

Research Article

***Bugula tsunamiensis* n. sp. (Bryozoa, Cheilostomata, Bugulidae) from Japanese tsunami marine debris landed in the Hawaiian Archipelago and the Pacific Coast of the USA**

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Abstract

A new species of the cheilostome bryozoan genus *Bugula* Oken, 1815, *Bugula tsunamiensis*, is described from Japan, having rafted across the North Pacific Ocean on numerous objects released into the ocean by the 2011 Great East Japan Earthquake and Tsunami, and landing in the Hawaiian Islands and on the Pacific Coast of the United States. This is the second species of the *Bugula uniserialis* Hincks, 1884 group to be reported from Japan. We elevate the Japanese species *Bugula scaphoides constricta* Yanagi and Okada, 1918 to full species status, *B. constricta*, based upon distinctions from the stem species. We suggest that *Bugula uniserialis* reported from the Galapagos Islands is an undescribed species.

Key words: ocean rafting, North Pacific, *Bugula uniserialis*, *Bugula constricta*, *Bugula scaphoides*, *Bugula pedunculata*

Introduction

Bryozoans are a diverse group of filter-feeding organisms well represented in a wide variety of marine environments and habitats, and capable of taking advantage of oceanic rafting objects (Winston 1982; Thiel and Gutow 2005; Goldstein et al. 2014). Arborescent forms appear to be particularly well-adapted to life on potentially space-limited rafting substrates (McCuller and Carlton 2018).

The Bugulidae (Cheilostomata) are a widespread group of arborescent bryozoans that have recently undergone substantial taxonomic revision. Changes

include: the resurrection of the genera *Bugulina* Gray, 1848 and *Crisularia* Gray, 1848; the description of the new genus *Virididentula* Fehlaue-Ale, Winston, Tilbrook, Nascimento and Vieira, 2015; and the restriction of the species assigned to *Bugula* Oken, 1815 with a redefinition of that genus (Fehlaue-Ale et al. 2015). Characteristics shared by all *Bugula* species include a lack of spines on the ancestrula and distal portion of autozooids, and an ooecium with both an ento- and ectooecium that is attached to the inner distal portion of zooids (Fehlaue-Ale et al. 2015). Fehlaue-Ale et al. (2015) distinguish two morphologically defined groups within *Bugula sensu stricto*:

the *Bugula neritina* (Linnaeus, 1758)–*B. minima* Waters, 1909 group; and the *Bugula uniserialis* Hincks, 1884 group. The latter has a branching pattern with long proximal portions of zooids and thus appears to have a uniserial growth pattern.

A number of species within the Bugulidae have been recorded from Japan. Species in the *Bugula neritina-minima* group include: *B. minima* (Okada and Mawatari 1938); *B. neritina* (Okada 1929; Mawatari 1981); *B. subglobosa* Harmer, 1926 (Mawatari 1963), and *B. vectifera* Harmer, 1926 (Okada and Mawatari 1938). The *Bugula uniserialis* group is represented by only one species, *B. scaphoides constricta* Yanagi and Okada, 1918. Species of *Bugulina* in Japan include *B. californica* (Robertson, 1905) (Okada and Mawatari 1938; Mawatari 1981; Nandakumar et al. 1993); *B. flabellata* (Thompson in Gray, 1848) (Mawatari 1981), and *B. stolonifera* (Ryland, 1960) (Scholz et al. 2003). At least three species of *Bugula* that are considered *incertae sedis* were reported in Japan: *B. hexacantha* Ortmann, 1890, *B. lophodendron* Ortmann, 1890, and *B. umbelliformis* Yanagi and Okada, 1918 (Okada and Mawatari 1938). Other species within the Bugulidae include: *Crisularia pacifica* (Robertson, 1905) (Grischenko et al. 2007); *Halophila johnstoniae* Gray, 1843 (Ortmann 1890); *Dendrobeatia japonica* (Ortmann, 1890), and *Viridentula dentata* (Lamouroux, 1816) (Okada and Mawatari 1937; Mawatari 1987).

Bugula species that are widely distributed and that have been previously recorded as rafting include *Bugulina flabellata*, *Bugula neritina*, and *Bugula minima* (Stevens et al. 1996; Winston et al. 1997; Astudillo et al. 2009). None of these species are in the *B. uniserialis* group.

