

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

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(Kobe University Research Center for Inland Seas)

1. PROJECT INFORMATION

Title	Marine Algae arriving on JTMD (Japanese Tsunami Marine Debris) and their invasion threat to the Northwestern Pacific coasts
Award period	(August 1, 2015–) April 1, 2016 – March 31, 2017
Amount of funding	1,119,327 yen (Year 1); 1,724,220 yen (Year 2); 4,087,943 yen (Year 3); 1,272,300 yen (Year 3 supplementary for database)
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2. YEAR 3 PROGRESS SUMMARY

a. Describe progress.

H. Kawai and T. Hanyuda made field collections of macroalgae on the Pacific coast of Tohoku (several localities in Iwate and Miyagi Prefectures from 20th to 22nd July) for the genetic analyses and field guide. We collected ca. 50 species of macroalgae from natural habitats.

H. Kawai made field surveys at Kodiak, Sitka (AK) and Vancouver I. (BC) for detecting new introductions of marine macroalgae that originated from JTMD: Kodiak and Sitka, Alaska from 10 July to 17 July, 2016 with S. Lindstrom and H. Maki, and Vancouver Island, British Columbia, Canada from 16 September to 21 September, 2016 with S. Lindstrom for detecting new introductions of macroalgae originated from JTMD, and for collections from native macroalgae populations. H. Kawai plan to visit Grays Harbor in March 2017 for detecting new introductions at the selected long term monitoring site in Westport.

We have genetically analyzed the specimens on JTMD, and compared them with those from natural habitats in Tohoku collected in 2015 and 2016, as well as NW America (mostly Washington and Oregon coast, sent from Gayle Hansen). For the genetic study, we used the following gene DNA sequences depending on taxa: chloroplast *psbC*, *rbcL*, *atpH-atpI* region, mitochondrial *cox1*, *cox3*, *cob-cox3* region, and nuclear 18S rDNA, rDNA ITS region and 28S rDNA.

The taxa we have examined are as follow.

Green algae: *Ulva* spp. (*U. compressa*, *U. lactuca*, *U. linza*, etc.), *Blidingia* spp.

Brown algae: *Feldmannia mitchelliae*, *Ectocarpus* spp., *Kuckuckia* sp., *Desmarestia japonica*, *Desmarestia* sp. *D. viridis*, *Petalonia* spp. (*P. fascia*, *P. zosterifolia*), *Saccharina japonica*, *Scytosiphon* spp. (*S. lomentaria*, *S. gracilis*).

Red algae: *Chondrus* spp. (*C. giganteus*, *C. yendoi*), *Grateloupia* spp. (*G. livida*, *G. turuturu*, etc.), *Palmaria palmate* and *Palmaria* cf. *mollis*.

Based on the results, we have reexamined the species level identifications based on morphology and have updated the list of taxa.

In the genetic study, we have analyzed the molecular phylogenetic and geographic distributions of representative haplotypes (genetic types) of the following taxa: *Ulva* spp., *Ulva lactuca*, *Ulva pertusa*, *Blidingia* spp. (Ulvophyceae); *Analipus japonicus*, *Costaria costata*, *Feldmannia mitchelliae*, *Mutimo cylindricus*, *Petalonia fascia*, *Desmarestia* spp. *Ectocarpus* spp., *Saccharina japonica*, *Scytosiphon lomentaria*, (Phaeophyceae); *Grateloupia turuturu*, *Palmaria palmata*/*P. mollis* (Rhodophyta)

We have presented oral talks at the PICES 2016 Annual Meeting (San Diego) and 9th Asia-Pacific Conference on Algal Biotechnology (Bangkok) along with a poster, and presented posters at the 11th EMECS Conference (St. Petersburg, Russia), and presented posters at the Marine Science Day 2016 (Newport, Oregon), under co-authorship with Gayle Hansen.

- Kawai, H., Hanyuda, T., Hansen, G. 2016. Species diversity and the threat of introduced macroalgal species arriving on Northwestern American shores via Japanese Tsunami Marine Debris (JTMD). 11th EMECS Conference, Azimut Hotel, St. Petersburg, Russia. 22-27 August, 2016. (Poster).
- Hanyuda, T., Hansen, G., Kawai, H. Genetic diversity and biogeography of the macroalgal species associated with the Japanese tsunami marine debris. 2016. PICES 2016 Annual Meeting. Omni San Diego Hotel, San Diego, CA, USA. 2-13 November, 2016. (Oral by Kawai, H.)
- Kawai, H., Hanyuda, T., Hansen, G. 2016. The threat of introduced macroalgal species arriving on Northwestern American shores associated with Japanese tsunami marine debris (JTMD). 9th Asia-Pacific Conference on Algal Biotechnology. Century Park Hotel, Bangkok, Thailand. 15-18 November, 2016. (Oral by Kawai, H.)
- Hansen, G., Hanyuda, T., Kawai, H. 2016. Marine algae carried across the North Pacific on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington, USA. Marine Sciences Day, Hatfield Marine Science Center. Newport, Oregon. 11 April, 2016. (Poster).
- Hansen, G., West, J.A., Hanyuda, T., Zuccarello, G.C. 2016. The Pink Crust, a new-to-science species on hard plastic debris. Marine Sciences Day, Hatfield Marine Science Center. Newport, Oregon. 11 April, 2016. (Poster).
- Hanyuda, T., Kawai, H., Hansen, G. 2016. Determining the source of the Seal Rock debris boat. Marine Sciences Day, Hatfield Marine Science Center. Newport, Oregon. 11 April, 2016. (Poster).

We have completed the macroalgal portion of the JTMD database, providing emendation and supplementary information to the draft database produced by Luran Liggan (UBC) for the PICES portion. Part of the work (red algae) was carried out by Dr. S. Kawaguchi (Prof. Emeritus, Kyushu Univ.) through an independent contract with PICES. We have completed a total of 40 species (6 of green algae, 14 of brown algae and 20 of red algae).

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

The condition of the JTMD samples were often not good enough for extracting DNA for reliable genetic works. However, considering the wide range of coasts that JTMD stranded and limited number of specialists who could assist the onsite samplings, it was rather difficult to improve this issue, and we believe we could get reasonable data from available resources.

3. ABSTRACT

In order to evaluate the invasion threat of JTME macroalgal species, we have undertaken the following research projects under collaboration with Gayle Hansen: 1) Identify JTMD macroalgae based on morphology and genetic markers, and compare them with native populations in Japan and Northeastern Pacific coasts; 2) Detect new algal invasions through visual searches for high profile JTMD species in anticipated habitats, and genetic screening for Japanese genetic types that could function as indicators of

JTMD macroalgal species recruitment; 3) Establish long term monitoring sites for detecting future introduction of JTMD macroalgal species, and provide a field guide to identify them. We did not find apparent JTMD macroalgal species during the survey either in natural habitat and artificial structures including floating piers in Alaska, British Columbia, Washington and Oregon. We have selected several localities as recommended long term monitoring sites for detecting new invasion of JTMD macroalgae.

4. PROJECT DESCRIPTION

a) Research Purpose

After being carried across the North Pacific on currents from Japan, marine debris items from the Great Tohoku Tsunami of 2011 (JTMD) are expected to arrive on the North American Pacific Northwest coasts (Alaska, Washington and Oregon) and Hawaii, bearing Japanese marine algae and benthic animals. The macroalgal species are often healthy and reproductive, and many can be expected to establish recruits to invade our shores.

In order to evaluate the invasion threat of these algal species, the following project is proposed: 1) Identify JTMD macroalgae based on morphology and genetic markers, and compare them with native populations in Japan and Northeastern Pacific coasts; 2) Detect new algal invasions through visual searches for high profile JTMD species in anticipated habitats, and genetic screening for Japanese genetic types that could function as indicators of JTMD macroalgal species recruitment; 3) Establish long term monitoring sites for detecting future introduction of JTMD macroalgal species, and provide a field guide to identify them. We will collaborate with and support Gayle Hansen's research activities as research partner; 4) Establish a database of biogeographical information, life histories and invasion histories, etc. for assessment of invasion risk in collaboration with PICES researchers.

b) Objectives

- 1) Identify JTMD macroalgal species collected from Washington and Oregon coasts and deposited in Gayle Hansen's laboratory, by morphological and genetic studies using appropriate gene markers (e.g., chloroplast *rbcL* and *rbcS*, mitochondrial *cox1* and *cox3*, and nuclear rDNA ITS) for identifying the macroalgae at species level. For reference, we use macroalgal specimens collected in Japan, especially from the Pacific coast of Tohoku.
- 2) For detecting new algal invasions, we will visit selected localities in Alaska, British Columbia, Washington and Oregon. At these locality we will collect targeted specimens (basically the taxa identified as JTMD macroalgae) for genetic comparisons with Japanese and North American local populations of these species.
- 3) Select suitable localities for detecting new introduction of JTMD macroalgae, and provide a field guide for identifying them.

c) Methods

Genetic analyses

Macroalgal specimens sampled from JTMD were first sent to Gayle Hansen at Hatfield Marine Science Center. They were primarily identified based on morphology and a part of the specimens quickly dried and preserved in silicagel were sent to Kobe University laboratory. Genomic DNA was extracted from fresh or silica gel-dried algal tissue of field-collected specimens and unialgal culture strains housed in the Kobe University Macroalgal Culture Collection (KU-MACC) 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*, *cob-cox3* region, and nuclear 18S

rDNA and its ITS region and 28S rDNA were carried out using the KOD FX (ToYoBo, Osaka, Japan) PCR enzyme and the TaKaRa PCR Thermal Cycler Dice (Takara Bio, Kusatsu, Japan). 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). The molecular phylogenetic analyses used published and newly determined sequence data. Alignments were prepared using the program MAFFT v.6 (Katoh 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 or mitochondrial haplotypes, or nuclear genotypes.

d) Results

Genetic identification of JTMD macroalgae

Marine macroalgae collected from JTMD were first identified based on morphology by Gayle Hansen in the laboratory. However, sometimes there are taxonomic difficulties due to damage to the specimens during the landing of the debris, sampling, and transportation to the laboratory. Furthermore, definitive species level identification can be impossible because of their unsuitable life history stages (juvenile or premature lacking reproductive structures) and poorly-defined morphological boundaries between related species. In addition, there is the possibility that some specimens sampled from the debris originated by settlement of propagules from native American populations before landing of the debris. In order to confirm the morphological identifications, and also to resolve these uncertainties, we have examined the JTMD macroalgae using selected genetic markers. For comparison, we also obtained genetic data from the Japanese and North American local (native) population of the species identified from JTMD. These genetic data will be used as baseline information for distinguishing species newly introduced by JTMD from native species.

