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Phycologia

Online ISSN: 2330-2968

DOI: 10.1080/00318884.2019.1661158

Publisher: Taylor and Francis

Published Article URL: <https://www.tandfonline.com/doi/full/10.1080/00318884.2019.1661158>

Version of Record available: <https://doi.org/10.1080/00318884.2019.1661158>

This is an Accepted Manuscript of an article published by Taylor & Francis in Phycologia on 2019-10-27, available online:  
<https://www.tandfonline.com/10.1080/00318884.2019.1661158>.



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**New species of *Galene* and *Howella* gen. nov. (Halymeniaceae, Rhodophyta)  
from the mesophotic zone off Bermuda**

CRAIG W. SCHNEIDER<sup>1</sup>, THEA R. POPOLIZIO<sup>2</sup>, LESLEIGH G.K. KRAFT<sup>3</sup> AND GARY W. SAUNDERS<sup>3</sup>

*<sup>1</sup>Department of Biology, Trinity College, Hartford, Connecticut 06106, USA*

*<sup>2</sup>Department of Biology, Salem State University, Salem, Massachusetts 01970, USA*

*<sup>3</sup>Centre for Environmental & Molecular Algal Research, Department of Biology,  
University of New Brunswick, Fredericton, New Brunswick E3B 5A3, Canada*

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**CONTACT** Craig W. Schneider [cschneid@trincoll.edu](mailto:cschneid@trincoll.edu)

Right Header: Schneider *et al.*: Bermuda mesophotic Halymeniaceae

**ARTICLE HISTORY**

*Received 29 May 2019; revised 18 August 2019; accepted 25 August 2019*

## ABSTRACT

The mesophotic zone off the coast of Bermuda has been explored for macroalgae beginning with the R/V *Seahawk* cruises of the 1980s and most recently on the Nekton XL Catlin cruise of the R/V *Baseline Explorer* in 2016. In this paper, we present two new members of the Halymeniaceae discovered on these missions based upon a combined ML analysis of mitochondrial (COI-5P), plastid (*rbcL*) and nuclear genes (LSU), as well as morphological and anatomical characteristics. *Howella gorgoniarum* gen. et sp. nov. conspicuously grows on the base of soft corals, and represents one of three species in the new genus. Two species of the non-monophyletic *Thamnoclonium*, *T. latifrons* Endlicher & Diesing and *T. lemmanianum* Harvey from South Africa and Australia, are moved to *Howella* based upon their molecular phylogenetic placements. First collected offshore of Bermuda in 1960 and misidentified as *Halymenia hancockii*, *Galene leptoclados* sp. nov. represents the first species in its genus collected outside of the western Pacific.

## KEYWORDS

COI-5P, *Galene leptoclados* sp. nov., *rbcL*, *Howella gorgoniarum* gen. et sp. nov., LSU, *Thamnoclonium*, western Atlantic.

## INTRODUCTION

The mesophotic zone, the area of our world's oceans where light-dependent communities survive in extreme light-limited habitats, has developed unusual biota where eurybathic organisms mix with species living only in these deep-water environments. Depending upon the clarity of the seawater, mesophotic zone depths vary; for example, from 30 to 100 m in the warm temperate environments offshore of the southeastern United States (Schneider 1976) to over 250 m in the clear tropical waters

of the Caribbean (Littler *et al.* 1985). Two deep-water expeditions off the coast of Bermuda, the first in the 1980s prior to DNA sequencing as a regular tool for phylogenetic studies, and a recent Nekton scientific cruise in 2016, have turned up a number of species that have been recognised as new to science (Wynne & Schneider 1996; Schneider & Searles 1997, 1998; Dixon *et al.* 2015; Richards *et al.* 2018; Schneider *et al.* 2018, 2019). The first papers from the 2016 cruise demonstrate that the offshore mesophotic zone hosts a community of red algae (Rhodophyta) that is quite different than the extensively studied nearshore euphotic zone. Algal collections in the mesophotic zone of the western Atlantic summarised by Ballantine *et al.* (2016) highlight the large number of species at their physiological limits for photosynthesis, many described as new to science. Using DNA sequencing, the mesophotic collections included *Sporolithon mesophoticum* J.Richards, P.W.Gabrielson & C.W.Schneider from 178 m, the deepest living algal specimen collected off Bermuda (Richards *et al.* 2018). Discoveries also found increased biogeographic ranges for genera of the Kallymeniaceae (Gigartinales). One new species, *Nothokallymenia erosa* C.W.Schneider, Popolizio & G.W.Saunders, represented a genus previously known only from the northeastern Atlantic, while another, *Psaromenia septentrionalis* C.W.Schneider, Popolizio & G.W.Saunders, provided evidence of a deep-water community with genetic connections to the Indo-Pacific (Schneider *et al.* 2019). A member of the Halymeniaceae (Halymeniales) from these same collections, *Cryptonemia abyssalis* C.W.Schneider & Popolizio, was genetically more similar to an undescribed species of the genus from the Mediterranean and Pacific (unpublished data) rather than its five congeners living in the waters around the Bermuda platform (Schneider *et al.* 2018).

