Anthropocene invasion of an ecosystem engineer: resolving the history of *Corophium volutator* (Amphipoda: Corophiidae) in the North Atlantic

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Resolving the natural histories of species is important for the interpretation of ecological patterns, as it provides evolutionary context for the interactions between organisms and their environment. Despite playing an integral role on the intertidal mudflats of the North Atlantic as an abundant food source for predators and as an ecosystem engineer that alters the soft sediment environment, no previous studies have provided empirical evidence to determine the biogeographical origin of the amphipod *Corophium volutator*. To resolve its status as introduced or indigenous in Europe and North America, we analyzed sequence data for two mitochondrial loci and two nuclear markers, aiming to determine whether the present range of *C. volutator* is the result of unresolved taxonomy, persistence in glacial refugia, natural trans-Atlantic dispersal, or human-mediated introduction. Our results demonstrate a reduced genetic diversity in North American populations that is a subsample of diversity in European populations, with coalescent analyses of mitochondrial and nuclear DNA supporting different models of multiple introductions from Europe to the Bay of Fundy and Gulf of Maine in North America. These results suggest that *C. volutator* was introduced to North America prior to the first surveys of local biota in the 20th Century, which has broad implications for interpretations of community and ecosystem interactions in the North Atlantic intertidal. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **115**, 288–304.

ADDITIONAL KEYWORDS: historic introduction – mitochondrial DNA – nuclear DNA – phylogeography.

INTRODUCTION

Biological introductions are a significant driver of human-caused environmental change that can disrupt ecosystem processes such as primary productivity, decomposition, hydrology, geomorphology, nutrient cycling, and disturbance regimes (Vitousek *et al*., 1997; Ehrenfeld, 2010; Simberloff, 2011; Vila *et al*., 2011). To manage and predict the future impact of introductions requires an understanding of ecosystems' natural states; however, our knowledge of what is natural is biased by the lack of both the quantity and quality of baseline data (Ruiz *et al*., 2000). This is particularly problematic in environments where primary studies of local biota lag behind introduction vectors associated with global exploration and trade, making the extent of historic introductions unknown and potentially underestimated. The common assumption that species with no documented introduction history are native has been challenged by Carlton (1996), who suggests that species that cannot be reliably assigned a status of native or introduced by observational data may have been influenced by historic anthropogenic dispersal. In many cases, molecular evidence has revealed species once considered to be widespread or cosmopolitan to be the product of historic introductions or unresolved taxonomy (Geller, Darling & Carlton, 2010). Resolving the biogeographical origins of species with uncertain histories is critical for understanding the impact of human activity on community ecology, natural diversity, and evolution (Grosholz, 2002; Carlton, 2003; Carlton, 2009).

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Marine life in the North Atlantic has a particularly suspect natural history because extensive exploration and trade between Europe and the new world pre-dated biological surveys by centuries (Carlton, 2003). Species that are indigenous to the North American Atlantic had to either persist in glacial refugia during Pleistocene glaciations approximately 20 000 years ago or colonize the North American coast via long distance dispersal from Europe, where glaciations were less severe (Hewitt, 2000; Wares & Cunningham, 2001). Although natural long-distance dispersal across the Atlantic is hindered by vast expanses of open water, mid-Atlantic islands such as Iceland and Greenland provided stepping-stones between Europe and North America for species capable of larval dispersal or rafting via ocean currents (Ingolfsson, 1992). A disjunct range (present in Europe and America but not in between) is inconsistent with the stepping-stone pattern expected to result from natural dispersal, and can be explained by one of four models proposed by Haydar (2012): (1) persistence in glacial refugia on both coasts with no post-glacial colonization of intermediate islands; (2) unresolved taxonomy with morphologically similar but genetically distinct species on each coast; (3) natural trans-oceanic dispersal without the colonization of intermediate islands; and (4) human-mediated introduction. To our knowledge, no studies have yet found evidence for models 1 or 3 in species with a disjunct distribution in the North Atlantic, suggesting that species with similar distributions may be either introduced or phylogenetically unresolved.

In the absence of direct paleontological, archaeological or observational evidence, molecular tools can be used to explicitly test models of population history. Within species, genetic data may be sufficient to rule out one or more hypotheses, although this does not always provide the ability to distinguish between competing models (Haydar *et al.*, 2011). If a disjunct distribution is the result of persistence on both coasts during the Pleistocene glaciations, each coast should harbour one or more monophyletic lineages as a result of restricted gene flow following vicariance (Haydar, 2012). Under this circumstance, colonization from southern glacial refugia to northern regions is expected to cause serial founder events that result in decreasing genetic diversity as a function of distance from refugial populations (Vainio & Vainola, 2003; Maggs *et al.*, 2008; Provan & Bennett, 2008), although the level of diversity on either coast could vary depending on the severity of bottlenecks. Natural trans-Alantic dispersal could result in either a pattern of uniform outward range expansion if dispersal arose from a rare event or admixture amongst colonized populations in the presence of ongoing gene flow. Although both natural models predict a signal of uniform expansion from the source populations, anthropogenic dispersal has the potential to facilitate multiple introductions followed by the admixture of genetically distinct lineages (Brawley *et al.*, 2009; Blakeslee *et al.*, 2010). Introduced species tend to undergo more severe bottlenecks than species that colonize new habitat naturally (Nei, Maruyama & Chakraborty, 1975; Sakai *et al.*, 2001; Hanfling & Kollmann, 2002), and different introduction processes can lead to a variety of genetic patterns in introduced populations (Voisin, Engel & Viard, 2005). Using molecular tools to differentiate between these patterns can provide insight into evolutionary relationships in communities and ecosystems in which introduction vectors predate historical observations.

Many introduced species in the North-West Atlantic intertidal have altered community dynamics through direct interactions (e.g. *Styela clava*, *Botrylloides diegensis*, and *Membranipora membranacea*: Berman *et al.*, 1992; *Mytilopsis leucophaeata* and *Ostrea edulis*: Carlton, 1999; *Carcinus maenas*, Carlton & Cohen, 2003; *Littorina littorea* and *Fucus serratus*: Brawley *et al.*, 2009), but some introduced species that alter the physical environment have the potential to cause change to the entire ecosystem (e.g. *Hediste diversicolor*; Einfeldt, Doucet & Addison, 2014). The littoral amphipod *Corophium volutator* (Pallas, 1766) is an ecosystem engineer that affects environmental conditions for all other members of the soft sediment community by constructing and moving water through U-shaped burrows, altering substrate stability, permeability, water content, nutrient cycling, and oxidation rates (Meadows & Tait, 1989; Meadows, Tait & Hussain, 1990; Mouritsen, Mouritsen & Jensen, 1998). Although important as an abundant and nutritious prey item for many species of economically valuable fish (McCurdy *et al.*, 2005), migratory shorebird (Peer, Linkletter & Hicklin, 1986), and other invertebrates (Pihl & Rosenberg, 1984), *C. volutator's* nontrophic impact may overshadow these interactions by influencing ecosystem structure and function via niche construction (Widdows & Brinsley, 2002).

