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ORIGINAL PAPER

Multiple introductions and invasion pathways for the invasive ctenophore *Mnemiopsis leidyi* in Eurasia

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Abstract The introduction and spread of non-indigenous species (NIS) in marine ecosystems accelerated during the twentieth century owing to human activities, notably international shipping. Genetic analysis has proven useful in understanding the invasion history and dynamics of colonizing NIS and identifying their source population(s). Here we investigated sequence variation in the nuclear ribosomal Internal Transcribed Spacer region of the ctenophore *Mnemiopsis leidyi*, a species considered one of the most invasive globally. We surveyed four populations from the native distribution range along the Atlantic coasts of the United States and South America, as well as six populations in the introduced range from the Black, Azov, Caspian and Baltic seas. Allelic and nucleotide diversity of introduced populations were comparable to those of native populations from which they were likely drawn. Introduced populations typically exhibited lower genetic differentiation ($F_{ST} = -0.014$–0.421) than native populations ($F_{ST} = 0.324$–0.688). Population genetic analyses supported the invasion of Eurasia from at least two different pathways, the first from the Gulf of Mexico (e.g., Tampa Bay) to the Black Sea and thence to the Caspian Sea, the second from the northern part of the native distribution range (e.g., Narragansett Bay) to the Baltic Sea. The relatively high genetic diversity observed in introduced populations is consistent with large inocula and/or multiple invasions, both of which are possible given ballast water transport and the extensive native distribution of the ctenophore in the Atlantic Ocean.

Keywords Non-indigenous species (NIS) · Genetic diversity · Internal transcribed spacer (ITS) · *Mnemiopsis leidyi* · Invasive species · Ctenophore

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Introduction

The introduction and spread of non-indigenous species (NIS) beyond their native range accelerated during the latter half of the twentieth century owing to a variety of human activities (e.g., Streftaris et al. 2005; Wonham and Carlton 2005; Ricciardi 2006; Leuven et al. 2009). Governments have begun to develop management programs to address adverse

ecological, health and economic effects of NIS (Vasarhelyi and Thomas 2003; Thomas et al. 2009; Biosecurity New Zealand 2009). These efforts focus predominately on controlling the vector(s) of invasion in an attempt to reduce the rate at which new NIS are introduced. However, effective management strategies also require knowledge of the source(s) of NIS and their pathway(s) of entry. This information may be obtained using importation records of specific commodities and the 'fellow traveller' NIS associated with them, or by tracking the source of vessels or aircrafts entering a country (see Ruiz and Carlton 2003). The use of genetic markers to compare successfully established NIS provides a post hoc alternative to these approaches. Genetic tools can provide information on the source(s) of an invasion and they can estimate inoculum size. These tools can also determine whether an invasion consisted of one or more introduction events, and whether admixtures occur in the introduced range (e.g., Rius et al. 2008; Gillis et al. 2009; Rollins et al. 2009).

The number of individuals and species introduced depends heavily on the nature of the introduction vector (Wilson et al. 2009). These two components have been classified as propagule pressure and colonization pressure, respectively (Lockwood et al. 2009). Colonization pressure is likely to be low with the importation of commodities such as fruit, whereas entire communities involving variable population densities of different NIS may be introduced with a major vector such as ballast water or hull fouling. Indeed, shipping is recognized as the leading vector of NIS introductions into marine ecosystems (Molnar et al. 2008).

Introductions may involve a small to moderate number of founder individuals drawn from a limited part of the native and/or introduced ranges (Wilson et al. 2009). These limitations can be and often are reflected in reduced genetic diversity and divergence of the founding population from the source population owing to founder effects (e.g., see Dlugosch and Parker 2008). If the founding population remains small over several generations, genetic diversity may be further eroded by genetic drift (Hauser et al. 2002; Spielman et al. 2004; Frankham 2005). In extreme cases, demographic bottlenecks associated with colonization may result in severely impoverished genetic diversity in introduced populations (Dlugosch and Parker 2008). For example, the spread of rapa whelk *Rapana venosa* from its native range in southeast Asia to Europe, North America and South America resulted in a drastic reduction in genetic diversity from 11 to 23 mitochondrial haplotypes in native populations to a single haplotype in the invaded range (Chandler et al. 2008). Similarly, the fishhook waterflea *Cercopagis pengoi* experienced a significant reduction in genetic diversity during its spread from the Black Sea to the Baltic Sea and thence to the Great Lakes. While the former populations contained five mitochondrial haplotypes, all introduced populations were fixed for the same haplotype (Cristescu et al. 2001).