In this paper, we discuss two additional members of the Halymeniaceae that were collected in the mesophotic zone off Bermuda. Using mitochondrial (COI-5P), plastid (*rbcL*) and nuclear (LSU) genes, as well as morphological characteristics, we can now taxonomically elucidate species that are

either only rarely found because of collection limitations associated with their exceptionally deep-water habitats, or others that are commonly collected yet consistently lack reproduction. Prior to the regular use of molecular systematics in the 1990s, workers reluctantly designated new taxa even for commonly collected specimens that were known only in the vegetative condition (Taylor 1974). With the advent of DNA sequencing, even for collections without known reproductive characteristics, phylogenetic placement is now routine (Cianciola *et al.* 2010), offering phylogeneticists a more detailed look at evolutionary relationships within a genus or family. Of course, at times this leads to erection of new species where they are best distinguished solely by genetic information; thus, truly cryptic species have increasingly appeared in the literature (see Schneider *et al.* 2017). Such species are distinguished from their closest congener by genetic sequences that are similar to the percent differences found in species with morphological characteristics, including reproduction, to separate them.

One of the taxa discussed here was collected dozens of times from the 1980s to the present, invariably found lacking reproduction, causing us earlier to continue to search for additional specimens to better describe our specimens taxonomically. More recent collections in the mesophotic zone have been sequenced and genetically compared with other Halymeniaceae, allowing us to provide a robust phylogenetic placement as well as describe their unusual clustering of cystocarps at the apices of branches. The second species we discuss in this paper is based upon a recent collection from 90 m that allowed us to examine herbarium records from deep-water in Bermuda where it had also been collected earlier and identified as *Halymenia hancockii* W.R.Taylor.

## **MATERIAL AND METHODS**

### **Standard methods**

Collections from the mesophotic zone off the coast of Bermuda were made beginning on the 1983 and 1985 R/V *Seahawk* cruises using Scuba and surface-supplied air (Searles & Schneider 1987), and on the 2016 cruise of the R/V *Baseline Explorer* by a team of nine technical divers (Global Underwater Explorers, High Springs, Florida, USA) equipped with closed-circuit JJ-CCR CE Edition rebreathers (JJ-CCR ApS, Copenhagen, Denmark) modified to GUE configuration, and Triton 1000-2 class submersibles (Vero Beach, Florida, USA) with mechanical arms. Voucher specimens are deposited in the herbaria under each species (herbarium abbreviations follow the online *Index Herbariorum*, <http://sweetgum.nybg.org/ih/>). Collection site locations were recorded with a Garmin™ eTrex H GPS (Olathe, Kansas, USA) or, on the R/V *Baseline Explorer*, using a Beier Radio DP1 (dynamic positioning, Beier Integrated Systems, Mandeville, Louisiana, USA) to receive shipboard GPS. Live specimens chosen for DNA analysis were photographed using a Canon Powershot s90 digital camera (Canon Inc., Tokyo, Japan), then fragments of individuals were dried on silica gel for DNA extraction, and preserved with 4-5% formaldehyde in sea water for anatomical study. Finally, the specimens were pressed fresh on herbarium paper as permanent vouchers. Thin sections were made with an American Optical freezing microtome model 880 (San Diego, California, USA) and mounted in 30% corn syrup with acidified 1% aniline blue in a ratio of 20:1. Photomicrographs were taken using Zeiss Axioskop 40 microscope or Zeiss Stemi 2000–CS stereomicroscope (Oberkochen, Germany) equipped with a Spot Idea 28.2–5MP digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA).

