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Research Article

Possible origins of planktonic copepods, *Pseudodiaptomus marinus* (Crustacea: Copepoda: Calanoida), introduced from East Asia to the San Francisco Estuary based on a molecular analysis

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Abstract

The calanoid copepod *Pseudodiaptomus marinus* Sato, 1913 is native to the coasts of East Asia, but has occurred in the San Francisco Estuary (SFE) as a non-indigenous species since 1986. Genetic analysis of the mitochondrial cytochrome *b* locus (400 bp) was used to infer the possible origin(s) of the introduced SFE population by comparison between the SFE population and populations from potential donor areas in Japan, Korea, and China. We detected 39 haplotypes from 131 individuals from 7 localities. The SFE population showed a high haplotype diversity and significant F_{st} values among the localities, suggesting that multiple introductions from several localities in Japan may have occurred, although introductions from Korea could not be ruled out. However, based on the morphological and molecular data the population from Xiamen, China, previously identified as *P. marinus*, was found to be a separate species. Possible vectors for *P. marinus* include ship ballast water or aquaculture. Our results also suggest the origin of *P. marinus* in the Asian continent and subsequent isolation and expansion of populations in Japan.

Key words: aquaculture, ballast water, calanoid, introduction, mitochondrial cytochrome *b,* population

Introduction

Introductions of non-indigenous marine organisms with ballast water have caused substantial environmental and economic problems in the world's oceans (Carlton et al. 1990; Nichols et al. 1990; Carlton 1992; Travis 1993; Ruiz et al. 1997). Estuaries and bays along the Pacific coast of the USA, notably the San Francisco Estuary (SFE), have received thousands of non-indigenous aquatic organisms via ballast water and other vectors (Carlton 1992; Cohen and Carlton 1997, 1998; Carlton et al. 2017). Since 2000, ships entering California have been required to exchange ballast water with oceanic water during the voyage to limit the introduction of non-indigenous organisms to waters of the state. Although ballast exchange seems effective in removing exotic zooplankton (Choi et al. 2005), some non-indigenous species had already become established in the region by 2000, including *Oithona davisae* Ferrari and Orsi, 1984 (year first detected in SFE: 1963), *Sinocalanus doerrii* (Brehm, 1909) (1978), *Limonoithona sinensis* (Burckhardt, 1913) (1979), *Pseudodiaptomus marinus* Sato, 1913 (1986), *P. forbesi* (Poppe and Richard, 1890) (1987), *Acartiella sinensis* Shen and Lee, 1963 (1993), *Tortanus dextrilobatus* Chen and Zhang, 1965 (1993), and *L. tetraspina* Zhang and Li, 1976 (1993) (Orsi et al. 1983; Orsi and Ohtsuka 1999), some of which are considered invasive.

The above eight non-indigenous copepod species originating from East Asia remain in the estuary and no extant copepod species have been extirpated from the SFE. These species have had numerous effects on extant zooplankton, although impacts are difficult to detect given the overwhelming influence of the clam *Potamocorbula amurensis* (Schrenck, 1861), introduced in 1986 (Nichols et al. 1990). For example, the previously abundant copepod *Eurytemora affinis* (Poppe, 1880) (cf. *E. carolleeae* Alekseev and Souissi, 2011) plummeted in abundance in 1987, roughly coincident with the introductions of *P. marinus* and *P. forbesi*, but also with the spread of the clam which had a devastating effect on abundance (Kimmerer et al. 1994; Kimmerer and Lougee 2015). Note that *E. affinis* was itself apparently introduced to the SFE (Lee 2000), perhaps with the deliberate introduction of striped bass *Morone saxatilis* (Walbaum, 1792) from the U.S. east coast during the nineteenth century (Orsi 2001). Other effects of the introduced copepods include reduction of food resources for fish through the strong escape responses of some introduced species (Meng and Orsi 1991), and small size of others making detection difficult and feeding inefficient (Gould and Kimmerer 2010; Sullivan et al. 2016). In addition, the introduction of the predatory copepod *Acartiella sinensis* in 1993 (Orsi and Ohtsuka 1999) increased mortality of copepod nauplii in the low-salinity zone of the estuary (Slaughter et al. 2016) and may have reduced the abundance of several other species, notably *P. forbesi* (Kayfetz and Kimmerer 2017). Subsequent to their introductions into SFE, several copepod species became established in the Columbia River system (Sytsma et al. 2004; Cordell et al. 2008; Dexter et al. 2015). However, no introductions of copepods to the SFE have been reported since 1993, possibly because of California's regulations requiring at-sea exchange of ballast water. Despite increased public awareness and obvious concerns over the spread of non-native species, few studies of planktonic copepods introduced from East Asia to North America have concentrated on spread and local ecological dynamics.

