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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 [1]

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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* Popolizio, 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. *Helminthocladia kempii* sp. nov. (Nemaliales, Liagoraceae) basée sur *H. calvadosii sensu auct.* d'atlantique de l'ouest. Depuis la récolte initiale dans les Keys de Floride et dans les iles des Bermudes au milieu du 19e *Helminthocladia calvadosii sensu auct.* (localité type : Calvados, France) a aussi été identifié dans les Caraïbes et dans des localités aussi septentrionales que le nord du Brésil. Jusqu'a présent, les récoltes d'Atlantique est et ouest n'ont pas été comparées en utilisant une approche d'alpha taxonomie assistée par des outils moléculaires. De récentes collectes de *H. calvadosii* aux Bermudes présentent globalement un aspect distinct des plantes conspécifiques en provenance de l'Atlantique ouest, et sont plus similaires a *H. reyesii*

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(localité type : îles Canaries). En utilisant le marqueur mitochondrial COI-5P, nous avons élucidé les relations entre les spécimens de *H. calvadosii* en provenance des Bermudas et ceux proche de la localité type vérifiant ainsi leur placement générique au sein des Liagoraceae et démontrant leur trait distinctif. Sur la base de leurs caractéristiques végétatives et reproductives, nous concluons que les spécimens historiquement identifié comme *H. calvadosii* aux Bermudes représentent une nouvelle espèce que nous proposons de nommer *Helminthocladia kempii* Popolizio, C.W. Schneid. *et* Chengsupanimit sp. nov.

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

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 vouchering 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

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*
Cumagloia andersonii (Farl.) Setch. *et* 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
Helminthocladia australis 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

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

Name	*Voucher*	***BOLD***	*GenBank*	*Collector(s)*	*Collection site*
	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
Helminthocladia calvadosii (Duby) Setch.	LLG3069	CNEM002-10	HQ603218	Y. Galdu, L. Le Gall & A. Besnier	Beniguet, Finistère, Brittany, France
Helminthocladia dotyi Womersley	GWS002626	**ABMMC8944-10**	**KC355084**	G.W. Saunders	Remarkable Cave near Port Arthur, Tasmania, Australia
Helminthocladia hudsonii J. Agardh	LLG3000	CNEM003-10	HQ603219	L. Le Gall, J.M. Utge & Y. Turpin	Lampaul île Ségal, Finistère, Brittany, France
Helminthocladia kempii 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
Helminthocladia rhizoidea Doty *et* I.A. Abbott	HADB03858	—	HQ423009	A. Kurihara	Malaekahana Beach Park, Oahu, Hawaii

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* (Kützing, 1866; Hauck, 1885, as *H. purpurea* (Harv.) J. Agardh; Gayral, 1966; Dixon & Irvine, 1977; O'Dwyer & Afonso-Carrillo, 2001) are shown to have prominent irregularly or pectinately branched main axes (see Kützing, 1866, pl. 62c, as *Nemalion purpureum* (Harv.) Chauv.; Hauck, 1885, Fig. 17a, as *H. purpurea*; O'Dwyer & Afonso-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).

