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ORIGINAL PAPER

Cryptic invasion of northeast Pacific estuaries by the Asian polychaete, *Hediste diadroma* (Nereididae)

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Abstract The estuarine polychaete, *Hediste diadroma* (Nereididae), of eastern Asia was found at all six sites sampled in October 2009 in northeast Pacific estuaries, including the Columbia River, along a 300-km stretch of coastline in Washington and Oregon, USA. The immature worms were returned to the laboratory in Japan where 13 of them were cultured to sexual maturity after 5–7 months. These worms had the diagnostic, epitoke-specific spinigers that distinguish *H. diadroma* from all other *Hediste* species. Their egg sizes (140–170 μm), paragnath numbers on the proboscis, and chromosome number ($2n = 28$) were also consistent with those previously reported for the Japanese population of *H. diadroma*. The widespread distribution of *H. diadroma* indicates that it is a successful invader of the North American Pacific and it may have long escaped detection because of its morphological similarity to the native species, *H. limnicola*. The long pelagic life of *H. diadroma* larvae could have facilitated its successful trans-ocean dispersal with ballast water of ships.

Introduction

Hediste species are euryhaline and a predominant burrowing macrobenthic component of northern hemisphere estuaries (Scaps 2002; Sato and Nakashima 2003; Sato 2004). Five nominal species are currently known: *H. diversicolor* (O. F. Müller 1776) occurs on European and the North American coasts of the Atlantic (Smith 1977); *H. limnicola* (Johnson 1903) occurs along the North American Pacific coast (Smith 1958); and *H. japonica* (Izuka 1908), *H. diadroma* Sato and Nakashima, 2003, and *H. atoka* Sato and Nakashima, 2003 occur in eastern Asia (Sato and Nakashima 2003; Sato 2004). Though these five species are morphologically very similar to one another (often indistinguishable) at sexually immature stages, there are marked differences in reproductive and developmental characteristics and diploid chromosome numbers between them (Table 1).

Hediste limnicola is unique in characteristics such as hermaphroditism, self-fertilization, viviparity (embryos are brooded in the adult coelom where they develop into juveniles) and the diploid chromosome number of 26 (Table 1) and known only from the North American Pacific coast (from Vancouver Island, Canada, to southern California, USA) (Smith 1958; Khlebovich 1996). No other northeast Pacific *Hediste* species was previously known. *Hediste limnicola* is also unique in its habitats, including not only brackish waters but also a freshwater lake (Johnson 1903; Smith 1950, 1958).

We collected immature "*H. limnicola*" from estuaries in Oregon and Washington in October 2009 and reared them

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Table 1 Comparison of reproductive, developmental, and chromosomal characteristics of five *Hediste* species

Geographic range and species (diploid chromosome number)	Reproductive mode	Epitoke-specific chaetae	Egg diameter (µm)	Salinity favorable for early development	Remarks on early development	References
Eastern Asia						
H. japonica (28)	Swarming	Absent	180–210	22–30	10-day planktonic trochophore/nectochaeta	Izuka (1908); Sato and Nakashima (2003); Tosuji et al. (2004); Tosuji and Sato (2006)
H. diadroma (28)	Swarming	Present	130–170	22–30	1-month planktonic trochophore/nectochaeta	Kagawa (1955); Sato and Tsuchiya (1987), (1991); Sato and Ikeda (1992); Sato and Nakashima (2003)
H. atoka (28)	Female spawns in burrow	Absent	200–250	9–21	No planktonic larval life, hatching out at 3-chaetiger juvenile	Sato and Tsuchiya (1987), (1991); Sato and Ikeda (1992); Sato (2004)
North American Pacific coast						
H. limnicola (26)	Hermaphrodite, self-fertilization	Absent	120–170 210[a]	15–20	Viviparity, emerging from parent coelom at 15–30-chaetiger juvenile	Smith (1950); Fong and Pearse (1992); Khlebovich (1996); Tosuji et al. (2010)
North Atlantic coasts						
H. diversicolor (28)	Female spawns, broods embryos in burrow	Absent	200–250	5–27	10-week demersal trochophore/nectochaeta	Dales (1950); Smith (1964); Christensen (1980); Bartels-Hardege and Zeeck (1990); Scaps (2002)

