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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

Chitons (Mollusca: Polyplacophora) rafted on tsunami debris from Japan to the shores of Washington, Oregon, and Hawai‘i

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Abstract

Floating maritime objects torn from their anchorage by the March 2011 Tōhoku tsunami transported Japanese near-shore biota to the Pacific coast of North America and the Hawaiian Islands. This Japanese tsunami marine debris (JTMD) biota included five species of chitons: *Mopalia seta* Jakovleva, 1952, *Placiphorella stimpsoni* (Gould, 1859), *Acanthochitona achates* (Gould, 1859), *Acanthochitona rubrolineata* (Lischke, 1873), and an undescribed species with close affinities to *Acanthochitona defilippii* (Tapparone-Canefri, 1874), here referred to as *Acanthochitona* sp. A. The last of these was the most common chiton on the tsunami debris. Our identifications are supported by morphological characters and analyses of mitochondrial 16S and COI gene sequences from the retrieved specimens, supplemented by new samples of North West Pacific specimens. *A. rubrolineata*, a former synonym of *A. achates*, is here revived as a valid species; it is, surprisingly, weakly supported as having close affinities to an Hawaiian endemic chiton, *A. viridis* (Pease, 1872).

Key words: North Pacific, species invasions, oceanic transport, rafting, biofouling, anthropogenic debris, amphi-Pacific distributions, DNA barcode

Introduction

The higher latitude regions of the North Pacific Ocean are regarded as biodiversity hotspots due in part to their tremendous kelp productivity (Estes and Steinberg 1988; Estes et al. 2005). In turn, these regions have been ranked among the greatest biogeographic engines for exporting cold-temperate species over evolutionary time to other marine provinces (Briggs and Bowen 2013). Despite this, and while on a geological timescale plate movements and climate shifts have led to biotic exchanges (Vermeij 2005), the Neogene fossil history of the North Pacific reveals rare trans-Pacific invasions prior to human activities. Instead, since the Miocene, the North Pacific has been subject to increasing geographical restriction (and thus eastern versus western vicariance) for taxa that previously were amphi-Pacific in distribution

(Vermeij 1989). Thus contemporary North West and North East Pacific shallow-water biotas are substantially diverged, and this is especially true for taxa with relatively brief planktonic larval durations.

It is thus not surprising that the shallow water chiton faunas of the temperate North West Pacific (Saito 2000; Sirenko 2013) and North East Pacific (Kaas and Van Belle 1985a–1994; Eernisse et al. 2007) share a number of genera but have few species in common. While several chiton species are found in both Alaska and Far Eastern Russia, their range does not extend further south than boreal latitudes (Kaas and Van Belle 1985b, 1990, 1994; Sirenko 2013). Of the few temperate, shallow-water chiton species reported to have an ostensibly amphi-Pacific distribution, recent molecular comparisons have revealed instead either species complexes or strong phylogeographic breaks between those of the Eastern and Western Pacific. (Eernisse and colleagues, unpublished observations). A similar comparison of Japanese and Hawaiian chiton faunas (Saito 2000; Schwabe and Pittman 2014) reveals that the Hawaiian Islands share seven of their eight intertidal and shallow water genera with Japan, but also have no species in common.

While having diverged over time, the biotas of the Western, Central, and Eastern North Pacific may still potentially be connected by means of planktonic larval dispersal or by ocean rafting. Chitons, however, have limited capacity for long-distance dispersal in the plankton (Eernisse and Reynolds 1994; Buckland-Nicks et al. 2001; Todt et al. 2008). Most chiton species are broadcast spawners but have only lecithotrophic (non-feeding) larvae. Pearse (1979) reported a maximum of a 19-day planktonic life for chiton larvae, far shorter than the 1.25-year transit time calculated from the arrival date of the first piece of current-transported Japanese tsunami debris to reach North America in 2012 (Table S1 in Carlton et al. 2017). The species that do not broadcast spawn brood their embryos, which develop into late-hatching larvae (Eernisse 1988); in these a planktonic larval stage is very brief or entirely lacking.

Chitons could be transported via rafting on floating macroalgae or on pumice or terrestrial vegetation (such as trees) cast into the sea. However, very few chiton species are known to inhabit pneumatocyst-bearing algae. Hobday (2000) reported a single specimen of the chiton *Stenoplax* sp. on one of 50 rafts of the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh, 1820, found floating 33 km off the southern California coast. Hobday (2000) estimated the maximum lifetime of rafted *Macrocystis* kelp to be about 100 days, also too short for transoceanic dispersal. Fraser et al. (2011) found two species of subantarctic chitons, *Onithochiton neglectus* Rochebrune, 1881 (a brooder) and *Plaxiphora boydeni* Murdoch, 1982, attached to the southern bull kelp *Durvillaea antarctica* (Chamisso) Hariot, 1892, which had drifted for several weeks between 390 and 600 km from subantarctic islands to the South Island of New Zealand. We have found no reports of chitons rafted on pumice or terrestrial vegetation. Floating pumice or wood would need to be near shore at the time of chiton recruitment, and also would have had to develop a biota sufficient to support chiton grazing during long-term transport. Both wood and pumice have the additional liability of sinking due to waterlogging or biofouling weight (Wolff 1979; Sirenko 2004; Thiel and Gutow 2005).

Anthropogenic vectors, such as the movement of commercial shellfisheries stocks, or biofouling on ships' hulls, in sea chests, or in ballast water, may conceivably move chitons along coastlines and across and between oceans. There appear to be few reports of chitons associated with any of these vectors. Smith et al. (1999) reported "Polyplacophora" from ship's ballast water; we have examined drawings (provided by L.D. Smith to J.T. Carlton, May 2017) of the single larva upon which this report was based and are unable to confirm that is a chiton. We have found no published papers in which chitons have been reported in ship sea chests or vessel hull fouling, although it would not be surprising if rare accounts exist. Carlton (1999), after observing that the chiton *Chaetopleura apiculata* (Say in Conrad, 1834) had crawled aboard a vessel's anchor, after the anchor had been on the seabed in 10 meters of water for 14 hours at Cape Cod, Massachusetts, speculated that "In earlier maritime history, with longer coastal (ship) residencies, anchors would have been available for colonization on and in bottom sediments, including mixed rubble and rock bottoms, for long periods of time. In turn, anchors may remain wet over considerable distances due to wave splash." The transport of commercial oyster stocks led to the introduction of a New Zealand chiton to Australia, and chitons have also been found associated with Pacific oysters intentionally introduced to the North American coast, as noted in the Discussion.

Ocean rafting on anthropogenic debris, especially on long-lasting plastic or other non-biodegradable debris, provides another mechanism for marine species dispersal. The buoys, docks, and other objects released by the disastrous Japanese Tōhoku tsunami of March 2011 often supported diverse nearshore marine animal and plant communities before being set adrift (Carlton et al. 2017). Here we report on five species of chitons found on Japanese Tsunami Marine Debris (JTMD). We have examined all known material of JTMD chitons, evaluating their morphology and sequencing two mitochondrial DNA gene regions for most specimens. One species, a member of the *Acanthochitona defilippii* species-complex indigenous to the western Pacific, appears to be undescribed. We also provide images of species that could be confused with JTMD Japanese chitons. Our species accounts summarize some of the challenges we have encountered in identifying the JTMD chitons. Our study has revealed the need for further work on chiton genera and species in the northwestern Pacific Ocean in the vicinity of Japan.

Methods

We received samples from nine JTMD objects (identified as such through multiple lines of evidence; see Carlton et al. 2017) landing between 2012 and 2015 in North America and the Hawaiian Islands (Supplementary Material Table S1). Each object was assigned a unique identification number preceded by JTMD-BF- (Japanese Tsunami Marine Debris – BioFouling-#). Two JTMD items were large floating docks from the Port of Misawa, Aomori Prefecture, Honshu. Five items were sections of pontoons (floats) from marinas or other harbor facilities. One item was a fender torn loose from a pier or dock, and the last object was a large metal cylinder, of the same apparent origin. All nine items supported a distinctive Japanese marine invertebrate fauna characteristic of the cold-temperate Tōhoku coastline.

