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Mitochondrial DNA reveals multiple Northern Hemisphere introductions of *Caprella mutica* (Crustacea, Amphipoda)

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Abstract

***Caprella mutica* (Crustacea, Amphipoda) has been widely introduced to non-native regions in the last 40 years. Its native habitat is sub-boreal northeast Asia, but in the Northern Hemisphere, it is now found on both coasts of North America, and North Atlantic coastlines of Europe. Direct sequencing of mitochondrial DNA (cytochrome *c* oxidase subunit I gene) was used to compare genetic variation in native and non-native populations of *C. mutica*. These data were used to investigate the invasion history of *C. mutica* and to test potential source populations in Japan. High diversity (31 haplotypes from 49 individuals), but no phylogeographical structure, was identified in four populations in the putative native range. In contrast, non-native populations showed reduced genetic diversity (7 haplotypes from 249 individuals) and informative phylogeographical structure. Grouping of *C. mutica* populations into native, east Pacific, and Atlantic groups explained the most among-region variation (59%). This indicates independent introduction pathways for *C. mutica* to the Pacific and Atlantic coasts of North America. Two dominant haplotypes were identified in eastern and western Atlantic coastal populations, indicating several dispersal routes within the Atlantic. The analysis indicated that several introductions from multiple sources were likely to be responsible for the observed global distribution of *C. mutica*, but the pathways were least well defined among the Atlantic populations. The four sampled populations of *C. mutica* in Japan could not be identified as the direct source of the non-native populations examined in this study. The high diversity within the Japan populations indicates that the native range needs to be assessed at a far greater scale, both within and among populations, to accurately assess the source of the global spread of *C. mutica*.**

Keywords: biological invasions, COI gene, Crustacea, marine non-native species, phylogeography, population genetics

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Introduction

The volume and rate of oceanic shipping are increasing the global dispersal of non-native marine species. When new populations of a species are detected, it can be difficult to establish the source population(s) or introduction pathways, particularly when the species has previously been identified as introduced in several locations. Clarification is rarely possible using traditional methods which are based on our understanding of the biology and ecology of a species (Holland 2000), and is further confounded by the complicated network of shipping and international trade (Endresen *et al*. 2003). Consequently, the introduction histories of many non-native populations remain unclear. One solution is to use genetic characters, which are often maintained in the introduced populations and can be used to detect the most likely introduction history of a species (Zardus & Hadfield 2005; Simon-Bouhet *et al*. 2006).

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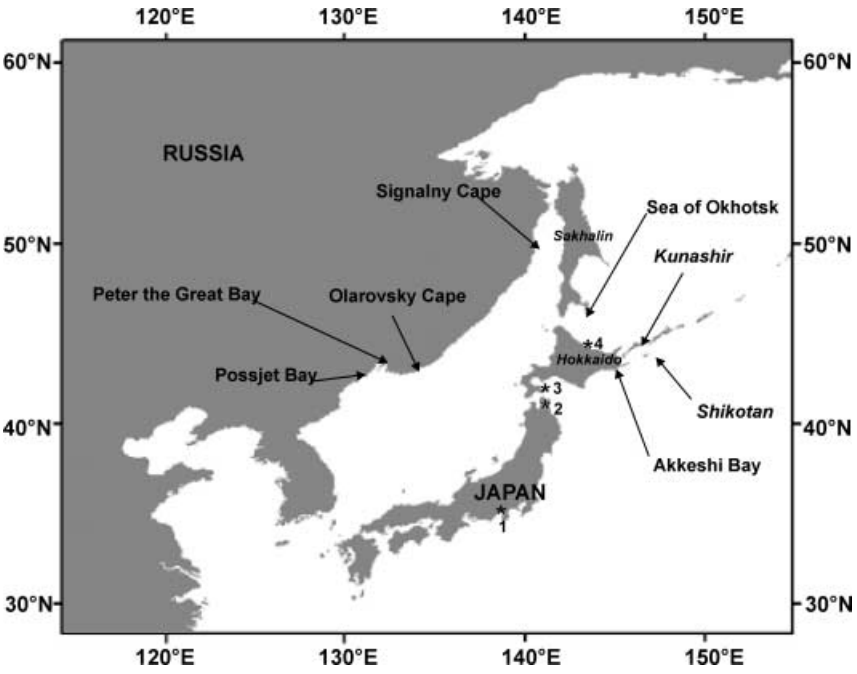

Fig. 1 Native range of *Caprella mutica*. Labels in capitals refer to countries, those in italics refer to islands, arrows indicate locations where *C. mutica* has been found previously (source; Schurin 1935; Vassilenko 2006). Asterisks show locations where *C. mutica* were collected for this study: 1- Port of Nagoya (aquarium sample); 2- Mutsu Bay; 3- Usujiri; 4- Saroma Lake.

