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Yamasaki, Hiroshi
Department of Chemistry, Biology & Marine Science, Faculty of Science, University of the Ryukyus

Hiruta, Shimpei F.
Department of Natural History Sciences, Faculty of Science, Hokkaido University

Kajihara, Hiroshi
Department of Natural History Sciences, Faculty of Science, Hokkaido University

Dick, Matthew H.
Department of Natural History Sciences, Faculty of Science, Hokkaido University

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Two Kinorhynch Species (Cyclorhagida, Echinoderidae, *Echinoderes*) Show Different Distribution Patterns Across Tsugaru Strait, Northern Japan

Hiroshi Yamasaki[1]*, Shimpei F. Hiruta[2], Hiroshi Kajihara[2], and Matthew H. Dick[2]

[1]*Department of Chemistry, Biology & Marine Science, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Nakagami, Okinawa 903-0213, Japan*
[2]*Department of Natural History Sciences, Faculty of Science, Hokkaido University, N10 W8, Sapporo 060-0810, Japan*

We investigated the geographic population structures of two intertidal kinorhynch species, *Echinoderes sensibilis* and *Echinoderes* sp. A, in the vicinity of Tsugaru Strait between Honshu and Hokkaido Islands, Japan, to examine whether the distribution or connectivity of populations of either species has been constrained by the strait. For each species, we examined the geographic distribution of COI haplotypes, constructed a median-joining haplotype network, and calculated statistics of genetic variation and connectivity. Tsugaru Strait is the northern range limit for *E. sensibilis*, which comprises a large, evolutionarily stable metapopulation that appears to have undergone a reduction in size followed by expansion; connectivity is low among most local populations, including across Tsugaru Strait. A divergent haplotype lineage showing no variation occurred only at Horozuki, suggesting recent immigration there from outside the study area. *Echinoderes* sp. A underwent a severe population bottleneck followed by rapid expansion. It occurred at all sampling sites on both sides of the strait, with high connectivity between populations across the strait. There is a zone of secondary contact between moderately divergent, presumably previously allopatric lineages in eastern Hokkaido. Present-day conditions in the strait have existed only for the past 8000 years, and differences in these species' distributions and apparent connectivity across the strait may relate to conditions existing in the strait when the species underwent population expansions or shifts in range; these historical events were not necessarily concurrent between the species, and occurred more than 8000 years ago. We discuss dispersal mechanisms for kinorhynchs, which could include suspension transport or rafting.

Key words: dispersal barrier, Kinorhyncha, phylogeography, intertidal organism, COI, population history

INTRODUCTION

The connectivity, or degree of genetic exchange, among populations of marine organisms is a topic of high current interest, as it is relevant to evolutionary biology (Palumbi, 1994; Lindsay, 2012), biodiversity (Knowlton, 2000), management and conservation biology (Palumbi, 2003; Selkow et al., 2008; Treml et al., 2008; Burton, 2009; Cowen and Sponaugle, 2009), and predicting the effects of climate change on marine ecosystems (Harley et al., 2006). Factors reducing connectivity, leading to evolutionary and possibly adaptive divergence of populations, include low innate dispersal ability, isolation by distance, and marine dispersal barriers (Bernardi, 2000; Riginos and Nachman, 2001; Hare et al., 2005). It can be difficult to predict the degree of connectivity among populations of a particular species based on assumptions concerning the relative importance of these factors, and empirical results have not matched expectations in some studies (e.g., Kyle and Boulding, 2000; Marko, 2004; Lessios and Robertson, 2006).

In the sea, physical impediments to the dispersal of benthic or planktonic marine organisms as adults, propagules, or larvae are often inconspicuous, as they can involve current patterns, depth, differences in temperature or salinity, or the distribution of suitable habitat, all of which require oceanographic effort to detect. As potential dispersal barriers, however, straits are an obvious exception, because they are directly evident from charts. While they are as at least as common a feature of marine landscapes as islands, the extent to which, and scales at which, straits act as barriers or impediments to the dispersal of marine organisms at various scales have not been well explored.

The best-studied example of a strait (or complex of straits) acting as a dispersal barrier is the Central American Seaway between North and South America. This seaway began to restrict gene flow between the Caribbean and Eastern Pacific 25–20 Ma, but became completely closed by the Isthmus of Panama approximately 3.5–3.0 Ma (Jackson and O'Dea, 2013). Molecular and paleontological data show that shallowing of the Seaway caused populations of various marine organisms to diverge on opposite sides of the isth-

* Corresponding author. Tel.   : +81-98-895-8826;
  Fax   : +81-98-895-8386;
  E-mail: h.yamasaki@meiobenthos.com

mus at different times between 12 Ma and 3 Ma, depending on species' depth range (Knowlton and Weigt, 1998; Lessios, 2008).

It is much less clear how smaller straits affect the dispersal of marine organisms at local scales, but there is evidence that they do play a role. For example, it is difficult to explain high diversity and endemism in two bryozoan genera (*Monoporella* and *Cauloramphus*), and an east–west cline in morphology in *Cauloramphus ascopher*, in the western Aleutian archipelago, Alaska (Dick, 2008; Dick et al., 2011) without invoking the numerous straits between Aleutian Islands and island groups as impediments to dispersal. In northern Japan, morphologically indistinguishable intertidal populations of the cheilostome bryozoan *Cauloramphus multispinosus* showed a COI divergence of 11% between Pacific and Sea of Japan populations on Hokkaido Island, separated from one another by Tsugaru Strait (Dick et al., 2013). Other studies have shown Tsugaru Strait to comprise a boundary between local nearshore populations, indicating it might have played a role in the differentiation of those populations by limiting E–W (*Littorina sitkana*; Nohara, 1999) or N–S dispersal (*Ceramium kondoi*; Yang et al., 2008).

These observations led us to investigate Tsugaru Strait as a model system for studying the extent to which a local strait can restrict the dispersal of intertidal and littoral (nearshore benthic) organisms. Tsugaru Strait (41°26′N, 140°36′E) (Fig. 1) is small but locally prominent; it separates Honshu and Hokkaido Islands north to south, and the North Pacific from the marginal Sea of Japan east to west. It is 73 km long between the western and eastern necks, is 18 km and 20 km wide at the two narrowest points, and its shallowest sill depth is 130 m. Currents are predominantly oceanic rather than tidal and are directionally biased: a branch of the warm Tsushima Current running northward in the Sea of Japan flows from west to east through the strait as the Tsugaru Current, which merges with the cold Oyashio Current flowing southward in the Pacific, forming a gyre east of the strait (Conlon, 1981; Sugimoto, 1990; Rosa et al., 2007).

Our long-term goal is to use a comparative approach to understand factors affecting the degree of dispersability across Tsugaru Strait by studying the phylogeography of around 10 nearshore marine species in the vicinity of the strait, chosen so as to represent variation in habitat (e.g., sandy or rocky; infaunal, epifaunal, or sessile) and life-history traits affecting dispersability (e.g., planktonic larval stage vs. direct development). The present study examined the phylogeography in northern Japan of two kinorhynch species, *Echinoderes sensibilis* Adrianov et al., 2002 and *Echinoderes* sp. A, with the specific goal of learning whether Tsugaru Strait has affected the geographical pattern of either species.