The particle-feeding calanoid copepod *Pseudodiaptomus marinus*, whose adults and late copepodids are epibenthic during the daytime and planktonic at night, was originally described by Sato (1913) from Hokkaido, Japan. It is naturally distributed in estuaries and coastal marine waters in East Asia including China (Shen and Lee 1963; Chen and Zhang 1965), Korea (Eyun et al. 2007) and Japan (Hirota 1962, 1964; Tanaka 1966; Tanaka and Hue 1966; Uye et al. 1982; Walter 1986). This species has been progressively introduced into bays and estuaries around the world. The first such report was from Hawaii (Jones 1966). More recently it has been introduced to coastal bays along the west coast of North America. It was detected in Mission Bay and Agua Hedionda Lagoon, Southern California, in 1986 (Fleminger and Kramer 1988) and in the SFE the same year (Orsi and Walter 1991), and has also been reported from Tomales Bay, California (Kimmerer 1993) and the Pacific coast of Mexico (Jimenez-Perez and Castro-Longoria 2006). Recently the species has been spreading into European coastal waters of the Atlantic Ocean, North Sea, and Mediterranean Sea (de Olazabal and Tirelli 2011; Brylinski et al. 2012; Sabia et al. 2014; Lučić et al. 2015). This distributional expansion seems to be partly through its tolerance to a wide range of environmental factors (Liang et al. 1996; Liang and Uye 1997). In the SFE, the species has remained abundant since its introduction, providing food for larval and juvenile fish (Bryant and Arnold 2007). Furthermore, *P. marinus* may be expanding its range from the SFE to other Northeast Pacific regions: it was collected at relatively high densities and frequencies of occurrence from ballast tanks of 44 ships entering Puget Sound during 2001–2007 that listed the San Francisco estuary as the ballast source (Cordell et al. 2008).

The objective of this study was to clarify the possible origins of the San Francisco Estuary population of *P. marinus* by employing a molecular analysis to compare between populations in the receiver and possible donor areas. This study may be informative for efforts to limit future range extensions of invasive copepods around the world.

Material and methods

Sampling

Samples of *Pseudodiaptomus marinus* were collected between June 2007 and October 2008 from three regions, Japan, Korea (East Asia) and the San Francisco Estuary; and from Xiamen, China on September 20, 2008. A NORPAC net (diameter 45 cm; mesh size 0.3 mm) or a small conical net (diameter 30 cm; mesh size 0.1 mm) were used for collection during the day and night (Supplementary material Table S1; see Figure 3). Samples were preserved in 99.5% ethanol immediately after collection, and copepods were identified under a binocular microscope.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following manufacturer-recommended protocols. Polymerase chain reaction amplifications were carried out with ASTEC PC-812 (ASTEC, Fukuoka, Japan) using a TaKaRa Ex Taq (TaKaRa BIO, Shiga, Japan) Reaction Kit. A portion of the mitochondrial cytochrome *b* (cyt-*b*) locus (400 bp) was amplified using the primers UCYTB151F (5'-TGT GGR GCN ACY GTW ATY ACT AA -3') and UCYTB270R (5'- AAN AGG AAR TAY CAY TCN GGY TG -3') (Merritt et al. 1998). Thermal cycling was performed as follows: a pre-dwell at 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 30 s at 50 °C, and 1 min at 72 °C, and post-dwell at 72 °C for 4 min. Amplified PCR products were purified by PEG purification (Lis 1980), cycle-sequenced from both strands using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), alcohol-precipitated, and sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Sequences were uploaded to GenBank (Accession No. AB920868–AB920906; see Supplementary material Table S2).