[a] Fong and Pearse (1992) reported the egg diameter, using materials collected from Watsonville Slough in 1987–1989, as 210 µm, which is much larger than the 120–170 µm reported by Smith (1950) in the specimens he collected from the Salinas River estuary in 1948, where he observed viviparous development

in aquaria to sexual maturity to study their unique hermaphroditism. However, to our surprise, all mature adults obtained were dioecious and reproduced by epitokous metamorphosis with a diagnostic characteristic of the western Pacific endemic, *H. diadroma* (Sato and Nakashima 2003; Sato 2004). In the present paper, we report the occurrence of *H. diadroma* in northeast Pacific estuaries as a possible exotic invader, which may have been confused with the American native, *H. limnicola.*

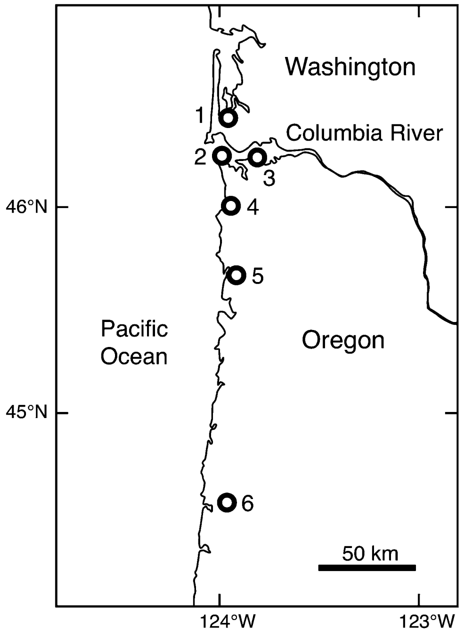

Fig. 1 Sampling sites of *Hediste diadroma: 1* Willapa Bay, *2* Trestle Bay, *3* Tongue Point, *4* Seaside, *5* Wheeler, *6* Toledo

Materials and methods

We collected *Hediste* worms from estuarine mudflat sediment at six sites along a 300-km stretch of coastline in Washington and northern Oregon, USA (Fig. 1), on October 28–31, 2009. Salinity of the interstitial water was measured using a hand refractometer (Atago, Tokyo, Japan) from water samples collected in mudflat depressions at each sampling site. Salinities were 4–18 (Table 2).

We shipped 70 of these worms to Kagoshima University, Japan, where they were cultured to sexual maturity in March–May 2010 (5–7 months after collection). Each worm was cultured individually in a 9-cm-diameter, 6-cm-deep Petri dish containing 50 % Jamarin-U artificial seawater (Jamarin Lab., Osaka, Japan) diluted to a salinity of 17 with distilled water, which is favorable for the reproduction of *H. limnicola* (Fong and Pearse 1992). The dishes were held at 18 °C in an incubator with a 12-h light/12-h dark photoperiod. The seawater was changed every 2 or 3 days, and the worms were fed commercial dry fish food (Tetramin). Mature adults, just before or just after spawning, were fixed and then preserved in 80 % ethanol. The sex of each individual was determined by the presence of

Table 2 Collection sites of young worms of *Hediste diadroma* on October 28–31, 2009, and summary of epitokes obtained in March–May 2010 under laboratory culture

