

**North Pacific Marine Science Organization (PICES)**  
**PICES-MoE project on “Effects of marine debris caused by the Great Tsunami of 2011”**  
**Year 3 Final Scientific Report**

**1. PROJECT INFORMATION**

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<b>Title</b>	<i>Japanese Tsunami Marine Debris (JTMD) and Alien Species Invasions:  Molecular Identification Species on JTMD and DNA Barcoding of Japanese Vouchers</i>
<b>Award period</b>	<i>April 1, 2016 – March 31, 2017</i>
<b>Amount of funding</b>	\$72,000 CAN (\$ USD)
<b>Report submission date</b>	January 31, 2017
<b>Lead Author of Report*</b>	Jonathan Geller, Moss Landing Marine Laboratories

**Principal Investigator(s), Co-Principal Investigators and Recipient Organization(s):**

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**2. YEAR 3 PROGRESS SUMMARY**

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The MLML Molecular Ecology laboratory was funded through a subaward from Williams College, which lead institution for the PICES ADRIFT project called "Japanese Tsunami Marine Debris (JTMD) and Alien Species Invasions." This was the third of three subawards, referred to here as Year 1, Year 2, and Year 3. Tasks assigned to MLML in Year 3 within the joint proposal were to process:

- **JTMD samples** for genetic analyses delivered to MLML between April 1, 2016 and July 1, 2016. The July cut-off was to ensure sufficient time prior to the January 31, 2017 report deadline.
- **Year 3 Japanese fouling samples:** 30-day "soaks" and 90-day "soaks" received by July 1, 2016 or as close to that date as possible. Priority and focus was to be on barcoding species not found in Year 2 Japanese samples.
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- **Mussels:** additional funds were provided to support graduate student research on potential invasion of *Mytilus galloprovincialis* in Oregon in connection with JTMD landings.

**a. Describe progress.**

No new JTMD samples (i.e, collections from debris) were delivered to MLML between April 1, 2016 and January 31, 2017. No Japanese fouling samples were delivered to MLML between April 1, 2016 and July 1, 2016. However, one shipment arrived Aug 11, 2016 and another arrived Oct 17, 2016. Despite late delivery, this report includes a majority of samples arrived in 2016 (Year 3) as well as a catch-up on samples from 2015 (Year 2), received February 2016. We experienced no impediment to mussel collections in Oregon.

**b. Describe any concerns or challenges you may have about your project's progress.**

As noted, delivery of vouchers did not meet proposed deadlines. Early reporting deadlines in advance of the project completion date of March 31, 2017 further compressed time available for molecular analysis. In general, coordination between ADRIFT project components was not optimal. We nonetheless accomplished the molecular processing of most identified taxa among delivered vouchers. We will continue to analyze additional replicates until final project completion (March 31, 2017).

**3. ABSTRACT**

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More than 300 species have been collected from debris from the 2011 Tohoku tsunami, many of which are challenging to identify by even well trained taxonomists. Genetic analysis provides a tool to assist in identification and can also be used for monitoring North American waters for potential JTMD associated invaders. Efficient DNA barcoding requires expert identification of voucher specimens that are sufficiently well preserved for molecular analysis. In Years 2 and 3, to overcome highly variable preservation status of beached JTMD specimens, we focused on fouling organisms collected in Japan in habitats that might have contributed to the rafting assemblage. To this purpose, we sequenced the mitochondrial COI gene from 130 morphospecies from 293 specimens collected in Miyako, Kesenuma, and Matsushima in 2015 and 2016 from settling panels deployed for 1 to 3 months. Sequences were aligned to Genbank sequences from putative conspecific, congeneric, confamilial, or consuperfamilial specimens. Species identifications were considered confirmed when new sequences were within monophyletic clades with putative conspecifics. Identifications were reassigned when sequences fell into clearly defined clades lacking putative conspecifics. Species identifications were provisionally accepted when sequences from putative species that lack records in Genbank were nonetheless phylogenetically related to relevant higher taxa. Apparent species misidentifications within Genbank records were also noted. In this way, we generated sequences for 125 unique species from the Japanese fouling community, including 38 for which no prior sequence existed. Mussels (n=500) collected in Oregon were identified by species-specific alleles at a nuclear locus, and were all native *Mytilus trossulus*. Species, and to a lesser extent haplotypes, not now known in North America can be a signature of tsunami related invasion if detected in North America in the near future.

## 4. PROJECT DESCRIPTION

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### a) Research Purpose

The purpose of the genetic component of the joint Williams College-Smithsonian Institution (SERC)-Oregon State University project "Japanese Tsunami Marine Debris (JTMD) and Alien Species Invasions" was to generate DNA sequences from taxonomically validated specimens and use these sequences in a program to detect Japanese species in North American waters. This purpose has a clear relevance to the overall ADRIFT project, which seeks to assess ecological risks associated with potential colonization of Japanese species via tsunami debris.

This purpose and plan -a transition from DNA barcoding to environmental assessment- was clearly expressed in the Year 1 and 2 proposals. To this end, in addition to voucher specimen collections, settling panels and plankton were retrieved from Oregon to Alaska in Year 2. A demonstration of metagenetic analysis of plankton was proposed and approved in Year 2: "we will analyze in Year 2 at least one community sample using metagenetics ... to be able to elucidate a broader and deeper understanding of site-specific diversity that may reveal the presence of JTMD species." However, further analysis of panels and plankton was not supported in Year 3. Therefore, the chief purpose for genetic analysis has been creation of a database for future investigations of the field environment or identification of individual samples.

### b) Objectives

The objective of the genetic component "Molecular Identification Species on JTMD and DNA Barcoding of Japanese Vouchers" was to obtain DNA sequences that can identify species associated with the 2011 Tohoku tsunami.

### c) Methods

*Collections.* Specimens were collected from JTMD objects in 2014 or earlier as described by Chapman (OSU) and Carlton (Williams College) in their Year 1,2, and 3 reports. Organisms on drift were collected live or dead. Regardless of living condition when found, tissues were typically not preserved fresh. Rather, they may have been collected dead, died in transit, frozen, dried, or stored in formalin and/or ethanol in unknown concentrations. Individual or bulk specimens were shipped to Williams College to be examined and sorted, and specimens or tissue subsamples were subsequently shipped to Moss Landing Marine Laboratories. Samples in Japan were collected by Hideki Takami, Hisatsugu Kato, Michio Otani in 2015 and 2016 after 1 and 3 month deployment of settling panels. Samples were frozen in bulk and later sorted, identified by Michio Otani, distributed to vials and shipped to Moss Landing Marine Laboratories. Plates and plankton were collected at the following sites: San Francisco Bay CA, Humboldt Bay CA, Yaquina Bay OR, Willapa Bay WA, Grays Harbor WA, Neah Bay WA, Prince Rupert BC, Ketchikan AK.

*DNA extractions and PCR.* DNA extractions of vouchers used the MagJet Genomic DNA extraction kit (ThermoFisher K2721) following the manufacturer's instructions. Briefly, tissues were mechanically homogenized, lysed in Proteinase-K, and nucleic acids bound to magnetic beads for washing and elution. DNA was extracted from plankton using a similar method contained in the PowerSoil DNA extraction kit (MoBio), with DNA bound to silica resin in columns rather than magnetic beads. PCR was used to amplify the mitochondrial cytochrome c oxidase subunit 1 gene using standard primers and methods (Geller et al. 2013).

*DNA sequencing and sequence analysis.* PCR products from JTMD were indexed with Ion Torrent library tags and individual sample tags (short DNA strands), pooled, ligated to Ion Torrent specific adaptors, and sequenced on an Ion Torrent PGM sequencer. PCR products from Japanese vouchers were purified and Sanger-sequenced by Elim Biopharmaceuticals (Hayward), or purified at MLML using Ampure beads (Agencourt) prior to sequencing by Elim Biopharmaceuticals. Sequence editing and analysis were performed within the Geneious software package (Biomatters, Ltd., Auckland, NZ). Ion Torrent sequences were demultiplexed and assembled into contiguous sequences. Forward and reverse Sanger sequences were assembled, and trimmed of primers and low quality bases. Sequences were compared to related sequences in Genbank to ascertain taxonomic identities where prior records existed. For Japanese fouling community samples, sequences were aligned to Genbank sequences of putative conspecific, congeneric, confamilial, or consuperfamilial specimens. Species identification were considered confirmed when new sequences were within monophyletic clades with putative conspecific sequences. Identification was reassigned when sequences fell into clades of sequences of other species. Species identifications were provisionally accepted when sequences without conspecific records in Genbank were phylogenetically placed among putatively related taxa. Apparent misidentifications within Genbank records were also noted.

