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Genetic identification of macroalgal species on Japanese tsunami marine debris and genetic comparisons with their wild populations[☆]

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ABSTRACT

Since 2012 a huge amount of marine debris caused by the 2011 Great East Japan Earthquake and Tsunami has been arriving on Northeastern Pacific shores. Often healthy macroalgae were attached to them, which may become introduced to the Northwestern Pacific coasts and disturb their ecosystems. In order to elucidate the diversity of those macroalgae, and to establish a basis for detecting their new introduction to Northwestern Pacific coasts, we have examined their species diversity by morphology and genetic identifications. We have obtained gene sequences for 205 specimens, and identified 49 species as Japanese Tsunami Marine Debris (JTMD) macroalgae. Most of them are known to be distributed in Japan and showed identical or very closely related genetic types to those of Tohoku, and confirmed to be originated from the area. Several species such as *Ceramium sungminbooi*, *Ectocarpus croaniorum*, *Polysiphonia koreana*, etc. have not been reported from Japan, but this is explained by the shortage of taxonomic information.

1. Introduction

Due to the globalization of ship transport systems, fisheries, and aquaculture activities, anthropogenic trans-oceanic introductions of marine organisms have accelerated and pose a threat to coastal ecosystems. Globally, more than two hundred seaweed species are believed to have been spread outside their native ranges by human-mediated means (Curiel et al., 1998; Boudouresque and Verlaque, 2002; Smith et al., 2002; Streftaris et al., 2005; Uwai et al., 2006; Hanyuda et al., 2016). However, trans-oceanic introductions by marine debris such as logs and floats have not been regarded as a significant threat, because the survival rates of the organisms attached on those items were estimated to be relatively low, and the amount of debris crossing over the oceans is relatively small.

The 2011 Great East Japan Earthquake and huge tsunami that followed caused massive damage to coastal areas in the Tohoku region. The Government of Japan estimated that 5 million tons of debris was carried into the Pacific by the massive tsunami wave (MoE, 2012). It is estimated that 70% of the debris immediately sank close to shore, leaving 1.5 million tons floating in the North Pacific with the potential to arrive on North American and Hawaiian coastlines. Since 2012 a huge amount of marine debris, which we call 'Japanese Tsunami Marine Debris' (JTMD), has been arriving on Northeastern Pacific shores. This included two large concrete floating docks, originally from

Misawa Fishing Port, Aomori Pref., Japan, that were found on beaches in Oregon (June 2012) and Washington (January 2013) with a number of non-native species attached, and > 200 small boats, many of which have been confirmed as lost during the tsunami. Often healthy macroalgae were attached to them, which may become introduced to the Northwestern Pacific coasts and disturb their ecosystems. There is a significant threat of new introductions of marine benthic organisms, not only because of the very large amount of debris, but also because such a transport across the ocean of healthy macroalgal communities including herbivores and carnivores, has not been experienced before.

Because of the great morphological plasticity and rather limited morphological features of many macroalgae, species level taxonomy, especially of minute species, is often difficult. In addition to such taxonomic difficulty, additional factors have made the precise taxonomy (species level identification) of JTMD macroalgal species difficult. Although we have been able to sample and process algal material from some newly arrived debris items, more often than not the items remain on the beach for several days before they are discovered and sampled for our study. Therefore, in order to elucidate the diversity of the macroalgae associated with JTMD, and to establish a basis for detecting and characterizing their new introduction to Northwestern Pacific coasts, we have examined the species diversity of the JTMD macroalgae both by morphology and by using genetic markers (also see Hansen et al., in this issue).

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The origins of a major part of the larger debris such as floating docks exceeding 10 m long (i.e., the Misawa floating docks) and intact boats were relatively easily identified, because they had markings indicating the installation location or boat names. However, it was relatively difficult to identify the origin of the smaller floating docks shorter than several meters, or derelict unmarked boats. These could be of various origins from different regions, and not JTMD. For example, the derelict boat spotted off Seal Rock, Oregon, in April 2015 was seriously damaged and the origin could not be identified by boat name or other markings. This boat was colonized by a number of macroalgae, and also many animals including live yellowtail jacks and striped beakfish. Yellowtail jack is a warm water species on Japanese coasts, and there was a possibility that the boat originated from some warmer water region, and not from the tsunami. Therefore, genetic data of JTMD macroalgae were also used for elucidating the origin of some debris.