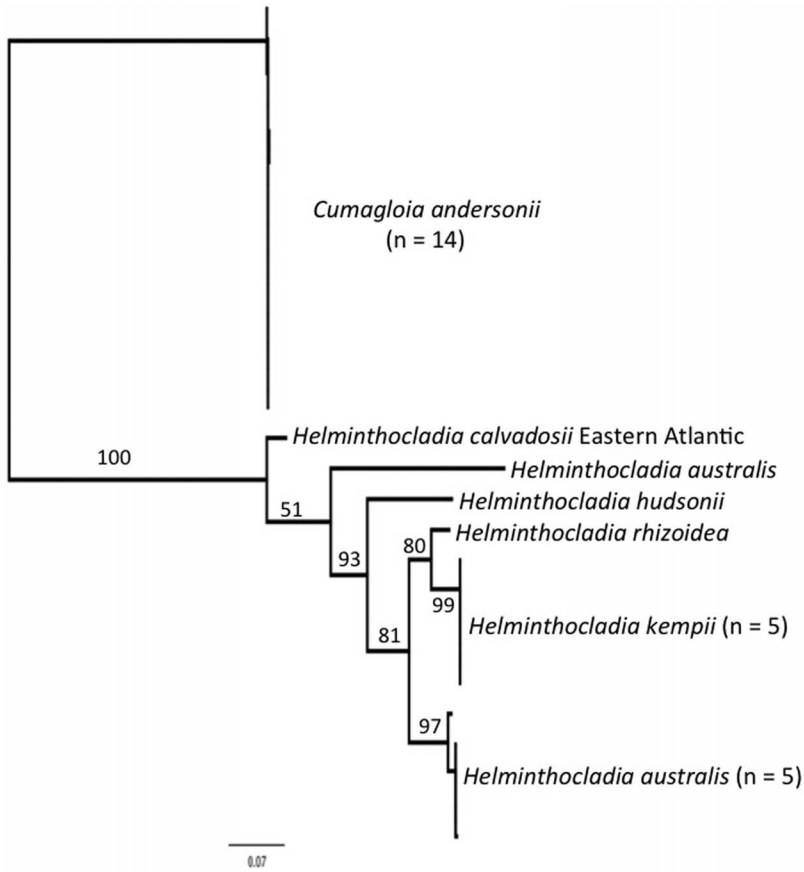


Fig. 1. Maximum likelihood phylogeny generated from the COI-5P alignment resolving the Bermudian collections assigned here to *H. kempii* sp. nov. as sister to *H. rhizoidea* and only distantly related to *H. calvadosii*. Bootstrap support (% of 500 replicates) is indicated along the branches. Refer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

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

	Helminthocladia calvadosii	*Helminthocladia 'calvadosii'*	*Helminthocladia kempii* sp. nov.	*Helminthocladia reyesii*
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. × length µm)	3-6 × 80-150	nd	3-12 × 35-70	5-22 × 60-200
Size 15 mm fr. apex (diam. × length µm)	to 12 × to 160	nd	11-27 × 70-175	20-50 × 200-350
Cortical fascicles				
Number of cells, base to apex	4-6	3-5 [a]	3-6	6-8
Length of cortical fasicles (µm)	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. × length in µm)	7.5-9.0 × 20-30	7.5-10.0 × 25-35 [a]	7-10 × 18-35	7.5-20.0 × 52-100
Subterminal cell (diam. ×length µm)	13.0-17.5 × 15-20	9.5-12.0 × 15-25 [a]	12-15 × 15-22	6-19 × 11-28 [b]
Apical cell (diam. × length µm)	(15-)20-26(-32) × 27-36(-50)	15-23 × 25-35(-52) [a]	10-20 × 20-35	20-35 × 36-60
Reproduction				
Gametophyte	monoecious	monoecious	dioecious	monoecious or dioecious

[a] calculated from Guimarães *et al.* (1990)
[b] calculated from O'Dwyer & Afonso-Carrillo (2001)
nd = no data

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

	Helminthocladia calvadosii	*Helminthocladia 'calvadosii'*	*Helminthocladia kempii* sp. nov.	*Helminthocladia reyesii*
Spermatangium diam. (µm)	3-5	2.5-5.0 [a]	2-5	2-3
Spermatangial clusters	paniculate	paniculate	paniculate	paniculate
Carpogonial branch (diam. × length µm)	8-10 × 21-23	7.5-11 × 20-22 [a]	7.5-10.0 × 30-38	10-13 × 24-43
Number carpogonial branch cells	(2-)3(-4)	3-4 [a]	3-4	3(-4)
First carpogonial division	oblique or transverse	oblique	transverse or oblique	transversely oblique
Post-fertilization fusion of carpogonial 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. × length µm)	4-8 × 12-15	2-4 × 9-15 [a]	3.5-4.5 × 7-9	3-5 × 10-16
Mature carposporophyte diam. (µm)	100-270	85-130	50-80	140-250
Carposporangia (diam. × length µm)	7-9 × 15-20	3.5-6.5 × 7.0-12.5 [a]	3-5 × 7-9	5-7 × 11-20
Seasonal appearance	May-Oct	Apr-Sept	Jan-Apr	(Mar-) Apr-Jul (-Aug)
References	Dixon & Irvine (1977) O'Dwyer & Afonso-Carrillo (2001)	Guimarães *et al.* (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

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 μm) than *H. reyesii* (to 400 μm), 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:

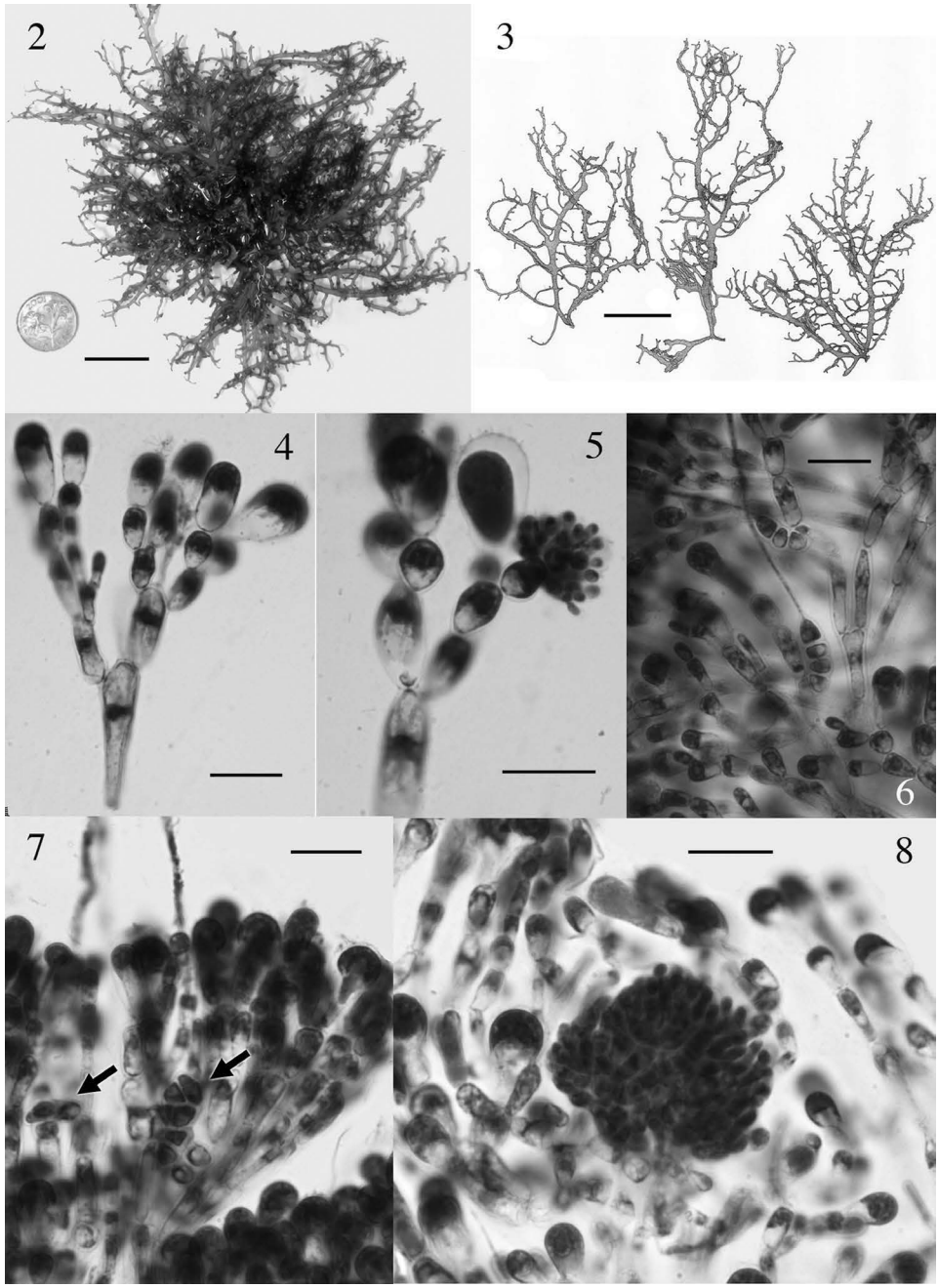
***Helminthocladia kempii* Popolizio, C.W. Schneid. *et* Chengsupanimit sp. nov.**
Figs 2-11

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).