Not all introduced populations exhibit impoverished genetic diversity when compared with their source populations. A number of recent studies have documented introduced populations with unexpectedly high levels of genetic diversity, suggesting that multiple introductions from different sources and/or major introduction events involving high propagule pressure can mitigate the effects of genetic bottlenecks during colonization (Roman 2006; Roman and Darling 2007; Taylor and Keller 2007; Dlugosch and Parker 2008; Gillis et al. 2009). An introduced population of the green crab *Carcinus maenas* in Japan, for example, possessed higher mitochondrial genetic diversity than its source population (Darling et al. 2008).

The ctenophore *Mnemiopsis leidyi* is perceived to be one of the most invasive species globally (Lowe et al. 2000), owing to its broad ecological and physiological tolerance. Its native distribution spans the western Atlantic Ocean from Massachusetts to southern Argentina (GESAMP 1997). The species is a simultaneous hermaphrodite capable of self-fertilization (Harbison and Miller 1986), and its life span ranges from several months to 1 year (Shiganova and Dumont 2011). *M. leidyi* was introduced into the Black Sea in the early 1980s. From the Black Sea, it spread north to the Sea of Azov, south to the Sea of Marmara and then to the Aegean Sea (Shiganova et al. 2001; Shiganova 1993; Kideys and Niermann 1994). Inter-basin spread to the Caspian Sea occurred in the late 1990s, reportedly due to the release of ballast water from the Black-Azov basin (Ivanov et al. 2000). In 2006 it was found in the Baltic and North seas (Faasse and Bayha 2006; Javidpour et al. 2006, 2009; Boersma et al. 2007). In 2009, *M. leidyi* was reported along the French coast of the

Mediterranean Sea, in the northern Adriatic Sea (Shiganova and Malej 2009), in the southern Levantine Sea (Galil et al. 2009), and in the western Mediterranean (Boero et al. 2009; Fuentes et al. 2009). The source of each of these invasions has not been ascertained. Given that key environmental factors such as temperature, salinity and productivity vary widely among these systems, it is not clear whether these invasions occurred *via* a single invasive genotype or whether multiple sources and/or genotypes were involved.

There have been few studies of ctenophore population genetics and phylogenetics (e.g., Podar et al. 2001), mainly owing to a lack of well developed molecular markers of high resolution. Our attempts to verify *M. leidyi* invasion pathways using mitochondrial markers failed. In addition, mtDNA has a slow rate of evolution in early metazoans such as Porifera and Anthozoa (Huang et al. 2008). Therefore, our focus in this study is on the nuclear ribosomal Internal Transcribed Spacer (ITS), a region widely used in phylogenetic analyses at the species and population levels in vertebrates and invertebrates (Coleman and Vacquier 2004; Kochzius et al. 2008).

In this study, we compare the genetic diversity of *M. leidyi* in both native and introduced ranges, and infer sources of the introduced populations using sequence variation in the ITS. Additionally, we test the hypothesis that Eurasian populations have been seeded by a single invasion of the Black Sea, with secondary 'hub and spoke' spread to other seas in Eurasia.

Materials and methods

Sample collection and DNA extraction

A total of 190 *M. leidyi* individuals were sampled from four native (Narragansett Bay; York River, Virginia; Tampa Bay, Florida; Peninsula Valdes coast, Argentina) and six introduced populations (north-eastern and central Black Sea; Sea of Azov; southern and middle Caspian Sea; Baltic Sea, Kiel Bay). Surface collection of *M. leidyi* individuals were made from 2006 to 2009 using hand-held jars or zooplankton nets (Raskoff et al. 2003). Individuals were preserved separately to avoid cross contamination of the tissue in 95% ethanol and stored at 4°C prior to genetic analysis.

Genomic DNA was isolated from gelatinous, lobe tissue of the ctenophores using the automatic robot DNA extraction protocol described by Elphinstone et al. (2003). The universal primer pair (ITS5F and ITS4R, White et al. 1990), which anneals at the 3′ end of 18S rDNA and the 5′ end of 28S rDNA, was used to amplify ITS-1, 5.8S rDNA, and ITS-2. PCR amplifications were carried out in a 40-µl reaction volume, with about 50 ng of genomic DNA, 1 unit of *Taq* DNA Polymerase (QIAGEN), 1× PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of dNTPs, and 0.4 µM of each primer. PCR was performed with an initial denaturing step at 95°C for 1 min, followed by 35 amplification cycles (95°C for 30 s, 50°C for 30 s, 72°C for 50 s), and a final elongation step at 72°C for 7 min.