Despite the central role of *C. volutator* in the intertidal community, there is no direct observational or fossil evidence to indicate its biogeographical origin in the North Atlantic. Although listed as amphi-Atlantic by Bousfield (1973), no studies have found *C. volutator* in Iceland or Greenland, the stepping-stone landmasses between Europe and North America. This disjunct distribution is not likely the result of natural dispersal. Because of a brood-rearing life history, *C. volutator* has a limited dispersal potential that is further restricted by current boundaries, causing strong patterns of genetic differentiation along coastlines at small spatial scales (Wilson,

Boates & Snyder, 1997). In North America, hydrological patterns that create barriers to dispersal have been attributed to maintaining strong genetic subdivision of *C. volutator* between the Bay of Fundy and the Gulf of Maine (Einfeldt & Addison, 2013). A similar pattern of genetic subdivision is exhibited by the polychaete *H. diversicolor*, which was introduced to North America from European populations and commonly occurs with *C. volutator* on both sides of the Atlantic (Einfeldt *et al*., 2014). Despite suggestions that *C. volutator* may have been introduced to North America (Chapman, 1999; Carlton & Ruiz, 2005) that conflict with the statement by Wilson *et al*. (1997) suggesting that preliminary (and unpublished) genetic investigations show a long period of separation between the Bay of Fundy and European populations, the hypothesis that *C. volutator* was introduced to North America has not been empirically tested. Other contentions over the biogeography of *C. volutator* have been resolved as more detailed morphological information within the genus became more readily available, reducing any chances of misidentification. *Corophium volutator* was considered to be introduced in the Mediterranean (Crawford, 1937; Segerstrale, 1959) but subsequent to Bellan-Santini *et al*. (1982) calling for confirmation of its presence, no reliable records of *C. volutator* have been presented from this area.

In the present study, we used DNA sequence data from mitochondrial and nuclear markers to test models of population history for the ecosystem engineer *C. volutator*. To determine whether its disjunct distribution in the North Atlantic is the result of unresolved taxonomy, glacial persistence, natural trans-oceanic colonization or anthropogenic introduction, we compared patterns of diversity between North American and European populations and tested whether North American populations result from single or multiple colonization events.

MATERIAL AND METHODS

SAMPLE COLLECTION, DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Samples of *C. volutator* were collected from 16 sites spanning the species' range on the Atlantic coast of Europe (Fig. 1) and shipped in 24% ethanol to Canada. Specimens were stored in 95% ethanol at −20 °C prior to DNA extraction and sequencing.

DNA was extracted from specimens of *C. volutator* using a cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1987), with the remaining tissue stored in 95% ethanol at −80 °C as a voucher. Eggs and larvae were excised from the brood pouches of gravid females prior to DNA extraction to avoid amplification of nuclear DNA from multiple individuals.

To investigate phylogeographical patterns in *C. volutator* and compare them with those found in species with known introduction histories and published mitochondrial sequence data, we amplified two mitochondrial DNA fragments: the 5' end of cytochrome *c* oxidase subunit I (*COI*) and a fragment spanning a 143-bp portion of the 3' end of *COI*, an intergenic spacer, and 376 bp of the 5' end of *COII* (*SUBI*). Because inference based on a single matrilineal gene tree can potentially provide a biased view of an organism's evolutionary history, we also sequenced the anonymous unlinked nuclear loci *CV1* and *CV2*. Amplification for all markers was performed using primers and protocols described in Einfeldt & Addison (2013).

Amplicons were checked in 1% agarose gels, sequenced with 3730xl DNA Analyzer technology (Applied Biosystem) using forward, reverse or both primers at the Genome Québec Innovation Centre (Montréal, Québec), and aligned, edited, and trimmed to standard length in SEQUENCHER, version 5.0 (Gene Codes). We combined these new European data with the North American data set analyzed by Einfeldt & Addison (2013; GenBank accession numbers JX991308–JX992554).

POLYMORPHISM AND PHYLOGENETIC ANALYSIS

The disjunct range of *C. volutator* in the North Atlantic can be explained by one of four hypotheses, which have different predictions for overall levels of polymorphism and phylogenetic relationships between alleles in the North-West Atlantic (NWA) and North-East Atlantic (NEA) (Table 1). If this range is the result of a taxonomic artefact, divergence at the *COI* marker between sequences from populations in the NWA and the NEA should be greater than 0.16 substitutions per site, which is indicative of species level differentiation at this locus in crustaceans (Lefébure *et al*., 2006). Reciprocal monophyly with high diversity at all loci in the NWA and the NEA would suggest that *C. volutator* persisted in glacial refugia on both coasts. Intermediate or high diversity in the NWA at all loci with alleles identical to or descended from those found in the NEA would indicate natural transoceanic dispersal from the NEA to the NWA. By contrast, a pattern of low diversity in the NWA with alleles being a subsample of those found in the NEA but non-monophyletic would suggest a recent and likely human-mediated introduction.

Allelic phases of nuclear (n)DNA were inferred using PHASE, version 2.1.1 (Stephens, Smith & Donnelly, 2001; Stephens & Scheet, 2005) for 10 000 iterations after a burn-in of 1000, with all haplotype

Table 1. Potential causes of the disjunct distribution of *Corophium volutator* in the North Atlantic and predictions of genetic consequences

Cause of disjunct range	Diversity in North America	Genetic pattern
Taxonomic artefact	High	Reciprocal monophyly; divergence > molecular threshold for species
Persistence in glacial refugia	High	Reciprocal monophyly; divergence < molecular threshold for species
Natural trans-oceanic dispersal	High/intermediate	No reciprocal monophyly, alleles descended from source diversity
Human-mediated introduction	Low	Alleles are subsample of source diversity

reconstructions included in analyses having a posterior probability $P > 0.95$ consistently over three runs. Statistical parsimony implemented in TCS, version 1.21 (Clement, Posada & Crandall, 2000) was used to map haplotype networks of mitochondrial (mt)DNA sequences. We calculated measures of genetic diversity in European populations to compare with data published for North American populations (Einfeldt & Addison, 2013). Haplotype and nucleotide diversity were calculated using DNASP, version 5.1 (Librado & Rozas, 2009), and nuclear markers were confirmed to be unlinked using GENEPOP, version 1.2 (Raymond & Rousset, 1995; Rousset, 2008). Observed and expected heterozygosities and fixation indices were calculated using ARLEQUIN, version 3.11 (Excoffier, Laval & Schneider, 2005), with Fu and Li's F and Tajima's D calculated to test for departures from neutrality based on allelic states or segregating sites, respectively. The significance of these neutrality tests was assessed by performing 10 000 bootstrap replicates.