We report here a new species of *Bugula* within the *B. uniserialis* group occurring on objects released into the North Pacific Ocean in March 2011 due to the Great East Japan Earthquake and Tsunami. These objects drifted across the ocean to the Central Pacific (Hawaiian Archipelago) and the Pacific coast of the USA.

Materials and methods

Bryozoan samples were obtained from JTMD (Japanese Tsunami Marine Debris) objects (see Carlton et al. 2017) landing between 2012 and 2016 in North America and the Hawaiian Islands. Each object was assigned a unique identification number preceded by JTMD-BF (BioFouling-number) (Supplementary material Table S1).

Bryozoan specimens (received dried or in 95% ethanol) were loose in submitted samples or were removed from their substrate with a scalpel and placed in

voucher collections. A number of colony fragments from BF samples intercepted in Hawaii, Oregon, and Washington were photographed with a Leica EZ4 HD (Leica Microsystems, Wetzlar, Germany) and LAS EZ (Leica Microsystems, Wetzlar, Germany) imaging software, and measured using Fiji (Schindelin et al. 2012). For scanning electron microscopy (SEM), specimens were cleaned in sodium hypochlorite solution, rinsed in tap water, and then replaced in ethanol to prevent degradation during transport. Samples were then air dried and coated with Au-Pd using an Anatech USA Hummer 6.6 Sputtering System (Anatech, Hayward, California USA) at 15mA and viewed under a JEOL JSM-7100FLV field emission SEM (JEOL USA Inc., Peabody, Massachusetts USA) at 5.0kV accelerating voltage. Images were retained as TIFF files.

Specimens of *Bugula* from JTMD-BF-339 (Table S1) were removed from storage in 95% ethanol and rinsed in distilled water. DNA was then extracted from ~25 mg subsamples of colonies using the DNEasy blood and tissue kit (Qiagen, Venlo, Netherlands; Catalog No. 69504) following the manufacturer's protocol. A 313 bp fragment of the cytochrome *c* oxidase subunit I (COI) mitochondrial gene was amplified by polymerase chain reaction (PCR) using primers described by Geller et al. (2013) and Leray et al. (2013), modified to contain T7 and T3 sequencing primer sequences (underlined): T3_intLCO (5'- AAT TAA CCC TCA CTA AAG GGG GWA CWG GWT GAA CWG TWT AYC CYC C -3') and T7_jgHCO (5'- TAA TAC GAC TCA CTA TAG GGT AIA CYT CIG GRT GIC CRA ARA AYC A -3'). Each PCR reaction consisted of 25 µL of Green GoTaq (Promega, Madison, Wisconsin, USA; Catalog No. PRM7123) master mix (400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, and 3 mM MgCl₂), 0.2 µM of each primer, 0.2 mg/ml BSA, and water to a volume of 50 µL. The PCR program was 3 min at 94 °C followed by 30 cycles of 94 °C for 60 sec, 47 °C for 45 sec, and 72 °C for 90 sec. PCR products were purified using Agencourt AMPure beads (Beckman Coulter, Brea, California USA; Catalog No. A63880) according to the manufacturer's protocol and submitted to Elim Biopharmaceuticals (Hayward, California, USA) for dideoxy chain termination sequencing using T7 and T3 primers. Forward and reverse sequences were assembled and primer sequencers removed using Geneious 10.1 (Biomatters, Auckland, New Zealand). Novel sequences were aligned to representative COI from *Bugula* species found in Genbank in March 2017, and phylogenetic trees generated using FastTree 2.1.5 (Price et al. 2010) using the GTR substitution model. Genbank voucher specimens from JTMD-BF-339 are deposited at the Royal British Columbia Museum, Victoria, Canada.

Results

Class Gymnolaemata Allman, 1856
Order Cheilostomata Busk, 1852
Family Bugulidae Gray, 1848
Genus *Bugula* Oken, 1815

Bugula tsunamiensis n. sp.

(Figures 1–3)

Material. JTMD-BF-23, 131, 134, 168, 196, 210, 212, 223, 226, 240, 241, 250, 253, 254, 264, 290, 304, 339, 352, 353, 356, 390, 398, 402, 410, 413, 414, 415, 471, 526, 530, 531, 532, 533, 555, 578, 626, 652, 668, 669, 670, 671, 672 (Table S1). These rafted objects include material washed ashore from Japan in Washington, Oregon, California, and Hawaii, between 2013 and 2016.