Species introductions are often rapid events in which populations are usually subjected to founder effects during colonization, followed by rapid expansion (Sakai *et al*. 2001). When studying introductions using molecular data, it is important to include populations from both native and non-native habitats in order to compare recent gene pools with those of longer-established populations. A low genetic divergence between native and non-native populations may enable identification of the source population (Berg *et al*. 2002); while a high genetic divergence between native and non-native populations may indicate limited gene flow (Zardus & Hadfield 2005), long-term isolation (Stevens & Hogg 2003; Stevens *et al*. 2006), different selection pressures in the non-native habitat (Filchak *et al*. 2000), or different source populations not sampled. Low genetic divergence between non-native populations (compared to that between native populations) may imply similar source populations, recent introduction events, introductions of a few individuals or that the populations were the result of stepping-stone introductions. High genetic divergence would suggest different source populations and/or limited gene flow (Holland 2001; Conner & Hartl 2004). Therefore, in conjunction with ecological studies, molecular approaches can be used to trace the biogeographical history of non-native species. For example, the origins of non-native species (e.g. Hebert & Cristescu 2002; Zardus & Hadfield 2005), mechanisms of introduction (e.g. Pollux *et al*. 2003; Turon *et al*. 2003; Voisin *et al*. 2005) and linkage between non-native populations (e.g. Martel *et al*. 2004; Zardus & Hadfield 2005) have been established previously using molecular data.

The marine amphipod *Caprella mutica* Schurin (1935), a native species of northeast Asia, is now widely distributed in the Northern Hemisphere between latitudes 35°N and 70°N (Ashton *et al*. 2007). This is a considerable extension to its native East Asian latitudinal range between 40°N and 50°N (see Fig. 1). The first non-native population of *C. mutica* was recorded in Humboldt Bay on the Pacific coast of North America in the 1970s (Marelli 1981). Since then, numerous populations have become established in Europe and on the east coast of North America (MIT. Sea Grant 2003; Ashton *et al*. 2007). Beyond the dates of detection, the introduction history of the non-native populations is unknown. Within the native range, ports in Japan and Russia both have important shipping links to North America and Europe and could be source regions for the introduced populations of *C. mutica*. In particular, Japan has been described as the source for a number of introductions of marine species globally (Cohen & Carlton 1995; Lambert & Lambert 1998) and ports in Japan were also considered important sources for species introduced via ballast water (Drake & Lodge 2000).

The cytochrome *c* oxidase subunit I (COI) gene has been useful in elucidating the invasion histories of species for many taxa (e.g. Mollusca, Renard *et al*. 2000; Gastropoda, Simon-Bouhet *et al*. 2006; Insecta, Grapputo *et al*. 2005; Crustacea, Zardus & Hadfield 2005). The COI gene was used to successfully determine multiple origins and incursions of the Atlantic barnacle, *Chthamalus proteus*, in the Pacific (Zardus & Hadfield 2005) and provided evidence of a European source for American populations of the Chinese mitten crab, *Eriocheir sinensis* (Hänfling *et al*. 2002). However,

Table 1 Sample information including ocean basin, source country, site location and description and number of COI sequences obtained per site (*n*). Samples in italics are non-*Caprella mutica* sequences (outgroups). Each population has a unique location code

Ocean Basin	Country	Site	Location code	Site description	*n*
Sea of Japan	Japan	Port of Nagoya, Honshu	Jap1	Aquarium	19
		Mutsu Bay, Honshu	Jap2	Natural habitat	4
		Usujiri, Hokkaido	Jap3	Natural habitat	10
		Saroma Lake, Hokkaido	Jap4	Natural habitat	16
Pacific	USA	Santa Barbara	SaB	Nearshore oil platform	20
Atlantic	Belgium	Zeebrugge	Bel	Harbour	10
	Canada	Passamaquoddy Bay	Can1	Mussel farm	18
		Chaleur Bay	Can2	Mussel farm	10
	England	Poole	Eng	Harbour	2
	France	Le Havre	Fra	Harbour	22
	Germany	Helgoland	Ger	Harbour	20
	Ireland	Betraghboy Bay	Ire1	Aquaculture	17
		Dun Laoghaire Bay	Ire2	Harbour	8
	Norway	Raunefjorden	Nor	Harbour	19
	Scotland	Various sites	Scot	Salmon farms	103
Outgroups					
Pacific	*Australia*	*Hobart, Tasmania*	*Caprella acanthogaster*	*Salmon farm*	2
	New Zealand	*Akaroa*	*Caprella equilibra*	*Salmon farm*	3

no such data exists for *C. mutica*. The present study investigated genetic variation in the COI gene in geographically isolated native and non-native populations of *C. mutica*. Genetic variation of *C. mutica* populations in the Northern Hemisphere was investigated at three geographical spatial scales: (i) Northern Hemisphere (native vs. non-native); (ii) ocean basin (native, non-native Atlantic and Pacific); and (iii) coastline (native, non-native east Pacific, east and west Atlantic). These data were used to contribute to our understanding of the introduction history of *C. mutica* in the Northern Hemisphere.