2. Materials and methods

2.1. Identification of JTMD macroalgae based on genetic markers

Macroalgal specimens sampled from JTMD were first sent to Gayle Hansen at Hatfield Marine Science Center, OR, USA. They were primarily identified based on morphology and a portion of the specimens were quickly dried and preserved in silica-gel, and sent to the Kobe University laboratory (Table S1). Genomic DNA was extracted from dried algal tissue using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) or QuickExtract Plant DNA Extraction Solution (Epicentre, Madison, WI, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplifications of the chloroplast *psbC*, *rbcL*, *atpH-atpI* region, mitochondrial *cox1*, *cox3*, *trnA-trnN* region, and nuclear 18S rDNA, 28S rDNA, rDNA ITS region were carried out using the KOD FX (ToYoBo, Osaka, Japan) PCR enzyme and the TaKaRa PCR Thermal Cycler Dice (Takara Bio, Kusatsu, Japan) (Table S2). After PEG purification (Lis, 1980), PCR products were sequenced using the CE DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) and the CEQ8000 DNA analysis system (Beckman Coulter) according to the manufacturer's instructions, or were sequenced by a DNA sequencing service (FASMAC, Atsugi, Japan). In order to identify species names, the determined DNA sequences were compared with sequences deposited in DNA databases using the Basic Local Alignment Search Tool (BLAST) from the NCBI GenBank database.

2.2. Genetic comparisons of JTMD macroalgal species with their wild populations

Genomic DNA was extracted from fresh or dried algal tissue of field-collected specimens from Japan, the USA, and the other regions, and from unialgal culture strains housed in the Kobe University Macroalgal Culture Collection (KU-MACC) using the above methods (Tables S3–S14).

The molecular phylogenetic analyses for several datasets (1. *Ulva* species, 2. *Ectocarpus* and *Kuckuckia* species, 3. *Scytosiphon lomentaria*) were performed using published and newly determined sequence data (Tables S3, S4 and S5). Alignments were prepared using the program MAFFT v.6 (Kato and Toh, 2008) and then manually adjusted prior to phylogenetic analyses. For ML analysis, we used RAxML GUI v.1.31 (Silvestro and Michalak, 2012), conducting 10,000 Rapid Bootstrap searches followed by an ML search, with the GTR + G model for each codon position of each organelle gene or for each position of each nuclear region. To examine genetic relationships among the haplotypes or genotypes, statistical parsimony networks were created using TCS v.1.21 (Clement et al., 2000) based on the DNA sequences of chloroplast and/or mitochondrial haplotypes.

3. Results

3.1. Identification of JTMD macroalgae based on genetic markers

Table S1 shows the taxonomic list of macroalgal specimens collected from JTMD and identified based on gene DNA sequences (selected depending on taxa: 18S rDNA, 28S rDNA, rDNA ITS, *rbcL*, *rbcL-S*, *psbC*, *atpH-I*, *cox1*, *cox3*, *cob-cox3*, *trnA-trnN*) and published sequence data of the corresponding taxa. Based on the genetic analyses, we have identified the following species.

Ulvophyceae (Chlorophyta)

Blidingia minima, *Bryopsis plumosa*, *Cladophora albida*, *Cladophora vagabunda*, *Cladophora* cf. *parriaudii*, *Codium fragile*, *Ulva compressa*, *U. lactuca*, *U. linza*, *U. pertusa* (= *U. australis*?), *U. prolifera*, *U. simplex*, *Ulva* sp. (*Ulva* sp. 4 in Ogawa et al., 2013).

Phaeophyceae (Heterokontophyta)

Alaria crassifolia, *Anelopus japonicus*, *Costaria costata*, *Desmarestia japonica*, *Ectocarpus crouaniorum*, *Ectocarpus* spp., *Feldmannia irregularis*, *F. mitchelliae*, *Kuckuckia spinosa*, *Mutimo cylindricus*, *Petalonia fascia*, *P. zosterifolia*, *Petroderma maculiforme*, *Punctaria latifolia*, *Saccharina japonica*, *Scytosiphon gracilis*, *S. lomentaria*, *Scytosiphon* sp., *Sphacelaria rigidula*, *Undaria pinnatifida*.

Rhodophyta

Bangia cf. *fuscopurpurea*, *Ceramium sungminbooi*, *Chondrus giganteus*, *C. yendoi*, *Colaconema* spp., *Grateloupia livida*, *G. turuturu*, *Neodilsea yendoana*, *Palmaria palmata* auct. japon., *Polysiphonia koreana*, *P. morrowii*, *Polysiphonia* sp., *P. yezoensis*, *Pyropia* sp., *Schizymenia dubyi*, *Tsunamiya transpacificca*.

3.2. Molecular phylogeny of dominant JTMD macroalgal species

3.2.1. *Ulva* spp.

