Aquatic Invasions (2018) Volume 13, Issue 1: 31–41
DOI: https://doi.org/10.3391/ai.2018.13.1.04

Special Issue: *Transoceanic Dispersal of Marine Life from Japan to North America and the Hawaiian Islands as a Result of the Japanese Earthquake and Tsunami of 2011*

Research Article

Porifera (Sponges) from Japanese Tsunami Marine Debris arriving in the Hawaiian Islands and on the Pacific coast of North America

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Received: 17 March 2017 / Accepted: 12 December 2017 / Published online: 15 February 2018

Handling editor: Amy Fowler

Co-Editors' Note:
This is one of the papers from the special issue of Aquatic Invasions on "Transoceanic Dispersal of Marine Life from Japan to North America and the Hawaiian Islands as a Result of the Japanese Earthquake and Tsunami of 2011." The special issue was supported by funding provided by the Ministry of the Environment (MOE) of the Government of Japan through the North Pacific Marine Science Organization (PICES).

Abstract

Twelve species of sponges (Calcarea and Demospongiae) were found on Japanese Tsunami Marine Debris (JTMD) that washed ashore in Oregon, Washington, and Hawai'i. All taxa but one determined to species level are amphi-Pacific, with three having type localities in California (*Leucosolenia eleanor* Urban, 1906, *Hymeniacidon sinapium* de Laubenfels, 1930, and *Mycale macginitei* de Laubenfels, 1930). *Haliclona xena* de Weerdt, 1986, known previously only from western Europe (and where it is regarded as introduced from an unknown region) is here newly reported from the Tohoku coast of Honshu, as is *Halisarca* "*dujardini* Johnston, 1842". Five species (*Mycale macginitei*, *Hymeniacidon sinapium*, *Ute* sp., *Haliclona xena* and *Halisarca* "*dujardini*") were observed only once. Multiple lines of evidence (including lack of colonization by uniquely Eastern Pacific sponge species, the arrival in Hawai'i of some of the same species whose only possible origin was Japan, and the low probability of coastal sponge larvae colonizing JTMD in the open ocean) indicate that the sponges on JTMD originate from the Western Pacific. Several species of sponges may have completed multiple generations on these long-distance rafts.

Key words: transoceanic dispersal, rafting, invasive species, cosmopolitan species complexes, *Haliclona xena*

Introduction

Transoceanic dispersal of shallow-water sponges can occur only by passive mechanisms, either naturally (such as by rafting on algae or seagrasses) or through human mediation (such as in ship fouling or by the movement of commercial shellfish). Indeed, the long history of moving commercial oysters (which frequently are harvested from rafts that support a diversity of sponge species; see Tanita 1958; Hoshino

1975) around the world may have altered the distribution of a number of sponge species. In contrast, the typically short duration of sponge larval life (24 to 48 hours) means that larvae are rarely if ever encountered in offshore plankton (Maldonado and Berquist 2002; Maldonado 2006), and thus natural recruitment over distances of more than a few kilometers per year is not favored.

The increasing amount of non-biodegradable marine debris in the world's oceans may serve as a novel vector for many shallow-water invertebrates. Monsoons, hurricanes, and tsunamis may eject debris into the ocean in the form of large pulses (Carlton et al. 2017). The March 11, 2011 Great Earthquake and Tsunami that impacted the Tohoku coast of northeast Honshu, Japan, released a vast number of objects, ranging from large docks and vessels to small buoys, into the Western Pacific Ocean. These objects began to come ashore in the Central Pacific (the Hawaiian Archipelago) and the Eastern Pacific (North America) in 2012 (Carlton et al. 2017).

We report here on the sponges (Phylum Porifera) that were collected on Japanese Tsunami Marine Debris (JTMD) that washed ashore from Washington to California and on two islands in Hawai'i.

Materials and methods

Morphological analysis

Forty-nine sponge samples (a total of 71 specimens) were obtained from a wide variety of JTMD objects (identified as such through multiple lines of evidence; see Carlton et al. 2017) landing between 2012 and 2016 (Supplementary material Table S1). Each object was assigned a unique identification number preceded by JTMD-BF- (Japanese Tsunami Marine Debris – BioFouling -). Sponges were either placed directly in 95% ethanol or were frozen (as part of larger bulk samples) and then later transferred to ethanol.

Samples were initially examined using a dissecting microscope in order to ensure separate species were under study. In some cases, up to three different species of calcareous sponges were found entwined around each other. Once separated, an estimate of volume in cubic centimeters per species was made when possible. Thin sections and spicule slides were made using standard techniques, which included paraffin embedding, sectioning at 100 µm, hydration, staining with basic fuchsin, and mounting in Permount (Fisher Scientific, Pittsburgh PA USA). A 1–2 mm^3 perpendicular section was dissolved with a drop of sodium perchlorite (Chlorox™), diluted with water and observed directly. This procedure insures that small elements are not lost to rinsing and that calcareous spicules are not dissolved. After measuring 30–50 spicules of each shape and size category, the spicule mix is carefully drained into a test tube, allowed to settle, rinsed with 90% alcohol, allowed to settle, evenly spread on a slide, dried, and mounted with Permount. Digital images were taken of slides.

Field records, statistical data, and images were stored in a MySQL (Oracle Corporation, USA) image database (available from the first author). Specimens > 5 cm^3 have been deposited at the Royal British Columbia Museum in Victoria BC. Smaller volumes, if any specimen was left, were archived as whole mounts. All permanent slide preparations were archived as well.