Materials and methods

Sample collection and DNA extraction

Specimens of *Caprella mutica* were collected from 26 sites in 10 countries in the Northern Hemisphere (Table 1), including four native sites in Japan (Fig. 1). It was not possible to obtain material from further sites in the native range. At each site, a maximum of 22 *C. mutica* were collected (dependent on density at the time of collection) and immediately preserved in 100% ethanol. Individuals were confirmed to be *C. mutica* by stereomicroscope using the characters described by Arimoto (1976). *Caprella acanthogaster* and *Caprella equilibra* were used as outgroups (Table 1).

Tissue for DNA extraction was removed from caprellid appendages (gnathopods, pereopods, antennae and gills) using a stereomicroscope, taking care to avoid contact with the gut. DNA was extracted using either the 'salting-out' procedure (Sunnucks & Hales 1996) or the GenElute Mammalian DNA Miniprep Kit (Sigma). DNA concentration was estimated using a Nanodrop Spectrophotometer (Nanodrop Technologies).

Mitochondrial DNA analysis

A 680-bp fragment of the mitochondrial DNA (mtDNA) COI gene was initially amplified using the universal primers COI2R (5′-GGRTARTCWGARTAWCGNCGWGGTAT-3′) and COI2F (5′-TTYGAYCCIDYIGGRGGAGGAGATCC-3′) (Otto & Wilson 2001). We then designed a genus-specific forward primer COI3F (5′-AGGAGATCCTATCCTTTACC-3′); the primer pair COI3F and COI2R amplified ~600-bp fragment. The polymerase chain reaction (PCR, Saiki *et al*. 1988) was run on a Biometra gradient thermocycler. The 10-μL reaction volume consisted of 1 μL (~20–50 ng) DNA, 1× PCR buffer (Roche), 1.75 mM $MgCl_2$, 0.2 mM dNTP, 0.4 μM of each primer and 0.3 U *Taq* DNA polymerase (Roche). Thermal cycling conditions were: 94 °C for 1 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min (modified from Witt & Hebert 2000). Negative controls were included with at least 1 per 10 reactions.

Agarose gel electrophoresis using SYBR stain (Invitrogen) was used to visualize PCR products, which were then purified using SAP/Exo (USB Corp.; Hanke & Wink 1994). Sequencing reactions were performed using BigDye Terminator chemistry (Applied Biosystems Inc.), with the

forward primer (COI3F) for all samples, and with the reverse primer (COI2R) for approximately 25 samples. Sequencing was performed using an ABI 3730 automated sequencer (Applied Biosystems Inc.) at the Allan Wilson Centre Genome Service, Massey University, New Zealand.

DNA sequence analysis

Sequences were verified as Caprellidae DNA using the GenBank BLASTN search (Altschul *et al*. 1990) and checked for open reading frames (using MACCLADE version 4.05; Maddison & Maddison 2000) to confirm the absence of nuclear copies (e.g. pseudogenes) or other unintended sequence types. All unique sequences for each species were deposited with GenBank (accession nos: DQ466220–466523). Preliminary analyses including three species in the genus *Cyamus* (Suborder Caprellidea) obtained from GenBank (*Cyamus erraticus,* accession no. DQ095135; *Cya. gracilis,* DQ095105; *Cya. ovalis,* DQ09150) identified the two *Caprella* species (*C. equilibra, C. acanthogaster*) as a divergent sister group to a monophyletic group containing all *C. mutica* sequences. *C. equilibra* and *C. acanthogaster* were subsequently used as outgroup taxa for the phylogenetic analyses. Sequences were edited and aligned using CONTIGEXPRESS (Vector NTI Advance 10, Invitrogen) and trimmed to a length of 563 bp.

Phylogenetic and statistical analysis

Chi-squared tests (implemented in PAUP* 4.0b10; Swofford 2002) were used to test the hypothesis of homogeneity of base frequencies among sequences. Bayesian phylogenetic analysis (BEAST version 1.4.5; Drummond & Rambaut 2007) was used to perform a Bayesian Markov chain Monte Carlo (MCMC) search in which a separate substitution model was applied to the 1st and 2nd codons vs. the 3rd codon (using the SRD06 model in BEAST). We ran 20 000 000 generations, sampling trees and parameters every 2000 generations, with the first 100 000 generations discarded as burn-in determined from plotting log-likelihood values against generation time in TRACER (version 1.3; Rambaut & Drummond 2004). The consensus (majority-rule) tree (sampled after the initial burn-in period) was obtained using TREEANNOTATOR (version 1.4.5; Drummond & Rambaut 2007), and visualized using FIGTREE (version 1.0; Drummond & Rambaut 2007). MODELTEST 3.7 (Posada & Crandall 1998) was used to determine the appropriate model parameters for maximum likelihood (ML) analysis in PAUP*. The HKY + I + Γ model [–ln L = 2070.06 (AIC); base frequencies set to A = 0.2626, C = 0.2087, G = 0.1679, T = 0.3608; Ti/tv ratio = 5. 1765; I = 0.4462; Γ = 3.1385] was found to be the best fit to the data; all other options in PAUP* remained as default for the ML heuristic analyses. ML bootstrap analyses were conducted with 500 replicates (Felsenstein 1985) using the same settings as the heuristic search. Relationships among mtDNA sequences were estimated via a haplotype network using the statistical parsimony method (Templeton *et al*. 1992) in TCS version 1.21 (Clement *et al*. 2000) while ensuring the alignment was free of missing or ambiguous sites (Joly *et al*. 2007).