Because the effects of sampling may lead to an underestimation of genetic diversity, we used rarefaction curves to estimate the total number of alleles or haplotypes present in the NWA and the NEA. ESTIMATE-S (Colwell *et al.*, 2012) was used to generate haplotype estimation curves through Monte Carlo resampling and randomization of sample order over 10 000 replicates. To account for the effects of rare haplotypes on total diversity, we employed the robust nonparametric Chao-2 indicator to estimate the number of haplotypes or alleles present in the NEA and NWA for each marker (Walther & Morand, 1998; Gotelli & Colwell, 2001; Foggo *et al.*, 2003).

COLONIZATION MODEL SELECTION

We found that the genetic diversity of *C. volutator* in the NWA is a subsample of diversity found in the NEA, suggesting that populations in the NWA were colonized from the NEA. However, it is not obvious whether colonization of the three genetically subdi-

vided regions in the NWA described by Einfeldt & Addison (2013) resulted from multiple introductions from the NEA or from dispersal along the coast after trans-Atlantic colonization to one region. Einfeldt & Addison (2013) report higher genetic diversity in the central region of the NWA, suggesting that it may be a contact zone between them. We tested six models of connectivity between the NEA and the NWA that reflect simple population genetic hypotheses and competing introduction histories consistent with either natural or anthropogenic dispersal (Fig. 2):

1. 'Single introduction pathway': asymmetric migration from the NEA to the middle region of the NWA with symmetric migration between adjacent regions in the NWA.
2. 'Two introduction pathways': asymmetric migration from the NEA to the northern and southern regions of the NWA with symmetric migration between adjacent regions in the NWA.
3. 'Three introduction pathways': asymmetric migration from the NEA to all regions of the NWA with symmetric migration between adjacent regions in the NWA.
4. 'Two populations': all samples in the NWA are of the same population, with asymmetric migration from the NEA to the NWA.
5. 'Open': symmetric migration between the NEA and the three NWA regions.
6. 'Panmixia': all samples are of the same population.

Models 5 and 6 provide simple models for comparison with other colonization models. Models 1 and 4 are consistent with either natural or anthropogenic trans-Atlantic dispersal, and thus provide little information regarding when colonization occurred. Models 2 and 3 reflect multiple introductions, which would require successful colonization of different areas within a suitably narrow timeframe for trans-Atlantic gene flow to outweigh local recruitment; a pattern that is unlikely to occur via natural trans-Atlantic dispersal, and is thus indicative of anthropogenic introduction.

To test which colonization models have the highest probability given the genetic data from *C. volutator* in the north Atlantic, we employed a model selection approach (Johnson & Omland, 2004; Beerli & Palczewski, 2010) to calculate the relative posterior probability of the six models using Bayesian inference under a coalescent framework with the program MIGRATE-N, version 3.4.4 (Beerli & Felsenstein, 2001). We translated the six models into migration matrices and estimated posterior values of migration and theta using a Bayesian Markov chain Monte Carlo search strategy using initial parameter values generated from an F_{ST} calculation. We examined effective sample sizes for each parameter to assess stationarity of the Markov chains. After preliminary trials, we applied a static heating scheme with six different temperatures for 10 concurrent (replicate) long chains totalling 40 000 000 steps, with each chain having 40 000 steps recorded over intervals of 100 steps after a burn-in of 10 000 generations. Each model was rerun for a total of 10 replicates to check for consistency of parameter estimates and model marginal log-likelihood (lmL). To evaluate support for each model, we calculated the mean Bezier thermodynamic approximation of lmL of each model, from which we calculated the natural log Bayes factors (LBF) with respect to the model with the highest mean Bezier lmL (Beerli & Palczewski 2010). Model choice probabilities were calculated *sensu* Kass & Raftery (1995). To check for consistency of parameter estimates, the best model for each data set was rerun for three times more replicates (30) and five times more recorded steps (20 000).

RESULTS

POLYMORPHISM AND DIVERSITY

We found 25 variable positions (24 transitions and one transversion) over 496 sequenced nucleotides of *COI* (GenBank accession numbers KP683379–KP683563), of which 80% (20/25) were synonymous. In *SUBI* (GenBank accession numbers KP683564–KP683770), we found 29 variable positions (24 transitions and five transversions) over 576 sequenced nucleotides, with 93% (13/14) of substitutions in the *COI* region being synonymous and 87% (13/15) of substitutions in the *COII* region being synonymous. In *CV1* (GenBank accession numbers KP683771–P683901), we found 16 variable positions (seven transitions and nine transversions) over 279 nucleotides. In *CV2* (GenBank accessions: KP683902–KP684029), we found 12 variable positions (four transitions and eight transversions) in 201 nucleotides. Tajima's *D* was not significantly different from zero for *COI* (0.2199, *P* > 0.1), *SUBI* (1.17273, *P* > 0.1), *CV1*

(−0.8868, *P* > 0.1), or *CV2* (−0.7734, *P* > 0.1). Fu and Li's *F* was not significantly different from zero for *COI* (0.5241, *P* > 0.1), *SUBI* (0.5657, *P* > 0.1), *CV1* (0.3470, *P* > 0.1), or *CV2* (−1.4484, *P* > 0.1). Mitochondrial DNA sequence data were concatenated into the marker *COI/SUBI* for all further analyses.

