1-1	S. Fredericq/19 Apr. 1999	Port Aransas, Aransas Co., Texas, USA	—	DQ374301
	LAF-20-vi-99-1-1 [00774]	B. Wysor/20 Jun. 1999	Galeta, STRI, Caribbean Panama	—	DQ374307
	LAF-1-ix-02-1-1 [B106]	E. Coppejans, D. Douterlungne, I. Razanakoto/1 Sept. 2002	Port de Fort Dauphin, North Fort Dauphin, Madagascar	—	DQ374294
	LAF-28-xii-02-1-1 [ST19]	A. Renoux/28 Dec. 2002	Jolly Beach, Antigua, Caribbean Sea	—	DQ374309
	LAF-4-x-2003-1-1 [TC532]	T.O. Cho/4 Oct. 2003	St. Lucie State Park, Florida, USA	—	DQ374303

	LAF-22-xi-03-1-1 [B6]	C.F.D. Gurgel/22 Nov. 2003	Praia Rasa, Buzios, Rio de Janeiro, Brasil	—	DQ374311
	LAF-19-iii-04-1-1 [B19]	P. Vroom/19 Mar. 2004	Kupikipikio Point (Black Point), O'ahu, Hawaii, USA	—	DQ374296
	CWS/CEL 10-23-2 [BDA0355]	C.W. Schneider, C.E. Lane/ 23 Aug.. 2010	St. George's Harbour, Bermuda (0- 1 m)	KP222744	—
	CWS/CEL 10-23-2 [BDA0356]	C.W. Schneider, C.E. Lane/ 23 Aug.. 2010	St. George's Harbour, Bermuda (0- 1 m)	KP222745	KP222805
	CWS/CEL 10-27-2 [BDA0429]	C.W. Schneider, C.E. Lane/ 24 Aug.. 2010	Tucker's Bay, Harrington Sound, Bermuda (0 m)	KP222743	—
	CWS/CEL 10-31-1 [BDA0470]	C.W. Schneider, C.E. Lane/ 25 Aug.. 2010	Horseshoe Bay, Bermuda Is., Bermuda (intertidal)	KP222746	—
	CWS/CEL 10-31-1 [BDA0471]	C.W. Schneider, C.E. Lane/ 25 Aug.. 2010	Horseshoe Bay, Bermuda Is., Bermuda (intertidal)	KP222747	—
	CWS/CEL/TRP 12-14-1 [BDA0627]	C.W. Schneider, C.E. Lane, T.R. Popolizio/18 Jan. 2012	Miss Vesey's Bay, Harrington Sound, Bermuda (0-1 m)	KP222749	—
	CWS/CEL/TRP 12-16-2 [BDA0631]	C.W. Schneider, C.E. Lane, T.R. Popolizio/18 Jan. 2012	Red Bay, Harrington Sound, Bermuda (0-1 m)	KP222748	—
	TRP 12-27-1 [BDA0759]	T.R. Popolizio/31 Jan. 2012	Cox's Bay, Bermuda Is., Bermuda (intertidal)	KP222751	—
	TRP 12-27-1 [BDA0760]	T.R. Popolizio/31 Jan. 2012	Cox's Bay, Bermuda Is., Bermuda (intertidal)	KP222750	—
<i>Centroceras hommersandii</i> Won, T.O. Cho et Fredericq	B105, as <i>Centroceras</i> sp.	E. Coppejans, O. De Clerck, J. Bolton, R. Anderson, F. Leliaert, J. Muylle, I. De Smet/20 Feb.1999	Trafalgar, Kwazulu Natal, South Africa	—	DQ374322
<i>Centroceras hyalacanthum</i> Kütz.	LAF-5-iii-03-1-1 [TC524]	T.O. Cho, B.Y. Won/5 Mar. 2003	Merritt Island, Brevard Co., Florida, USA	—	DQ374341
	LAF-17-xii-03-1-1 [B-5]	A. Renoux/17 Dec. 2003	Anse Babin, Guadeloupe, French West Indies, Caribbean Sea	—	DQ374342

