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Phylogeography of the ocean quahog (*Arctica islandica*): influences of paleoclimate on genetic diversity and species range

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Abstract The ocean quahog, *Arctica islandica* (Linnaeus, 1767), is a commercially important bivalve found on continental shelves throughout much of the North Atlantic. To assess genetic subdivision in this species, we sequenced 385 nucleotides of the mitochondrial cytochrome *b* (cyt *b*) gene from 83 specimens collected from 12 localities between September 1998 and July 1999 (based on preliminary data, the Internal Transcribed Spacers, ITS, of the nuclear ribosomal repeat were not useful). The cyt *b* data delimited 11 haplotypes with 0.26 to 8.1% nucleotide difference (coded by 36 variable nucleotide positions) among them. Only three haplotypes were detected in 39 specimens collected along the USA coastline, compared to five haplotypes from nine Icelandic individuals. The western Atlantic populations ranging from Penobscot Bay (Maine, USA) to southern Virginia showed relatively low diversity and appeared genetically similar in that region. Based on the presence of shared haplotypes, AMOVA analyses, and phylogenetic reconstructions, Icelandic populations appear to be more genetically similar to western Atlantic populations than eastern Atlantic populations. Specimens from the Faroe Islands ($n = 4$) show mixed affinities. These data are consistent with the hypothesis that a warm Holocene climatic optimum (ca. 7,500 years BP), and not glacial refugia, shaped the present-day genetic structure in *A. islandica*.

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Supplementary material: Appendix 1. *Arctica islandica*. Alignment of Cytb data for 11 haplotypes. Appendix 2. *Arctica islandica*. Alignment of ITS2 for four specimens. Available in electronic form on Springer-Verlag's server under http://link.springer.de/link/service/journals/00227

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Introduction

Arctica islandica, commonly called the ocean quahog, is a northern Atlantic clam that is commercially harvested in the USA, Canada, and Iceland. Larger specimens (60 to 90 mm in length) are often 80 to 100 years old and have a reported growth rate of $\leq$1 mm yr^{-1} (Forster 1981; Murawski et al. 1982; Kraus et al. 1992; Kennish et al. 1994; Witbaard et al. 1999). It takes an individual 8 to 15 years to reach maturity (Thompson et al. 1980; Rowell et al. 1990) and recruitment is often low and unpredictable (NEFSC 1998). This dioecious bivalve produces planktonic larvae that are present in waters off New England USA throughout much of the year (Loosanoff 1953; Mann 1985). Geographical differences in growth rates (compare Murawski et al. 1982; Ropes and Pyoas 1982; Kraus et al. 1992; Witbaard et al. 1999) and population size-structure (NEFSC 1995) have been reported, however little is known about the population genetic structure of this species. To assess genetic subdivision within *A. islandica*, we examined the nucleotide sequence of the mitochondrial cytochrome *b* (cyt *b*) gene and the Internal Transcribed Spacers (ITS) of the nuclear ribosomal repeat.

Present-day and historical distributions (Nicol 1951; Zatsepin and Filatova 1961; Raffi 1986; Funder and Weidick 1991; Salvigsen et al. 1992; Galkin 1998; Gulliksen et al. 1999) of *Arctica islandica* are shown in Fig. 1. This boreal amphi-Atlantic bivalve only occurs in the northern hemisphere. With a depth distribution of 15 to 256 m (Bush 1885; Merrill and Ropes 1969), *A. islandica* is most abundant in 30 to 60 m of water (authors' personal observations) throughout much of its range, but restricted to colder, deeper water at the southern end of its range (submergence, Franz and