Sequencing and cloning protocol

All PCR products were verified on 1% agarose gel and purified using Agencourt® Clean SEQ from Beckman Coulter®. Cleaned PCR products were directly sequenced with the reverse primer (ITS4R) using BigDye terminator sequencing chemistry with an ABI 3130XL genetic analyzer (Applied Biosystems). Sequences were inspected and aligned using CodonCode Aligner 2.0 (CodonCode Corporation, Dedham, MA). All nuclear fragments that contained double nucleotide calls (overlapping peaks) were cloned using Cloning and Amplification Kit (pSMART® GC HK, Lucigen®) to verify the sequence of both alleles in all heterozygous individuals.

Population genetic and phylogenetic analyses

Standard diversity indices including allelic diversity (h) and nucleotide diversity (π) (Nei 1987) were assessed using Arlequin version 3.1 (Excoffier et al. 2005). Genetic diversity within populations was measured with three additional indices including the number of alleles (N_a), observed (H_o) and expected heterozygosity (H_e) calculated using the GENEPOP (online version http://genepop.curtin.edu.au) and Arlequin (version 3.1). The Markov chain method was used to estimate the probability of significant deviation from Hardy–Weinberg equilibrium (HWE) using GENEPOP. To test neutral evolution of the marker, we calculated Tajima's D (Tajima 1989), and Fu and Li's (1993) D^*, using DnaSP version 5 (Librado and Rozas

2009). The degree of population subdivision was determined from pairwise F_{ST} using Arlequin. Hierarchical structure of nucleotide variation was assessed using an analysis of molecular variance (AMOVA) based on 10,000 random permutations in Arlequin. Populations were grouped with a priori expectations based on their geographical origin. We grouped populations from North America (Florida, Narragansett Bay, and York River), South America (Peninsula Valdes), Ponto-Caspian region (Sea of Azov, Black Sea and Caspian Sea) and Europe (Baltic Sea). To investigate population genetic similarity and clustering, a UPGMA tree was reconstructed based on Nei's (1972) genetic distance between populations excluding population PV, due to monomorphism. Nei's genetic distance was calculated using POPGENE version 1.44 (Yeh et al. 2000), while the tree was constructed using MEGA version 4 (Tamura et al. 2007).

Phylogenetic relationship among alleles was reconstructed using the neighbor joining algorithm in MEGA version 4 (Tamura et al. 2007). An ITS sequence of the ctenophore *Bolinopsis* sp. (GenBank accession no. U65480) was used as outgroup. A network of allele sequences was generated using TCS 1.0 (Clement et al. 2000). The program estimates genealogical relationships among sequences using the 95% statistical parsimony algorithm (Templeton et al. 1992).

Results

The DNA fragment comprising the complete ITS1, 5.8S rDNA and ITS2 regions was sequenced for 190 individuals of *M. leidyi*. The length of the sequenced fragment was 619 base pairs (bp): 233 bp for ITS1, 158 bp for 5.8S, and 228 bp for ITS2. No insertion/deletions (indels) were detected in any of the amplified individuals.

Allelic diversity (h) of introduced (0.794) and native (0.790) populations of *M. leidyi* was relatively high, whereas nucleotide diversity (π) (0.002 and 0.002, respectively) was relatively low. We detected a total of 13 alleles (Accession nos. GU062750–GU062762), defined by four variable ITS1 sites and three variable ITS2 sites (Table 1), with 1.7 and 1.3% variation, respectively. No variable site was observed for 5.8S. The 13 alleles resulted in 26 genotypes across all populations (Supplementary Table 1). Nine alleles were detected in native locations, whereas invaded sites had seven of these alleles plus four unique alleles not detected in native populations. All introduced populations had allelic diversity equal to or higher

Table 1 Population code, sample size (N), number of alleles (N_a), number of genotypes (N_g), nucleotide (π) and allelic (h) diversity, observed (H_o) and expected (H_e) heterozygosity, and P-value for Hardy–Weinberg equilibrium (HWE) analysis