### **Molecular methods**

Five mesophytic halymeniacean specimens from Bermuda and St. Croix (Table S1) were assigned to genetic groupings using COI-5P and/or *rbcL*-3P sequences as outlined previously in Saunders &

Moore (2013) following DNA extraction (Saunders & McDevit 2012). To place these species into a wider phylogenetic context COI-5P, LSU and *rbcL* sequences were generated when possible for the novel genetic groups from Bermuda, as well as for a variety of related Halymeniaceae (Table S1; some generated here, others from previous work in the Saunders lab) following published protocols (Saunders & Moore 2013). To supplement our analyses, *rbcL* data from nine related species were downloaded from GenBank (Table S1). Ultimately, three alignments were generated using the MUSCLE algorithm in Geneious 10.2.4 (<https://www.geneious.com>; Kearse *et al.* 2012) and subsequently refined by eye (LSU only): COI-5P with 22 sequences of 661 bp (base pairs); *rbcL* with 34 sequences and 1358 bp; and 24 LSU sequences with an alignment of 2002 sites after variable regions were removed. The gene alignments were analysed independently with maximum likelihood (GTR+I+G) using RAxML (Stamatakis 2014) with partitioning by codon (for the two protein coding genes) and 500 bootstrap replicates. Since no conflicts were detected between the individual gene topologies, a concatenated COI-5P, LSU and *rbcL* alignment was constructed (34 sequences, 4021 bp) and analyses as described above, but with partitioning by gene and codon and with 1000 bootstrap replicates. This resulting topology was rooted along the branch between *Eiploea/Gelinaria/Halymenia* (serving as outgroup taxa) and the remaining Halymeniaceae.

## RESULTS

Two COI-5P sequences were successfully generated for four mesophytic halymeniacean collections from Bermuda (Table S1). The sequences of these two specimens (BDA1999, 2000) are identical and highly divergent from all other species included in our study (6.6% divergent from nearest neighbor *Galene* sp. 1LH), indicative of a distinct species. This genetic group is sister to a clade containing known and undescribed species of the genus *Galene*. For the other two specimens (BDA0443, 0444),

*rbcL*-3P revealed a second genetic group whose sequences were identical for this marker (n = 800 bp), but again the nearest neighbor was 4.5% divergent (across the entire *rbcL*), which unequivocally indicates a distinct species. A specimen from St. Croix (STX071) for which 600 bp of *rbcL*-3P was determined also joined this genetic group that formed a branch including two species of *Thamnoclonium* from Australia (GENT O241) and South Africa (GENT KZN2134) (Table S1). Our phylogenetic results indicate that the two offshore Bermuda taxa need to be described as new to science, and that *Thamnoclonium* is polyphyletic. We therefore propose the following taxonomic treatment.

***Galene leptoclados* C.W.Schneider, Popolizio & G.W.Saunders sp. nov.**

**Figs 2-8**

DESCRIPTION: Plants light rosy-red and erect to 6 cm tall, composed of ligulate, bifurcate to irregularly branched blades; one or more proximally tapering blades on simple to branched stipes to 2 mm long, arising from small discoidal holdfasts (Fig. 2); proliferous blades arising from margins in older proximal portions of plants as well as terminally at wound sites (Fig. 3), occasionally directly from blade surfaces; blades increasing in breadth and becoming more lightly pigmented distally, to 1.2 cm wide and 34–46  $\mu\text{m}$  thick in upper blade portions, the margins entire; blades initially bearing a two-layered cortex on both blade surfaces with outer cortical cells elongate to quadrate in transverse section, 4.5–8.3  $\mu\text{m}$  diam., irregularly polygonal in surface view forming a compressed surface with thick cell walls (Fig. 4); inner cortical layer remaining at the margins, but laminally mostly becoming stretched by connection to the filamentous medulla (Figs 5, 6); medulla comprised of a taut mucilaginous matrix with attenuate intertwining, elongate filaments, 1.5–3.0  $\mu\text{m}$  diam., directly



connected to small-bodied ganglial cells as an anastomosing network, these cells not staining darkly (Fig. 7); hemispherical cystocarps protruding from the blade surfaces, to 400 µm in diam., occasionally adjacent and overlapping; carposporangia irregular to pyriform, to 9.5 µm diam.; spermatangial mother cells formed in small isolated patches over single and adjacent cortical cells, 3.6–7.2 µm diam. (Fig. 8); tetrasporangia unknown.

HOLOTYPE: *Craig W. Schneider (CWS)/Thea R. Popolizio (TRP)* 16-13-6 [BDA 1999], 31 July 2016, ledge north northeast of St. George's I., Bermuda, western Atlantic Ocean, 32°28.96750' N, 64°35.07183' W, depth 90 m [deposited in UNB] (Fig. 2); isotypes MICH (male), Herb. CWS [BDA2000].