<i>CWS/CEL</i> 09-5-9	C.W. Schneider, C.E. Lane/ 15 Mar. 2009	Lagoon Park, Ireland Is. South, Bermuda (1 m)	KP222782	—
<i>CWS/CEL</i> 10-27-2 [BDA0428]	C.W. Schneider, C.E. Lane/ 24 Aug. 2010	Tucker's Bay, Harrington Sound, Bermuda (0 m)	KP222787	—
<i>CWS/CEL</i> 10-30- 11 [BDA0457]	C.W. Schneider, C.E. Lane/ 25 Aug.. 2010	Lagoon Park, Ireland Is. South, Bermuda (0-1 m)	KP222784	—
<i>CWS/CEL</i> 10-30- 12b [BDA0461]	C.W. Schneider, C.E. Lane/ 25 Aug.. 2010	Lagoon Park, Ireland Is. South, Bermuda (0-1 m)	KP222785	—
<i>CWS/CEL</i> 10-30- 12b [BDA0462]	C.W. Schneider, C.E. Lane/ 25 Aug.. 2010	Lagoon Park, Ireland Is. South, Bermuda (0-1 m)	KP222783	—
<i>CWS/CEL/TRP</i> 12-10-13 [BDA0589]	C.W. Schneider, C.E. Lane, T.R. Popolizio/18 Jan. 2012	Cove east of Spanish Pt., Bermuda Is., Bermuda (0-1 m)	KP222788	—
<i>CWS/CEL/TRP</i> 12-10-13 [BDA0590]	C.W. Schneider, C.E. Lane, T.R. Popolizio/18 Jan. 2012	Cove east of Spanish Pt., Bermuda Is., Bermuda (0-1 m)	KP222789	KP222803
<i>CWS/CEL/TRP</i> 12-18-15 [BDA0653]	C.W. Schneider, C.E. Lane, T.R. Popolizio/19 Jan. 2012	Tobacco Bay, St. George's Is., Bermuda (0-2 m)	KP222786	—
<i>TRP</i> 12-171-3 [BDA1810]	T.R. Popolizio/10 Dec. 2012	Capt. Williams' Bay, Bermuda Is., Bermuda (tide pool)	KP222790	—
<i>TRP</i> 12-171-3 [BDA1811]	T.R. Popolizio/10 Dec. 2012	Capt. Williams' Bay, Bermuda Is., Bermuda (tide pool)	KP222791	—
<i>CWS/CEL/TRP</i> 13-8-17 [KW090]	C.W. Schneider, C.E. Lane, T.R. Popolizio/29 May 2013	Cow Key Channel, Key West, Florida, USA (1-2 m)	KP222793	—
<i>CWS/CEL/TRP</i> 13-8-17 [KW091]	C.W. Schneider, C.E. Lane, T.R. Popolizio/29 May 2013	Cow Key Channel, Key West, Florida, USA (1-2 m)	KP222795	—
<i>CWS/CEL/TRP</i> 13-8--23 [KW099]	C.W. Schneider, C.E. Lane, T.R. Popolizio/29 May 2013	Cow Key Channel, Key West, Florida, USA (1-2 m)	KP222794	—