Diagnosis. Recumbent to erect colonies, tan to red in ethanol; alternating elongate zooids (ca. 0.61 mm long and 0.14 mm wide) and large avicularia (ca. 0.14 mm long) with a length to width ratio of 1.45–1.70, attached by a short peduncle to a long peduncle cushion; zooids may have more than one radicle fiber.

Description. Zoarium delicate, recumbent to erect, biserial and alternating with a uniserial appearance, with bifurcation type 3 (see Vieira et al. 2012), often curling strongly inwards distally, color tan to red in ethanol. Number of zooids between branch bifurcations is low (1–3) at proximal portions and higher (5–7) at distal portions of the colony. Zooids elongate (mean \pm standard deviation; 0.61 mm \pm 0.07 mm), opesia occupying approximately half of zooid length, tubular proximally and truncate distally, with a step-like process between. Radicle fibers originate basally just above step-like process, often short at proximal portions of the colony and sometimes proximal zooecia have two radicles (Figure 1D); radicles often kept the proximal portions recumbent against the substrate. Avicularia monomorphic and large, with a length to width ratio of 1.45–1.70:1, attached by a short peduncle to a long peduncle cushion at the extreme proximal end on the outer edge of each zooid (Figure 2). Ooecia attached at the inner distal edge, sub-spherical, curved inwards (Figure 1C). No ancestrula observed.

Remarks. A majority of specimens of this species were branches or branch fragments randomly distributed or tangled in clumps of other arborescent bryozoans or hydroid stolons. In some samples, colonies were attached by radicles to various substrates, including captured sand, foraminiferans, other bryozoans, or the gooseneck barnacle *Lepas* sp. A few specimens had ovicells (JTMD-BF-131, 264, 402, 555).

Of note is the striking intracolony (Table S2; Figure 1B) and intercolony morphological variation

(Figure 3) of *B. tsunamiensis*, potentially due to the different routes and thus the broad sweep of environmental conditions experienced, that their debris items took across the North Pacific Ocean. For example, avicularia length, zooid length and frontal membrane length were larger on average for specimens that arrived on objects intercepted on the Pacific Coast of the United States, and smaller for specimens on objects that landed in Hawaii (Figure 3). *Bugula tsunamiensis* also demonstrates the widest known range of avicularia size of any *Bugula* species. *Bugula tsunamiensis* appears to follow the “temperature-size rule” (see Atkinson 1994) which has been found to be closely followed by cheilostome bryozoans (O’Dea and Okamura 1999; Okamura et al. 2011).

Phylogenetics. Sequences of 313 bp from the two specimens of *Bugula tsunamiensis* were identical, were 33.5% GC, and have been deposited in Genbank as Accession Number MF593127. A BLAST search against the nucleotide database at Genbank recovered *Bugula migottoi* Vieira, Winston and Fehlaue-Ale, 2012 at 86% pairwise identity as top match. No other Bryozoa were in the top 100 BLAST hits, likely resulting from high levels of divergence seen in this genus (Figure 4). A maximum likelihood tree (Figure 4) including COI sequences of *Bicellaria*, *Bugula*, *Bugulina*, *Crisularia*, and *Viridentula* placed *B. tsunamiensis* in a strongly supported clade containing species retained in the genus *Bugula* by Fehlaue-Ale et al. (2015). The phylogenetic tree presented here is entirely compatible with the combined COI and 16S rRNA tree in Fehlaue-Ale et al. (2015). We conclude *B. tsunamiensis* is distinct genetically from known members of Bugulidae for which data were available, and is contained in the genus *Bugula*.

Etymology. The species name, which is derived from the Japanese word “tsunami,” memorializes the tragedy of the Great East Japan Earthquake and Tsunami of 2011.

Type Material. Holotype deposited at the National Museum of Nature and Science, Tokyo, NSMT-Te 1208 (JTMD-BF-555, identical in every respect to specimens shown in Figures 1 and 2; colony data for BF-555 shown in Table S2). Paratype deposited at the National Museum of Natural History, Smithsonian Institution, NMNH 1437663 (JTMD-BF-264). The holotype specimen is from the biofouling on the hull of the fishing vessel *Daini Katsu Maru*, lost from Ogatsu, Miyagi Prefecture, on March 11, 2011, and washing ashore at Alan Davis Beach, O’ahu, on April 22, 2015. The *Daini Katsu Maru* was returned to Japan aboard the Japanese training vessel *Miyagi Maru* in March 2016 to be part of a memorial exhibit.