Locality of original samples			Salinity	Date of fixation of epitokes (number of epitokes)	Mean egg diameter ±SD[c] (μm)
Site[a]	Bay or Estuary	Location			
1	Washington:	Southern end of Willapa Bay	18	2 Mar (1 female)	
	Willapa Bay	46°22′31.07″N, 123°57′06.58″W			
2	Oregon:	Hammond: Trestle Bay (Jetty Lagoon)	10	2 Mar (1 male)	
	Columbia River Estuary	46°13′30.03″N, 123°59′29.70″W		7 Apr (1 female)	
3	Oregon:	Astoria: Tongue Point	4	11 Mar (2 males)	
	Columbia River Estuary	46°11′46.54″N, 123°46′44.00″W		16 Mar (1 female)	166 ± 5.5
				19 May (1 female)	152 ± 7.2
4	Oregon:	Seaside	12	13 Mar (2 females)[b]	151 ± 4.6
	Necanicum River Estuary	46°01′02.60″N, 123°55′18.70″W		16 Mar (1 spent)	
				27 Apr (1 female)	155 ± 4.8
5	Oregon:	Wheeler	10	2 Apr (1 female)	160 ± 7.2
	Nehalem River Estuary	45°41′15.05″N, 123°53′20.63″W			
6	Oregon:	Toledo: Toledo State Airport	14	2 Mar (1 spent)	
	Yaquina River Estuary	44°35′50.57″N, 123°56′15.78″W			

[a] Corresponding to site numbers in Fig. 1

[b] Well-spread metaphase chromosome plates of 32 mitotic cells were obtained from one of the females

[c] Diameter of 25 eggs was measured for each of the five females

coelomic eggs or spermatozoa. The body length (*L*) and the anterior maximum body width excluding the parapodia (*W*) were measured in the fixed mature specimens. The paragnaths in each of the groups on the proboscis were counted under a stereomicroscope.

The numbers of epitoke-specific chaetae, which occur only in mature *H. diadroma* (Sato and Nakashima 2003), were counted on every fifth parapodium of two mature adults (a female and a spent worm of indeterminate sex). Diameters of 25 eggs, which were obtained from the coelom of each of the five females, were measured using a calibrated micrometer on a light microscope.

To determine the diploid chromosome number, well-spread metaphase chromosome plates of mitotic cells were prepared according to the method of Tosuji et al. (2010) using a regenerating tail of each of the six females from the cultures. Here, we describe the result from only one female where a sufficient number of well-spread plates were obtained.

Results

Three mature males, eight mature females, and two spent worms from all six sampling sites were obtained in March–May 2010 under laboratory culture (Table 2). The specimens were incomplete or complete with a regenerating tail tip, up to 43 mm in *L* and 2.0–2.5 mm in *W*.

All specimens were identified as *Hediste diadroma* according to the keys of Sato and Nakashima (2003) and