Members of the phylum Kinorhyncha are benthic marine meiofauna (up to about 1.1 mm long) that primarily inhabit the upper layer of sediment or interstices among sessile organisms such as ascidians, barnacles, and algae. Kinorhynchs are distributed worldwide from equatorial to polar regions, and comprise approximately 200 species (Sørensen and Pardos, 2008; Herranz and Pardos, 2013; Herranz et al., 2014; Neuhaus, 2013; Sørensen, 2014). Because they lack locomotory cilia, spend their lives in sediment, and are gonochoristic, with eggs laid in the sediment and undergoing direct development (Kozloff, 1972), kinorhynchs should have low innate dispersal ability (Artois et al., 2011). In general, species distributions appear to be regional and limited to a few hundreds of kilometers in extent; only one nominal species (*Campyloderes vanhoeffeni*) has a putative cosmopolitan distribution, although it may represent a species complex (Sørensen and Pardos, 2008; Artois et al., 2011; Neuhaus and Sørensen, 2013).

Echinoderes sensibilis and *Echinoderes* sp. A both occur intertidally in northern Japan. The former was originally described from intertidal pools on the Kii Peninsula, Pacific coast of central Honshu Island, Japan (Adrianov et al., 2002), but was subsequently reported farther east from the Boso Peninsula (Murakami, 2003) and occurs as far north as Tsugaru Strait (this study). *Echinoderes* sp. A was first detected through molecular data in a preliminary study and initially appeared to be restricted to Hokkaido; though very similar to *E. sensibilis*, there are morphological differences (Yamasaki, 2013), and the two differ in COI (cytochrome oxidase *c* subunit I) sequence by 14.3%.

MATERIALS AND METHODS

Sampling and molecular methods

We conducted sampling for the two *Echinoderes* target species
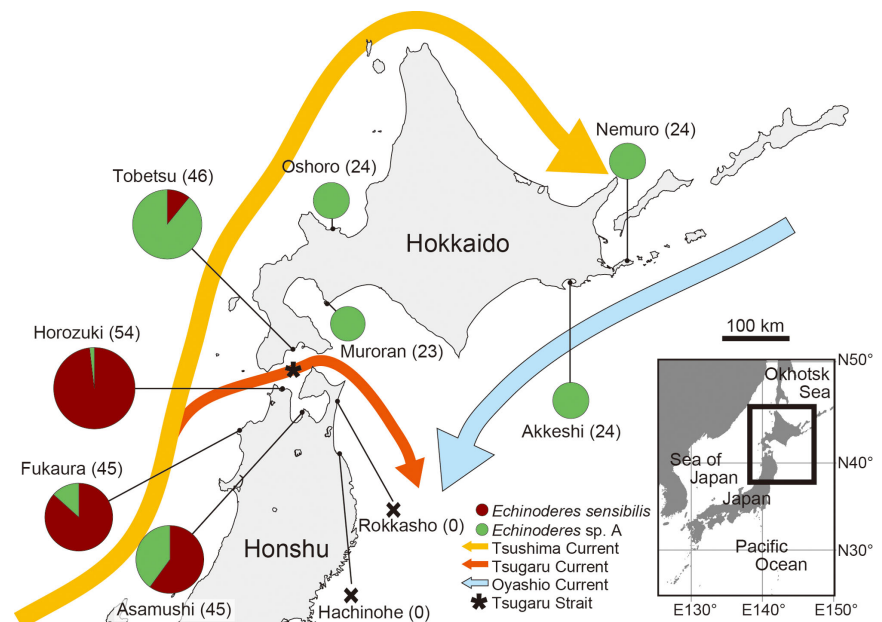
Fig. 1. Map of northern Japan showing sampling localities for *Echinoderes* and ocean currents around Hokkaido Island. Small black circles indicate sampling sites; larger circles indicate the proportions of *E. sensibilis* and *Echinoderes* sp. A detected at each site, with the size of the circle proportional to the total number of individuals analyzed; samples sizes are indicated in parentheses. An X indicates a site at which no *Echinoderes* individuals were detected, despite sampling in appropriate habitat.

at 10 sites (five on Hokkaido, five on Honshu) (Fig. 1, Table 1) in 2011 and 2012. At each site, we obtained sediment debris and organic detritus by washing the surfaces of rocks from intertidal tide pools, along with the attached algae. We extracted kinorhynchs from the debris and detritus by the bubbling and blot method (Sørensen and Pardos, 2008) and preserved them in 99% EtOH. In the laboratory, we extracted total genomic DNA from single individuals by using a DNeasy Tissue Kit (Qiagen, Tokyo), following the protocol of Yamasaki et al. (2013). We recovered the exoskeleton of each specimen from the lysis buffer by centrifugation and mounted it on a glass slide in Hoyer's medium or Fluoromount G as a morphological voucher. We PCR amplified part of the coding region of COI using primer pair LCO (5′-GGTCAACAAATCATAAA-GATATTGG-3′) and HCO (5′-TAAACTTCAGGGTGACCA-AAAAATCA-3′) (Folmer et al., 1994). PCR thermal cycling conditions were 95°C for 1 min; 35 cycles of 95°C for 30 sec, 45°C for 1 min 30 sec, and 72°C for 1 min 30 sec; and 72°C for 7 min. We determined nucleotide sequences by direct sequencing with a BigDye Terminator Kit ver. 3.1 (Life Technologies, USA) and a 3730 DNA Analyzer (Life Technologies, USA), using the same primer pair as above. Sequences were deposited in GenBank under accession numbers AB904214–AB904498. Morphological vouchers were deposited in the invertebrate collection of the Hokkaido University Museum, Sapporo, Japan under catalog numbers ZIHU04459–04716. In this study, identification to species (*E. sensibilis* or *Echinoderes* sp. A) was based entirely on the COI sequence rather than morphology.

Data analysis

We used MEGA version 5 software (Tamura et al., 2011) to align nucleotide sequences, DAMBE 5.2 (Xia and Xie, 2001) to examine haplotype variation, and NETWORK 4.6.1.0 (http://www.fluxus-engineering.com) to construct median-joining (MJ) haplotype networks (Bandelt et al., 1999). To estimate the extent of genetic diversity in each population, we calculated the haplotype diversity (h) and nucleotide diversity (π) (Nei, 1987) with Arlequin 3.5.1.2 software (Excoffier et al., 2005). With the same software, we

also calculated Tajima's D (Tajima, 1989) and Fu's F_S statistics (Fu, 1997) to infer the extent of population expansion. To estimate the extent of divergence between populations, we calculated average pairwise K2P (Kimura 2-parameter) genetic distances (Kimura, 1980) using MEGA 5. Finally, to estimate the relative degree of genetic isolation, we calculated pairwise F_{ST} values among populations for both species using ARLEQUIN 3.5.1.2. We tested the significance of F_{ST} values with 1000 permutations in ARLEQUIN 3.5.1.2. We did not calculate population statistics for *Echinoderes* sp. A at the Horozuki site because we obtained only one specimen there.

