

**Collections from the mesophytic zone off Bermuda reveal three species of Kallymeniaceae (Gigartinales, Rhodophyta) in genera with transoceanic distributions**

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COLLECTIONS FROM THE MESOPHYTIC ZONE OFF BERMUDA REVEAL THREE SPECIES  
OF KALLYMENIACEAE (GIGARTINALES, RHODOPHYTA) IN GENERA WITH  
TRANSOCEANIC DISTRIBUTIONS<sup>1</sup>

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**A molecular survey of red algae collected by technical divers and submersibles from 90 m in the mesophotic zone off the coast of Bermuda revealed three species assignable to the Kallymeniaceae. Two of the species are representative of recently described genera centered in the western Pacific in Australia and New Zealand, *Austrokallymenia* and *Psaromenia*, and the third from the Mediterranean Sea and the eastern Atlantic, *Nothokallymenia*. A phylogenetic analysis of concatenated mitochondrial (COI-5P) and chloroplast (*rbcL*) genes, as well as morphological characteristics, revealed that two are shown to be new species with distant closest relatives (*N. erosa* and *P. septentrionalis*), while the third represents a deep water western Atlantic species now moved to an Australasian genus (*A. westii*).**

***Key index words:* *Austrokallymenia*, *A. westii*, Bermuda, COI-5P, Kallymeniaceae, mesophotic zone, *Nothokallymenia*, *N. erosa* sp. nov., *Psaromenia*, *P. septentrionalis* sp. nov., *rbcL*, western Atlantic**

***Abbreviations:* COI-5P, 5' region of the cytochrome oxidase subunit 1 gene; Mya, million years ago; *rbcL*, large subunit ribulose-1,5-bisphosphate carboxylase/oxygenase gene**

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A recent phylogenetic monograph of the diverse red algal family Kallymeniaceae (Gigartinales) provided genetic evidence of a much larger number of genera (Saunders et al. 2017) than previous known (Schneider and Wynne 2007, 2013; Wynne and Schneider 2010, 2016). The several new genera elucidated by Saunders et al. (2017) were part of an “explosion of molecularly-assisted taxonomic modifications” that were revealed in an extensive barcode and phylogenetic study

of specimens centered in Australia and western Europe. The authors focused mostly on sorting out taxa above the species level in order to present a “contemporary genus-level taxonomic framework” built on the principle of monophyly for other workers to later fill in species. One genus previously placed in synonymy with *Kallymenia* was resurrected (*Euhymenia*; but see Wynne 2018), several species were moved to newly described genera removing polyphyletic or paraphyletic groupings, and ten new genera were erected to house them (Saunders et al. 2017).

Many of the species discovered in the mesophotic zone off Bermuda in 2016 on the Nekton XL Catlin cruise of the R/V *Baseline Explorer* are new to science and a few have already been presented (Richards et al. 2018, Schneider et al. 2018). They were collected in areas of descending, low relief hermatyptic coral reefs or rhodolith covered areas from 60-178 m depths by technical divers or mechanical arms from two-man submersibles. In this report, we recognize an additional two new species and one new combination, all members of the Kallymeniaceae with their closest known relative from distant seas and not previously known in the western Atlantic Ocean. Interestingly, each of the three species represents a genus that has been introduced to science during the present decade (D’Archino et al. 2010, Saunders et al. 2017).

Since our collaborative work began more than a decade ago, we have reported on a great number of algal genera from Bermuda with genetic biogeographic connections to the Indo-Pacific region, in most cases from Australia. These include *Asteromenia* (Saunders et al. 2006), *Crassitegula* (Schneider et al. 2006, 2014a), *Halopeltis* (Schneider et al. 2012), *Meredithia* (Schneider et al. 2014b), *Ethelia* (Dixon et al. 2015) and *Dasya* (Schneider et al. 2017). Some of these studies describe Bermuda taxa whose closest known genetic relative is from the Indo-Pacific rather than any known species from the Atlantic (*Crassitegula*, *Halopeltis* and *Meredithia*). The species of *Austrokallymenia* and *Psaromenia* reported here can be added to this latter group with genetic ties to the Indo-Pacific region.