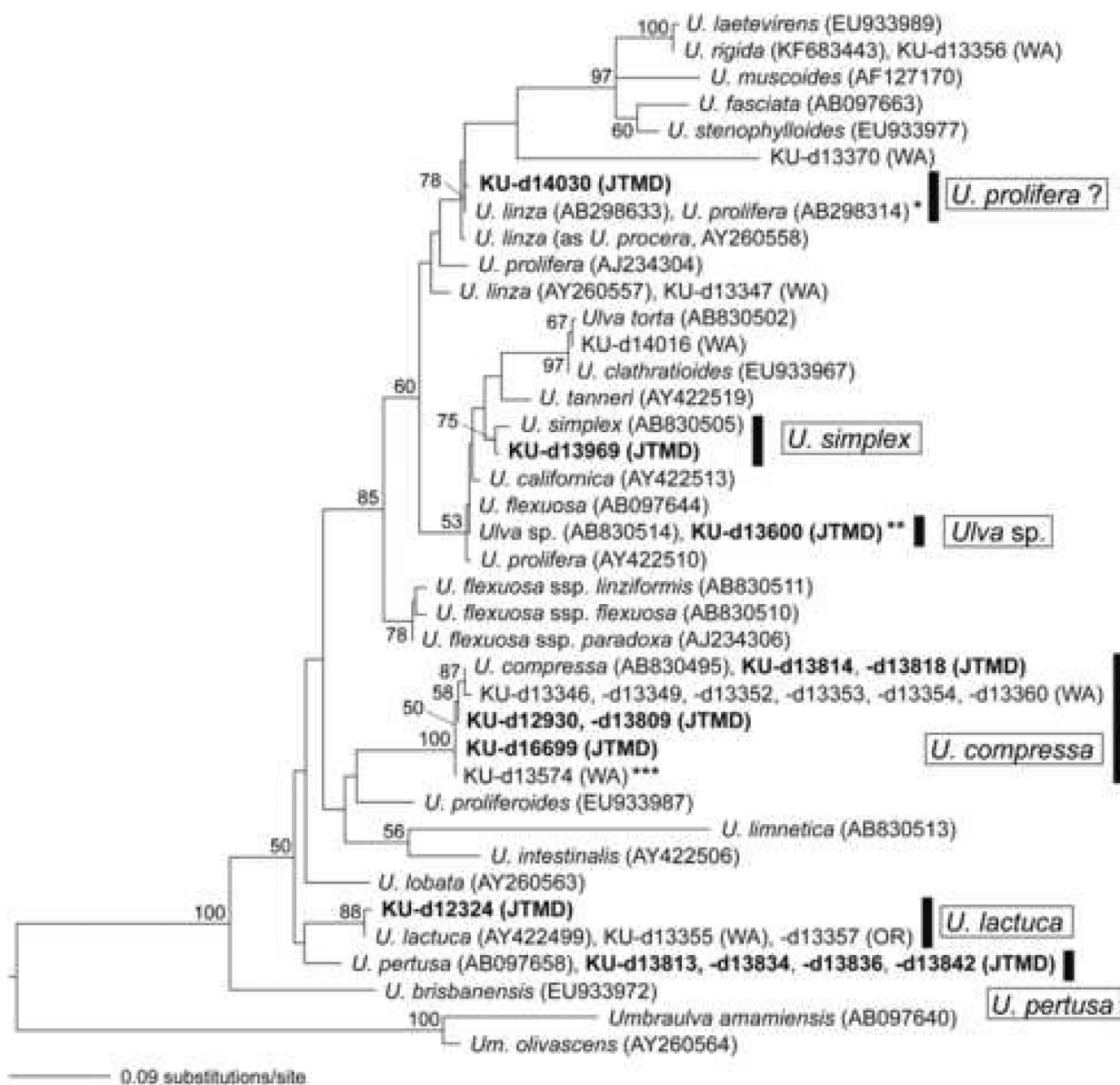
Molecular phylogeny of *Ulva* species indicated that at least 6 lineages, perhaps corresponding to independent species, were attached to JTMD (Fig. 1, Table S3). They included *Ulva compressa*, *U. lactuca*, *U. linza*, *U. pertusa* (= *U. australis*?), *U. prolifera*, *U. simplex* and *U. sp.* (= *U. sp.4* in Ogawa et al., 2013). Among the taxa, *U. simplex* and *U. sp.4* have not been reported from Northeastern Pacific coasts. In contrast, *U. compressa*, *U. lactuca* and *U. prolifera* have broad distributional ranges on Atlantic and Pacific coasts, and are distributed in both Japan and the NE Pacific (Pacific Coast of Northeastern Asian).

3.2.2. *Ectocarpus* spp.

Molecular phylogeny of the JTMD specimens of filamentous brown algae morphologically referable to *Ectocarpus* species (and *Kuckuckia*) indicated that at least 4 lineages corresponding to independent species of *Ectocarpus* and one lineage corresponding to *Kuckuckia* were found on JTMD (Fig. S1, Table S4). Among the lineages, clade A corresponded to *Ectocarpus crouaniorum*, however, the species level taxonomy of *Ectocarpus* is still not clarified and it is difficult assign appropriate species name to the other clades.