Corophium volutator exhibited lower genetic diversity in North America than in Europe, with common mtDNA (*COI/SUBI*) haplotypes and nDNA (*CV1* and *CV2*) alleles in the NWA representing a non-monophyletic subsample of those found in the NEA (Fig. 1, Table 2). We observed a total of 39 haplotypes for concatenated *COI/SUBI* mtDNA with three common haplotypes in the NWA shared with the NEA, five singleton haplotypes unique to the NWA (present at the North and South range edges), and 26 haplotypes unique to the NEA. The NWA had significantly fewer haplotypes than the NEA (χ^2 = 16.1, d.f. = 1, *P* < 0.001). The nuclear marker *CV1* had 34 alleles, with seven NWA alleles shared with the NEA, two singleton alleles unique to the NWA, and 25 alleles unique to the NEA. The nuclear marker *CV2* had a total of 16 alleles, with four NWA alleles shared with the NEA, one allele unique to the NWA, and 11 alleles unique to the NEA. Both *CV1* and *CV2* had significantly fewer alleles in the NWA compared to the NEA (*CV1*: χ^2 = 12.9, d.f. = 1, *P* < 0.001; *CV2*: χ^2 = 37.7, d.f. = 1, *P* < 0.001).

To evaluate the thoroughness of our sampling, we estimated the expected number of haplotypes in the NEA and the NWA using the Chao2 parameter (Table 3). For concatenated mtDNA, we found that the expected maximum number of haplotypes in the NWA was 17 [95% confidence interval (CI) = 10–57], which is an order of magnitude less than the 766 (95% CI = 79–767) expected in the NEA. For *CV1*, the expected maximum number of haplotypes in the NWA was 19 (95% CI = 11–59), which is an order of magnitude less than the 297 (95% CI = 79–1523) expected in the NEA. For *CV2*, the expected maximum number of haplotypes in the NWA was five (95% CI did not deviate from this value), which is considerably less than the 18 (95% CI = 15–34) expected in the NEA. These results suggest that our sampling was sufficient to capture most genetic diversity in the NWA, although additional sampling of populations in the NEA would reveal a great deal more genetic variation for all three loci.

COLONIZATION MODEL SELECTION

Parameter estimates of MIGRATE-N analyses using mtDNA and nDNA reached convergence, with little to no variation shown when replicating analyses for three times more replicates or five times more recorded steps (see Supporting Information,
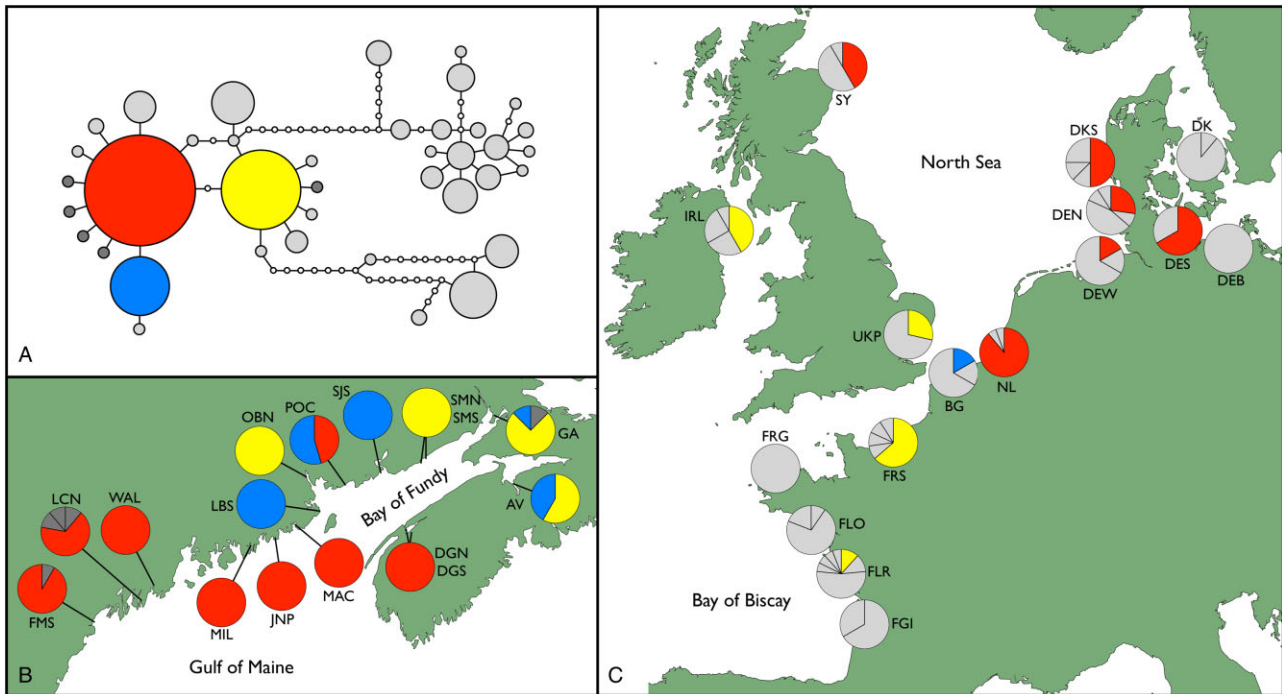
Figure 1. A, haplotype network for concatenated *COI/SUBI* mitochondrial DNA for *Corophium volutator*. Circle area is proportional to the number of haplotypes sequenced. B, sampling locations and haplotype distribution of *COI/SUBI* for *Corophium volutator* in the North-West Atlantic (NWA) (Einfeldt & Addison, 2013). C, sampling locations and haplotype distribution of *COI/SUBI* for *Corophium volutator* in the North-East Atlantic (NEA). Red, blue, and yellow haplotypes are shared between the NWA and the NEA; dark shaded haplotypes were found once, only in the NWA; light shaded haplotypes were found only in the NEA.

Table S1). In every run, effective sample sizes for each parameter were greater than 1000. Independent colonization model testing using Bayes factors calculated from marginal log-likelihood outputs for both data sets supported models of multiple colonization pathways, although the models with the highest probability were different for each marker type (Table 4). Analyses of mtDNA (*COI/SUBI*) supported a model of two colonization pathways to the NWA from the NEA (LBF = 0.0; *P* = 0.8122) over a single colonization model (LBF = −4.5; *P* = 0.1779) and all other models (*P* < 0.01). Analyses of nDNA (*CV1* and *CV2*) supported a model of three introduction pathways from the NEA (LBF = 0.0; *P* = 0.9277) over a model of symmetric migration between the three regions of the NWA and the NEA (LBF = −5.1; *P* = 0.0720) and all other models (*P* < 0.01). For both data sets, the panmixia model was the least supported, and the model of two populations with asymmetric migration to the NWA from the NEA was the second least supported. The rank order of models 1, 2, 3, and 5 differed between data sets; although the top three models for mtDNA represent colonization from the NEA to the NWA, this was not the case for nDNA. Although the faster rate of evolution of mtDNA can

lead to geographically structured phylogenies without corroboration of nuclear gene sequences in populations with relatively short periods of isolation (Hare, 2001; Zink & Barrowclough, 2008), this does not fully explain the disagreement between our analyses. These differences highlight the importance of considering multiple markers and marker types in reconstructing population histories, and suggest that further investigations using additional loci may better resolve the colonization pathways followed by *C. volutator* from the NEA to the NWA.