*Plankton metagenetics.* Genomic DNA was quantified using Picogreen and standardized to 5 ng  $\mu\text{L}^{-1}$ . The COI gene was amplified, in triplicate, using primers with adapters for Nextera barcode indices. Triplicates were pooled and purified with Agencourt Ampure beads. Purified, barcoded amplicons were pooled evenly by mass and sequenced on an Illumina MiSeq instrument. Plankton metagenetic sequences were clustered into OTU using USEARCH 1.8.

#### **d) Results**

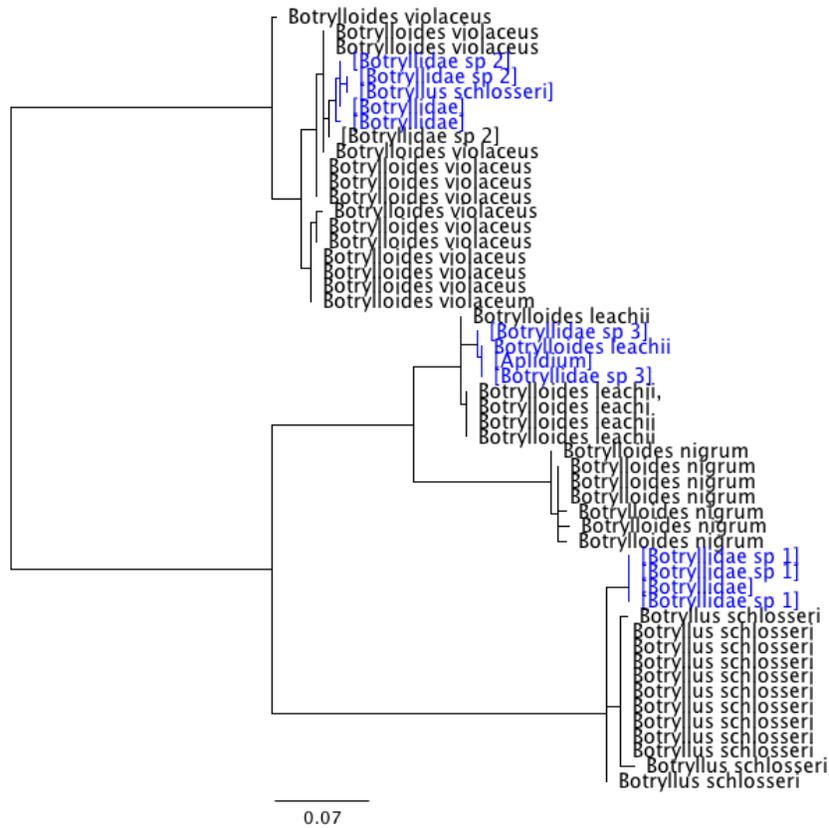
##### *Year 1: JTMD Voucher sequencing.*

In total, 294 specimens were sequenced on the Ion Torrent PGM instrument. 191 templates were from PCR reactions with low yield and insufficient numbers of reads obtained. From the remainder, 29 morphological identifications comprising seven species were confirmed by comparing sequences to Genbank or the MLML invasive species genetic database. For example, 19 specimens identified only as the amphipod *Jassa* were refined to *Jassa marmorata*. Fifty-six specimens had no match to Genbank or the MLML invasive species database at a similarity of 95% or greater. These were initially morphologically identified as: Capitellidae, *Hydroides ezoensis*, Ampithoidae, *Caprella*, *Aetea* sp. B, *Alcyonidium*, *Bugula*, *Bugula neritina*, *Jellyella tuberculata*, *Membranipora*, *Membraniporine* sp. 2, *Scruparia*, *Tricellaria*, *Tubulipora misakiensis*, *Tubulipora* sp. A, *Tubulipora* sp. B, *Watersipora*, Ascidiacea, and "anemone." BLAST results showing 94% or greater similarity to Genbank or MLML records were *Jassa marmorata*, *Ampithoe lacertosa*, *Semibalanus cariorus*, and *Watersipora subtorquata*. We correlate low PCR and sequencing success to tissue quality (dried, discolored) or size (not visible or miniscule), and this was a major reason to shift focus to fresh material from Japan.

##### *Year 2 and 3: Japanese fouling community sequencing.*

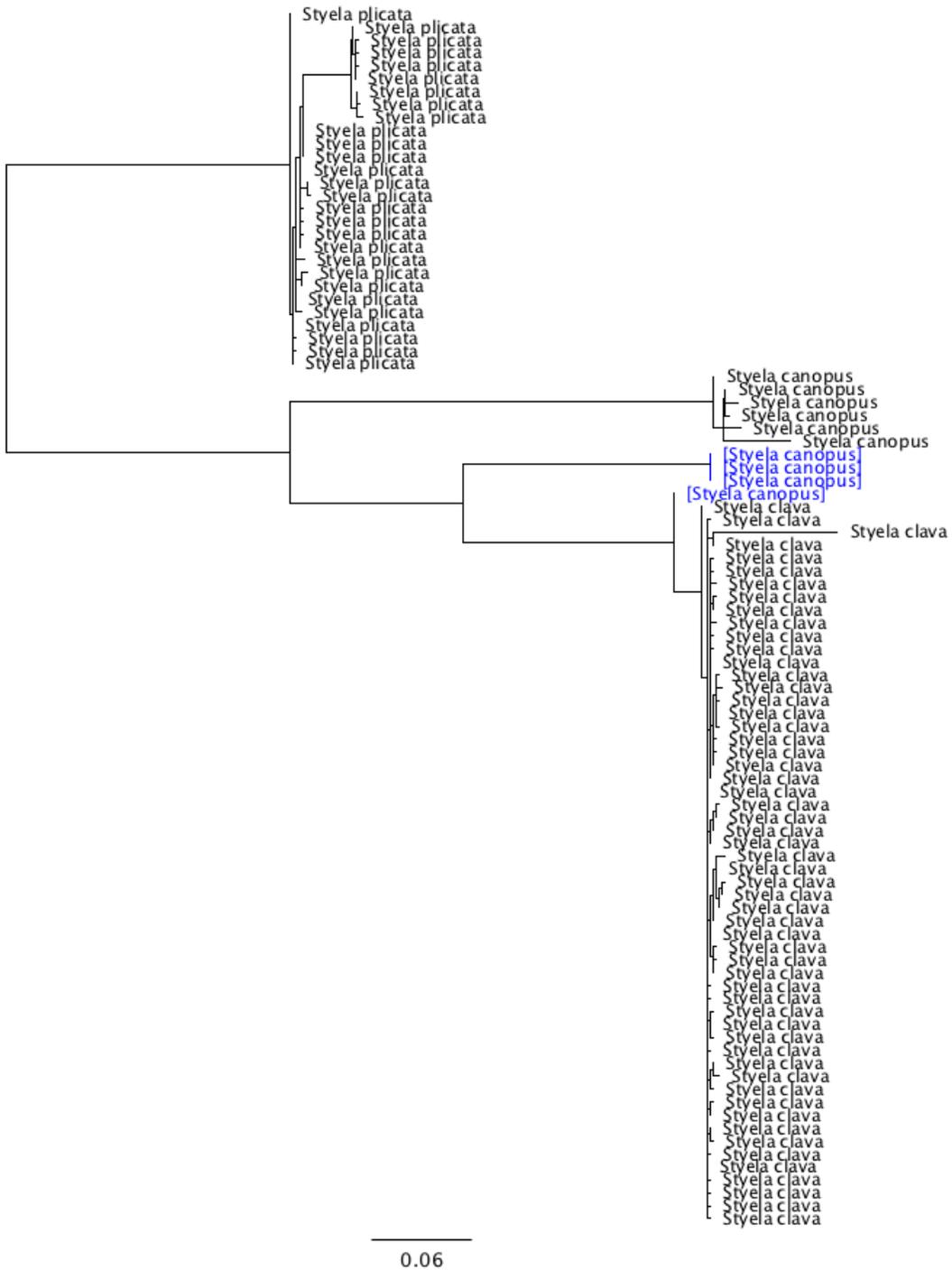
As noted earlier, we sequenced the mitochondrial COI gene from 130 morphospecies from 293 specimens that were collected in Miyako, Kesenuma, and Matsushima in 2015 and 2016 from settling panels deployed for 1 to 3 months. In this way, we generated sequences for 125 unique species from the Japanese fouling community, including 38 for which no prior sequence existed. Appendix 1 contains a list of specimens sequenced, their *a priori* morphological identification, and the genetic identification made here.