3.2.3. *Scytosiphon lomentaria*

Scytosiphon lomentaria is a common ephemeral brown alga growing on diverse substrates including artificial structures in the intertidal zone. The species also has broad distributional ranges in temperate and cold water seas. Therefore, in order to clarify the origin of the *S. lomentaria* individuals collected from JTMD, we have compared the *cox3* gene sequences of the specimens from JTMD and from Japan and North American coasts. We also added some specimens collected worldwide



*KU-d13970, -d13592, -d13597, -d13598 (JTMD), KU-d13348 (WA), -d13350 (OR), -d13358 (WA), -d13359 (WA), -d13362 (WA), -d13364 (WA), -d13368 (WA) had identical sequence as AB298633.

**KU-d13351, -d13361, -d13365, -d13366, -d13367, -d13372 (WA) had identical sequence as AB830514.

***KU-d13832, -d13853, -d13854, -d13855, -d13862, -d13863 (JTMD) had identical sequence as KU-d13574.

Fig. 1. Molecular phylogeny of *Ulva* spp. Maximum likelihood tree based on the DNA sequences of nuclear rDNA ITS1, 5.8S, and ITS2 (488 bp). Numbers at nodes indicate bootstrap values in maximum likelihood analysis. Only bootstrap values > 50% are shown.

for comparisons in order to clarify the global biogeography of this species.

In the molecular phylogenetic analysis, JTMD *Scytosiphon* included two independent lineages (Fig. S2, Table S5). They were one genetically close to those from Hokkaido (Hanasaki and Setana) and the other genetically close to those from Europe (Naples, Italy and Sleat Head, Ireland). Specimens from Oregon and Washington belonged to three independent lineages, which were genetically close to those from various localities in Japan. This may suggest that intercontinental introductions of the species have frequently occurred and the original distributional pattern has been disturbed. Species level taxonomy of *Scytosiphon lomentaria* needs further examination, because it is possible that these lineages correspond to independent species, or some intra-species level taxa such as subspecies or variety.

3.3. Genetic comparisons of JTMD macroalgal species with their wild populations

3.3.1. *Ulva pertusa*

Ulva pertusa is one of the commonest intertidal green algae on temperate Japanese coasts. Because *Ulva australis* showed DNA sequences identical to *Ulva pertusa*, and *U. australis* has nomenclatural priority, it was suggested to synonymize *U. pertusa* to *U. australis* (Kirkendale et al., 2013). However, *Ulva pertusa* is widely introduced to Australia as a non-indigenous species, perhaps from Japan (Hanyuda et al., 2016). We consider that there remains some possibility that the *U. australis* in Kirkendale et al. (2013) was actually based on an introduced population of *U. pertusa* instead of native *U. australis*. A project to compare the type materials of *U. australis* with *U. pertusa* is in

progress, so that in the present manuscript we use *U. pertusa*.

Ulva pertusa is already introduced to Northeastern Pacific coasts from Oregon to California, so that even if new introductions of the species by JTMD occur to these coasts, the impact on the local ecosystems might not be significant. On the other hand, the derelict boat spotted off Seal Rock, Oregon bore a number of macroalgal species including *U. pertusa*, but their origin was uncertain. The haplotype (chloroplast *atp1-H* and mitochondrial *trnA-trnN* DNA sequence) of the *Ulva pertusa* specimen agreed with that of the specimens collected from two localities in Iwate Pref., Japan among the 51 haplotypes worldwide (Fig. S3, Table S6).

3.3.2. *Petalonia fascia*

Petalonia fascia is a common ephemeral brown alga growing on diverse substrates including artificial structures in the intertidal zone. This species has broad distributional ranges in temperate and cold water seas. Since the gametophytes (erect thalli) grow rather rapidly, it is possible they newly attached to the JTMD from local populations of the species after the arrival of the JTMD to NE Pacific coasts. If the JTMD becomes cast ashore or stays near the shore for some days, zooids (zoospores) from mature crustose sporophytes of the local population may also attach on JTMD, and become emergent within a couple of weeks. Therefore, in order to clarify the origin of the *P. fascia* individuals collected from JTMD, we compared the *cox3* gene sequences of the specimens from JTMD and from the coasts of Japan and North America. We also included some specimens collected worldwide for comparisons in order to clarify the global biogeography of the species.