PARATYPE SPECIMENS EXAMINED: Bermuda – *John J. Frederick (JJF)* 60-652 [as ?*Halymenia hancockii*], 10 Aug. 1960, cystocarpic, station no. 137 off Nonsuch I., 32°20.20000' N, 64°37.40000' W, depth 63.8 m [MICH 657514] (Fig. 3); *JJF* 60-668 [as ?*H. hancockii*], 10 Aug. 1960, station no. 136, off south shore, 32°20.78333' N, 64°36.80000' W, depth 60 m [MICH 657513]; *JJF* 60-907 [as ?*H. hancockii*], 7 Sept. 1960, station no. 198, Challenger Bank, 32°05.61667' N, 65°01.10000' W, depth 60.3 m on a rhodolith [MICH 657511].

ETYMOLOGY: *leptocladus* (Gr., f.) translated literally as “thin branches”, for its recognition as the thinnest, most delicate, member of the genus.

DISTRIBUTION: At present, endemic to the mesophotic zone from depths of 60–90 m off Bermuda, western Atlantic Ocean.

***Howella* C.W.Schneider, Popolizio, L.G.Kraft & G.W.Saunders *gen. nov.***

DESCRIPTION: Plants erect and cartilaginous, consisting of single to clustered blades, with one or more blades arising from short stipes off discoidal or host-penetrating holdfasts, associated with sponges or epizoic on soft corals; erect axes simple to alternately or dichotomously to irregularly branched, flattened into narrow ligulate to slightly expanded blades, margins entire to dentate, and undulate to crenate and lacerated, with or without marginal proliferations, the axes often penetrating expanding blades with short proximal or percurrent midribs; blades structurally consisting of a cortex of one to several layers, the multiaxial medulla composed of dense anastomosing filaments between the cortical layers; reproductive structures borne in small, leaf-like proliferations arising from the blades or apically in wart-like clusters.

TYPE SPECIES: *Howella gorgoniarum* C.W.Schneider, Popolizio & G.W.Saunders *sp. nov.*

EPONOMY: An honorific name for Marshall Avery Howe (1867–1936), a botanist and curator at the New York Botanical Garden in the early 20th century (Wynne 2000), an early researcher of the Bermuda algal flora (Howe 1918) including work on the Halymeniales (Collins & Howe 1916), as well as floras from other areas in the western Atlantic Ocean.

***Howella gorgoniarum* C.W.Schneider, Popolizio & G.W.Saunders *sp. nov.***

**Figs 9-14**

DESCRIPTION: Cartilaginous plants epizoic above the bases of soft corals as clusters of blades to 3.5 cm tall arising from short stipes off small to large common discoidal holdfasts (Fig. 9) that penetrate the outer layer of soft corals (Fig. 10); blades ligulate, simple to bifurcate, to 2–6 mm wide and 70–300  $\mu\text{m}$  thick, the undulate margins entire to denticulate and erose; stipes slightly penetrating into the base of blades as short midribs (Fig. 9) that thicken the blades more than 3 times the areas lacking midribs; in basal portions lacking midribs, blades thickest centrally and thinning to the margins; cortex consisting of 2–3(–5) layers, the outer cortex in surface view a mosaic of small to large circular clusters of rounded irregular cells 4.5–9.0  $\mu\text{m}$  in longest dimension (Fig. 11), and rounded elongate rectangular to square, 4.5–7.5  $\mu\text{m}$  long in transverse section (Figs 12, 13); inner cortical layers slightly larger, elongating transversely to twice the size of the outer cortex; medulla connected to inner cortical cells, at maturity comprised of a tight matrix with densely packed, intertwining and swirling, often parallel, elongate filaments (Fig. 13), 1.5–6.0  $\mu\text{m}$  diam.; sporangia unknown; gametophytes monoecious, spermatangia borne from elongated mother cells raised above the outer cortex; cystocarps borne in wart-like clusters at apices of branches thickening the blades (Fig. 14), 150–200  $\mu\text{m}$  diam.; carposporangia obpyriform to irregular, to 19  $\mu\text{m}$  long.

HOLOTYPE: *Craig W. Schneider* 10-29-10 [BDA0443], 24 Aug. 2010, west of High Point, Bermuda I., 32°15.31200' N, 65°02.19600' W, depth 35–36 m, deposited in UNB (Fig. 9); isotype Herb. CWS [BDA0444].