<i>Centroceras illaqueans</i> C.W. Schneid., Popolizio et C.E. Lane <i>Centroceras</i> <i>micracanthum</i> Kütz.	<i>CWS/CEL/TRP</i> 13-16-5 [KW265]	C.W. Schneider, C.E. Lane, T.R. Popolizio/31 May 2013	Big Coppitt Key, Florida, USA (0-1 m)	KP222792	—
	<i>CWS/CEL</i> 09-2-9 (Holotype)	C.W. Schneider, C.E. Lane/ 15 Mar. 2009	Capt. Williams' Bay, Bermuda Is., Bermuda (tide pool)	KP222781	KP222802
	LAF-8-iii-97-1-1 [TC426]	M.H. Hommersand/8 Mar. 1997	Bridge to Key Biscayne, Miami- Dade Co., Florida, USA	—	DQ374335
	LAF-21-ix-99-1-1 [01024]	B. Wysor/21 Sept. 1999	Galeta (STRI), Caribbean Panama	—	DQ374337
	<i>CWS/CEL</i> 09-16-5	C.W. Schneider, C.E. Lane/ 17 Mar. 2009	Tucker's Bay, Harrington Sound, Bermuda (0-1 m)	KP222771	—
	<i>CWS/CEL</i> 09-16-6	C.W. Schneider, C.E. Lane/ 17 Mar. 2009	Tucker's Bay, Harrington Sound, Bermuda (0-1 m)	KP222772	—
	<i>CWS/CEL</i> 10-11- 4a [BDA0084]	C.W. Schneider, C.E. Lane/ 19 Aug. 2010	Ferry Reach, St. George's Is., Bermuda (0-1 m)	KP222774	—
	<i>CWS/CEL</i> 10-14- 24 [BDA0179]	C.W. Schneider, C.E. Lane/ 21 Aug. 2010	Tobacco Bay, St. George's Is., Bermuda (0-2 m)	KP222777	—
	<i>CWS/CEL</i> 10-14- 24 [BDA0180]	C.W. Schneider, C.E. Lane/ 21 Aug. 2010	Tobacco Bay, St. George's Is., Bermuda (0-2 m)	KP222778	—
	<i>CWS/CEL</i> 10-30- 12a [BDA0460]	C.W. Schneider, C.E. Lane/ 25 Aug. 2010	Lagoon Park, Ireland Is. South, Bermuda (0-1 m)	KP222769	—
	<i>CWS</i> 12-10-9 [BDA0583]	C.W. Schneider/18 Jan. 2012	Cove east of Spanish Pt., Bermuda Is., Bermuda (0-1 m)	KP222779	—
	<i>CWS</i> 12-10-9 [BDA0584]	C.W. Schneider/18 Jan. 2012	Cove east of Spanish Pt., Bermuda Is., Bermuda (0-1 m)	KP222770	KP222804
	<i>CWS/CEL/TRP</i> 12-11-12 [BDA0611]	C.W. Schneider, C.E. Lane, T.R. Popolizio/18 Jan. 2012	Spanish Pt., Bermuda Is., Bermuda (0-2 m)	KP222773	—

	<i>CWS/CEL/TRP</i> 12-18-14 [BDA0652]	C.W. Schneider, C.E. Lane, T.R. Popolizio/19 Jan. 2012	Tobacco Bay, St. George's Is., Bermuda (0-2 m)	KP222775	—
	<i>TRP</i> 12-28-2 [BDA0778]	T.R. Popolizio/1 Feb. 2012	Old Ferry Crossing, St. George's Is., Bermuda (0-1 m)	KP222776	—
	<i>TRP</i> 12-134-4 [BDA1561]	T.R. Popolizio/3 Oct. 2012	Off Red Hole, St. David's Is., Bermuda (17 m)	KP222768	—
	<i>TRP</i> 12-153-6 [BDA1682]	T.R. Popolizio/17 Nov. 2012	North Rock, north of Bermuda Is., Bermuda (12 m)	KP222766	—
	<i>TRP</i> 12-153-6 [BDA1683]	T.R. Popolizio/17 Nov. 2012	North Rock, north of Bermuda Is., Bermuda (12 m)	KP222767	—
	<i>TRP</i> 12-169-1 [BDA1795]	T.R. Popolizio/4 Dec. 2012	Rockfish Shoals, north of Somerset Is., Bermuda (9 m)	KP222765	—
	<i>TRP</i> 12-173-9 [BDA1825]	T.R. Popolizio/12 Dec. 2012	Bowen Pt., Shelly Bay, Bermuda Is., Bermuda (0-2 m)	KP222780	—
	<i>CWS/CEL/TRP</i> 13-6-29 [KW039]	C.W. Schneider, C.E. Lane, T.R. Popolizio/27 May 2013	Cow Key Channel, Key West, Florida, USA (1-3 m)	KP222796	—
	<i>CWS/CEL/TRP</i> 13-16-2 [KW263]	C.W. Schneider, C.E. Lane, T.R. Popolizio/31 May 2013	Big Coppitt Key, US-1, boat launch seawall, Florida, USA (0-1 m)	KP222797	—
	<i>CWS/CEL/TRP</i> 13-16-9 [KW270]	C.W. Schneider, C.E. Lane, T.R. Popolizio/31 May 2013	Big Coppitt Key, US-1, boat launch seawall, Florida, USA (0-1 m)	KP222799	—
	<i>CWS/CEL/TRP</i> 13-16-25 [KW288]	C.W. Schneider, C.E. Lane, T.R. Popolizio/31 May 2013	Big Coppitt Key, US-1, boat launch seawall, Florida, USA (0-1 m)	KP222798	—
<i>Centroceras minutum</i> Yamada	LAF-26-vi-02-1-1 [TC580]	?/26 Jun. 2002	Horn I., Queensland, Australia	—	DQ374321
<i>Centroceras rodmanii</i> Won, T.O. Cho et Fredericq	LAF-1-i-95-1-1 [TC435]	M.H. Hommersand/1 Jan. 1995	Cocholgue, Bahia Concepcion, Prov. Chile	—	DQ374333