Chitons were placed directly in 95% ethanol or were frozen (as part of larger bulk samples) and then later transferred to ethanol. Other chiton specimens from Japan, Russia, South Korea, and the North East Pacific in the authors' possession were used for morphological and molecular comparisons. Molecular and morphological data for some of this comparative material have been previously published (Okusu et al. 2003; Kelly and Eernisse 2007, 2008).

JTMD chitons are in the first author's (DJE) collection at California State University, Fullerton. Comparative material is in the DJE collections or has been donated to the Santa Barbara Museum of Natural History (SBMNH).

Morphological analyses

The light micrographs presented here, all by A. Draeger, are the result of focus stacking of serial images, performed by Helicon Focus 6.2.2 (Helicon Soft Inc., Kharkiv, Ukraine). Where indicated in the figure captions, some of the micrographs are mosaics of contiguous stacks of serial images. The specimens in the serial photos were lighted by LED sources, with a white-balance reference target photographed at the end of each photo session. The white balance of the stacks of images from each photo session was set at the time when the 12 bit Olympus RAW files were processed into 16 bit TIF format using Olympus Viewer 3 version 1.4.2 (Olympus Corporation, Shinjuku, Tokyo, Japan). Subsequent to focus stacking, the output images were adjusted in brightness and contrast using ImageJ 1.51j, an open source Java image processing program (https://www.versioneye.com/java/net.imagej:ij/1.51j) to better show structures, and thus the image colors should be considered as only approximations of the true specimen coloration. The grayscale images are the isolated green channel of 16 bit TIF images (using ImageJ), which was found to better show structural details in low contrast subjects for the highest magnification photos.

Phylogenetic and molecular analyses

Two congeneric outgroup species from the eastern Atlantic or Mediterranean were included for the *Acanthochitona* analysis, selected because they were among the most proximal species to our ingroup species in more inclusive analyses across Acanthochitonidae of COI (n = 362) or 16S (n = 175) sequences (D. Eernisse, unpublished). Three species of *Dendrochiton* were used as the most proximal known outgroup of *Mopalia* (Kelly and Eernisse 2008). In order to place the JTMD material in a broader context, selected additional 16S and COI sequences were either downloaded from GenBank or newly sequenced from non-JTMD material; these are summarized in Table S3. *Acanthochitona* sp. B treated here represents a population from Vancouver Island that is believed to represent another distinct Japanese species introduced with oyster culture (R. Harbo, E. Pilgrim, D. Eernisse, in preparation).

Kelly and Eernisse (2008) analyzed 21 of 24 known species of *Mopalia* with a combined dataset of four gene regions (16S and COI mtDNA, 18S and 28S rDNA). Missing from their analysis were two North West Pacific species, *M. schrencki* Thiele, 1909 and *M. middendorffii* (Schrenck, 1861), and one northeastern Pacific species, *M. egretta* Berry, 1919. We provide here new 16S sequences of these latter three species; COI sequences for these species are not yet available. The new *M. schrencki* and *M. middendorffii* 16S sequences were provided to us by Anton Chichvarkhin (Far Eastern Federal University, Vladivostok, Russia) from localities in Far Eastern Russia. We added a 16S sequence for *M. egretta* from our own collection.

Genomic DNA (gDNA) was extracted from a strip of tissue (approximately 25 mg) sliced off the side of each chiton foot. The DNA extraction was carried out using the GeneJET Genomic DNA Purification Kit by ThermoFisher Scientific (Waltham, Massachusetts, USA). Standard primers were used for the PCR amplification of mitochondrial gene regions, including LCOI490 (5’ - GGT CAA CAA ATC ATA AAG ATA TTG G - 3’) and HCO2198 (5’ - TAA ACT TCA GGG TGA CCA AAA AAT CA - 3’) for a portion of the cytochrome oxidase subunit I (COI) gene (Folmer et al. 1994), and 16Sar (5’- CGC CTG TTT ATC AAA AAC AT - 3’) and 16Sbr (5’ - GCC GGT CTG AAC TCA GAT CAC GT - 3’) for a portion of the mitochondrial large subunit or 16S rDNA (16S) gene (Palumbi 1996). Both COI and 16S were amplified using 20 µL reactions with 3 mM $MgCl_2$, 200 pM dNTPs, 1 Unit Taq polymerase (Qiagen, Valencia, CA, USA). Thermal cycling conditions started with denaturation at 94 °C for 2.5 min, continuing at 94 °C for 30 sec, 46 °C for 30 sec and 72 °C for 1 min for 35 cycles. Final extension was 72 °C for 10 min. PCR products were cleaned with Qiaquick PCR kit and sequenced using ABI Big Dye 3.1 (Carlsbad, CA, USA). Sequenced products were purified with DyeEx 96 Kits from Qiagen and run on ABI Prism 3730xl DNA Analyzer; DNA reads were assembled in Sequencher v. 4.8 (Gene Codes, Ann Arbor, MI, USA).

Alignment of sequences was carried out using MAFFT v. 7 (Katoh 2013) with the FFT-NS-I, E-INS-I, or Q-INS-I (slow, very slow, or extremely slow methods), depending on the alignment size, using the most refined (slowest) method that was feasible for a given set of sequences. The phylogenetic analysis results presented here for the *Acanthochitona* species complex were performed on a combined gene data set of separately aligned COI and 16S sequences, with the data set limited to those taxa (new or from GenBank) that had at least the 16S gene sequence available. The phylogenetic analyses employed the maximum likelihood criterion as implemented in RAxML (Stamatakis et al. 2008). The option of gamma model of rate heterogeneity was selected, and the best maximum likelihood tree was searched for, along with a bootstrap analysis (100 bootstrap replicates). We used the program PAUP* v. 4.0a152 (Swofford 2002) to calculate average, minimum, and maximum inter- and intraspecific uncorrected p pairwise distances for congeneric species. This allowed us to compare the amount of sequence divergence observed for COI and 16S and the extent of sequence divergence between our groupings treated as species.

Identified JTMD chitons with GenBank accession numbers are shown in Table S2. Non-JTMD chitons used for outgroup or reference sequences, and their GenBank accession numbers, are shown in Table S3.

Results

Chiton diversity on Japanese tsunami marine debris

We studied 40 chiton specimens belonging to two families, three genera, and five species, one apparently undescribed, from multiple JTMD biofouled landings recovered from Oregon, Washington, and Hawai‘i (Tables S1 and S2). A maximum of three species was found on one object (JTMD-BF-32) landing in Maui; all other objects supported one or two species. *Mopalia seta* Jakovleva, 1952 occurred only on JTMD docks arriving from the northern port of Misawa, Honshu, in 2012. *Placiphorella stimpsoni* (Gould, 1859) appeared on one pontoon section landing in Washington in 2015. Otherwise, three species of *Acanthochitona* made up the remainder of the chitons recovered over the period of 2012 to 2015, as detailed in the Systematic Account below.

Mitochondrial DNA sequences

We obtained mitochondrial COI and 16S sequences from most of the JTMD specimens. We have estimated phylogenetic relationships of these with the inclusion of selected other congeneric species, in order to both identify them to species and to provide a preliminary phylogenetic estimate for the JTMD species within their respective genera. We were unable to obtain sequences for two JTMD *Acanthochitona* specimens; our identifications for these are thus tentative, as they are based on morphology only (Table S2). For the purpose of documenting a commonly employed estimate of the sequence divergence between what we have interpreted as species-level distances, we have presented uncorrected p distances separately for COI and 16S, also including congeneric non-JTMD sequences (Table S3), when appropriate. These calculated distances (discussed below) were consistent with previous studies (Kelly and Eernisse 2008) in that intraspecific distances were much less than 1 percent divergence for either COI or 16S, and interspecific distances were much greater.