ARLEQUIN 3.0 (Excoffier *et al*. 2005) was used to calculate pairwise F_{ST} measures between sites, and Φ-statistics to compare genetic differentiation among geographical regions using analysis of molecular variance (AMOVA). Total variation within the non-native populations was analysed by including all geographically isolated populations independently in an AMOVA. To analyse the geographical extent of population differentiation, three groupings were used for the AMOVA tests: (i) native vs. non-native (Japan vs. all other source regions), (ii) ocean basin province (native, east Pacific, Atlantic), and (iii) coastline (native, east Pacific, east Atlantic, west Atlantic).

Results

Sequence variability

Of 298 *Caprella mutica* individuals, 49 were from the putative native area in Japan and 249 from non-native populations (Table 1). Overall, base frequencies were biased towards A and T (A = 26%, T = 36%, C = 20%, G = 18%). We were unable to reject the hypothesis of homogeneity of base frequencies among sequences for all sites ($\chi^2_{153} = 5.05$, $P = 1.00$), the 170 variable sites ($\chi^2_{153} = 19.23$, $P = 1.00$), or the third codon sites ($\chi^2_{153} = 17.17$, $P = 1.00$).

With the *Caprella* outgroups included in the analysis, there were 170 polymorphic sites. Of these, 60 were variable among the 300 *C. mutica* sequences (Table S1, Supplementary material); 13.33%, 3.33% and 83.33% of the polymorphic sites were at the first, second and third codon positions, respectively. No insertions or deletions were detected in any of the sequences. Sequence divergence between all identified *C. mutica* haplotypes ranged from 0.2% to 3.4% (Table S2, Supplementary material). The levels of divergence among specimens identified as *C. mutica* do not suggest the presence of multiple 'cryptic' species (Witt & Hebert 2000; Hogg *et al*. 2006).

Thirty-one of the 38 unique haplotypes were observed in the native range, and none of these were shared between the native locations (Fig. 2). There were two amino acid changes among predicted protein sequences in the native range (Table S1), one in haplotype 1 (Threonine–Alanine) and one in haplotype 23 (Threonine–Isoleucine). Haplotypes F and G (unique to Santa Barbara) also had an amino acid change from Threonine to Isoleucine at a different position (node 503). In the Atlantic populations, there were five unique haplotypes (A–E) but no amino acid changes.

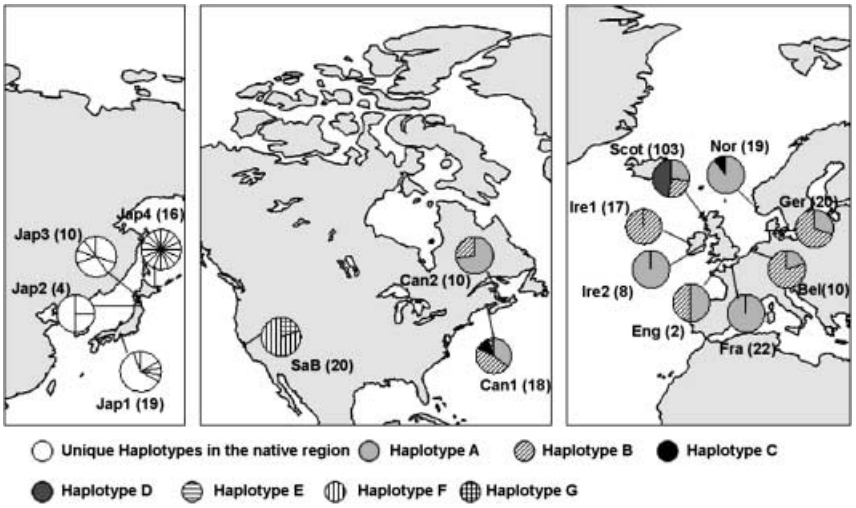

Fig. 2 Distribution of the 38 COI haplotypes of *Caprella mutica* in the Northern Hemisphere. Each site is represented by a pie chart showing population composition and relative haplotype frequency. Site codes correspond to Table 1, numbers in parentheses indicate sample size. All haplotypes in the native range are unique to a single location, represented by white pie fill. Pie fill in the non-native sites corresponds to the haplotypes in Table S1.