Genetic analysis

Three approximately 20 × 20 cm scrapings were taken from the sides of a floating dock (JTMD-BF-1) originating from the Port of Misawa, Aomori Prefecture, which landed on the central Oregon coast in early June 2012 (Table S1). The samples were preserved in 70% ethanol and sent to the Geller Laboratory at Moss Landing Marine Laboratories, Moss Landing, California USA. The ethanol was later decanted and samples were rinsed with distilled water, drained, and homogenized in an IKA A11 analytical mill (Wilmington, NC, USA). 10 g of homogenate were used in a MoBio PowerSoil DNA extraction kit (Qiagen, Germantown, Maryland, USA). Genomic DNA was quantified using Nanodrop ND-1000 (ThermoFisher, Waltham, Massachusetts USA). 5 ng of each total DNA extraction were amplified in PCR cocktails comprising a final concentration of 1× Green Go Taq Master Mix, 0.2 mg mL^{-1} BSA, 1.5 mM $MgCl_2$, and 0.2 µM of each primer in a 50 µL reaction. We used primers jgHCO2198 and jgLCO1490 from Geller et al. (2013). Reaction conditions consisted of an initial 3 minute melt at 94 °C, followed by 32 cycles of a 1 minute at 95 °C, 45 seconds at 47 °C, and 90 seconds at 72 °C. PCR amplicons were viewed on a 2% agarose gel stained with ethidium bromide. Samples were purified with 1.4 × the sample volume of Agencourt Ampure (Brea, California USA) beads, according to the manufacturer's protocol.

Samples were quantified using Picogreen High sensitivity DNA assay according to the manufacturer's protocol (Qiagen). 100 ng of sample were fragmented with the IonXpress Ion Shear enzyme kit (ThermoFisher). Samples were purified with 1.4 × the sample volume of Agencourt Ampure beads. Samples were then ligated with IonXpress barcodes and sequencing adapters, size selected for ca. 400 bp using an e-gel cassette, purified once more with 1.4 × Ampure beads.

Samples were quantified using the Agilent (Santa Clara, California, USA) Bioanalyzer high sensitivity chip assay and combined into an equimolar pool. Samples were run using the Ion Torrent 400 bp sequencing kit and v314 chip according to the manufacturer's protocol, yielding 500,000 reads passing filter. Reads were trimmed of primers and clustered into groups using a 95% similarity threshold using the software package Geneious v9 (Biomatters, Auckland, New Zealand).

Consensus sequences were compared to Genbank for any matches to COI sequences annotated as derived from Porifera. Candidate novel sequences were aligned with sponge sequences downloaded from Genbank, aligned with MAFFT (Katoh and Standley 2013). Maximum likelihood trees were constructed with FastTree (Price et al. 2010) from within Geneious.

Samples of individual sponges collected from a wide variety of JTMD objects (below) were also submitted for genetic analysis (by analytical techniques as described in McCuller et al. 2018), but failed to sequence.

Systematics and biogeography of North Pacific Porifera

Despite more than 100 years of research on marine sponges both in Japan (reviewed by Ise 2017) and on the Pacific coast of North America (reviewed by de Laubenfels 1932 and Lee et al. 2007), a great deal remains to be known about poriferan taxonomy and distribution in the North Pacific Ocean. Early workers regularly employed the names of Atlantic species for Pacific sponges, a legacy remaining to this day, resulting in an unknown number of shallow-water North Pacific species that likely remain undescribed as part of global species complexes. In the present work, we use quotation marks around such species names to indicate that they are not likely to represent the same North Atlantic taxa whose names they still bear.

Results

Twelve species of what we interpret to be Western Pacific sponges were found on JTMD. We discuss below the evidence suggesting that these populations arose in Japan, and were not likely to have been acquired after arrival in the Eastern or Central Pacific. Calcarea are represented by five species in three orders and four families; the Demospongia are represented by 7 species in five orders and six families. Two species reported here were found solely as DNA sequences from a metagenetic analysis of fouling biomass on the Misawa fisheries dock (JTMD-BF-1). The ecologically well-known but taxonomically challenging boring sponges (Clionaidae) were present as remnant spicules or empty holes, but as only a very small fraction of biofouled JTMD was intercepted and sampled in North America and Hawai‘i (Carlton et al. 2017), clionaids may have arrived alive on other objects.

All taxa but one which we were able to determine to species level are considered to be amphi-Pacific, with three having type localities in California (*Leucosolenia eleanor* Urban, 1906, *Hymeniacidon sinapium* de Laubenfels, 1930, and *Mycale macginitei* de Laubenfels, 1930). *Haliclona xena* de Weerdt, 1986, known previously only from western Europe (and to where it is regarded as introduced from an unknown source, as discussed below) is here newly reported from the Tohoku coast of Honshu. Also newly reported from Japan is *Halisarca* "*dujardini* Johnston, 1842", as discussed in the Systematic Account below. Five species (*Mycale macginitei*, *Hymeniacidon sinapium*, *Ute* sp., *Haliclona xena* and *Halisarca* "*dujardini*") were seen only once, arriving over a span of several years. In concert with patterns seen in other JTMD taxa (Carlton et al. 2017), diversity declined over time, with early 2012 to winter 2013 arrivals having up to six species per object. After the spring of 2013, with rare exception, most JTMD items arrived with only two species aboard.

Systematic Account

Class Calcarea

Calcarea were numerous; all samples consisted of small specimens.