RESULTS

Species distributions

We determined COI sequences for 124 specimens of *Echinoderes sensibilis* and 161 of *Echinoderes* sp. A (Table 1). *Echinoderes sensibilis* was the dominant species at three sites on northern Honshu (Fig. 1), detected at moderately (Asamushi) to greatly (Fukaura and Horozuki) higher frequency than *Echinoderes* sp. A, but occurred only at the most southern site (Tobetsu) on Hokkaido, within Tsugaru Strait. We detected *Echinoderes* sp. A at eight sampling sites. It was the dominant species on Hokkaido, where it was the sole species at four sites and co-occurred with *E. sensibilis* only at Tobetsu but at much higher frequency than the latter. We found no individuals of either *Echinoderes* species in appropriate habitat at the two sites located on the Pacific side of Honshu (Fig. 1).

Population structure of *Echinoderes sensibilis*

We identified 34 haplotypes (S01–S34; 571 bp) for *E. sensibilis* (Table 1). The aligned sequences showed 28 variable sites, 23 of which involved synonymous substitutions. Among the 34 haplotypes, 30 were unique to a single sampling site, three (S29, S31, S33) were detected at two sites,

Table 1. Sampling sites for *Echinoderes*, indicating sample sizes and haplotypes detected for two species. Private haplotypes (those detected only in a particular local population) are underlined.

Sampling site	Coordinates	*E. sensibilis* Sample size (% of sample)	*E. sensibilis* haplotypes (Number of individuals) [Number and % of private haplotypes]	*E.* sp. A Sample size (% of sample)	*E.* sp. A (Number of individuals) [Number and % of private haplotypes]
Nemuro	43°23′16″N 145°41′34″E	0	–	24 (100)	<u>H17</u> (1), <u>H18</u> (1), H19 (1), H20 (7), H45 (14) [2, 40%]
Akkeshi	43°00′53″N 144°49′56″E	0	–	24 (100)	<u>H01</u> (1), <u>H02</u> (1), <u>H03</u> (1), <u>H04</u> (1), <u>H05</u> (1), H20 (19) [5, 83%]
Muroran	42°18′48″N 140°58′05″E	0	–	23 (100)	<u>H12</u> (1), <u>H13</u> (1), <u>H14</u> (1), <u>H15</u> (1), <u>H16</u> (1), H35 (1), H45 (17) [5, 71%]
Oshoro	43°12′38″N 140°51′25″E	0	–	24 (100)	<u>H21</u> (1), <u>H22</u> (1), <u>H23</u> (1), <u>H24</u> (1), H37 (1), H45 (19) [4, 67%]
Asamushi	40°54′11″N 140°51′16″E	27 (60)	<u>S01</u> (1), <u>S02</u> (1), <u>S03</u> (1), <u>S04</u> (1), <u>S05</u> (2), S27 (16), S29 (5) [5, 71%]	18 (40)	<u>H06</u> (1), <u>H07</u> (1), <u>H08</u> (1), <u>H09</u> (1), H45 (14) [4, 80%]
Tobetsu	41°44′12″N 140°35′01″E	5 (11)	S33 (3), <u>S34</u> (2) [1, 50%]	41 (89)	<u>H25</u> (1), <u>H26</u> (1), <u>H27</u> (1), <u>H28</u> (1), <u>H29</u> (1), <u>H30</u> (1), <u>H31</u> (1), <u>H32</u> (1), <u>H33</u> (1), <u>H34</u> (1), H35 (1), <u>H36</u> (1), H37 (1), <u>H38</u> (1), <u>H39</u> (1), <u>H40</u> (1), <u>H41</u> (2), <u>H42</u> (1), <u>H43</u> (1), <u>H44</u> (1), H45 (20) [17, 81%]
Horozuki	41°13′35″N 140°32′51″E	53 (98)	<u>S21</u> (1), <u>S22</u> (1), <u>S23</u> (1), <u>S24</u> (1), <u>S25</u> (3), <u>S26</u> (1), S27 (2), <u>S28</u> (1), S29 (7), <u>S30</u> (13), S31(21), <u>S32</u> (1) [9, 75%]	1 (2)	H45 (1) [0]
Fukaura	40°45′59″N 140°03′51″E	39 (87)	<u>S06</u> (1), <u>S07</u> (1), <u>S08</u> (1), <u>S09</u> (1), <u>S10</u> (1), <u>S11</u> (1), <u>S12</u> (1), <u>S13</u> (1), <u>S14</u> (1), <u>S15</u> (2), <u>S16</u> (3), <u>S17</u> (1), <u>S18</u> (2), <u>S19</u> (1), <u>S20</u> (7), S27 (11), S31 (1), S33 (2) [15, 83%]	6 (13)	<u>H10</u> (1), <u>H11</u> (1), H19 (1), H31 (1), H35 (1), H45 (1) [2, 33%]
	Total	124	Total haplotypes = 34	161	Total haplotypes = 45

and one (S27) at three sites (Table 1). A haplotype network (Fig. 2A) shows two lineages differentiated by six nucleotide substitutions, here designated as clusters A (haplotype S30) and B (all other haplotypes), with an average K2P distance of 1.55% between these lineages. Cluster-B haplotypes occurred at all four sites, whereas cluster-A haplotypes were restricted to Horozuki (Fig. 2B).

Overall h and π values for *E. sensibilis* were both high (0.90 and 0.0071, respectively) (Table 2). All four local populations showed high h (> 0.5), and all but one (Horozuki) showed low π (< 0.005). Tajima's D statistics were not significantly different from zero. F_S values were negative and significant for the overall sample ($F_S = -15.72$, $P = 0.001$) and the sample from Fukaura ($F_S = -10.29$, $P < 0.001$).

Average pairwise COI divergences (K2P) among local populations of *E. sensibilis* ranged from 0.44% to 0.85% (Table 3). Pairwise F_{ST} values between populations were all significant, and were moderately high (0.206–0.502) for all comparisons except between Asamushi and Fukaura (0.085). The F_{ST} value between the Tobetsu population and the three Honshu populations combined (Asamushi + Horozuki + Fukaura) was also moderately high and significant ($F_{ST} = 0.258$, $P < 0.001$).