Genetic identifications of the macroalgal specimens on tsunami debris and from natural habitats collected in Washington and Oregon coast, provided by Dr. Gayle Hansen, used the following gene DNA sequences: *psbC*, *rbcL*, *atpH-atpI* region, mitochondrial *cox1*, *cox3*, *cob-cox3* region, and nuclear 18S rDNA, rDNA ITS, 28S rDNA. We have obtained DNA sequence data from ca. 130 specimens out of ca. 130 JTMD and NW American specimens collected and sent to Kobe University.

List of specimens examined for genetic analyses are given as Appendix I.

Field study for detecting new introductions of JTMD macroalgae, and selection of observation sites for long term monitoring

In order to detect newly introduced macroalgal species on North American coasts, we have made field surveys (observations and collections) at selected localities in Alaska, British Columbia, Washington and Oregon. We concentrated on the macroalgal vegetation on floating artificial structures, such as floating docks, because it is known that they are preferred habitat for both intertidal and subtidal macroalgae, and new introduction of non-indigenous species are often first noticed on such habitats.

H. Kawai and H. Hanyuda visited several localities in Grays Harbor and Willapa Bay (WA) in 2015 with Gayle Hansen, and H. Kawai visited Kodiak, Sitka (AK) and Vancouver I. (BC) in 2016. Based on our observations, we concluded that the piers in Sitka, Alaska and Westport in Grays Harbor, Washington are suitable sites for long term monitoring considering their accessibility, relatively rich macroalgal species

diversity on the docks, and abundant arrival of JTMD to the area. We have made some surveys in natural habitat in Sitka and Vancouver Islands where a large amount of JTMD have been stranded, but because of dense populations of native macroalgal species, it was considered to be difficult to recognize non-indigenous species if the population is small. We did not find apparent JTMD macroalgal species during the survey either in natural habitat and artificial structures including floating piers. Therefore, non-indigenous Japanese macroalgal species found at these sites in the near future are likely caused by JTMD.

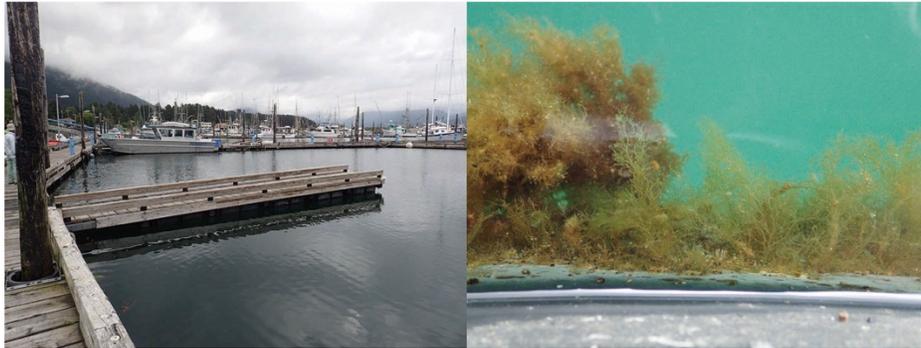


Fig. 1 Piers in Sitka, Alaska and macroalgal vegetation on the floating dock (recommended long term monitoring site).



Fig. 2 Piers at West Port, Grays Harbor, Washington and macroalgal vegetation on the floating docks (recommended long term monitoring site).

Molecular phylogeny and biogeographical analyses of representative taxa

1) Taxonomy of *Ulva* species

Molecular phylogenetic analyses of *Ulva* species of JTMD specimens based on nuclear ITS1-5.8S-ITS2 rDNA sequences revealed that at least 7 species were included: *U. compressa*, *U. flexosa*?, *U. lactuca*, *U. linza*, *U. pertusa/australis*, *U. prolifera* and *U. simplex*. Among them, *U. simplex* has not been reported from Northwestern American coasts.

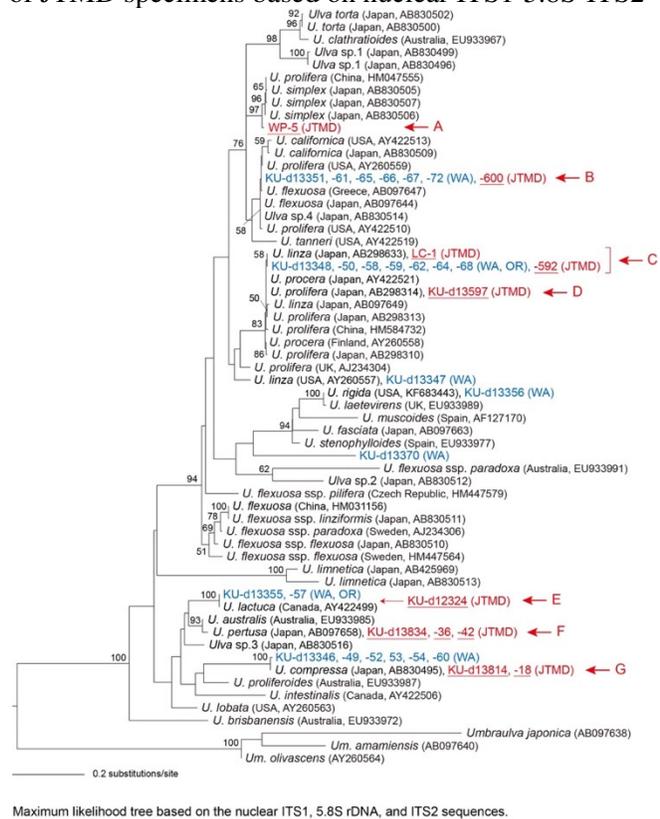


Fig. 3. Molecular phylogenetic tree of *Ulva* species based on ITS1, 5.8S and ITS2 regions of rDNA sequences (ML tree). JTMD specimens are shown in red letters, and field-collected NW American specimens are shown in blue letters.

2) *Ulva lactuca* (green algae, Ulvales)

Ulva lactuca is distributed both in Japan and Pacific North America, but the JTMD specimens were genetically distinct from those in Hokkaido, BC, Washington, and Oregon.

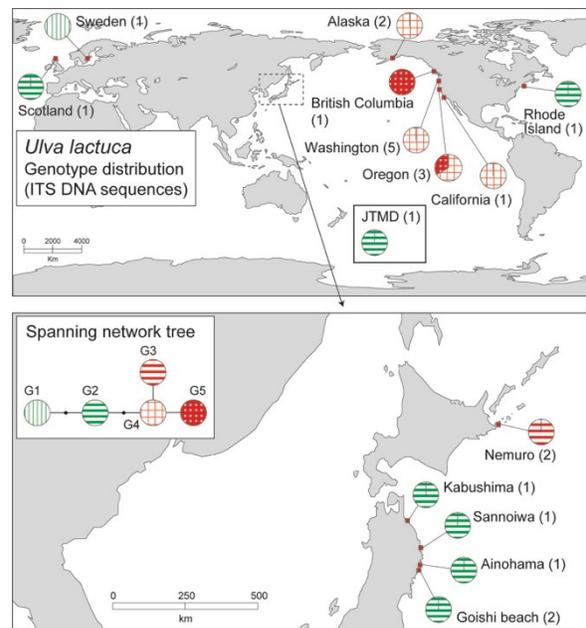


Fig. 4. Spanning network tree based on the ITS region sequence data of *Ulva lactuca*, and the locality of *U. lactuca* samples and the distribution of ITS genotypes.

3) *Ulva pertusa/australis* (green algae, Ulvales)

Ulva pertusa, a common intertidal/subtidal species in Japan, was collected from several JTMD. The species has been introduced world-wide, and has already been recorded from WA and Oregon. Therefore, our study of the species was mainly focused on determining the origin of the large JTMD whose origin was unclear, such as the derelict (unidentified) boat. We have examined the *atpI-H* and *cob-cox3* DNA sequences of *Ulva pertusa* (= *U. australis*) specimens collected from a derelict (unidentified) boat from off-shore at Seal Rock, Oregon on 9 April, 2015, and compared to those of specimens from Northern Japan based on Hanyuda et al. (2016, Phycol. Res. 64: 102–109.) and newly collected specimens from Tohoku. The haplotype of the Seal Rock boat specimens agreed with a haplotype found in Aino-hama, Iwate, although this haplotype has not been found in other areas including central/southern Honshu (data not shown because it is under submission to a journal). Therefore, it is strongly suggested that the unidentified boat originated from Tohoku as JTMD.