Order Clathrinida
Family Clathrinidae

Clathrina sp.

Material. BF1 (0.0025 cm^3); BF23 (0.2805 cm^3, 2 pieces); BF40 (1.0005 cm^3, 2 pieces); BF223 (0.0255 cm^3); BF329 (0.0085 cm^3). Frequently as a mass of brownish tubes mixed in with *Leucosolenia*.
Remarks. Identification was based on brown anastomosing tubes and a small leaf-like piece (possibly representing an immature stage before anastomosing tubes have been formed) with equiangular, equilateral triactines within the expected size range (up to 180 µm). Klautau and Valentine (2003) have discussed the confusion in *Clathrina* species. Recruitment on these JTMD objects, which were released into the ocean in March 2011, may have occurred between autumn and spring, with growth during early spring, a distinguishing character of certain *Clathrina* species (Johnson 1978b).

Distribution. The material in hand is similar to *Clathrina coriacea* (Montagu, 1814), a widespread North Atlantic (including Mediterranean) species (Klautau and Valentine 2003). A *Clathrina* species also given this European name is reported from both Japan (Ise 2017) and the Pacific coast of North America (Johnson 1978a; Austin and Ott 1996; Lee et al. 2007). Whether the European clade is introduced to New Zealand (Cranfield et al. 1998), or is an undescribed Pacific member of the complex, is unclear. *C. coriacea* is reported from intertidal and subtidal habitats.

Order Leucosoleniida
Family Leucosoleniidae

Leucosolenia eleanor Urban, 1906

Material. BF1 (in the mussel *Mytilus galloprovincialis* Lamarck, 1819 beds on the dock, 0.0405 cm^3, 4 pieces); BF8 (0.0805 cm^3, 2 pieces); BF23 (0.0325 cm^3); BF40 (5.2705 cm^3, 3 pieces); BF131 (0.0015 cm^3); BF240 (0.0015 cm^3); BF329 (0.0015 cm^3); BF356 (0.5005 cm^3, 3 pieces); BF455 (0.4005 cm^3); additional material of very small specimens also from BF23 (0.0015 cm^3) and BF223 (0.0015 cm^3) discussed below.

Remarks. With one exception (BF40), all samples were less than 0.085 cm^3 in volume. Individuals were wrapped around the base of *Sycon* "*raphanus*" or entwined with *Clathrina* sp. This species is similar to the North Atlantic *L. variabilis* Haeckel, 1870, but the growth form consists of a mass of anastomosing tubes, oxea length is greater than 200 µm and triactines and quadriradiates are slightly smaller. The single large specimen (BF40) was a mat of small and large erect tubes. Two very small specimens from BF23 and BF223 have shorter (< 200 µm) oxeas, but the triradiates and quadriradiates are also smaller than expected and may represent young colonies of *L. eleanor*.

Distribution. Originally described from Monterey, California, *L. eleanor* occurs from British Columbia to southern California (Bakus and Abbot 1980; Austin and Ott 1996; Lee et al. 2007). It is also reported from Japan (Bakus and Abbott 1980; Ise 2017). Whether this species is native or introduced to the Western Pacific (if the same species) is not known. Bakus and Abbott (1980) report it from the intertidal to 26 m, as well as fouling on vessel hulls.

Order Leucosolenida
Family Sycettidae

Sycon "*raphanus* Schmidt, 1862"

Material. BF1 (0.045 cm^3); BF23 (0.0355 cm^3); BF356 (0.045 cm^3, 3 pieces); BF402 (0.0125 cm^3).

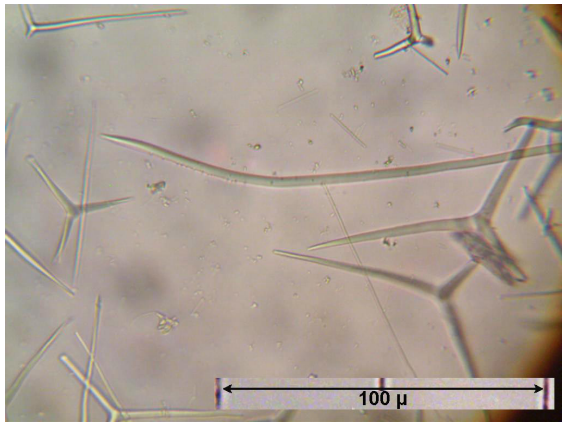

Figure 1. *Sycon* "*raphanus*", (BF-1, dock from Aomori Prefecture). Microphotograph by D. Elvin.

Remarks. This species is said to occur on both sides of the North Pacific Ocean (Hozawa 1929; Austin 1985). It is closely related to *Sycon ciliatum* (Fabricius, 1780), a North Atlantic species, also reported in Japanese and North American literature. The two species differ genetically (Voigt et al. 2012) as well as in gross morphology: the radial choanocyte tubes are distinctly separate in *S. ciliatum*, but are at least 90% attached in *S. raphanus*. Long thin diacts (up to 1350 µm) have a bend at 100 µm with short spines (Figure 1). Schmidt's original description shows a drawing of a diact with such a bend, but it is not detailed enough to show small spines. We have not seen this feature discussed in more recent literature.

Three groups of small individuals (1–2 mm^3) demonstrated variations:

***Sycon* sp. variety A.** (BF1 (0.0015 cm^3); BF8 (0.0025 cm^3)) One 1.5 mm specimen was found entwined in a mass of *Leucosolenia eleanor*. It may be a young *S.* "*raphanus*" but was too small to permit spicule preparation. Distinct features, however, include a stalk 45% of the total specimen height (in contrast to a small stalk for *S.* "*raphanus*") and possibly an oscular collar at right angles to the oscular crown.