Population genetic structure

In the native range, high genetic variability was identified, no major clades were resolved and haplotype groups did not represent clear source locations for non-native populations (Figs 3 and 4). Haplotype diversity was lower at Port of Nagoya (Jap1, $h \pm SD = 0.621 \pm 0.121$; Table S3, Supplementary material) and Usujiri (Jap3, $h \pm SD = 0.867 \pm 0.085$; Table S3) compared to the 100% diversity observed in samples from the other two Japanese sites. Nucleotide diversity was fairly constant at all four native sites ($\pi = 0.009–0.014$; Table S3). Pairwise F_{ST} values were small between all native sites (–0.015–0.258; Table S4, Supplementary material), and statistically significant between Port of Nagoya, Usujiri and Saroma Lake (Jap1, Jap3 and Jap4; Table S4). The nonsignificance of pairwise F_{ST} values with Mutsu Bay (Jap2) was most likely due to the small sample size.

The non-native haplotypes clustered into four groups: (1) A and B, (2) C, (3) D, and E (4) F and G; (Fig. 3). The low bootstrap support most likely reflected the low genetic divergence between these intraspecific sequences. The four groups were also apparent in the haplotype network, constructed without weighting (Fig. 4) and the non-native haplotypes were consistently found at the tips of the network. A total of seven haplotypes were identified in the non-native populations (Figs 2 and 3), and there was considerable genetic differentiation between non-native populations (total non-native $\Phi_{ST} = 0.612$). Haplotypes F and G, found only in Santa Barbara, were the most divergent from all other haplotypes, with genetic distance (HKY + I + Γ model) ranging from 1.2% to 3.4% when compared to all other haplotypes (Table S2). The five haplotypes present in the Atlantic (A–E) were highly differentiated from those at Santa Barbara (2.4–3.2% divergence, Table S2; 0.782–0.994 significant pairwise F_{ST} values, Table S4). Passamaquoddy Bay (Can1) had the highest site haplotype diversity followed by Scotland ($h \pm SD = 0.677 \pm 0.075$ & 0.642 ± 0.022, respectively, Table S3).

F_{ST} values were greater between native and non-native sites ($P < 0.05$, Table S4). The exception to this was the nonsignificance of the F_{ST} between England and Mutsu Bay (Jap2), most likely due to the small sample sizes at both these locations. AMOVA tests resulted in significant genetic differences within regions, among sites within regions, and among regions at all geographical levels (Table 2). All three population groupings were significant (native vs. non-native, ocean basin, and coastline). Grouping by ocean basin (i.e. native, east Pacific and Atlantic) explained the most (59%) among-region variance ($\Phi_{CT} = 0.592$, $P < 0.001$; Table 2).

Discussion

Attempts to reduce the increasing frequency and problematic impacts of marine invasions are frequently hindered by the lack of information regarding the patterns and modes of introduction of non-native species. The aim of this study was to investigate the introduction history of native and non-native populations of *Caprella mutica* in the Northern Hemisphere.

A high level of genetic diversity, but no phylogeographical structure, was found in the Japanese populations of *C. mutica*. The haplotype network and low F_{ST} values suggest large and diverse populations. High genetic diversity in the native range has been observed as a characteristic of invasive species (Ehrlich 1986, 1989), and may be a result of the widespread and gregarious nature of many invasive species in their native habitats (Duda 1994). The Japanese haplotypes are phylogenetically distinct from the North American and European haplotypes indicating that these were not the source of the introduced populations sampled. However, given the high haplotype diversity in the populations from Japan, these populations can not be ruled out as a potential source (it may just be that the sampled individuals do not have shared haplotypes) or that other areas in Japan are the source of the introduced populations.