## MATERIALS AND METHODS

### *Standard methods*

Collections from the mesophotic zone were made on the Nekton 2016 XL Catlin cruise of the R/V *Baseline Explorer* by technical ‘rebreather’ divers or by mechanical arms from submersibles. The difficulty of securing sparsely distributed specimens of red-bladed algae from these deep reefs limited the number of samples of any species brought to the ship’s deck. Collection site locations were marked using a Beier Radio DP1 (dynamic positioning, Beier Integrated Systems, Mandeville, Louisiana, USA) to receive ship board GPS. Live specimens chosen for DNA analysis were photographed using a Canon Powershot s90 digital camera (Canon Inc., Tokyo, Japan) and dried/pressed onto herbarium paper as permanent vouchers. Fragments of these vouchers were dried on silica gel for DNA extraction, and additional fragments were preserved in 4-5% Formalin in seawater for anatomical study. Thick sections were cut by hand with a razor blade and thin sections (30-50  $\mu\text{m}$ ) were made with an American Optical freezing microtome model 880 (San Diego, California, USA). Sections were mounted in 30% corn syrup with acidified 1% aniline blue in a ratio of 20:1 with a few drops of Formalin as a medium preservative. Herbarium specimens were scanned on an Epson ET-2650 scanner (Seiko Epson Corporation, Suwa, Nagano, Japan) and photomicrographs were taken using Zeiss Axioskop 40 microscope (Oberkochen, Germany) equipped with a model 11.2 Spot InSight 2 digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA). The digital images were composed in Adobe Photoshop<sup>TM</sup>CS6 v. 13.0.1 (Adobe Systems, San Jose, California, USA). Voucher specimens are deposited in UNB, MICH, the Bermuda Natural History Museum (BAMZ) and CWS’s personal herbarium. Unless otherwise noted, specimens cited under the taxa, as well as numbers from the *Phycotheca Boreali-Americana* (*P.B.-A.*) exsiccata, are

found in the first author's herbarium. Herbarium abbreviations follow the online *Index Herbariorum* <<http://sweetgum.nybg.org/ih/>> and standard author initials are from Brummitt and Powell (1992).

### *Molecular methods*

Specimens were assigned to genetic groupings after generating COI-5P sequences. To place these species into a wider phylogenetic context, a *rbcL* sequence was generated for a representative of each genetic group and these data, along with the COI-5P sequences, were analyzed with 70 other Kallymeniaceae (Fig. 1, Table S1). DNA extraction (Saunders and McDevit 2012) and marker amplification followed published protocols (Saunders and Moore 2013). Sequence data were edited and contigs produced in Geneious 9.1.3 (<http://www.geneious.com>; Kearse et al. 2012). Sequences generated here and those produced previously by our lab, as well as data from other labs downloaded from GenBank (Table S1), were aligned in Geneious. Two individual gene alignments were prepared for phylogenetic analyses: COI-5P (69 sequences, 664 bp) and *rbcL* (73 sequences, 1358 bp). The gene alignments were analyzed independently with maximum likelihood (GTR+I+G) using RAxML (Stamatakis 2014) with partitioning by codon in Geneious. Since no conflicts were detected between individual gene topologies, a concatenated COI-5P + *rbcL* alignment was constructed (73 sequences, 2022 bp). This tree (Fig. 1) was rooted along the branch between the *Meredithia* – *Psaromenia* clade and the remaining Kallymeniaceae as these two groups are unequivocally reciprocally monophyletic (Saunders et al. 2017), therefore representing the best choice of outgroup for one another (Saunders et al. 2018). Support was assessed with 500 bootstrap replicates.

## RESULTS AND DISCUSSION

### *Molecular observations*

DNA barcoding with COI-5P uncovered three Bermuda species of Kallymeniaceae from the mesophotic zone in Bermuda (Table S1). Based upon the concatenated COI-5P + *rbcL* phylogeny, these species aligned with *Austrokallymenia* (n = 2 specimens), *Nothokallymenia* (n = 2 specimens) and *Psaromenia* (n = 3 specimens) with solid support in their respective genera. Each is discussed in detail below.