DISCUSSION

The reduction of genetic diversity and the pattern of shared ancestry between disjunct populations of *C. volutator* in the north Atlantic suggest that this species was recently introduced from Europe to North America. Genetic diversity of both nuclear and mitochondrial markers was significantly reduced in the NWA, and common alleles in the NWA represented a nested subset of diversity found in the NEA, indicating that populations in the NWA are descended from ancestors in the NEA. Our coalescent analysis of nuclear DNA sequences strongly supports a model of
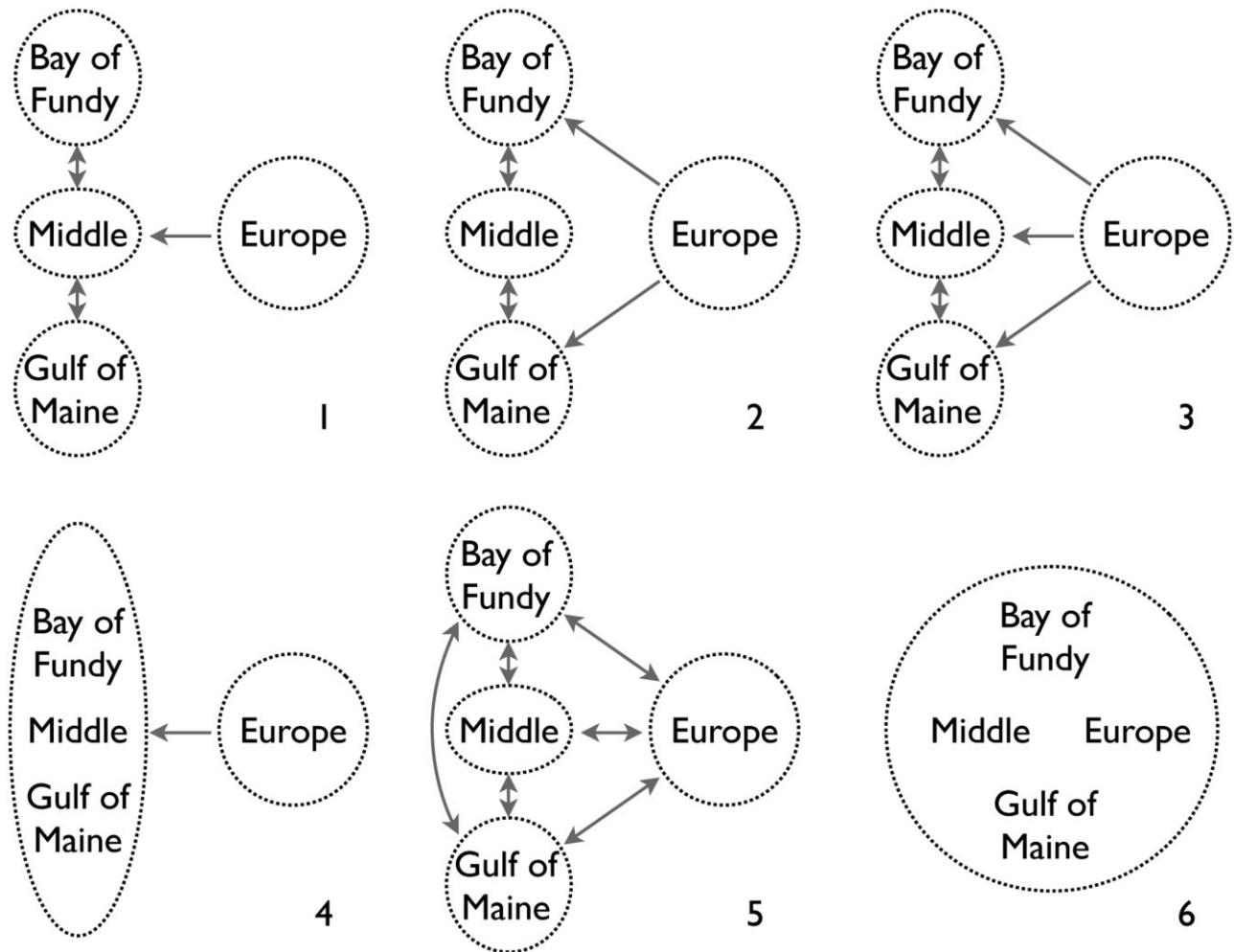
Figure 2. Diagrams of migration models tested using concatenated mitochondrial DNA (*COI/SUBI*) and nuclear DNA (*CV1* and *CV2*) of *Corophium volutator* in MIGRATE-N. Numeric labels correspond to models described in the Introduction and Table 4. Dotted lines depict population groupings of four biogeographical regions: the Bay of Fundy, the Gulf of Maine, a Middle region in North America, and Europe. Arrows depict directionality of gene flow among populations.

unidirectional gene flow following multiple colonization pathways from the NEA to the NWA, supporting the hypothesis that populations of *C. volutator* in the Bay of Fundy and the Gulf of Maine were independently established from European populations. This pattern of multiple introductions is unlikely to have occurred via natural dispersal, suggesting that shipping practices during the Age of Discovery played a key role in establishing populations in North America. This conclusion challenges assumptions about community and ecosystem level interactions in the soft sediment intertidal of the NWA because the relationships between *C. volutator*, its predators, competitors, and the environment are likely the product of a much more recent evolutionary history than previously assumed.