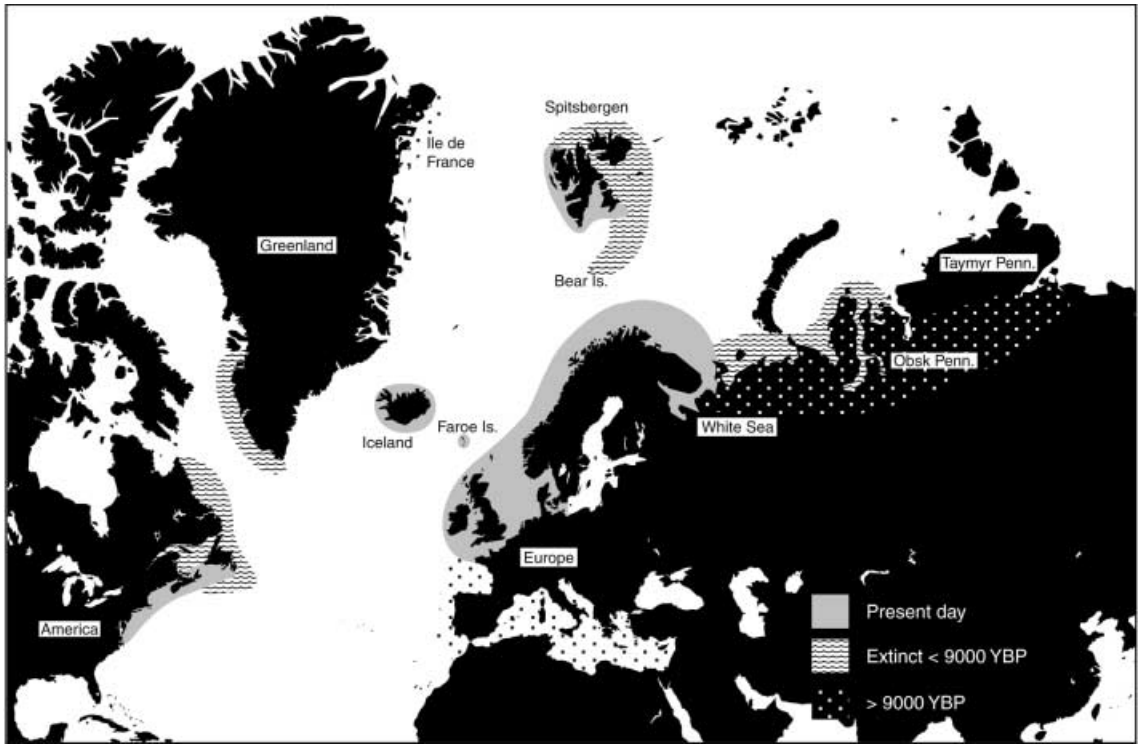

Fig. 1 *Arctica islandica*. The present-day distribution is highlighted in gray. The recent past (<9,000 years BP), but presently extinct distribution is represented by wavy lines. Regions of known occurrence >9,000 years BP are denoted by dots. Pleistocene deposits are reported from Île de France (Bennike and Weidick 2000). Mediterranean populations died out around 9,800 years BP (Froget et al. 1972). Temporal changes in distribution in northern Siberia are uncertain. Shell remains on the Taymyr Peninsula date back to the Pleistocene, but shells west of the Obsk Peninsula may be considerably younger (<10,000 years BP; Zatsepin and Filatova 1961). Data for high latitude archipelagoes (e.g. Franz Josef Land and Novaya Zemlya) are lacking. Figure compiled from several references (Nicol 1951; Zatsepin and Filatova 1961; Raffi 1986; Funder and Weidick 1991; Salvigsen et al. 1992; Galkin 1998; Gulliksen 1999)

Merrill 1980a). Fossil and subfossil (incompletely fossilized shells) evidence shows that the range of *A. islandica* has changed throughout the Quaternary (1.8 million years BP to present). During this time, the southern edge of eastern Atlantic populations extended into the Mediterranean and Bay of Biscay (Froget et al. 1972; Raffi 1986). The Mediterranean population became extinct about 9,800 years BP (Froget et al. 1972). The known northern limit occurred off Spitsbergen during the Holocene warm period (ca. 8,000 years BP; Salvigsen et al. 1992) and Ilê de France, Greenland 2 million years BP (Bennike and Weidick 2000). To the best of our knowledge, no extant populations of *A. islandica* occur off the coast of Greenland (Jensen 1912; Ockelmann 1958), but subfossil shells dated to 5,000–8,000 years BP exist on the west coast (Funder and Weidick 1991). The ocean quahog was thought to be extinct around Spitsbergen (Peacock 1989; Salvigsen et al. 1992), and numerous bivalve reports between 1869 and 1984 fail to mention *A. islandica* (see Rozycki 1987), but recent reports suggest otherwise (Gulliksen et al. 1999). This bivalve is known to have expanded its range (e.g. Barents Sea; Galkin 1998) and we suspect that a warming trend during the latter half of this century (Dyer et al. 1984; Galkin 1998) has allowed *A. islandica* to re-inhabit Spitsbergen. Shells of *A. islandica* have been found in river banks in the Taymyr Peninsula of Russia and in the northern part of the western Siberian Lowlands (Zatsepin and Filatova 1961). However, post-Pleistocene distributions (<10,000 years BP) have only reached as far as the Obsk Peninsula (Zatsepin and Filatova 1961). Only limited information on the prehistoric distribution of this quahog along the American coast exists; the earliest report of *A. islandica* is from the Gulf of Maine 10,590 years BP (Dyke et al. 1996). The recent discovery of *A. islandica* shells on the Pacific USA coast (Coan 1998) is most probably due to the species being used as fishing bait (Chapman and Miller 1999), and an older record from the Bering Sea (Leche 1883) was probably a printing error (Jensen 1912; Zatsepin and Filatova 1961).