***Sycon* sp. variety B.** (BF1 (0.0025 cm^3); BF209 (0.0125 cm^3)) This material (2 mm^3 specimens) may be *S.* "*raphanus*", but some of the quadriradiate basal rays are very long.

***Sycon* sp. variety C.** (BF23 (0.0015 cm^3)) A single 1 mm specimen possesses two sizes of thick diacts in addition to the long thin ones. Triacts and particularly the quadracts are unequally paired or have sagittal rays and long basals.

Distribution. A well-known European species, the name *Sycon raphanus* has been applied to a *Sycon*

species on both the Pacific coast of North America (Austin 1985) and Japan (Hozawa 1929; Ise 2017). Goldstein et al. (2014) reported "*Sycon* spp." from several marine debris objects drifting in the mid-North Pacific in 2011–2012 between 3,500 and 4,600 km from North America.

Family Grantiidae

***Leucandra* sp.**

Material. BF23 (0.0255 cm^3); BF356 (0.0025 cm^3); BF402 (0.0055 cm^3).

Remarks. Specimens were small white crunchy mounds (5 × 5 × 1 mm^3) composed of thick diacts (275 µm) and triacts (125 µm) but unfortunately consisting of insufficient material to pursue to species. The oxeas and triacts are reminiscent of *Leucandra heathi* Urban, 1906 but are lacking the large spicules expected in mature specimens. No quadriradiates were found. Ise (2017) lists at least 30 species of *Leucandra* in the Japanese fauna.

***Ute* sp.?**

Material. BF402 (0.020 cm^3).

Remarks. This very small specimen is a solid tube/oval vase, 5 mm tall and 2 mm in diameter. There is no stalk and no coronal collar, but it does have parallel tracts of large thick diacts laying parallel to the surface (measuring up to 629 µm). This last feature is characteristic of the genus *Ute*. Triradiate and quadriradiate basal rays averaged 158 µm and 100 µm, respectively. More material would be required for confirmation. Ise (2017) notes two species of Japanese *Ute*, *U. armata* Hozawa, 1929, and *U. pedunculata* Hozawa, 1929.

Class Demospongiae
Order Clionaida
Family Clionaidae

***Cliona* sp.**

Material. BF168 (clionaid-excavated substrata in shells of the barnacle *Megabalanus rosa* Pilsbry, 1916); BF197 (in oyster shell with remnant tissue); BF369 and BF 677 (both of the latter, clionaid-excavated substrata in oyster (Ostreidae and Gryphaeidae) shells).

Remarks. BF197 represents a *Cliona* that has bored into the shell of the Japanese oyster *Crassostrea gigas* (Thunberg, 1793), leaving a few clionaid tylostyles but no fresh material. No further identification of this material is possible at this time. *Cliona* excavations were observed in barnacle and oyster shells on a number of objects (examples above), but the sponges were either dead prior to the tsunami, or failed to survive the transoceanic voyage.

Distribution. Hoshino (1981b) and Ise (2017) review several *Cliona* species known from Japan.

Order Suberitida

Halichondria and *Hymeniacidon* species are relatively tough, such that their skeletons would be expected to survive on floating debris for some time after the individuals have died.

Family Halichondriidae

***Halichondria* "*panicea* Pallas, 1766"**

Material. BF1 (1.4005 cm^3, 3 pieces); BF23 (48.000 cm^3); BF172 (2.000 cm^3); BF196 (18.000 cm^3); BF208 (2.000 cm^3)

Remarks. This is a species group with varieties related to microfloral and other differences. The species is defined by existence of an ectosome, mostly vertically multispicular skeleton tracts in the choanosome, and otherwise random distribution of oxeas (160–420 µm) of wide (> 50%) range in size. The ectosome may slough off under fouling conditions (Barthel and Wolfrath 1989). Additional material (BF1, 23, 172) represents small specimens (0.001 cc or less) in which the spicules conform to the shape and size of *H.* "*panicea*", but the sample is too small for thick section confirmation. Two community metabarcode sequences derived from BF-1 were 93.8% and 93.9% similar to sequences of *H.* "*panicea*" in GenBank (KC869423 and EF095183) from Morrow et al. (2013) and Itskovich et al. (2007) respectively (Figure 6). As no sequences (other than the present one) of the *H. panicea*-group are available from Honshu, it is not surprising that our material does not align completely with populations from elsewhere in this global species complex.

Three additional sets of specimens of *Halichondria* may prove to be within the range of variation of *H.* "*panicea*" (*sensu lato*):

***Halichondria* sp. variety A** (0.001 cm^3). This specimen was on a valve of the Japanese barnacle *Megabalanus rosa* from a buoy found at Long Beach, Washington in 2004. This is a pre-JTMD specimen, as discussed below. Two very small specimens whose structure could not be determined. They both have interesting sinuous diacts (Figure 2) with lengths of 235–240 µm. It may further be related to *H. surrubicunda* (Hoshino, 1981), but no definite determination can be made without larger specimens.

***Halichondria* sp. variety B** (BF229, 10 cm^3). A large amount of material that appears to have been thinly attached has the halichondriid spicule structure, a tough growth form, fibrous matter of thick multispicular

tracts and otherwise confused architecture, but not the "crumb-of-bread" type of *H.* "*panicea*". Oxea length is too long to associate this specimen with *H. bowerbanki* Burton, 1930.