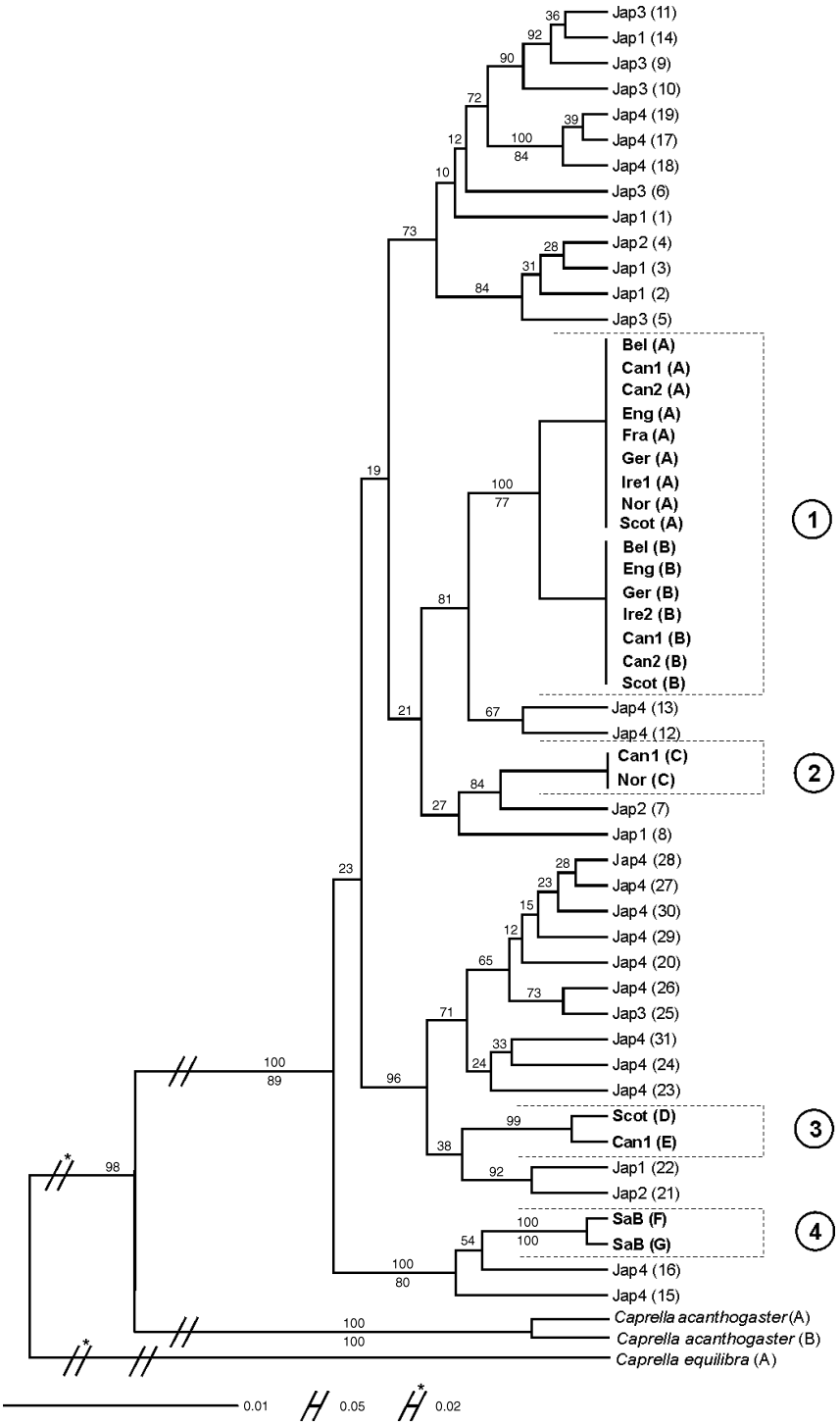

Fig. 3 Phylogeny of 15 *Caprella mutica* populations from its native and non-native range, constructed using a Bayesian phylogenetic approach. *Caprella acanthogaster* and *Caprella equilibra* haplotypes were included as outgroup taxa. Numbers above branches show Bayesian posterior probabilities, below indicate bootstrap support (500 replicates) greater than 70%. Population codes (e.g. Jap1) indicate geographical source and correspond to Table 1, characters in parentheses correspond to the haplotype numbers in Table S1, bold text indicates non-native locations. Dashed lines and numbers indicate groups referred to in the text.

The high level of genetic divergence between non-native populations of *C. mutica* in the Northern Hemisphere indicates significant differentiation between populations in different ocean basins and among populations in geographical regions. A total of seven haplotypes were found in the non-native populations, indicating considerably reduced genetic diversity relative to the native populations in Japan, where 31 unique haplotypes were observed. Introduced haplotypes consistently fell at the tips of the haplotype network, suggesting they were more recently derived than haplotypes at the interior of the network (Castelloe & Templeton 1994). Recent mutations in the non-native populations could be responsible for the positioning of these haplotypes at the tips of the network. Alternatively,

Table 2 Results of the AMOVA tests comparing variation in COI sequences of *Caprella mutica* grouped at three geographical levels: (A) native vs. non-native, (B) ocean basin (native, non-native east Pacific and Atlantic), and (C) coastline (native, non-native east Pacific, east and west Atlantic)

Group	Source of variation	d.f.	SS	Variance components	Percentage of variation	Φ_{ST}	Φ_{SC}	Φ_{CT}
A Native vs. non-native	Among regions	1	155.610	1.46553	30.01			0.300’
	Among sites within regions	13	441.875	1.84307	37.75		0.539*	
	Within sites	281	442.340	1.57416	32.24	0.678*		
	Total	295	1039.824	4.88277				
B Ocean basin	Among regions	2	415.594	3.43525	59.21			0.592’
	Among sites within regions	12	181.890	0.79207	13.65		0.334*	
	Within sites	281	442.340	1.57416	27.13	0.728*		
	Total	195	1039.824	5.80149				
C Coastline	Among regions	3	422.480	2.45480	50.31			0.503’
	Among sites within regions	11	175.004	0.85031	17.43		0.351*	
	Within sites	281	442.340	1.57416	32.26	0.677*		
	Total	295	1039.824	1.87927				

*Significant at $P < 0.0001$; statistical probabilities derived from 1023 permutations.