Based on geographical and hydrodynamic information, there are two potential barriers to present-day gene flow. A distance of 3,000 km separates the Icelandic population from the nearest North American population (i.e. off Nova Scotia). Second, present-day current patterns around Cape Cod, Massachusetts may limit dispersal and act as a barrier to many species (Franz and Merrill 1980a, b). The existence of a smaller size structure in natural populations of *Arctica islandica* from Maine (NEFSC 1995) raised the possibility that they are genetically distinct in that area. When reared under laboratory conditions, however, individuals from this

region grew relatively rapidly and attained more typical quahog sizes (Kraus et al. 1992), suggesting that the observed size variation was environmental in nature.

Both climatic optima and minima have been purported to cause range shifts in North Atlantic species (e.g. Vermeij 1989a, b, 1991a, b; Hewitt 1996; Cunningham and Collins 1998). For recent taxa, however, it has been postulated that repeated extinction and recolonization events associated with glaciation have reduced haplotypic variation in the northern ranges of boreal and arctic species (e.g. Hewitt 1996). In contrast, climatic optima would have had the opposite effect on present-day genetic patterns. Haplotypic diversity would be reduced at the southern limit of a range due to extinction associated with thermal stress, whereas northern ranges of species would expand, with a subsequent increase in genetic diversity. This study is the first to examine these alternative predictions for a subtidal marine organism.

We sequenced 385 nucleotides of the mitochondrial cyt *b* gene (5′ region) from 83 specimens of *Arctica islandica* collected from 12 localities throughout the known range. Because *A. islandica* appears to be a fairly distinct bivalve (the "genus" *Arctica* contains only one extant species), a close outgroup for this study was wanting. However, based on current knowledge of bivalve phylogeny (Adamkewicz et al. 1997; Slack-Smith 1998), we have chosen to use *Mercenaria campechiensis* (the southern quahog), *Calyptogena* sp., *Pitar morrhuana* (the false quahog), and *Spisula solidissima* (the Atlantic surfclam) as outgroup taxa. Additionally, we examined the ITS region for its utility in detecting population subdivision in a subset of individuals representative of the sampled range. The ITS region showed either no variation (ITS-1, $n = 18$) or had multiple copies which confounded sequencing (ITS-2, $n = 12$). Therefore, phylogeographic analysis focused exclusively on the cyt *b* data.

Materials and methods

Specimens of *Arctica islandica* (Linnaeus, 1776) were collected between September 1998 and July 1999 from 12 sites encompassing the known extant range, except the extreme eastern region along the European Arctic coast (Table 1). Specimens were identified at the time of collection by one of the authors, or identified by a local expert (see "Acknowledgements") with subsequent verification of a voucher specimen (deposited at The Smithsonian Institution, Museum of Natural History, Washington D.C., USNM 894359 and 905028–905035). Some samples from the southern New England area were frozen at −70 °C prior to use. Material obtained during the 1999 National Marine Fisheries Service (NMFS) clam survey or mailed to us by colleagues was preserved in ethanol. Initially, five randomly chosen specimens (only four available from Faroe Islands) were sequenced for each locality (six stations from the NMFS cruise were chosen to represent geographical and depth variation covered by the cruise). Based on preliminary results (see below), additional representatives were included for some northern localities. All specimens were obtained by dredge.

Whole genomic DNA was extracted from finely minced adductor muscle with a modified CTAB protocol (Doyle and Dickson 1987). Tissue was incubated for 15 min at 55 °C in 600 µl CTAB with 25 µl 10 mg ml^{-1} proteinase K, homogenized with a pestle, and incubated for an additional 15 min. Following extractions with saturated phenol and then chloroform:iso-amyl alcohol (24:1), DNA was ethanol-precipitated and resuspended in 50 µl TE. Use of mantle and foot tissue was initially tried but repeatedly resulted in poorer quality DNA, possibly due to high mucus content. For the *Calyptogena* sp. specimen, DNA was extracted using GeneReleaser (BioVentures, Inc., Murfreesboro, Tennessee) in accordance with the manufacturer's recommendations.

