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Notes on the marine algae of the Bermudas. 15. *Dichotomaria huismanii* (Galaxauraceae, Rhodophyta), a new species in the *D. marginata* complex from the western Atlantic

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Notes on the marine algae of the Bermudas. 15. *Dichotomaria huismanii* (Galaxauraceae, Rhodophyta), a new species in the *D. marginata* complex from the western Atlantic

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Abstract: Using plastid-encoded *rbcL* and mitochondrial COI-5P sequence data, the species in Bermuda formerly recognized as *Dichotomaria marginata* was found to be a cryptic species in a wide complex of species all falling under the morphologically broad species concept for this supposed pantropical species. The new species, *Dichotomaria huismanii*, shows subtle anatomical and morphological differences when compared to *D. marginata*, and at present is only known from Bermuda. Bermuda specimens were compared with our isolates of *D. marginata* from St. Croix (Virgin Is.), as well as *rbcL* sequenced specimens with those from Guadeloupe and Puerto Rico in GenBank as *D. marginata*. Morphological and molecular characterization of *D. marginata* is provided along with the new species.

Keywords: Bermuda; COI-5P; *Dichotomaria*; *D. huismanii* sp. nov.; *D. marginata*; Galaxauraceae; *rbcL*; Rhodophyta.

Introduction

In a circumscription of the nemalialean genus *Galaxaura*, Papenfuss et al. (1982) taxonomically linked many earlier described pairs of species as heteromorphic sporophyte and gametophyte generations of the same species. Subsequently, Huisman et al. (2004) used LSU rDNA sequences to show that *Galaxaura* was polyphyletic, moving a clade of species to the newly resurrected

genus *Dichotomaria*. Along with four others, they re-assigned two species originally included by Lamarck (1816) in his new genus *Dichotomaria*, *Dichotomaria marginata* (J. Ellis et Solander) Lamarck and *Dichotomaria obtusata* (J. Ellis et Solander) Lamarck, species known during the 20th century as *Galaxaura*, both being widely reported in the western Atlantic as well as other seas. Pantropical *D. obtusata* has been shown to be a complex of species, but authentic material from the West Indies has not been sequenced and compared with the various genetic species now known from distant seas (Wiriadamrikul et al. 2014). Bermuda specimens designated as *D. obtusata* var. *major* W.R. Taylor (Taylor 1960) were based on the larger habits they displayed in the islands as compared to the nominate variety, and sequences we have generated from recent collections match those derived from isotype material of *D. obtusata* var. *major* by Wiriadamrikul et al. (2014). Whether they are representative of the species remains to be seen, as material of *D. obtusata* from the region of the type locality (Bahamas Is.) needs to be collected and sequenced for comparison.

The second species presently known from Bermuda, *Dichotomaria marginata*, is likewise considered pantropical (Papenfuss et al. 1982, Kurihara and Huisman 2006, Guiry and Guiry 2015) and, until recently, has been an easily recognizable member of the genus due to its flattened axes with thickened margins. First described in the genus *Corallina* from the Bahamas by Ellis and Solander (1786), *Corallina marginata* J. Ellis et Solander was later moved as the generitype to *Dichotomaria* by Lamarck (1816), and subsequently into other genera of the Galaxauraceae over its long taxonomic history. When Huisman et al. (2004) analyzed their molecular and anatomical results, they discovered that there were at least three distinct genetic species at the time known as *D. marginata* from Puerto Rico, eastern Africa and Australia. As the type specimen from the Bahamas was apparently lost over the two and a half centuries since its collection, the Ellis and Solander illustration of *C. marginata* (1786, pl. 22 figure 6) was lectotypified by Papenfuss et al. (1982, footnote 3),

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thus disallowing an anatomical comparison of the genetic species with the morphological type from the western Atlantic Ocean. Nevertheless, Huisman et al. (2004) suggested that the material from Puerto Rico most reasonably would be the best fit for the species given its proximity to the type locality just over 500 km to its northwest in the Atlantic. For the other two genetic species, Huisman et al. (2004) resurrected synonyms of *D. marginata* as independent species with flattened axes from the Indo-Pacific region, *D. australis* (Sonder) Huisman, J.T. Harper et G.W. Saunders (type locality=Victoria, Australia) and *D. tenera* (Kjellman) Huisman, J.T. Harper et G.W. Saunders (type locality=Mobasa, Kenya). These two, therefore, represented the first segregate species recognized in what had been a morphologically variable and pantropical *D. marginata* complex outlined by Papenfuss et al. (1982). A second Australian species, *D. spathulata* (Kjellman) A. Kurihara et Huisman (type locality=Fremantle, Western Australia), was later resurrected from the complex by Kurihara and Huisman (2006), and they suggested additional species might be segregated from *D. marginata* in the future. Interestingly, when Kurihara and Huisman (2006) segregated *D. spathulata*, they left specimens from the northern tropical region as representatives of *D. marginata* from Australia based on morphological evidence. Lacking *rbcl* sequences for these, this action awaits molecular evidence and analysis for confirmation.

Dichotomaria marginata was first reported as part of the Bermuda flora with its inclusion in the widely disseminated exsiccata *Phycotheca Boreali-Americana* (P.B.-A) as *Brachycladia marginata* (J. Ellis et Solander) F. Schmitz (Collins et al. 1913, no. 1930). This early collection was cited by Collins and Hervey (1917) and Howe (1918a) as *Galaxaura marginata* (J. Ellis et Solander) J.V. Lamouroux, and all subsequent reports in Bermuda followed their lead (see Schneider 2003). In the present study, we have made several recent collections of *D. marginata* in Bermuda and St. Croix, Virgin Is., to compare genetically with other species in the complex, in particular specimens attributed to this species from Puerto Rico analyzed by Huisman et al. (2004) and Guadeloupe by Wang et al. (2005) and Wiriadamrikul et al. (2014).

Materials and methods

Standard methods

Offshore collections from Bermuda and St. Croix, Virgin Is. were made using SCUBA, with site locations marked

with a Garmin™ eTrex H (Olathe, KS, USA). Small portions of each specimen were dried on silica gel for DNA extraction and other samples were preserved in 4–5% formalin in seawater for sectioning, the remainder being pressed onto herbarium paper as a permanent voucher. Liquid preserved samples were decalcified in 8% HCl, sectioned with a model 880 American Optical freezing microtome (San Diego, CA, USA), with sections 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, CA, USA). 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 composed in Adobe Photoshop™CS6 v. 13.0.1 (Adobe Systems, San Jose, CA, USA). Voucher specimens of some numbers are deposited in MICH, NY, the Bermuda Natural History Museum and CWS's personal herbarium, the holotype in the first. Herbarium abbreviations follow the online Index Herbariorum (<http://sweetgum.nybg.org/ih/>) and standard author initials were taken from Brummitt and Powell (1992).

Molecular methods

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, MO, USA) GenElute Plant Genomic Miniprep Kit according to the manufacturer's protocol, with 500 µl of modified lysis solution (50 µl 10% TWEEN 20 and 5 µl of 20 mg ml⁻¹ ProK), as well as 1 h 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. Specimens were likewise sequenced

for the plastid-encoded *rbcL* operon. Amplification and/or sequencing reactions for *rbcL* were conducted using the primers (F43-R753; F615-RrbcS start) and the thermal profile referenced in Wiriyadamrikul et al. (2014). All amplified DNA was treated with the Qiagen (Redwood City, CA, USA) QIAquick PCR Purification Kit following the manufacturer's protocol. 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, NY, USA).