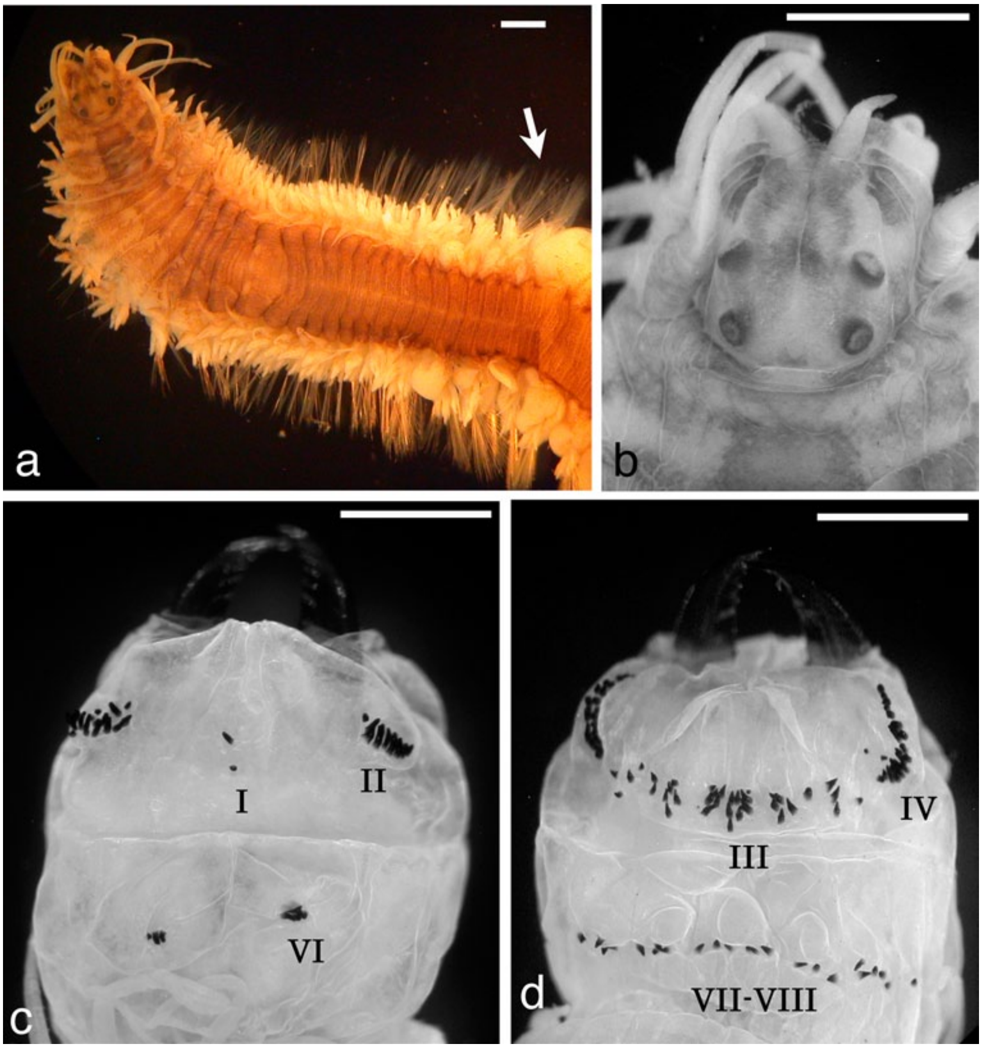

Fig. 2 *Hediste diadroma* epitokous males cultured for 4.5 months. **a**, **b** Male from Tongue Point: **a** dorsal view of anterior half with many sesquigomph spinigers in middle parapodia (*arrow*); **b** dorsal view of anterior end showing two pairs of large eyes in prostomium. **c**, **d** Male from Trestle Bay: **c** dorsal view of everted proboscis showing paragnaths in groups *I*, *II*, and *VI*; **d** ventral view of everted proboscis showing paragnaths in groups *III*, *IV*, and *VII—VIII*. Scale 1 mm

Sato (2004); the most important diagnostic characteristics are the delicate, transparent, epitoke-specific sesquigomph (intermediate between homogomph and heterogomph) spinigers (Figs. 2a, 3a, b). These spinigers are located posterior to all fascicles of atokous chaetae in both notopodia and neuropodia in middle chaetigers (around parapodia 20–60) of both mature males and females and were confirmed for all specimens (Figs. 2a, 3a, b; Online Resource 1). The morphology of the epitoke-specific sesquigomph spinigers and their positions were also consistent with those of Japanese specimens of *H. diadroma* (Sato and Nakashima 2003), though the maximum number of the sesquigomph spinigers in each fascicle of the American specimens (68 in notochaetae, 23 in upper neurochaetae, and 66 in lower neurochaetae) was much higher than that of the Japanese specimens (20, 8, and 19, respectively). No other *Hediste* species is known to possess the epitoke-specific sesquigomph spinigers. All specimens had two pairs of enlarged eyes on the prostomium (Fig. 2b), which is also a characteristic of epitokes of *H. diadroma* (Sato and Nakashima 2003).