***Halichondria* sp. variety C (**BF1, 0.900 cm^3). This small specimen from the Misawa 1 floating dock is white-cream in preservation. Its features are multi-spicular tracts, confused architecture, and oxeas averaging 253 µm and 51% range in length. It differs from the specimens above by having spicular papillae on the rugose surface. Based on the superficial surface appearance, this specimen appears to be a young *H. sitiens* (Schmidt, 1870), but larger, mature individuals would be required to confirm identification. Although this boreal-arctic species has not been reported from the Washington, Oregon, or California coasts, it has been noted sporadically at sites around the North Pacific. Hoshino (1981a) and Ise (2017) treat it as a cosmopolitan species found in Western Japan. Lambe (1894) lists it as *Eumastia sitiens* from Alaska. de Laubenfels (1957) reported a single specimen from Pearl Harbor as *Pellina sitiens*, which identification, however, Bergquist (1977) assigned to the Indo-Pacific *Pellina eusiphonia* Ridley, 1884 (now *Oceanapia eusiphonia*).

Distribution. de Laubenfels (1932) implied long ago that the application of the European name *Halichondria panicea* around the world indicated a probable and problematic species complex, a position supported by Lee et al. (2007) and others. *H.* "*panicea*" is reported along much of the Pacific coast (de Laubenfels 1932; Austin and Ott 1996; Lee et al. 2007) and in Japan (Hoshino 1981a; Ise 2017). Goldstein et al. (2014) reported *H. panicea* (as "*panacea*") from several marine debris objects drifting in the mid-North Pacific in 2009 between 1,000 and 2,100 km from the North America coast.

Hymeniacidon sinapium de Laubenfels, 1930

Material. BF226 (2.600 cm^3).

Remarks. This is a species that might also be typically expected in biofouling communities. Fuller and Hughey (2013) have reviewed aspects of the distribution and history of *H. sinapium* in the Western and Eastern Pacific Oceans. While they concluded that *H. sinapium* was introduced from Japan to California, a suite of historical, biogeographic, and vector evidence suggests that the reverse is more likely to be the case (J. T. Carlton, unpublished).

Distribution. Originally described from southern California with a curious habitat breadth (from the exposed surf-swept rocky shore to brackish oyster beds in Newport Bay) and since then expanding its range, presumably with warming coastal waters, into central California (Fuller and Hughey 2013). Reported from Japan, where a complex taxonomic history and confusion with *Halichondria japonica* (Kadota, 1922) accompanies this species (Fuller and Hughey 2013).

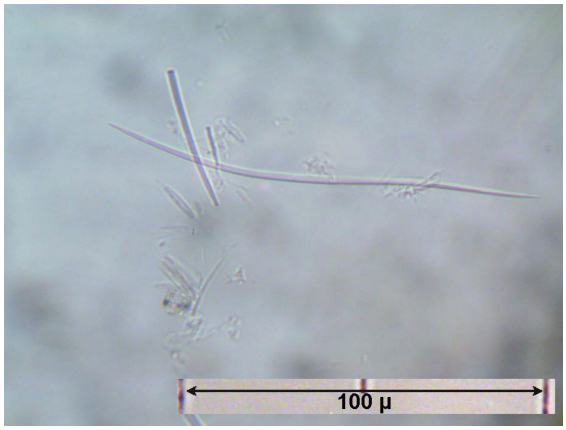

Figure 2. *Halichondria* sp., var. A, from a buoy ashore in Washington, 2004 (see text). Microphotograph by D. Elvin.

Order Poecilosclerida
Family Mycalidae

Mycale (*Mycale*) *macginitei* de Laubenfels, 1930

Material. BF1 (0.015 cm^3).

Remarks. One specimen of *Mycale macginitei* represents the only poecilosclerid species found on JTMD material.

Distribution. A presumably native California species first described from the brackish waters of Elkhorn Slough, Monterey Bay, and distributed from the Pacific Northwest to southern California (Austin and Ott 1996; Lee et al. 2007). It is also reported from Korea and Japan, including from Matsushima on the Tohoku coast (Hoshino 1981a), the latter coastline the source of the tsunami debris.

Order Haplosclerida
Family Callyspongiidae

Callyspongia sp.

Material. BF8 (45.000 cm^3); BF168 (1.000 cm^3); BF386 (0.200 cm^3).

Remarks. A large (11 × 10 cm and from 0.5 to 1 cm thick) specimen (BF8, with considerable foreign material embedded) is tough fibrous and encrusting (Figure 3); the dorsal surface is relatively smooth but has 1 mm projections of spicules bundles; oscules

1.5–3 mm without rims; pores 750 µm. The ventral surface is smooth; the specimen may have extended out from the float in leaf-like manner. Spicules are oxeas 90–101 ± 7–113 µm × 4–5 µm (with a 7% standard deviation and 23% range). Larger oxeas with have hastate ends were noted. The skeletal structure of 60 µm multispicular (5 spicule width) branching tracts in all directions with thinner individual spicules in between (Figure 4); multispicule sheaths surround 200 µm diameter canals giving both the ectosome and choanosome a "honey-comb" appearance (Figure 5). These characters are consistent with those of *Callyspongia murex* (Hoshino, 1981), but the large amount of foreign material suggests a contaminated *Callyspongia* skeleton. *C. murex* has been observed to be fouling on floating fish cages (Raveendran and Harada 2001). Fresh material would be required to confirm species-level identification.