COI-5P barcode sequences from representatives in the Galaxauraceae, including some available through GenBank and those 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 (42 specimens, 538 sites) with Tamura-nei-corrected distances (default setting). The resulting tree (Figure 1) was used to demarcate species groupings. Based on these genetically related groups (=species), and with comparative data available from GenBank, one specimen from each species and/or geographic location was selected for phylogenetic analysis using *rbcL* sequences (Figure 2). The best models of evolution for the individual gene regions *rbcL* (62 taxa, 1249 sites) were determined in jModelTest 2 (volume 2.1.5; Darrriba 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 ML phylogeny was estimated using the RAxML graphical user interface (Silvestro and

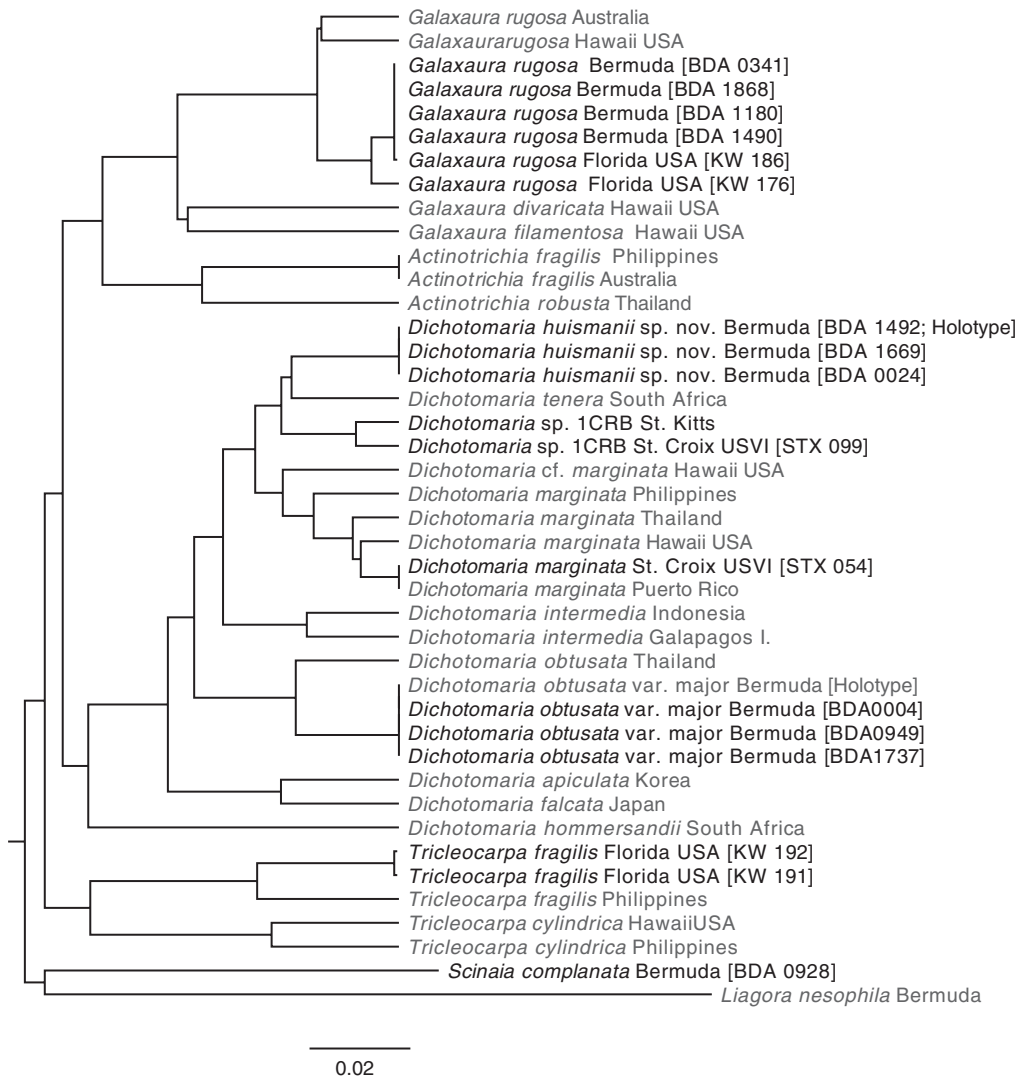


Figure 1: Species groups in the Galaxauraceae determined with UPGMA clustering of the COI-5P genetic barcode, with *Scinia* and *Liagora* as outgroups. Sequences generated for this study are in bold text.

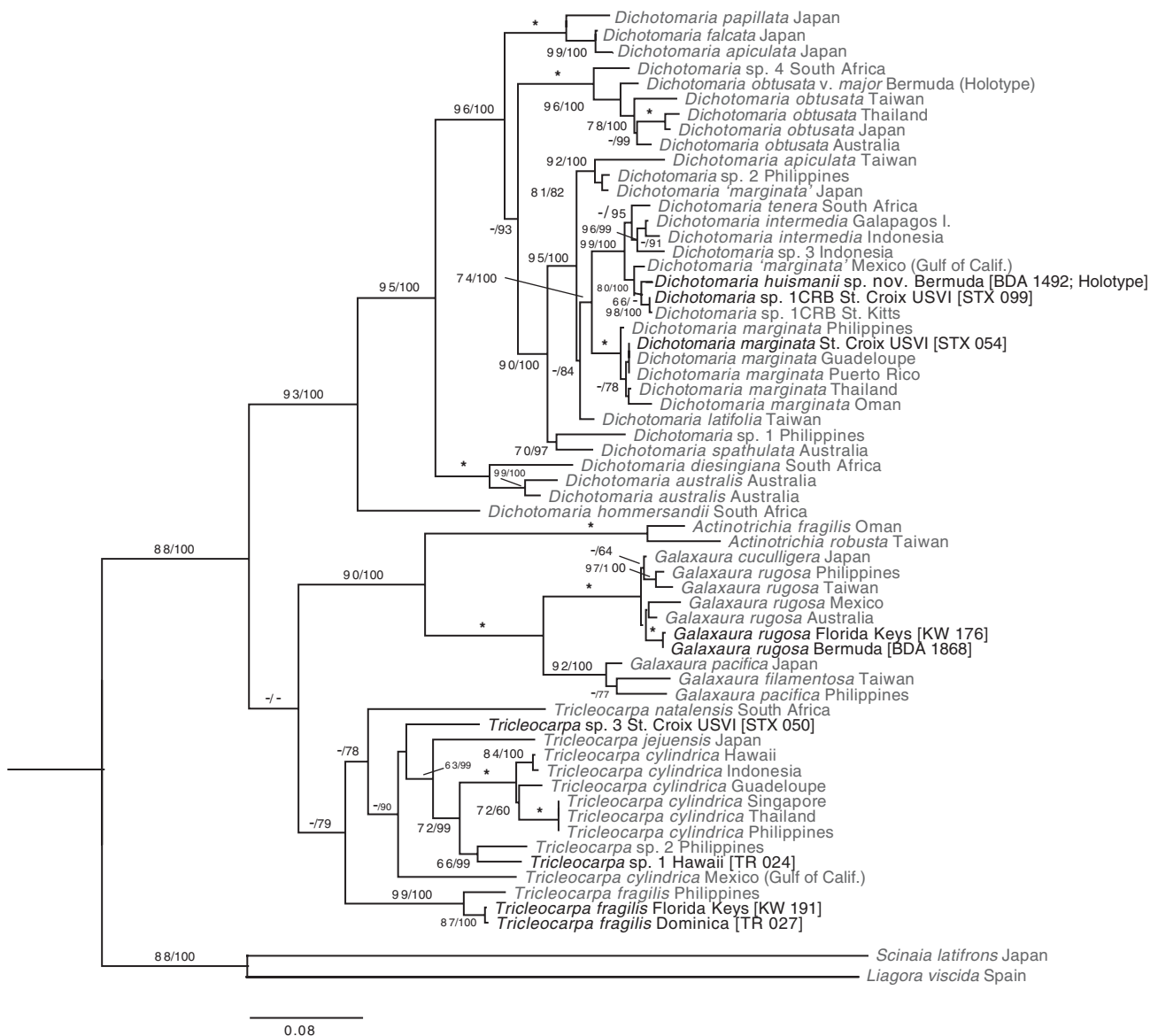


Figure 2: Phylogeny of the Galaxauraceae based on maximum-likelihood analyses of *rbcL* sequences. Branch values are bootstrap supports for ML (1000 replicates) followed by Bayesian posterior probabilities expressed as a percentage of support out of 1. An asterisk (*) indicates 100% support for both robustness metrics while a dash (-) indicates support values <60%. Sequences generated for this study are in bold text.