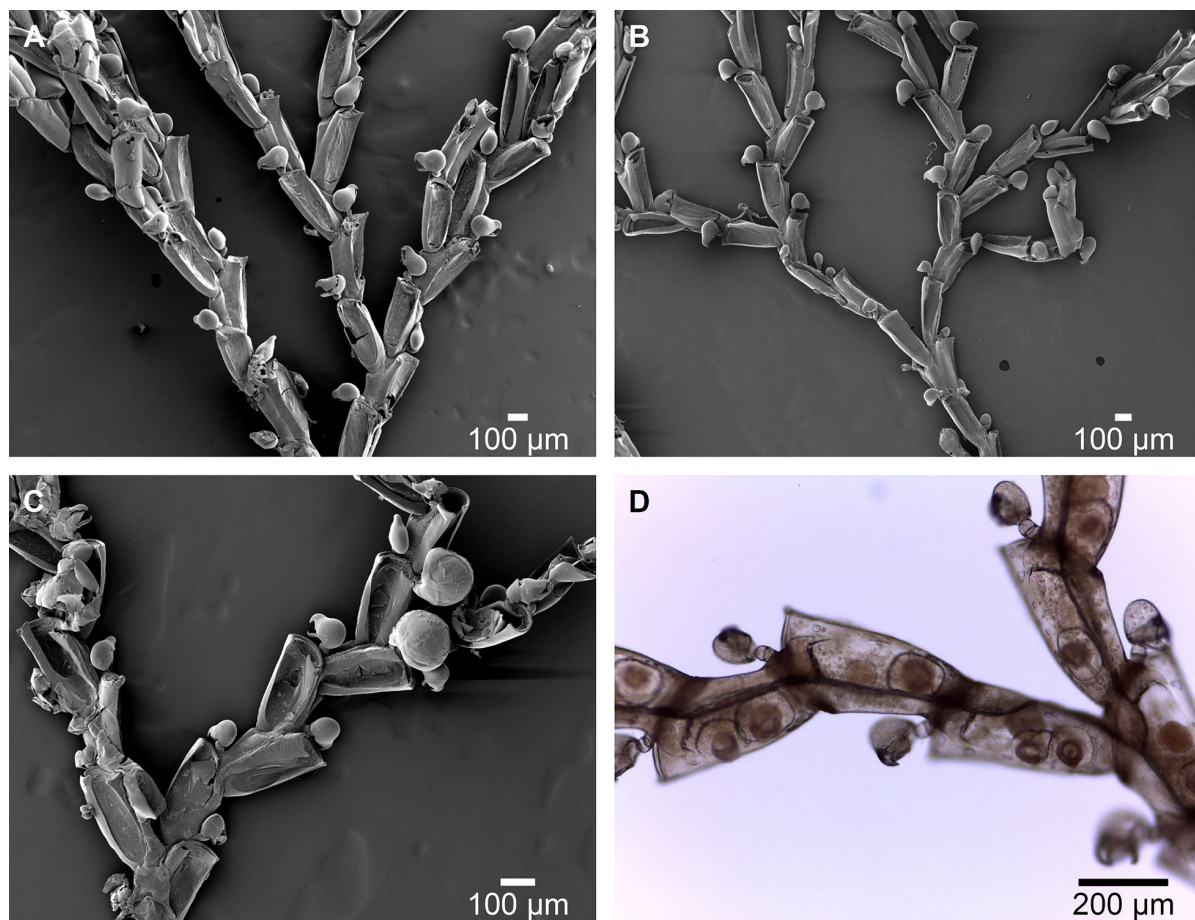


Figure 1. *Bugula tsunamiensis* new species. (A) colony, JTMD-BF-131, (B) colony showing wide range in avicularia sizes, JTMD-BF-168; (C) colony fragment with ovicells, JTMD-BF-264; (D) basal side, internode bifurcation point, and zooids with two rhizoids, JTMD-BF-210. Microphotographs by Megan I. McCuller.