We obtained sequences for two genera, *Acanthochitona* and *Mopalia*; sequencing of JTMD *Placiphorella* is in progress. The treatment of *Acanthochitona* species was far more challenging because there were multiple species represented that have only subtle morphological distinctions, and comprehensive regional and global treatments of this species-rich genus, with more than 80 species (Marshall and Gofas 2017), are lacking. In contrast, the estimated 24 members of *Mopalia* are restricted to the North Pacific and most of these have been the subject of detailed morphological studies in Japan (Saito and Okutani 1991) and western North America

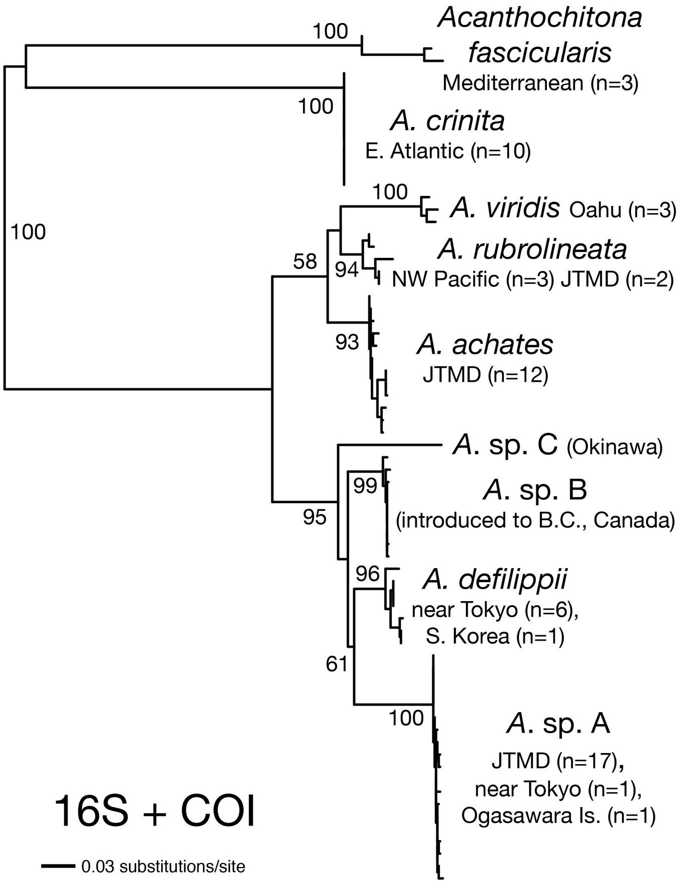

Figure 1. Best phylogram result found in RAxML analysis of combined 16S and COI data sets including known northwestern Pacific species of *Acanthochitona*, plus two European species as proximal outgroups within this large genus. Of 73 total terminal taxa in the combined data set, two ingroup and two outgroup taxa with missing 16S were deleted, and five ingroup and two outgroup taxa missing COI sequences were included in this result based on their 16S sequence only. Details for individual vouchers are summarized in Tables S2 and S3. For simplicity of presentation, bootstrap proportions > 50% are shown only for interspecific nodes.

(Eernisse et al. 2007); a complete monograph for the genus is available as well (Kaas and Van Belle 1994). They have also been featured in studies of DNA barcoding performance (Kelly et al. 2007), population connectivity (Kelly and Eernisse 2007), and phylogeny as estimated with multiple gene markers (Kelly and Eernisse 2008).

We calculated a maximum likelihood phylogenetic estimate (Figure 1) for a combined COI + 16S data set for all JTMD specimens of *Acanthochitona*, together with selected other congeneric specimens. The result is a surprising diversity of mitochondrial lineages that we have interpreted as distinct species because of not only robust support for the molecular differences but also because of morphological distinctions, some of which will be addressed in subsequent studies in collaboration with Japanese, Korean, and Chinese colleagues. Based on the sequence correspondence of the JTMD specimens to our limited sampling from Japan to date, the JTMD specimens could be assigned to one of three species also present in Japan. Our studies have further revealed that one of the species, *A. rubrolineata*, is distinct from *A. achates*, despite having been considered a junior synonym of the latter (Saito 1995). Another surprising result was our finding a degree of weak support for *A. rubrolineata* being evolutionarily closer to the Hawaiian endemic species, *A. viridis*, than either of these species is to *A. achates*, although further multi-locus studies are needed to test this suggestion.

Furthermore, a third species referred to here as *Acanthochitona* sp. A is likely a new species whose affinities include *A. defilippii* and two other potentially new species (*Acanthochitona* sp. B and *Acanthochitona* sp. C in Figure 1). Details of *A*. sp. B will be forthcoming, whereas *A*. sp. C, now based upon a single eroded presumed juvenile specimen (University of Florida 445778) from the subtidal of White Beach, Okinawa Island, Japan, will require new material before its status can be evaluated. We have not yet obtained samples from further north, on the northeastern coast of Honshu adjacent to the earthquake's epicenter, but based on the relative

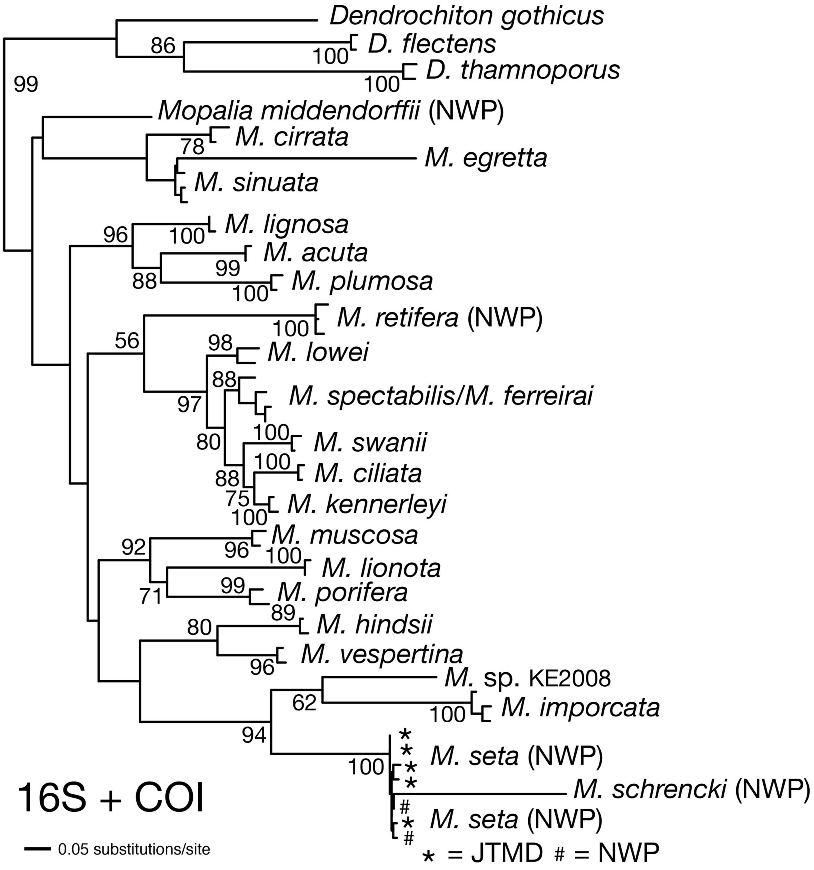

Figure 2. Best phylogram result found in RAxML analysis of combined 16S and COI data sets for all but one known species of *Mopalia* (*M. phorminx*) with selected outgroup (*Dendrochiton*) species. Of 55 total terminal taxa in the analysis, four with missing 16S were deleted, and four ingroup taxa missing COI sequences were included in this result based on their 16S sequence only. Details for individual vouchers are summarized in Tables S2 and S3. For simplicity of presentation, bootstrap proportions > 50% are shown only for interspecific nodes.

frequency of the three species we recovered from JTMD material, we expect that *Acanthochitona* sp. A, *A. achates*, and *A. rubrolineata*, listed in descending order of expected abundance, should be present on that coast. We have used molecular and morphological evidence to identify a limited number of *Acanthochitona* specimens from the vicinity of Tokyo. We have identified the most common species among this material as *A. defilippii*, but we also found one specimen each of two of the three JTMD species of *Acanthochitona*: *A. rubrolineata* and *A*. sp. A.