Distribution. *Callyspongia murex* is a Japanese species (Ise 2017) described from Matsushima on the Tohoku coast from intertidal and subtidal rocky substrate (Hoshino 1981a).

Family Chalinidae

***Haliclona xena* De Weerdt, 1986**

Material. BF1: A community metabarcode sequence (Figure 6), with 100% pairwise identity over 247 bp, to a GenBank sequence (JN242209) of *Haliclona xena* from the Netherlands, the latter deposited by Redmond et al. (2011).

Remarks. *Haliclona xena* was described as a new species from The Netherlands, albeit recognized at the time as a probable introduction (and thus the name, derived from the Greek *xenos*, for strange or outsider) from an unknown source (de Weerdt 1986). It remains generally regarded as non-native in western Europe (van Soest et al. 2007, 2012). It has not, until now, been reported from elsewhere in the world. No additional specimens of *Haliclona* were collected on the Misawa dock (JTMD-BF-1) that landed in June 2012 in central Oregon. However, the dock supported more than 75 square meters of biofouling, which was only partially sampled.

Also in hand is a small sample (0.005 cm^3) of a chalinid sponge from BF-207, a buoy that floated into the harbor of Charleston in Coos Bay, in southern Oregon. This sponge was white, with a rugose surface, encrusting on a mass of dead hydroids. The sample is too small to permit identification beyond family level. This specimen may have died (but remained intact) shortly before collection, as the matrix bore considerable diatoms and debris.

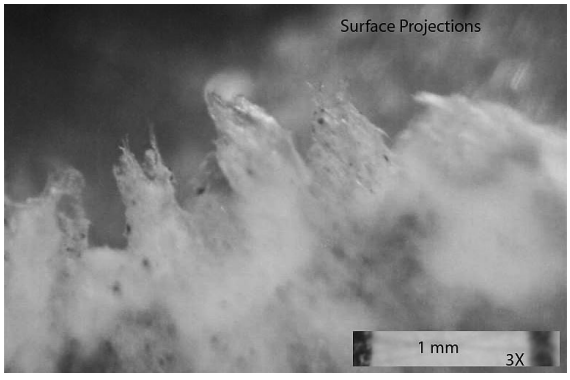

Figure 3. *Callyspongia* sp., with projecting spicule bundles (BF-8). Microphotograph by D. Elvin.

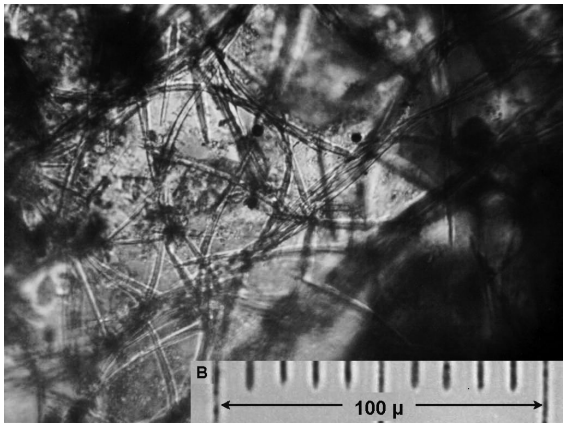

Figure 4. *Callyspongia* sp., with multispicular branching tracts with thinner spicules in between (BF-8). Microphotograph by D. Elvin.

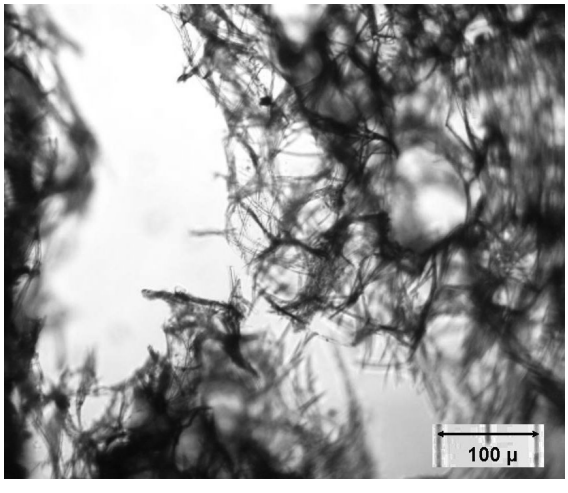

Figure 5. *Callyspongia* sp., showing honey-comb appearance (BF-168, a buoy washed ashore in Washington, 2014). Microphotograph by D. Elvin.