Michalak 2011) 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. Stationarity was attained after the first 425,000 generations (burnin=4250 trees) and posterior probabilities were estimated based on the remaining trees. The *rbcL* phylogenetic tree (Figure 2) includes *Liagora* and *Scinaia* as outgroups, and was manipulated for presentation using FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>). Specimens used in our molecular analyses are recorded in Table 1.

Results and discussion

Our *rbcL* results demonstrated that two of our St. Croix isolates (STX032, STX054) were a perfect genetic match with *Dichotomaria marginata* from Puerto Rico (coll. H. Ruiz, GenBank KC134339) and Guadeloupe (coll. S. Mege, AY680019), but all of the Bermuda sequences and a second species from St. Croix and St. Kitts were genetically distinct (Table 1, Figure 2). For COI-5P, our two identical St. Croix *D. marginata* specimens (STX032, STX054) were again a perfect match to that from Puerto Rico (Figure 1; this gene was not sequenced for the Guadeloupe isolate). The COI-5P sequence of a second genetic

species in the *D. marginata* complex, *Dichotomaria* sp. 1CRB, was 5.5% different from *D. marginata* from St. Croix and Puerto Rico, and only 0.6% different from specimens under the same name collected in Bermuda. All of the many Bermuda specimens were genetically identical, differing by 4.8% of their nucleotide base pairs (bp) from *D. marginata* specimens from Puerto Rico and St. Croix in COI-5P sequences, and 2.4% from Guadeloupe, Puerto Rico and St. Croix specimens in *rbcL* sequences.

Using *D. marginata* specimens from St. Croix that genetically matched those from Guadeloupe and Puerto Rico, we completed a morphological assessment of characteristics and found them to conform to descriptive accounts of this species from the geographic area including the type locality. The protologue and other early accounts offered little in the way of specifics other than overall morphology, Ellis and Solander (1786, p. 115) fully describing it as a “Dichotomous coralline with flat smooth branches, scarcely jointed, and a raised border”. In fact, this would describe any of the flattened *Dichotomaria* specimens we have located in the western Atlantic, and is thus of little use in distinguishing similar species with flattened axes in the Caribbean and surrounding areas. It would seem obvious, then, that 20th century and later accounts of *D. marginata* in the western Atlantic (e.g. Kjellman 1900, Børgesen 1916, Taylor 1928, 1960, Littler and Littler 2000, Dawes and Mathieson 2008) could have included observations and measurements from more than a single species with flattened axes.

Therefore, our St. Croix *D. marginata* specimens that genetically match *rbcL* sequences from Guadeloupe and Puerto Rico, the latter being a genetic standard for *D. marginata* set by Huisman et al. (2004), can be used to provide a set of morphological characteristics to distinguish our other genetic species from Bermuda and the Caribbean. The report of *D. marginata* by Collins and Hervey (1917) was made using widely distributed Bermuda specimens (*P.B.-A.* no. 1930) and therefore represents the segregate species we describe and differentiate in the *D. marginata* complex below.

Dichotomaria marginata **(J. Ellis et Solander) Lamarck 1816,** **p. 146 (Figures 3–6)**

Basionym

Corallina marginata J. Ellis et Solander 1786, p. 115, pl. 22 figure 6 (Figure 3).

Description

Rosy-brown plants to 10 cm tall and 13 cm across, composed of dichotomously branched flattened axes with thickened margins, these showing faint annulations; axes mottled in appearance when dried; internodes 4.7–13.7 mm long and 1.6–2.6 mm wide; axes branching dichotomously at angles of 72–89°; cortex of sporophytes composed of two layers of cells, outer cortical assimilatory cells subspherical to ovoid, 20–40 µm long and (21–) 33–49 µm diameter, and an inner layer of flared stalk cells each bearing 1–2 assimilatory cells, 15–36 µm long and 5–10 µm diameter (at narrowest point); stalk cells borne typically in pairs though occasionally singly on the outer surface of the inner cortex; stalk cells most commonly unicellular, rarely two-celled; inner cortex consisting of typically one, occasionally two, layers of transversely ovoid to rectangular cells, 50–115 µm diameter and 25–68 µm high; subcortical cells subspherical at margins; medullary filaments extending from subcortical cells, 7–13 µm diameter; gametangia and tetrasporangia not seen in our sequenced specimens.

Type locality

“... on the shore of one of the Bahama islands”, West Indies, western Atlantic Ocean (Ellis and Solander 1786).

Distribution

Formerly reported as pantropical, genetically verified specimens are known from Guadeloupe, Puerto Rico and St. Croix, USVI (West Indies).

Selected collections

St. Croix, Virgin Is. – T.R. Popolizio (TRP)/C.E. Lane (CEL)/E.D. Salomaki (EDS) 13-24-1 [STX032] (Figure 4), TRP/CEL/EDS 13-25-9 [STX054] (see Table 1 for collection details).

Remarks

As discussed above, two St. Croix collections genetically matched other sequenced specimens from Guadeloupe and Puerto Rico, as well as the description of *Dichotomaria marginata* provided by Papenfuss et al. (1982). This has allowed us to identify STX032 and STX054 (Figures 1 and 2) as true representatives of *D. marginata* in the West

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 nos.	Collectors/date	Collection site	COI-5P	rbcl
Galaxauraceae					
<i>Actinotrichia fragilis</i> (Forsskål) Børgesen	GWS 000151	J. Huisman/17 Aug. 1995	Ningaloo Reef, Western Australia, Australia	KT886155	
<i>Actinotrichia robusta</i> Itono	– CNU 32154	T. Schils, K. Pauly and E. Demeulenaere/28 Oct. 2004 20 Jan. 2006	Bandar Khayran, Capital Area, Oman		EU095253
<i>Dichotomaria apiculata</i> (Kjellman) Kurihara et Masuda	– CNU 32279 SAP 095732	S.-L. Liu and S. M. Lin/ 1 Apr. 2003 K. Lewmanomont/6 Apr. 2010 16 Jun. 2002	Bonbon, Cagayan de Oro, Philippines 3rd Nuclear Power Outlet, Kenting National Park, Taiwan Similan I., Phang Nga, Thailand Reihoku, Amakusa, Kumamoto Prefecture, Japan	KC609385 KC609386	AY688011 AB117635
<i>Dichotomaria australis</i> (Sonder) Huisman, J.T. Harper et G.W. Saunders	TUNG 05369 CNU 32163 SAP 101111 SAP 101108	S.-M. Lin and S.-L. Liu/21 Jul. 2002 3 Dec. 2002 A. Kurihara and S. Shimada/28 Nov. 2003 A. Kurihara and S. Shimada/ 4 Dec. 2003 M. Hommersand/9 Jul. 1993	Sail Rock, Kenting National Park, Taiwan Sungsan, Jeju, South Korea No. 16 Beach, Rye, Victoria, Australia Jervis Bay, New South Wales, Australia	KC768178	AY688020 AB258443 AB258440
<i>Dichotomaria diesingiana</i> (Zanardini) Huisman, J.T. Harper et G.W. Saunders	–		Sharks Bay, Port Alfred, Cape Province, South Africa		AY688026
<i>Dichotomaria falcata</i> (Kjellman) Kurihara et Masuda	– CNU 032192	24 Jul. 2001 K.M. Kim/31 Jul. 2009	Toji, Shimoda, Shizuoka Prefecture, Japan Oura Bay, Shimoda, Japan	KF752527	AB117629
<i>Dichotomaria hommersandii</i> S.-L. Liu et S.-M. Lin	–	M. Hommersand/20 Jul. 1993	Riet River, Port Alfred, Cape Province, South Africa	KC253901	JX072968
<i>Dichotomaria huismanii</i> C.W. Schneider, Popolizio et Spagnuolo	CWS/CEL/TRP 10-5-13 [BDA 0024] TRP 12-117-5 [BDA 1492] (holotype)	C.W. Schneider, C.E. Lane, T.R. Popolizio/19 Aug. 2010 T.R. Popolizio/20 Sep. 2012	Off Frick's Beach, Tucker's Town, Bermuda I., Bermuda (10–12 m) Hog Breaker, north shore Bermuda I., Bermuda (12 m)	KU321660 KU321661	KU321674
<i>Dichotomaria intermedia</i> (R.C.Y. Chou) Wiriyadamrikul, M.J. Wynne et S.M. Boo	TRP 12-151-11 [BDA 1669] WRT 1934-164 CNU 032199	T.R. Popolizio/16 Nov. 2012 W.R. Taylor/17 Jan. 1934 K.M. Kim/24 Oct. 2009	Southwest of North Rock, Bermuda I., Bermuda (12 m) Isabela Island, Galapagos I., Ecuador Gerupuk, Lombok, Indonesia	KU321659 KF752532	KF752506 KF752509
<i>Dichotomaria latifolia</i> ined. (<i>Galaxaura latifolia</i> Tak. Tanaka)	–	S.-L. Liu and W.L. Wang/ 25 Jan. 2003	Tahsianglian, Taipei County, Taiwan	KF752529	AY688021