Mature eggs (oocytes) from five females were 140–170 μm in diameter (Table 2). The egg size was consistent with that of *H. diadroma* (Sato and Nakashima 2003). No significant difference in numbers of paragnaths (Fig. 2c, d) between the North American and Japanese populations was found for all groups (unpaired *t* test, $P = 0.14–1$, Table 3). Hermaphroditism and viviparity, which are diagnostic

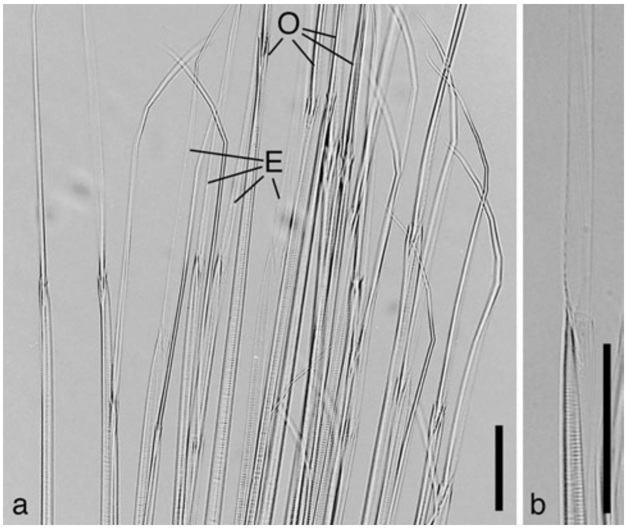

Fig. 3 Epitoke-specific sesquigomph spinigers in the notopodial fascicle of chaetiger 22 of an epitokous female of *Hediste diadroma* originating from Seaside (fixed 7 months after collection). **a** Many transparent epitoke-specific sesquigomph spinigers (*E*) added behind the ordinary homogomph spinigers (*0*). **b** Enlarged part around base of blade of an epitoke-specific sesquigomph spiniger. Scale 0.05 mm

Table 3 *Hediste diadroma* size and paragnath numbers in North American (present study) and Japanese (Sato and Nakashima 2003) mature adults (epitokes)

Locality	*n*	Body width (mm)	Mean paragnath number ± SD (range)						
			I	II[a]	III	IV[a]	V	VI[a]	VII–VIII
North America	10–12	2.0–2.5	2.1 ± 1.0 (1–4)	27.1 ± 5.7 (20–36)	40.0 ± 7.1 (25–49)	47.7 ± 5.9 (37–60)	0 ± 0 (0–0)	10.6 ± 1.4 (8–13)	25.3 ± 4.7 (21–37)
Japan[b]	171–187	1.7–5.1	1.8 ± 1.1 (1–8)	29.4 ± 6.9 (18–52)	39.2 ± 7.3 (22–61)	43.5 ± 8.8 (25–76)	0 ± 0 (0–0)	9.9 ± 2.4 (3–17)	23.7 ± 5.0 (11–41)
P[c]			0.36	0.31	0.74	0.14	1	0.38	0.32

Seven groups of paragnaths in different areas on proboscis are shown as I to VII–VIII

[a] Total number on right and left sides of proboscis

[b] All original data pooled from 10 sites

[c] Significance of difference in mean paragnath number between two localities was tested by unpaired *t* test, which was applicable because their variances were not significantly different for all groups (*F* test, $P = 0.1–1.0$)