SHARED GENETIC DIVERSITY

Introduced populations typically experience strong genetic bottlenecks, with introduced diversity representing a subsample of that found in the species' native range (Bastrop, Jürss & Sturmbauer, 1998; Slade & Moritz, 1998; Ashton *et al*., 2008; Audzijonyte *et al*., 2008; Darling *et al*., 2008; Dlugosch & Parker, 2008; Slothouber Galbreath *et al*., 2010; Lejeusne *et al*., 2011). Although introductions from multiple sources can alleviate these bottlenecks, these mixed populations still exhibit a pattern of subsampling, with alleles identical to or closely descended from those found in more phylogenetically diverse indigenous populations (Geller *et al*., 1997; Frankham, 2004; Hauber *et al*., 2011; Lacoursière-Roussel *et al*.,

Table 2. Concatenated mitochondrial and nuclear DNA diversity for *Corophium volutator* populations surveyed: sampling sites, number of individuals sequenced per locus (N), number of segregation sites (S), number of haplotypes or alleles (H), haplotype diversity (h), observed heterozygosity (H_O), expected heterozygosity (H_E), and mean nucleotide diversity (π)

Site	Population	Abbreviation	COI/SUBI					CV1						CV2					
			N	S	H	h	π	N	S	H	H_O	H_E	π	N	S	H	H_O	H_E	π
1	Nieuwpoort	BG	6	19	3	0.6000	0.0093	28	5	5	0.5714	0.5688	0.0034	16	5	5	0.5000	0.5333	0.0042
2	Warnow	DEB	10	0	1	0.0000	0.0000	12	2	3	0.6667	0.6212	0.0039	12	4	4	0.3333	0.4545	0.0054
3	Sylt	DEN	11	20	5	0.7636	0.0088	14	5	5	0.5714	0.7582	0.0074	10	5	4	0.6000	0.6444	0.0063
4	Salzhaff	DES	6	18	2	0.5333	0.0090	14	6	9	0.8571	0.8791	0.0075	10	6	4	0.6000	0.5333	0.0060
5	Dorum-Neufeld	DEW	6	18	3	0.6000	0.0088	6	5	5	1.0000	0.9333	0.0086	6	5	5	0.6667	0.9333	0.0096
6	Roskilde	DK	9	19	2	0.2222	0.0040	14	2	3	0.5714	0.7912	0.0025	12	4	4	0.6667	0.7576	0.0075
7	Skallingen	DKS	8	19	4	0.7500	0.0089	12	4	5	0.8333	0.7879	0.0064	12	6	4	0.5000	0.5606	0.0070
8	St-Estephe	FGI	6	17	2	0.5333	0.0085	10	3	3	0.2000	0.6444	0.0046	12	3	3	0.6667	0.5303	0.0065
9	Loire	FLO	21	25	3	0.4667	0.0067	16	5	7	0.4444	0.8758	0.0059	36	5	5	0.1667	0.2603	0.0024
10	Le Platin	FLR	17	18	7	0.7206	0.0073	20	4	7	1.0000	0.8737	0.0063	8	4	4	0.6000	0.6444	0.0059
11	Guillec	FRG	12	0	1	0.0000	0.0000	20	4	5	0.7000	0.7368	0.0043	22	3	4	0.3636	0.4632	0.0025
12	Seine	FRS	11	19	5	0.6182	0.0076	24	6	7	0.8333	0.8732	0.0059	18	1	2	0.1111	0.1111	0.0006
13	Ireland	IRL	12	15	4	0.7576	0.0070	16	3	4	0.3750	0.6167	0.0039	16	5	4	0.7500	0.6917	0.0082
14	Netherlands	NL	18	18	3	0.2157	0.0034	26	8	11	0.8462	0.8831	0.0076	12	5	5	0.6250	0.8083	0.0048
15	Scotland	SY	12	20	3	0.6212	0.0096	14	3	4	0.7143	0.7582	0.0037	16	6	5	0.8750	0.6833	0.0056
16	United Kingdom	UKP	7	17	2	0.4762	0.0076	12	5	7	0.7143	0.9121	0.0064	22	5	7	0.7273	0.6840	0.0056
	Europe		172	52	34	0.9254	0.0108	258	16	31		0.8525		240	9	15			0.0060
	North America		138	8	8	0.6452	0.0011	252	5	9			0.0055	191	4	7			0.0084

Table 3. Observed and expected number of haplotypes and alleles in Europe (North-East Atlantic; NEA) and North America (North-West Atlantic; NWA) for *Corophium volutator*

Marker	Region	Populations sampled	$H_{observed}$	$H_{maximim-expected}$	Lower 95%	Upper 95%
COI/SUBI	Europe	16	34	216.3	79.4	765.7
	Bay of Fundy	9	4	4.9	4.1	16.0
	Gulf of Maine	8	5	10.3	5.9	37.5
	North America	17	8	17.4	9.8	56.9
CV1	Europe	16	31	296.5	78.9	1522.6
	Bay of Fundy	16	8	13.6	8.9	43.3
	Gulf of Maine	8	4	4.0	4.0	5.5
	North America	24	9	18.6	10.8	58.9
CV2	Europe	16	15	17.7	15.4	33.9
	Bay of Fundy	14	5	5.0	5.0	5.8
	Gulf of Maine	5	3	3.0	3.0	3.2
	North America	19	7	5.0	5.0	5.0

Non-overlapping confidence intervals indicate a higher genetic diversity in Europe than in North America, suggesting unique North American haplotypes and also that alleles are likely among the many remaining unsampled in Europe.

2012; Cahill & Viard, 2014). By contrast, natural post-glacial trans-Atlantic dispersal is expected to result in a distribution including the intermediate landmasses of Iceland and Greenland and one or more monophyletic lineages on each side of the Atlantic (Krebes, Blank & Bastrop, 2011; Panova *et al.*, 2011; Palsson *et al.*, 2014) If *C. volutator* was recently introduced to the NWA from the NEA, we expect genetic diversity in the NWA to be a subsample from more phylogenetically diverse populations in the NEA. Consistent with this prediction, we found that all genetic diversity in the NWA was identical to or recently descended from that found in the NEA (Fig. 1). All concatenated mtDNA haplotypes (*COI/SUBI*) and nDNA alleles (*CV1* and *CV2*) for which more than a single copy was found in the NWA were shared with the NEA, with the exception of a single *CV2* allele for which six copies were detected in the NWA only. The five concatenated mtDNA haplotypes and two *CV1* alleles detected only in the NWA were found in only one instance. Our rarefaction analyses show that the expected number of alleles in the NEA is more than an order of magnitude greater than in the NWA for concatenated mtDNA and *CV1*, and 3.5 times greater for *CV2* (Table 3), suggesting that we have sampled the NWA sufficiently to capture all common variants but that a considerable amount of unsampled diversity exists in the NEA. The genetic diversity found in the NWA populations of *C. volutator* thus represents a subset of diversity in the NEA, with a small number of exceptions that could be an artefact of the limitations of sampling diverse indigenous populations. These findings are consistent with genetic studies of species with docu-

mented introductions; because of the practical limits of completely sampling diverse indigenous populations, it is common for unique diversity to be found in introduced ranges (Dawson, Gupta & England, 2005; Miura *et al.*, 2006; Roman, 2006; Blakeslee, Byers & Lesser, 2008).