In the spanning network tree analyses, the haplotypes based on mitochondrial *cox3* gene suggested the occurrence of two genetic groups: group A comprised of populations in Honshu, the Southern Hemisphere, and a part of Europe; and group B comprised of populations in Hokkaido, Pacific and Atlantic America, and Europe (Fig. 2, Table S7). The haplotypes H1–H3 were somewhat distant from H4–H8, but sometimes found mixed in Honshu. In contrast, the haplotypes H9–H15 were rather close. The haplotypes of the specimens on JTMD (15 individuals) were all of group A (H2, H5, H6 and H8), whereas field-collected specimens from Washington, Oregon and California were all of group B. Therefore, all of the JTMD specimens of *P. fascia* were concluded to have originated from Japan. Although H1, H2 and H5 were dominant in the population in Tohoku from Aomori to Fukushima, half of the JTMD specimens showed H6 and the rest were H2, H5 and H8. This may be explained by the small number of specimens examined of the specimens collected from the Tohoku area.

3.3.3. *Analipus japonicus*

Analipus japonicus is a common brown alga growing on intertidal rocks or benthic animals such as barnacles and mussels of cold temperate to colder seas in the North Pacific. This species was only found on the Misawa floating dock as JTMD. The species is distributed on both sides of the North Pacific, from the Tohoku region to Hokkaido in Japan and from Alaska to California on American coasts. Genetic comparison using *cox3* DNA sequences (Fig. S4, Table S8) revealed that North American (H6) and Japanese populations (H1–H5) are genetically relatively distant, although Japanese populations show relatively high genetic divergence. JTMD *Analipus* showed H2. H1–H3 and H4/H5 were separated by 6–8 bp but they were collected from adjacent localities. This genetic distance was similar to the that between H4/H5 and H6.

3.3.4. *Mutimo cylindricus*

Mutimo cylindricus is an annual brown alga originally distributed in Northeast Asia. It was suggested to be introduced to Santa Catarina Island, California (Kogishi et al., 2010 as *Cutleria cylindrica*, later transferred to *Mutimo* in Kawai et al., 2012). The California population was suggested to originate from a parthenogenetic population in Tohoku, because of the haplotype similarity, and also by a life-history

feature: The California population was dominated by female gametophytes, and the occurrence of asexual gametophytes reproducing by parthenogenetic reproduction was reported from rather limited areas in Japan in Tohoku (haplotype K). *Mutimo cylindricus* found on JTMD was shown to have a distinct genetic type (haplotype X), and is likely a sexual lineage since parthenogenetic populations was only reported from limited localities in Japan (Kitayama et al., 1992) (Fig. S5, Table S9). Therefore, although *M. cylindricus* is already distributed in California, if the JTMD individuals become introduced to the Northeastern Pacific coasts, they may show a different propagation pattern from the California population, and may cause serious impacts to the local ecosystem.

3.3.5. *Costaria costata*

Costaria costata is an annual kelp species, only found on the Misawa floating dock as JTMD species. This species is distributed on both sides of the North Pacific, from Hokkaido to Tohoku in Japan (and Pacific Russia), and from Alaska to California on American coasts. Genetic comparison using *cox3* DNA sequences (Fig. S6, Table S10) indicated that Japanese populations showed three haplotypes (H1 – H3) and North American populations included two haplotypes (H4 and H5). JTMD *Costaria* was the H2 haplotype, and was genetically distinct from Northeastern Pacific populations, although the genetic distance was relatively small (2 bp).

3.3.6. *Saccharina japonica*

Saccharina japonica is a biannual kelp species distributed on Pacific coasts of Hokkaido and Tohoku, and only found on the Misawa floating dock as JTMD species. *Saccharina* species are important ecological elements of cold temperate coasts, and since they are genetically rather closely related and easily form hybrids (Funano, 1978, 1980; tom Dieck, 1992), there is concern about genetic contamination of native populations if the species becomes introduced. The genetic analysis using *cox3* DNA sequences (Fig. S7, Table S11) indicated that JTMD specimens included two haplotypes (H1, H2), although we have identified only H1 in our field collections. This is explained by the very limited number of field specimens examined in the present analyses.

3.3.7. *Undaria pinnatifida*

Undaria pinnatifida was found only on the Misawa floating dock stranded on the Oregon coast. The species is an annual kelp constituting important ecological element of temperate seas in Japan. The original distributional range of the species is in the Northwestern Pacific (Northeast Asia), but has been introduced to wide ranges both in the Northern and Southern Hemispheres. On Northeastern Pacific coasts, although the species has been introduced to California and Pacific Mexico, it has not spread north to Oregon. Therefore, there is concern about new introductions of non-indigenous species if *U. pinnatifida* became introduced, because it often causes considerable disturbance to local ecosystems and fisheries. In the genetic analysis using *cox3* DNA sequences (Fig. S8, Table S12), the specimen collected from JTMD showed haplotype B, and the same haplotype was found in the collection from the reconstructed floating dock at the Misawa Fishing Port. This haplotype was genetically close but independent from those of the introduced populations in Los Angeles, California (haplotype J) and Baja California, Mexico (haplotype A).