Population structure of *Echinoderes* sp. A

We identified 45 haplotypes (H1–H45; 590 bp) for *Echinoderes* sp. A (Table 1). The aligned sequences showed 42 variable sites, 38 of which involved synonymous substitutions. Among the 45 haplotypes, 39 were each unique to a single sampling site, four (H19, H20, H31, H37) were detected at two sites, one (H35) at three sites, and one (H45) at all sites except
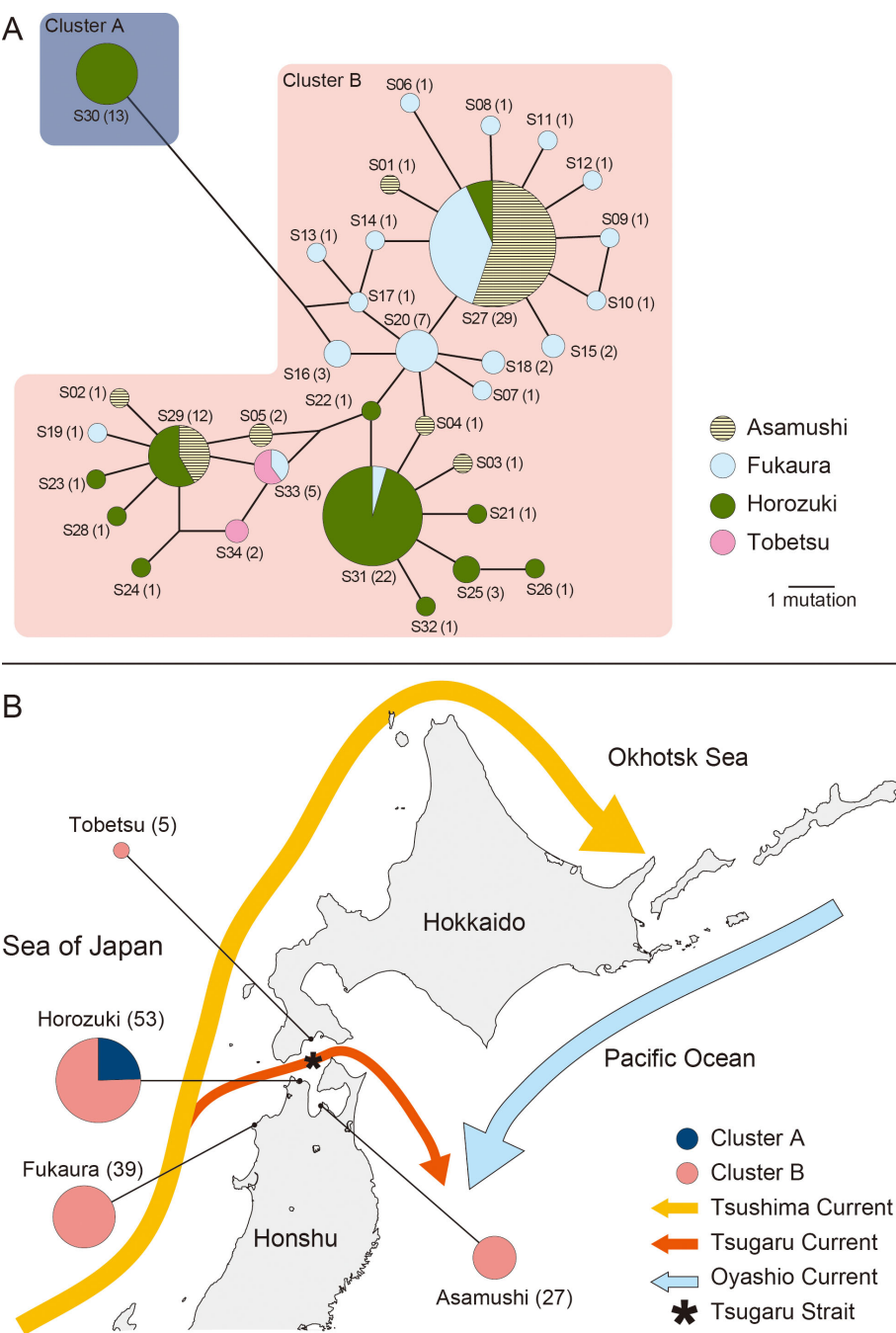

Fig. 2. **(A)** Median-joining network for COI haplotypes detected in *Echinoderes sensibilis* in northern Japan. Each circle represents a haplotype, with the size of the circle proportional to the frequency of the haplotype among four local populations; variable filling inside circles indicates the proportion of haplotype representation by locality (see key at lower right). Line lengths between circles are proportional to the number of nucleotide changes. **(B)** Frequencies of COI haplotypes in clusters A and B at each locality for *Echinoderes sensibilis*. Aside from the site markers, the size of each circle is proportional to sample size, which is given in parentheses.

Table 2. Haplotype diversity (h), nucleotide diversity (π), Tajima's D, and Fu's F_S for four populations of *Echinoderes sensibilis*.

Population	Sample size	Haplotype diversity h (SD)	Nucleotide diversity π (SD)	Tajima's D (P-value)	Fu's F_S (P-value)
Asamushi	27	0.63 (0.095)	0.0044 (0.0027)	0.27 (0.644)	0.11 (0.548)
Tobetsu	5	0.60 (0.175)	0.0011 (0.0012)	1.22 (0.948)	0.63 (0.535)
Horozuki	53	0.77 (0.041)	0.0085 (0.0047)	0.71 (0.807)	0.56 (0.617)
Fukaura	39	0.90 (0.025)	0.0041 (0.0025)	−1.25 (0.098)	−10.29 (< 0.001)
Overall	124	0.90 (0.014)	0.0071 (0.0040)	−0.61 (0.328)	−15.72 (0.001)

Akkeshi (Table 1). A haplotype network (Fig. 3A) shows two lineages separated by four substitutions (average pairwise K2P distance between the lineages = 1.11%), each lineage having one major haplotype (H20 or H45) from which other haplotypes are divergent generally by one or two substitutions to form a star-like topology. We designated these lineages as the eastern cluster (haplotypes H01–H05, H18, H20; detected only at Nemuro and Akkeshi in eastern Hokkaido) and the western cluster (all other haplotypes; detected in western Hokkaido and northern Honshu, but co-occurring with eastern-cluster individuals at Nemuro) (Fig. 3A, B).

Overall h for *Echinoderes* sp. A was moderately high (0.69) and π was moderately low (0.0045) (Table 4). The pattern of values differed between the eastern and western clusters, with the former having low h and π, and the latter having moderately high h and low π. There was variation among sampling sites, with four (Akkeshi, Muroran, Oshoro, Asamushi) of seven sites having low h and π, two (Tobetsu, Fukaura) having high h and low π, and one (Nemuro) having moderately high h and π. Tajima's D and Fu's F_S values for *Echinoderes* sp. A overall, for the eastern and western clusters, and for local populations except Nemuro (and excluding Horozuki) were significant ($P < 0.05$ or $P < 0.01$) negative values.

Pairwise F_{ST} values (Table 5) were high (0.595–0.933) and significant between Akkeshi and all other local populations (excluding Horozuki), and moderately high (0.228–0.250) and significant between Nemuro and the western sites except for Fukaura (0.075; not significant). Pairwise comparisons among populations other than Akkeshi and Nemuro generally gave low, non-significant F_{ST} values (–0.001 to 0.022); Fukaura was again an exception, with a low, significant value (0.083) in the Muroran comparison and a moderately high (0.112), significant value in the Asamushi comparison. K2P distances between populations ranged from 0.05% to 1.12% and were higher for comparisons involving Akkeshi and Nemuro than for other comparisons, due to the occurrence of the divergent eastern clade only at those sites.

DISCUSSION

Inferred population histories

The two species we investigated have somewhat different geographi-

Table 3. Average pairwise K2P distances in percent (above diagonal) and F_{ST} values (below diagonal) among populations of *Echinoderes sensibilis*. **, $P < 0.01$. Sample sizes are indicated in parentheses.