PCR amplification used standard protocols (Palumbi 1996) and controls for contamination (e.g. negative controls, autoclaving,

Table 1 *Arctica islandica*. Distribution of haplotypes. Sample sizes and number of individuals per haplotype are given for each locality sampled. Samples collected from September 1998 to July 1999. Depth for FA sample was 97 m, Collection depth is not known for SE, NO, IC, NS, and Maine (USA). Collection details for other USA samples given in NEFSC (1999)

Haplotype	Collection locality												GenBank Accession No.[c]
	Kristineberg and Tjärnö, Sweden (SE)	Bergen, Norway (NO)	Faroe Islands (FA)	Iceland (IC)	Yarmouth, Nova Scotia (NS)	Maine, USA	Georges Bank, USA Stratum 621[b]	South of Cape Cod, USA, Stratum 41[b]	Eastern Long Island, USA, Stratum 33[b]	Northern New Jersey, USA, Stratum 26[b]	Delaware, USA, Stratum 17[b]	Virginia, USA, Stratum 9[b]	
P	1	–	–	2	12	7	3	3	4	1	4	4	AF202095
Q	–	–	–	4	6	2	–	2	1	4	1	–	AF202096
R	–	–	–	1	1	–	2	–	–	–	–	1	AF202097
S	4	4	2	–	–	–	–	–	–	–	–	–	AF202100
T	–	–	1	–	–	–	–	–	–	–	–	–	AF202098
U	–	–	1	–	–	–	–	–	–	–	–	–	AF202099
V	–	–	–	1	–	–	–	–	–	–	–	–	AF202102
W	–	–	–	1	–	–	–	–	–	–	–	–	AF202103
X	–	–	–	–	1	–	–	–	–	–	–	–	AF202101
Y	–	1	–	–	–	–	–	–	–	–	–	–	AF202105
Z	1	–	–	–	–	–	–	–	–	–	–	–	AF202104
Sample size	6	5	4	9[a]	20	9	5	5	5	5	5	5	

[a] One Icelandic specimen displayed heteroplasmy and was not included in the analysis
[b] Numbers refer to "strata" recognized by NMFS/NOAA during Cruise 9903
[c] The outgroup taxa: *Mercenaria campechiensis*, *Calytogena* sp. (specimen from Alvin Dive 3446, Kodiak Seep), *Pitar morrhuana* and *Spisula solidissima* have GenBank Accession Nos. AF205080–AF205083, respectively

etc.). For cyt *b* amplifications, the cycling profile employed an initial denaturation of 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 46 °C for 30 s, extension at 70 °C for 30 s, and a final extension at 72 °C for 5 min. The cyt *b* primers [CytB 397F (5′-YWYTRCCTTggRggRCARATATC-3′) and CytB 811R (5′-gCRWAYARAAARTAYCAYTCWgg-3′)] were constructed from an alignment of four mollusks and, using the PCR parameters above, these primers were found to work on a variety of bivalve species. The entire ITS region was amplified with ITS-F (5′-TTgATTACgTCCCTgCCCTTT-3′) and ITS-R (5′-TTTCAC-TCgCCgTTACTAAgg-3′) primers (Martel et al. 1999) using the profile of initial denaturation at 94 °C for 2 min, 40 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were purified with QIAquick PCR purification kit (QIAGEN Inc., Valencia, California). DNA sequencing reactions of the cyt *b* fragment used the forward primer CytB 402F (5′-CCTTggggCAgATATCTTTTTg-3′) and the reverse primer CytB 811R. (Sequencing reactions with CytB 397F were inconsistent presumably due to the primer's degenerate nature, and thus it was modified to form CytB 402F.) The ITS region was sequenced using the amplification primers, a new primer constructed from preliminary ITS1 data (ITS 501F – 5′-CAg-ggAAgCCTgCTCCTATgACggAC-3′), a modified version of Hillis and Dixon's (1991) 5.8c (5.8c A.i. – 5′-gTgCgTTCAAggTg-TCgATgTTCAg-3′), and 5.8d (Hillis and Dixon 1991). BigDye cycle sequencing chemistry (PE Biosystems, Foster City, California) was used. Excess dye terminators were separated from the sequencing reaction with the aid of CentriSep spin columns (Princeton Separations, Adelphia, New Jersey) or with laboratory-made Sephadex (G-50 Sigma, St. Louis, Missouri) columns. Fragments were visualized on an ABI 377 automated sequencer. All cyt *b* fragments were sequenced in both directions and assembled using the AutoAssembler program Version 2.1 (PE Biosystems). Sequences were deposited in GenBank (Table 1). Although the single Swedish specimen with Haplotype P and the sequence of Haplotype X from one Nova Scotia specimen seem aberrant (see below), the results were confirmed by repeating the DNA extraction, PCR, and sequencing of the specimens concerned.