3.3.8. *Grateloupia turuturu*

Grateloupia turuturu is an annual red alga with branched membranous thalli. The original distributional range of this species is Northeast Asia, but has been introduced to various areas in Europe, Northwestern and Northeastern American coasts, etc. In the Northwestern American coasts, it has been reported from California and Baja California, Mexico, but it has not been reported from Oregon. Based on the *rbcL* DNA sequence, the specimen collected from JTMD had the same haplotype as that reported from Baja California (Fig. S9, Table S13). However,

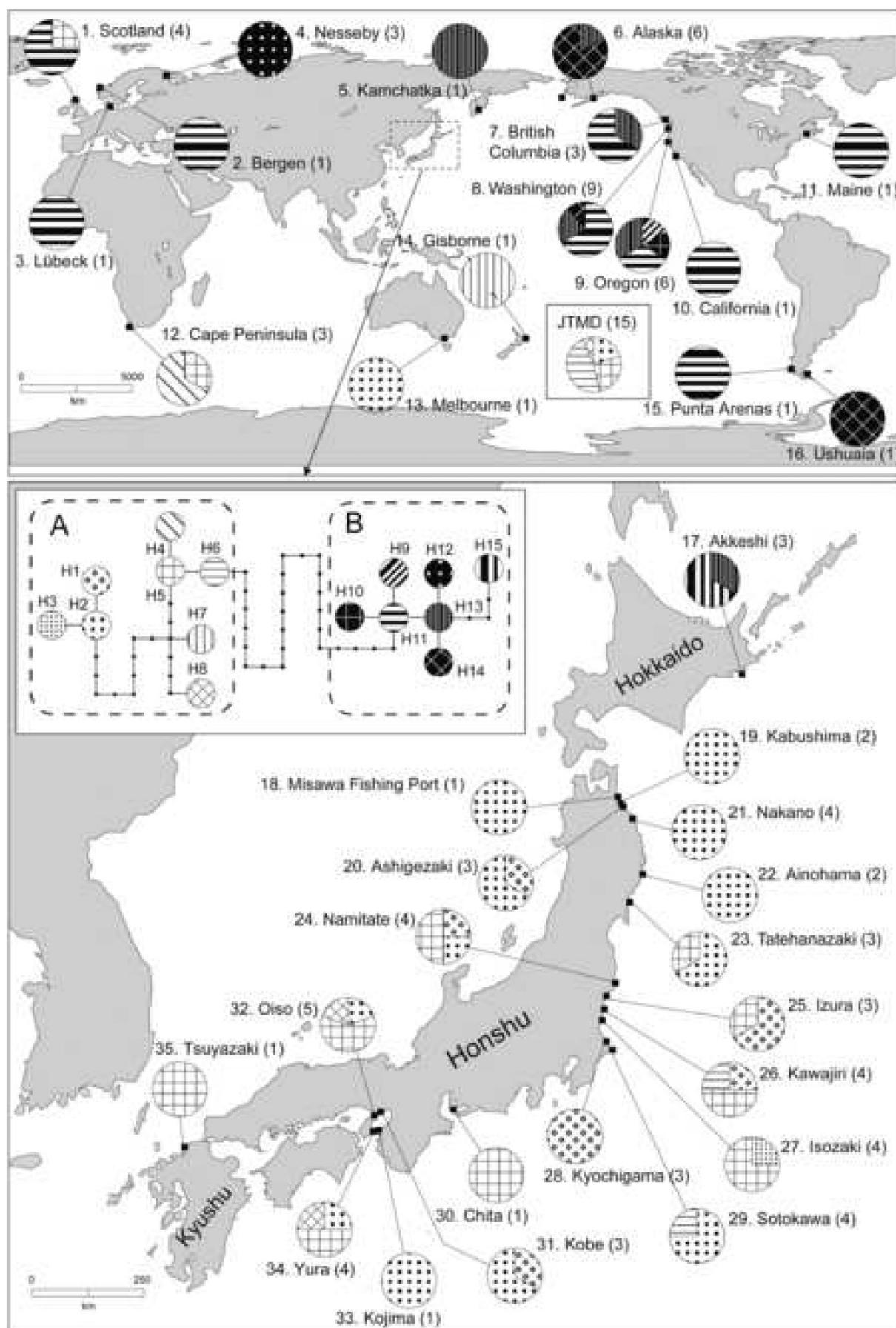


Fig. 2. Geographical distribution of haplotypes in *Petalonia fasciá*. Spanning network tree based on the *cox3* DNA sequence (718 bp).