PARATYPE SPECIMENS EXAMINED: **Bermuda** – *CWS/Richard B. Searles (RBS)* 85-9-1, 8 June 1985, northeast of Northeast Breakers, north of St. Catherine's Point, St. George's I., 32°31.20' N, 64°39.60' W, depth 37 m (Fig. 10); *CWS/RBS* 85-10-2, 8 June 1985, northeast of Northeast Breakers,

*loc. cit.*, depth 29 m; *CWS/RBS* 85-11-3, 10 June 1985, east of Mills Breaker, north of Great Head, St. David's I., 32°25.00' N, 64°33.50' W, depth 30–34 m; *CWS/RBS* 85-20-24, 16 June 1985, southwest of Long Bar, west of High Point, Bermuda I., 32°13.70' N, 65°01.00' W, depth 24–27 m; *CWS/Christopher E. Lane (CEL)* 10-12-32 [BDA0128, 0129], 20 Aug. 2010, Middle Buoy, Eastern Blue Cut Channel, north of Daniel's Head, 32°23.34333' N, 64°53.33167' W, depth 9–10 m; *TRP* 12-101-18 [BDA 1413], 22 Aug. 2012, *Cristobal Colon* wreck off Bermuda I., 32°29.10333' N, 64°43.20333' W, depth 16 m; *TRP* 12-102-18 [BDA 1436, 1437], 23 Aug. 2012, North Rock, off north shore Bermuda I., 32°28.44333' N, 64°46.12500' W, depth 10 m; *TRP* 12-148-3 [BDA 1622], 5 Nov. 2012, *Pelinaion* wreck off south shore Bermuda I., 32°21.34833' N, 64°38.61500' W, depth 18 m; *TRP* 12-154-1 [BDA1687], cystocarpic, male, 19 Nov. 2012, west of Ireland I. North, 32°21.061'N, 64°57.267'W, depth 20 m. **St. Croix, USVI** – *TRP/CEL/Eric D. Salomaki* 13-25-24 [STX071], 20 Nov. 2013, Sprat Hole, Cables Reef, 17°44.24667' N, 64°53.84167' W, depth 13–14 m.

**ETYMOLOGY:** *gorgoniarum* (f., gen. pl.), for its growth near the bases of living soft whip and fan corals, thus “of the gorgonians” (= alcyonarians).

**DISTRIBUTION:** At present, known from deep waters off Bermuda and St. Croix (USVI), western Atlantic Ocean.

## **DISCUSSION**

In their account of *Galene*, D'Archinio *et al.* (2014) noted that the species assigned to the new genus were virtually indistinguishable in vegetative and reproductive characteristics from species of

*Cryptonemia* and that the two genera could best be discriminated using sequence data. Using *rbcL* sequence analysis, D'Archino *et al.* (2014) assigned three species to *Galene*, one a reassignment for *Cryptonemia rotunda* (Okamura) Kawaguchi from Japan and Korea (Kawaguchi 1993), the other two new species endemic to New Zealand. In our field log, the name assigned for the collection of *G. leptoclados* aboard ship was entered as *Cryptonemia*, a genus we were studying at the time (Schneider *et al.* 2018). After genetic sequencing, we discovered the mesophotic Bermuda specimens represented a unique species of the southern hemisphere genus *Galene*. Our Atlantic collections are associated with *Galene* despite the genetic differences they have with the other members of the genus. The overall morphological/anatomical similarities of *G. leptoclados* to other members of *Galene* do not warrant the erection of a new genus for the Bermuda plants at this time. In the future, should additional species be assigned to the genus that confuse the current genetic relationships, or unique reproductive characteristics are discovered for *G. leptoclados*, the new species could eventually be segregated at the generic level. At this point, however, we follow a conservative approach in describing the Bermuda specimens as the fourth species in *Galene*. D'Archino *et al.* (2014) reported an undescribed species from New Zealand, and we have discovered an additional four genetic species from Western Australia, Lord Howe I. (2) and South Africa (Fig. 1). These latter four will be discussed in a subsequent paper, but the *rbcL* gene of *G. leptoclados* has 96% similarity to the genetic species from South Africa (*G. sp.* 1SAfr) and for COI-5P, 93% similarity to the undescribed species from Lord Howe I. (*G. sp.* 1LH).

Collections of *Galene leptoclados* (Fig. 3) were first made in 1960 off Bermuda by lockout divers of the U.S. Navy from 60 to 64 m depths while surveying the offshore Challenger and Argus Banks. Frederick (1963) tenuously identified these specimens as ‘?*Halymenia hancockii*’, a new distributional record for Bermuda, no doubt based upon their thin blades, bifurcated apices,

occasional marginal and laminal proliferations, single-layered cortexes and their deep-water habitat (Taylor 1942). But our examination of these specimens in MICH showed that the blades do not taper to both apices and bases (lanceolate), a characteristic of *H. hancockii*, but rather have blades expanded distally to broadly rounded apices. Furthermore, the blades of ‘?H. hancockii’ had obvious stellate ganglial cells connecting the thin network of medullary filaments similar to the new species of *Galene* described here (Fig. 7), rather than lacking them as noted for *H. hancockii* (Taylor 1942; Schneider & Searles 1991; Godínez-Ortega *et al.* 2019). Therefore, we recognise the 1960 Bermuda ‘?H. hancockii’ specimens as *G. leptoclados* due to their anatomical and morphological matches to our sequenced specimens.