#### *Systematic treatment*

***Austrokallymenia westii* (Ganesan) C.W. Schneider & G.W. Saunders comb. nov.** (Figs 2A-C)

*Basionym:* *Kallymenia westii* Ganesan, *Boletín del Instituto Oceanográfico, Universidad de Oriente* vol. 15 (1976), pp. 169-170, figs 1-12, 14, 16-21.

*Selected specimens observed:* Bermuda – *W.G. Farlow s.n.* as *Kallymenia perforata*, 1881, Cooper’s I., drift [FH; MICH 660484, 660487; Herb. CWS]; *A.J. Bernatowitz* 53-512 as *K. perforata*, 25 Jul. 1953, half mile south of Castle Harbour, depth 91.5 m [MICH 660485]; *J.J. Frederick (JJF)* 60-588 as *K. perforata*, 19 Jul. 1960, Challenger Bank Sta. 116, 32°06’21”N, 65°01’38”W, depth 50.8 m [MICH 660505]; *JJF* 60-844 as *K. perforata*, 1 Sept. 1960, Sta. 173 off Gurnet Rock, 32°19’N, 65°39’W, depth 50.2 m [MICH 660501]; *C.W. Schneider (CWS)/T.R. Popolizio (TRP)* 16-12-1 [BDA1991], 29 Jul. 2016, deep ledge north northeast of St. George’s I., 32°28’58.05”N, 64°35’04.31”W, depth 90 m; *CWS/TRP* 16-13-2 [BDA1995], 31 Jul. 2016, *loc. cit.*, depth 90 m. North Carolina – *R.B. Searles (RBS)/CWS s.n.*, as *K. perforata*, R/V *Eastward* Sta.17683, 4 Aug. 1971, 33°46.5’N, 76°51.1’W, depth 40 m; *RBS* 85-31-10, as *K. westii*, 28 Jul. 1985, east of Cape Fear, 33°32’N, 77°25’W, depth 30-33 m. US Virgin Islands – *D.L. Ballantine* 2966, as *K. westii*, R/V *Oregon II* Sta. 46048, 11 Aug. 1987, St. Thomas, shelf south Charlotte Amalie, depth 36 m.

*Distribution:* Bermuda, North Carolina, Florida, south in Caribbean Sea to Venezuela (type locality).

*Remarks:* COI-5P and *rbcL* sequences (Table S1) of freshly collected plants from the mesophotic off Bermuda previously known as *Kallymenia westii* show them embedded in a genetic grouping with Indo-Pacific species of *Austrokallymenia* (Fig. 1). The name for this recently described new genus alluded to its southern hemisphere distribution (Saunders et al. 2017), and here we add to it a species from the Atlantic Ocean and northern hemisphere. In their work, Saunders and co-workers (2017, p. 117) considered *K. westii* a perforate, polycarpogonial species similar in many characteristics to one of their new Australian species *Leiomenia lacunata*, but without available DNA from the type locality it was retained in *Kallymenia*. Among the species that Saunders et al. (2017) transferred from *Kallymenia* to *Austrokallymenia*, they selected the only perforate species as the generitype, *A. cribrogloea*, from Australia. Their analyses showed that *A. cribrogloea* was a complex of at least three genetic groups in need of additional study (Saunders et al. 2017). Interestingly, Littler and Littler (2000) reported *A. cf. cribrogloea* (as *K. cf. cribrogloea*) for the first time in the Atlantic Ocean from deep water in the Lesser Antilles, but as yet this material has not had its identity confirmed by gene sequencing. As it turns out, the closest known relative of *A. westii*, now the second perforate species in the genus, is the undescribed imperforate *Austrokallymenia* sp.1\_SAfr (Fig. 1). These two are 4% divergent in their *rbcL* sequences, however, *A. westii* displays a similar level of divergence from several Australian species (Fig. 1). Some perforate *Kallymenia* species (Abbott and McDermid 2002) have yet to be analyzed genetically, and as more are sequenced, it is possible that relationships could change within *Austrokallymenia* and the Kallymeniaceae in general as was the case with the addition of *A. westii*.