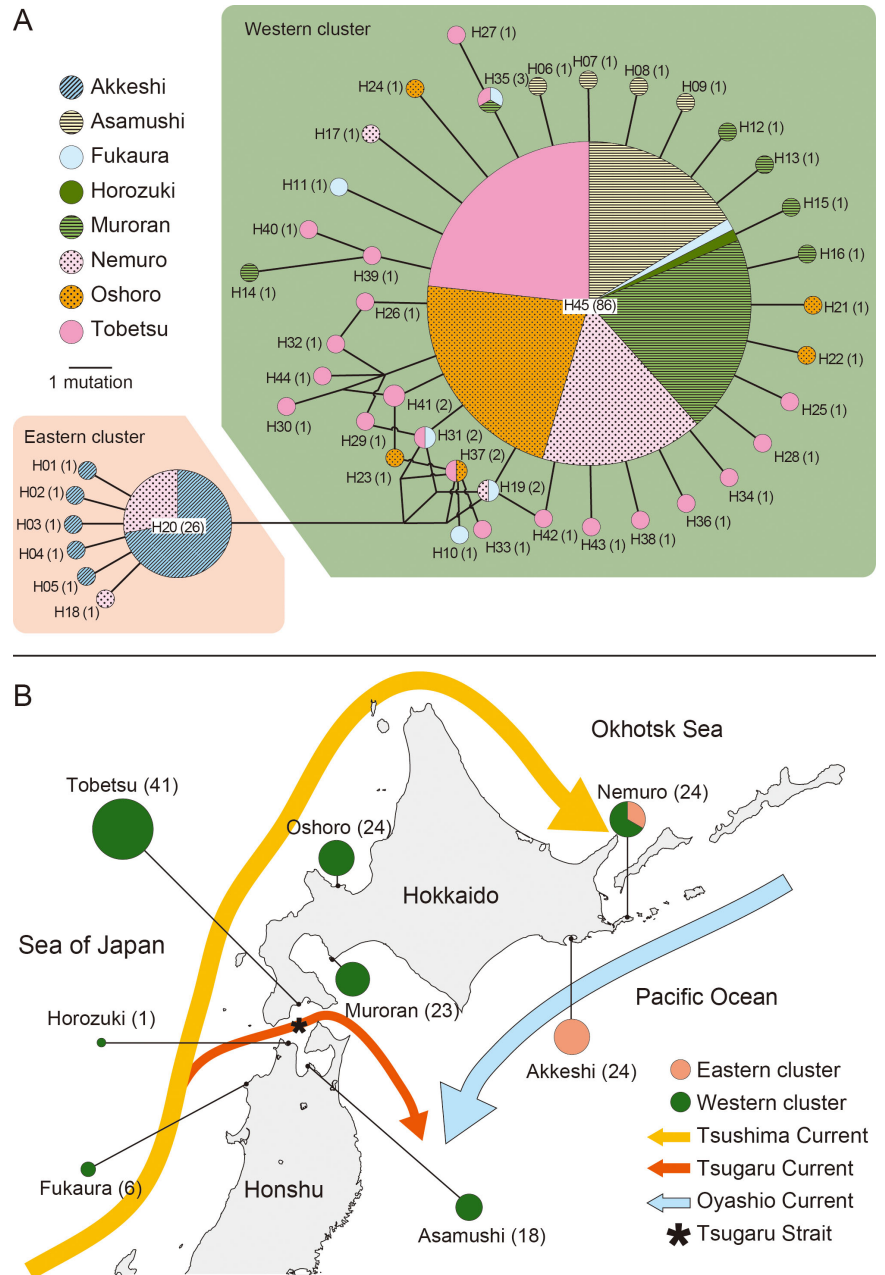|	Asamushi (27)	Tobetsu (5)	Horozuki (53)	Fukaura (39)
Asamushi (27)	–	0.55	0.85	0.44
Tobetsu (5)	0.380**	–	0.83	0.62
Horozuki (53)	0.206**	0.260**	–	0.82
Fukaura (39)	0.085**	0.502**	0.266**	–

Fig. 3. **(A)** Median-joining network for COI haplotypes detected in *Echinoderes* sp. A. in northern Japan. Each circle represents a haplotype, with the size of the circle proportional to the frequency of the haplotype among eight local populations; variable filling inside circles indicates the proportion of haplotype representation by locality (see key at upper left). Line lengths between circles are proportional to the number of nucleotide changes. **(B)** Frequencies of COI haplotypes in the eastern and western clusters at each locality for *Echinoderes* sp. A. Aside from site markers, the size of each circle is proportional to sample size, which is given in parentheses.

Table 4. Haplotype diversity (h), nucleotide diversity (π), Tajima's D, and Fu's F_S for eight populations of *Echinoderes* sp. A, and for pooled populations in the Eastern and Western clusters.

Population	Sample size	Haplotype diversity h (SD)	Nucleotide diversity π (SD)	Tajima's D (P-value)	Fu's F_S (P-value)
Nemuro	24	0.59 (0.08)	0.0052 (0.0031)	0.88 (0.826)	2.39 (0.882)
Akkeshi	24	0.38 (0.13)	0.0007 (0.0007)	−2.00 (0.006)	−4.98 (< 0.001)
Muroran	23	0.46 (0.13)	0.0012 (0.0010)	−2.20 (< 0.001)	−4.68 (< 0.001)
Oshoro	24	0.38 (0.13)	0.0010 (0.0009)	−1.94 (0.008)	−3.78 (0.002)
Asamushi	18	0.41 (0.14)	0.0008 (0.0008)	−1.85 (0.007)	−3.49 (< 0.001)
Tobetsu	41	0.77 (0.07)	0.0023 (0.0016)	−2.26 (< 0.001)	−24.48 (< 0.001)
Fukaura	6	1.00 (0.01)	0.0040 (0.0029)	−1.39 (0.047)	−3.93 (0.002)
Horozuki	1	–	–	–	–
Eastern cluster	32	0.34 (0.11)	0.0006 (0.0007)	−2.10 (0.002)	−6.78 (< 0.001)
Western cluster	129	0.56 (0.06)	0.0015 (0.0012)	−2.63 (< 0.001)	-3.40×10^{21} (< 0.001)
Overall	161	0.69 (0.04)	0.0045 (0.0027)	−1.92 (0.006)	−26.64 (< 0.001)

Table 5. Average pairwise K2P distances in percent (above diagonal) and F_{ST} values (below diagonal) among populations of *Echinoderes* sp. A. *, $P < 0.05$; **, $P < 0.01$. Sample sizes are indicated in parentheses.