Population code	N	N_a	N_g	$\pi \pm$ SD	$h \pm$ SD	H_o	H_e	HWE P-value	Tajima's D	Fu and Li's D^*
AZ	30	7	13	0.0021 ± 0.0002	0.711 ± 0.052	0.70	0.70	0.882	0.343	0.895
BL	20	6	9	0.0022 ± 0.0002	0.789 ± 0.036	0.50	0.76	**0.002**	1.044	1.029
BLA	16	5	6	0.0019 ± 0.0002	0.760 ± 0.041	0.62	0.76	**0.001**	1.140	0.945
NC	40	8	11	0.0021 ± 0.0001	0.727 ± 0.037	0.65	0.72	**0.039**	1.341	0.960
SC	20	6	8	0.0021 ± 0.0002	0.677 ± 0.058	0.50	0.70	**0.011**	0.714	1.026
BA	20	5	6	0.0010 ± 0.0003	0.394 ± 0.094	0.30	0.39	0.092	−0.170	0.915
FL	11	5	8	0.0018 ± 0.0002	0.701 ± 0.066	0.63	0.76	0.559	0.943	−0.141
NB	14	5	8	0.0013 ± 0.0003	0.471 ± 0.103	0.50	0.47	0.568	0.120	−0.240
YR	14	4	4	0.0009 ± 0.0009	0.458 ± 0.096	0.57	0.45	0.725	−1.182	−1.933
PV	5	1	1	0.0000 ± 0.0000	0.000 ± 0.000	0.00	0.00	0.000	–	–

Bold numbers correspond to populations deviating significantly from HWE. All measures of neutrality tests (i.e., Tajima's D and Fu and Li's D*) were not significant ($P > 0.10$). Population sources: *AZ* Sea of Azov, Yasenskaya Bay; *BL* Black Sea, transect from Blue Bay; *BLA* Black Sea, near Gelendzhik; *NC* North Caspian Sea, Makhachkala coast; *SC* South Caspian Sea, Sari and Noor coasts; *BA* Baltic Sea, Kiel–Kiel Fiord; *FL* Tampa Bay, Florida; *NB* Narragansett Bay, Rhode Island; *YR* York River, Virginia; and *PV* Peninsula Valdes coast, Argentina

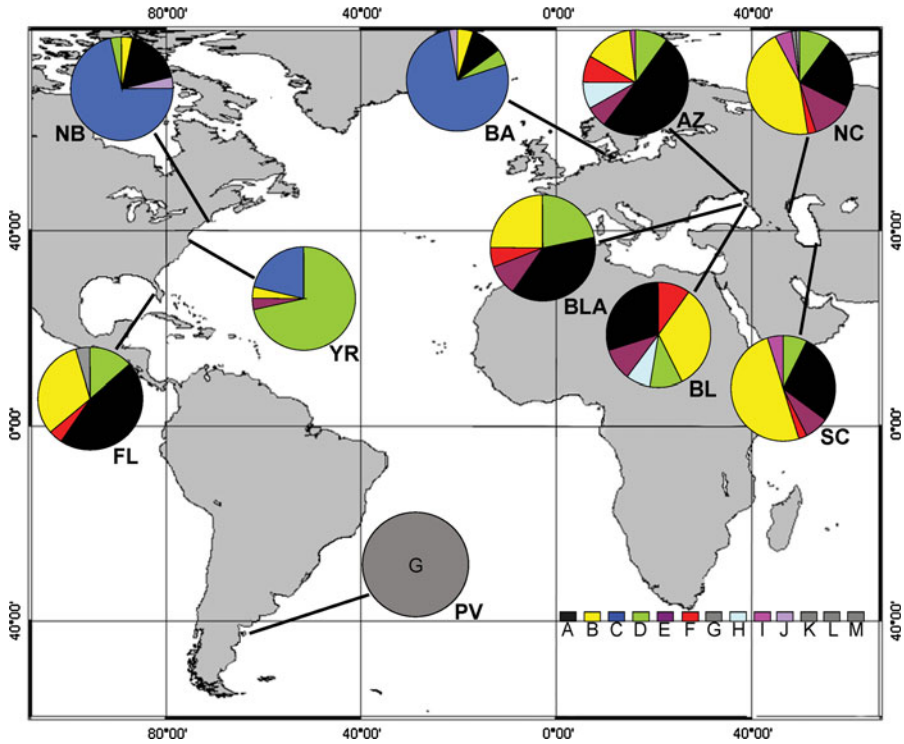

Fig. 1 Allele distribution map of *Mnemiopsis leidyi*. Each *shade* indicates a different allele. Private alleles (G, K, L and M) have similar *shade*. Population codes are described in Table 1

Table 2 Alleles (A–M) found in the 10 surveyed *Mnemiopsis* populations and their polymorphic sites at the corresponding nucleotide position of the sequence