COLONIZATION MODELS

Analyses of gene flow using mitochondrial and nuclear DNA support different models, although both marker types provide evidence of unidirectional colonization following multiple colonization pathways from the NEA to the NWA. Natural trans-Atlantic dispersal is expected to facilitate bilateral gene flow between both coasts (e.g. *Asterias rubens*: Harper, Addison & Hart, 2007); however, the model of symmetric gene flow between populations was not the highest ranked by analyses using either marker type. Although natural dispersal could potentially result in unidirectional colonization if driven by predominant currents, southern habitat that first became available during glacial recession would likely be colonized first, with serial founder events from northwards range expansion along the NWA predicted to cause a pattern of decreasing genetic diversity in more northern populations. By contrast, analyses using both concatenated mtDNA and nDNA show no evidence of southern refugia and rank models consistent with natural dispersal via a single colonization pathway lower than one of two models reflecting multiple colonization pathways.

Although each marker type supports a different colonization model, the models ranked highest by

Table 4. Comparison of models using log Bayes factors (LBF) from Bezier approximated log marginal likelihood (Bezier lmL) estimated in MIGRATE-N for nuclear and mitochondrial DNA from *Corophium volutator*

Model	Description of model	COI/SUBI lmL (10 run average)	LBF	Probability	Rank	CV1 + CV2 lmL (10 run average)	LBF	Probability	Rank
1	'Single introduction pathway': asymmetric migration from the NEA to the middle region of the NWA, symmetric migration between adjacent regions in the NWA	−3738.51	−3.0	0.1779	2	−2893.71	−29.7	<0.0001	4
2	'Two introduction pathways': asymmetric migration from the NEA to the northern and southern regions of the NWA, symmetric migration between adjacent regions in the NWA	−3736.99	0.0	0.8122	best	−2886.79	−15.8	0.0003	3
3	'Three introduction pathways': asymmetric migration from the NEA to all regions of the NWA, symmetric migration between adjacent regions in the NWA	−3741.41	−8.8	0.0098	3	−2878.88	0.0	0.9277	best
4	'Two populations': all samples in the NWA are of the same population, asymmetric migration from the NEA to the NWA	−3812.32	−150.7	<0.0001	5	−2964.72	−171.7	<0.0001	5
5	'Open': symmetric migration between the NEA and the three NWA regions	−3748.86	−23.7	<0.0001	4	−2881.44	−5.1	0.0720	2
6	'Panmixia': all samples are of the same population	−4197.46	−920.9	<0.0001	6	−3177.69	−597.6	<0.0001	6

NEA, North-East Atlantic; NWA, North-West Atlantic.

analyses of nDNA and mtDNA are consistent with multiple introduction pathways from the NEA to the NWA. Analyses using mtDNA support a model of two introduction pathways: to the Bay of Fundy and to the Gulf of Maine, with a contact zone between the two regions. By contrast, analyses using nDNA support a model of separate introduction pathways to the Bay of Fundy, the Gulf of Maine, and the region between them. For natural colonization to produce a pattern of multiple introduction pathways, colonization to each region in the NWA would have to occur within relatively similar time frames for migration across the Atlantic to outweigh along-shore dispersal rates of early colonists, which would favour the success of the first lineages to colonize the NWA. Although a scenario of multiple colonization events is not impossible via natural trans-Atlantic dispersal, it is much more likely to occur via human-mediated dispersal (Geller *et al.*, 2010).

DISPERSAL MECHANISMS AND INTRODUCTION VECTORS

Natural long-distance dispersal by marine invertebrates can occur via planktonic larval dispersal (Thorson, 1950; Scheltema, 1971) and rafting on ice, seaweed or other debris (Highsmith, 1985). The brood-rearing life history of *C. volutator* rules out planktonic dispersal as a potential mechanism of dispersal. *Corophium volutator* lives in close association with sediment and has not been observed rafting on seaweed (Ingolfsson, 1995), but can survive freezing in ice blocks that facilitate rafting (Macfarlane *et al.*, 2013). However, this mechanism would have directed dispersal from Europe to North America along predominant currents that pass the intermediate land-masses of Iceland and Greenland. Despite soft-sediment invertebrates that commonly co-occur with *C. volutator* in Europe being found in Iceland and Greenland (Ingolfsson, 1996), *C. volutator* is not found on either intermediate land mass. *Corophium volutator* experiences linear population decreases during winter and higher rates of mortality in colder temperatures, likely as a result of the high energetic costs maintaining a low freezing point of body fluids (Drolet, Kennedy & Barbeau, 2013), making ice rafting an unlikely vector for natural dispersal of *C. volutator* from the NEA to the NWA. Known mechanisms of natural trans-Atlantic dispersal thus do not adequately explain the disjunct distribution of *C. volutator* across approximately 5000 km of open ocean.

The pattern of multiple colonization events from the NEA to the NWA is consistent with human-mediated transport during early European exploration and trade in the New World. Although subject to more trade and exploration by Europeans much earlier than the Pacific Coast of North America, the NWA has only half as many documented invasions, which is partially attributable to temporal biases in data availability (Ruiz *et al.*, 2000; Carlton, 2003). Shipping pressure from European explorers, traders, and colonists intensified after the establishment of settlements during the first decade of the 1600s in the Gulf of Maine and Bay of Fundy, although the first recorded biological surveys began in the late 1600s (Denys, 1672) and focused mainly on conspicuous terrestrial vertebrate and plant species. *Corophium volutator* was not first described until 1766 by Pallas on the coast of Norway, and was first recorded in North America by Huntsman & Sparks (1924), who noted its high abundance and unknown distribution in the NWA. This delay between initial shipping pressure and scientific surveys that took note of invertebrates in littoral soft sediment habitat may have allowed *C. volutator* to be introduced and proliferate in the NWA without these events being observed directly.