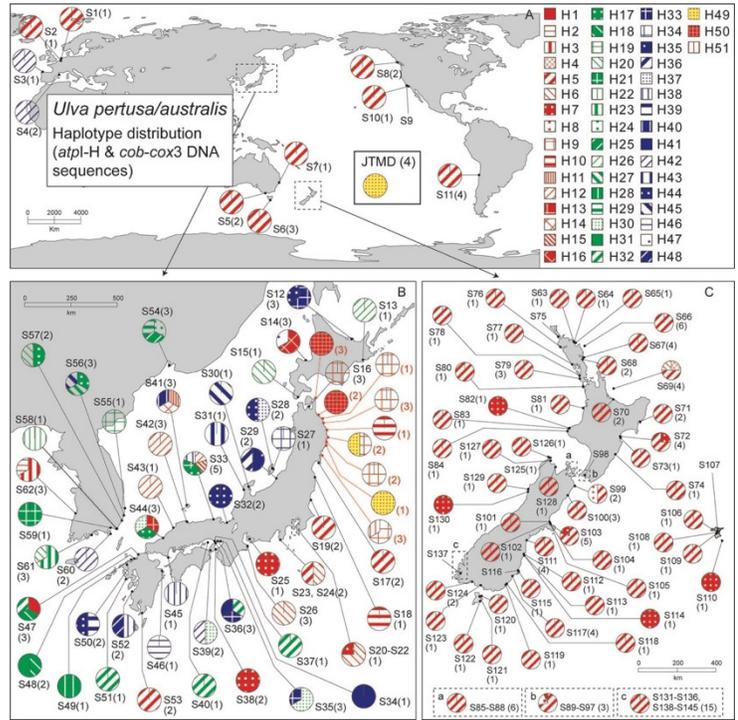
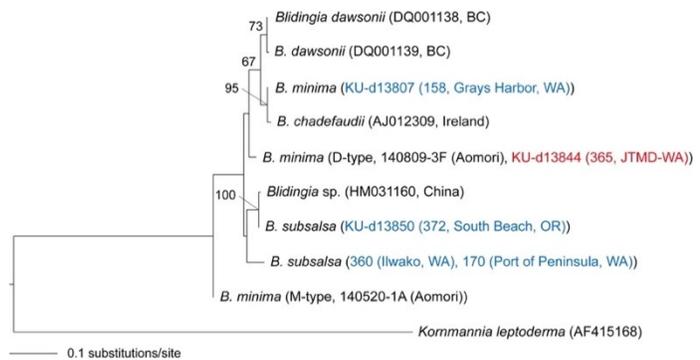


Fig. 5. Geographical distributions of genetic types revealed from *atpI-H* and *cob-cox3* DNA sequences.

4) *Blidingia minima* (green algae, Ulvales)

Blidingia spp. are upper intertidal green algae, which are often difficult to identify at the species level solely based on morphology. We have examined *Blidingia* specimens collected in WA (KU-d13844), and confirmed that they have a sequence identical to *B. minima* collected at Aomori, Tohoku, and identified as *B. minima*.

Fig. 6. Maximum likelihood tree based on the nuclear rDNA ITS region sequences (571 bp). Numbers at nodes indicate bootstrap values in maximum likelihood analysis. Only bootstrap values >50% are shown.



Maximum likelihood tree based on the nuclear ITS1, 5.8S rDNA, and ITS2 sequences.

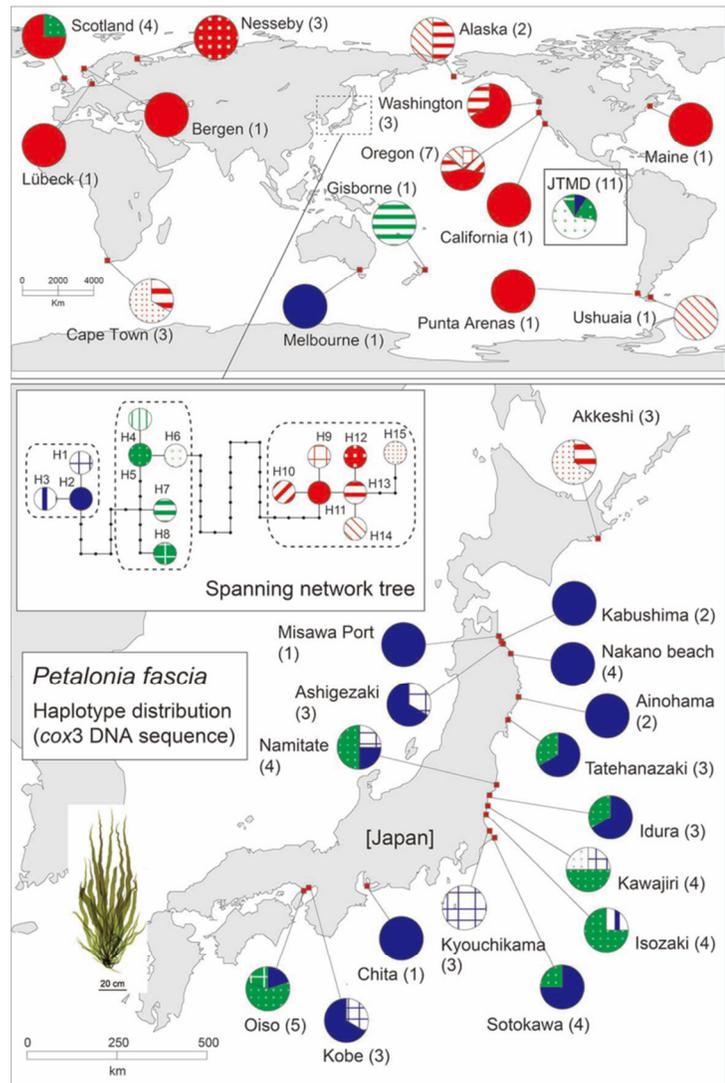
5) *Petalonia fasciata* (brown alga, Ectocarpales s.l.)

Petalonia fasciata is a common ephemeral brown alga growing on diverse substrates including artificial structures in the intertidal zone, and is frequently found on JTMD. The species has broad distributional ranges in temperate and cold water seas. Since the gametophytes (erect thalli) grow rather rapidly, it is difficult to determine the origin of the individuals on JTMD. If the JTMD becomes cast ashore or stays near the shore for some days, zoids (zoospores) from mature crustose sporophytes of the natural population may also attach on JTMD, and become emergent within a couple of weeks. Therefore, in order to clarify the origin of the *P. fasciata* individuals collected from JTMD, we have compared the *cox3* gene sequences of the specimens from JTMD and from the field in Japan and North American coasts. We also added some specimens collected worldwide for comparisons in order to clarify the global biogeography of the species.

In the spanning network analyses, the haplotypes based on mitochondrial *cox3* gene suggested the occurrence of two genetic groups: group-a comprised of populations in Honshu, Southern Hemisphere and a part of Europe; and group-b comprised of populations in Hokkaido, Pacific and Atlantic America, and Europe. The haplotypes H1/H2, H7 were somewhat distant from H3-H6, but sometimes found mixed in Honshu, Japan (i.e. Tatehanazaki, Oiso/Suma). In contrast, the haplotypes H8-H13 were rather close.

The haplotypes of the specimens on JTMD were all of group-a, whereas field-collected specimens from Washington, Oregon and California were all of group-b. Therefore, the *P. fasciata* specimens were considered to have originated from Japan. Although H2 was dominant in Aomori and Iwate populations, JTMD specimens showed rather high genetic divergence (four haplotypes: H2, H4, H5 and H7). This may be explained by the lack of specimens from the southern Tohoku Area.

Fig. 7. Spanning network tree based on the *cox3* sequence data of *P. fasciata*, and the locality of *Petalonia fasciata* samples and the distribution of *cox3* haplotypes. H1: KU-d13951 (Kobe, Hyogo Pref.), 150225-B16 (Ashigezaki, Aomori Pref.), 160214-A4 (Namitate, Ibaraki Pref.), 160214-B1 (Idura, Ibaraki Pref.), 160214-B2 (Idura, Ibaraki Pref.), 160214-C4 (Kawajiri, Ibaraki Pref.), 160215-B1 (Kyouchikama, Ibaraki Pref.), 160215-B2 (Kyouchikama, Ibaraki Pref.), 160215-B3 (Kyouchikama, Ibaraki Pref.); H2: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H3: KU-d13951 (Kobe, Hyogo Pref.), 150225-B16 (Ashigezaki, Aomori Pref.), 160214-A4 (Namitate, Ibaraki Pref.), 160214-B1 (Idura, Ibaraki Pref.), 160214-B2 (Idura, Ibaraki Pref.), 160214-C4 (Kawajiri, Ibaraki Pref.), 160215-B1 (Kyouchikama, Ibaraki Pref.), 160215-B2 (Kyouchikama, Ibaraki Pref.), 160215-B3 (Kyouchikama, Ibaraki Pref.); H4: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H5: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H6: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H7: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H8: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H9: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H10: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H11: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H12: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H13: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H14: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.); H15: KU-d367 (Melbourne, Australia), KU-d13944 (Ashigezaki, Aomori Pref.), 150225-B15 (Ashigezaki, Aomori Pref.), 150421-A1 (Ainohama, Iwaki Pref.), 150421-A2 (Ainohama, Iwaki Pref.), 150325-A11 (Oiso, Hyogo Pref.), KU-d5672 (Kobe, Hyogo Pref.), KU-d5673 (Kobe, Hyogo Pref.), KU-d13928 (Tatehanazaki, Miyagi Pref.).