Allele	ITS2			ITS1				Populations										Total
	64	193	212	416	507	574	583	AZ	BL	BLA	NC	SC	BA	FL	NB	YR	PV	
A	C	A	A	A	C	A	C	7.89	3.68	3.15	4.73	2.89	1.05	2.10	2.36			27.85
B	C	A	T	G	C	A	C	2.89	3.42	2.10	9.47	5.00	0.52	1.84	0.52	0.26		26.02
C	C	G	A	G	C	A	C						7.89		5.26	1.57		14.72
D	C	A	A	G	C	A	C	1.05	1.05	1.84	2.10	0.78	0.52	0.78	0.26	5.26		13.64
E	T	A	A	A	C	A	C	1.05	0.78	0.78	2.63					0.26		5.50
F	C	A	T	A	C	A	C	0.78	1.05	0.52	0.52	0.52		0.78				4.17
G	C	A	A	G	T	A	C										2.63	2.63
H	C	A	T	G	C	T	C	1.31	0.52									1.83
I	C	A	T	G	C	A	A	0.26			1.05	0.52						1.83
J	C	G	A	A	C	A	C						0.26		0.26			0.52
K	C	A	T	A	C	A	A							0.26				0.26
L	T	A	T	G	C	A	C				0.26							0.26
M	T	A	A	G	C	A	C				0.26							0.26

Populations as in Table 1, and the relative frequency of alleles for each population and in total

than that of native populations (Fig. 1; Table 2). Results from neutrality tests were not significant ($P > 0.10$) for Tajima's D, and Fu and Li's D^*, indicating neutral evolution for ITS (Table 1).

Populations from Black (BL, BLA) and Caspian (NC, SC) seas showed significant differences between observed and expected heterozygosity and departed significantly from Hardy–Weinberg equilibrium, while

all other populations were in Hardy–Weinberg equilibrium (Table 1).

Genetic structure of *M. leidyi* populations in the Baltic Sea (BA) and Narragansett Bay (NB) was very similar, with shared alleles of similar frequencies (Fig. 1; Table 2). By contrast, all populations from the Black (BL), Azov (AZ) and Caspian (NC, SC) seas shared similar alleles with the Florida (FL) population and they shared one extra allele with the York River (YR) population (Fig. 1). Ctenophore populations in Florida and the Black and Azov seas were dominated by allele A followed by allele B, whereas allele B was dominant in the Caspian Sea (Fig. 1; Table 2). All native populations along the US coast shared alleles B and D, though different alleles dominated at each site. Individuals from the native South American population (PV) were homozygous for the private allele G (Fig. 1).

Native populations had relatively high population differentiation with F_{ST} values ranging from 0.324 (FL and YR) to 0.688 (YR and PV). Based on allele distribution and pairwise F_{ST} values, little gene flow was inferred between South and North American populations. Gene flow was also limited among populations within North America. (Fig. 1; Table 3). Introduced populations typically exhibited less population differentiation than native populations. For example, F_{ST} values ranged between −0.014 and 0.421 in introduced populations. Two populations of SC and BA were the most distinct ($F_{ST} = 0.421$), while there was little differentiation between those in the north (NC) and south (SC) Caspian Sea ($F_{ST} = -0.014$) (Table 3). Comparisons of F_{ST} values among native and introduced populations revealed that those in the Baltic Sea (BA) and Peninsula Valdes (PV) coast ($F_{ST} = 0.711$) were the most genetically divergent (Table 3). Conversely, populations in Florida (FL) and the Black Sea (BL) were very similar ($F_{ST} = -0.027$), as were those in the Baltic Sea (BA) and Narragansett Bay (NB) ($F_{ST} = -0.018$) (Table 3). The highest F_{ST} value (0.481–0.711) for all populations was observed when compared to PV (Table 3). Average F_{ST} values for native and introduced populations were 0.498 and 0.151, respectively, indicating that the genetic differentiation among introduced populations is lower than that among native populations.