Sequence analysis

Using ARLEQUIN v. 2.0 (Schneider et al. 2000) haplotype (Hd) and nucleotide (π) diversities were measured for each population; population pairwise *F*st was used to examine genetic differences between pairs of sampling areas. Haplotype networks were reconstructed using TCS v. 1.21 to find gene genealogies and regional distributions of mtDNA haplotypes (Clement et al. 2000). Mismatch distribution analyses to explore demographic patterns of populations were performed by DNAsp v.5 (Librado and Rozas 2009). In addition, two neutrality tests, Tajima's *D* (Tajima 1989) and Fu's *F*s (Fu 1997), were performed using ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). Haplotype diversity was calculated for each sample, and approximate 90% confidence intervals were determined through bootstrap resampling (function *boot* in R version 3.2.3, R Development Core Team 2015).

Results

Genetic and haplotype diversity

Thirty-nine haplotypes were found in a total of 131 individuals from seven localities (Table 1; see also Supplementary Table S1). Haplotype (H, 0.929) and nucleotide (π, 0171) diversities were highest in Haeui Is., Korea, followed by SFE (0.858 (H), 0.0046 (π)).

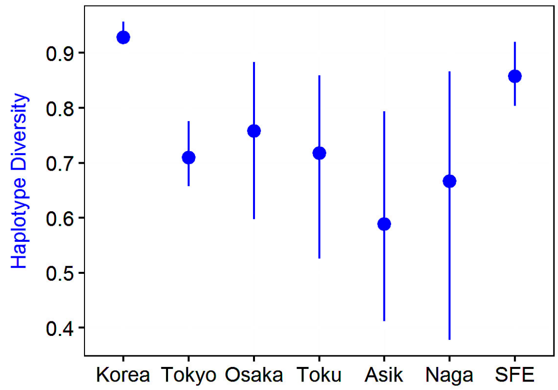

Figure 1. Haplotype diversity H of *Pseudodiaptomus marinus* in each sample with bootstrap 90% confidence intervals. Abbreviated sites are Tokushima Port (Toku), Ashikara, Ariake Sea (Asik), Nagasaki Port (Naga), and San Francisco Estuary (SFE).

In Japan, these values ranged from 0.588 to 0.758 and from 0.0017 to 0.0038, respectively (Figure 1, Table S1). Variability was high partly because of small sample sizes from the Japanese locations (Table S1). Bootstrap confidence intervals did not overlap between Korea and any other sample, while confidence intervals for SFE overlapped with those from three of the Japanese samples.

Differences in morphology and sequence divergences between 11 specimens from near Xiamen, China and *P. marinus* from the SFE indicated that the Chinese specimens were a different congener (see Discussion for details).

Haplotype network

Thirteen haplotypes from 21 cyt-*b* sequences were obtained from Haeui Is., Korea (Figure 2, Table 1). Haplotypes H1 and H2 were also found in localities in Japan and the SFE, but the remaining 11 haplotypes were unique to Korea (Figure 3, Table 1). Moreover, nine of these 11 haplotypes were genetically closely related to each other, and were at least two or three mutational steps distant from all haplotypes collected from Japan and the SFE (Figure 3).

Twelve haplotypes were detected in 27 sequences from the SFE (Figure 3, Table 1) and 20 haplotypes were detected from the five Japanese localities (Figures 2, 3, Table 1). Though most haplotypes collected from Japan and SFE were unique, seven haplotypes (H1–H7) were found in more than one individual, and three of them, H1, H2 and H5, were detected in 16, 45 and17 individuals, respectively.

Table 1. Haplotype numbers and source locations. See Supplemental Information for accession numbers. Numbers in columns 1–7 indicate the number of individual copepods with each of those haplotypes by location. "Others" lists the remaining haplotypes from each location; numbers in parentheses are the number of copepods with the haplotype if more than one.

Location	Haplotype							
	1	2	3	4	5	6	7	Others
Haeui Is., Korea	1	2						20(2), 21, 22(5), 23, 24(3), 25–29, 38
Tokyo Bay, Japan	9	5			6			11
Osaka Port, Japan	1	11		1	2	1	1	32–36
Tokushima Port, Japan		7	2	1			1	37, 39
Asikari, Ariake Sea, Japan		11	2	1				8–10
Nagasaki Port, Japan		6		1			1	30, 31
SFE, USA	5	3			9	1		12–16, 17(2), 18, 19

Table 2. Pairwise F_{st} estimates among seven populations based on the cytochrome *b* sequences.