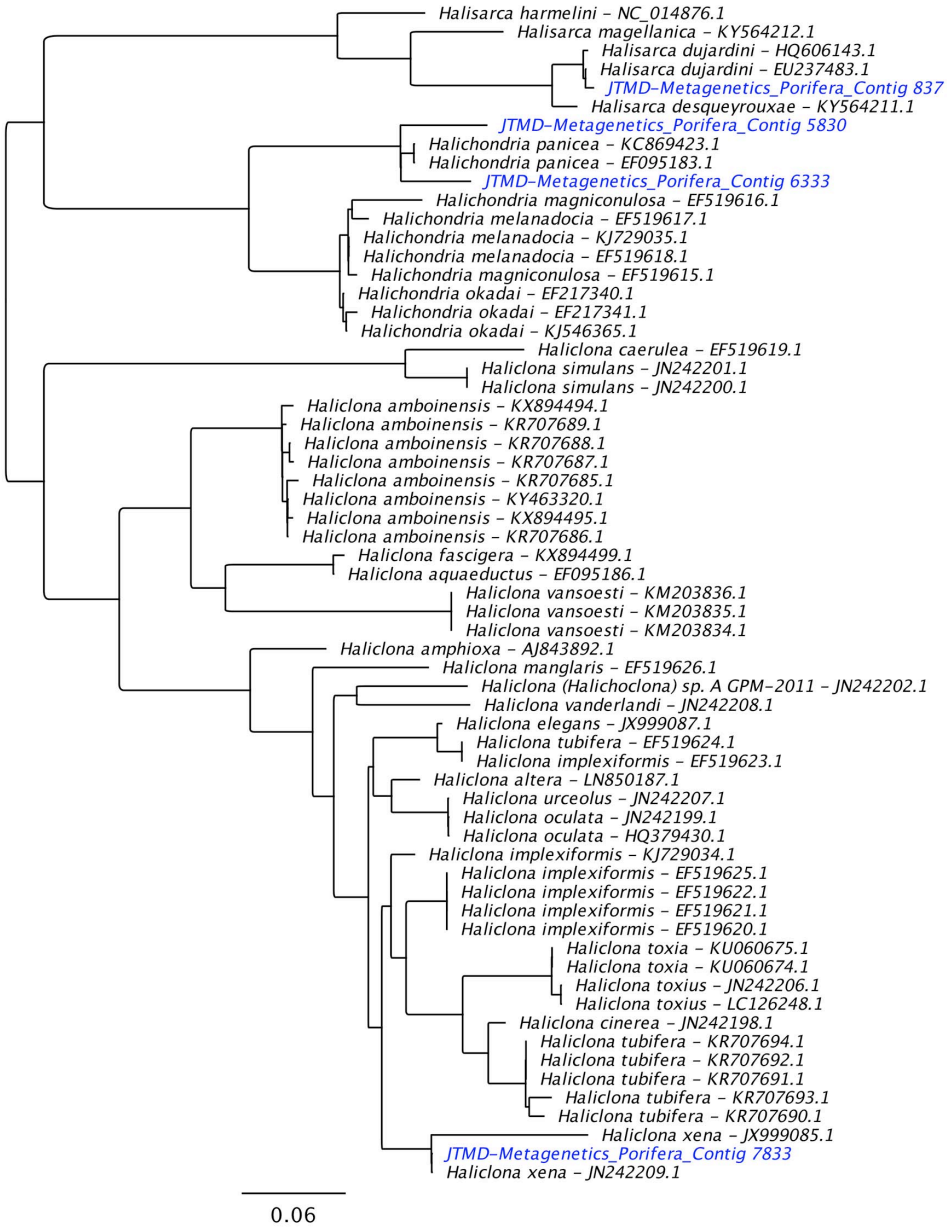

Figure 6. Maximum likelihood tree of sponges in the genera *Halichondria*, *Haliclona*, *and Halisarca*. The placement of JTMD sponges is shown in blue. Accession numbers are listed after taxonomic names given in Genbank annotations.

Order Chondrillida
Family Halisarcidae

***Halisarca* "*dujardini* Johnston, 1842"**

Material. BF1: A community metabarcode sequence (Figure 6) with 99.7% pairwise identity to Genbank (EU237483) and 99.4% pairwise identity over 319 bpto Genbank HQ606143 attributed to *Halisarca dujardini* by Kayal and Lavrov (2008) and Ereskovsky et al. (2011) respectively.

Remarks. No additional specimens of halisarcids were recovered from the Misawa fisheries dock (JTMD-BF-1) that landed in June 2012 in central Oregon. However, as noted above, the dock presented more than 75 square meters of biofouling, only a fraction of which was sampled. A metagenetic community sample from this dock yielded a sequence essentially identical to records of *Halisarca dujardini*, a complex of an unknown number of species from the

North Atlantic Ocean (where the stem species is regarded as occurring) and from the North Pacific, as well as from the South Atlantic and South Pacific (Ereskovsky et al. 2011; Alvizu et al. 2013). In the North Pacific, *Halisarca* "*dujardini*" has been recorded from Peter the Great Bay in the Sea of Japan (East Sea) (Ereskovsky et al. 2011); other species of *Halisarca* are also reported to the north of Japan (Sirenko 2013) and to the south (Lim et al. 2016). While Ise (2017) does not list any *Halisarca* species from Japan, we suggest that *Halisarca* "*dujardini*" is present and undetected on the Tohoku coast.

Discussion

We report here the first evidence of the long-distance transport of marine sponges on rafted materials from the Western Pacific Ocean to the Pacific Northwest coast of North America and the Hawaiian Islands. While sponges have been found on marine debris (of unknown origin and date of entry into the sea) on the high seas (for example, Goldstein et al. 2014), we know of no previous reports of the successful landfall of sponge-bearing overseas debris in the Central and Eastern Pacific Ocean. The discovery of *Halichondria* sp. on a Japanese buoy landing on the coast at Long Beach, Washington state, in 2004 suggests that marine debris at scales far smaller than that generated by the tsunami, may have been playing a role in transoceanic crossings for some time, although such events appear to be so rare that no records of such appear in the scientific or historical literature for the Pacific coast of North America (Carlton et al. 2017).