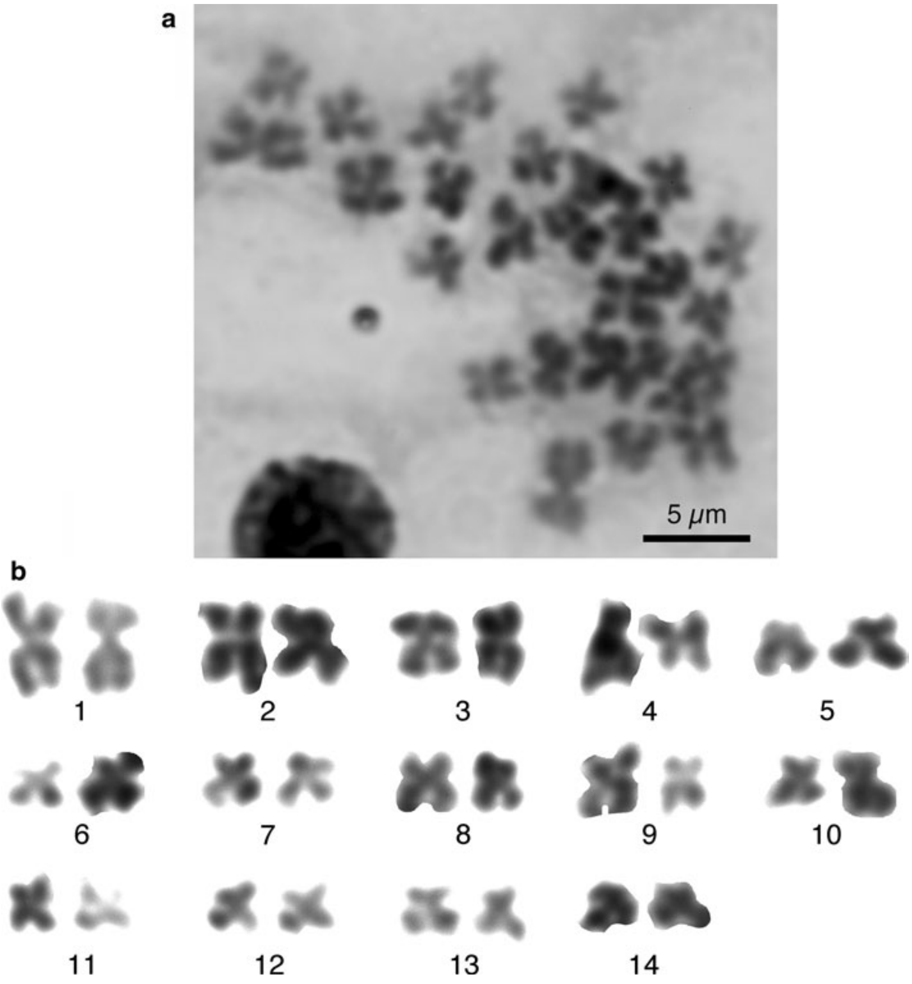

Fig. 4 Mitotic metaphase spread of chromosomes (**a**) and karyotype (**b**) from female *Hediste diadroma* from Seaside. Heterogametic XY chromosome pair characteristic of *H. diadroma* males (Tosuji et al. 2004) not present

characters of *H. limnicola* (see Smith 1958), did not occur among the mature worms.

Well-spread metaphase chromosome plates of 32 mitotic cells were obtained from a female from Seaside (Table 2). A diploid (2*n*) chromosome number of 28 (Fig. 4) was observed in most spreads (56 %), while smaller (27–23; 41 %) and larger numbers (29; 3 %) were also observed. The variation was probably due to artifacts of preparation. Chromosome sets of a representative spread comprised metacentric and submetacentric pairs (Fig. 4b). The chromosome number ($2n = 28$) and the karyotypes agreed with *H. diadroma* (Sato and Ikeda 1992; Tosuji et al. 2004) and differed from *H. limnicola* ($2n = 26$) (Tosuji et al. 2010).

Discussion

Our results indicate that *Hediste diadroma* occurred at all six sampling sites in Washington and Oregon. These sites are located not only in the major international shipping area, i.e., the Columbia River estuary, but also in places remote from that, e.g., Toledo, Oregon (Online Resource 2), and Willapa Bay, Washington (Online Resource 3). Thus, *H. diadroma* is established as an exotic invader and has spread among Washington and Oregon estuaries where it escaped previous detection due to its similarity to the native northeast Pacific species, *H. limnicola*. *Hediste diadroma* is among the most common estuarine polychaetes in Japan with probable distribution in China (Sato and Nakashima 2003; Sato 2004). This is the first record of *H. diadroma* outside of Asia. In the present study, there was a big difference in the number of epitoke-specific sesquigomph spinigers per fascicle in the American and Japanese specimens. Since this is a labile character in *H. diadroma* (M. Sato, unpubl. data), it is unlikely that this reflects any marked population differentiation among the specimens.