Population	1	2	3	4	5	6	7
1. Tokyo Bay, Japan	–						
2. Osaka Port, Japan	0.12663**	–					
3. Tokushima Port, Japan	0.22124**	0.00015	–				
4. Asikari, Ariake Sea, Japan	0.24239***	–0.00206	–0.03183	–			
5. Nagasaki Port, Japan	0.18036*	–0.03969	–0.01807	0.00019	–		
6. Haeui Is., Mokpo, Korea	0.45056***	0.39126***	0.36198***	0.39570***	0.34778***	–	
7. SFE, USA	0.07805*	0.09094**	0.17231***	0.19106***	0.10269*	0.41771***	–

P value was generated by 10,100 times of permutation (*P < 0.05, **P < 0.01, ***P < 0.001).

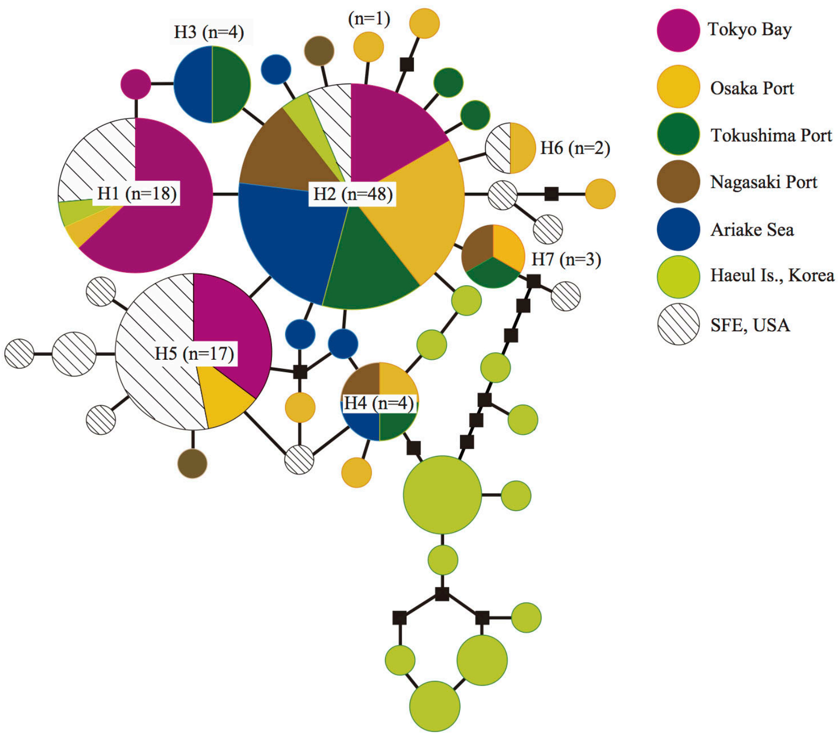

Figure 2. Haplotype network of *Pseudodiaptomus marinus* based on mitochondrial cytochrome *b*. Each circle represents a single haplotype (see Table 1). Closed small squares indicate a missing haplotype (one nucleotide difference for each link in the network). Haplotypes H1–7 are shared by several populations in San Francisco Estuary, Japan, and Korea; haplotypes H8–39 are each unique to one population. Circle size is proportional to haplotype frequency.