Hierarchical analysis of molecular variance (AMOVA) indicated that most of the genetic variation was partitioned within populations (69.2%), followed by variation among groups (21.5%) (Table 4). Genetic distance in populations from the Black, Azov and Caspian seas and Florida was relatively minor, and these populations were separated from the clade containing the Baltic Sea and Narragansett Bay

Table 3 Population subdivision according to pairwise F_{ST} values

	AZ	BA	BL	BLA	NC	SC	FL	NB	YR
BA	0.399								
BL	**0.011**	0.387							
BLA	**0.005**	0.395	**−0.012**						
NC	0.088	0.386	**0.011**	**0.033**					
SC	0.076	0.421	**0.002**	**0.030**	**−0.014**				
FL	**0.010**	0.413	**−0.027**	**−0.020**	**0.019**	**0.004**			
NB	0.330	**−0.018**	0.321	0.324	0.338	0.362	0.337		
YR	0.349	0.469	0.322	0.264	0.322	0.365	0.324	0.434	
PV	0.503	0.711	0.487	0.502	0.481	0.522	0.528	0.680	0.688

Differences that are not significant are highlighted in bold. Population codes correspond to Table 1

Table 4 Analysis of molecular variance (AMOVA) for *M. leidyi*

Source of variation	Sum of squares	Variance components	Percentage of variation	*P*-value
Among groups	23.71	0.099	21.5	0.002
Among populations within groups	12.50	0.043	9.3	<0.001
Within populations	118.02	0.319	69.2	<0.001

Populations are grouped based on their geographical distribution; [(BA), (PV), (AZ, BL, BLA, NC, SC), (NB, YR, FL)]

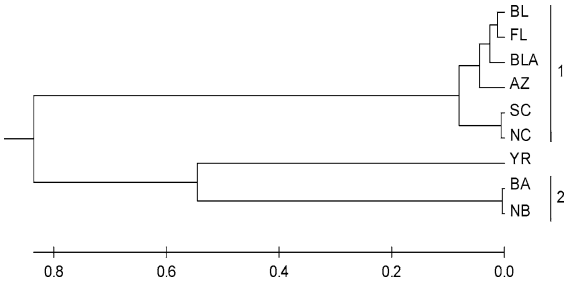

Fig. 2 Genetic distances of *Mnemiopsis leidyi* populations from native and introduced habitats, as determined by a UPGMA tree based on Nei's genetic distance (Nei 1972). Black, Azov and Caspian Seas populations cluster separately from Baltic Sea population. The two recovered clusters suggest two likely invasion pathways between native North American (Atlantic Ocean) populations and introduced Eurasian ones. The South American population (*PV*) was excluded from this analysis because no polymorphism was detected in that population

(UPGMA reconstruction; Fig. 2). These patterns were in agreement with pairwise F_{ST} analysis (Table 3).

The neighbor joining tree did not show any distinct phylogenetic or phylogeographic structure, indicating a close evolutionary history of all alleles. The allele network exhibited limited mutational steps among different alleles. Both results indicated recent geographical expansion of *M. leidyi* populations (Fig. 3).

Discussion

In this study we explored genetic diversity of the ctenophore *M. leidyi* in its native and introduced ranges to determine the invasion history, source(s) of invasions, and number of invasion waves. Results from allele distributions, pairwise F_{ST} values, and cluster analysis reject the hypothesis that the Eurasian invasion resulted from a single introduction into the Black Sea followed by secondary, 'hub and spoke' invasions throughout Eurasia. Rather, our results support the alternative view that at least two separate invasions following different pathways were involved in the invasion of Eurasia. We suggest that an initial invasion from the Gulf of Mexico region (e.g., Florida) to the Black Sea was followed by a second introduction from a more