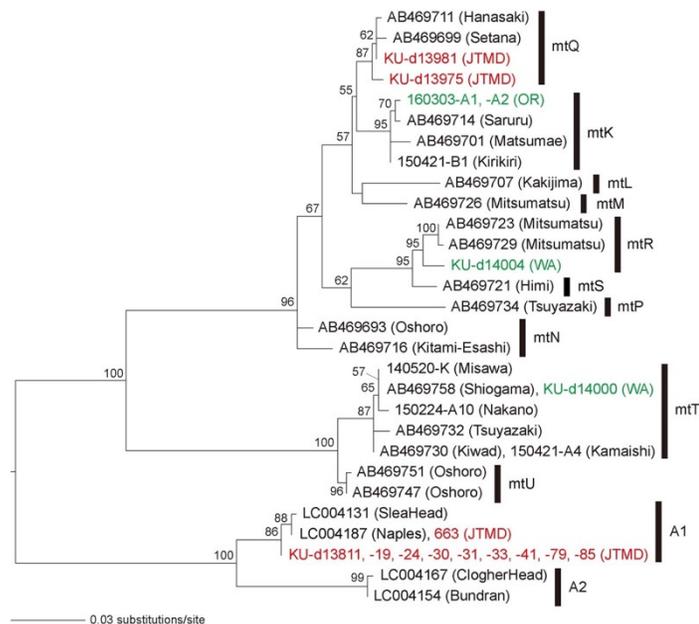


Pref.), KU-d13930 (Tatehanazaki, Miyagi Pref.), KU-d13912 (Kabushima, Aomori Pref.), KU-d13913 (Kabushima, Aomori Pref.), 160214-A3 (Namitate, Ibaraki Pref.), KU-d13952 (Nakano Beach, Iwate Pref.), KU-d13953 (Nakano Beach, Iwate Pref.), KU-d13954 (Nakano Beach, Iwate Pref.), KU-d13955 (Nakano Beach, Iwate Pref.), 160323-A1 (Yura, Hyogo Pref.), 160215-A2 (Sotokawa, Chiba Pref.), 160215-A3 (Sotokawa, Chiba Pref.), 160215-A4 (Sotokawa, Chiba Pref.), 170104-C1 (Kojima, Osaka Pref.), 150225-A11 (Misawa Fishing Port, Aomori Pref.), LC-4 (JTMD); H3: 160213-B4 (Isozaki, Ibaraki Pref.); H4: KU-d13175 (Platboom Bay, South Africa), KU-d13179 (Platboom Bay, South Africa); H5: KU-d13960 (Oiso, Hyogo Pref.), KU-d13961 (Oiso, Hyogo Pref.), 150325-A10 (Oiso, Hyogo Pref.), KU-d4674 (Kyle of Lochalsh, Scotland), KU-d12650 (Chita, Aichi Pref.), KU-d13927 (Tatehanazaki, Miyagi Pref.), KU-d13857 (JTMD), KU-d13867 (JTMD), 630 (JTMD), KU-d12935 (JTMD), 160214-B3 (Idura, Ibaraki Pref.), 160215-A1 (Sotokawa, Chiba Pref.), 160214-C1 (Kawajiri, Ibaraki Pref.), 160214-C3 (Kawajiri, Ibaraki Pref.), 160214-A1 (Namitate, Ibaraki Pref.), 160214-A2 (Namitate, Ibaraki Pref.), 160323-A2 (Yura, Hyogo Pref.), 160323-A4 (Yura, Hyogo Pref.), 160213-B1 (Isozaki, Ibaraki Pref.), 160213-B2 (Isozaki, Ibaraki Pref.), 160213-B3 (Isozaki, Ibaraki Pref.), KU-d13167 (Cape Town, South Africa); H6: KU-d12830 (JTMD), KU-d12831 (JTMD), KU-d12832 (JTMD), WP-4 (JTMD), KU-d13593 (JTMD), KU-d13594 (JTMD), KU-d13822 (JTMD), 160214-C2 (Kawajiri, Ibaraki Pref.); H7: KU-d289 (Gisborne, NZ); H8: KU-d13812 (JTMD), 150325-A9 (Oiso, Hyogo Pref.), 160323-A3 (Yura, Hyogo Pref.); H9: KU-d13326 (Jetty Fishery Marina, OR); H10: KU-d14011 (Brighton, OR); H11: KU-d12829 (Boiler Bay, OR), KU-d914 (Leubeck, Germany), KU-d3371 (Patagonia, Argentina), KU-d3501 (Portland, ME), KU-d4649 (Helmsdale, Scotland), KU-d807 (Bergen, Norway), KU-d4675 (Buckie, Scotland), KU-d12618 (Santa Cruse, CA), KU-d13610 (Grays Harbor, WA), KU-d13325 (Brighton, OR), KU-d4681 (Buckie, Scotland), KU-d13616 (Grays Harbor, WA), KU-d14216 (Grays Harbor, WA), 160306-A1 (Grays Harbor, WA), 160302-A2 (Grays Harbor, WA), KU-d15395 (Tofino, BC), KU-d15421 (Banfield, BC), KU-d14003 (Port of Peninsula, WA); H12: KU-d12986 (Nesseby, Norway), KU-d12987 (Nesseby, Norway), KU-d12988 (Nesseby, Norway); H13: KU-d8811 (Newport, OR), KU-d4877 (St. Paul Island, AK), KU-d13228 (Akkeshi, Hokkaido Pref.), KU-d13661 (Willapa Bay, WA), KU-d20 (Kamchatka, Russia), KU-d15420 (Banfield, BC), KU-d14009 (Jetty Fishery Marina, OR), KU-d14044 (Port of Peninsula, WA); H14: KU-d13327 (Jetty Channel, OR), KU-d3418 (Fego, Argentina), KU-d4866 (St. Paul Island, AK), KU-d15185 (Kodiak, AK), KU-d15186 (Kodiak, AK), KU-d15211 (Kodiak, AK), KU-d15174 (Kodiak, AK); H15: KU-d13265 (Akkeshi, Hokkaido Pref.), KU-d13271 (Akkeshi, Hokkaido Pref.)

6) *Scytosiphon lomentaria* (brown alga, Ectocarpales s.l.)

Scytosiphon lomentaria is a common ephemeral brown alga growing on diverse substrates including artificial structures in the intertidal zone, and is frequently found on JTMD. *S. lomentaria* is taxonomically very close to *Petalonia fascia* and has similar life history and ecological characters. 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 the field in Japan and North American coasts. We also added some specimens collected worldwide for comparisons in order to clarify the global biogeography of the species.

Our results show that JTMD *Scytosiphon* included two independent lineages, one genetically close to those from Hokkaido (mtQ) and the other genetically close to those from Europe (A1). Field-collected specimens from



Oregon and Washington belonged to three independent lineages (mtK, mtR, mtT), which were genetically close to various localities in Japan. This may suggest that the intercontinental introductions of this species have frequently occurred and the original distributional pattern has been disturbed. Species level taxonomy of *Scytosiphon lomentaria* needs further examination, so it is possible that these lineages correspond to independent species, or some intra-species level taxa such as subspecies or variety.

Fig. 8. Maximum likelihood tree based on the mitochondrial *cox3* gene sequences (500 bp). Numbers at nodes indicate bootstrap values in maximum likelihood analysis. Only bootstrap values >50% are shown.

7) *Ectocarpus* spp. (brown algae, Ectocarpales)

Filamentous brown algae are frequently encountered on JTMD, but they are generally difficult to identify solely based on morphology. In addition, the species level taxonomy of *Ectocarpus* and related taxa is rather confused because although around ten phylogenetic entities, perhaps corresponding to species, are identified worldwide, many of them do not have formally correct names at present.

In the present project we examined their *cox3* gene sequences and found that they belong to 8 to 9 *Ectocarpus* species and one *Kuckuckia* species. Two of the *Ectocarpus* taxa had sequences identical or very close to *E. croaniorum* and *E. siliculosus*, and were identifiable to these taxa, but it was difficult to identify all of them to the species level by genetic study because taxonomic revision of the genus *Ectocarpus* has not been completed and species names are not assigned to some clades. However, it is noteworthy that some of the clades (plausible species) of JTMD specimens have not been recorded from NW American coasts.

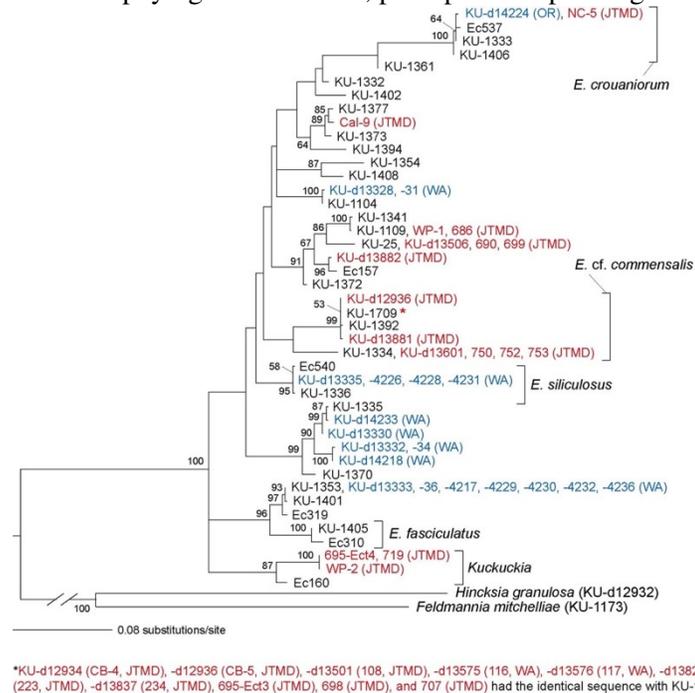


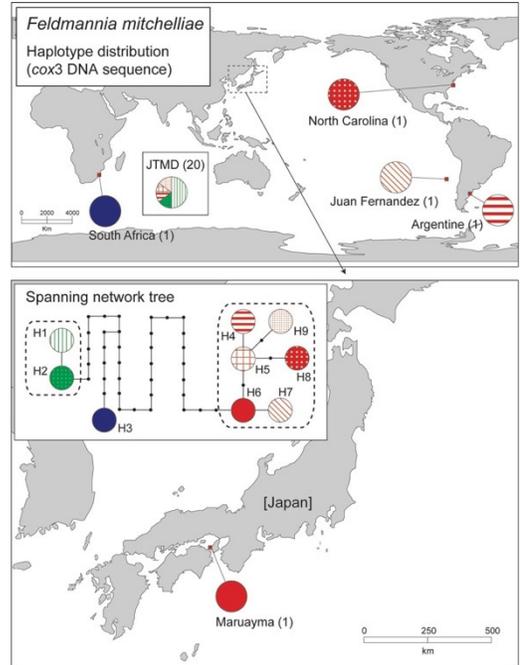
Fig. 9. Maximum likelihood tree based on the mitochondrial *cox3* gene sequences (665 bp). Numbers at nodes indicate bootstrap values in maximum likelihood analysis. Only bootstrap values >50% are shown.