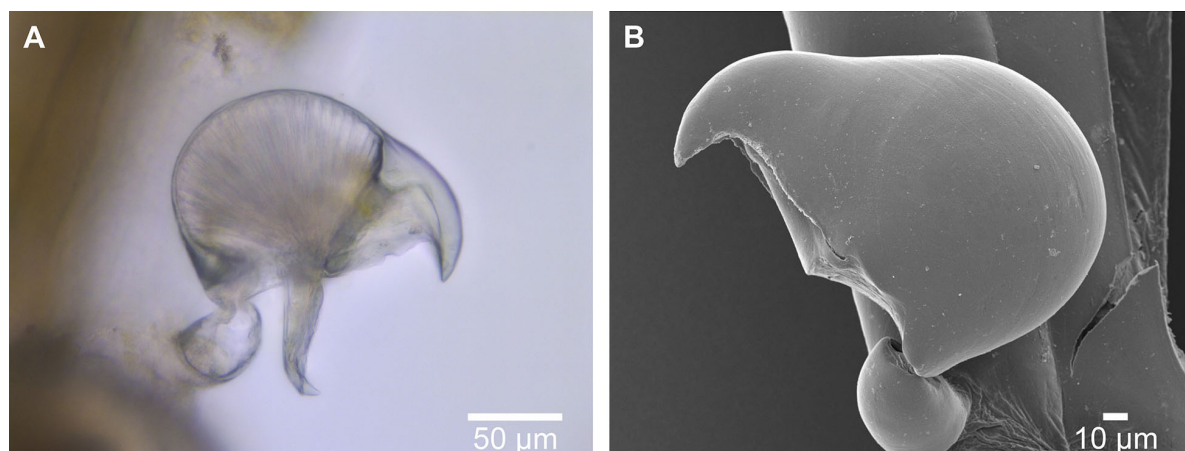


Figure 2. Avicularia of *Bugula tsunamiensis* new species, JTMD-BF-168. Microphotographs by Megan I. McCuller.

Figure 3. Mean measurements of *Bugula tsunamiensis* new species morphological characters by object intercept location. Zooid length (ZL) and width (ZW), frontal membrane length (FML), avicularia length (AvL) and width (AvW), and ovicell width (OvW). Error bars represent standard deviations of measurements of specimens noted in Table S2. HI, Hawaii; OR, Oregon; WA, Washington.

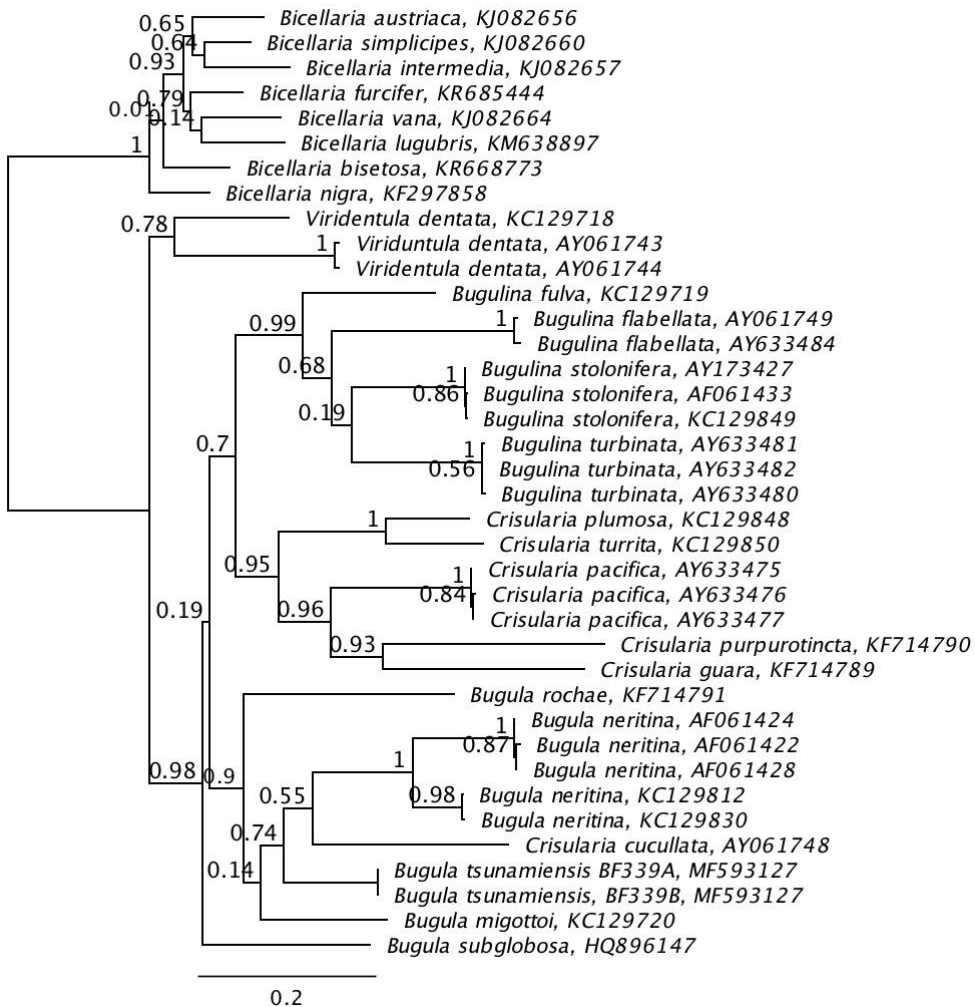
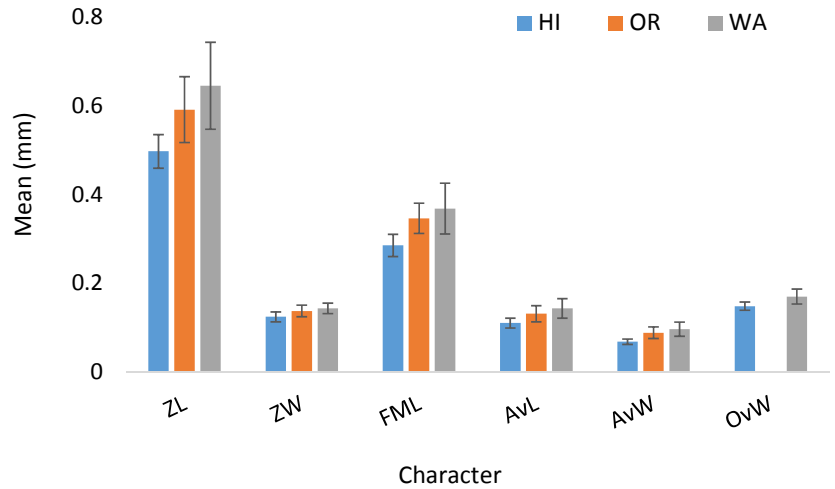