Alignment of the data was produced with the Clustal X program (Thompson et al. 1994) and, in the case of cyt *b* verified by amino acid sequence. The aligned databases were deposited in TREEBASE (http://phylogeny.harvard.edu/treebase) and are available as an electronic appendix (http://link.springer.de/link/service/journals/00227). Phylogenetic analyses were conducted using the PAUP* phylogenetic software package 4.0 Version b2 (Swofford 1999). Additionally, MacClade Version 3.0 (Maddison and Maddison 1992) was used for various character statistics and tree length calculations. The detailed descriptions of the phylogenetic analyses are given in the relevant subsections in "Results". Differences between observed haplotypes were illustrated by drawing a parsimony network following Avise et al. (1979).

We used the AMOVA program (analysis of molecular variance, Version 1.55; Excoffier 1995) to estimate the degree and statistical significance (employing 1,000 permutations) of genetic subdivision between sampled populations (Excoffier et al. 1992). Based on results from the phylogenetic analyses, three separate analyses were used to test for structure within the species: (1) without partitioning populations into groups, (2) partitioning populations into two groups, western Atlantic and eastern Atlantic, with the Icelandic population considered eastern Atlantic, and (3) western and eastern Atlantic groups, with Iceland considered western Atlantic. Populations in these analyses are the same as the localities in Table 1. For simplicity, the limited Faroe Islands sample was treated as belonging to the eastern Atlantic group in analyses 2 and 3 (although two haplotypes appear to be western Atlantic, see below).

Results

The aligned cyt *b* data set consisted of 385 nucleotide positions with no indels for 83 individuals of *Arctica*

Table 2 *Arctica islandica*. Variable positions in 11 haplotypes (P to Z). Numbers refer to positions along the 385 basepair cyt *b* fragment. *Numbers in bold* indicate first positions, whereas all others are third positions. *Bases in bold* refer to amino acid changes

	22	31	37	46	**53**	58	64	79	97	100	112	121	133	**143**	**146**	148	**149**	154	157	**161**	187	190	229	**230**	262	**263**	292	295	**311**	316	325	328	334	337	**347**	358
P	T	T	G	T	G	G	G	G	T	C	C	T	T	T	C	G	G	G	T	A	A	T	C	C	T	C	C	G	T	C	G	G	C	C	T	A	T
Q	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T	–	–	–	–	–	–
R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S	C	–	–	–	–	–	–	–	–	T	–	C	–	T	–	–	A	–	–	–	–	C	–	–	–	–	–	–	T	–	–	–	–	–	–	–
T	–	–	–	–	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–	–
U	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T	–	–	A	–	–	–	C	–	–	–	–	–	–	–	T	–	–	–	–	–	–
V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	–
W	–	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	G	G	–	T	T	C	T	T	A	G	T	–	C	T	–	G	C
X	–	C	A	C	A	A	A	T	–	–	T	C	C	C	G	A	A	–	–	G	G	–	T	T	C	T	T	A	G	T	–	C	T	–	G	C
Y	C	–	–	–	–	–	–	–	–	T	–	–	C	–	T	–	–	A	–	–	–	C	–	–	–	–	–	–	–	T	–	–	–	C	–	–
Z	C	–	–	–	–	–	–	–	C	T	–	–	C	–	T	–	–	A	–	–	–	C	–	–	–	–	–	–	–	T	–	–	–	–	–	–

islandica and the four outgroup taxa. The entire data set included 201 (52.2%) variable positions of which 107 (27.8%) were parsimony informative. The number of variable positions was 36 (9.4%) with 10 (2.6%) parsimony-informative characters when just ingroup taxa were considered. The 11 cyt *b* *A. islandica* haplotypes identified (Table 2) displayed between 0.26 and 8.1% difference, and no second position changes were found. Of the 83 specimens included herein, no evidence of heteroplasmy was detected (e.g. Hoeh et al. 1991), but one specimen had at least two distinct cyt *b* haplotypes (as judged by the presence of multiple sequencing peaks). We suspect that this Icelandic individual had haplotypes similar to X and W. Because further analysis on this individual is needed, this specimen was not included in the phylogeographic analysis.