	Nemuro (24)	Akkeshi (24)	Muroran (23)	Oshoro (24)	Asamushi (18)	Tobetsu (41)	Fukaura (6)	Horozuki (1)
Nemuro (24)	–	0.74	0.43	0.41	0.41	0.48	0.51	0.37
Akkeshi (24)	0.595**	–	1.12	1.08	1.10	1.14	1.09	1.06
Muroran (23)	0.250**	0.916**	–	0.11	0.10	0.18	0.25	0.06
Oshoro (24)	0.244**	0.922**	0.006	–	0.09	0.17	0.24	0.05
Asamushi (18)	0.241**	0.933**	−0.003	0.005	–	0.16	0.24	0.85
Tobetsu (41)	0.228**	0.848**	0.001	−0.001	0.002	–	0.62	0.83
Fukaura (6)	0.075	0.871**	0.083*	0.091	0.112**	0.022	–	0.82
Horozuki (1)	–	–	–	–	–	–	–	–

cal population structures, although both appear to have undergone population reduction followed by expansion. Overall, *Echinoderes sensibilis* showed high h and π (> 0.5 and > 0.005, respectively) (Table 2), indicative of a large, evolutionarily relatively stable population (Grant and Bowen, 1998). The haplotype network (Fig. 2A) bears this out, with four common haplotypes (S27, S29, S30, S31) interconnected by three to 11 mutational steps. Consistent with this inferred history is the broad known range of the species, which extends from Tsugaru Strait at least 1200 km southward to the Kii Peninsula and probably beyond that. Nonetheless, a significant large, negative Fu's F_S value (Table 2) indicates less than expected variation, suggesting that the overall population sampled underwent a past reduction in size followed by expansion (Ray et al., 2003). In this interpretation, the four common haplotypes in clusters A (S30) and B (S27, S29, S31) in the haplotype network (Fig. 2A) represent ancestral variation retained during the population reduction, whereas haplotypes divergent by one or two mutations, forming a star-like topology with each of the core haplotypes, represent variation accumulated during expansion. The simplest explanation for divergent cluster A, which comprised a single common haplotype (S30) detected only at Horozuki, is that this haplotype immigrated from an allopatrically divergent lineage occurring outside the study area, with the migration so recent that no genetic variation has accumulated.

Overall, *Echinoderes* sp. A showed high h and low π, indicative of a population bottleneck followed by rapid expansion (Grant and Bowen, 1998). The haplotype network (Fig. 3A) is dominated by a common, widespread haplotype (H45), detected in 53% of the individuals sampled, and at seven of eight sampling sites. A less common and widespread haplotype (H20) contributed to a separate cluster restricted to eastern Hokkaido. Each cluster shows a star-like topology. Significantly large, negative Tajima's D and Fu's F_S values overall and for each cluster (Table 4) indicate less variation than expected and corroborate a population reduction followed by expansion. The simplest explanation for the limited distribution of the eastern cluster is that it represents an allopatrically diverged lineage, formerly restricted farther to the northeast, whose range is expanding southwestward along the Kurile Archipelago, forming a zone of secondary contact with the western lineage at Nemuro. Haplotype and nucleotide diversities for the eastern cluster (Table 4) are both low, and both lower than for the western cluster, suggestive of a founder event associated with a range expansion. The apparent lack of western-cluster haplotypes at Akkeshi is interesting, and suggests that the western lineage went locally extinct there prior to an influx of eastern-cluster haplotypes; the eastern lineage arrived in Akkeshi long enough ago that we detected five private haplotypes (H01–H05; Table 1) occurring there but not at Nemuro.

Connectivity among populations

For *E. sensibilis*, connectivity was restricted (range of F_{ST}, 0.206–0.502) among most of the four local populations sampled (Table 3), but less so between Fukaura and Asamushi ($F_{ST} = 0.085$), which is difficult to explain because Horozuki lies between Fukaura and Asamushi yet shows less connection to either population. Degree of isolation across Tsugaru Strait between Tobetsu on Hokkaido and the three stations on northern Honshu increased with increasing straight-line distance, in the order Horozuki ($F_{ST} = 0.260$; 60 km), Asamushi (0.380; 90 km), Fukaura (0.502; 135 km).

For *Echinoderes* sp. A, there was high connectivity among most of the populations in western Hokkaido and northern Honshu, with non-significant F_{ST} values ranging from −0.003 to 0.006 (Table 5). The exception was Fukaura

on the Sea of Japan with somewhat higher values (F_{ST}, 0.083–0.112) in comparisons with Oshoro, Muroran, and Asamushi. Connectivity was reduced between Nemuro and all sites in western Hokkaido and northern Honshu (F_{ST}, 0.228–0.250) except Fukaura (0.075), and quite low between Akkeshi and all other sites (F_{ST}, 0.595–0.933). The low sample size at Fukaura may have been a factor in the somewhat aberrant values in comparisons, although two of its F_{ST} values were significant ($P < 0.01$). Fukaura is also the farthest southwestern extent of the known range of *Echinoderes* sp. A, and the low frequencies of this species at Horozuki and Fukaura might indicate these localities really are near the limit of the range. The high, significant values in most comparisons involving Nemuro and Akkeshi were undoubtedly due to the occurrence of haplotypes in the divergent eastern lineage only at those sites.

Tsugaru Strait as a dispersal barrier

Tsugaru Strait has a complex geological and paleoceanographic history. Present-day oceanographic conditions in the strait have existed only for roughly the past 8000 years; during at least one previous interval (95–27 ka) the Tsushima Current (and thus also the Tsugaru Current) did not exist, and during another interval (17–10 ka) the cold Oyashio Current flowed westward through the strait (Oba, 2006). Tsugaru Strait became much shallower during glacial maxima throughout the Pleistocene, but it remains controversial whether the strait was ever completely closed by a land bridge (Fujii, 1990; Ohshima, 1990; Yashima and Miyauchi, 1990; Keigwin and Gorbarenko, 1992) due to uncertainty over the magnitude of sea level drops during glacial maxima.

The lowest average inter-population K2P distances for either species were in the range of 0.09–0.18% in comparisons of local populations of *Echinoderes* sp. A among Oshoro, Asamushi, Muroran, and Tobetsu (Table 5); a COI divergence rate of 1.6% Myr^{-1}, the average of five published COI rate values for marine invertebrates (Dick et al., 2009), gives divergence times of 56–113 ka. While this estimate is extremely rough, it suggests that the observed ranges and geographical population structures of the two kinorhynchs were shaped over a much longer time span than during the past 8000 years. This means that any role Tsugaru Strait played in determining these patterns likely occurred when conditions in the strait were different from those today.

Moderately high F_{ST} values (0.260–0.502) for *E. sensibilis* between Tobetsu and the Honshu sites indicate currently limited genetic exchange across the strait. This in itself does not clearly implicate the strait as a causative factor in the range boundary, however, because F_{ST} values are similarly moderately high in comparisons bewtween Horozuki and the other two sites on Honshu (Fukaura, F_{ST} = 0.266; Asamushi, F_{ST} = 0.206). The main evidence that Tsugaru Strait has somehow restricted *E. sensibilis* dispersal is that it constitutes the northern range boundary of this species. We detected *E. sensibilis* on Hokkaido only at Tobetsu, the site closest to any of the Honshu sites (60 km from Horozuki), and it may be that *E. sensibilis* occasionally disperses across the strait but establishes only ephemeral populations there. Of the two haplotypes observed among five individuals at Tobetsu, however, one (S33) was shared only with the distant Fukaura population, and one was a private haplotype, suggesting that dispersal was not from Horozuki and was not recent.

A possible explanation for the northern distributional boundary of *E. sensibilis* at Tsugaru Strait is that this is a warm-adapted species that reaches a limit of temperature tolerance at Tsugaru Strait. However, while Nemuro and Akkeshi in eastern Hokkaido show average summer and winter sea surface temperatures 5–6°C lower than in the vicinity of Tsugaru Strait, our study sites in northern Honshu and southwestern Hokkaido all fall within the same 2°C isotherm interval in summer and winter, and annual mean salinity nearshore varies little, from 33.5 to 34.5‰ (Tomczak and Godfrey, 2003). There is thus no obvious environmental explanation why we failed to detect *E. sensibilis* at Oshoro or Muroran in southern Hokkaido.