Sequences from each putative morphospecies was aligned and phylogenetically analyzed. Some examples of phylogenetic analysis and types of results are given below. By noting inclusion of novel sequences in unambiguous clades, some low resolution morphological identifications could be determined. For example, specimens variously identified as Botryllidae, Botryllidae sp1, and so on, were determined as *Botrylloides violaceus*, *Botrylloides leachii*, or *Botryllus schlosseri*. Conversely, one specimen positively identified morphologically as *Botryllus schlosseri* was shown to be *Botrylloides violaceus*. Finally, sequences that might indicate contamination of tissues or DNA were uncovered, such the morphological identification of a specimen as *Aplidium* that was genetically *Botrylloides leachi* (Fig. 1).



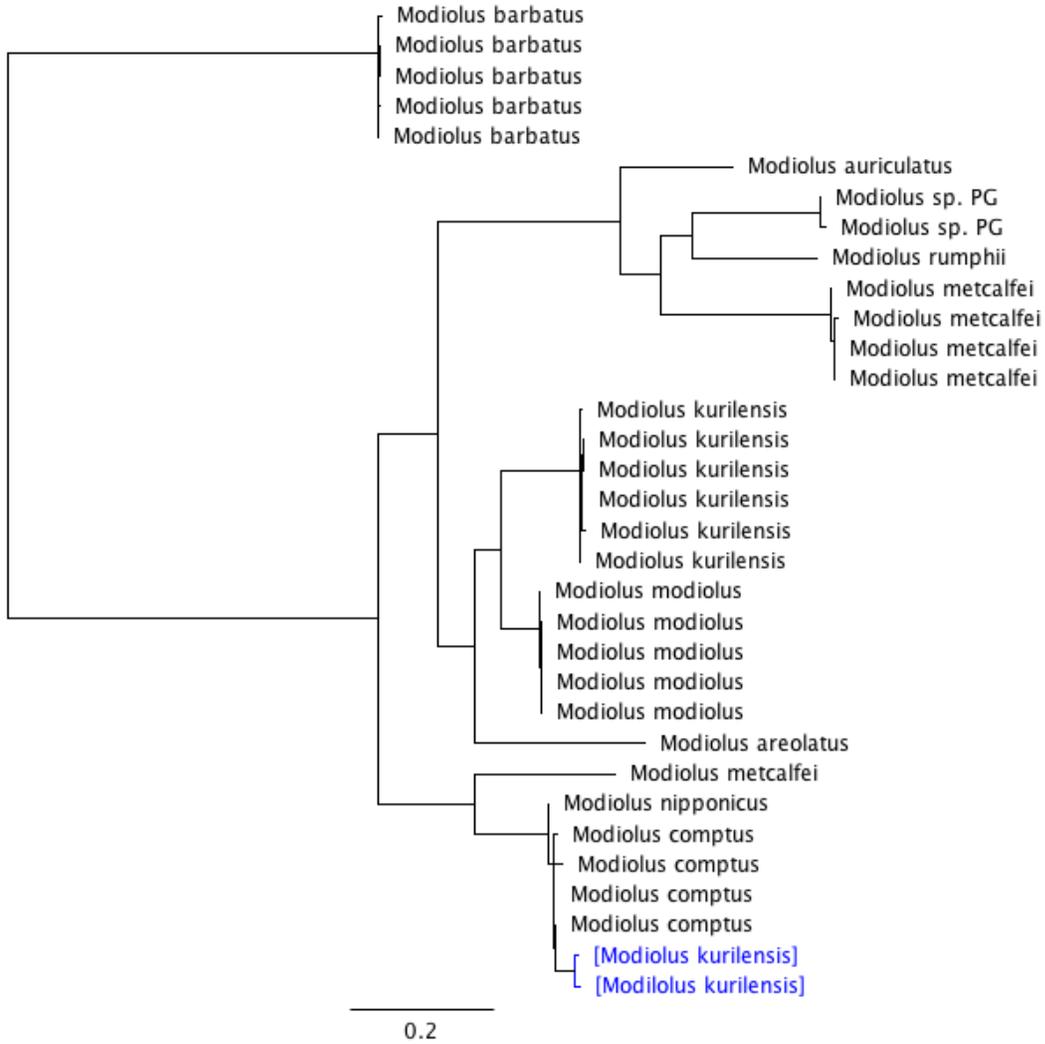
**Figure 1.** Maximum likelihood tree of COI sequences for specimens morphologically identified as Botryllidae, and one identified as *Aplidium*. Blue font surrounded by brackets indicates a Japanese fouling voucher; all other records are from Genbank or MLML database.

In other cases, morphological identifications suggested hidden diversity within nominal species. No specimens identified by morphology as *Styela canopus* clustered with Genbank entries for this species, but did so as sister to *S. clava*. Thus, these specimens may be *S. clava* or a cryptic species related to *S. clava* (Fig. 2).



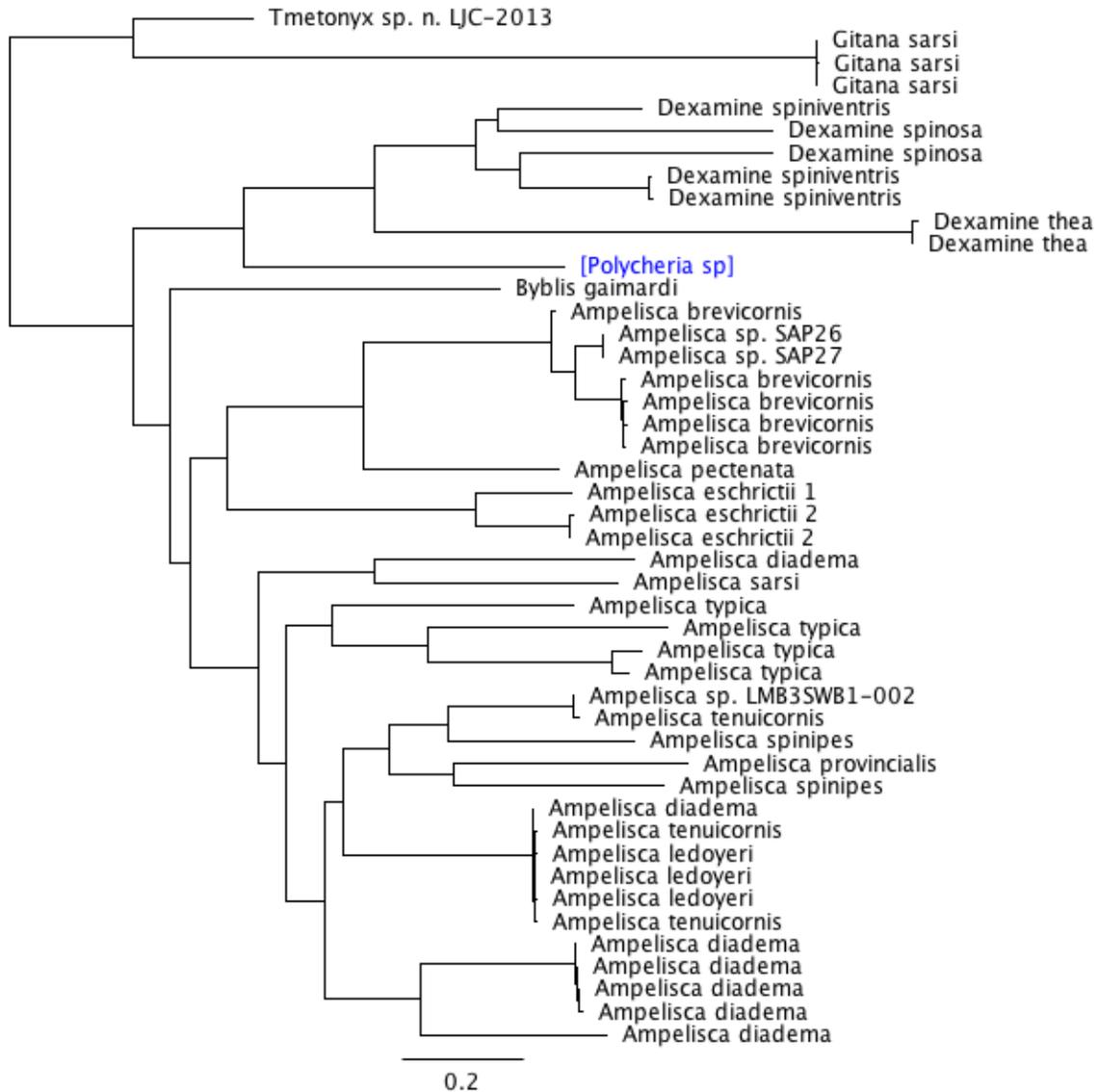
**Figure 2.** Relationships of Japanese specimens identified as *Styela canopus* to existing Genbank records.