	LAF-ix-98-1-1 [B22]	N. Arakaki/Sept. 1998	Desembocadura del Bio-Bio, Concepción, Chile	—	DQ374334
<i>Centroceras tetrachotomum</i> Won, T.O. Cho et Fredericq	LAF-25-i-01-1-1 [TC415]	M.H. Hommersand/25 Jan. 2001	Lambert Bay, Western Cape, South Africa	—	DQ374323
<i>Centroceras</i> sp.	ARS03786		Hawaii	HQ422953	—
<i>Ceramium brevizonatum</i> var. <i>caraibicum</i> H.E. Petersen et Børgesen		C.F. Gurgel/13 Feb 1998	Yucatan, Mexico	—	AF259491
<i>Corallophila huysmansii</i> (Weber Bosse) R.E. Norris	ARS03236	A. Sherwood	Kure Atoll, Northwestern Hawaiian Islands, USA	HQ422782	—
<i>Corallophila eatoniana</i> (Farl.) T.O. Cho, H.-G. Choi, G.I. Hansen et S.M. Boo	TC047	T.O. Cho/16 Jul. 1998	Friday Harbor, Washington, USA	—	AY945765
<i>Gayliella transversalis</i> (Collins & Herv.) T.O. Cho et Fredericq	LAF-30.x.03.1.1	T.O. Cho/30 Oct. 2003	Key West, Florida, USA	—	AY945777
<i>Microcladia borealis</i> Rupr.	TC004	T.O. Cho & G.I. Hansen/12 Jul. 1998	Otter Crest, Oregon, USA	—	AY945770

Table 2. Morphological comparison of *Centroceras* species in the ‘*C. clavulatum*-complex’ from Bermuda (nd = no data available).

Characteristics	<i>C. arcii</i>	<i>C. gasparrinii</i>	<i>C. hyalacanthum</i>	<i>C. illaqueans</i>	<i>C. micracanthum</i>
Mature axis diameter (μm)	160 – 260 (–300)	120 – 220	110 – 170	140 – 210	130 – 240
No. segments between indeterminate branches	7 – 14	10 – 12	8 – 15	11 – 12	9 – 15
No. periaxial cells at node	14–16	12 – 16	10 – 13	10 – 12	14 – 16
Branch pairs in notches of branches	Present	Present	Present	Present	Unusual
Spine in notches of branches	Present	Present	Present	Present	Present
No. spines at nodes	5 – 12	5 – 8	6 – 10	0 – 4	2 – 8
Spine length (μm)	80 – 130	33 – 75	40 – 68	33 – 39	48 – 118
Spine diameter (μm)	24 – 40	14 – 25	15 – 18	12 – 14	15 – 40
Acropetal cell length (μm)	18–35	9 – 20	15 – 21	25 – 38	14 – 32
Acropetal cell diameter (μm)	3 – 8	4 – 6	2 – 5	3 – 4	2 – 7
No. cells in descending cortical files	8 – 27	12 – 35	7 – 11	8 – 9	5 – 13 (–17)
Smallest median descending tier cells ($l \times d$ in μm)	9 – 43 x 3 – 10	11 – 15 x 4 – 10	21 – 28 x 2 – 5	10 – 13 x 7 – 8	14 – 78 x 5 – 12

Largest median descending tier cells (<i>l</i> x <i>d</i> in μm)	8 – 54 x 6 – 17	12 – 18 x 5 – 11	19 – 33 x 5 – 10	20 – 25 x 5 – 14	18 – 116 x 2 – 10
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Tetrasporangia

length (μm)	38 – 70	nd	38 - 55	nd	nd
diameter (μm)	26 – 50	nd	35 - 48	nd	nd
no. involucral cells	0 – 3	nd	nd	nd	nd

Fig. 1. Consensus tree derived from maximum-likelihood analyses of COI-5P sequences. Values above branches are bootstrap supports for ML (1000 replicates) followed by Bayesian posterior probabilities in %. An asterisk (*) indicates full support for both robustness metrics (refer to Table 1 for GenBank numbers).