*Galene leptoclados* differs from the present three species in the genus by its thin (merely 34–46 µm thick), ligulate, bifurcate blades (Figs 5, 6). The described species of the genus, *G. meridionalis* D’Archino & Zuccarello, *G. profundae* D’Archino & Zuccarello and *G. rotunda* (Okamura) D’Archino & Zuccarello, are all characterised by broad blades (obovate, elliptical or circular to irregular) and are variously between 70 and 300 µm thick. Only *G. profundae* is found in deep waters like *G. leptoclados*, but this species has a broad vertical distribution from 8 to 77 m off the coast of New Zealand. The new species has only a single-layered cortex (Figs 5, 6), a feature shared with only *G. profundae* at present. In overall habit, *G. leptoclados* is more similar to *Cryptonemia lacunicola* C.W.Schneider, C.E.Lane & G.W.Saunders, a recently described species from shaded inland sinkholes in Bermuda but never below 10 m depths (Schneider *et al.* 2018, figs 18-20). Aside from their defining sequence differences and the environments from which they were collected, *G. leptoclados* is less regularly branched (Figs 2, 3) and markedly thinner than *C. lacunicola*.

The multigene analysis of members of the Halymeniaceae showed the new genus and species, *Howella gorgoniarum*, aligning in a clade (Fig. 1) with two Indo-Pacific species of *Thamnoclonium*, *T. lemmanianum* Harvey (1855) and *T. latifrons* Endlicher & Diesing (1845). Although *T. latifrons* was previously considered a synonym of the generitype *T. hisutum* Kützing [= *T. dichotomum* (J.Agardh) J.Agardh], Huisman *et al.* (2011) showed using *rbcL* analyses that South African specimens of *T. latifrons* represented an independent species closely related to *T. lemmanianum*. The clade with these two *Thamnoclonium* species does not group with *T. dichotomum* in our multigene alignment (Fig. 1). As the genus *Thamnoclonium* was not monophyletic, Huisman *et al.* (2011) suggested that a segregate genus was warranted for *T. latifrons* and *T. lemmanianum*. Because these two species lie in the same molecular clade with *H. gorgoniarum* quite apart with the generitype, both *T. latifrons* and *T. lemmanianum* are here reassigned to the genus *Howella*:

***Howella latifrons* (Endlicher & Diesing) C.W.Schneider, L.G.Kraft & G.W.Saunders *comb. nov.***

BASIONYM: *Thamnoclonium latifrons* Endlicher & Diesing 1845, *Botanische Zeitung* 3: p. 289

(Type locality: Port Natal, Durban, South Africa).

DISTRIBUTION: This species is presently restricted to the Indian Ocean coast of South Africa (De Clerck *et al.* 2005). Reports of *T. claviferum* J.Agardh from the Atlantic coast of Africa need molecular investigation to confirm their identity as *T. claviferum*, *T. dichotomum* or *Howella latifrons*.

***Howella lemmaniana* (Harvey) C.W.Schneider, L.G.Kraft & G.W.Saunders *comb. nov.***

BASIONYM: *Thamnoclonium lemmanianum* Harvey 1855, *Transactions of the Royal Irish Academy* 22: p. 538 (Type locality: Fremantle, Western Australia, Australia).

DISTRIBUTION: This distinctive species is restricted to Western Australia where it is apparently a rare species (Scott *et al.* 1984).

First discovered offshore of Bermuda in the early 1980s, *Howella gorgoniarum* specimens are restricted to deep waters from 9 to 37 m and are almost always found growing above the bases of living gorgonians (various soft corals of the Alcyonacea) (Fig. 10). The narrow-branched ligulate blades range from 2 to 6 mm wide with undulate margins bearing occasional fine teeth (Fig. 9), and are often gnawed by herbivores. As mature small plants, *H. gorgoniarum* is somewhat reminiscent of juvenile stages of some *Cryptonemia* spp. in Bermuda (Schneider *et al.* 2018), however *rbcL* sequencing has shown *Howella* to be allied with *Thamnoclonium* (*sensu lato*) and *Carpopeltis* but with only moderate support. *Thamnoclonium* is known mostly from the Indo-Pacific, but *T. dichotomum* has been reported north of the equator from Ghana in the northeastern Atlantic Ocean (Lawson & John 1987, as *T. claviferum*). As with other halymeniacean genera including *Carpopeltis*, *Codiophyllum* and *Spongophloea*, *Thamnoclonium* is often intimately associated with sponges (Scott *et al.* 1984, Womersley 1994, Huisman *et al.* 2011). *Howella* presently supports three species, two from the Indo-Pacific formerly in *Thamnoclonium* likewise associated with sponges, but the new species from the western Atlantic only associates with soft corals.