Shipping is the most common introduction vector in marine systems, and has been responsible for the movement of organisms mainly via ballast water or fouling on hulls and anchors (Ruiz *et al.*, 2000). Despite their tendency to dwell in burrows, adult *C. volutator* swim during flood tide (Drolet & Barbeau, 2009) and were found in a ballast tank in Norway (Gollasch *et al.*, 2002). However, how common this occurrence is remains unknown; a survey of macro-invertebrates in 62 ships arriving in North American ports found no *C. volutator* in any of 67 ballast tanks (Briski *et al.*, 2012). Although ballast water is a potential vector for anthropogenic dispersal, it was likely not adopted sufficiently early to account for the high abundance of *C. volutator* observed in North America during the early 1900s. Prior to the adoption of ballast tanks in 1880, ships controlled buoyancy with semi-dry ballast (i.e. rock and sediment loaded into damp holds that was collected from heaps in the intertidal at different tidal heights), enabling global introductions of benthic marine invertebrates (Bax *et al.*, 2003; Minchin *et al.*, 2009). The preferred habitat of *C. volutator* in the littoral soft sediment of estuaries and river mouths that provided safe harbor for vessels in the Atlantic would likely have enabled the inadvertent movement of many individuals along with this substrate. Although modern ballast tanks may enable contemporary transport of *C. volutator*, semi-dry ballast is a more likely vector for the initial introduction of *C. volutator* from the NEA to the NWA, and may have had a greater influence on the soft sediment community than previously assumed.

IMPLICATIONS FOR ECOLOGY

Whether a species is introduced or indigenous across its range has repercussions for studies of local population dynamics, trophic and community interactions, and evolution. Introduced species are often considered threats to marine biodiversity and as harmful to indigenous populations (Elton, 1958; Vitousek *et al.*, 1997; Ruiz, Fofonoff & Hines, 1999; Chapin *et al.*, 2000; Bax *et al.*, 2003); particularly when these invaders are ecosystem engineers (Crooks, 2002; Cuddington & Hastings, 2004). In the Bay of Fundy, *C. volutator* has been viewed as an integral member of the soft sediment ecosystem and populations are used as an index of ecological health (Hawkins, 1985; Murdoch, Barlocher & Laltoo, 1986), which is a perspective at odds with how invasive ecosystem engineers are commonly viewed. A history of anthropogenic introduction challenges the notion that the stability of this species alone is informative to overall ecosystem function and health, and calls to question previous interpretations of ecosystem dynamics. Our results suggest that contemporary ecological studies of the mudflats in the Bay of Fundy and Gulf of Maine should consider that this system might still be recovering from or adapting to a shift in community structure following the introduction of *C. volutator*.

Resolving the history of apparently important prey items provides valuable evolutionary context to ecological studies because using abundance as a metric of value overlooks the potential flexibility of predators' responses to a changing community structure. Although it is not known whether *C. volutator* displaced more common invertebrates or provided a new energetic contribution to the soft sediment ecosystem, there is some evidence that *C. volutator* is a preferred prey item simply as a result of its widespread availability. The migratory semipalmated sandpiper *Calidris pusilla* (Linnaeus 1766) was considered to be dependent on *C. volutator* to provide sufficient energy for annual migrations by doubling its weight when feeding on mudflats in the Bay of Fundy (Hamilton, Barbeau & Diamond, 2003). In contrast to the assumption that *C. volutator* is a necessary component of semipalmated sandpipers' diet, a more recent study by the same research group suggests that these birds may consume different prey items based on availability (MacDonald, Ginn & Hamilton, 2012). Contrasting findings of prey preference are also found for the Atlantic sturgeon *Acipenser oxyrhinchus oxyrhinchus* (Mitchill 1815) in the Bay of Fundy. Pearson *et al.* (2007) report targeted feeding on aggregations of *C. volutator*, whereas Maclean, Dadswell & Stokesbury (2013) found that sturgeon have a strong preference for polychaetes. Both the semipalmated

sandpiper and sturgeon have been present in the NWA longer than we suggest *C. volutator* has been, implying that any apparent reliance on *C. volutator* may be a response to changes in prey availability after the arrival of *C. volutator*. These shifts in diet may have consequences for predators beyond nutritive value; elevated concentrations of mercury in semipalmated sandpipers associated with feeding in the Bay of Fundy (Didyk *et al.*, 2005) are considered to be influenced mostly by polychaete worms that are at a higher trophic level and have greater bioaccumulation factors than *C. volutator* (Sizmur *et al.*, 2013).

In addition to direct interactions, the introduction of an ecosystem engineer can indirectly affect indigenous biota by physically altering the environment (Wallentinus & Nyberg, 2007; Mermillod-Blondin & Rosenberg, 2011). Environmental alterations may be beneficial for some species and detrimental to others, making it difficult to assess the impact of introduced species on local biota. *Corophium volutator* significantly alters the physical environment by reducing soft-sediment erosion (Mouritsen *et al.*, 1998) and sedimentation of re-suspended particles through secretions used to construct burrows (Meadows *et al.*, 1990), increasing turbidity through the construction of these burrows (De Deckere, van de Koppel & Heip, 2000; Biles *et al.*, 2002), and increasing CO_2/O_2 fluxes and denitrification through burrow ventilation (Pelegri & Blackburn, 1994). Bioturbation caused by *C. volutator* results in dramatic increases in the aqueous concentration of sediment-bound contaminants, which subsequently are accumulated by filter-feeders (Ciarelli *et al.*, 1999), representing a potential concern for shellfish fisheries. *Corophium volutator* has a negative impact on the successional development of salt marsh vegetation by preventing the establishment of seedlings (Gerdol & Hughes, 1993), thereby decreasing the area covered by salt marshes and expanding soft-sediment habitat. Within mudflats, the presence of *C. volutator* can have a negative impact on the densities of other infaunal species (Commito, 1982; Hughes & Gerdol, 1997) and increase diatom species richness by reducing the biomass of dominant taxa in biofilm (Hagerthey, Defew & Paterson, 2002). These significant impacts on the soft sediment ecosystem fundamentally alter habitat qualities, and may have led to a restructuring of the soft sediment infauna when *C. volutator* became established in the NWA.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Parameter estimates for best model choices for *Corophium volutator* concatenated mitochondrial and nuclear DNA analyses in MIGRATE-N: mutation-scaled effective population size (θ and mutation scaled effective immigration rate (M). Posterior distributions are summarized by the mode and 95% credibility intervals under standard settings, with three times more replicates, and with five times more recorded steps. BoF, Bay of Fundy; GoM, Gulf of Maine; EUR, Europe.