*Austrokallymenia westii* was first collected by W.G. Farlow from the drift at Cooper's I., Bermuda in 1881 (Fig. 2B). His specimens were later used by Børgesen (1910) and Collins and

Hervey (1917) to make the first reports of it (as *Kallymenia perforata*) for Bermuda. Several of Farlow's early collections can be seen in FH and MICH on the Macroalgal Herbarium Portal site (<http://macroalgae.org/portal/collections/index.php>). The Farlow collections are small (young) and vary from imperforate (Fig. 2B) to slightly perforated. Collins and Hervey (1917) also based their report of the species entirely on the Farlow collections noting that the plants they had seen showed "much variability as to the amount of perforation" from quite imperforate to others "little more than a network." Frederick (1963, as *K. perforata*) reported on specimens collected on the offshore Argus and Challenger Banks in 1960-1961 at depths of 50-62 m, these also demonstrating a range of perforations.

Whether the representatives of *Austrokallymenia westii* with few perforations or those highly networked represent the same genetic species we cannot determine without additional genetic sequences. Saunders et al. (2017) noted that their collections of *Leiomenia lacunata* had blades that varied from "few to numerous perforations", and all forms were a genetic match to the holotype. Our recent 90 m large collections (to 22 cm tall) contain relatively few perforations (Fig. 2A) and are similar in habit and anatomy to specimens from Venezuela selected by Ganesan (1976, figs 1, 3) for the type collection as well as those found in deep waters off the coast of North Carolina (Schneider and Searles 1973, as *Kallymenia perforata*; Schneider and Searles 1991, as *K. westii*). However, looking at archival material in MICH and digitized specimens of this species from throughout its geographic range in the Macroalgal Herbarium portal, there is a continuum of perforation density between the two extremes. Until more specimens from throughout the western Atlantic are sequenced, we can only suggest that what is considered *Austrokallymenia westii* could possibly be a complex of at least two species or a phenotypically variable species in regards to perforation density. Unfortunately, we did not collect highly perforate or networked specimens on the 2016 Nekton cruise, thus we could not test whether *A. westii* is one or more perforate species in Bermuda and the

western Atlantic. For the present, a morphological comparison of our recent specimens with the type of *K. westii* shows that the weakly perforate type material (Ganesan 1976, fig. 1) is similar to specimens from Bermuda and North Carolina (Schneider and Searles 1991), not the highly perforate/networked specimens found throughout its range in the western Atlantic including some found in Venezuela (Fig. 2C). There are no morphological or anatomical characteristics of Bermuda and North Carolina specimens to distinguish them from *A. westii*. Like the 19<sup>th</sup> century Farlow collections of *A. westii* in Bermuda, Ganesan (1976, fig. 3) demonstrated an imperforate paratype specimen along with the protologue of his new species. Since *A. westii* has only been collected in deep water throughout its range and all of the specimens we are aware of were preserved in Formalin-seawater prior to drying on herbarium paper, we were unable to find Venezuelan specimens that could provide DNA sequences. Unfortunately, the sequenced specimens from Bermuda were vegetative, disallowing a check as to whether they were, like the type, polycarpogonial. Despite these issues, we are comfortable in taking the conservative approach in aligning our mesophotic Bermuda specimens with *A. westii* from the Caribbean until proven otherwise by sequence data.