While there is no genetic or distributional evidence that Tsugaru Strait has acted as a N-S dispersal barrier for *Echinoderes* sp. A, our results are not informative as to whether the strait currently inhibits dispersal. Dispersal across the strait could have preceded the past 8000 years, the interval during which conditions currently existing in the strait have prevailed, with the low F_{ST} values between the Hokkaido (Tobetsu, Muroran, Oshoro) and northern Honshu (Fukaura, Asamushi) populations due to widespread, common haplotype H45 (Table 1; Fig. 3B) and reflecting geologically recent but currently restricted gene flow. Historical factors that could produce low F_{ST} values include geologically recent shared ancestry of geographically fragmented populations, or an extensive, geologically recent range expansion from a single local population (Whitlock and McCauley, 1998; Templeton, 1998). High proportions of private haplotypes per site (33–81% at sites with more than one specimen; Table 1) indicate that gene flow among populations may in fact be less than the low F_{ST} values suggest.

Both *E. sensibilis* and *Echinoderes* sp. A appear to be confined to the intertidal and nearshore subtidal zones. This raises the question how these sediment-dwelling, direct-developing, nearshore species disperse at all across the strait, which is 18 km and 20 km across at its two narrowest points. One possibility is that individuals dislodged from the sediment by wave action become suspended in the water column and are transported by wind-generated, tidal, or oceanic currents. In fact, suspension is a common occurrence for meiofauna (Palmer, 1988; Boeckner et al., 2009), including kinorhynchs (Palmer and Gust, 1985), and transport rates of up to 10 km/day have been documented for suspended meiofauna (Hagerman and Rieger, 1981). The other possibility is dispersal by rafting. Drifting wood or clumps of detached algae or marine grasses can harbor diverse assemblages of sessile and motile macrobenthic invertebrates (Highsmith, 1985; Thiel and Gutow, 2005) and can transport them hundreds to thousands of kilometers (Jokiel, 1990; Fraser et al., 2011). Kinorhynchs could potentially be transported in sediment bound in holdfasts, in crevices in the rafts themselves, or among sessile animals attached to the rafts.

The question remains as to why the distributions of the two species differ with respect to Tsugaru Strait. A mechanistic hypothesis is that unknown differences in the natural histories of these species affect their susceptibility to disper-

sal by suspension transport or rafting. However, historical scenarios could also explain the differences between the two species in geographical pattern and estimated connectivity among populations. For example, assume that the low stand sea level during the last glacial maximum (roughly 19–26 ka) approached or exceeded –130 m, the present sill depth, and that a shoal area or land bridge formed between Hokkaido and Honshu, permitting uninterrupted N–S dispersal of intertidal species along the Pacific and Sea of Japan coasts. Also assume that *Echinoderes* sp. A was a more northerly distributed, cold-adapted species and *Echinoderes sensibilis* a more southerly distributed, warm-adapted species. In this scenario, if cooler sea-surface temperatures during the glacial maximum caused the ranges of both species to shift southward, *Echinoderes* sp. A but not *Echinoderes sensibilis* would have dispersed southward along the shores of the land bridge. As the climate subsequently warmed, Tsugaru Strait reappeared and both species' ranges shifted northward. Assuming that neither species has dispersed well across the strait under conditions subsequent to the last glacial maximum, *Echinoderes* sp. A would have been left with a distribution on both sides of the strait, whereas *Echinoderes sensibilis* would have stopped at the strait, possibly with only infrequent, temporary incursions across. This scenario shows how differences in the apparent connectivity of populations across the strait could be due to historical factors—e.g., differences between the two species in historical ranges, climatic adaptation, and the magnitude and timing of population reductions and expansions.

We are aware of some deficiencies in our study. Broader geographical sampling might have clarified whether the eastern cluster of *Echinoderes* sp. A is in fact distributed along the Kurile Archipelago northeast of Hokkaido; detected the southern extent of the range of *Echinoderes* sp. A; and possibly detected the source of the cluster-A population of *E. sensibilis* at Horozuki. In addition, our study is based on a single, uniparentally inherited mitochondrial locus, which can give a non-representative view of demographic history, or a misleading one if selection is involved (Hare, 2001). On the other hand, mtDNA loci appear to be more robust and sensitive indicators of patterns of population history than nuclear loci (Zink and Barrowclough, 2007). Future work with broader geographical coverage and additional markers can test our results.

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REFERENCES

Adrianov AV, Murakami C, Shirayama Y (2002) Taxonomic study of the Kinorhyncha in Japan. 3. *Echinoderes sensibilis* n. sp. (Kinorhyncha: Cyclorhagida) from Tanabe Bay. Zool Sci 19: 463–473

Artois T, Fontaneto D, McInnes S, Todaro MA, Sørensen MV, Zullini A (2011) Ubiquity of microscopic animals? Evidence from the morphological approach in species identification. In "Biogeography of Microscopic Organisms: Is Everything Small Everywhere?" Ed by D Fontaneto, Cambridge University Press, Cambridge, pp 244–283

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16: 37–48

Bernardi G (2000) Barriers to gene flow in *Embiotoca jacksoni*, a marine fish lacking a pelagic larval stage. Evolution 54: 226–237

Boeckner MJ, Sharma J, Proctor HC (2009) Revisiting the meiofauna paradox: dispersal and colonization of nematodes and other meiofaunal organisms in low- and high-energy environments. Hydrobiologia 624: 91–106

Burton RS (2009) Molecular markers, natural history, and conservation of marine animals. BioScience 59: 831–841

Conlon DM (1981) Dynamics of Flow in the Region of the Tsugaru Strait. Technical Report, Coastal Studies Institute, Louisiana State University, Baton Rouge

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Annu Rev Mar Sci 2009.1: 443–466

Dick MH (2008) Unexpectedly high diversity of *Monoporella* (Bryozoa: Cheilostomata) in the Aleutian Islands, Alaska: taxonomy and distribution of six new species. Zool Sci 25: 36–52

Dick MH, Hirose M, Mawatari SF (2013) Molecular distance and morphological divergence in *Cauloramphus* (Cheilostomata: Calloporidae). In "Bryozoan Studies 2010, Lecture Notes in Earth System Sciences 143A" Ed by A Ernst, P Schäfer, J Scholz, Springer-Verlag, Berlin, pp 29–44

Dick MH, Lidgard S, Gordon DP, Mawatari SF (2009) The origin of ascophoran bryozoans was historically contingent but likely. Proc Roy Soc B 276: 3141–3148

Dick MH, Mawatari SF, Sanner J, Grischenko AV (2011) Cribrimorph and other *Cauloramphus* species (Bryozoa: Cheilostomata) from the northwestern Pacific. Zool Sci 28: 134–147

Excoffier L, Laval G, Schneider S (2005) Arlequin (ver. 3.0): An integrated software package for population genetics data analysis. Evol Bioinform Online 1: 47–50