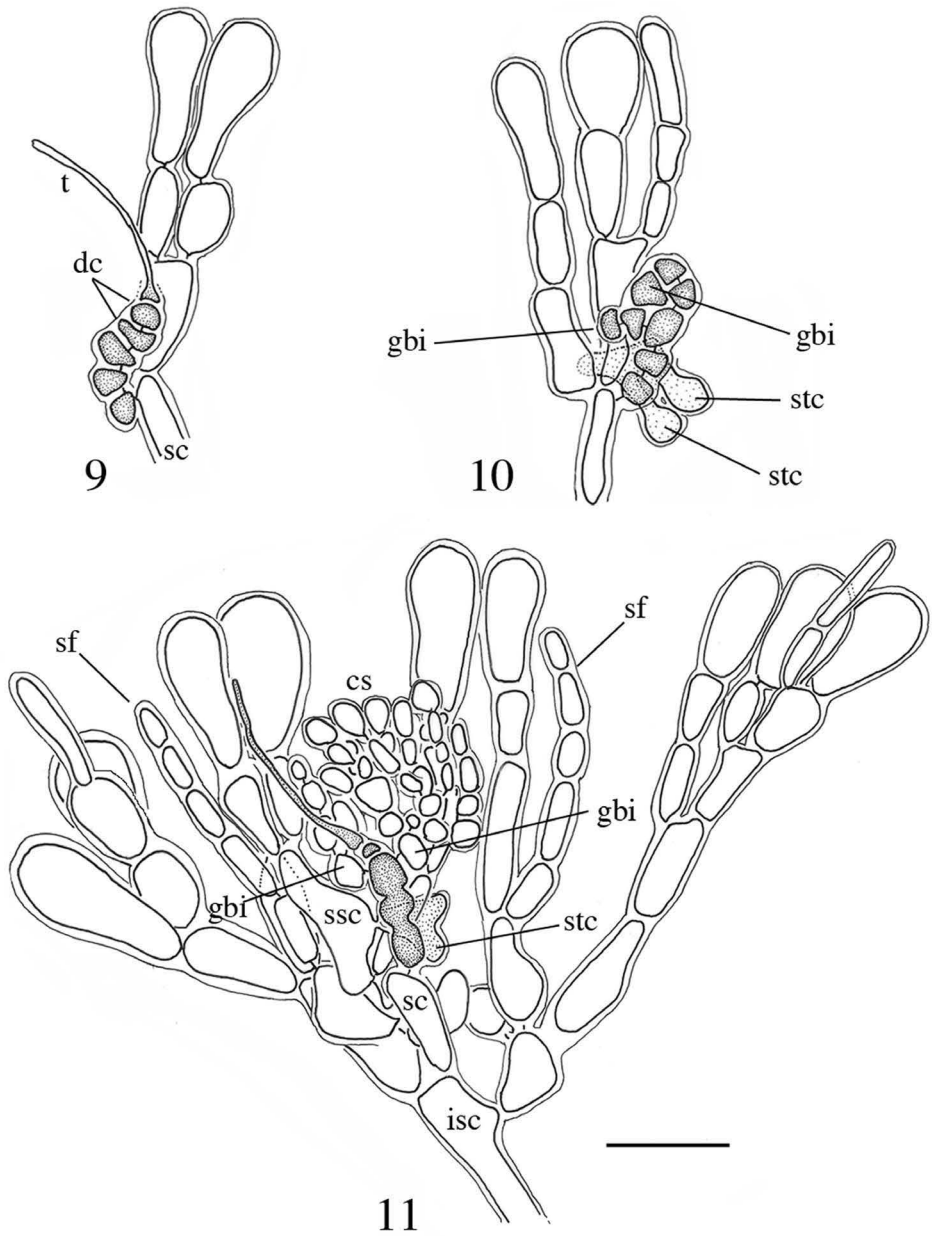
Etymology: Named for the Rev. Alexander Ferrie Kemp (1822-1884), a Scottish-born Canadian Presbyterian minister posted to Bermuda during the 1850s when he collected and published the first report on the algae of the islands (Kemp, 1857), including the first collection of the red alga that now bears his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



Figs 2-8. *Helminthocladia kempii* sp. nov. **2.** Holotype specimen, *CWS* 12-10-14 (BDA0591). Scale bar = 2 cm. **3.** Isotype specimens. Scale bar = 2 cm. **4.** Cortical fascicle, *CWS* 12-10-14. Scale bar = 25 µm. **5.** Spermatangial cluster on cortical fascicle, *CWS* 12-10-14. Scale bar = 25 µm. **6.** Three- and four-celled carpogonial branches with extended trichogynes, *CWS/CEL* 03-31-18. Scale bar = 25 µm. **7.** Post-fertilization carpogonia with early gonimoblast initials (arrows) and withering trichogynes, *CWS/CEL* 03-31-18. Scale bar = 25 µm. **8.** Carposporophyte with weakly investing sterile involucral filaments, *CWS/CEL* 03-31-18. Scale bar = 25 µm.



Figs 9-11. *Helminthocladia kempii* sp. nov. Scale bar = 25 µm. **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.

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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