Figure 4. FastTree 2.1.5 maximum likelihood COI phylogeny for *Bugula tsunamiensis* and closely related Bugulidae. Members of the genus *Bicellaria* were included as an outgroup to root the tree. Support values (proportion of 1000 trees) were generated by resampling the data with 1000 replicates. The scale bar indicates branch length in number of substitutions per site. Genbank accession numbers are given after species names; however nomenclature follows Fehlaue-Ale et al. (2015) rather than sequence authors.

Type Locality. According to Article 76.1.1 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999), “If capture or collection occurred after transport by artificial means, the type locality is the place from which the name-bearing type, or its wild progenitor, began its unnatural journey”. At this time we are only able to designate a relatively broad region, Honshu, Japan, as the type locality.

New Species Registration. The electronic pdf version of this article represents a published work under the codes of the International Commission on Zoological Nomenclature (ICZN). The online version of this article is archived and available from digital repositories. The new species name in the electronic version thus constitutes publication under the ICZN codes based upon the electronic edition alone. This work and the species name have been registered in ZooBank, the ICZN online registration.

Publication LSID: urn:lsid:zoobank.org:pub:DA36E402-4A4A-4F57-AEE2-32F585CFC6C0

Species name: urn:lsid:zoobank.org:act:E9C17D86-AB13-4840-8FCB-BCA545474264

Discussion

Species morphologically similar to Bugula tsunamiensis

Bugula tsunamiensis is a member of the *B. uniserialis* group (see Vieira et al. 2012; Fehlaue-Ale et al. 2015). Only one member of the *Bugula uniserialis*-group has been previously reported from Japan, *Bugula constricta*. Here, we elevate *B. constricta* to full species status from *Bugula scaphoides constricta* based upon a distinctive indentation at the base of the zooecial outer later wall and “the shorter spinous process at the outer angle of [the] zooecial aperture” (Yanagi and Okada 1918), characters not found in the Indo-West Pacific *Bugula scaphoides* Kirkpatrick, 1890. *Bugula constricta* is a deep water species collected from Sagami Bay, central Honshu, in 620 meters (Yanagi and Okada 1918); it apparently has not been found again. *Bugula constricta* differs from *B. tsunamiensis* by the former’s small avicularia and ovicells with reticulated pattern (characters also shared with *B. scaphoides*).