Table 1 (continued)

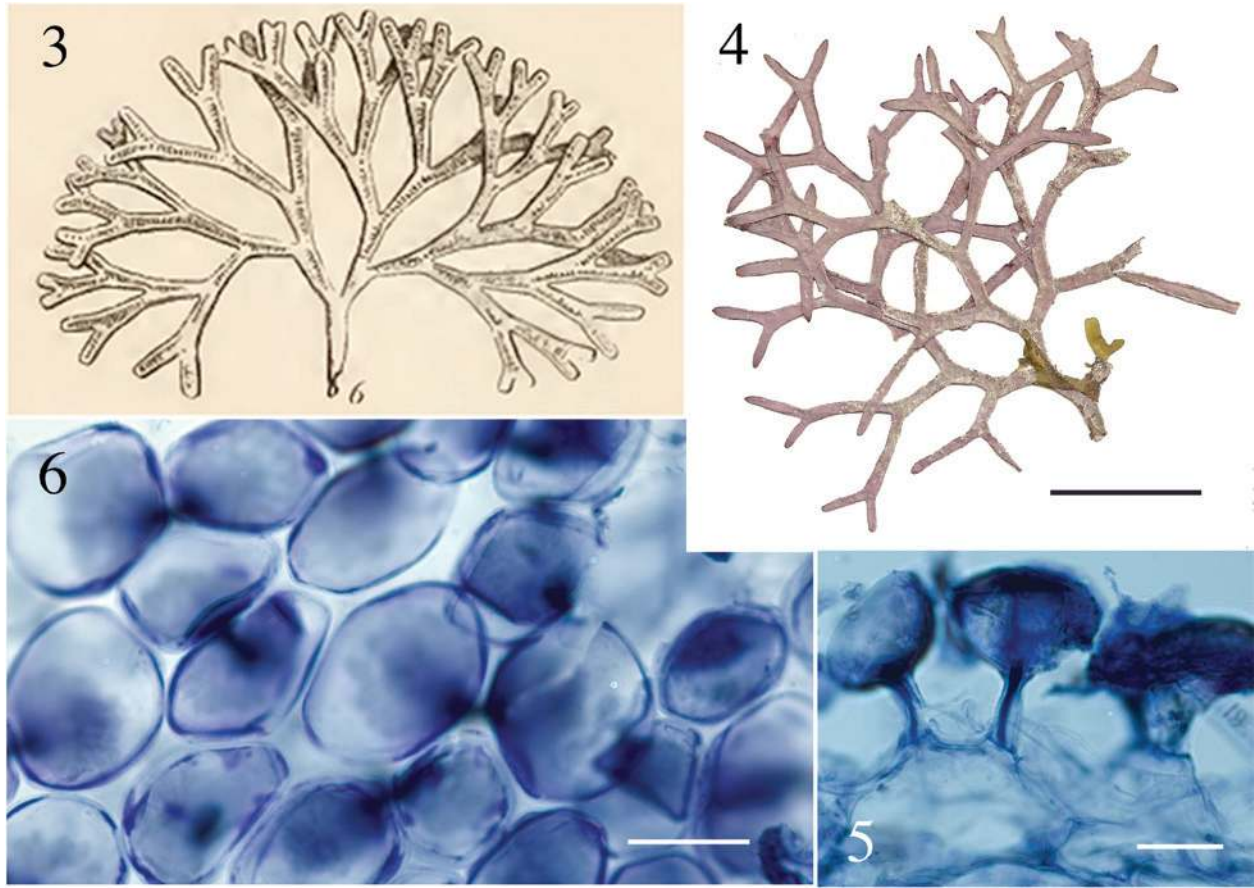
Species	Voucher nos.	Collectors/date	Collection site	COL-5P	rbcl
<i>Dichotomaria marginata</i> (J. Ellis et Solander) J.V. Lamouroux	–	S. Mege/27 May 1995	Anorde Rocks, St. Prorjono, Guadeloupe, West Indies		AY688019
	–	J. Hughey/29 Jul. 1996	Puerto Libertad, Sonora, Gulf of California, Mexico		AY688022
	GR 20002006	G. Richards/26 Sep. 2000 19 Jul. 2001	Mirbat, Dhofar, Oman		KF752510
	–	S.L. Liu and L.M. Liao/7 Nov. 2002	Ookataura, Hachijo Island, Tokyo, Japan		AB117630
	–		Sulpa I., Cebu, Philippines		AY688017
	GWS 001893	H. Ruiz/20 Oct. 2003	Lajas, Turrumote Reef, La Parguera, Puerto Rico	KC130147	KC134339
	CNU 032166	E.C. Yang/14 Dec. 2003	Camotes I., Cebu, Philippines	KF752533	
	ARS 02613	A. Sherwood/21 Mar. 2007	Makapu, Oahu I., Hawaii, USA	HQ423119	
	CNU 032231	J. Wiriyadamrikul/5 Apr. 2010	Similan I., Phang Nga, Thailand	KF752534	KF752512
	TRP/CEL 13-24-1 [STX 032]	T. Popolizio, C. Lane and E. Salomaki/19 Nov. 2013	Alien Nation Reef, Frederiksted, St. Croix, US Virgin Is. (15–16 m)	KU360125	
	TRP/CEL 13-25-9 [STX 054]	T. Popolizio, C. Lane and E. Salomaki/20 Nov. 2013	Cables Reef, west end St. Croix, US Virgin Is. (13–14 m)	KU321662	KU321676
<i>Dichotomaria</i> cf. <i>marginata</i>	ARS 03619	A. Sherwood/23 Mar. 2008	Hale O Lono Harbor, Molokai I., Hawaii, USA	HQ422899	
<i>Dichotomaria obtusata</i> (J. Ellis et Solander) Lamarck	–	C.-S. Lin/15 Jun. 2002	Tiaoshih, KNP, Taiwan		AY688024
	SAP 095741	15 Jul. 2002	Itoman, Okinawa Island, Japan		AB117632
	SAP 101115	A. Kurihara and S. Shimada/13 Nov. 2003	Parakeand Bay, Rottneest Island, Australia		AB258447
	CNU 021562	J. Wiriyadamrikul/8 Apr. 2012	Surin Islands, Phang Nga, Thailand	KF752536	KF752514
<i>Dichotomaria obtusata</i> v. <i>major</i> (W.R. Taylor) M.J. Wynne	WRT 1949-288 (holotype)	W.R. Taylor/15 May 1949	Tucker's Town Bay, Hamilton I., Bermuda	KF752537	KF752515
	CWS/CEL/TRP 10-5-3 [BDA 0004]	C.W. Schneider, C.E. Lane, T.R. Popolizio/19 Aug. 2010	Off Frick's Beach, Tucker's Town, Bermuda I., Bermuda (10–12 m)	KU321671	
	TRP 12-42-7 [BDA 0949]	T.R. Popolizio/14 Mar. 2012	Middle buoy, Eastern Blue Cut channel, blue hole, north of Daniel's Head, Somerset Is., Bermuda (16 m)	KU321672	
	TRP 12-160-2 [BDA 1737]	T.R. Popolizio/1 Dec. 2012	Wreck of the Hermes, south shore Bermuda I., Bermuda (23 m)	KU321673	
<i>Dichotomaria papillata</i> (Kjellman) Kurihara et Masuda	SAP 095740	18 Jun. 2002	Reihoku, Amakusa, Kumamoto Prefecture, Japan		AB117631
<i>Dichotomaria spathulata</i> (Kjellman) Kurihara et Huisman	SAP 101113	A. Kurihara and S. Shimada/16 Nov. 2003	Green Island, Rottneest I., W.A., Australia		AB258445