A maximum likelihood phylogeny (Figure 2) was estimated for a combined COI + 16S data set of selected representatives (Tables S2 and S3) of all but one recognized *Mopalia* species, rooted with three species of *Dendrochiton* included to represent the most proximal known outgroup of *Mopalia* (Kelly and Eernisse 2008). Both COI and 16S sequences were obtained or were previously available from all recognized species of *Mopalia* except for three species with only one each 16S sequence available (as noted above), and one with only COI sequences (*M. phorminx* Berry, 1919) that was not included in the presented results (see Kelly and Eernisse 2008 for the affinities of *M. phorminx*). Besides morphological confirmation that the largest three of the five total *Mopalia* specimens unambiguously matched *M. seta* (see systematic account below), all five JTMD *Mopalia* specimens had nearly identical sequences to each other and to previously confirmed specimens of *M. seta* (although 16S was missing from one of the five specimens). Average pairwise intraspecific uncorrected p distances for all *M. seta* specimens included in our phylogenetic analysis were 0.1% for COI and 0% for 16S. In contrast, the average interspecific uncorrected p distances between *M. seta* and every other available species of *Mopalia* were 13.4% (maximum 16.5%; minimum 11.4%) for COI and 3.2% (maximum 4.7%; minimum 1.8%) for 16S.

As expected, the co-occurring and morphologically similar species, *M. schrencki*, had the closest (1.8%) distance for 16S, but the corresponding COI distance is unknown because no *M. schrencki* COI sequence was available. We suspect that our coding of COI sites for *M. schrencki* as missing for our combined gene analysis, or only having a single *M. schrencki*

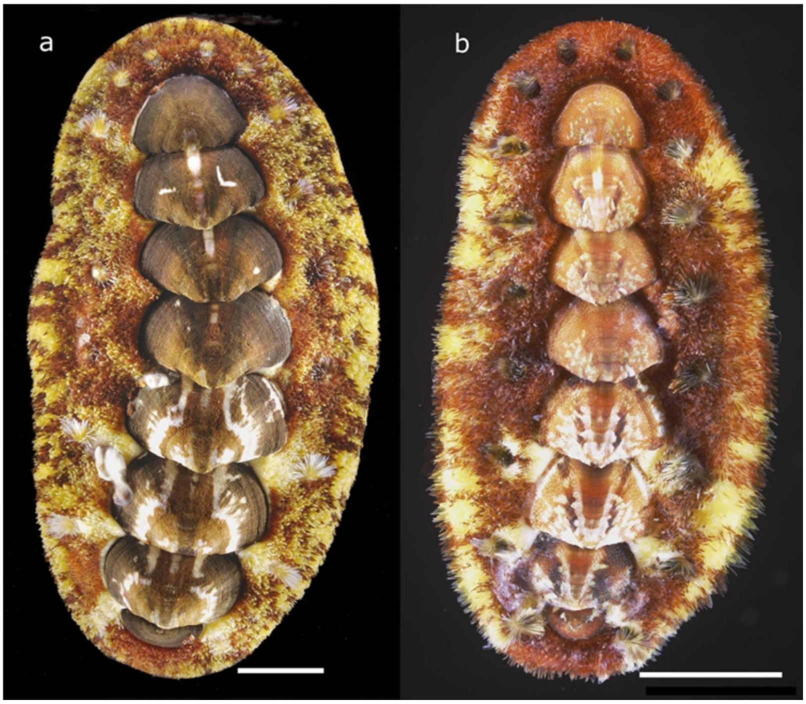

Figure 3. (a) Mosaic image of *Acanthochitona achates* from JTMD-BF-18, one of the two more common chiton species in the tsunami debris reaching Hawai'i (b) Mosaic image of *A. rubrolineata* from JTMD-BF-18. Scales equal 5 mm.

16S sequence, could be why *M. schrencki* appears to be nested within *M. seta* in our phylogenetic analysis (Figure 2). We predict that sequencing more individuals of this species for both genes will result in a reciprocal monophyletic result, supporting *M. seta* and *M. schrencki* as sister species. An analogous situation is apparent for *M. egretta*, which is nested within *M. sinuata*, and is also presently represented by only a single 16S (and no COI) sequence. For the purpose of the present study, sampling of COI and 16S has unambiguously supported our morphological identification of all JTMD *Mopalia* specimens as *M. seta*.

Systematic Account

The following classification follows Sirenko (2006).

Order Chitonida Thiele, 1909
Suborder Acanthochitonina Bergenhayn, 1930
Acanthochitonidae Pilsbry, 1893

Two recognized and one undescribed species of *Acanthochitona* were identified from the JTMD. Along the Pacific coast of North America *Acanthochitona avicula* (Carpenter, 1864) is the northernmost member of the genus, found as far north as Orange County, California (Watters 1990). The Hawaiian chiton fauna includes the endemic *Acanthochitona viridis* (Pease, 1872) (Figure 5b) and *A. armata* (Pease, 1872) (Anseeuw 2011; Schwabe and Pittman 2014). *A. viridis* is the only Hawaiian *Acanthochitona* that is similar to any of our three JTMD *Acanthochitona* species. The comparative specimens of *A. viridis* we studied were collected in November 2011 from a rocky intertidal habitat in Hawai'i, before the first recorded Tōhoku tsunami debris landed in Hawai'i in November 2012 (Table S1 in Carlton et al. 2017).

Acanthochitona Gray, 1821
(type: *Chiton fascicularis* Linnaeus, 1767)

***Acanthochitona achates* (Gould, 1859)**

(Figures 1, 3a, 4a, b, e, f, i, j)

Chiton (*Acanthochaetes*) *achates* Gould, 1859: 165 (unfigured)

Material.—Hawai'i: Maui, Ahihi Kinau, 4 specimens, JTMD-BF-32; Hawai'i: Oahu, 4 specimens, JTMD-BF-257; Hawai'i: Oahu, Makapuu, 1 specimen, JTMD-BF-27; Hawai'i: Oahu, Mokuleia, 2 specimens, JTMD-BF-20; Oregon: Clatsop Beach, 1 specimen, JTMD-BF-18, 1 specimen (de4409), JTMD-BF-8; Washington: Olympic National Park (based on morphological identification only; no DNA sequences obtained).

Remarks.—*Acanthochiton achates* ranges from southern Hokkaido southward along eastern and western Japan as well as the Inland Sea to the eastern Korean Peninsula and the Amami Islands and south to the Kinmen and Matsu Islands (Taki 1938; Wu 2004). It was also recently reported from

Viet Nam (Sirenko and Saito 2017). Although the tsunami was centered near the northern end of this species' range, it was the second most common species on tsunami debris, with 11 of the 13 specimens collected coming from JTMD landing in the Hawaiian Islands. The tsunami debris arriving there is presumed to have first drifted north and east, eventually south to tropical latitudes and west, following the North Pacific gyre current system (Figure S1 in Carlton et al. 2017).