Origin of JTMD sponge populations

Multiple lines of evidence suggest that the sponges on the JTMD examined here originate from the Western Pacific, and may thus have completed multiple generations on these long-distance rafts:

1. With rare exception, JTMD items appear to have had low residency time in Eastern Pacific waters, such that colonization by larvae of coastal North American species on JTMD was observed only in a few cases, and these largely involved settlement by < 1.0 mm barnacles (Carlton et al. 2017). In concert with this, and critically, sponge larvae (typically with only a few hours duration in the water column, and rarely more than 24–48 hours) are unlikely to be encountered in the offshore ocean as the JTMD approached the North American coast, nor, even if acquired, would these have grown to the at times even smaller sizes observed in the apparently short time between object acquisition in the Eastern Pacific and shore landing.
2. A key element of biogeographic evidence comes from JTMD landings in the Hawaiian Islands, which include taxa here identified as *Leucosolenia eleanor, Clathrina* sp. and *Sycon* "*raphanus*". The sole possible origin of these sponges on these objects is from Japan (the routes of JTMD objects arriving in Hawaii not including the nearshore waters of North America). We conclude that it is an improbable coincidence that the same species arriving on JTMD landing in North America happened to be the same as those arriving in Hawaii, and yet the former would represent Eastern Pacific colonists.
3. Species diversity declined over time. Arriving in the summer of 2012 on the Misawa fisheries dock (BF-1) in Oregon were at least seven species (*Clathrina* sp., *Leucosolenia eleanor*, *Sycon* "*raphanus*", *Halichondria* "*panicea*", *Mycale macginitei*, *Haliclona xena*, and *Halisarca* "*dujardini*"). Arriving in the spring of 2013 on a tsunami vessel landing in Washington were five species (the first four noted on BF-1 and *Leucandra* sp.). After the spring of 2013, sponge diversity was primarily represented as only one living species, and rarely two or three species, per object, presumably due to increased mortality over time (there was no change in the types of material arriving over this period of time, all being largely plastic-based). If sponges were being acquired by JTMD objects after arrival along the North American coast, there would be no reason for diversity to decline.
4. The absence of unique Eastern Pacific sponge species on JTMD suggests that recruitment was rare to absent after objects arrived in North America (and the same may be said for the Hawaiian arrivals). That is, it would appear improbable that all the species on JTMD (with the exception of uniquely Japanese taxa) would, coincidentally, also be those known from both sides of the North Pacific Ocean.

It thus seems probable that the sponges arriving on JTMD self-recruited *in situ* over time (including over years). It may also be possible that coastal species, while surviving transoceanic transport, grew little if at all in the environmental conditions of the open ocean.

The discovery of Haliclona xena *in Japan*

The chalinid *Haliclona xena* has long been regarded as non-native in western Europe, yet of unknown provenance (de Weerdt 1986; van Soest et al. 2007, 2012). Our discovery of it in the biofouling community

on a fisheries dock originating from the Port of Misawa, Aomori Prefecture, Japan, is the first report of *H. xena* outside of the Northwest Atlantic Ocean. The long history of sponge research in both the western and eastern North Atlantic precludes the possibility that *H. xena* originated from those regions. In turn, the only major source (outside of the Atlantic Ocean) of non-native marine species in *H. xena*'s type locality of The Netherlands is the Western North Pacific. Nearly 30 species of Japanese invertebrates and algae have been introduced to The Netherlands (Wolff 2005; Gittenberger et al. 2010; Buschbaum et al. 2012; Hobbs et al. 2015). The presence of *H. xena* in the biofouling community originating from northeast Honshu suggests that this sponge's homeport may be Japan, where more than 40 additional species of *Haliclona* (*sensu lato*) occur (Ise 2017).

Conclusions

We note that none of the taxa observed here that may be unique to Japan, including *Callyspongia* sp., *Leucandra* sp., and *Ute* sp., can yet be added to the list of North American species, as they are, or were, known only from rafted objects, and we have no evidence yet of their establishment on the Pacific coast of North America. This said, sponge invasions around the world are well-documented (Cohen and Carlton 1995; Carlton and Eldredge 2009; van Soest et al. 2007; Longo et al. 2007). As noted earlier, sponge samples were obtained from only a very small fraction of the biofouled Japanese tsunami marine debris that arrived and landed in the Central and Eastern Pacific, and thus the successful introduction of novel species from the Western Pacific would not be entirely unexpected. Investigations of biofouling communities along the Pacific Northwest coast, and in the Hawaiian Islands, would do well to keep potential new invasions of Western Pacific species in mind when identifying sponges.

Acknowledgements

Thanks to the many collectors (see Carlton et al. 2017) who recovered JTMD objects and biological samples from Washington, Oregon, California and Hawaii. We thank John Anderson and Nancy Treneman for providing access to, and samples from, a 2004 Japanese buoy landing in Washington. Deborah A. Carlton and Megan I. McCuller carefully sorted sponge samples from bulk collections. We thank Melinda Wheelock and Tracy Campbell for laboratory assistance. We thank two reviewers for helpful comments. Funding for JTMD research was generously provided by Oregon Sea Grant, the National Science Foundation, and the Japanese Ministry of the Environment (MoE) through the North Pacific Marine Science Organization (PICES).

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Supplementary material

The following supplementary material is available for this article:

Table S1. JTMD Objects: BF numbers, landing site locations, dates and object types, and prefecture and city origins if known, and recorded species of sponges.

This material is available as part of online article from: http://www.aquaticinvasions.net/2018/Supplements/AI_2018_JTMD_Elvin_etal_Table_S1.xlsx