8) *Feldmannia mitchelliae* (brown algae, Ectocarpales)

Feldmannia species are filamentous brown algae resembling *Ectocarpus*, but morphologically primarily distinguished from *Ectocarpus* in the chloroplast morphology (discoid vs. ribbon-shaped). However, the vegetative morphology is rather plastic and species level taxonomy is often difficult. In the present study, we have identified the JTMD specimens as *F. mitchelliae* by *cox3* gene sequences. JTMD *F. mitchelliae* included several haplotypes of *cox3*, but unfortunately data of the genetic types of local populations in NE Pacific coasts are not available at present.

Fig. 10. Spanning network tree based on the *cox3* sequence data of *Feldmannia mitchelliae*, and the locality of *F. mitchelliae* samples and the distribution of *cox3* haplotypes.

H1: KU-d13808 (JTMD), KU-d13821 (JTMD), KU-d13838 (JTMD); H2: KU-d13828 (JTMD), KU-d13883 (JTMD); H3: KU-1181 (Ballito Natal, South Africa); H4: Cal-15 (JTMD), KU-d12826 (JTMD), KU-2317 (Las Grudas, Argentina); H5: KU-d12825 (JTMD); H6: KU-1773 (Maruyama, Hyogo Pref.); H7: KU-1195 (Radio Island, NC); H8: KU-1193 (Isla Santa Clara, Chile)

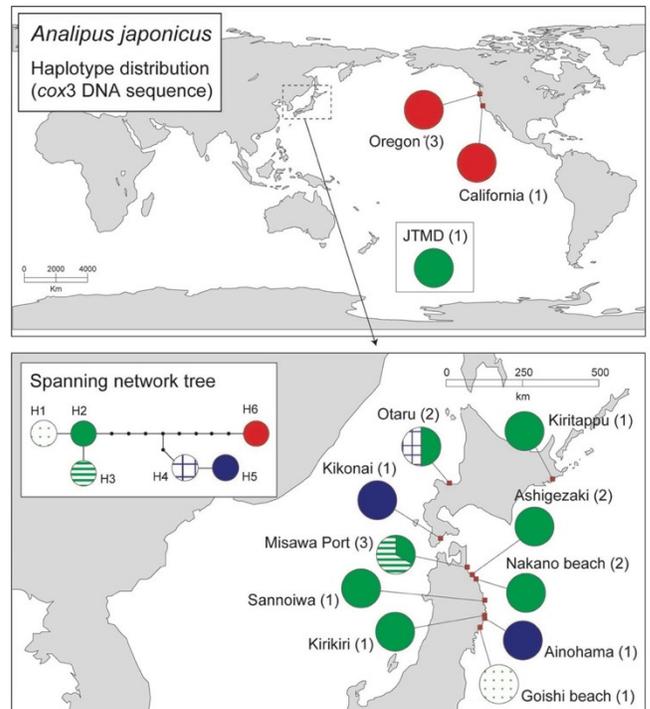


9) *Analipus japonicus* (brown algae, Ralfsiales)

Analipus japonicus is a common brown alga growing on intertidal rocks or on mussels in cold temperate and colder seas. This species was found on the the Misawa floating dock as JTMD. Upright thalli are annual but the basal part of the thallus is perennial. The species is distributed on both sides of the North Pacific, from Tohoku region to Hokkaido in Japan and from Alaska to California on American coasts. Genetic comparison using *cox3* DNA sequences has revealed that North American and Japanese populations are genetically distinct, although they are relatively closely related and Japanese populations show high genetic divergence.

Fig. 11. Spanning network tree based on the *cox3* sequence data of *Analipus japonicus*, and the locality of *Analipus japonicus* samples and the distribution of *cox3* haplotypes.

H1: 150520-B15 (Goishi Beach, Miyagi Pref.); H2: KU-883 (Otaru, Hokkaido), 140520-O (Misawa Fishing Port, Aomori Pref.), 140520-2L, 150225-B19 (Ashigezaki, Aomori Pref.), 150607-A28 (Sannoiwa, Iwate Pref.), 150421-B13 (Kirikiri, Iwate Pref.), 140519-1E, 150224-A19 (Nakano Beach, Iwate Pref.), KU-d5925, (Kiritappu, Hokkaido), GIH5778 (JTMD); H3: 150225-A17, 150605-A8 (Misawa Fishing Port, Aomori Pref.); H4: KU-d13672 (Otaru, Hokkaido); H5: KU-d701 (Kikonai, Hokkaido), 150421-A14 (Ainohama, Iwate Pref.); H6: KU-d8806 (Newport, OR), KU-d8838 (Newport, OR), GIH4203 (OR), EU681432 (Bodega Bay, CA)

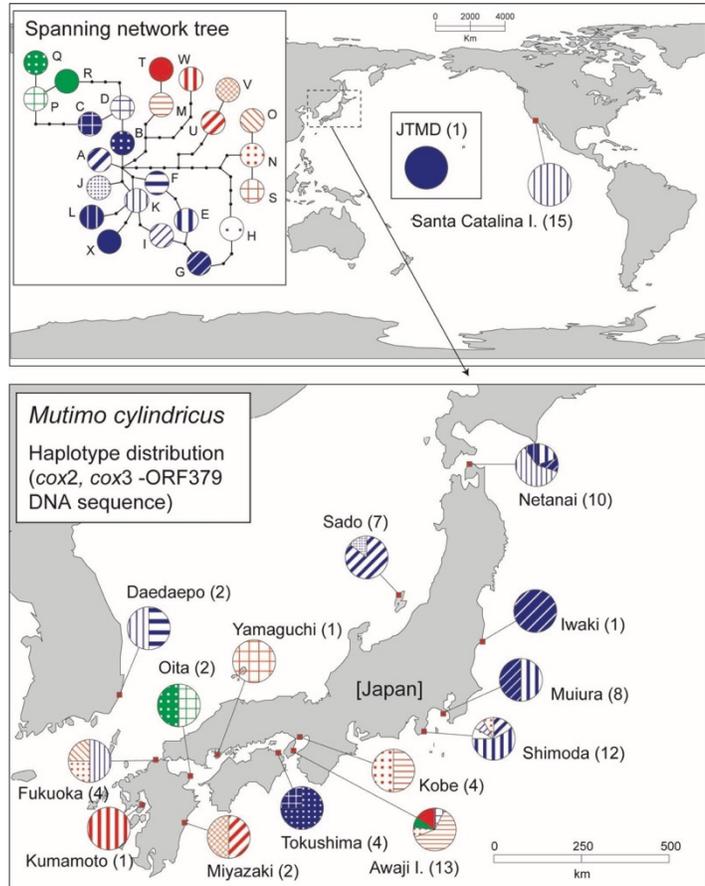


10) *Mutimo cylindricus* (brown algae, Tilopteridales)

Mutimo cylindricus is an annual brown alga originally distributed in Far-east Asia. It was shown to be introduced to Santa Catalina Island, California (Kogishi et al. 2010 as *Cutleria cylindrica*, later transferred to *Mutimo* in Kawai et al. 2012), perhaps from Tohoku, Japan. The California population was dominated by female, asexual gametophytes reproducing by parthenogenesis. *Mutimo cylindricus* found on JTMD was shown to have a distinct genetic type, and is likely a sexual strain. Therefore, new introductions may show different propagation patterns from the California population.

Fig. 12. Spanning network tree based on the *cox2* and *cox3*-ORF379 sequence data of *Mutimo cylindricus*, and the locality of *M. cylindricus* samples and the distribution of haplotypes.

HA: Sado Island, Niigata Pref. (6); Shimoda, Shizuoka Pref. (2); HB: Shishikui, Tokushima Pref. (3); HC: Shishikui, Tokushima Pref. (1); HD: Awaji Island, Hyogo Pref. (1); HE: Netanai/Ohma, Aomori Pref. (2); Miura, Kanagawa Pref. (4); Shimoda, Shizuoka Pref. (7); HF: Dadaepo, Pusan (1); HG: Netanai/Ohma, Aomori Pref. (1); Miura, Kanagawa Pref. (4); Iwaki, Fukushima Pref. (1); HH: Shimoda, Shizuoka Pref. (1); HI: Shimoda, Shizuoka Pref. (1); HJ: Sado Island, Niigata Pref. (1); HK: Netanai/Ohma, Aomori Pref. (6); Shikanoshima, Fukuoka Pref. (2); Dadaepo, Pusan (1); Santa Catalina Island, CA (15); HL: Netanai/Ohma, Aomori Pref. (1); HM: Maiko, Kobe, Hyogo Pref. (2); Awaji Island, Hyogo Pref. (8); HN: Shimoda, Shizuoka Pref. (1); Maiko, Kobe, Hyogo Pref. (2); Awaji Island, Hyogo Pref. (1); Shikanoshima, Fukuoka Pref. (1); HO: Shikanoshima, Fukuoka Pref. (1); HP: Onyu Island, Oita Pref. (1); HQ: Onyu Island, Oita Pref. (1); HR: Awaji Island, Hyogo Pref. (1); HS: Suouoshima, Yamaguchi Pref. (1); HT: Awaji Island, Hyogo Pref. (2); HU: Miyazaki, Miyazaki Pref. (1); HV: Miyazaki, Miyazaki Pref. (1); HW: Otsuku Island, Kumamoto Pref. (1); HX: JTMD (1, 716)