Another outcome example is the reassignment of sequences from the morphological prior identification to an ambiguous genetic assignment. Specimens identified as *Modiolus kurelensis* were not related to Genbank records of this name, but ambiguously to *Modiolus comptus* or *M. nipponicus* (Fig. 3).



**Figure 3.** Specimens identified as *Modiolus kurilensis* are related to *M. nipponicus* or *M. comptus*.

Finally, in many cases Genbank is sparse for records closely related to a Japanese voucher, and phylogenetic analysis may only show that the novel sequence fits among confamilials or consuperfamilials. In these cases, there is no genetic evidence that contradicts the morphological identification, which is thus provisionally accepted. For example, the amphipod called *Polycheria* fits at the base of other members of the family Dexaminidae (Fig. 4) and so is plausibly *Polycheria*.



**Figure 4.** Morphologically identified *Polycheria* was phylogenetically basal to other Dexaminidae among the Gammaridea, and therefore this identification was accepted.

*Year 2 and 3: Mussel identifications.*

All 500 mussels collected in Yaquina Bay and Coos Bay (250 each) were identified as *Mytilus trossulus*, a native of the Northeastern Pacific Ocean, using the method of Inoue et al. (1995).

*Year 2: Plankton metagenetics*

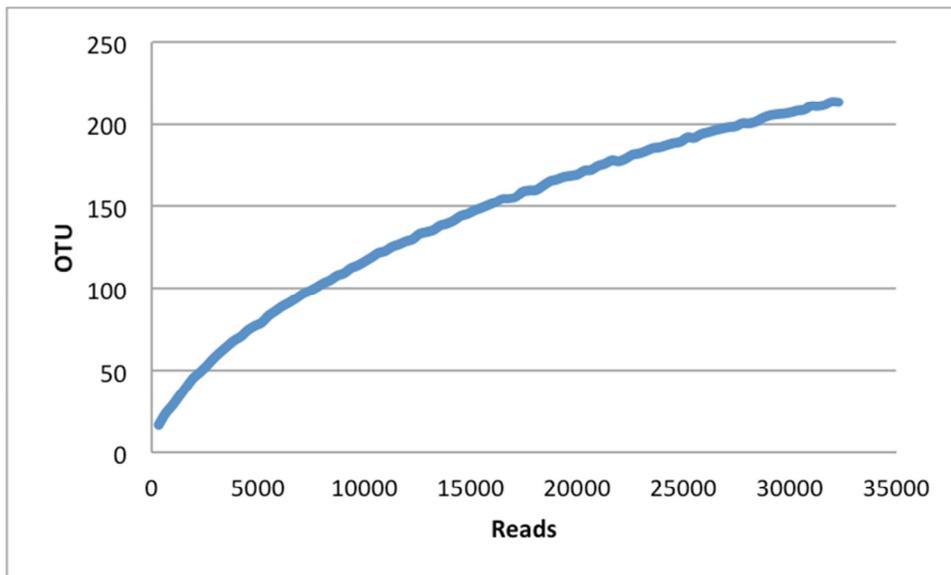
Although the ADRIFT scientific advisory panel later decided that metagenetic analysis was not to be continued in Year 3, 99 plankton samples, 39 from British Columbia and 60 from Oregon and Washington, were received at MLML for DNA extractions for potential future PCR and sequencing-based detection of JTMD-associated species. One sample from Yaquina Bay, OR was processed and analyzed in Year 2, as per the approved Year 2 scope of work.

211,466 reads were analyzed with the 64 bit version of USEARCH 1.861 (Edgar 2015). Three sites in Yaquina Bay (Hog's Marina, Port of Newport, and Embarcadero) yielded 209 OTU from 64,972 paired-end reads of COI. These clustered into 209 OTU using a 97% similarity threshold, and OTUs matched Genbank at 95% or better for 66 OTU. Removing bacteria and unidentified phytoplankton left 63 OTU (Table 1). Clustering was also performed with unpaired reads to increase available reads (since not all reads could be paired), which increased the number of OTU to 297. Rarefaction analysis shows that OTU accumulation had not reached an asymptote, suggesting that deeper sequencing will greatly increase the number of taxa recovered (Figure 1).

**Table 1.** OTU identified COI sequences from pooled zooplankton samples from Yaquina Bay Oregon.

Species	Taxon	Species	Taxon
<i>Anthopleura elegantissima</i>	Anthozoa	<i>Hematodinium sp.</i>	Dinoflagellate
<i>Angulus nuculoides</i>	Bivalve	<i>Proto-peridinium cf.</i>	Dinoflagellate
<i>Hiatella sp.</i>	Bivalvia	<i>Pyrocystis lunula</i>	Dinoflagellate
<i>Kellia suborbicularis</i>	Bivalvia	<i>Aplysiopsis enteromorphae</i>	Gastropoda
<i>Melanochlamys diomedea</i>	Bivalvia	<i>Assimineia grayana</i>	Gastropoda
<i>Mytilus californianus</i>	Bivalvia	<i>Dendronotus venustus</i>	Gastropoda
<i>Mytilus trossulus</i>	Bivalvia	<i>Diaulula sandiegensis</i>	Gastropoda
<i>Neaeromya rugifera</i>	Bivalvia	<i>Doris montereyensis</i>	Gastropoda
<i>Hemigrapsus oregonensis</i>	Brachyura	<i>Flabellina verrucosa</i>	Gastropoda
<i>Lophopanopeus bellus</i>	Bryachyura	<i>Gastropterion pacificum</i>	Gastropoda
<i>Pandalus jordani</i>	Caridea	<i>Hermisenda crassicornis</i>	Gastropoda
<i>Amphibalanus improvisus</i>	Cirrepedia	<i>Limacina helicina</i>	Gastropoda
<i>Amphibalanus sp.</i>	Cirrepedia	<i>Littorina plena</i>	Gastropoda
<i>Balanus crenatus</i>	Cirrepedia	<i>Lottia pelta</i>	Gastropoda
<i>Balanus glandula</i>	Cirrepedia	<i>Margarites pupillus</i>	Gastropoda
<i>Chthamalus dalli</i>	Cirrepedia	<i>Olivella biplicata</i>	Gastropoda
<i>Pollicipes polymerus</i>	Cirrepedia	<i>Olivella baetica</i>	Gastropoda
<i>Evadne nordmanni</i>	Cladocera	<i>Rictaxis punctocaelatus</i>	Gastropoda
<i>Podon leuckartii</i>	Cladocera	<i>Stiliger fuscovittatus</i>	Gastropoda
<i>Acartia californiensis</i>	Copepoda	<i>Williamia peltoides</i>	Gastropoda
<i>Acartia sp.</i>	Copepoda	<i>Merluccius productus</i>	Hake
<i>Acartia tonsa</i>	Copepoda	<i>Clytia sp.</i>	Hydrozoa
<i>Calanus pacificus</i>	Copepoda	<i>Obelia dichotoma</i>	Hydrozoa
<i>Centropages abdominalis</i>	Copepoda	<i>Poseidonemertes collaris</i>	Nemertea
<i>Ctenocalanus vanus</i>	Copepoda	<i>Ophiopholis kennerlyi</i>	Ophiuroidea
<i>Cyclops kikuchii</i>	Copepoda	<i>Dictyosiphon sp.</i>	Phaeophyta
<i>Eucalanus californicus</i>	Copepoda	<i>Ectocarpus fasciculatus</i>	Phaeophyta
<i>Oithona similis</i>	Copepoda	<i>Ectocarpus siliculosus</i>	Phaeophyta
<i>Orthione griffenis</i>	Copepoda	<i>Myrionema balticum</i>	Phaeophyta
<i>Paracalanus parvus</i>	Copepoda	<i>Myrionema strangulans</i>	Phaeophyta
<i>Pseudocalanus mimus</i>	Copepoda	<i>Chone magna</i>	Polychaeta

<i>Attheya longicornis</i>	Diatom	<i>Leitoscoloplos pugettensis</i>	Polychaeta
<i>Berkeleya fennica</i>	Diatom	<i>Nereis vexillosa</i>	Polychaeta
<i>Ditylum brightwellii</i>	Diatom	<i>Platynereis sp.</i>	Polychaeta
<i>Eucampia zodiacus</i>	Diatom	<i>Polydora cornuta</i>	Polychaeta
<i>Fragilaria striatula</i>	Diatom	<i>Scoloplos acmeceps</i>	Polychaeta
<i>Grammonema striatula</i>	Diatom	<i>Thaleichthys pacificus</i>	Smelt
<i>Melosira nummuloides</i>	Diatom	<i>Citharichthys stigmaeus</i>	Speckled Sanddab
<i>Pseudo-nitzschia pungens</i>	Diatom		
<i>Stephanopyxis turris</i>	Diatom		



**Figure 5.** Yaquina Bay plankton. Rarefaction of OTUs recovered with increasing read abundance, using 165,809 unique reads, clustered at a 97% similarity threshold, and omitting any OTU with group size of 1 read.