***Nothokallymenia erosa* C.W. Schneider, Popolizio & G.W. Saunders sp. nov.** (Figs 3A-E)

*Description:* Rosy-red plants composed of irregular ligulate blades to 3.5 cm long from small discoidal holdfasts (Fig. 3A), stipes lacking; blades 135-250  $\mu\text{m}$  thick, margins with irregular indentations of varying sizes (Fig. 3B); blade structure multiaxial with cell layers increasing in size from the outermost pigmented cell layer to the innermost non-pigmented cell layer connecting to the filamentous medulla (Fig. 3C); pigmented cortex composed of a single layer, the small cells globose to ovoid and sub-rectangular to irregular in surface view, transversely elongated in section, 7-12  $\mu\text{m}$  in diameter; inner cortex of two layers of elongated cells increasing inwardly in size and stretching and thinning, some appearing stellate, the innermost 28-67  $\mu\text{m}$  in length and connecting to the

filamentous medulla; medulla composed of a spiderweb-like network of elongated filaments 2-5  $\mu\text{m}$  in diameter, connected to occasional large darkly staining stellate ganglia (Fig. 3D); sporangia in scattered loose sori on blade surfaces under and displacing outer cortical cells (Fig. 3E), obovoid to ellipsoidal in shape, irregularly cruciately divided, 12-17  $\mu\text{m}$  in diameter and 17-22  $\mu\text{m}$  long; gametangia unknown.

*Diagnosis:* Distinguished from the generitype, *N. crouaniorum*, by its COI-5P (GenBank MH777606) and *rbcL* (GenBank MH777609) sequence data, its ligulate blades with erose margins and smaller-sized tetrasporangia formed in sori.

*Holotype* (designated here): *CWS/TRP* 16-13-4 [BDA1997], tetrasporic, 31 Jul. 2016, deep ledge north northeast of St. George's I. (NNE02), 32°28'58.05"N, 64°35'04.31"W, Bermuda, western Atlantic Ocean, depth 90 m, bottom temp. 19.4°C, deposited in UNB (Fig. 3A).

*Etymology:* *erosa* (L, f.), for its irregularly dentate margins.

*Distribution:* At present, endemic to the mesophotic zone off Bermuda, western Atlantic Ocean.

*Remarks:* *Nothokallymenia* was recently segregated from *Kallymenia* with the generitype, *N. crouaniorum*, from the Northeast Atlantic and three undescribed genetic species from the Mediterranean Sea (Saunders et al. 2017). The mesophotic *N. erosa* from Bermuda represents the second described species in the genus and the first from the western Atlantic. After molecular analysis, Robuchon et al. (2014, as *Kallymenia crouaniorum*) found *N. crouaniorum* as a consistent understory species in the *Laminaria hyperborea* community of Atlantic European waters confused with, and historically identified as, *K. reniformis*. *Nothokallymenia crouaniorum* is comprised of blades that are large (to 20 cm tall, 15 cm broad), thick (to 320  $\mu\text{m}$ ), and deeply lobed to laciniate (Robuchon et al. 2014). The new species has an internal anatomy that is remarkably similar to *N. crouaniorum* (Fig. 3C), including their large darkly staining stellate ganglia (Fig. 3D), but *N. erosa*

has thinner (to 250  $\mu\text{m}$ ) ligulate blades with erose margins (Fig. 3B). Although both species have irregularly cruciate tetrasporangia, those of *N. erosa* are smaller (12-17  $\mu\text{m}$  diam.) than those of the generitype (18-24  $\mu\text{m}$  diam.) and are found in loose patches (sori) on blade surfaces (Fig. 3E), while those of *N. crouaniorum* were scattered over the blades (Robuchon et al. 2014, Saunders et al. 2017). The *rbcL* for *Nothokallymenia erosa* was 4% divergent from an unpublished species from Brittany, France (KM896874).