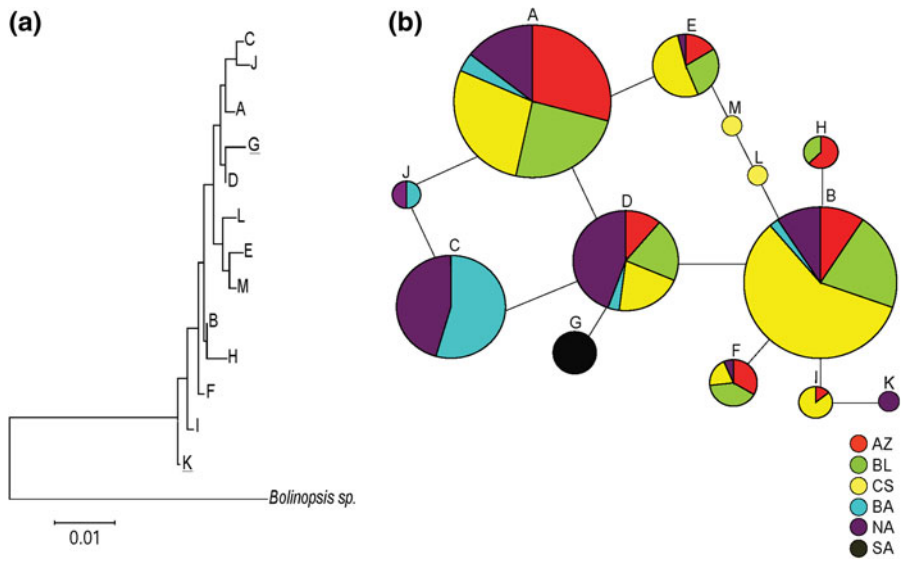

Fig. 3 Phylogenetic and network relationship between the 13 alleles identified at the ITS region. **a** Neighbor joining phylogenetic tree based on nucleotide divergence calculated using Tamura–Nei model. The non-invasive alleles, G and K are *underlined*. **b** Network relationships among alleles for native and invasive populations, inferred by statistical parsimony. *Circles* in the network (A–M) correspond to sampled alleles described in Table 2 and Fig. 1. The size of the *circles* corresponds to the frequency of the allele among all samples. *Shades* are showing different sampling locations: *AZ* Sea of Azov, *BL* Black Sea, *CS* Caspian Sea, *BA* Baltic Sea, *NA* North America, *SA* South America

northerly Atlantic region (e.g., Narragansett Bay) to the Baltic Sea. Subsequent to colonization of the Black Sea, invasion of the Sea of Azov would be relatively straightforward given the natural connection between these basins (Shiganova et al. 2001). In addition, the Volga-Don Canal, which links the Don River and the Sea of Azov to the Volga River and the Caspian Sea, allows commercial vessels to move between these basins. Discharge of contaminated ballast water from the Black/Azov Sea likely accounts for invasion of the Caspian Sea (Shiganova et al. 2004, 2005).

Molecular marker and phylogenetic pattern

Although our analyses of genetic structure of native and introduced ctenophore populations are based on a single marker, the resolution of the nuclear ITS locus was sufficient to reconstruct the invasion history. The length of ITS and its underlined allelic and nucleotide diversity varies greatly among different marine invertebrates, even at the intraspecies level. In the marine mollusk *Tridacna crocea*, ITS1 showed considerable (29%) variation including insertions/deletions (indels) (Yu et al. 2000). Conversely, ITS1 and ITS2 showed no length variation and had only 0.9 and 2.2% variation, respectively, in the sponge *Crambe crambe* (Duran et al. 2004). Previous genetic study of *M. leidyi* along the Netherlands coast indicated very low variation at ITS1, with only one nucleotide difference among sequences (Faasse and Bayha 2006). We found four polymorphic ITS1 sites which resulted in 1.7% variation, mainly due to the wider geographical coverage of our study. Although the global nucleotide diversity was rather low ($\pi = 0.002$), we found relatively high allelic diversity ($h = 0.814$), which enabled us to reconstruct the invasion history of *M. leidyi*.

Neighbor joining phylogenetic reconstruction and the network analysis demonstrate very close evolutionary relationships among alleles and no evident phylogeographic structure. A relatively recent geographic expansion along the Atlantic coast of North America could explain the lack of phylogeographic structure and the strong relatedness among alleles as only a few mutation steps were observed among them.

Genetic diversity and population differentiation

Most of the introduced populations, except those from the Azov and Baltic seas, showed significant deviation from Hardy–Weinberg equilibrium (Table 1). We detected heterozygote deficiency in these populations (Table 1), possibly as a result of inbreeding and/or population admixture (i.e., Wahlund effect). *M. leidyi* is a simultaneous hermaphrodite capable of self-fertilization (Harbison and Miller 1986; Frankham et al. 2002). Self-fertilization is likely to occur during the initial colonization phase, when population size would be comparatively low (Shiganova et al. 2007). Alternatively, population admixture resulting from multiple introductions from different source populations could temporally contribute to the observed departure from Hardy–Weinberg equilibrium.

A number of studies have reported that introduced populations have higher genetic diversity than the native populations from which they were drawn (e.g., Brown and Stepien 2009; Gillis et al. 2009). These patterns may result from populations being seeded by multiple introduction events from genetically distinct sources (Kelly et al. 2006; Roman and Darling 2007). It is possible, therefore, that multiple introductions can account for patterns observed with respect to genetic diversity in introduced populations of *M. leidyi*. Introduced populations had similar allelic diversity to putative source populations, although those in the Black and Caspian seas had higher diversity, including the private alleles L and M, than any surveyed source.