Gould's type description lacks illustration and is insufficient to distinguish between *Acanthochitona* species. Pilsbry (1893) examined one of Gould's type specimens and noted that erosion had obscured the characteristics. Thiele (1909) and Taki (1938) redescribed *A. achates* based on different material, although it is unclear if either of these authors examined type specimens. Taki (1938) provided detailed descriptions, comparisons, and illustrations of *A. achates* and the similar *A. rubrolineata* based on examination of a much larger number of specimens from the vicinity of Japan. We found that JTMD specimens that conformed to Taki's descriptions for either *A. achates* or *A. rubrolineata* also separated in our combined COI + 16S analysis (Figure 1). Taki also provided a key to distinguish between these species, but the comparisons in his key are subjective, requiring the user to be familiar with both species in order to apply them.

Watters (1990) and Schmidt-Petersen et al. (2015) have concluded that the characteristics of megalaesthetes, micraesthetes, and perinotum spicules are taxonomically relevant within *Acanthochitona*. Although the characteristics of the micraesthetes are best examined with SEM, the placement of megalaesthetes, the characteristics of the tegmental granules, and the characteristics of the larger perinotum spicules are visible by light microscopy on alcohol-preserved specimens (Figure 4). We contrast some microstructural differences between the *A. achates* and *A. rubrolineata* (Table 1).

Acanthochitona rubrolineata (Lischke, 1873)

(Figures 1, 3b, 4c, d, g, h, k, l)

Chiton rubro-lineatus Lischke, 1873: 24 (unfigured)
Chiton rubro-lineatus Lischke, 1874: 73–74 pl. 5, fig. 12

Material.—Hawai'i: Maui, Ahihi Kinua, 1 specimen, JTMD-BF-32; Oregon: Clatsop Beach, 1 specimen, JTMD-BF-18.

Remarks.—*Acanthochitona rubrolineata* ranges from southern Hokkaido southwards along the eastern and western shores of Japan as well as the Inland Sea, and along the western and southern shores of South Korea to China's Shandong Peninsula (Taki 1938). Jakovleva (1952) reported it to be present "along almost the entire Soviet coast" of the Sea of Japan. Only two specimens of *A. rubrolineata* have been collected from JTMD, one landing in Oregon and the other on Maui, Hawai'i.

In 1874 Lischke augmented his 1873 species description, mentioned aspects of the sculpture and perinotum, and provided an illustration of the specimen's dorsum. However, his description does not include the microscopic details useful for distinguishing between *Acanthochitona* species. Thiele (1909), initially unable to access the sole Lischke specimen, redescribed this species based on other specimens and provided the first illustrations of the insertion plates and perinotum spicules. Later, after access to the Lischke specimen, he concluded that the newer material he had used was indeed conspecific with the holotype. Taki (1938) remarked that the Lischke type specimen at the Löbbecke Museum, Düsseldorf, Germany, had been lost, and he redescribed and illustrated *A. rubrolineata* based on examination of 43 specimens from around Japan. Our examination of photos of an *A. rubrolineata* specimen currently at the Löbbecke Museum revealed several discrepancies between that specimen and the Lischke description and illustration, but direct observation could provide a more conclusive comparison.

We based the identification of the two JTMD specimens upon their general body form and coloration as depicted by Taki, as well as upon the results of our combined COI + 16S phylogenetic estimate, which supported both specimens grouping with previously acquired specimens of *A. rubrolineata,* one from the Vladivostok region of Russia and one from near Tokyo, and these four grouped apart from *A. achates* (Figure 1). As mentioned for *A. achates*, Taki's (1938) key for distinguishing these species depends on subjective comparisons, and he cautioned that "a sensible taxonomist" could err identifying specimens of the two species. However, we found subtle microstructural differences for distinguishing *A. achates* and *A. rubrolineata* that Taki did not mention (Figure 4a-l, and Table 1).

Acanthochitona sp. A

(Figures 1, 5a)

Material.—Hawai'i: Maui, Ahihi Kinau, 2 specimens, JTMD-BF-32; Hawai'i: Oahu, 6 specimens, JTMD-BF-257; Hawai'i: Oahu, Makapuu, 5 specimens, JTMD-BF-27; Hawai'i: Oahu, Mokuleia, 3 specimens, JTMD-BF-20; Oregon: Clatsop Beach, 1 specimen, JTMD-BF-130; Washington: Olympic National Park, 1 specimen (de4403), JTMD-BF-20; Hawai'i: Oahu, Mokuleia (based on morphological identification only; no DNA sequences obtained).

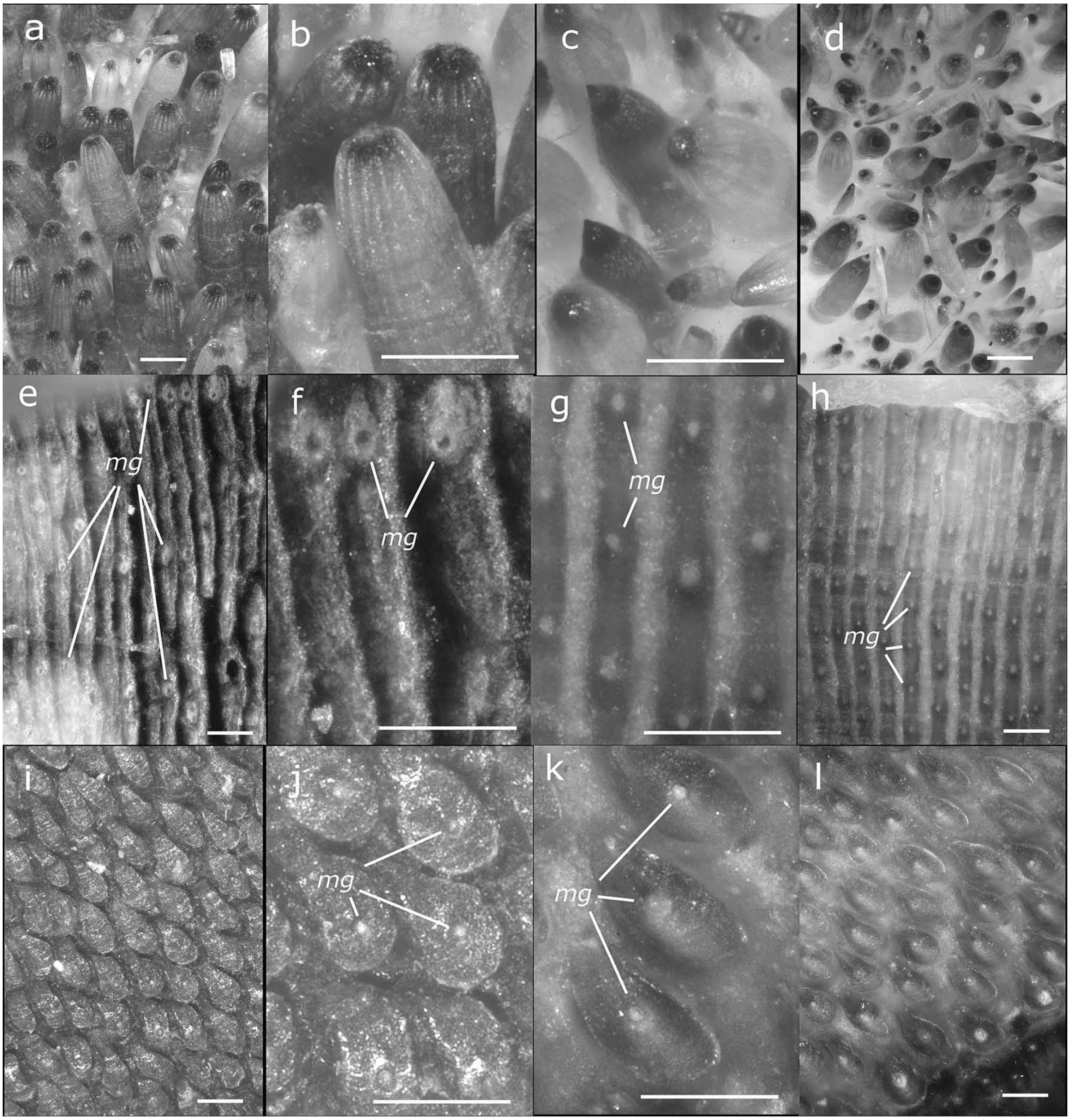

Figure 4. Light micrographs of *Acanthochitona* microstructures of the specimens in Figure 3. See Table 1 for comparison details. (a, b) *A. achates* perinotum, (e, f, i, j) *A. achates* Valve IV, (c, d) *A. rubrolineata* perinotum, (g, h, k, l) *A. rubrolineata* Valve III. (a-d) perinotum spicules near body midpoint (not the tufts of spines). (e-h) intermediate valve jugal longitudinal ribbing and megalaesthete pattern adjacent preceding valve. The jugal ribbing can be eroded smooth in specimens. (i-l) intermediate valve pleurolateral area granular sculpture. The girdle is located beyond the lower right corner of the images. (j, k) showing location of megalaesthete on granule, granule outline and granule spacing. *mg*=megalaesthete. Scales equal 100 µm.