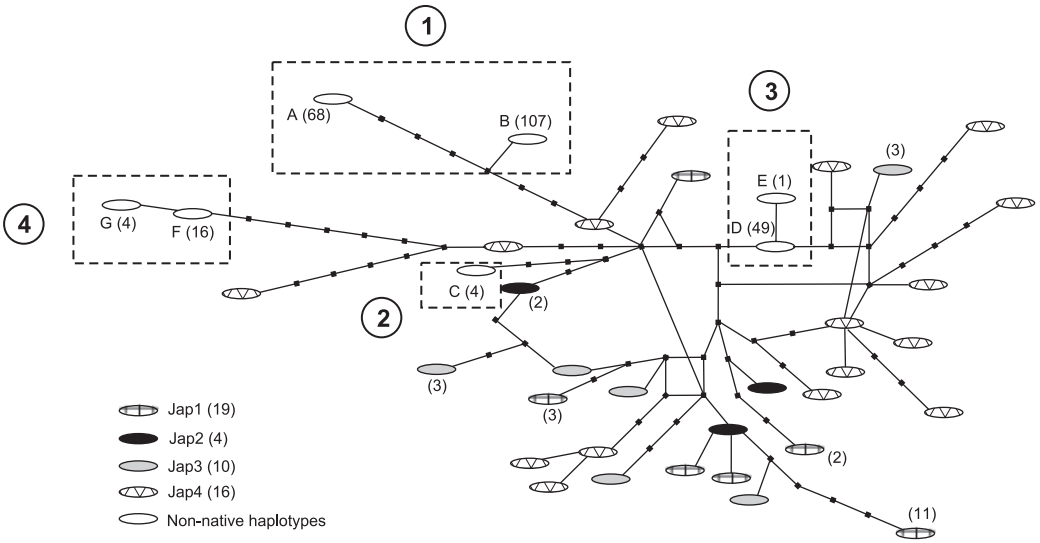

Fig. 4 Haplotype network for the 38 COI haplotypes (from 298 individuals) of *Caprella mutica* from its native and non-native range, estimated by statistical parsimony (Templeton *et al*. 1992). Lines show most-parsimonious relationships between individual haplotypes, represented by ovals. Nodes along each branch designate the number of base pair differences between haplotypes. Ovals representing native haplotypes have a fill pattern corresponding to the geographical source (see legend). Non-native haplotypes are labelled corresponding to Table S1 (A–G). Frequency of hapolotypes are in parentheses, no parentheses indicates a frequency of 1. Dashed lines and numbers indicate groups referred to in the text.

these haplotypes could be present in the native range but not sampled in this study (this is considered likely given the low number of individuals and sites sampled in the native range).Collectively, the Atlantic populations have a comparatively high genetic diversity and most of the populations from this ocean basin share haplotypes. The unique haplotypes present on the Pacific coast of North America indicate strong genetic differentiation and no gene flow between the Atlantic and Pacific populations. The geographical distribution of the haplotypes and differentiation between ocean basins suggests multiple introduction pathways in the Northern Hemisphere.

Independent pathways to the east and west coasts of America have been suggested for non-native populations of *Botryllus schlosseri* (Stoner *et al*. 2002). In our study, the low genetic diversity in the Pacific (Santa Barbara) *C. mutica*

population indicates one, or possibly two, introduction events of a small number of individuals, either directly from the native region (but unidentified here) or from other established populations on the Pacific coast of America. A non-native population of *C. mutica* was described from the west Pacific at least 20 years before the species was discovered in other regions (Carlton 1979). These data indicate that introductions of *C. mutica* to the northwest Pacific are likely to be independent to those on the North Atlantic coasts.

The sharing of haplotypes among distant locations in the Atlantic reveals recent connections between the non-native populations in this region. This is in marked contrast to the lack of any identified haplotype being shared between the four Japanese populations, and could be further supported by the location of the introduced haplotypes at the tips of the haplotype network (although likely to be affected by low sample sizes). On the Atlantic coast of North America, the high haplotype diversity in the Canadian Passamaquoddy Bay population suggests that this may be the primary introduction site to the Atlantic, with either a large number of individuals contributing to the founder population in a single introduction event, or several introduction events increasing the genetic diversity. However, introduction records do not support this as the primary introduction site, with individuals being identified in Europe in 1995 (Platvoet *et al*. 1995) and Atlantic North America in 2003 (MIT. Sea Grant 2003). Further north in Chaleur Bay, haplotypic diversity was reduced, with only two haplotypes present (A and B). These haplotypes were dominant in both the Passamaquoddy Bay and eastern Atlantic populations. Thus, two possible introduction pathways are suggested for Chaleur Bay: (i) northward transport from Passamaquoddy Bay, as has been observed for several introduced species such as the green crab, *Carcinus maenas* (Audet *et al*. 2003) and *Codium fragile* spp. *tomentosoides* (Bird *et al*. 1993); and (ii) *trans*-Atlantic transfer from Europe. The presence of haplotype C at both Passamaquoddy Bay and Raunefjorden, Norway, provides further evidence of potential *trans*-Atlantic introductions. There are examples of Crustacea being introduced in both directions across the Atlantic, for example the crabs *Car. maenas* from Europe to North America (Geller *et al*. 1997) and *Hemigrapsus sanguineus* in the opposite direction (Breton *et al*. 2002). The alga *Co. fragile* ssp. *tomentosoides* may also have been introduced across the Atlantic from Western Europe (Carlton & Scanlon 1985).