Although just below the apices *Howella gorgoniarum* has a somewhat loose arrangement of medullary filaments, at maturity it is densely packed with narrow, mostly parallel, swirling filaments between the cortical layers (Fig. 13). This compact medulla is a feature shared with only some members of the Halymeniaceae including *Thamnoclonium* (Womersley 1994). The other two members of the genus, *H. latifrons* and *H. lemnniana*, also have densely packed medullary filaments (De Clerck *et al.* 2005, as *T. dichotomum*; Huisman *et al.* 2011), thus this is a consistent feature of the



new genus. The generitype differs from the Western Australian *H. lemmaniana* in its smaller size and more robust teeth, but more importantly by its lack of terete axes from which the alternate branches flatten into blades in the latter species (Harvey 1859, pl. 114; Kützing 1869, Tab. 48, as *T. lemmanianum*). Furthermore, *H. lemmaniana* has spinous wart-like protuberances on the blade surfaces and percurrent midribs unlike the new species. *Howella latifrons* has long been considered a synonym of *Thamnoclonium dichotomum* (Papenfuss 1968, Chiang 1970), but Huisman *et al.* (2011) recognised it as a separate entity restricted to South Africa. From the generitype, *H. latifrons* dramatically differs as its branches are also covered with pronounced, wart-like protuberances, these containing living symbiotic sponge tissue (De Clerck *et al.* 2005, as *T. dichotomum*).

The transfer of two Indo-Pacific species to *Howella* afforded us the benefit of including additional reproductive features to the description of the new genus. Although we have made numerous collections of *H. gorgoniarum* over several decades, reproductive gametophytes have been found only rarely. Both *H. lemmaniana* and *H. latifrons* have gametangia and cystocarps formed in small, leaf-like proliferations arising from the blades (Huisman 2000; De Clerck *et al.* 2005, as *T. dichotomum*), whereas the new Atlantic species has cystocarps that are formed within the branch axes at their apices in the same area as raised spermatangial sori.

In habit, *Howella gorgoniarum* is similar in its overall clustered habit to *Carpopeltis maillardii* (Montagne & Millardet) Y.M.Chiang (Coppejans *et al.* 2009, p. 191), but that Indo-Pacific species has larger, more dense clusters of small irregularly dichotomous blades than the new species. Both *C. maillardii* and *H. gorgoniarum* have short proximal midribs extending from their stipes into the blades expanding both surfaces of the lower blades. Despite these morphological similarities with *H. gorgoniarum*, the *rbcL* data of *C. maillardii* (GenBank AY294400; Taiwan) place it closer to

*Grateloupia* and related genera, not *Howella*. Sequence data and identification of *C. maillardii* is needed from the type locality (Réunion, southwest Indian Ocean).

Based upon a 600 bp *rbcL*-3P sequence from a collection made in St. Croix, USVI, we are tentatively extending the geographic range of *Howella gorgoniarum* to the Caribbean Sea. Like the Bermuda specimens, the St. Croix isolate was found growing on a living gorgonian, and its characteristics, including a densely packed medulla, were consistent with specimens collected far to the north in Bermuda.

Similar biogeographic patterns are seen for *Galene* and *Howella* as other members of the Bermuda flora, genera ranging from the southern Indo-Pacific to the North Atlantic (Schneider *et al.* 2019). Along with the new species from deep-water in Bermuda, two species of *Thamnoclonium* have been reassigned to *Howella*, both with strict Indo-Pacific distributions. However, with only three presently known species, the biogeographic history of *Howella* is difficult to accurately assess. Hommersand (2007) suggested that genera of the Halymeniaceae, along with several other red algal families, had their origins in the Tethys Sea and since have dispersed throughout tropical seas and radiated during the latter half of the Cenozoic Era. More species are known in the genus *Galene* than *Howella*. Based on the relaxed molecular clock used by Bringloe & Saunders (2018) for the genes COI-5P and *rbcL*, *G. leptoclados* most recently segregated from the South African genetic species *Galene* sp. 1SAfr clade approximately 3.5–4.0 Mya (data not shown). A detailed molecular clock analysis is necessary for more precise dating of species divergences. However, with the descriptions of *G. leptoclados* and *H. gorgoniarum* from Bermuda, we have added to the pattern of genera with Indo-Pacific origins occurring today in the western Pacific and eastern Indian Oceans and the warm-temperate/subtropical western Atlantic Ocean (Schneider *et al.* 2019).