Table 1 (continued)

Species	Voucher nos.	Collectors/date	Collection site	COI-5P	rbcl
<i>Dichotomaria tenera</i> (Kjellman)	–	M. Hommersand/24 Jul. 1993	Palm Beach, Natal, South Africa		AY688023
Huisman, J.T. Harper et G.W. Saunders	CNU 032161	S.M. Boo/17 Aug. 2005	Rocky Bay, Kwazulu Natal, South Africa	KF752540	
<i>Dichotomaria</i> sp. 1	CNU 032178	S.M. Boo/10 Mar. 2005	Pagudpud, Ilocos Norte, Philippines		KF752522
<i>Dichotomaria</i> sp. 2	CNU 032180	S.M. Boo/11 Mar. 2005	Pangil, Ilocos Norte, Philippines		KF752523
<i>Dichotomaria</i> sp. 3	CNU 023023	S.M. Boo/6 Jun. 2012	Lombok, Indonesia		KF752520
<i>Dichotomaria</i> sp. 4	CNU 032175	S.M. Boo/17 Aug. 2005	Rocky Bay, Kwazulu Natal, South Africa		KF752524
<i>Dichotomaria</i> sp. 1CRB	MJW 10514	M.J. Wynne/24 Nov. 1995	Sand Bank Bay, St. Kitts, Lesser Antilles	KU321657	KF752521
	TRP/CEL 13-29-2	T. Popolizio, C. Lane and	Salt River Wall, north shore St. Croix, US	KU321658	KU321675
	[STX 099]	E. Salomaki/21 Nov. 2013	Virgin Is., Lesser Antilles (18–22 m)		
<i>Galaxaura cuculligera</i> Kjellman	SAP 095742	18 Jun. 2002	Reihoku, Amakusa, Kumamoto Prefecture, Japan		AB117633
<i>Galaxaura divaricata</i> (Linnaeus) Huisman et R.A. Townsend	ARS 00995	B. Pauahi/18 Mar. 2004	Manele Bay, Lanai I., Hawaii, USA	HQ422677	
<i>Galaxaura filamentosa</i> R.C.Y. Chou	–	S.-M. Lin/17 Apr. 2001	Five Caves, Orchid Is., Taiwan		AY688006
<i>Galaxaura pacifica</i> Tak. Tanaka	ARS 03487	A. Sherwood/11 Oct. 2007	Sandy Beach, Oahu I., Hawaii, USA	HQ422797	
	SAP 095744	15 May 2002	Higashi Port, Haha-jima, Bonin I., Japan		AB117638
<i>Galaxaura rugosa</i> (J. Ellis et Solander) J.V. Lamouroux	–	L.-M. Liao/19 Feb. 2003	Bulusan, Sorsogon, Philippines		AY688004
	GWS 000144	J. Huisman/28 Nov. 1995	Barrow I., Western Australia, Australia	KT886158	
	–	M. Hommersand/27 Feb. 1998	Las Playas Piedras, Bahia San Carlos, Mexico		AY688003
	–	S.-L. Liu and C.-S. Lin/11 Apr. 2002	Chiupeng, KNP, Taiwan		AY687998
	–	L.-M. Liao/19 Feb. 2003	Bulusan, Sorsogon, Philippines		AY688000
	ARS 02608	A. Sherwood/17 Mar. 2007	Kaaawa Beach Park, Oahu I., Hawaii, USA	HQ423122	
	CWS/CEL/TRP 10-22-7	C. Schneider, C. Lane and	Capt. Williams' Bay, south shore	KU321663	
	[BDA 0341]	T. Popolizio/23 Aug. 2010	Bermuda I., Bermuda (intertidal)		
	TRP 12-72-11 [BDA 1180]	T.R. Popolizio/14 Jun. 2012	Annie's Bay, St David's I., Bermuda (0–3 m)	KU321665	
	TRP 12-117-3 [BDA 1490]	T.R. Popolizio/20 Sep. 2012	Hog Breaker, north shore Bermuda I., Bermuda (12 m)	KU321666	
	CWS/CEL/TRP 13-12-9	C. Schneider, C. Lane and	Sand Key Lighthouse Reef, Key West, FL, USA (6–8 m)	KU321667	KU321678
	[KW 176]	T. Popolizio/30 May 2013			
	CWS/CEL/TRP 13-12-19	C. Schneider, C. Lane and	Sand Key Lighthouse Reef, Key West, FL, USA (6–8 m)	KU321668	
	[KW 186]	T. Popolizio/30 May 2013			
	TRP 13-20-1 [BDA 1868]	T. Popolizio/18 Sep. 2013	Whalebone Bay, St. George's I., Bermuda (0–1 m)	KU321664	KU321677
	SAP 101116	A. Kurihara and S. Shimada	Rottneest Island, Western Australia		AB258448

Table 1 (continued)

Species	Voucher nos.	Collectors/date	Collection site	COI-5P	rbcl
<i>Tricleocarpa cylindrica</i> (J. Ellis et Solander) <i>Huisman et Borowitzka</i>	–	S. Fredericq/29 Mar. 1995	Guadeloupe, West Indies		AY688012
	–	M. Hommersand/26 Feb. 1998	Guyamas, Gulf of California, Mexico		AY688015
<i>Tricleocarpa fragilis</i> (Linnaeus) <i>Huisman et R.A. Townsend</i>	CNU 32194	E.C. Yang/15 Aug. 2007	Minahasa, Manado, Indonesia		KC768127
	CNU 32185	S.M. Boo/17 Jul. 2009	Kaui Island, Hawaii, USA	KC768161	KC768126
	CNU 32277	S.M. Cho/2 Feb. 2010	Bulusan, Sorsogon, Philippines	KC768177	KC768144
	CNU 21692	K. Lewmanomont/11 Apr. 2012	Haad Thaptawan, Phang Nga, Thailand		KC768128
<i>Tricleocarpa fragilis</i> (Linnaeus) <i>Huisman et R.A. Townsend</i>	CNU 32298	S.M. Boo/15 Oct. 2012	Sentosa Island, Singapore		KC768145
	CWS 07-1-1 [TR 027]	C. Schneider/30 Mar. 2007	Champagne Reef, Roseau, Dominica, Lesser Antilles		KU321680
	CNU 32276	P.J. Geraldino/23 Jan. 2008	San Francisco, Surigao del norte, Philippines	KC768176	KC768143
<i>Tricleocarpa jejuensis</i> <i>Wiriyadamirikul, Geraldino, Huisman, Lewmanomont et S.M. Boo</i>	CWS/CEL/TRP 13-13-1 [KW 191]	C. Schneider, C. Lane and T. Popolizio/30 May 2013	East Dry Rocks Reef, Key West, FL, USA (7 m)	KU321669	KU321679
	CWS/CEL/TRP 13-13-1 [KW 192]	C. Schneider, C. Lane and T. Popolizio/30 May 2013	East Dry Rocks Reef, Key West, FL, USA (7 m)	KU321670	
	CNU 29837	A. Kurihara/12 Jun. 2012	Awase, Okinawa, Japan		KC768121
	CNU 32169	S.M. Boo/9 Aug. 2005	Lala Neck, Kwazulu-Natal, South Africa		KC768122
<i>Tricleocarpa</i> sp. 1 <i>Tricleocarpa</i> sp. 2 <i>Tricleocarpa</i> sp. 3	CWS 02-23-1 [TR 024]	C. Schneider/8 Aug. 2002	Sunset Beach Park, O'ahu, Hawaii, USA		KU321682
	CNU 32236	S.M. Boo/10 Mar. 2005	Pagudpud, Ilocos Norte, Philippines		KC768132
	TRP/CEL/EDS 13-25-6 [STX 050]	T. Popolizio, C. Lane and E. Salomaki/20 Nov. 2013	Cables Reef, west end St. Croix, US Virgin Is., Lesser Antilles (13–14 m)		KU321681
Liagoraceae					
<i>Liagora nesophila</i> Popolizio, C.W. Schneider et C.E. Lane	TRP 12-32-9 [BDA 0846]	T.R. Popolizio/13 Feb. 2012	Walsingham Bay, Castle Harbor, Bermuda I., Bermuda (0–1 m)	KR005345	KR014263
	–	C. Rodriguez-Prieto/12 Aug. 2005	El Golfet, Caleta de Palafrugell, Spain		GUJ57678
Scinaiceae					
<i>Scinaia complatata</i> (Collins) Cotton	TRP 12-40-7 [BDA 0928]	T.R. Popolizio/13 Mar. 2012	Gurnet Rock, mouth of Castle Harbor, Bermuda (13 m)	KU321656	KR005364
	SAP 101117	S. Arai/17 Dec. 2001	Tsurumi, Ooita Prefecture, Japan		AB258449