Haplotypes A and B dominated the populations in the eastern Atlantic (Europe), with the highest diversity present in the Scottish populations (although this was also the most intensively sampled region). Thus, Scotland may be a primary introduction site in the eastern Atlantic, with subsequent erosion of genetic diversity occurring during introduction events from Scotland to other sites within the region. The presence of haplotype C in Norway, suggests a further primary introduction site in Europe, with potential *trans*-Atlantic links to Passamaquoddy Bay, as mentioned above.

The presence of only one haplotype in the French and Irish *C. mutica* populations may represent recent steps in the spread of this species. Founding populations may have consisted of a small number of individuals in a single introduction event derived from a single source location (Holland 2000; Zardus & Hadfield 2005). The populations in France and Ireland could be derived from any of the Atlantic populations, as haplotypes A and B are common to all of them, with the exception of Norway.

Shipping routes have existed across the Atlantic for more than 500 years (Carlton 1989), and shellfish transfers have also historically been made across the Atlantic (Loosanoff 1975). Both of these vectors are likely to be responsible for introductions of *C. mutica* globally. In the Pacific, oyster spat introductions were considered the most likely vectors for the introduction of *C. mutica* to San Francisco Bay (Cohen & Carlton 1995). Ballast water and the movement of oil platforms (or similar structures) across the ocean (Rodríguez & Suaréz 2001; Page *et al*. 2006) may also be introduction vectors. Because *C. mutica* does not have a free-swimming planktonic larval stage, it has limited dispersal abilities, and consequently, secondary dispersal within regions is also most likely to be human mediated or associated with drift-weed (Ashton 2006). Aquaculture is an important industry in Canada, Scotland and Norway (FAO Fisheries Statistics 1999–2006), with movements of species and equipment facilitating the transfer of the caprellid both within and between these regions, as might be inferred by the sharing of haplotypes A, B and C. The unregulated movement of recreational boats provides a further dispersal mechanism, as excessive hull fouling of many small boats is commonplace (Ashton *et al*. 2006). The global spread of *C. mutica* presented here is most likely due to human-mediated dispersal, in particular shipping, aquaculture activities and recreational boating (Ashton 2006). The risk of this species, in particular, spreading further is extremely high. In addition to its spread throughout the Northern Hemisphere examined here, this species has now been identified morphologically from the first southern hemisphere site (Port of Timaru, New Zealand; Inglis *et al*. 2006) now making this a 'globally' introduced species.

Genetic analyses of non-native species in both their native and introduced ranges can reveal details of an invasion which cannot be elucidated from distributional information. The level of diversity of the COI gene of sampled populations of *C. mutica*, while not establishing a single Asian source population, has enabled several conclusions regarding its introduction history to be made. This lends support to the conclusion of Voisin *et al*. (2005) that a variety of processes can be involved in the widespread success of an introduced species, including multiple source populations, introduction events and introduction pathways. Sequencing the COI gene of *C. mutica* has allowed inferences to be made concerning introduced populations on a global scale. Including more

individuals from more populations (and within populations) in the native range would increase the likelihood of accurately identifying source populations for the global spread of *C. mutica*. Furthermore, use of a nuclear marker (i.e. biparental inheritance) would potentially allow larger effective sampling of the population(s).

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Supplementary material

The following supplementary material is available for this article:

Table S1 The 60 variable nucleotide sites among the 38 *Caprella mutica* haplotypes. *Caprella mutica* haplotype 1 is used as a reference sequence

Table S2 Genetic distance based on sequence variation in the mtDNA COI sequences (563 aligned sites) among the 38 identified *Caprella mutica* haplotypes and two outgroup taxa (Cap1- *Caprella acanthogaster*; Cap2- *Caprella equilibra*)

Table S3 Sample number (*n*), haplotype diversity (*h*), and nucleotide diversity (π) for COI sequences of Caprella mutica. Site codes correspond to Table 1

Table S4 Pairwise comparisons of F_{ST} values for COI sequences of *Caprella mutica* from 15 sites in the native and non-native range

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