### e) Discussion

The DNA sequences generated herein provide tools for detection and monitoring Japanese species beyond their natural biogeographic limits. Methods such as metabarcoding, as illustrated here, or probing of environmental samples by qPCR (Mackie and Geller (2010), will allow interrogation of large volumes of biomass. Metabarcoding of the Yaquina Bay sample did not reveal Japanese species not known previously as invaders. Similarly, all mussels identified in Coos Bay and Yaquina Bay were native. These results provide a baseline with which to compare future measurements: appearance of taxa identified genetically or morphologically from JTMD or the Japanese fouling community will be signals of a tsunami related invasion.

The absence of *Mytilus galloprovincialis* from Oregon was striking given the abundance of this species on JTMD objects. This species is well established in California, but our ongoing study of its distribution indicates a northern boundary currently below the Oregon border. Thee potential saltatory appearance of *M. galloprovincialis* in Oregon sites is another tsunami-related invasion signal to monitor. Population genetic comparisons of any such Oregon invasions to California populations will provide a second level of testing.

The results of phylogenetic analyses presented in Appendix 1 reveal many cases of potential cryptic species. Too many specimens that were barcoded were not fully identified to the species level such that DNA barcodes yet lack a specific assignment. Parallel morphological vouchers were sent to Williams College in 2016 to be further distributed to taxonomic experts. As these specimens are identified or described, this project will contribute to refinement of taxonomic knowledge of the Japanese biota and, not unimportantly, to a suite of species that are common invaders world-wide.

#### **f) Challenges**

Two issues challenged the success of this project. The first was unreliable and undetermined quality of specimens received from JTMD objects. The majority appear to have unknown histories of morbidity, storage condition, or preservation. Changing focus to fresh material from the Japanese fouling communities dramatically enhanced success of DNA barcoding. The second challenge was the dependence on other laboratories for timely collection and delivery of samples, as MLML was funded only for laboratory analyses. Shortened timelines due to delayed receipt of funding and early deadlines <12 months from initiation of research contributed to this issue. Requests for progress and final reports were not always realistically aligned to the actual research timetable.

#### **g) Achievements**

The major achievement is the establishment of a DNA barcode dataset for many taxa delivered or potentially delivered to North America by JTMD. These sequences provide a framework for detection and association of new invasions with JTMD. Although the analysis of environmental samples (plates and plankton) collected under ADRIFT could not be fully analyzed herein for lack of available funding, their eventual analysis provides a baseline for comparison for future studies.

#### **h) Literature Cited**

Inoue, K., Waite, J.H., Odo, S., and Harayama, S. 1995. Interspecific variations in adhesive protein structures of *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus*. *Biological Bulletin* 189:370-375.

Geller, J., Meyer, C.P., Parker, M., and Hawk, H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources* 13: 851-861. (DOI: 10.1111/1755-0998.12138)

Mackie, J. and Geller, J.B. 2010. Experimental parameters affecting quantitative PCR of *Artemia franciscana*, a model for a marine planktonic target, in natural plankton samples. *Limnology and Oceanography Methods* 8:337-347

## **5. OUTPUTS**

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#### **a. Completed and planned publications**

Publications or planned publications are listed in the Williams College report. I have no separate publications resulting from ADRIFT to report.

#### **b. Poster and oral presentations at scientific conferences or seminars**

- Departmental seminar, Moss Landing Marine Laboratories (November 2014)
- International Conference on Marine BioInvasions, Sydney, Australia (January 2016);
- PICES Science Meeting, San Diego, California (November 2016);

- Western Society of Naturalists, Monterey, CA (November 2016), presentation by M. Wheelock.

### **c. Education and outreach**

Melinda Wheelock, a graduate student has been supported and conducted research on *Mytilus galloprovincialis*.

## **6. RESEARCH STATUS AND FUTURE STEPS/PLANS**

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I envision three major continuing themes for my laboratory related to this project.

1. The phylogenetic analyses reveal many cases of potential cryptic species, new species, and confused taxonomy. I expect to collaborate with Dr. Carlton to further parse these results and refer sequences and specimens to taxonomic analyses for further investigation. Sequences with confident morphological identifications will be deposited into Genbank. The remaining time and funds through March 31 will be spent in this effort.

2. In Year 2, hundreds of plankton and settling plates samples were collected and archived but not analyzed. We possess the capacity but not funding to complete these analyses and will seek to complete this work, some day.

3. Independent of ADRIFT, my laboratory monitors California waters for invasive species. We will be attentive to signals of JTMD in these ongoing studies.

Geller; PICES final report

Appendix 1: Morphological and Genetic Identification of Japanese Fouling Community Species

(M1= 1 month deployment; M3=3 month deployment; K=Kesennuma; M=Miyako, MS=Matsushima)