Populations of *M. leidyi* in the Black and Caspian seas exhibited very similar allelic diversity, and the low F_{ST} values suggest high genetic affinity of these populations (Tables 1, 3). Moreover, populations of the ctenophore collected from the northern and southern Caspian Sea—across which profound thermal and salinity gradients exist—also exhibited little population differentiation (Table 4). Low frequency alleles were not detected in the populations sampled from the Black Sea (Fig. 1). Such a genetic pattern is likely due to the recent demographic decline of the Black Sea population after introduction of its predator *Beroe ovata*. Since low frequency alleles tend to be lost first when the population size is shrinking (Evans

et al. 2004), we surmise that predation by *B. ovata* may have caused a loss of rare alleles in the Black Sea population.

Native populations exhibited high population differentiation with F_{ST} values ranging from 0.324 to 0.688. Introduced populations showed the same or less population differentiation with F_{ST} values ranging from −0.014 to 0.421. The lowest F_{ST} values between native and introduced populations were found between Florida and Black Sea ($F_{ST} = -0.027$) and the Baltic Sea and Narragansett Bay ($F_{ST} = -0.018$). The UPGMA tree based on Nei's genetic distance recovers two clades, supporting the population differentiation based on F_{ST} values. These results indicate that the invasion of *M. leidyi* into Eurasian seas has likely occurred through more than a single source. However, these patterns must be interpreted with caution because undersampling in the native range may result in inappropriate assignment of source populations or misidentification of the number of source populations contributing to introduced populations (see Muirhead et al. 2008). We are confident that our narrow coverage in South America has not affected our interpretation of invasion pathways for *M. leidyi*. Individuals from PV in Argentina were all homozygous for a single private allele, indicating that although the sample size of this population is low, it clearly was not the source of any Eurasian populations. The global genetic differentiation between Eurasian populations and PV was 0.534. According to Muirhead et al. (2008), with $F_{ST} \sim 0.5$ and with five individuals sampled from the putative source, the probability that the Eurasian populations were not derived from PV is 95%.

Invasion pathways

Ballast water is a potent vector for introducing NIS globally (Carlton 1985). *M. leidyi* was first recorded in the North and Baltic seas in 2006 (Faasse and Bayha 2006; Javidpour et al. 2006). Faasse and Bayha (2006) suggested that due to higher than normal sea surface temperatures in recent years, expanding populations in Dutch ports of the southern North Sea could serve as a source of *M. leidyi* in the northeastern North Sea (Skagerrak and Kattegat) and possibly the westernmost part of the Baltic Sea. Alternatively, they proposed that the Baltic Sea could be colonized by individuals arriving *via* the Kiel Canal from the North Sea. Genetic evidence from our study supports invasion of Baltic Sea from northern part of native range in North America (e.g., Narragansett Bay; Fig. 2; Table 4), though three other possibilities exist. First, it is possible that the species was transported from the Black Sea in ballast water by ships traversing internal waterways in Europe, as has apparently occurred with other invaders (e.g., Cristescu et al. 2001). Secondly, the Volga River cascade provides a direct link between the Caspian and Baltic seas, and ships could carry the species from south to north. These two scenarios seem very unlikely, however, as genetic differentiation between populations in the Black/Caspian seas and the Sea of Azov and that in the Baltic Sea was much greater than that between Narragansett Bay and the Baltic Sea (Table 3; Fig. 2). A third possibility is that the species was originally introduced into the North Sea from North America and transshipped to the Baltic Sea shortly thereafter (Faasse and Bayha 2006). If this hypothesis is correct, we would expect that genetic differentiation between the North and Baltic seas and Narragansett Bay would be very low. Additional sampling is required to test this possibility.

Further expansion of *M. leidyi* can be anticipated (Richardson et al. 2009). Global warming may facilitate natural dispersal of *M. leidyi* in the North Atlantic Ocean (Oliveira 2007), while cultural eutrophication and fishing pressure appear to promote gelatinous zooplankton blooms to the detriment of other marine species (Purcell et al. 2007; Richardson et al. 2009). Moreover, presence of *M. leidyi* in key European ports predisposes the species to being loaded with ballast water and transported elsewhere. Given the adverse ecological and economic consequences associated with *M. leidyi* invasions, care must be taken by commercial vessels to reduce the likelihood of further invasions. The International Maritime Organization passed ballast water guidelines in 1993 that include not loading knowingly contaminated ballast water, and exchange of ballast water on the open ocean whenever possible (IMO 1993). Both of these prescriptions would seem to reduce the likelihood of spreading *M. leidyi* to additional areas.

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