***Psaromenia septentrionalis* C.W. Schneider, Popolizio & G.W. Saunders sp. nov.** (Figs 4A-F)

*Description:* Rosy-red plants composed of subdichotomously branched ligulate blades, arising to 13 cm tall from small discoidal holdfasts, stipes lacking, the blades bearing marginal proliferations that eventually develop into fusiform to lanceolate blades, 300-500  $\mu\text{m}$  thick (Fig. 4A); structure multiaxial, the cortex with cell layers increasing in size from the outermost pigmented cell layer to the innermost non-pigmented cell layer connecting to the filamentous medulla (Fig. 4B); pigmented cortex composed of one (-two) layers, the outermost cells polyhedral in surface view, subglobose or elongated to irregular in section, 3.5-7.5  $\mu\text{m}$  in diameter; inner cortex of three layers of subglobose cells increasing inwardly in size and elongating parallel to the blade surface, the innermost 33.5-67.5  $\mu\text{m}$  in diameter and connecting to the filamentous medulla; subsurface cortical cells often with clusters of refractive globules; medulla composed of a loose network of elongated filaments 7-12  $\mu\text{m}$  in diameter, connected to stellate ganglia morphed from the innermost cortical cells (Figs 4C, 4D); monocarpogonial supporting cells bearing a single 3-celled carpogonial branch and 3-4 sterile subsidiary cells (Fig. 4E), carposporophytes formed in groups within a cystocarp, in part separated by vegetative filaments, distally producing carpospores towards the center of the cystocarp (Fig. 4F); carpospores obpyriform to spherical and irregular, 9.5-17.0  $\mu\text{m}$  in longest dimension; cystocarps

formed over blades, at times on margins, when fully developed raising one surface or the other as pustule-like, irregular cystocarps to 1.3 mm in diameter, formed singly or at times in clusters of 3-4, ostioles lacking; spermatangia and tetrasporangia unknown.

*Diagnosis:* Distinguished from the generitype, *Psaromenia berggrenii*, by its COI-5P (GenBank MH777603) and *rbcL* (GenBank MH777607) sequence data, its geographic location, lack of a mottled appearance and monocarpogonial supporting cells.

*Holotype* (designated here): *CWS/TRP* 16-13-1 [BDA1993], female/cystocarpic, 31 Jul. 2016, deep ledge north northeast of St. George's I. (NNE02), 32°28'58.05"N, 64°35'04.31"W, Bermuda, western Atlantic Ocean, depth 90 m, bottom temp. 19.4°C, deposited in UNB (Fig. 4A); isotypes – BAMZ, Herb. CWS (*CWS/TRP* 16-13-1 [BDA1994]), MICH (*CWS/TRP* 16-13-3 [BDA1996]).

*Etymology:* *septentrionalis* (L, f.), northern, for its being the first species of *Psaromenia* formally described from north of the equator.

*Distribution:* At present, endemic to the mesophotic zone off Bermuda, western Atlantic Ocean.

*Remarks:* *Psaromenia septentrionalis* represents the second species formally described in the genus, and the first described from the northern hemisphere (D'Archino et al. 2010). It is also the first species of this genus reported from the Atlantic Ocean. Its closest relative in our analysis is *Psaromenia* sp.1\_LH, an undescribed Australian species in the genus (Schneider et al. 2014b, Saunders et al. 2017).

D'Archino et al. (2010) named their monotypic genus *Psaromenia* from New Zealand for the distinctive mottled appearance of the blades. The new species from Bermuda does not share this characteristic, all the blades showing even coloration (Fig. 4A). The internal structure of the new species is similar to that of a majority of genera and species in the Kallymeniaceae. A complete outer layer or layers of pigmented smaller cortical cells sits outside an inner layer of enlarged cortical cells

(Fig. 4B). The polyhedral outer cortical cells form in groups or clusters somewhat reminiscent of a rosette pattern. As the enlarged inner cortical cells mature, they become separated (Fig. 4C), often widely, as they appear pulled apart by the medullary filaments they are connected to (Womersley and Norris 1971), thus they represent a transitional layer between the cortex and medulla. At maturity, the transitional cells in *P. septentrionalis* are star-shaped (stellate) ganglia and are lightly staining (Fig. 4D). Overall, the anatomical arrangement of the new species is similar to that found in *P. berggrenii* (D'Archino *et al.* 2010).

*Psaromenia septentrionalis* has monocarpogonial supporting cells (Fig. 4E), whereas *P. berggrenii* is basically polycarpogonial with some monocarpogonial supporting cells (D'Archino *et al.* 2010). In *P. septentrionalis*, we have not seen connecting filaments directly produced by post-fertilization fusion cells but have discovered clustered cystocarps leading to speculation that both exist. Supporting cells may act as auxiliary cells in *P. septentrionalis* as is the case for several Kallymeniaceae such as *Callophyllis*, *Euthora* and *Pugetia* among others (Norris 1957). In the few post-fertilization systems that we discovered, young gonimoblast filaments were issued directly from lobate fusion cells. Two fusion cells that we observed were in proximity to each other in the inner cortex-outer medulla, but we did not observe discernable connecting filaments entering or leaving the two systems.