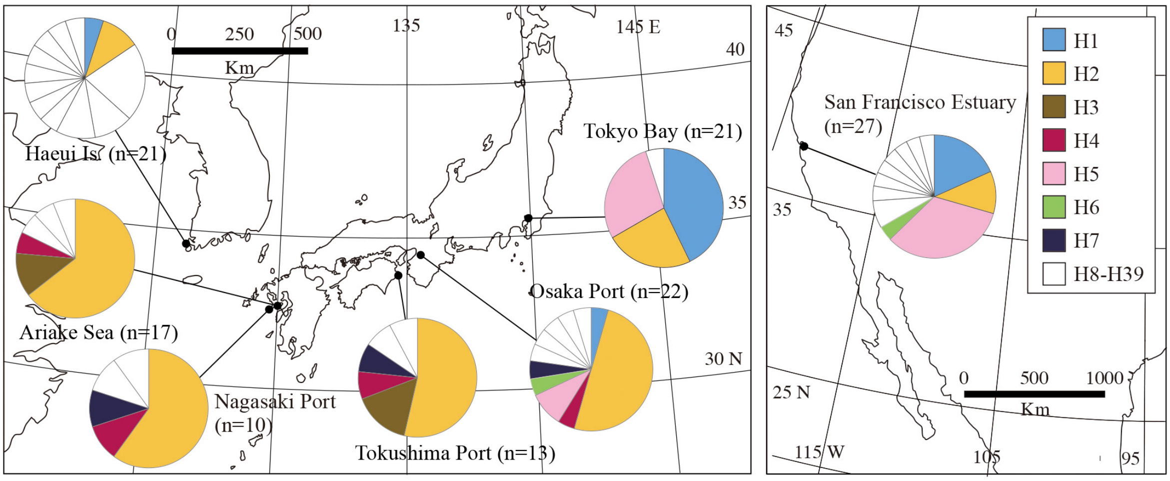

Figure 3. Geographic distribution of haplotypes for *Pseudodiaptomus marinus* based on mitochondrial cytochrome *b* (see Table 1).

Four haplotypes (H1, H2, H5 and H6), were shared between Japanese and SFE populations (Figures 2, 3, Table 1). H2 in particular was found in all localities of Japan. H1 and H2 were also detected from Korea, while H5 and H6 were found only in Japan and SFE (Table 1). H6 was found in one individual each from Osaka Port and SFE (Table 1). Eight haplotypes collected from the SFE were not detected in populations from Japan or Korea in this study (Table 1).

Haplotype composition in samples from Korea, Japan and SFE

In addition to its ubiquity in all sampling locations, haplotype H2 was most abundant in the Japanese samples with a frequency of about 24% in Tokyo Bay and 50% at other Japanese localities (Figure 3). In contrast, H2 made up only ~ 11% of the haplotypes in the SFE. Haplotypes H1, H2, and H5 were highly frequent in SFE (second, third and highest frequency, respectively).

Four haplotypes were detected in 21 cyt-*b* sequences from Tokyo Bay, where three haplotypes, H1, H2 and H5, were detected at frequencies of approximately 43, 24 and 29%, respectively. Eleven haplotypes were detected from Osaka Port from 22 sequences. Haplotype H2 made up half of these sequences, while all but one other haplotype were represented by a single individual, including haplotypes H1 and H5.

Haplotype H2 comprised over 50% of the samples from Tokushima Port, Ariake Sea and Nagasaki Port (Figure 3). These populations had unique haplotypes, and also shared haplotypes not detected from Tokyo Bay, SFE, or Korea. Examples were H3 from Tokushima Port and Ariake Sea, and H7 from Tokushima Port, Nagasaki Port and Osaka Port.

Thirteen haplotypes were detected from 21 sequences from Haeui Is., of which 11 haplotypes were apparently endemic. None of them were dominant in the population. H1 and H2, which were found in Tokyo Bay, Osaka Port and SFE, were also detected at low frequency from Haeui Is. (Figure 2).

Discussion

High genetic diversity of P. marinus *in the SFE*

Our genetic analysis of *P. marinus* from the introduced population in the SFE provides some ideas concerning the origin and mode of introduction. The high haplotype diversity in the SFE population contrasts with the general pattern by which diversity in an introduced population is depressed compared to that in the donor area as a result of bottlenecks and founder effects. Such an effect has been demonstrated in genetic studies of marine algae (cf. Uwai et al. 2006; Kogishi et al. 2010; Kawai et al. 2016). The *P. marinus* population of Osaka Port, Japan most closely resembled that of SFE in haplotype composition, because both shared four dominant haplotypes H1, H2, H5 and H6. However, F_{st} showed significant genetic differentiation between these populations (Table 2). In addition, the SFE sample differed significantly in F_{st} from samples from all other populations in Japan and Korea (Table 2). Therefore, it is likely that the SFE population could have been repeatedly introduced from various sources in Japan, with additional introductions possible from Korea and other areas in East Asia not analyzed here.

The origin of *P. marinus* in the SFE can be partially deduced from our genetic data. First, haplotype H2 was found at all localities in the SFE, Japan, and Korea. In contrast, H1 was unique to the SFE, Tokyo Bay, Osaka Port and Haeui Is., H5 to the SFE, Tokyo Bay and Osaka Port, and H6 to the SFE and Osaka Port. Second, eight haplotypes from the SFE were absent in samples from other populations in Japan and Korea. High haplotype diversity in an introduced population suggests multiple introductions from several donor areas (Voisin et al. 2005). Therefore, in the SFE, *P. marinus* may have been repeatedly introduced during the > 14 years between its establishment in 1986 and 1999 resulting in a highly diverse population (cf. Orsi and Walter 1991). This species has been detected in ballast water of both bulk carriers and container ships reaching the SFE from Asian ports following open-ocean exchange of ballast water, providing opportunities for repeated introduction (Choi et al. 2005). Since populations in Tokyo and Osaka Bays share more common haplotypes with the SFE population than others, these appear to be the most likely donor areas, at least among the surveyed sites. Northern China may be an additional donor area, but it is uncertain whether *P. marinus* occurs in these waters (see below).