Specimen	Morphological Assignment	Genetic result
M3_K-13.06	Actiniaria	Aiptasia possibly pulchella
M3_K-13.07-11	Actiniaria	Aiptasia possibly pulchella
M3_S-10.01	Amathia distans	Amathia distans provisionally accept
M3_S-10.02	Amathia distans	Amathia distans provisionally accept
M3_S-10.03	Amathia distans	Amathia distans provisionally accept
M3_M-13.05	Amphibalanus amphitrite	Amphibalanus amphitrite confirm
M3_M-27.05	Amphibalanus amphitrite	Caprella mutica
M3_S-77.01	Amphibalanus amphitrite	Amphibalanus amphitrite confirm
M3_S-63.01	Amphibalanus eburneus	Amphibalanus eburneus confirm
M3_M-8.01	Amphibalanus improvisus	Amphibalanus improvisus confirmed
M3_M-8.02	Amphibalanus improvisus	Amphibalanus improvisus confirmed
M3_M-8.03	Amphibalanus improvisus	Amphibalanus improvisus confirmed
M3_S-41.01	Amphitrite sp.	Amphitrite sp. Provisionally accepted
M3_S-41.02	Amphitrite sp.	Amphitrite sp. Provisionally accepted
M3_M-13.01	Ampithoe sp. 1	Ampithoe tarasovi
M3_M-13.02	Ampithoe sp. 1	Ampithoe tarasovi
M3_M-36.05	Ampithoe sp. 1	Ampithoe tarasovi
M3_S-17.01	Ampithoe sp. 2	Ampithoe sp
M3_S-17.02-04	Ampithoe sp. 2	Ampithoe sp
M3_K-64.02	Ampothoe sp1	Fistulobalanus albicostatus
M3_M-19.01-02	Anaitides sp.	Phyllocidae, possible conflict with Anaitides in Genbank
M3_S-42.01	Anamixis sp.	Ampithoe tarasovi
M3_S-42.02	Anamixis sp.	Ampithoe tarasovi
M3_S-42.03	Anamixis sp.	Ampithoe tarasovi
M3_M-43.01-02	Anoplodactylus crassus	Anoplodactylus crassus provisionally accepted
M3_S-51.01	Anoplodactylus crassus	Not Anoplodactylus crassus, possibly Ascorhyncus
M1_S-47.01	Anthopleura sp.	Anthopleura, probably midori but also very similar to elegantissima (low COI variation in Anthozoa)
M3_S-43.06	Aoroides longimerus	Aoroides longimerus provisionally accepted but not near Aoroides columbiae
M3_S-43.02-06	Aoroides longimerus	Aoroides longimerus provisionally accepted but not near Aoroides columbiae
M3_M-24.01-04	Aoroides sp.	Aoroides longimerus provisionally accepted but not near Aoroides columbiae
M3_K-4.01	Aplidium sp.	Aiptasia sp possibly pulchella
M3_K-4.02	Aplidium sp.	Botrylloides leachii
M3_K-4.03	Aplidium sp.	Aplidium, possibly fuscum
M3_M-51.01	Arabella sp.	Unknown polychaete, distant from Arabella genbank records
M3_S-39.01	Arcuatula senhousia	Arcuatula senhousia confirm (as Musculista)
M3_S-39.02	Arcuatula senhousia	Arcuatula senhousia confirm (as Musculista)
M3_S-39.03	Arcuatula senhousia	Arcuatula senhousia confirm (as Musculista)
M3_M-30.01	Ascidia sp.	Nemertean contaminant?
M3_M-35.02	Ascidia sp.	Nemertean contaminant?
M3_S-4.03	Ascidia sydneyensis	Halichondria, contaminant?
M3_S-4.05	Ascidia sydneyensis	unknown; contaminant?
M3_S-2.01	Ascidia zara	Ascidia zara confirm
M3_S-2.02	Ascidia zara	Ciona savignyi
M3_S-2.03	Ascidia zara	Ascidia zara confirm
M3_K-1.07	Balanus trigonus	Balanus trigonus confirmed
M3_K-1.09	Balanus trigonus	Balanus trigonus confirmed
M3_K-1.10	Balanus trigonus	Balanus trigonus confirmed
M3_S-12.01	Botryllidae sp. 1	Botryllus schlosseri
M3_S-12.02	Botryllidae sp. 1	Botryllus schlosseri
M3_S-12.03	Botryllidae sp. 1	Botryllus schlosseri
M3_M-26.01	Botryllidae sp. 2	Botrylloides violaceus
M3_M-26.02	Botryllidae sp. 2	Botrylloides violaceus
M3_M-26.03	Botryllidae sp. 2	Botrylloides violaceus
M3_M-40.01	Botryllidae sp. 3	Botrylloides leachii
M3_M-40.02	Botryllidae sp. 3	Botrylloides leachii
M1_M-28.01	Botryllidae gen. sp. 4	Botrylloides violaceus
M1_M-28.02	Botryllidae gen. sp. 4	Botrylloides violaceus
M1_S-23.03	Botryllidae sp.	Botrylloides violaceus
M16_M-40.1	Botrylloides violaceus	Botrylloides violaceus confirm
M16_M-40.2	Botrylloides violaceus	Botrylloides violaceus confirm
M16_M-40.3	Botrylloides violaceus	Botrylloides violaceus confirm
M3_M-29.01	Botryllus schlosseri	Botrylloides violaceus

M16_M-41	<i>Botryllus</i> sp.	<i>Botryllus</i> schlosseri
M3_S-52.01-03	<i>Brachystomia minutiovum</i>	<i>Brachystomia minutiovum</i> provisionally accept; near Pyramidellidae in Genbank tree
M3_S-26.01	<i>Bugula neritina</i>	<i>Bugula neritina</i> confirm (note cryptic species exist)
M3_S-26.02	<i>Bugula neritina</i>	<i>Bugula neritina</i> confirm (note cryptic species exist)
M3_S-26.03	<i>Bugula neritina</i>	<i>Bugula neritina</i> confirm (note cryptic species exist)
M1_S-3.01	<i>Bugula stolonifera</i>	<i>Bugula stolonifera</i> confirmed
M1_S-3.02	<i>Bugula stolonifera</i>	<i>Bugula stolonifera</i> confirmed
M1_S-3.03	<i>Bugula stolonifera</i>	<i>Bugula stolonifera</i> confirmed
M3_M-11.01	<i>Caprella equilibra</i>	<i>Caprella equilibra</i> confirmed
M3_M-11.02	<i>Caprella equilibra</i>	<i>Caprella equilibra</i> confirmed
M3_M-11.03	<i>Caprella equilibra</i>	<i>Caprella equilibra</i> confirmed
M3_M-27.02	<i>Caprella mutica</i>	<i>Caprella mutica</i> confirmed
M3_M-27.03	<i>Caprella mutica</i>	<i>Caprella mutica</i> confirmed
M3_M-27.04	<i>Caprella mutica</i>	<i>Caprella mutica</i> confirmed
M16_MS-4.1	<i>Caprella penantis</i>	<i>Caprella</i> sp., not <i>penantis</i> cf Genbank KC146253
M16_MS-4.2	<i>Caprella penantis</i>	<i>Caprella</i> sp., not <i>penantis</i> cf Genbank KC146254
M16_MS-4.4	<i>Caprella penantis</i>	<i>Caprella</i> sp., not <i>penantis</i> cf Genbank KC146255
M3_S-27.03	<i>Caprella scaura</i>	<i>Caprella</i> sp., 91% similar to <i>scaura</i>
M3_S-27.04	<i>Caprella scaura</i>	<i>Caprella</i> sp., 91% similar to <i>scaura</i>
M3_S-27.05	<i>Caprella scaura</i>	<i>Caprella</i> sp., 91% similar to <i>scaura</i>
M3_M-14.01	<i>Celleporina Porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M3_M-14.02	<i>Celleporina Porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M3_M-14.03	<i>Celleporina Porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M16_M-18.1	<i>Celleporina porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M16_M-18.2	<i>Celleporina porosissima</i>	<i>Botrylloides violaceus</i>
M16_M-18.3	<i>Celleporina porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M16_MS-2.2	<i>Celleporina porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M16_MS-2.5	<i>Celleporina porosissima</i>	<i>Celleporina poroissima</i> provisionally accept
M3_S-37.06	<i>Chlamys farreri nipponensis</i>	<i>Azumapecten farreri</i>
M3_S-37.07	<i>Chlamys farreri nipponensis</i>	<i>Azumapecten farreri</i>
M3_S-37.08	<i>Chlamys farreri nipponensis</i>	<i>Azumapecten farreri</i>
M3_S-80.01-02	<i>Chlamys</i> sp.	<i>Azumapecten farreri</i>
M1_K-24.01	<i>Chthamalus challenger</i>	<i>Chthamalus sinensis</i> or <i>neglectus</i> ; Genbank ambiguous but not <i>challenger</i>
M3_S-1.01	<i>Ciona intestinalis</i> type A	<i>Ciona intestinalis</i> confirm
M3_S-1.02	<i>Ciona intestinalis</i> type A	<i>Ciona intestinalis</i> confirm
M3_S-1.03	<i>Ciona intestinalis</i> type A	<i>Ciona intestinalis</i> confirm
M3_S-3.01	<i>Ciona savignyi</i>	<i>Ciona savignyi</i> confirm
M3_S-3.02	<i>Ciona savignyi</i>	<i>Ciona savignyi</i> confirm
M3_S-3.03	<i>Ciona savignyi</i>	<i>Ciona savignyi</i> confirm
M3_K-2.01	<i>Cirolana harfordi japonica</i>	<i>Cirolana harfordi japonica</i> but <i>japonica</i> is probably a distinct species
M3_K-2.02	<i>Cirolana harfordi japonica</i>	<i>Cirolana harfordi japonica</i> but <i>japonica</i> is probably a distinct species
M3_S-77.02	<i>Cirolana harfordi japonica</i>	<i>Cirolana harfordi japonica</i> but <i>japonica</i> is probably a distinct species
M3_S-71.01	<i>Colomastix</i> sp.	<i>Colomastix</i> provisionally accept
M3_S-71.02-06	<i>Colomastix</i> sp.	<i>Colomastix</i> sp provisionally accept
M3_S-30.01	<i>Crassostrea gigas</i>	<i>Crassostrea gigas</i> confirm
M3_S-30.02	<i>Crassostrea gigas</i>	<i>Crassostrea gigas</i> confirm
M3_S-30.03	<i>Crassostrea gigas</i>	<i>Crassostrea gigas</i> confirm
M3_S-34.01	<i>Cymodoce japonica</i>	<i>Cymodoce japonica</i> provisionally accept
M3_S-34.02	<i>Cymodoce japonica</i>	<i>Cymodoce japonica</i> provisionally accept
M3_S-34.03	<i>Cymodoce japonica</i>	<i>Cymodoce japonica</i> provisionally accept
M1_S-17.01	<i>Cypsiphimedia mala</i>	<i>Cypsiphimedia mala</i> provisionally accept
M3_S-29.01	<i>Diadumene lineata</i>	<i>Diadumene lineata</i> confirm
M3_S-29.02	<i>Diadumene lineata</i>	<i>Diadumene lineata</i> confirm
M3_S-29.03	<i>Diadumene lineata</i>	<i>Diadumene lineata</i> confirm
M3_S-20.02	<i>Didemnum</i> sp.	<i>Didemnum</i> sp
M3_S-20.03	<i>Didemnum</i> sp.	<i>Didemnum</i> sp
M3_S-20.04	<i>Didemnum</i> sp.	<i>Didemnum</i> sp
M1_M-18.02	<i>Diplosoma listerianum</i>	<i>Diplosoma listerianum</i> confirmed
M1_M-18.03	<i>Diplosoma listerianum</i>	<i>Diplosoma listerianum</i> confirmed
M1_M-1.02	<i>Distaplia dubia</i>	<i>Distaplia dubia</i> provisionally accept; not near <i>Distaplia colligans</i> or other Claveliniidae
M1_M-1.03	<i>Distaplia dubia</i>	<i>Distaplia dubia</i> provisionally accept; not near <i>Distaplia colligans</i> or other Claveliniidae
M1_M-1.04	<i>Distaplia dubia</i>	<i>Distaplia dubia</i> provisionally accept; not near <i>Distaplia colligans</i> or other Claveliniidae
M3_S-68.01	<i>Escharella takatukii</i>	<i>Celleporaria brunnea</i> , distant from <i>Escharella immersa</i>
M3_S-31.01	<i>Eualus leptognathus</i>	<i>Eualus leptognathus</i> provisionally accept
M3_S-31.02	<i>Eualus leptognathus</i>	<i>Eualus leptognathus</i> provisionally accept
M3_S-31.03	<i>Eualus leptognathus</i>	<i>Eualus leptognathus</i> provisionally accept
M1_S-40.01	<i>Eudendrium</i> sp.	Hydrozoa; distant from <i>Eudendrium</i> records, closer to <i>Bouganvillia</i>