The Columbia River is a major entry point for Asian ships bound for several ports in Oregon and Washington, with a marked increase in the number of ballasted ships from Japan, Korea, Taiwan, and China during 1980s; net tonnage of ballasted ships from these countries entering the Columbia River approximately doubled between 1976 and 1990 (Cordell et al. 1992). The life cycle of *H. diadroma* includes the following: (1) benthic adults in euryhaline estuaries, (2) reproductive swarming of mature adults around river mouths, (3) small eggs (130–170 μm in diameter) developing into free-swimming larvae under relatively high salinity (favorable salinity 22–30), which is comparable to the typical salinity (25–30) of ballast water samples from Japanese ships entering Coos Bay, Oregon, in 1986–1991 (J. Carlton, personal communication), (4) planktonic larval life lasting 1 month or more at 15 °C, and (5) upstream migrations and settlement of larvae into brackish waters (Kagawa 1955; Inamori and Kurihara 1979; Sato and Tsuchiya 1987, 1991; Sato 1999; Sato and Nakashima 2003; Tosuji and Sato 2006). The long pelagic larval life of *H. diadroma* possibly facilitated its dispersal to North America with ballast water traffic.

Since the life cycles of *H. diversicolor, H. atoka,* and *H. limnicola* do not include pelagic larval stages, they appear to be restricted to brackish (or fresh) waters where they reproduce without epitokous swarming. *Hediste japonica* produces epitokous swarms for reproduction similar to *H. diadroma*, differing from the latter in the absence of the epitoke-specific sesquigomph spinigers (Sato and Nakashima 2003). However, *H. japonica* larval dispersal seems to be limited due to its relatively short (10 days at 15 °C) pelagic larval life (Izuka 1908; Tosuji and Sato 2006).

Human-mediated introductions of marine and estuarine species to North America have increased exponentially over the past 200 years with greater shipping from the Indo-West Pacific to the Pacific coast (Ruiz et al. 2000). Wonham and Carlton (2005) documented 123 introduced marine and estuarine species established in the cool-temperate area of the North American Pacific coast (from northern California to British Columbia), with 43 species (35 %) from Asia, including common species from Japanese estuaries, e.g., spionid polychaetes (*Pseudopolydora kempi* and *P. paucibranchiata*), a gastropod (*Batillaria attramentaria* [=*B. cumingi*]), and bivalves (*Laternula marilina*, *Venerupis philippinarum* [= *Ruditapes philippinarum*]), which were first collected from the North American Pacific coast between the 1920s and 1990s. Our results confirm predictions by Carlton and Geller (1993) and Carlton (2009) that there are potentially many more introduced estuarine species that are cryptic, going unrecognized or mistaken as native species.

Fong and Garthwaite (1994) found high levels of heterozygosity in "*H. limnicola,*" collected from Oregon and California, USA, in 1989, and they concluded that some outcrossing must occur in this species since such high heterozygosity would not be expected to accompany the known reproductive mode (i.e., self-fertilizing hermaphroditism) of this species. The present study, however, suggests that their "*H. limnicola*" may have included the Asian cryptic invader, *H. diadroma*. The invasion by marine and estuarine species via ballast water on the Oregon coast has accelerated since 1980 (Wonham and Carlton 2005).

Additional surveys will be necessary to determine how far *H. diadroma* has spread in North America or its impact on the native *H. limnicola* populations. Tosuji and Sato's (2012) molecular methods for distinguishing *Hediste* species at morphologically indistinguishable immature stages are allowing us to pursue these questions.

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