Introduction of P. marinus *from China unlikely*

Pseudodiaptomus marinus has been reported from the coast of mainland China off Xiamen (Chen and Zhang 1965) and in river estuaries of the northern Liuchow Peninsula (Shen and Lee 1963). Therefore, we examined the morphological and genetic features of 19 copepod specimens from Xiamen that resembled *P. marinus*. We found some conspicuous differences in the morphology of the female urosome, in particular the genital double-somite, between the Chinese specimens and our *P. marinus* specimens (Ohtsuka et al. *in prep.*). In addition, a molecular analysis using the mitochondrial gene cyt-*b* of 11 specimens from Xiamen showed that the genetic distance (uncorrected *p*-distances) between the SFE and Chinese populations ranged from 17.3 to 18.5%. In contrast, the intraspecific variation within *P. marinus* was 0–2.8%, suggesting that the Chinese population is a separate undescribed species (Ohtsuka et al. *in prep.*). Interspecific genetic distances between *P. marinus* and two congeners (*P. inopinus* Burckhardt, 1913 and *P. forbesi*) were 17.0 to 26.4% (Ohtsuka et al. *in prep.*). None of 27 specimens collected from SFE exhibited the same sequences as the Chinese specimens. Therefore, it is very unlikely that the undescribed Chinese species of *Pseudodiaptomus* contributed to the population identified as *P. marinus* in the SFE. Future studies are needed assessing whether *P. marinus sensu stricto* occurs in Chinese waters, and whether not only brackish species but coastal species have been introduced from China to any other area.

Introduction of P. marinus *into SFE via ballast water or aquaculture?*

The SFE has received a number of planktonic and benthic organisms from brackish waters of China rather than coastal waters (Carlton et al. 1990; Orsi and Ohtsuka 1999), because international trading ports in China are generally located near the mouths of large rivers (Ohtsuka and Hiromi 2009). The following six brackish copepod species introduced to the SFE from East Asia most likely originated from China, because they have not been found in Japanese waters: *Sinocalanus doerrii*, *Limnoithona sinensis*, *Pseudodiaptomus forbesi*, *Acartiella sinensis*, *Tortanus dextrilobatus*, and *L. tetraspina* (Orsi and Ohtsuka 1999). Among these species, only *T. dextrilobatus* has also been recorded from Korean waters (Ohtsuka et al. 1992).

In contrast, two coastal species, *O. davisae* and *P. marinus*, also occur in Japan and were first recorded from the SFE in 1963 and 1986, respectively (Orsi and Ohtsuka 1999). Anthropogenic introduction of *O. davisae* into the SFE has been suggested but the vector was not specified (Ferrari and Orsi 1984). In the case of *P. marinus*, vectors via both ship ballast water and aquaculture activities are possible. *Pseudodiaptomus marinus* was found in the ballast tanks of bulk carriers and container ships reaching SFE from Japan and other countries (Choi et al. 2005), suggesting that the species could have been introduced to SFE before exchange of ballast water in the ocean was mandated in 2000. Orsi and Walter (1991) hypothesized two possibilities for the introduction of *P. marinus* into SFE: (1) direct introduction by way of discharge of ballast water originating from Asia; (2) indirect introduction by currents from San Diego or Tomales Bay where Pacific oysters shipped in from Japan were cultured. Fleminger and Kramer (1988) also proposed oyster aquaculture as a vector for non-native *P. marinus* in two small Southern California estuaries lacking international seaports.