Figures 3–6: *Dichotomaria marginata*.

(3) Lectotype of *Corallina marginata* (= *Dichotomaria marginata*): Ellis and Solander illustration (1786, pl. 22 figure 6). (4) Sample from St. Croix (STX032), scale bar=2 cm. (5) Section through outer cortex showing subcortical cell bearing a pair of assimilatory outer cortical cells, scale bar=25 μ m. (6) Surface view of outer cortical assimilatory cells (STX054), scale bar=25 μ m.

Indies, a species with a type locality in the Bahamas (Silva et al. 1996). All of the genetic collections made in St. Croix identified as *D. marginata* have the anatomy of sporophytes defined for the species, unfortunately none bearing tetrasporangia. Thus, we cannot provide tetrasporangial size and shape nor the anatomy or reproduction in gametophytes of these genetically significant *D. marginata* vouchers. Although there are reports about these features in earlier literature (e.g. Børgesen 1916, Howe 1918b, Taylor 1960, Papenfuss et al. 1982), without a molecular tie to this species where crypsis is now reported, we are hesitant to link that information here.

Similar to what Huisman et al. (2004) found for the species, tetrasporic St. Croix representatives of *D. marginata* have “the presence of a distinctly thickened margin (particularly noticeable when dried)” and a “cortex of the tetrasporophyte bearing paired subspherical cells (at least some with a small terminal spine)” (Figure 5). As was pointed out in the Ellis and Solander (1786) protologue, the flattened axes of *D. marginata* are more pronounced

when removed from the water, the first collections being washed up and dried on a Bahamian beach. Our specimens have three, or occasionally four, layers of cells including stalk and outer assimilatory cells. The assimilatory cells of St. Croix *D. marginata* are subspherical to ovoid in cross-section (Figure 5), and appear circular to ellipsoidal in surface view (Figure 6). Some, but not a majority, of the assimilatory cells are apiculate, these cells usually paired on stalk cells that are mostly paired on outer subcortical cells (Figure 5). The subcortical cells are transversely ovoid to rectangular, and occasionally subspherical at the margins (Figure 5).

Howe (1918b) was the first to consider *Galaxaura occidentalis* Børgesen (type locality=Virgin Islands) as the gametophyte generation of *G. marginata*, and Børgesen (1920) and subsequent workers (Taylor 1960, Papenfuss et al. 1982) followed his supposition. Howe (1918a) mentioned gametophytic specimens in his account of *G. marginata* in the Bermuda flora, but it remains unclear whether he was referring to actual Bermuda gametophytes or

rather to the gametophytes he was working on at the same time from the West Indies (Howe 1918b). We have been able to observe the gametophytic holotype of *G. occidentalis* [in C], most reasonably the alternate generation of the sporangial *D. marginata*, but this requires genetic confirmation. It would be helpful to find sporophytes and gametophytes of *D. marginata* in a single Bahamian or West Indian population to confirm this supposition, both generations matching genetic sequences already linked to this species from the West Indies.

A second genetic species, *Dichotomaria* sp. 1CRB, was collected in St. Croix and St. Kitts (Figures 1 and 2, Tables 1 and 2), and the St. Croix isolate represents the gametophytic stage of an undescribed species in the *D. marginata* complex. The St. Croix specimen has anatomical similarities with the type of *G. occidentalis*; unfortunately, it is genetically distinct from local and regional *D. marginata*. We only have two representatives of this species from the West Indies, thus until additional specimens are obtained and sequenced, *Dichotomaria* sp. 1CRB will remain undescribed. Whether its gametophytic stage represents what was described as *G. occidentalis* requires further investigation. Nevertheless, its sequence demonstrates that there are at least two species in the *D. marginata* complex in the West Indies.

Dichotomaria huismanii C.W. Schneid., Popolizio et Spagnuolo sp. nov. (Figures 7–13)

Description

Rosy-brown plants to 8 cm tall and 12 cm across, composed of dichotomously branched, flattened axes with thickened margins above, the axes subterete below; axes mottled in appearance when dried, annulations, when present, faint; internodes 3–9 mm long and 1.2–2.0 mm wide; axes branching dichotomously at angles of (32–) 41–54 (–68°); cortex of sporophytes composed of two layers of cells, outer cortical assimilatory cells subspherical to ovoid, 28–50 µm length and 22–48 µm diameter, and an inner layer of flared stalk cells, each bearing one or two assimilatory cells, 23–45 µm length and 5–13 µm diameter at narrowest point; stalk cells borne typically in pairs though occasionally alone on the outer layer of the inner cortex; stalk cells unicellular; inner cortex consisting of two layers of transversely ovoid to rectangular cells, 24–44 (–70) µm long and (38–) 58–89 µm diameter;

subcortical cells subspherical at margins; medullary filaments extending from subcortical cells, closely adherent and parallel, running longitudinally the length of the axis, 13–20 µm diameter; tetrasporangia and gametophytes unknown.

Etymology

The epithet “*huismanii*” honors Dr. John M. Huisman, the foremost expert on the Nemaliales today, for, among other things, his prolific systematic work on the order over the past three decades, culminating in his recognition of the *D. marginata* complex using genetic techniques (Huisman et al. 2004, Kurihara and Huisman 2006).

Holotype

T.R. Popolizio 12-117-5 [BDA1492], 20 Sept. 2012, Hog Breaker, north shore Bermuda I., 32°27′47.7″N, 64°49′48.9″W, Bermuda, western Atlantic Ocean, depth 12 m [MICH] (Figure 8); isotype Herb. CWS.

Paratypes

Bermuda – *A.B. Hervey, P.B.-A.* no. 1930 [Collins et al. 1913, as *Brachycladia marginata*], 22 Jan. 1913, near low water mark south shore, Bermuda I. [Herb. CWS] (Figure 7); *CWS/CEL* 10-5-13 [BDA0024], 19 August 2010, reef off Frick’s Beach, 32°19′56.0″N, 64°40′20.7″W, Tucker’s Town, Bermuda I., depth 10–12 m (Figure 9); *TRP/CWS* 12-151-11 [BDA1669], *TRP/CWS* 12-158-3 [BDA1709], *TRP/CWS* 12-170-9 [BDA1805] (see Table 1 for collection details).

Distribution

Endemic to Bermuda as presently known.