Carposporophytes of *Psaromenia septentrionalis* form in the pattern as described for other members of the Kallymeniaceae by Womersley and Norris (1971) as a mass of slender gonimoblast cells with carposporangia separated into what appear as distinct 'loculi' separated by vegetative filaments (Fig. 4F). The gonimoblast filaments produce distal carposporangia towards the center of cystocarps producing at maturity a mass of sporangia and intermixed vegetative filaments. Externally, older wart-like pericarps of *P. septentrionalis* are reminiscent of those in the genus *Cirrulicarpus* as

described and illustrated by Hansen (1977a, 1977b), however they have not been shown to form in ring-like clusters as does the latter genus.

Males were not seen on cystocarpic plants of *Psaromenia septentrionalis* and are thus far unknown, therefore it is possible that this species is dioecious as is *P. berggrenii* (D'Archino et al. 2010). After observing more than 200 specimens from depths of 3-25 m, D'Archino et al. (2010) never found tetrasporangial plants for *Psaromenia berggrenii*. In our limited collections from offshore Bermuda, we likewise did not find sporophytes.

In the molecular analyses of D'Archino et al. (2010), *Psaromenia* formed a clade with *Meredithia*, at that time geographically restricted to the Atlantic, while their new genus was considered endemic to New Zealand. Since then, and with this report of *P. septentrionalis*, neither *Psaromenia* or *Meredithia* remains restricted to either the Atlantic or Indo-Pacific Oceans (Schneider et al. 2014b).

## CONCLUSION

What has been discovered in the mesophotic zone of the world's oceans represents a few narrow snapshots in time and place from a vast landscape, only a tiny fraction of which has been visited by scientists (Ballantine et al. 2016), Unfortunately, the expense and technical difficulties necessary to work in mesophotic habitats, as well as the massive footprint of such areas in our oceans, precludes a worldwide systematic survey of the biota of these environments. Nevertheless, the "snapshots" taken have uncovered interesting species living at the limits of light penetration (McDermid and Abbott 2006, Sherwood et al. 2010, Ballantine et al. 2016). Using genetic sequences from specimens collected at a depth of 90 m off Bermuda, we have uncovered three species assignable to the family Kallymeniaceae. Two were described as new species of *Nothokallymenia* and

*Psaromenia* and the third required a new combination for a western Atlantic species now recognized by molecular data to be representative of the newly described *Austrokallymenia*. These three species focus attention on the great depth at which they were found and highlight the uncovered diversity in the oceans' mesophotic zone.

There are interesting biogeographic links for the three species reported in this paper, two from the Pacific and the other from the Atlantic Ocean and Mediterranean Sea. An alliance between Atlantic and Pacific species is one that we have observed repeatedly for other species that we have discovered in Bermuda. For example, we demonstrated a transoceanic biogeographic pattern for the kallymeniacean *Meredithia* (Schneider et al. 2014b), and since then additional species in the genus have been discovered in the western Atlantic and Indo-Pacific regions (Ballantine et al. 2015, Saunders et al. 2017). *Meredithia crenata* from Bermuda and *M. pulchella* from Puerto Rico are distant to the eastern Atlantic *M. microphylla* and join species from Norfolk I., Australia, the Philippines and Cocos (Keeling) I. The two species of *Crassitegula* in Bermuda are sister to species from Western Australia and Lord Howe I. (Schneider et al. 2014a). *Asteromenia peltata* from the western Atlantic genetically joins species from Western Australia and Cocos I., while *A. bermudensis* groups with a new species from Norfolk I. (Saunders et al. 2006). Genetic sequences of *Halopeltis pellucida* from Bermuda and *H. willisii* from North Carolina show that these species do not group together as sister species from the Atlantic Ocean, rather they join two different Indo-Pacific region clades within this widespread genus (Schneider et al. 2012).