M1_S-40.02	Eudendrium sp.	Hydrozoa; distant from Eudendrium records, closer to Bouganvillia
M1_S-40.03	Eudendrium sp.	Hydrozoa; distant from Eudendrium records, closer to Bouganvillia
M3_S-9.03	Eulalia sp.	Hydrozoan (epibiont?)
M3_M-32.01	Eulalia viridis japonensis	Eulalia viridis or clavigera
M3_M-49.01	Fistulobalanus albicostatus	Fistulobalanus albicostatus confirmed
M3_M-49.02	Fistulobalanus albicostatus	Fistulobalanus albicostatus confirmed
M3_M-49.03	Fistulobalanus albicostatus	Fistulobalanus albicostatus confirmed
M3_K-22.06	Gammaropsis japonica	Gammaropsis japonica provisionally accept
M3_K-22.07	Gammaropsis japonica	Gammaropsis japonica provisionally accept
M3_K-22.09	Gammaropsis japonica	Gammaropsis japonica provisionally accept
M3_S-44.02-06	Gitanopsis sp.	Gitanopsis sp provisionally accept
M16_M-31	Gordonodius zelleri	Not Gordomodius? Seems deeply contained within Leucothoe tree.
M3_M-12.01	Halecium pusillum	Halecium pusillum provisionally accept
M3_M-12.02	Halecium pusillum	Halecium pusillum provisionally accept
M3_M-12.03	Halecium pusillum	Halecium pusillum provisionally accept
M3_S-22.01	Halichondria sitiens	Halichondria sp. (same as MLML sp 1)
M3_S-22.02	Halichondria sitiens	Halichondria sp. (same as MLML sp 1)
M3_S-22.03	Halichondria sitiens	Halichondria sp. (same as MLML sp 1)
M3_K-35.02	Halichondria sp.	Halichondria (same as sitiens herein)
M3_M-39.01	Halichondria sp.	Halichondria (same as sitiens herein)
M3_M-39.02	Halichondria sp.	Halichondria (same as sitiens herein)
M3_S-66.01	Haliclona sp.	Haliclona sp
M3_S-40.01	Halosydna brevisetosa	Halosydna brevisetosa of China not Canada
M3_S-40.02	Halosydna brevisetosa	Halosydna brevisetosa of China not Canada
M3_S-62.01	Harmothoe sp.	Harmothoe provisionally accept
M3_S-56.01	Hemigrapsus takanoi	Hemigrapsus takanoi
M3_S-56.02	Hemigrapsus takanoi	Hemigrapsus takanoi
M3_S-56.03	Hemigrapsus takanoi	Hemigrapsus takanoi
M3_S-32.02	Heptacarpus rectirostris	Heptorostris rectirostris provisionally accepted
M3_S-32.03	Heptacarpus rectirostris	Heptorostris rectirostris provisionally accepted
M3_S-32.04	Heptacarpus rectirostris	Heptorostris rectirostris provisionally accepted
M3_M-2.01	Hermilepidonotus helotypus	Halosydna brevisetosa of China not Canada
M16_M-16.1	Hiatella orientalis	Botrylloides violaceus
M16_M-16.5	Hiatella orientalis	Botrylloides violaceus
M3_M-9.02	Hydroides ezoensis	Hydroides ezoensis confirmed
M3_M-9.03	Hydroides ezoensis	Hydroides ezoensis confirmed
M3_M-9.04	Hydroides ezoensis	Hydroides ezoensis confirmed
M3_M-23.02-06	Ianiropsis serricaudis	Probably not Ianiropsis; it is not close to I. epilittoralis
M16_MS-10.3	Jassa marmorata	Quasitetrastramma stimpsoni; contaminant
M16_MS-10.4	Jassa marmorata	Jassa sp, not marmorata; cf GU048162
M16_MS-10.5	Jassa marmorata	Jassa sp, not marmorata; cf GU048162
M3_M-16.01	Jassa slatteryi	Jassa slatteryi confirmed
M3_M-16.02-06	Jassa slatteryi	Jassa slatteryi confirmed
M16_MS-11.1	Jassa staudei	Jassa sp, not staudei
M16_MS-11.2	Jassa staudei	Jassa sp, not staudei
M16_MS-11.3	Jassa staudei	Jassa sp, not staudei
M3_S-23.01	Lepidonotus elongatus	Lepidonotus elongatus provisional cf Caprella sp 2 (Genbank KC146254)
M3_S-23.02	Lepidonotus elongatus	Lepidonotus elongatus provisional cf Caprella sp 2 (Genbank KC146254)
M3_S-23.03	Lepidonotus elongatus	Lepidonotus elongatus provisional cf Caprella sp 2 (Genbank KC146254)
M3_S-14.02	Leucothoe nagatai	Leucothoe nagatai provisionally accept
M3_S-14.03	Leucothoe nagatai	Leucothoe nagatai provisionally accept
M3_S-14.04	Leucothoe nagatai	Leucothoe nagatai provisionally accept
M3_S-57.06-08	Liljeborgia serrata	Liljeborgia serrata provisionally accept, closest Gammaridean in Genbank is Cyclocaris
M16_M-27	Lirularia iridescens	Lirularia iridescens confirmed
M3_K-44.01	Maera pacifica	Maera pacifica provisionally accept; closest Genbank record is M. loveni
M3_K-44.02	Maera pacifica	Maera pacifica provisionally accept; closest Genbank record is M. loveni
M3_K-44.03	Maera pacifica	Maera pacifica provisionally accept; closest Genbank record is M. loveni
M3_K-44.07-06	Maera pacifica	Maera pacifica provisionally accept; closest Genbank record is M. loveni
M3_S-69.01	Maera sp.	Maera sp. Closest to M. loveni in genbank
M3_S-48.01	Marphysa sp.	Marphysa sp. Provisionally accept
M3_S-48.02	Marphysa sp.	Marphysa sp. Provisionally accept
M1_K-14.01	Megabalanus rosa	Megabalanus rosa confirmed
M1_K-14.02	Megabalanus rosa	Megabalanus rosa confirmed
M1_K-14.03	Megabalanus rosa	Megabalanus rosa confirmed
M16_M-36	Megasyllis nipponica	Megasyllis nipponica conflict in Genbank\
M3_S-15.01	Melita rylovae	Melita rylovae provisionally accept
M3_S-15.02	Melita rylovae	Melita rylovae provisionally accept