Remarks

The new species is presently very rare in Bermuda and, when found, there are few individuals in the population. When Collins and Hervey (1917) first reported *Galaxaura marginata* from Bermuda, they cited only their shallow water collection from Gravelly Bay (*P.B.-A.* no. 1930; Figure 7), but clearly there were enough specimens available at the time to make herbarium specimens for

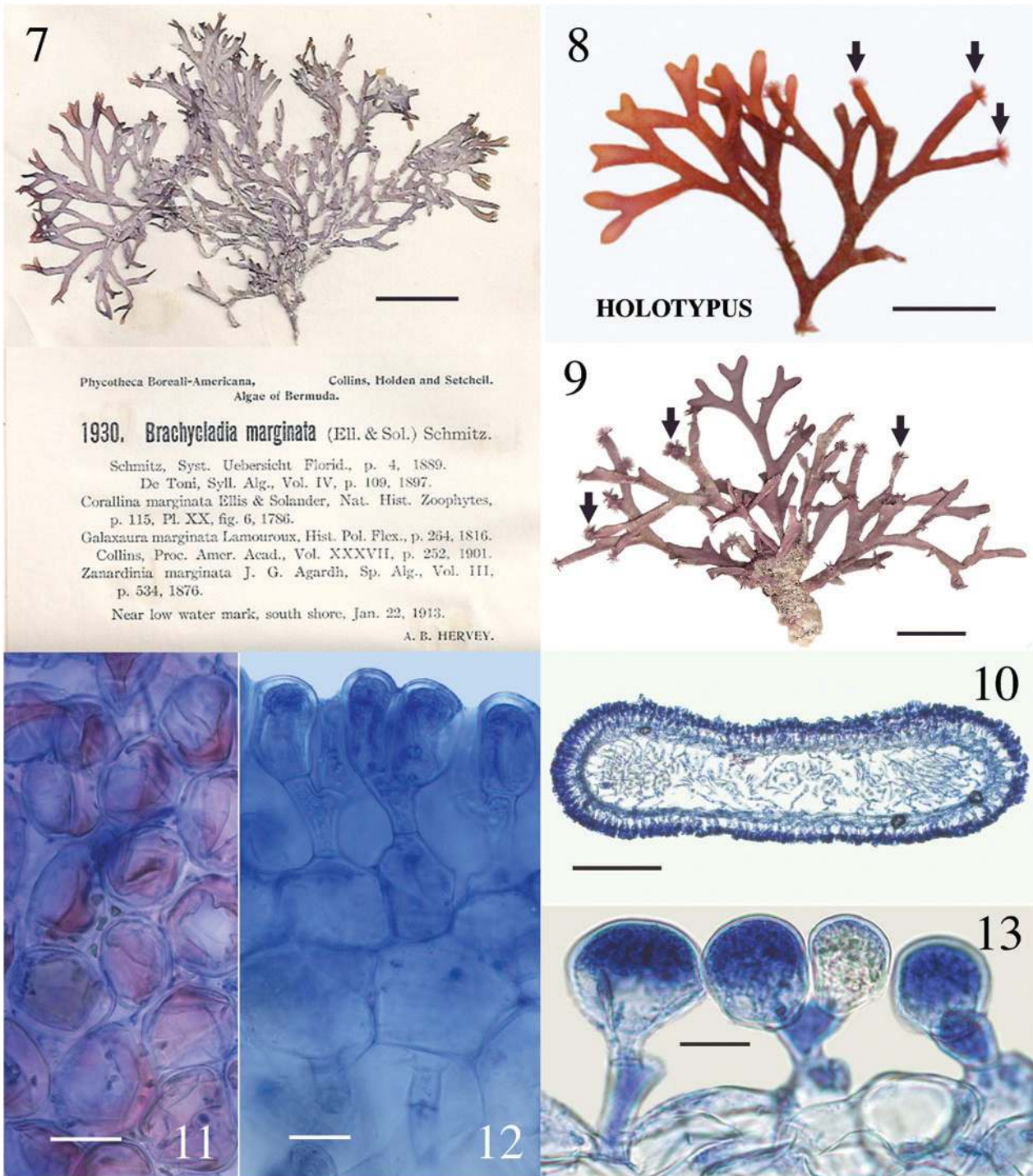
Table 2: Morphological characters of sequenced isolates used for genetic comparison of western Atlantic species in the *Dichotomaria marginata* complex.

Species	<i>Dichotomaria marginata</i>	<i>Dichotomaria huismanii</i> sp. nov.	<i>Dichotomaria</i> sp. 1CRB
Plant height (cm)	To 10	To 8	To 11
Plant width (cm)	To 13	To 12	To 14
Axis in transverse section	Flattened throughout	Subterete below, flattened above	Flattened throughout
Branch angles	72–89°	(32–) 41–54 (–68)°	41–89°
Internode length (mm)	4.7–13.7	3–9	4.8–13.3
Internode width (mm)	1.6–2.6	1.2–2.2	1.0–2.1
Annulations	Faint	Faint when present	Faint to strong
<i>Sporophyte</i>			
Cortical cell layers	3, rarely 4, including stalk cells	4 including stalk cells	4 including stalk cells
Outer cortical cells	Ovoid to subspherical in section, circular to ellipsoidal in surface view	Ovoid to subspherical in section, irregularly polygonal in surface view	Ovoid to lanceolate in section, polygonal in surface view
Outer cortical cell height (µm)	20–40	28–50	34–50
Outer cortical cell diameter (µm)	(21–) 33–49	22–48	17–34
Apiculae on outer cortical cells	Rare	Absent	Numerous
Stalk cell length (µm)	15–36	23–45	25–41
Stalk cell, center diameter (µm)	5–10	5–14	7–15
Subcortical cell length (µm)	25–68	21–48 (–70)	27–43
Subcortical cell diameter (µm)	50–115	(32–) 60–93	21–30
Medullary filament diameter (µm)	7–13	13–26	6–15
Type locality	Bahamas Is., West Indies	Bermuda	–
Collection region of specimens	St. Croix, USVI, West Indies	Bermuda	St. Kitts, St. Croix

each of the 80 distributed sets of the exsiccata they were ultimately placed in (Fahey and Doty 1955). Collins and Hervey (1917) did not distinguish their collections in any feature from the standard characteristics of *G. marginata* taken from earlier sources available to them at the time. Interestingly, all of the *Dichotomaria huismanii* specimens we have collected show many truncated branch tips that have been grazed by herbivores (Figures 8 and 9). Often on these plants, closely adherent medullary filaments that run longitudinally through the axes emerge from the truncated tips, and occasionally from branch nodes, as caespitose tufts of loose, uniseriate filaments, appearing much like a dense epiphytic growth of an acrochaetoid alga (Figures 8 and 9; we sequenced these tufts and they were a 100% match to the macroscopic plant on which they form). Taylor (1960) may have observed the same for *D. marginata* when he mentioned “branches occasionally tipped with a brush of deciduous hairs”, but he made no mention of herbivory. It is worth noting that all of our recent collections from Bermuda are much smaller (to 4 cm tall, 7 cm across) and less robust than those distributed in 1913 fascicles of *P.B.-A* (8 cm tall, 12 cm across; Figure 7). The reduced habit appears to be a trend seen in many seaweeds collected in Bermuda during the latter part of the 20th and first part of the 21st centuries, these being smaller than herbarium collections made in the

early years of the 20th century. We believe increased fish herbivory in Bermuda may be responsible for the more diminutive plants of today, as was shown for two small *Botryocladia* spp. growing on the reefs compared to large, fully formed, non-grazed plants in display tanks of the Bermuda Aquarium (Schneider and Lane 2008). Increased herbivory could also account for the fewer individuals found today in Bermuda populations as compared with the distant past, as well as a lack of specimens from shallow waters, the only habitat reported for this species by Collins and Hervey (1917).

Thus far, the new species is known only from Bermuda and represents the only flattened member of the genus in the local flora. *Dichotomaria huismanii* is genetically distinct from *D. marginata* in the West Indies (4.8% bp differences in COI-5P sequences, 2.4% in *rbcL*), the islands of Bermuda being isolated from the closest populations of the genotype by a distance >1350 km to the northeast of the Bahamas. St. Kitts/St. Croix isolates (*Dichotomaria* sp. 1CRB, Table 1) are sister to the new species in the COI-5P tree (Figure 1), but show 4.1% bp differences with it (22 nucleotide differences out of 538). The *rbcL* clade where *D. huismanii* is resolved (Figure 2) also includes a specimen identified as “*D. marginata*” from the Gulf of California (GenBank AY688022). The *rbcL* sequences from Bermuda and Mexico are 0.6% different from each other (there is no COI-5P data for the Mexican



Figures 7–13: *Dichotomaria huismanii* sp. nov.