Bugula scaphula Tilbrook, Hayward, and Gordon, 2001 from Vanuatu differs from *B. tsunamiensis* in having globular ovicells, smaller zooids (about 0.45 mm long and 0.15 mm wide), and smaller, more elongate avicularia (about 0.125 mm long and 0.074 mm wide). *Bugula rochae* Vieira, Winston and Fehlaue-Ale, 2012 from Brazil, noted below, has more

compact, distinctly smaller avicularia with a stronger hook to the rostrum, as well as smaller ovicells.

Several new species of *Bugula* in the *uniserialis* group were described by Vieira et al. (2012). *Bugula tsunamiensis* overlaps in measurements in a number of characters with several of these species, particularly in terms of avicularia length; *B. tsunamiensis* possesses avicularia which may approach the small avicularia size of *Bugula gnoma* Vieira, Winston and Fehlaue-Ale, 2012, or which are as large (and larger) as those of *Bugula biota* Vieira, Winston and Fehlaue-Ale, 2012. The only species that grows in a recumbent fashion is *B. gnoma*, but that species has smaller avicularia on average (0.076 mm) than the smallest avicularia of *B. tsunamiensis* (0.083 mm) (Vieira et al. 2012). Both *Bugula ingens* Vieira, Winston and Fehlaue-Ale, 2012 and *B. rochae* have avicularia with a cuspidate peduncle, absent in *B. tsunamiensis*. Additionally, *B. rochae* has a small peduncle cushion as compared to the long cushion of *B. tsunamiensis* and the length to width ratio of the avicularia of *B. ingens* is smaller (1.3–1.55:1) than that of our new species (1.45–1.70:1). *Bugula tsunamiensis* is most similar to *B. biota*, but the former has a long peduncle cushion, recumbent growth, and lacks the abfrontal V-shaped indentation on the avicularia.

Bugula tsunamiensis bears some similarity (L. M. Vieira, personal communication, 2017) to *Bugula pedunculata* O’Donoghue, 1925 (Vieira et al. 2012). *Bugula pedunculata* was described from specimens from “rocks” (likely intertidal) in La Jolla in San Diego, California, near the Scripps Institution of Oceanography. It has not been reported since its original description. Between 1925 and 1928, O’Donoghue loaned a specimen of *B. pedunculata* to Anna B.

Hastings, who synonymized it with *Bugula uniserialis* (Hastings, 1930). *Bugula uniserialis* had been described from “weed” from “Western Australia”, without any further location details (Vieira et al.’s (2012) remark that Hastings synonymized *B. pedunculata* with *B. minima* is in error). The name *Bugula uniserialis* has been applied since to many populations around the world, but is now recognized as a global species complex (Vieira et al. 2012). Hastings (1930) believed O’Donoghue’s La Jolla material to be similar to specimens she identified as *B. uniserialis* from the Galapagos Islands; no additional specimens identified as *B. uniserialis* have been noted since Hasting’s report. We suggest here that the latter may represent an undescribed species, unless the Eastern Pacific name *B. pedunculata* would apply to those populations as well.

Based upon O’Donoghue’s description and two line drawings (O’Donoghue 1925), which are

unaccompanied by measurements, *B. pedunculata* is a distinct, albeit related species to *B. tsunamiensis*. *Bugula pedunculata* has smaller avicularia, more elongate ovicells, and is less branched than *B. tsunamiensis*. Further, *B. tsunamiensis* lacks the thin, distinctive peduncle on the ooecium that inspired O'Donoghue's name. We have attempted to locate O'Donoghue's specimens of *B. pedunculata*, which were loaned to O'Donoghue by Trevor Kincaid of the University of Washington (Seattle, Washington, USA). At the time Charles O'Donoghue described *B. pedunculata*, he was at the University of Manitoba (Winnipeg, Manitoba, Canada), but later moved to the University of Reading (Berkshire, England). Anna Hastings (to whom O'Donoghue loaned material, as noted above) was based at the Natural History Museum in London (England). None of these four institutions have O'Donoghue's or any other material of *B. pedunculata* (Melissa Frey, Burke Museum, University of Washington, Seattle, Washington USA; Alan Kohn, Department of Biology, University of Washington Seattle, Washington USA; Brenda Hann, University of Manitoba, Winnipeg, Manitoba, Canada; Amanda Callaghan (University of Reading, Berkshire, England), and Mary Spencer Jones, Natural History Museum, London, England, personal communications).