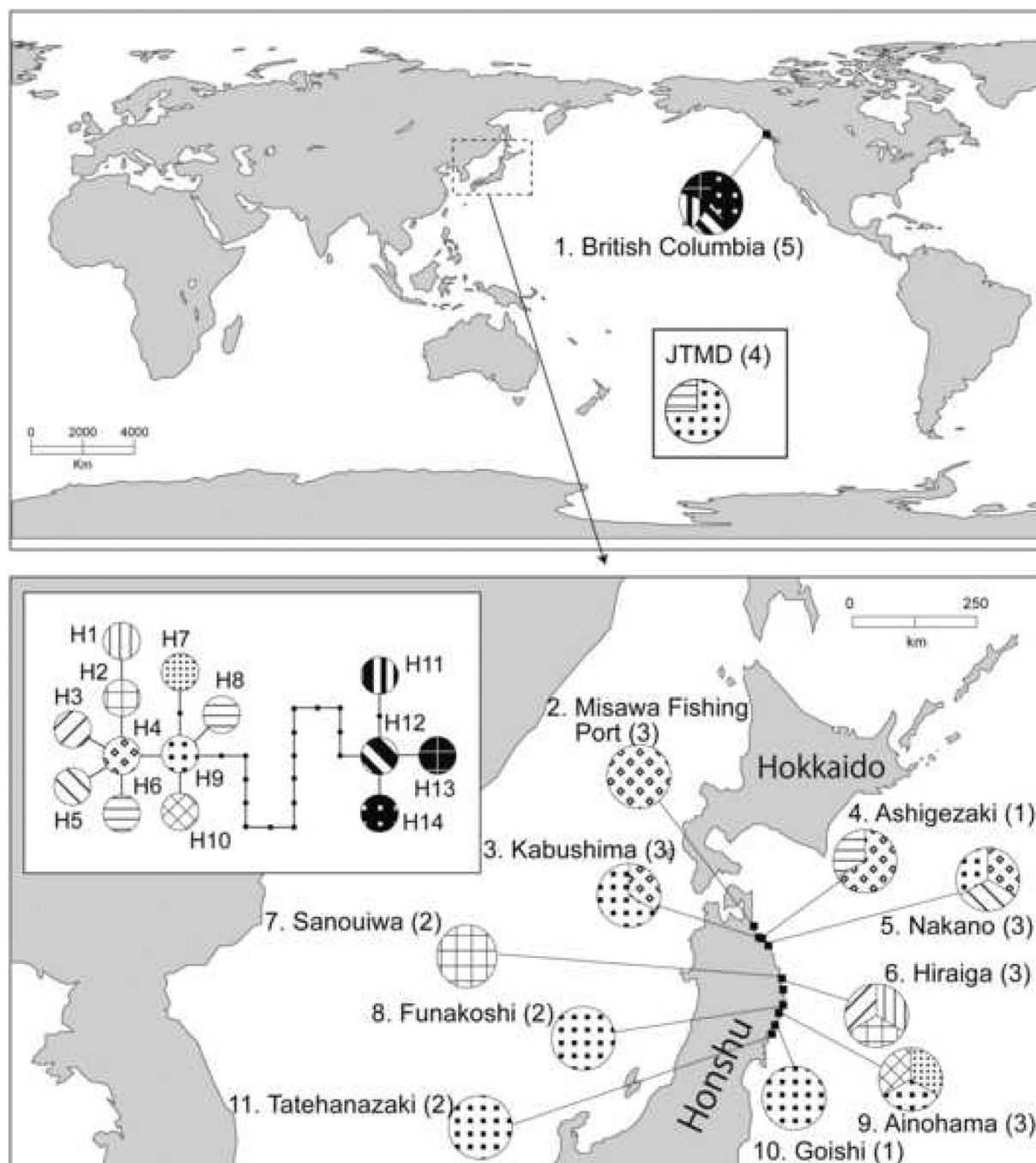


Fig. 3. Geographical distribution of haplotypes in *Palmaria palmata* auct. japon. (Japan and JTMD) and *P. mollis* (Canada). Spanning network tree based on the *cox1* DNA sequence (618 bp).

resolution of this gene region is considered to be rather low, because it is a conserved region. Therefore, it is necessary to use another gene region of faster mutation rate for distinguishing the JTMD specimen from existing California/Baja California populations.

3.3.9. *Palmaria palmata* (auct. japon.)