(7) Bermuda P.B.-A. no, 1930, as *Brachycladia marginata*, scale bar=2 cm. (8) Holotype specimen [BDA1492]; truncated axes with emergent medullary filaments (arrows), scale bar=1 cm. (9) Paratype specimen [BDA0024]; truncated axes with emergent medullary filaments (arrows), scale bar=1 cm. (10) Transverse section of axis [BDA1709], scale bar=200 μ m. (11) Surface view of outer cortical assimilatory cells [BDA0025], scale bar=25 μ m. (12) Section through axis showing two subcortical cell layers bearing pairs of ovoid outer cortical assimilatory cells and medullary connections [BDA0025], scale bar=20 μ m. (13) Section through cortex showing subcortical cells bearing pairs of subspherical outer cortical assimilatory cells [BDA1492], scale bar=20 μ m.

isolate). The Bermuda and Mexico species are each 0.6% different in *rbcl* bp from the two Caribbean sequences of *Dichotomaria* sp. 1CRB (Table 1).

Aside from the geographic separation and genetic difference, a few morphological characteristics present themselves as means for differentiating *D. huismanii* from its cryptic counterpart in the western Atlantic, *D. marginata*. In gross morphology, *D. huismanii* is difficult to distinguish from *D. marginata*, but subtle differences can be found anatomically. The dimensions of cortical cells, thallus height and width, and the length and width of internodes all demonstrate a great amount of overlap between the two species (Table 2). But unlike *D. marginata*, none of the assimilatory cells on *D. huismanii* are apiculate (Figures 11–13), a character that may be of use in western Atlantic populations of *Dichotomaria* after a more thorough examination of isolates is made from the region of the Bahamas, throughout the Caribbean and south to Brazil. The new species has four cortical cell layers including stalk and outer assimilatory cells (Figure 12) and in general has greater diameter medullary filaments (13–20 μm) than the generitype *D. marginata* (7–13 μm), which mostly shows three cortical cell layers (Figure 5). The dichotomous axes of *D. huismanii* branch at markedly narrower angles than St. Croix specimens of *D. marginata*, 32–68° vs. 72–89°. Whether any of these anatomical character differences will hold up as more isolates from the Caribbean are sequenced remains to be seen.

Using the chloroplast *rbcl* gene, along with the western Atlantic species mentioned above, *D. huismanii* clusters with two Indo-Pacific species, *D. tenera* and *D. intermedia* (Figure 2). The only detailed description of *D. tenera* since its genetic analysis and resurrection by Huisman et al. (2004) is found in De Clerck et al. (2005) based on specimens from Kwazulu-Natal, South Africa, the geographic area from which Huisman et al. (2004) used isolate sequences to segregate it from Caribbean *D. marginata*. These Indian Ocean specimens have somewhat broader flattened branches (1.5–3.0 mm) than *D. huismanii* (1.2–2.2 mm), and unlike the new species have axes that are hirsute above the holdfasts and produce outer cortical cells that are apiculate at the margins (De Clerck et al. 2005). Specimens of *D. tenera* from Mauritius are reported to have outer cortical cells that are 38–42 μm tall and 27–30 μm diameter (Børgesen 1942, as *Galaxaura tenera* Kjellman), well within the range of these cell sizes in *D. huismanii*. Their branch angles (measured from Børgesen 1942, figure 24) are from 50–75°, a slightly wider angle range than that of the new species reported here (Table 2). A specimen attributed to *D. tenera* from South Africa and pictured by Kylin (1938), as *G. tenera*, has much narrower branch angles, 40–55°, than the Mauritian sample.

The second species that genetically clusters with *Dichotomaria huismanii* (Figure 2), *D. intermedia* (R.C.Y. Chou) Wiriyadamrikul, M.J. Wynne et S.M. Boo (type locality=Galapagos Is.), is considerably larger (to 23 cm) than the new species (to 8 cm). Despite its overall large size, *D. intermedia* has similar cell dimensions for outer cortical cells on tetrasporophytes, 30–50 μm tall and 25–35 μm diameter (Wiriyadamrikul et al. 2014), as compared to those for *D. huismanii*, 28–50 μm tall and 22–48 μm diameter (Table 2), but unlike the new species, these cells are apiculate.

Morphological comparisons of *Galaxaura* and *Dichotomaria* species from locations other than their type localities with species from different parts of the world create similar problems to those we have already alluded to for genetic data from distant locations. For example, prior to the availability of molecular sequencing and comparison of species, Chou (1945) used Pacific Costa Rican samples of *G. stupocaulon* Kjellman (type locality=Brazil) to characterize this species, and make comparisons with other members that would be included in the *D. marginata* complex today (Huisman et al. 2004, Wang et al. 2005, Kurihara and Huisman 2006, Wiriyadamrikul et al. 2014). Oliveira (1977) noted the “great similarity” of *Galaxaura angustifrons* Kjellman (type locality=Brazil) from Brazil to *G. veprecula* Kjellman (type locality=Madagascar) from the Philippines and Ecuador based on Chou’s (1947) observations and measurements. *G. veprecula* was subsequently placed in synonymy with *G. tenera* by Papenfuss and Chiang (1969), species later subsumed in *G. marginata* (Papenfuss et al. 1982). Finally, *G. tenera* was resurrected as *Dichotomaria tenera* by Huisman et al. (2004) based on LSU sequence data (and the morphology of terminal cortical cells) of specimens from South Africa. It seems obvious from these convoluted pathways that historical taxonomic decisions involving *Galaxaura* and *Dichotomaria* species need to be reassessed from the fresh perspective of DNA sequencing, as has been done in selected recent studies (Huisman et al. 2004, Wang et al. 2005, Kurihara and Huisman 2006, Wiriyadamrikul et al. 2014). Accordingly, what has been combined in the past under *D. marginata*, has already begun fragmenting back to a greater number of species.

For their Indian Ocean and Australian specimens of *Dichotomaria* “*marginata*”, Huisman et al. (2004) were able to resurrect species names for flattened taxa from those areas with specimens that did not genetically match the sequence of true *D. marginata* from Puerto Rico. The resurrected *D. australis* and *D. tenera* had been among a large number of species that, over the years and culminating with the Papenfuss et al. (1982) circumscription, were placed in synonymy with *D. marginata*. In the western Atlantic, there are early names presently considered junior

synonyms of *D. marginata*: *G. angustifrons*, *G. frutescens* Kjellman (type locality=Brazil), *G. occidentalis* and *G. stupocaulon*. None of these taxa were ever reported from Bermuda (Taylor 1960). The above three species presently in the *D. marginata* complex with type localities in Brazil do share some resemblance to both *D. marginata* and *D. huismanii*, and are presently considered synonyms of the former. Observations for these species on type specimens is critical to linking anatomical measurements/characteristics to recently collected genetic species from near their type localities in this highly cryptic complex of species. As none of the historical species were described locally, we choose to describe our Bermuda specimens as a new species from the northern limit of distribution of the genus in the western Atlantic. Genetic information from other areas to the south may prove this species to have a more widespread distribution than presently delimited.

The separation and description of a new cryptic species from Bermuda for records that in the past were considered a part of a pantropical species' range with a western Atlantic type is similar to other nemalialean examples already genetically distinguished in the islands. What was identified in Bermuda as *Helminthocladia calvadosii* (J.V. Lamouroux ex Duby) Setchell for over a century was shown to be *H. kempii* Popolizio, Chengsupanimit et C.W. Schneider (Popolizio et al. 2013), and what was known as *Liagora ceranoides* J.V. Lamouroux since the early 1900s in the islands was recently described as *L. nesophila* Popolizio, C.W. Schneider et C.E. Lane (Popolizio et al. 2015).

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