The ecological characteristics, including habitat use, of *P. marinus* should be considered for inferring the mode of introduction. The species has been expanding its distribution around the world by unintentional introduction (Sabia et al. 2014), and has been found recently in the North Sea (Brylinski et al. 2012) and the Adriatic Sea (de Olazabal and Tirelli 2011; Lučić et al. 2015). In the Seto Inland Sea,

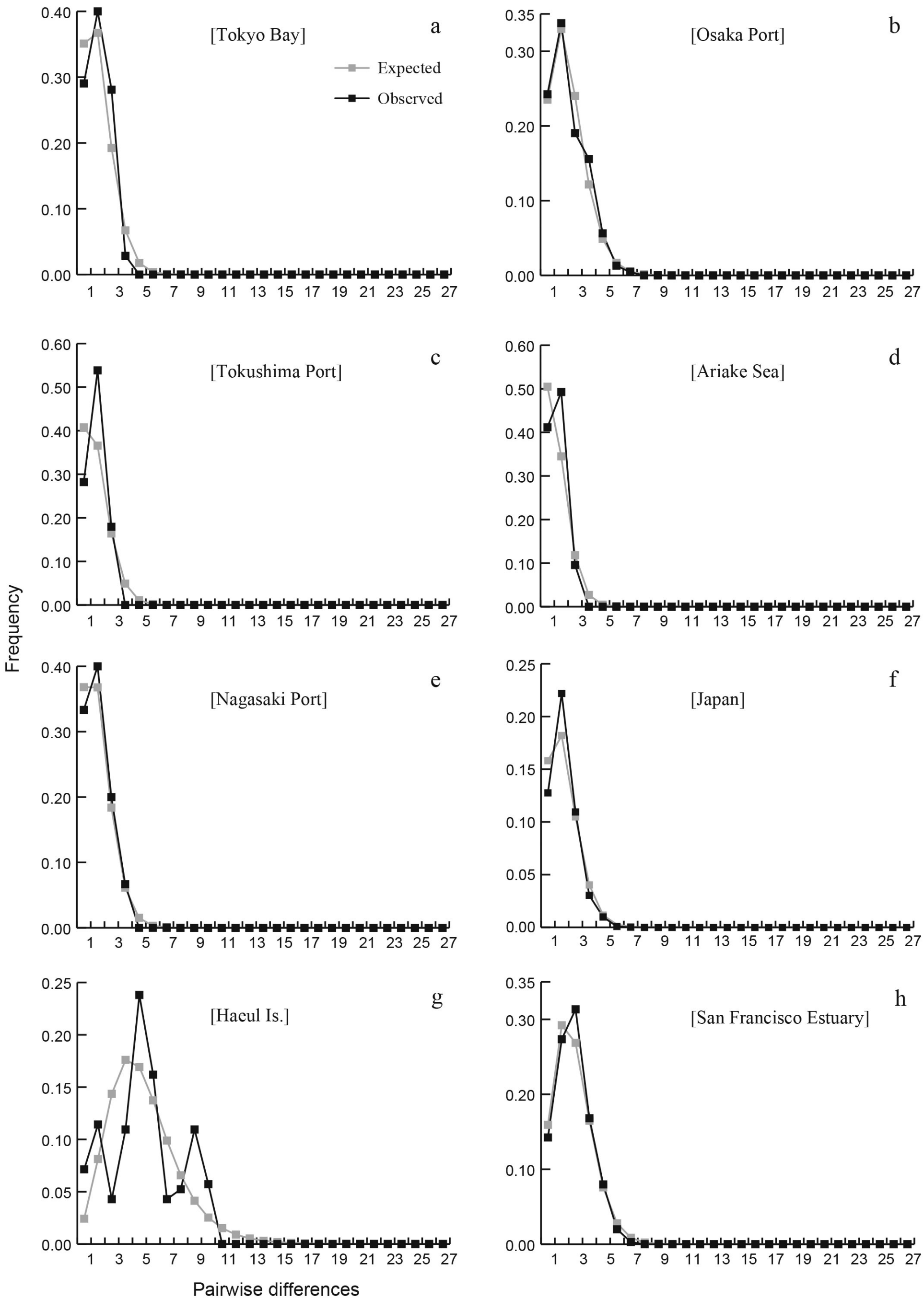

Figure 4. Mismatch distribution analysis of populations in Japan (a–f), Korea (g) and San Francisco Estuary (h). "Japan" indicates all Japanese localities combined.