Palmaria palmata (auct. japon.) was collected on the Misawa floating dock. *Palmaria palmata* is an annual foliose red alga distributed throughout wide ranges of colder seas, however, there remain taxonomic problems in the species level taxonomy. *P. palmata* (auct. japon.) may be conspecific with *Palmaria mollis* distributed on the Northwestern coast of America, and is considered to constitute an important ecological element due to its relatively large size and

abundance. Comparisons of *cox1* gene haplotypes of Japanese and JTMD derived specimens suggested that Japanese *P. palmata* collected on the tsunami debris agreed with the specimens from Northern Tohoku, and were genetically rather distant by about 20 bp in *cox1* sequences from a local population of *P. mollis* in British Columbia, Canada (Fig. 3, Table S14). If Japanese *P. palmata* and *P. mollis* are independent species, there is concern about a new introduction of a non-indigenous species, and if they are conspecific, there is concern about genetic contamination of native populations. Even if they are concluded to be independent species, considering their close genetic relationship there is still possibility that they could form hybrids and cause genetic contamination by introgression. *P. mollis* is used for food (as 'dulse') and sold commercially. Although the amount sold is not

great in North America, it is widely consumed in Northern Europe and on NW American coasts, so that its introduction could result in economic damage in addition to the risk of disturbance to local ecosystems.

4. Discussion

4.1. Identification of JTMD macroalgae based on genetic markers

In the present study we have obtained gene sequences useful for taxonomy for 203 specimens, and we have identified 49 species as JTMD macroalgae. The species level taxonomy of about 20 taxa (e.g., *Chondrus giganteus*, *Scytosiphon lomentaria*, *Ulva compressa*) based on morphology were not supported by the genetic identifications, therefore, we have re-examined the primary identification and corrected the names if necessary.

Genetic data were also used to confirm that macroalgal species sampled from JTMD originated from Japan, and not by secondary attachment of local macroalgae on NE Pacific coasts after the arrival of JTMD on the coasts and before their landing. Some ephemeral taxa such as filamentous brown algae (e.g. ecotocarpoids) and green algae (ulvoids) may be able to settle on JTMD and grow on them rather rapidly, so genetic examination was considered to be important. In addition, some JTMD species are known to be distributed on both sides of the Pacific by natural distribution or by anthropogenic introductions before the Great East Japan Earthquake. Therefore, it was necessary to compare the three entities (i.e., Japanese natural populations, JTMD macroalgae, and North American natural populations) by appropriate genetic markers.

Most of the JTMD macroalgal specimens identified using genetic markers are also known to be distributed in Japan and showed identical or very closely related genetic types to those collected in the Tohoku region. Therefore, we have concluded that they have attached on the substrates (JTMD) in Tohoku, and not in the American coasts after arriving to the area. In the case of *Ulva pertusa*, based on the genetic type of the specimen collected from an unidentified derelict boat cast ashore on the Oregon coast in April 2015 (sample code 230/KU-d13834 and 233/KU-d13836), we could identify that the boat originated from the Tohoku region.

Several species collected from JTMD have not been reported from Japan: *Ectocarpus crouaniorum*, *Polysiphonia koreana*, *Tsunamia transpacific*, etc. (Yoshida et al., 2015). However, species level taxonomy of Japanese *Ectocarpus* species is not well-studied. *Polysiphonia koreana* was relatively recently described from Korea (Bustamante et al., 2015), and it is likely that the species is also distributed in Japan, considering the geographical proximity and high similarity between the macroalgal floras of Korea and Japan. *Tsunamia transpacific* was described based on the JTMD collections (West et al., 2016), and has not been found in Japan, but considering the inconspicuous habit of the taxon, it is likely that the species is native in Japan. Therefore, in spite of the lack of the distributional information of these taxa in Japan, we consider them to have originated from Japan.

Our genetic analyses indicated that most JTMD macroalgae had haplotypes identical or very closely related to the populations in the Tohoku region, so they were confirmed to have originated from Japan, and not from secondary settlement. Among the JTMD taxa examined, some species are reported to be distributed on both NW Pacific coast (Western Coast of North America) and NE Pacific coast, and therefore their new introduction to the Northwestern American coast may not be regarded as a species level invasion. However, our genetic comparisons indicated that they are genetically distinct and may cause genetic contamination, e.g., *Analipus japonicus*, *Petalonia fascia*, *Scytosiphon lomentaria* and *Costaria costata*. Some species are already introduced to Northeastern American coasts by relatively recent anthropogenic

introductions (e.g. *Mutimo cylindricus*, *Undaria pinnatifida* and *Grateloupia turuturu*), but have not spread north to the coast of Oregon, and have different haplotypes. Therefore, new introductions will still pose risks of accelerating the dispersal of non-indigenous species and enriching the genetic divergence of the introduced populations, further increasing their competitiveness in local populations.

Genetic analyses have revealed the species diversity of the JTMD macroalgae in taxonomically difficult taxa such as *Ulva* spp. and *Ectocarpus* spp. Although the species level taxonomy of *Ectocarpus* is still not fully resolved, based on existing genetic data we consider that there are about a dozen of species in the genus. The fact that at least 7 taxa (corresponding to more than half of all taxa worldwide) have been found from JTMD suggest that their natural distributional pattern can be considerably disturbed by anthropogenic floating structures.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marpolbul.2017.06.053>.

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