Remarks.—While the geographic range of this potentially undescribed species remains to be delineated, three aspects suggest that it includes the Pacific coast of Honshu and Ogasawara Islands. One of the 19 JTMD specimens was collected from the floating dock (JTMD-BF-8) known to have originated from Misawa, Amori Prefecture, Honshu. The other 18 JTMD specimens were collected from objects that appear to have been permanently anchored structures that had been torn from their moorings in the Tōhoku region

Table 1. Microscopic characteristics differentiating *Acanthochitona achates* from *A. rubrolineata*. See Figure 4.

Characteristic	*A. achates*	*A. rubrolineata*
Perinotum large spicules (excluding fascicle spines)	some are blunt tipped, their longitudinal ribbing distinct (Figure 4a, b)	some are sharp tipped, their longitudinal ribbing vague (Figure 4c, d)
Jugal rib megalaesthete separation	inter-aesthete interval along the ribs average 6.7 times width of rib; interval irregular, 5 to 10 times the rib width (Figure 4e, f)	inter-aesthete interval along the ribs average 2.8 times width of rib; interval regular, 2.3 to 3.5 times the rib width (Figure 4g, h)
Pleurolateral granule megalaesthete location	distal to granule midpoint (Figure 4i, j)	at granule midpoint (Figure 4k, l)
Tegmental granule perimeter	somewhat irregular (Figure 4i, j)	smoothly curved (Figure 4s, l)
Tegmental granule narrowest separation	averaging 0.14 granule width (Figure 4i, j)	averaging 0.29 granule width (Figure 4k, l)

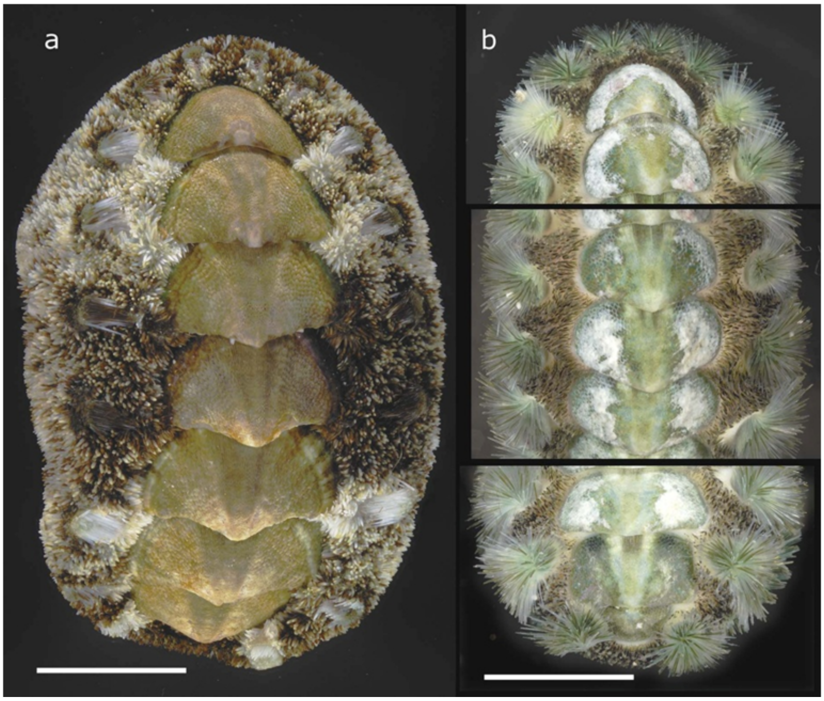

Figure 5. (a) Mosaic image of *Acanthochitona* sp. A, a species in the *A. defilippii* complex and the most common chiton species in the tsunami debris recovered from Hawai‘i, from JTMD-BF-27 (b) A moderately curled specimen of the Hawaiian indigenous *A. viridis*. Specimen acquisition unrelated to Tōhoku tsunami. White valve patches are bleached encrusting coralline algae. Scales equal 5 mm.

of Honshu where the tsunami heights and inundations were greatest (Mori et al. 2011). Additionally, two non-JTMD specimens have been identified as belonging to this species based on sequencing, one from a 2003 collection from near Tokyo, Tokyo Prefecture, Honshu, and one from Miyanohama, Chichijima Island in the Ogasawara Islands.

Some characteristics of the tegmental sculpture and perinotum spicules of *Acanthochitona* sp. A are similar to features present in either *A. achates* or *A. rubrolineata*, and the key morphological distinctions between *A.* sp. A and these other *Acanthochitona* species awaits the full species description. However, our combined COI + 16S phylogenetic estimate (Figure 1) places *A.* sp. A within a species complex of which *A. defilippii* is the only well documented member, and is tentatively represented here by all but one of the specimens collected from near the type locality of Tokyo Bay that we have sequenced and identified as *A. defilippii* (Table S3). Our other specimen from near Tokyo matched *A.* sp. A (see above).

Because *A.* sp. A was the most common species present on JTMD, and the other *Acanthochitona* species found on tsunami debris had been previously well-documented from the northern portion of Honshu (Taki 1938), and because the short planktonic duration of chiton larvae makes them unlikely in the open ocean, we see no reason to challenge the assumption that all chitons studied here originated from the Tōhoku shore struck by the tsunami rather than having been recruited later during a hypothetical southward debris drift pattern before crossing the Pacific.