Our new *Austrokallymenia* and *Psaromenia* species reported herein, as well as the other examples noted above, display *rbcL* divergence typically in the 2-5% range from their Pacific congeners, which indicates separation approximately 2-4 Mya based on the molecular clock used in Bringloe and Saunders (2018). This timeframe would allow for Pacific to Caribbean migrations prior to the closing of open waters between the Americas by the Isthmus of Panama approximately 2.8

Mya (O’Dea et al. 2016). A detailed molecular clock analysis is necessary to more precisely date species divergences which is not the focus of this paper. However, the tectonic closure of the Pacific-Caribbean passage created a vicariance event that allowed for speciation in macroalgal species and other marine biota over geologic time (Rosen 1975, Knowlton and Weigt 1998, McCartney et al. 2000), providing that there was a pathway of dispersal from the southern Pacific Ocean to the Americas. Therefore, with the discovery of *P. septentrionalis* in the western Atlantic, and the movement of *Kallymenia westii* to *Austrokallymenia*, we have added to the pattern of recurring speciation events between Australasia and the warm-temperate/subtropical western Atlantic Ocean.

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The authors report no conflicts of interest.

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## Figure Captions

FIG. 1. Combined COI-5P and *rbcL* RAxML analyses (GTR+I+G) 500 bootstrap replicates (only values > 70% shown). Generitypes are marked with asterisks (\*).

FIG. 2. *Austrokallymenia westii* (Ganesan) comb. nov. (A) Mature specimen from the mesophotic (CWS/TRP 16-12-1), scale bar = 5 cm. (B) Young imperforate specimens washed onshore in

Bermuda, 1881 (*W.G. Farlow s.n.*), scale bar = 2 cm. (C) Net-like, highly perforated specimen from Venezuela (MICH 660509), scale bar = 5 cm.

FIG. 3. *Nothokallymenia erosa* sp. nov. (CWS/TRP 16-13-4), holotype. (A) Holotype specimen showing position of the holdfast (arrowhead), scale bar = 1 cm. (B) Close-up of irregularly toothed (erose) margin, scale bar = 250  $\mu\text{m}$ . (C) Cross-section of blade with dark staining ganglionic cell (arrowhead), scale bar = 100  $\mu\text{m}$ . (D) Stellate medullary ganglia, scale bar = 100  $\mu\text{m}$ . (E) Surface view of a loose sorus of tetrasporangia displacing outer cortical layer, scale bar = 50  $\mu\text{m}$ .

FIG. 4. *Psaromenia septentrionalis* sp. nov. (CWS/TRP 16-13-1). (A) Holotype specimen, scale bar = 5 cm. (B) Cross-section at margin of thallus, scale bar = 100  $\mu\text{m}$ . (C) Inner cortical cell stretching with attached medullary filaments, scale bar = 50  $\mu\text{m}$ . (D) Fully developed stellate ganglia and attached medullary filaments, scale bar = 50  $\mu\text{m}$ . (E) Monocarpogonial supporting cell (sc) bearing a 3-celled carpogonial branch (branch cells 1 and 2 [c1, c2], carpogonium [cp]) and 3 sterile subsidiary cells (ss). It appears that the subsidiary cells are at early stage of fusion with carpogonial branch and supporting cell, scale bar = 25  $\mu\text{m}$ . (F) Section through a cystocarp demonstrating carposporophytes with carpospores separated by vegetative filaments, scale bar = 250  $\mu\text{m}$ .

### *Supplemental files*

**Table S1.** Table of specimens with corresponding GenBank numbers used in study.



**DSPACE**

<https://dspace.org/>

**Collections from the mesophytic zone off Bermuda  
reveal three species of Kallymeniaceae (Gigartinales,  
Rhodophyta) in genera with transoceanic distributions**

**Schneider, Craig, W.; Popolizio, Thea, R.; Saunders, Gary, W.**

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