## **ACKNOWLEDGEMENTS**

We thank crew and divers aboard the 1980s' R/V *Seahawk* cruises including Drs Chuck Amsler, Paulette Peckol and Rick Searles. The XL Catlin Deep Ocean Survey, Nekton's mission to the Northwest Atlantic and Bermuda aboard the R/V *Baseline Explorer (BEX)*, allowed for the 2016 collections. Our work on the *BEX* would have been impossible without the assistance of Capt. Larry Bennett and his crew, Brownies Global Logistics, Triton Submersibles and pilots, and the technical divers of Global Underwater Explorers led by Dr. Todd Kincaid. Christopher Flook of the Bermuda Institute of Ocean Sciences (BIOS) and Roger Simmons of the Bermuda Aquarium, Natural History Museum and Zoo (BAMZ) provided logistical support while in Bermuda. A specimen loan of *Halymenia hancockii* from MICH was kindly arranged by Dr. Michael Wynne. This is contribution no. 274 to the Bermuda Biodiversity Project (BBP) of BAMZ, Department of Environment and Natural Resources, and Nekton contribution No. 14.

## **FUNDING**

Field work for this project was supported by the National Undersea Research Program of the U.S. National Oceanic and Atmospheric Administration (NOAA SU-0683-2), and by the Nekton Foundation, Oxford, England with the support of XL Catlin and the Garfield Western Foundation. The genetic work at UNB was supported by Discovery and Accelerator grants to GWS from the Natural Sciences and Engineering Research Council of Canada, as well as funding from the Canada Foundation for Innovation, the New Brunswick Innovation Foundation and the Nekton Foundation.

## **ORCID**

Craig W. Schneider: <https://orcid.org/0000-0003-0506-3791>

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## Figure Captions

**Fig 1.** Concatenated *rbcL*, LSU and COI-5P ML tree of Halymeniaceae with partitioning by gene and codon for the two protein coding genes, and 1000 bootstrap replications (only bootstrap values > 70 shown). Sequences taken from GenBank are marked with asterisks (\*).

**Figs 2–8.** *Galene leptoclados* sp. nov.

**Fig. 2.** Holotype specimen [*CWS/TRP* 16-13-6 = BDA1999]. Scale bar = 2 cm.

**Fig. 3.** Bermuda collection from 1960 identified as “?*Halymenia hancockii*” [*JJF* 60-668 = MICH 657513] and now assigned to *Galene leptoclados*. Scale bar = 2 cm.

**Fig. 4.** Surface view of cortex at margin [*CWS/TRP* 16-13-6 = BDA1999]. Scale bar = 20 µm.

**Fig. 5.** Cross-section of median portion of blade, arrow showing 2-layered cortex at the blade margin [*CWS/TRP* 16-13-6]. Scale bar = 50 µm.

**Fig. 6.** Cross-section of median portion of blade, arrows showing inner cortex elongated adjacent to the medulla [*CWS/TRP* 16-13-6]. Scale bar = 50 µm.

**Fig. 7.** View of anastomosing medullary filaments, focused through the surface near the margin of the blade [*CWS/TRP* 16-13-6 = BDA1999]. Scale bar = 20 µm.

**Fig. 8.** Surface view of blade with small isolated patches of spermatangia covering cortex cells [*CWS/TRP* 16-13-6]. Scale bar = 50 µm.

**Fig. 9–14.** *Howella gorgoniarum* gen. et sp. nov.

**Fig. 9.** Holotype specimen [*CWS* 10-29-10 = BDA0443]. Scale bar = 1 cm.

**Fig. 10.** Clustered specimens growing above bases of a soft coral [*CWS/RBS* 85-9-1]. Scale bar = 2 cm.

**Fig. 11.** Surface view of the outer cortex with a mosaic of small to large circular clusters of rounded, irregularly shaped cells (arrows) [TRP 12-102-18]. Scale bar = 50  $\mu$ m.

**Fig. 12.** Distal portion of blade in cross-section at the margin [CWS/CEL/TRP 10-29-10]. Scale bar = 50  $\mu$ m.

**Fig. 13.** Cross-section of median portion of blade with densely packed medullary filaments [TRP 12-102-18]. Scale bar = 50  $\mu$ m.

**Fig. 14.** Cystocarps in wart-like clusters at branch apices [TRP 12-154-1]. Scale bar = 2 mm.

*Supplemental files*

**Table S1.** Collection details for isolates included in the molecular analyses of this study with newly generated GenBank accession numbers in bold type.

2019

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10.1080/00318884.2019.1661158

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