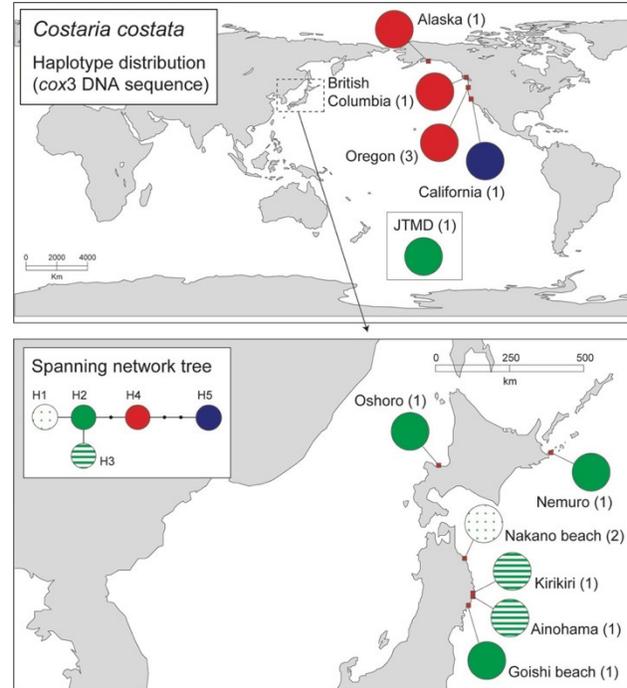


11) *Costaria costata* (brown algae, Laminariales)

Costaria costata is an annual kelp species, and found on the Misawa floating dock. 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 has revealed that North American and Japanese populations are genetically distinct, although they are relatively closely related.

Fig. 13. Spanning network tree based on the *cox3* sequence data of *Costaria costata*, and the locality of *Costaria costata* samples and the distribution of *cox3* haplotypes.

H1: 140519-1A (Nakano Beach, Iwate Pref.), 140519-1B (Nakano Beach, Iwate Pref.); H2: KU-d5836 (Oshoro, Hokkaido Pref.), KU-d15600 (JTMD), KU-d78 (Nemuro, Hokkaido Pref.), 150520-B16 (Goishi Beach, Iwate Pref.); H3: 150421-A15 (Ainohama, Iwate Pref.), 150421-B14 (Kirikiri, Iwate Pref.); H4: KU-679 (Vancouver, BC), KU-d15601 (Boilger Bay, OR), KU-d15602 (Boaidmon, OR), KU-d15603 (Green I., AK), KU-d8831 (Newport, OR); H5: KU-d14053 (Moss Landing, CA)

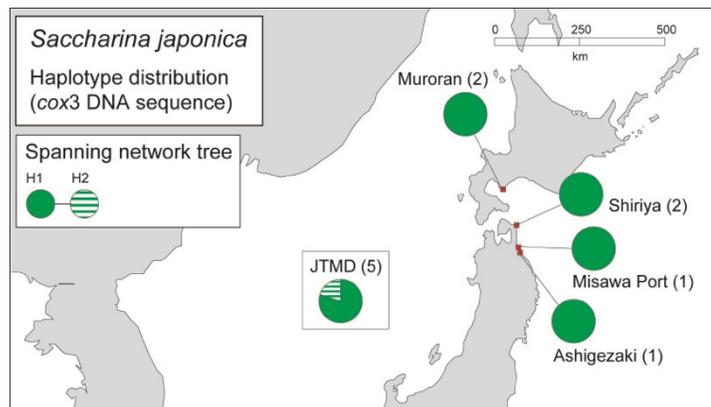


12) *Saccharina japonica* (brown algae, Laminariales)

Saccharina japonica is a biannual kelp species exceeding several meters in length, and constitutes one of the most important ecological elements in colder seas. *S. japonica* was found on the Misawa floating dock. The species is distributed in cold water regions of the Pacific Northwest of America, and in Tohoku and Hokkaido in Japan. The species is not distributed in NE Pacific, therefore there is concern about a new introduction of a non-indigenous species if it become introduced. In addition, *Saccharina* species are genetically very closely related and the occurrence of interspecies hybrids (and sometimes even hybrids between genera) are known. Therefore, there is concern about genetic contamination of native populations that could cause strong disruptions to the local ecosystems.

Fig. 14. Spanning network tree based on the *cox3* sequence data of *Saccharina japonica*, and the locality of *S. japonica* samples and the distribution of *cox3* haplotypes.

H1: KU-508 (Muroran, Hokkaido Pref.), AP011493 (Muroran, Hokkaido Pref.), KU-d12319 (JTMD), KU-d12320 (JTMD), KU-d12321 (JTMD), KU-d12323 (JTMD), 140520-I (Misawa Fishing Port, Aomori Pref.), 140520-2I (Ashigezaki, Aomori Pref.), 140809-1D (Shiriyu, Aomori Pref.), 140809-2I (Shiriyu, Aomori Pref.); H2: KU-d12356 (JTMD)



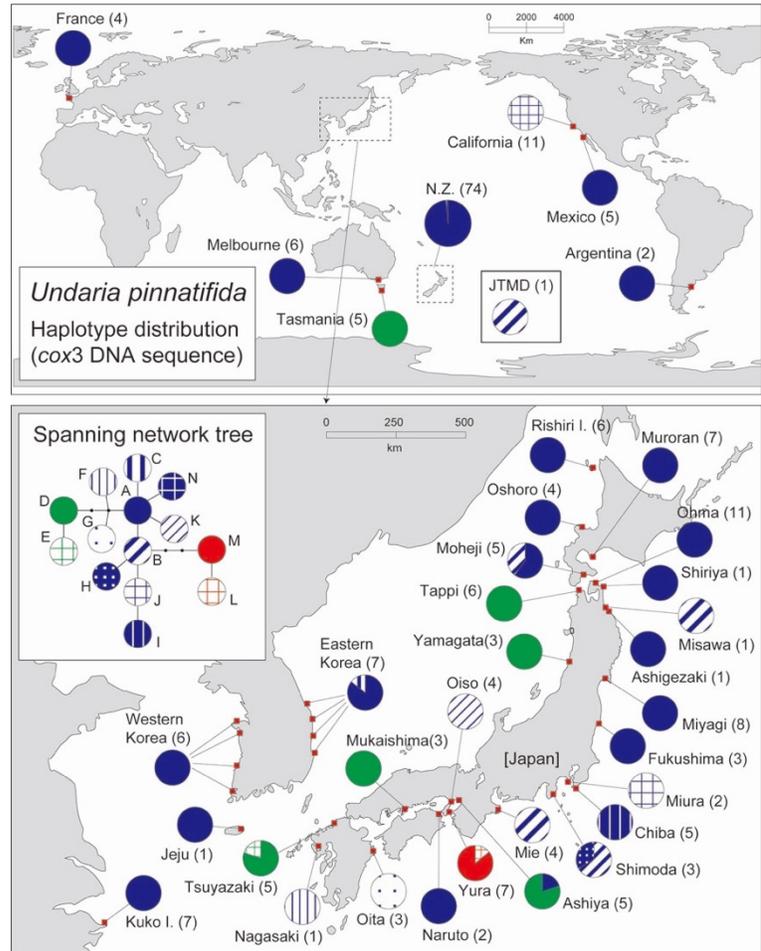
13) *Undaria pinnatifida* (brown algae, Laminariales)

Undaria pinnatifida was found on the Misawa floating dock. The species is an annual kelp constituting an important ecological element of temperate seas. The original distributional range of the

species is NW Pacific (Fareast Asia), but has been introduced and has established wide ranges both in the Northern and Southern Hemisphere. On Northwestern American coasts, the species has been introduced to California and Pacific Mexico, but has not spread north to Oregon. Therefore, there is concern about a new introduction of a non-indigenous species if it become introduced, because it could cause considerable disturbance to the local ecosystems, as well as fisheries.

Fig. 15. Spanning network tree based on the *cox3* sequence data of *Undaria pinnatifida*, and the locality of *U. pinnatifida* samples and the distribution of haplotypes.