M3_S-15.03	Melita rylovae	Melita rylovae provisionally accept
M3_S-53.01	Membranipora sp. 2	Conopeum sp
M3_S-25.06	Modiolus kurilensis	Modiolus nipponicus or comptus, not kurilensis
M3_S-25.08	Modiolus kurilensis	Modiolus nipponicus or comptus, not kurilensis
M3_S-25.09	Modiolus kurilensis	Modiolus nipponicus or comptus, not kurilensis
M3_S-11.03	Molgula manhattensis	Molgula manhattensis confirmed
M3_M-17.01	Monocorophium acherusicum	Monocorophium acherusicum confirmed
M3_M-17.02-05	Monocorophium acherusicum	Monocorophium acherusicum confirmed
M3_S-16.02-04	Monocorophium uenoi	Monocorophium uenoi provisionally accepted
M16_M-37	Musculus cupreus	Musculus cupreus provisionally accept
M16_MS-18	Musculus cupreus	Musculus cupreus provisionally accept
M3_S-38.01	Mytilus galloprovincialis	Mytilus galloprovincialis confirmed
M3_S-38.02	Mytilus galloprovincialis	Mytilus galloprovincialis confirmed
M3_S-38.03	Mytilus galloprovincialis	Mytilus galloprovincialis confirmed
M3_K-9.06	Nemertellina yamaokai	Megabalanus rosa; contaminant?
M3_K-9.07	Nemertellina yamaokai	Megabalanus rosa; contaminant?
M3_K-9.08	Nemertellina yamaokai	Megabalanus rosa; contaminant?
M16_M-6.3	Nemertellina yamaokai	Quasitetrastemma stimpsoni
M16_M-6.4	Nemertellina yamaokai	Quasitetrastemma stimpsoni
M16_M-6.5	Nemertellina yamaokai	Quasitetrastemma stimpsoni
M3_S-61.01	Nereiphylla castanea	Undetermined; Conflicting Genbank entries
M3_S-61.02	Nereiphylla castanea	Undetermined; Conflicting Genbank entries
M3_S-61.03	Nereiphylla castanea	Undetermined; Conflicting Genbank entries
M3_S-7.06	Nereis multignatha	Nereis neoneanthes; clusters with K-56-01
M3_S-7.07	Nereis multignatha	Nereis multignatha confirmed
M3_S-7.08	Nereis multignatha	Nereis multignatha confirmed
M3_K-56.01	Nereis neoneanthes	Nereis neoneanthes provisionally accepted
M3_M-33.01	Nereis pelagica	Nereis pelagica confirmed
M3_M-33.02	Nereis pelagica	Nereis multignatha
M3_M-33.03	Nereis pelagica	Nereis pelagica confirmed
M16_M-5.1	Nereis vexillosa	Nereis sp not vexillosa
M16_M-5.2	Nereis vexillosa	Nereis sp not vexillosa
M16_M-5.3	Nereis vexillosa	Nereis sp not vexillosa
M3_K-31.01	Nicolea sp.	Nicolea sp 1
M3_K-31.02	Nicolea sp.	Nicolia sp 1
M3_K-31.03	Nicolea sp.	Nicolia sp 2
M16_MS-1.1	Obelia sp.	Obelia possibly geniculata
M16_MS-1.4	Obelia sp.	Botrylloides violaceus; contaminant?
M3_S-13.06	Orchomene sp.	Orchomene sp provisionally accept, closest Genbank record is Ichnopus
M3_S-13.07	Orchomene sp.	Orchomene sp provisionally accept, closest Genbank record is Ichnopus
M3_S-13.10	Orchomene sp.	Orchomene sp provisionally accept, closest Genbank record is Ichnopus
M16_M-33	Pacificincola perforata	Pacificincola perforata provisionally accept
M3_S-60.01	Paradexamine sp.	Paradexamine sp. Provisionally accept
M3_S-19.01	Paranthura japonica	Paranthura provisionally accept
M3_S-19.03	Paranthura japonica	Paranthura provisionally accept
M3_S-19.04	Paranthura japonica	Paranthura provisionally accept
M3_K-23.06	Parapleustes sp.	Parapleustes sp provisionally accept, closest Genbank record is Parapleustes bicuspis
M3_K-23.07	Parapleustes sp.	Parapleustes sp provisionally accept, closest Genbank record is Parapleustes bicuspis
M3_K-23.09	Parapleustes sp.	Parapleustes sp provisionally accept, closest Genbank record is Parapleustes bicuspis
M3_K-27.01	Perforatus perforatus	Perforatus perforatus confirmed
M3_K-27.02	Perforatus perforatus	Perforatus perforatus confirmed
M3_K-27.03	Perforatus perforatus	Perforatus perforatus confirmed
M3_M-45.01	Perophora japonica	Perophora japonica confirmed
M3_M-45.02	Perophora japonica	Perophora japonica confirmed
M16_M-28.1	Perophora sp.	Perophora japonica confirmed
M3_S-6.02	Platynereis bicanaliculata	Neridae; is not near other Platynereis; closest Genbank record is Nereis heterocirrata
M3_S-6.04	Platynereis bicanaliculata	Neridae; is not near other Platynereis; closest Genbank record is Nereis heterocirrata
M3_K-24.06	Podocerus sp.	Podocerus sp provisionally accept
M3_M-21.01	Polycheria sp.	Polycheria provisionally accept
M3_S-54.01	Procehalothrix sp.	Cephalothrix simula
M3_S-54.03	Procehalothrix sp.	Cephalothrix simula
M3_S-5.01	Sabella sp.	Parasabella sp.
M3_S-5.03	Sabella sp.	Parasabella sp.
M3_S-5.04	Sabella sp.	Parasabella sp.
M3_M-3.05	Sakuraeolis sp.	Eubranchus
M3_M-36.02	Sakuraeolis sp.	Eubranchus
M3_M-36.03	Sakuraeolis sp.	Eubranchus

M3_S-45.06	Stenothoe sp. 1	Stenothoe provisionally accept
M3_S-45.07-11	Stenothoe sp. 1	Stenothoe provisionally accept
M3_M-5.02-06	Stenothoe sp. 2	Stenothoe sp 2; this is different from Stenothoe sp 1 herein
M3_S-50.01	Styela canopus	Styela, but not canopus or clava.
M3_S-50.02	Styela canopus	Styela, but not canopus or clava.
M3_S-50.03	Styela canopus	Styela, but not canopus or clava.
M3_M-50.01	Styela sp.	Styela not canopus, same as other Styela in voucher set
M3_M-50.02-06	Styela sp.	Styela clava, but based on a short read
M16_M-29.1	Styelidae gen. sp.	Botrylloides violaceus
M16_M-29.2	Styelidae gen. sp.	Styela clava
M3_M-10.01	Syllis sp.	Syllis vittata
M3_M-10.02	Syllis sp.	Syllis vittata
M3_M-10.03	Syllis sp.	Syllis vittata
M3_M-47.01	Synidotea hikigawaensis	Synidotea hikigawaensis provisionally accept
M16_MS-36.3	Tetrastemma nigrifrons	Quasitetrastramma stimpsoni
M16_MS-36.4	Tetrastemma nigrifrons	Quasitetrastramma stimpsoni
M16_MS-36.5	Tetrastemma nigrifrons	Quasitetrastramma stimpsoni
M1_S-31.01	Theora fragilis	Theora fragilis provisionally accepted
M3_S-35.01	Tricellaria inopinata	Tricellaria occidentalis; possible Genbank ambiguity
M3_S-35.02	Tricellaria inopinata	Tricellaria occidentalis; possible Genbank ambiguity
M3_S-35.03	Tricellaria inopinata	Tricellaria occidentalis; possible Genbank ambiguity
M3_M-31.01	Tricellaria inopinata	Tricellaria occidentalis; possible Genbank ambiguity
M3_K-50.02	Tricellaria inopinata	Tricellaria occidentalis; possible Genbank ambiguity
M16_M-45	Vilasina decorata	Vilasina decorata provisionally accept
M3_K-18.02	Watersipora cucullata	Watersipora subtorquata, in conventional use as the widespread invasive
M3_K-18.03	Watersipora cucullata	Watersipora subtorquata, in conventional use as the widespread invasive
M3_K-18.04	Watersipora cucullata	Watersipora subtorquata, in conventional use as the widespread invasive
M16_MS-30	Watersipora subatra	Watersipora subtorquata, in conventional use as the widespread invasive
M3_M-22.01-04	Zeuxo sp.	Zeuxo sp.