Japan, a possible donor area, the species occurred in waters with a temperature range of 8.9–28.2 °C and a salinity range of 28.6–32.3 (Liang et al. 1996; Liang and Uye 1997). In the SFE, the maximum abundance (up to 838 individuals m^{-3}) of *P. marinus* was recorded at salinities of 14.8–17.1 (Orsi and Walter 1991), but since its introduction it has been found at salinity < 1, and in at least 10% of monitoring samples at salinity > 6 (Kimmerer, unpublished). In southern California, USA, it occurred in waters of

14.0–22.0 °C and salinity 33.0–34.0 (Fleminger and Kramer 1988). In the Adriatic Sea, its occurrence was recorded at 8.9–25.3 °C and salinity 30.0–37.3 (de Olazabal and Tirelli 2011; Lučić et al. 2015), and in the North Sea at 5.6–19.0 °C and salinity 33.1–34.2 (Brylinski et al. 2012). In the Adriatic Sea, abundance was very low at < 1 to 73 individuals m^{-3} (de Olazabal and Tirelli 2011; Lučić et al. 2015).

The occurrence of *Pseudodiaptomus marinus* in wide ranges of water temperature (overall, 5.6–28.2 °C) and salinity (<1–37.3) and the observation of this species in ballast water (Choi et al. 2005) suggest it should be highly vulnerable to transport in ballast water originating in a variety of ports. The demersal daylight habit of *P. marinus* also makes it susceptible to introduction via shipment of benthic shellfish (Fleminger and Kramer 1988). *P. marinus* may have expanded its distribution in European waters via both ballast-water and aquaculture (Sabia et al. 2014). However, the most likely vector for introduction to the SFE is ballast water, given the lack of aquaculture facilities and the high volume of shipping traffic from Asia to the SFE.

Zoogeographical comments on P. marinus *in East Asia*

Finally, we comment briefly on the zoogeography of *P. marinus* in East Asia on the basis of our genetic results. All of the five Japanese populations exhibited a single peak in their mismatch distributions (Figure 4). In addition, both Tajima's *D* and Fu's *Fs* were negative (Table 3), suggesting that distributions of these populations have been expanding. The haplotype and nucleotide diversities of these Japanese populations were relatively low in comparison with those of the Korean population (Table S1), suggesting bottleneck effects. In contrast, the Korean population showed multiple peaks in the mismatch distribution analysis and a positive value in Tajima's *D*, although Fu's *Fs* was negative (Table 3). These results suggest that *P. marinus* originated on the Asian continent and underwent subsequent colonization and intermittent isolation of the populations in Japan during the geological period since the Miocene (Nishimura 1980, 1981). Originally *P. marinus* had a highly restricted distribution in East Asia (Sato 1913; Brodsky 1950; Hirota 1962, 1964; Tanaka 1966; Tanaka and Hue 1966; Uye et al. 1982; Walter 1986; Eyun et al. 2007; present study). Considering the present distributional pattern and habitat of the species, it could have originated from near the mouths of the enclosed brackish bay of the ancient East China Sea (cf. Nishimura 1980, 1981; Ohtsuka and Reid 1998). The brackish bay has been established since the Miocene, and played an important role as a center of speciation of brackish and coastal organisms (Nishimura 1980, 1981; Ohtsuka and Reid 1998; Orsi and Ohtsuka 1999). Ohtsuka and Reid (1998) proposed the origin and speciation of the brackish calanoid copepod subgenus *Tortanus* (*Eutortanus*) in East Asia in consideration of Nishimura's (1980, 1981) ideas. The same evolutionary scenario could be applied to *P. marinus*, since these copepods have similar distributional patterns and habitats. Success of colonization of a non-indigenous species likely depends on the number of organisms released and the characteristics of the receiving water body (Choi and Kimmerer 2008), as well as the life history, place of origin, and evolutionary history of the species (Orsi and Ohtsuka 1999).

Table 3. Summary statistics for neutrality tests.

Population	Tajima's *D*	Fu's *Fs*
Tokyo Bay, Japan	0.6734	0.15246
Osaka Port, Japan	2.04997**	−7.34372***
Tokushima Port, Japan	−1.57943*	−3.48915**
Asikari, Ariake Sea, Japan	−1.29156	−3.80785***
Nagasaki Port, Japan	−1.7411*	−2.25975*
Haeui Is., Korea	0.11848	−4.21352*
SFE, USA	−1.53464*	−6.54175***

P value was generated by 10,100 times of permutation (*P < 0.05, **P < 0.01, ***P < 0.001).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Sampling information (location, date, and sample size) and genetic diversity based on the cytochrome b sequences.

Table S2. Accession number for haplotypes.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2018/Supplements/AI_2018_Ohtsuka_etal_SupplementaryTables.xlsx