HA: Songjeong beach, Pusan (1); Cheongsapo, Pusan (1); Ayajin, Gosung, Gangwon Province (2); Anin, Gangreung, Gangwon Province (1); Suryeomri, Gyeongju, Gyeongbuk Province (1); Ilchulbong, Seongsan, Jeju (1); Namyangman, Gyeonggi Province (1); Oeyeondo, Boryeong, Chungnam Province (1); Daecheon, Boryeong, Chungnam Province (1); Jeongdori, Jindo, Jeonnam Province (1); Hoedong, Jindo, Jeonnam Province (1); Zoushan, Kuko Island (7); Rishiri Island, Hokkaido Pref. (6); Oshoro, Hokkaido Pref. (4); Moheji, Kamiiso, Hokkaido Pref. (3); Murooran, Hokkaido Pref. (7); Ohma, Aomori Pref. (11); Naruto, Tokushima Pref. (2); Ashiya, Hyogo Pref. (1); Onahama, Fukushima Pref. (3); Oga, Miyagi Pref. (8); Todos Santos Island, Baja California (5); Viaduct Basin, Auckland (2); Western boat ramp, Auckland (2); Akersten Street boat ramp, Nelson (1); Wakefield Quay, Nelson (2); Picton Marina, Picton (5); Chaffers Marina, Wellington (5); Scorching Bay, Wellington (5); Gisborne Port, Gisborne (6); Port of Napier, Napier (7); New Wharf, Kaikoura (3); Lyttelton Marina, Christchurch (4); Taylor's Mistake, Christchurch (4); Slipway, Timaru (3); North Mole, Timaru (3); Oamaru (5); Moeraki (3); Deborah Bay, Dunedin (3); Port Chalmers, Back Beach (3); Halfmoon Bay, Stewart Island (3); Big Glory Bay, Stewart Island (1); Below Foreshore Road, Bluff (4); Williamstown, Melbourne (6); Buhia Bustamente, Chubut, Argentina (1); Golfo, Nuero, Argentina (1); Brest, Brittany, France (2); Roscoff, Brittany, France (2); Shiriya, Aomori Pref. (1); Ashigezaki, Aomori Pref. (1); HB: Moheji, Kamiiso, Hokkaido Pref. (2); Hamashima, Mie Pref. (4); Shimoda, Shizuoka Pref. (2); Misawa Fishing Port, Aomori Pref. (1); JTMD (1, KU-d15599); HC: Haegeumgang, Geojeodo, Gyeongnam Province (C: 1), Oamaru (1); HD: Tappi, Aomori Pref. (6); Atsumi, Yamagata Pref. (3); Tsuyazaki, Fukuoka Pref. (4); Mukai-shima, Hiroshima Pref. (3); Ashiya, Hyogo Pref. (4); George's Bay, St. Helens, Tasmania (1); North Bay, Forestier, Tasmania (1); Mercury Passage/St. Helens, Tasmania (3); Triabunna, Tasmania (3); HE: Tsuyazaki, Fukuoka Pref. (1); HF: Saikai-Bashi, Nagasaki Pref. (1); HG: Tsurumi, Oh-ita Pref. (3); HH: Shimoda, Shizuoka Pref. (1); HI: Kominato, Chiba Pref. (5); HJ: Kan-non-zaki, Yokosuka, Kanagawa Pref. (2); Shinjuku, Zushi, Kanagawa Pref. (1); Moroiso, Misaki, Miura, Kanagawa Pref. (2); Monterey Marina, Monterey, CA (4); Cabrillo, San Pedro, CA (5); Santa Barbara, CA (2); HK: Oh-iso, Awaji Island, Hyogo Pref. (4); HL: Sumoto, Awaji Island, Hyogo Pref. (1); HM: Sumoto, Awaji Island, Hyogo Pref. (6); HN: Bluff Harbor (1)

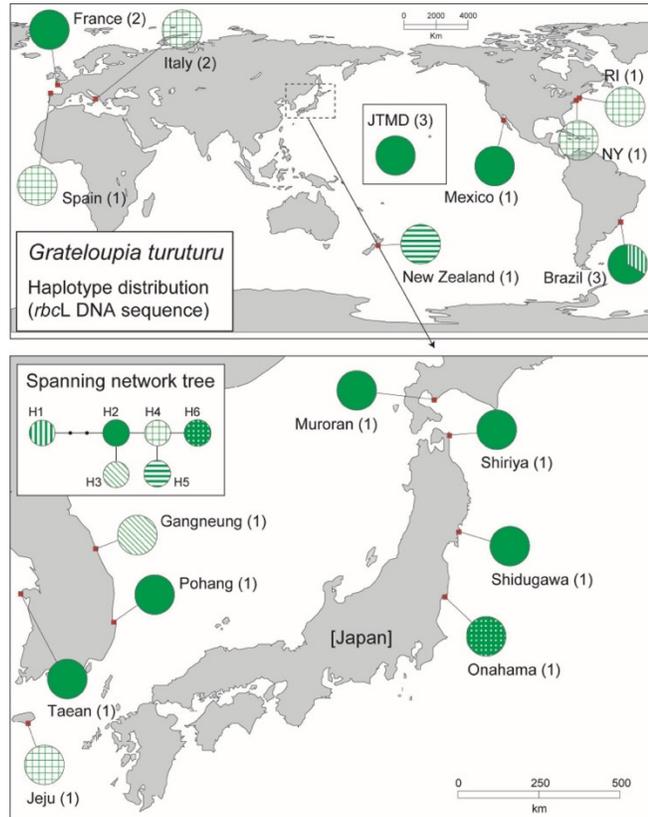


14) *Grateloupia turuturu* (red algae, Halymeniales)

Grateloupia turuturu is an annual red alga with branched membranous thalli. The original distributional range of this species is Far-east Asia, but it has been introduced to various areas in Europe, Northwestern and Northeastern American coasts, etc. On NW American coasts it has been reported from California and Baja California, but has not been reported from Oregon before. Based on the *rbcL* DNA sequence, the specimen collected from JTMD had the same haplotype as that reported from Baja California. However, resolution of this gene region is considered to be rather low, because it is a conserved region. Therefore, it is necessary to use some gene region with a faster mutation rate in order to distinguish the JTMD specimen from existing California/Baja California populations.

Fig. 16. Spanning network tree based on the *rbcL* sequence data of *Grateloupia turuturu*, and the locality of *G. turuturu* samples and the distribution of *rbcL* haplotypes.

H1: 150520-B15 (Goishi Beach, Miyagi Pref.); H2: KU-d12359 (JTMD), AF488821 (Wolpo, Korea), 710 (JTMD), 721 (JTMD), 140809-1E (Shiriya, Aomori Pref.), 160722-A14 (Shizugawa, Miyagi Pref.), AY100003 (Brittany, France), KP976176 (Santa Catarina, Brazil), KU976177 (Santa Catarina, Brazil), AF488820 (Muroran, Hokkaido Pref.), AJ868493 (Brittany, France), AJ868494 (Brittany, France), GU168561 (Taeon, Korea), JN808318 (Baja California, Mexico); H3: GU168564 (Gangneung, Korea); H4: AJ868495 (San Amaro, Spain), AF488818 (Bristol, RI), AF488819 (Montauk Park, NY), FN821950 (Venice, Italy), FN821951 (Taranto, Italy), KJ648568 (Jeju, Korea); H5: EF091853 (Wellington, NZ); H6: AB055475 (Onahama, Fukushima Pref.)



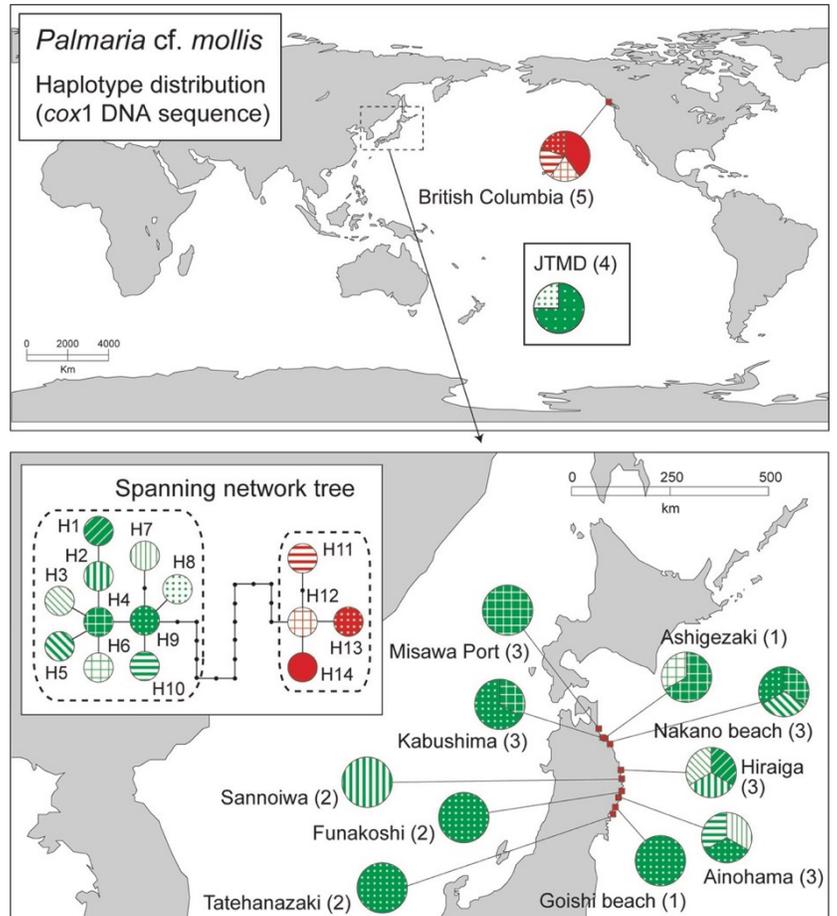
15) *Palmaria palmata/mollis* (red alga, Palmariales)

Palmaria palmata (auct. japon.) was collected on the Misawa floating dock. *Palmaria palmata* is an annual foliose red alga distributed in wide ranges of colder seas, however, there remains taxonomic problem in the species level taxonomy. *Palmaria palmata* (auct. japon.) can be conspecific to *Palmaria mollis* distributed in the Northwestern coast of America, and considered to constitute important ecological element by their relatively large size and abundance. In the comparisons of *cox1* gene haplotypes of Japanese and JTMD derived specimens suggested that Japanese *Palmaria palmata* collected on the tsunami debris agreed with the field-collected specimens from Northern Tohoku, and were genetically rather distant by about 20 bp in *cox1* sequences from a native (local) population of *P. mollis* in British Columbia. If the taxa (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 taxonomically concluded to be independent species, considering their close genetic relationship, there is still a possibility that they could cross to form hybrids and cause genetic contamination by introgression. *Palmaria mollis* is used for food (as 'dulse' and sold

commercially, although the amount is not great in North America, but widely consumed in Northern Europe) on NW American coasts, so that its introduction could cause economic damage in addition to the risks of disturbance to the local ecosystems.

Fig. 17. Spanning network tree based on the *cox1* sequence data of *Palmaria palmata*/*P. mollis*, and the locality of *Palmaria palmata*/*P. mollis* samples and the distribution of *cox3* haplotypes.