Folmer O, Black W, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294–299

Fraser CI, Nikula R, Waters JM (2011) Oceanic rafting by a coastal community. Proc Roy Soc B 278: 649–655

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–925

Fujii S (1990) Changes of the palaeoenvironment along the Japan Sea since the early Pleistocene. Quaternary Res 29: 173–182 (in Japanese with English summary)

Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J Hered 89: 415–426

Hagerman GM, Rieger RM (1981) Dispersal of benthic meiofauna by wave and current action in Bogue Sound, North Carolina, USA. Mar Ecol 2: 245–270

Hare MP (2001) Prospects for nuclear gene phylogeography. Trends Ecol Evol 16: 700–706

Hare MP, Guenther C, Fagan WF (2005) Nonrandom larval dispersal can steepen marine clines. Evolution 59: 2509–2517

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, et al. (2006) The impacts of climate change in coastal marine systems. Ecol Lett 9: 228–241

Herranz M, Pardos F (2013) *Fissuroderes sorenseni* sp. nov. and *Meristoderes boylei* sp. nov.: first Atlantic recording of two rare kinorhynch genera, with new identification keys. Zool Anz 243: 93–111

Herranz M, Sánchez N, Pardos F, Higgins RP (2014) New Kinorhyncha from Florida coastal waters. Helgoland Mar Res 68: 59–87

Highsmith RC (1985) Floating and algal rafting as potential dispersal mechanisms in brooding invertebrates. Mar Ecol Prog Ser 25: 169–179

Jackson JBC, O'Dea A (2013) Timing of oceanographic and biological isolation of the Caribbean Sea from the Tropical Eastern Pacific Ocean. Bull Mar Sci 89: 779–800

Jokiel PL (1990) Long-distance dispersal by rafting: reemergence of an old hypothesis. Endeavour NS 14: 66–73

Keigwin LD, Gorbarenko SA (1992) Sea level, surface salinity of the Japan Sea, and the younger Dryas Event in the northwestern Pacific Ocean. Quaternary Res 37: 346–360

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111–120

Knowlton N (2000) Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420: 73–90

Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. Proc Roy Soc Lond B 265: 2257–2263

Kozloff EN (1972) Some aspects of development in *Echinoderes* (Kinorhyncha). Trans Am Microsc Soc 91: 119–130

Kyle CJ, Boulding EG (2000) Comparative populations genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar Biol 137: 835–845

Lessios HA (2008) The great American schism: divergence of marine organisms after the rise of the Central American Isthmus. Ann Rev Ecol Evol Syst 39: 63–91

Lessios HA, Robertson DR (2006) Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. Proc Roy Soc B 273: 2201–2208

Lindsay SM (2012) Dispersal of marine organisms and the grand challenges in biology: an introduction to the symposium. Integr Comp Biol 52: 443–446

Marko PB (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Mol Ecol 13: 597–611

Murakami C (2003) Studies on kinorhynchs, reporting a new distributional area in Japan. Ann Rep Mar Biol Res Inst Jpn 2003: 43–50 (in Japanese)

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York

Neuhaus B (2013) Kinorhyncha (=Echinodera). In "Handbook of Zoology. Gastrotricha, Cycloneuralia and Gnathifera. Volume 1: Nematomorpha, Priapulida, Kinorhyncha, Loricifera" Ed by A Schmidt-Rhaesa, De Gruyter, Berlin, pp 181–348

Neuhaus B, Sørensen MV (2013) Populations of *Campyloderes* sp. (Kinorhyncha, Cyclorhagida): One global species with significant morphological variation? Zool Anz 242: 48–75

Nohara M (1999) Genetic and conchological variation in *Littorina sitkana* Philippi (Mollusca, Gastropoda) on northern Japanese coasts. Zool Sci 16: 309–317

Oba T (2006) Paleoenvironmental changes in the Japan Sea and off Kashima over the last 150 kyr based on oxygen and carbon isotopes of foraminiferal tests. J Geogr 115: 652–660

Ohshima K (1990) The history of straits around the Japanese Islands in the late-Quaternary. Quaternary Res 29: 193–208

Palmer MA (1988) Dispersal of marine meiofauna: a review and conceptual model explaining passive transport and active emergence with implications for recruitment. Mar Ecol Prog Ser 48: 81–91

Palmer MA, Gust G (1985) Dispersal of meiofauna in a turbulent tidal creek. J Mar Res 43: 179–210

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25: 547–572

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13 Suppl: S146–S158

Ray N, Currat M, Excoffier L (2003) Intra-deme molecular diversity in spatially expanding populations. Mol Bio Evol 20: 76–86

Riginos C, Nachman MW (2001) Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. Mol Ecol 10: 1439–1453

Rosa AL, Isoda Y, Uehara K, Aiki T (2007) Seasonal variations of water system distribution and flow patterns in the southern sea area of Hokkaido, Japan. J Oceanogr 63: 573–588

Selkow KA, Henzler CM, Gaines SD (2008) Seascape genetics and the spatial ecology of marine populations. Fish Fisheries 9: 363–377

Sørensen MV (2014) First account of echinoderid kinorhynchs from Brazil, with the description of three new species. Mar Biodivers, doi: 10.1007/s12526-013-0181-4 [Online first]

Sørensen MV, Pardos F (2008) Kinorhynch systematics and biology — an introduction to the study of kinorhynchs, inclusive identification keys to the genera. Meiofauna Mar 16: 21–73

Sugimoto T (1990) A review of recent physical investigations on the straits around the Japanese Islands. In "The Physical Oceanography of Sea Straits" Ed by LJ Pratt, Kluwer Academic Publishers, Dordrecht, pp 191–209

Tajima F (1989) The effect of change in population-size on DNA polymorphism. Genetics 123: 597–601

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2734

Templeton AR (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol Ecol 7: 381–397

Thiel M, Gutow L (2005) The ecology of rafting in the marine environment. II. The rafting organisms and community. Oceanogr Mar Biol Ann Rev 43: 279–418

Tomczak M, Godfrey JS (2003) Regional Oceanography: An Introduction. 2nd ed, Daya Publishing House, Delhi

Treml EA, Halpin PN, Urban DL, Pratson LF (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landscape Ecol 23: 19–36

Whitlock MC, McCauley DE (1998) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82: 117–125

Xia XH, Xie Z (2001) DAMBE: Data analysis in molecular biology and evolution. J Hered 92: 371–373

Yamasaki H (2013) Taxonomic, Phylogeographic, and Molecular Phylogenetic Studies in Phylum Kinorhyncha. PhD Thesis, Department of Natural History Sciences, Hokkaido University, Sapporo, Japan

Yamasaki H, Hiruta SF, Kajihara H (2013) Molecular phylogeny of kinorhynchs. Mol Phylogenet Evol 67: 303–310

Yang EC, Cho GY, Kogame K, Carille AL, Boo SM (2008) RuBisCO cistron sequence variation and phylogeography of *Ceramium kondoi* (Ceramiaceae, Rhodophyta). Bot Mar 51: 370–377

Yashima K, Miyauchi T (1990) The Tsugaru land bridge problem related to Quaternary coastal tectonics, northeast Japan. Quaternary Res 29: 267–275

Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. Mol Ecol 17: 2107–2121