Fig. 2. Phylogeny of *Centroceras* based on maximum-likelihood analyses of *rbcL* sequences. Values above branches are bootstrap supports for ML (1000 replicates) followed by Bayesian posterior probabilities in %. An asterisk (*) indicates 100% support for both robustness metrics while a dash (-) indicates support values <80% (refer to Table 1 for GenBank numbers).

Fig. 3. *Centroceras arcii* sp. nov. (A) Habit of one of two cards in holotype packet, *CWS/CEL* 09-13-6. (B) Habit displaying reflexive branching, *CWS/CEL/TRP* 12-21-10. (C) Axis with a branch pair in the notch of the main branch and single branch in penultimate notch, *CWS/CEL/TRP* 12-21-10. (D) Axial node with cortical initials bearing elongated acropetal cells, spines, and tetrasporangium, *CWS/CEL* 08-9-2. (E) Cross section of axis showing 13 periaxial cells, *TRP* 12-34-4. (F) Axial segment displaying staggered, basipetal filaments of cortical cells, *TRP* 12-70-4. Scale bars represent: A, 1.5 cm; B, 2 cm; C, 2 mm; D-F, 50 μ m. Ax, axial cell; C1–3, sequence of cortical initials; P, periaxial cell.

Fig. 4. *Centroceras gasparrinii*. (A) Axis with straight, pseudodichotomous branching, *CWS/CEL/TRP* 12-20-13. (B) Axial node with cortical initials bearing ovoid acropetal cells and a spine, *CWS/CEL/TRP* 12-14-1. (C) Cross section of axis showing 14 periaxial cells, *CWS/CEL/TRP* 12-14-1. (D) Axial segment displaying staggered, basipetal filaments of cortical cells, *CWS/CEL/TRP* 12-14-1. Scale bars represent: A, 1.5 mm; B, 15 μ m; C, 50 μ m; D, 25 μ m. Ax, axial cell; C1–2, sequence of cortical initials; P, periaxial cell.

Fig. 5. *Centroceras hyalacanthum*. (A) Axis with branch forming in the notch of the main axis, *CWS/CEL/TRP* 13-16-5. (B) Axial node with cortical initials bearing elongated acropetal cells and spines, *TRP* 12-171-3. (C) Cross section of axis showing 14 periaxial cells, *CWS/CEL* 10-30-12. (D) Axial segment displaying staggered, basipetal filaments of cortical cells, *TRP* 12-171-3. Scale bars represent: A, 1 mm; B & D, 25 μ m; C, 50 μ m. Ax, axial cell; C1–2, sequence of cortical initials; P, periaxial cell.

Fig. 6. *Centroceras illaqueans* sp. nov. (A) Habit, holotype, CWS/CEL 09-2-9. (B) Axis with straight, pseudodichotomous branching, CWS/CEL 09-2-9. (C) Cross section of axis showing 10 periaxial cells, CWS/CEL 09-2-9. (D) Axial node with cortical initials bearing elongated acropetal cells and single spine (arrowhead), CWS/CEL 09-2-9. (E) Axial segments displaying tiered, basipetal filaments of isodiametric cortical cells and lack of spines at nodes, CWS/CEL 09-2-9. Scale bars represent: A, 2 cm; B, 1 mm; C-E, 25 μ m. Ax, axial cell; C1–2, sequence of cortical initials; P, periaxial cell.

Fig. 7. *Centroceras micracanthum*. (A) Axis with reflexive, pseudodichotomous branching, *CWS/CEL/TRP* 13-16-25. (B) Axial node with cortical initials bearing elongated acropetal cells and spines, *CWS/CEL/TRP* 12-10-9. (C) Cross section of axis showing 15 periaxial cells, *CWS/CEL/TRP* 12-11-12. (D) Axial segment displaying staggered, basipetal filaments of cortical cells, *CWS/CEL/TRP* 12-10-9. Scale bars represent: A, 2 mm; B, 25 μ m; C & D, 50 μ m. Ax, axial cell; C1–2, sequence of cortical initials; P, periaxial cell.

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