H1: 150422-B5 (Hiraiga, Iwate Pref.); H2: 150607-A40 (Sannoiwa, Iwate Pref.), 150607-A41 (Sannoiwa, Iwate Pref.), 150422-B4 (Hiraiga, Iwate Pref.); H3: 150422-B6 (Hiraiga, Iwate Pref.); H4: KU-d13910 (Misawa Fishing Port, Aomori Pref.), 150605-A17 (Misawa Fishing Port, Aomori Pref.), 140520-2D (Ashigezaki, Aomori Pref.), 150225-B18 (Ashigezaki, Aomori Pref.), 150422-B6 (Kabushima, Aomori Pref.), 150224-A18 (Nakano beach, Iwate Pref.); H5: 150224-A8 (Nakano beach, Iwate Pref.); H6: 150225-B17 (Ashigezaki, Aomori Pref.); H7: 150421-A11 (Ainohama, Iwate Pref.); H8: KU-d12933 (JTMD); H9: 150421-A12 (Ainohama, Iwate Pref.), 150422-B4 (Kabushima, Aomori Pref.), 150422-B5 (Kabushima, Aomori Pref.), 150224-A7 (Nakano beach, Iwate Pref.), Cal-3 (JTMD), Cal-17 (JTMD), KU-d12822 (JTMD), 150520-B9 (Goishi beach, Miyagi Pref.), 150421-C5 (Funakoshi, Iwate Pref.), 150421-C6 (Funakoshi, Iwate Pref.), 150421-C7 (Funakoshi, Iwate Pref.), 150520-A14 (Tatehanazaki, Miyagi Pref.), 150520-A15 (Tatehanazaki, Miyagi Pref.); H10: 150421-A10 (Ainohama, Iwate Pref.); H11: GU224108 (Kelsey Bay, BC); H12: GU224111 (Port Hardy, BC); H13: GU224112 (Otter Point, BC); H14: GU224109 (Bamfield, BC), GU224110 (Ridley Island, BC)



Production of field guide for detecting new introduction of JTMD macroalgae

We are planning to produce a flier indicating the gross morphology and important anatomical features for identifying high-profile JTMD macroalgal species. We have collected intact specimens of the corresponding species, mostly in Tohoku, and prepared anatomical micrographs. We plan to complete preparation and print the field guide by the end of March 2017, and distribute it to local communities.

e) Discussion

Because of the great morphological plasticity and rather limited morphological features, species level taxonomy of macroalgae, especially those of minute species, are often difficult. In addition to such taxonomic difficulty, there are additional factors that made the precise taxonomy (species level identification) of JTMD macroalgal species difficult: In most cases the sampling of the JTMD specimens

was done by non-specialists in algal taxonomy, and the specimens are not properly prepared for taxonomic examination. Furthermore, the sampled specimens often deteriorated before they arrived at the laboratory. Therefore, in order to compensate for these problems and improve the accuracy of identifications, we have applied genetic analyses using selected genetic markers. Based on these analyses, we have obtained gene sequences useful for genetic taxonomy for ca. 190 specimens and have identified ca. 50 species as JTMD macroalgae. We have corrected the preliminary morphological identification of over 10 taxa (e.g., *Chondrus giganteus*, *Grateloupica turuturu*) by adding genetic data.

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 the NE Pacific coasts after arriving 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 that this examination was considered to be important. In addition, some JTMD species are known to be distributed on both side 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 population, JTMD macroalgae and North American natural population) by appropriate genetic markers.

Our genetic analyses indicated that most JTMD macroalgae had haplotypes identical with or very closely related to natural populations in the Tohoku region, so that 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 sides of NW and NE Pacific, and therefore their new introduction to the NW American coast may not be regarded as a species level invasion. However, our genetic comparisons have indicated that they are genetically distinct and may cause genetic contamination: e.g., *Blidingia minima*, *Analipus japonicum*, *Petalonia fascia* and *Costaria costata*. Some species are already introduced to NE American coasts by relatively recent anthropogenic introductions (e.g. *Mutimo cylindricus*, *Undaria pinnatifida* and *Grateloupia turuturu*), but have not been spread to the coasts of Oregon, and had different haplotypes. Therefore, these new introductions will still pose a risk of accelerating the dispersal of these non-indigenous species, enrich the genetic diversity of the introduced populations, and further increase their competitiveness within the local populations.

Genetic analyses have revealed the species diversity of the JTMD macroalgae in the 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 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.

Through the field surveys of selected natural populations from Alaska to Oregon, we have not found any conclusively introduced populations originated from JTMD. This may be because of the short time period after these possible introduction events, and we cannot evaluate the risk of new introductions by the JTMD. However, we consider that the long-term monitoring sites we have selected will be helpful in detecting new introductions if they ever occur on these coasts.

f) Literature Cited

- Clement, M., Posada, D., Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9: 1657–9.
- Katoh, K., Toh, H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* 9:286–98.
- Kawai, H., Kogishi, K., Hanyuda, T., Kitayama, T. 2012. Taxonomic revision of the genus *Cutleria* proposing a new genus *Mutimo* to accommodate *M. cylindrica* (Cutleriaceae, Phaeophyceae). *Phycol. Res.* 60: 241–248.

- Kogishi, K., Kitayama, T., Miller, K. A., Hanyuda, T., Kawai, H. 2010. Phylogeography of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in northeastern Asia, and the identity of an introduced population in California. *J. Phycol.* 46: 553–558.
- Lis, J. T. 1980. Fractionation of DNA fragments by polyethylene glycol induced precipitation. *Meth. Enzymol.* 65:347–53.
- Silvestro, D., Michalak, I. 2012. raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* 12:335–7.
- Uwai, S., Nelson, W., Neill, K., Wang, W. D., Aguilar-Rosas, L.E., Boo, S.M., Kitayama, T., Kawai, H. 2006. Genetic diversity in *Undaria pinnatifida* (Laminariales, Phaeophyceae) deduced from mitochondria genes – origins and succession of introduced populations. *Phycologia* 45: 687-695.

5. OUTPUTS

a. Completed and planned publications

- West, J.A., Hansen, G.I., Hanyuda, T., Zuccarello, G.C.. 2016. Flora of drift plastics: a new red algal genus, *Tsunamiya transpacific* (Stylonematophyceae) from Japanese tsunami debris in the northeast Pacific Ocean. *Algae* 31 (4): 289-301. <http://www.e-algae.org/>
- Hanyuda, T., Kawai, H., Hansen, G.I. (to be submitted in February 2017). Genetic identifications of macroalgae species on Japanese Tsunami Marine Debris (JTMD) and their genetic comparisons with wild populations in Northeastern Pacific coasts. *Marine Pollution Bulletin, Special Issue*
- Hansen, G.I., Hanyuda, T., Kawai, H. (to be submitted in February 2017). Marine algae carried across the North Pacific on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington. *Marine Pollution Bulletin, Special Issue*
- Hansen, G.I., Hanyuda, T., Kawai, H. (March 2017). An illustrated guide to the most invasive marine algal species on Japanese Tsunami Marine Debris.

b. Poster and oral presentations at scientific conferences or seminars

- Hansen, G., Hanyuda, T., Kawai, H. 2016. Marine algae carried across the North Pacific on Japanese Tsunami Marine Debris (JTMD) and their invasion threat to the coasts of Oregon and Washington, USA. 9th International Conference on Marine Bioinvasions. The Menzies Sydney Hotel, Sydney. 19-21 January, 2016.
- Kawai, H., Hanyuda, T., Hansen, G. 2016. Species diversity and the threat of introduced macroalgal species arriving on Northwestern American shores via Japanese Tunami Marine Debris (JTMD). 11th EMECS Conference, Azimut Hotel, St. Petersburg, Russia. 22-27 August, 2016. (Poster).
- Hanyuda, T., Hansen, G., Kawai, H. Genetic diversity and biogeography of the macroalgal species associated with the Japanese tsunami marine debris. 2016. PICES 2016 Annual Meeting. Omni San Diego Hotel, San Diego, CA, USA. 2-13 November, 2016. (Oral by Kawai, H.)
- Kawai, H., Hanyuda, T., Hansen, G. 2016. The threat of introduced macroalgal species arriving on Northwestern American shores associated with Japanese tsunami marine debris (JTMD). 9th Asia-Pacific Conference on Algal Biotechnology. Century Park Hotel, Bangkok, Thailand. 15-18 November, 2016. (Oral by Kawai, H.).
- 羽生田岳昭・Hansen Gayle I.・川井浩史「東日本大震災由来の漂着物に着生していた海藻類の種多様性及び遺伝的多様性」日本藻類学会第40回大会 日本歯科大学 2016年3月18-20日
- 羽生田岳昭「東日本大震災由来の漂着物に着生した海藻類の種及び遺伝的多様性について」2016年度藻類談話会 奈良女子大学理学部 2016年11月19日

H. Kawai gave lectures to citizens on the transcontinental introductions of seaweeds including the JTMD macroalgae, and introduced about ADRIFT project as follow:

- 川井浩史「海を渡る侵入者～外来種となった海藻類」ドキドキ齊塾遊学会. 大阪市立大学文化交流センター2014年10月3日
- 川井浩史「外来種としての海藻類」奈良女子大学共生科学研究センター国際シンポジウム「外来生物に関する近年の進展」奈良女子大学. 2014年11月8日
- 川井浩史「海藻類から見た海の環境：環境指標と外来種」神戸大学理学部サイエンスセミナー 神戸大学百年記念館大講堂 2015年7月25日

6. RESEARCH STATUS AND FUTURE STEPS/PLANS

We are planning to produce a flier indicating the gross morphology and important anatomical features for identifying high-profile JTMD macroalgal species in March. We are willing to continue monitoring for detecting new introduction of JTMD macroalgae at selected monitoring sites under collaboration with local phycologists. H. Kawai will visit Grays Harbor in March 2017 as a part of this activity.

H. Kawai will give presentations in the mini-symposium representing the results of ADRIFT project (Tokyo and Sendai), and the lecture in public event in May 2017.