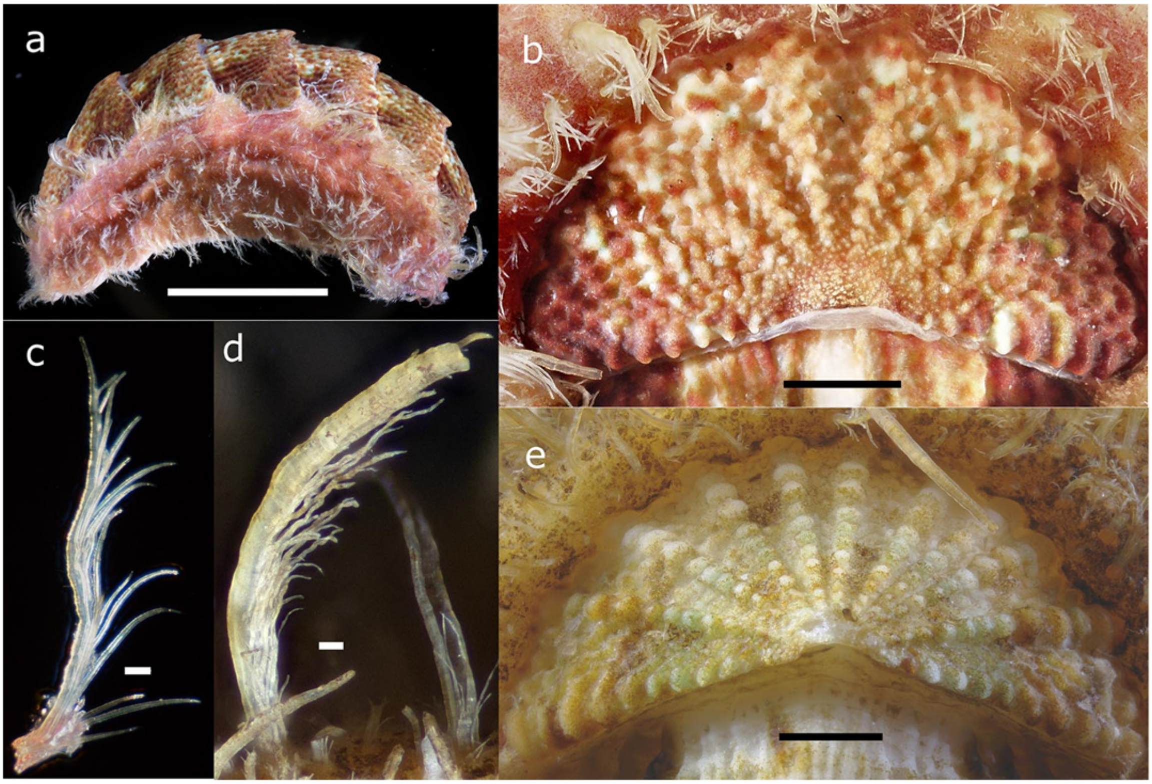

Figure 6. (a-c) *Mopalia seta* from JTMD-BF-1, (d, e) *M. schrencki*, specimen acquisition unrelated to Tōhoku tsunami. (a) Profile showing wide girdle. Scale equals 5 mm. (b) Head valve showing coarse interradial sculpture that invades the sculpture of the radial rows. Scale equals 1 mm. (c) perinotum seta showing smoothly curved filaments on the setal shaft. (d) perinotum seta showing sinuous filaments on the setal shaft. Scales for (c, d) equal 100 µm. (e) Head valve showing subdued interradial sculpture and radial rows of distinct ovoid pustules. Scale equals 1 mm.

Mopaliidae Dall, 1889
Mopalia Gray, 1821
(type: *Chiton hindsii* Sowerby MS in Reeve, 1847)

Mopalia seta Jakovleva, 1952
(Figures 2, 6a–c)

Mopalia seta Jakovleva, 1952: 72, text-fig. 32, pl. 4, figs. 3a, b

Material.—Oregon: Newport, 4 specimens, JTMD-BF-1; Washington: Olympic National Park, 1 specimen, JTMD-BF-8.

Remarks.—*Mopalia seta* ranges along the Russian coast of the Sea of Japan, along both sides of the southern half of Sakhalin Island, and from Hokkaido northward along the Kuril archipelago to Urup Island (Kaas and Van Belle 1994). Saito and Okutani (1991) found *M. seta* present on most of the Hokkaido coastline, with the southern range limit at Muroran, Hokkaido Prefecture. The presence of the five specimens on the dock originating from Misawa, Aomori Prefecture is thus a range extension for *M. seta* of approximately 180 km southwards onto Honshu (Saito and Okutani 1991; Saito 2000).

The identification of three specimens was based on setal morphology and valve sculpture as illustrated by Saito and Okutani (1991), by comparison to voucher specimens from Russia near the border with North Korea (previously collected, identified, and donated to DJE by Boris Sirenko, Zoological Institute, Russian Academy of Sciences, St. Petersburg), and by comparison of the specimens' COI and 16S gene sequences to sequences previously determined by Kelly and Eernisse (2008). Four of the five specimens, including two additional smaller specimens, had sequences that closely matched previously obtained 16S and COI sequences for *M. seta* whose identity was confirmed with setal morphology. We obtained only COI sequence for the fifth specimen, and this likewise matched the others.

The western Pacific *M. schrencki* Thiele, 1909 (Figure 6d, e) is similar to *M. seta* in its general morphology, its vertical distribution, and its geographic distribution (Saito and Okutani 1991; Kaas and Van Belle 1994). Compared with uncorrected p pairwise distance calculations for 16S sequences, *M. schrencki*

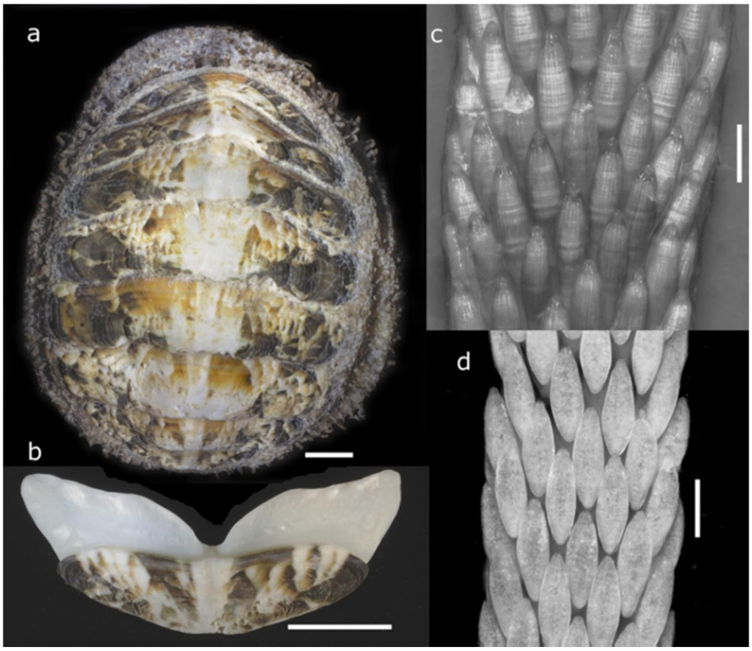

Figure 7. (a-c) *Placiphorella stimpsoni* from JTMD-BF-397*,* (d) *P. velata*, specimen acquisition unrelated to Tōhoku tsunami. (a) Mosaic dorsal view of the slightly curled specimen. The anteriorly protruding girdle flap was contracted and partly flexed ventrally, foreshortened in this photograph. The girdle color has been whitened by the residual stolons of a removed fouling hydrozoan. (b) Tail valve showing the characteristic bridge of articulamentum across the jugal sinus. Scales for (a, b) equal 5 mm. (c) light micrograph of the KOH cleaned, lightly brushed, setal scales. (d) A similarly prepared and photographed seta of the eastern Pacific *P. velata*. Scales for (c, d) equal 100 µm.

is also the most similar species to *M. seta* within *Mopalia* (average pairwise distance for *M. seta* vs. *M. schrencki* is 1.76%; average pairwise distance for *M. seta* vs. other *Mopalia* spp. is 3.22%; maximum observed distance is 4.65%; intraspecific pairwise distance for *M. seta* is 0.0% with no 16S sequence variation detected within *M. seta*). No specimen of *M. schrencki* was recovered from tsunami debris, but this species might be anticipated to be present at or near the coastline impacted by the tsunami.

From our examination of *M. seta* specimens from the tsunami debris and specimens of *M. seta* and *M. schrencki* previously collected and identified by Boris Sirenko from Vostok Bay, Russia, characteristics for differentiating between non-disarticulated specimens of the two species that were illustrated by Saito and Okutani but not explicitly mentioned are as follows. In *M. seta*, the head valve inter-radial sculpture is of coarse and irregular pustules and pits and this sculpture partially intergrades with the pustular sculpture of the radial ribs, making the rib pustules somewhat misshapen. This is in contrast to *M. schrencki* in which the inter-radial sculpture is of subdued elevations and depressions that do not intergrade with the rows of ovoid pustules that comprise the radial ribs (compare Figure 6b, e). Regarding the larger setae, the filaments attached to the shaft of each seta in *M. seta* are smoothly curved or mildly angular, in contrast to the sinuous filaments attached to the shaft of each seta in *M. schrencki* (compare Figure 6c, d).