Phylogenetic relationships of Bugula tsunamiensis

Of the species closely grouping phylogenetically with *B. tsunamiensis* (Figure 4: *B. migottoi*, *B. neritina*, *B. rochae*, *B. subglobosa* Harmer, 1926, and *Crisularia cucullata* (Busk, 1867), only *B. rochae* (discussed above) is in the *B. uniserialis* group. While the *Bugula* clade in Figure 4 is highly supported, the relationships of species within the clade are less certain. Thus it is plausible that *B. tsunamiensis* has a closer relationship to *B. rochae* than is suggested in Figure 4, or the uniserial appearance is evolutionarily labile.

Temporal patterns of arrival and possible biogeographic origins

Bugula tsunamiensis appeared on only two objects in 2013, but 41 times between 2014 and 2016; thus, approximately 95% of populations arrived in later years, suggesting that these objects took a longer path around the North Pacific Ocean. Fifteen of these objects (35%) also bore warm-water if not subtropical species that were acquired in post-tsunami southern ocean transit routes (Carlton et al. 2017). Thus while *B. tsunamiensis* may be native to the Tohoku region, it may be more common in warmer waters south of the Boso Peninsula.

Although *B. tsunamiensis* represents one of the more common bryozoan species on JTMD (McCuller and Carlton 2018), it is a delicate and somewhat inconspicuous species that may be easily overlooked. We note that there are few studies of the marine cheilostome Bryozoa of the Tohoku coast (Okada 1929; Okada and Mawatari 1937; Mawatari 1948; also see Hirose 2017), with only one study of the bryozoans from the Iwate Prefecture (Hirose et al. 2012), and none from Miyagi Prefecture. A majority of the bryozoan work in Japan has been focused on Hokkaido, middle and southern Honshu (Sagami Bay and Kii Peninsula), and the southern islands of Japan (Shikoku, Kyushu, and the Nansei Islands) (Grischenko et al. 2007, and summaries in Hirose 2017), but there are no similar species reported in those studies.

Designation of type location

In close concert with this new species of *Bugula* having been recovered to date only from rafted objects, West et al. (2016) have described a new red algal genus and species, *Tsunamia transpacific*, also known only from Japanese tsunami marine debris. We share the conundrum with authors since the early 1800s of first discovering undescribed species from either their means of conveyance or in regions to where they were conveyed (Carlton 2009). Lesueur (1823) described the ascidian *Styela plicata* from the hull of a vessel in the harbor of Philadelphia (Pennsylvania, USA); it is now known to be a Western Pacific species. At least three species of barnacles remain known only from ships' hulls (Newman and Ross 1976), and shipworm (teredinid bivalve) species were regularly first described in the 19th century from wooden ships far from the original homes of the species concerned (Turner 1966). Faubel and Gollasch (1996) described a marine flatworm, *Cryptostylochus hullensis*, from the fouling community on the hull of an automobile carrier that had arrived in the Port of Bremerhaven, Germany; they speculated that the species may have come from a distant warm-water port. Similarly, a long list of allochthonous freshwater and terrestrial invertebrates were first described from (and with type localities as) European greenhouses and botanical gardens (Lankester 1880; Lincoln 1979; Taiti and Ferrara 1991; Moore and Gibson 1985).

We suggest that *B. tsunamiensis* settled in nearshore waters of the Northwest Pacific Ocean on the vessels and other items sent to sea by the tsunami, and not after the arrival of these same objects in the Northeast Pacific. No similar species has been described from the Pacific coast of North America,

amongst a fairly well-known bryozoan fauna. Further, no adult invertebrates known solely from the Pacific coast of North America or Hawaii were on any of the objects (Table S1) that supported *B. tsunamiensis* (Carlton et al. 2017). It would appear unlikely that the sole species to do so would be a bryozoan whose life history includes non-feeding larvae of only a few hours (if that) duration in the water column, and thus not likely to colonize objects in open ocean waters.

We predict that *B. tsunamiensis* will be found in biofouling communities of Honshu, Japan, if not specifically in Miyagi and Iwate Prefectures (Table S1). More extensive work, similar to that of Vieira et al. (2012) in the Southwestern Atlantic Ocean, is needed to understand the diversity of Bugulidae in the Northwest Pacific Ocean.

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Supplementary material

The following supplementary material is available for this article:

Table S1. JTMD Objects with records of *Bugula tsunamensis* new species.

Table S2. Measurements (in mm) of *Bugula tsunamensis* new species according to object (BF) intercept location.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2018/Supplements/AI_2018_JTMD_McCuller_et al_SupplementaryTables.xlsx