Placiphorella Dall, 1879
(type: *Placiphorella velata* Carpenter MS, Dall, 1879)

Placiphorella stimpsoni **(Gould, 1859)**
(Figure 7a–c)

Chiton (*Molpalia*) [sic] *stimpsoni* Gould, 1859: 165 (unfigured)

Material.—Washington: Long Beach, 1 specimen, JTMD-BF-397.

Remarks.—Saito and Okutani (1989) discussed discrepancies between Gould's (1859) terse Latin description of *P. stimpsoni*, with type locality of "Hakodade Bay" (Hakodate Bay, Hokkaido), and surviving records, published sketches, and the two extant syntype specimens held at the Smithsonian Institution. Specimens were not illustrated until the work of Pilsbry (1892). The range of *P. stimpsoni* had in the meantime been extended to the Eastern Pacific by multiple authors (listed by Clark 1994). In their review of Japanese *Placiphorella*, Saito and Okutani (1989) described both *P. stimpsoni* and *P. velata* Carpenter MS, Dall, 1879 as having an amphi-Pacific distribution, and advised that the two species could be separated on the basis of the posterior area of their tail valve. In contrast, Clark (1994) restricted *P. stimpsoni* to the northwestern Pacific and *P. velata* to the northeastern Pacific, instead distinguishing specimens based on the size and sculpture differences of the scales on the setae as well as on tegmental coloration. Clark also disputed identifications by other authors of *P. stimpsoni* specimens reported from Far Eastern Russia. Likewise, Sirenko (2013) did not include it as present in Far Eastern Russia. Clark's (1994) reported distribution (see also Kaas and Van Belle 1994) agrees mostly with Saito and Okutani (1989) for its western Pacific distribution within Japan, extending from southern Hokkaido southwards along the Pacific coast of Japan to the south coast of Kyushu, but the range has been extended westward to Korea (Dell'Angelo et al. 1990).

The identification of this specimen relative to the other Japanese shallow water *Placiphorella* species and to the eastern Pacific *P. velata* was based on the criteria of Saito and Okutani (1989), Clark (1994), and Kaas and Van Belle (1994): the exceptional length, conspicuous longitudinal ribbing, and imbricate crowding of the setal scales; the rugose surface of the disarticulated head valve insertion plate teeth, and the bridge of articulamentum connecting the tail valve apophyses.

Discussion

Invasion potential of non-native chitons

Chitons are rarely reported as introduced species anywhere in the world. This may be due in part to challenges attendant with their successful transport, release, and establishment in a new region (discussed below), but also due to the difficulties, revealed in the current treatment, of distinguishing similar-looking taxa, and thus potentially overlooking invasions. The few known successful introductions include the New Zealand species *Chiton glaucus* Gray, 1828, introduced with commercial oysters to Tasmania, Australia in the early 20th century (Kershaw 1955; Ponder et al. 2002). The South American chiton *Chaetopleura angulata* (Spengler, 1797) has also been considered to have been introduced in the early 20th century to the Iberian Peninsula (Kaas 1954), expanding in subsequent decades into the Bay of Biscay (Arias and Anadon 2013). Dell'Angelo and Silva (2003) noted that they were unable to verify early incomplete reports of fossils that would currently be considered *C. angulata* from both South America and Portugal. Two additional South American species, *Tonicia atrata* (Sowerby, G.B. II, 1840) and *Chiton cumingsii* Frembly, 1827 have become established in Spain and the Canary Islands (Arias and Anadon 2013).

Hanna (1966) reported a single specimen of one of the species treated here, *Acanthochitona achates*, as having been collected by Trevor Kincaid "on the oyster beds of Puget Sound" (Washington) in 1948. However, between 1924 and 1948, Professor Kincaid's practice of sampling epizoic mollusks associated with commercial Pacific oysters (*Crassostrea gigas* Thunberg, 1793) imported to North America evolved from initially sampling newly laid-out beds in 1924 (Kincaid 1947) to sampling newly imported oyster seed cases coming directly from Kumamoto, Kyushu Island before the oysters were planted (Kincaid 1949). Thus, Hanna's report does not provide evidence that the chiton from oyster cases identified as *A. achates* was also surviving in Puget Sound. Kincaid made no reference in any of his writings to collections in 1948 on the oyster beds of Puget Sound.

The potential for the Japanese tsunami-debris chiton species to become established in the North East Pacific or the Hawaiian Islands is difficult to judge. None of the JTMD species we studied are brooders. Thus, as broadcast spawners, the larvae of these species would have to remain hydrodynamically captured to settle in a basin or harbor in order to potentially establish sustained populations. That this can be an effective scenario is revealed by the presence of an established population of a Japanese *Acanthochitona* (noted in the Methods and illustrated here in our Figure 1 as *A.* sp. B) believed to have been introduced with commercial oysters into a harbor in British Columbia (R. Harbo, E. Pilgrim, and D. Eernisse, in preparation).

Furthermore, the Pacific Coast of North America supports indigenous species of all three genera, suggesting potential climate and habitat compatibility. Native Eastern Pacific species of *Mopalia* and *Placiphorella* occur along the Pacific Northwest coast where JTMD species in these genera have arrived. While, as noted earlier, the nearest native west coast *Acanthochitona* species occurs far to the south, in southern California, *A. rubrolineata* has been reported (Taki 1938) to range to the cool temperate shores of northernmost Honshu. Specimens of three transported Japanese chiton species that appeared to have been in good health at the time of collection were present on the floating docks (JTMD-BF-1, 8) from Misawa, Amori Prefecture, at the northeastern end of Honshu. Aomori Prefecture experiences a harsh winter typical for a continental climate, which suggests that these *Acanthochitona* could find the Pacific Northwest compatible.

It is also noteworthy that the specimens of *Acanthochitona* sp. A, *A. achates* and *A. rubrolineata* that were collected from tsunami debris reaching Hawai'i all appeared to be in good health at the time they were collected, and yielded usable genomic DNA for sequencing. Little is known of the ability of these species to complete their life cycle under tropical conditions, but the reported range of *A. achates* and *A. rubrolineata* in the western Pacific (Taki 1938) extends into the Tsushima Warm Current (Ito et al. 2014). The live state of specimens of all three species, and the broad range of *A. achates* and *A. rubrolineata* in the western Pacific, suggest that these *Acanthochitona* species could also be compatible with the tropical conditions typical for Hawai‘i.

Conclusion

The present work is the first to document long-distance, multi-year transoceanic rafting of chitons. The chitons arriving in 2014 and 2015 were at least 3 and 4 years old, having survived drifting through the open North Pacific Ocean to the Hawaiian Islands and North America. Of no small interest is the observation that all chitons discovered on JTMD came from a similar, relatively large, class of debris objects: concrete-encased floating pontoon sections of similar size (Table S1), a large dock fender, a large metal cylinder that had been previously anchored or tied, and two docks. All of these appear to have been permanent, fixed port and harbor structures, apparently conducive to the settlement and growth of chitons. No chitons were found on the many hundreds of buoys, totes, vessels, post-and-beam wood, and other JTMD objects (Carlton et al. 2017) that supported varied and often dense populations of Japanese invertebrates, including a diverse assemblage of limpets. Without human-built coastal structures in the nearshore environment, we suspect that transoceanic rafting of chitons would be improbable. That such structures are proliferating on coastal zones around the world suggests that future rafting events are possible.

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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, prefecture and city origins if known, and recorded chiton species.

Table S2. Identified JTMD chitons with BF number (Table S1), DNA number, and GenBank accession numbers.

Table S3. Chitons included as outgroup or reference sequences but not associated with JTMD material.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2018/Supplements/AI_2018_JTMD_Eernisse_etal_SupplementaryTables.xlsx