Table 1 shows the number of haplotypes at each locality and the GenBank accession number of each haplotype. Haplotype frequency distributions can be biased by differences in sampling effort among geographical areas, and therefore, our inferences based on frequency data are limited. Due to the higher haplotypic diversity and the distinctiveness of Haplotype X, we sequenced more individuals from Iceland, Nova Scotia, and Maine in order to assess whether additional variation existed. It should be noted that, even with comparable sample sizes, Iceland ($n = 9$) has greater diversity than Maine ($n = 9$) or even all USA areas combined ($n = 39$; Table 1).

A parsimony network (sensu Avise et al. 1979) illustrates the differences between the observed haplotypes (Fig. 2). P is the most common haplotype among western Atlantic samples (64.4% of individuals excluding Iceland) and occupies a central position in the parsimony network. Based on coalescent theory (Crandall and Templeton 1993; Crandall et al. 1994) and using the heuristic method of Castelloe and Templeton (1994), one would infer that P is the ancestral haplotype. However, this conclusion is most likely incorrect. This particular method assumes that organismal sampling is not biased. As mentioned above, the present study does not include the northernmost populations, and sampling was more limited in the eastern Atlantic. Secondly, the method does not take into account mutational distances >1, therefore ignoring the importance of ancient haplotypic lineages. Because of these shortcomings, we used outgroup rooting to assign polarity (see below).

In addition to the parsimony network, we employed both likelihood and neighbor-joining (NJ) analyses to determine the evolutionary history of observed haplotypes. We used a likelihood approach to determine which model of DNA substitution was most appropriate (Huelsenbeck and Rannala 1997). We found no significant difference between NJ topologies produced using a Jukes–Cantor, Kimura-2-parameter, Tamura–Nei, Jukes–Cantor with gamma, Kimura-2-parameter with gamma, Tamura–Nei with gamma, or Log/Det correction models (for relevant models gamma shape parameter was estimated to be infinite and was set to a number >100). Therefore, the Log/Det model was arbitrarily chosen in NJ analyses. The resultant topology, including all four outgroups, is not shown, but was similar to the likelihood topology produced (Fig. 3A; see below).

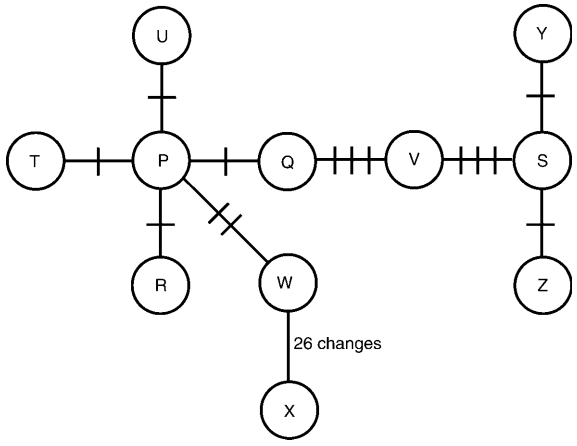

Fig. 2 *Arctica islandica*. Parsimony network of cyt *b* haplotypes. Lines crossing branches represent minimum observed number of nucleotide differences between haplotypes. Localities of haplotypes given in Table 1

Because likelihood can be a computationally expensive procedure, the NJ tree was used as the starting tree for a likelihood heuristic search (with Tree-Bisection-Reconnection, TBR, branch swapping). Relevant likelihood parameters (gamma, proportion of invariant sites, and the rate matrix) were optimized on this starting tree, and the values were set during the heuristic search. A general-time-reversible (GTR) model was employed, as all other commonly used models are a special case of the GTR model (Swofford et al. 1996). The best tree from the likelihood search had a ln likelihood score of −1,558.96462 (Fig. 3A).

Long outgroup branch lengths can cause spurious rooting of ingroup taxa (e.g. Wheeler 1990; Maddison et al. 1992; Halanych et al. 1999). Therefore, we repeated the likelihood (Fig. 3B; ln likelihood of −676.48697) and NJ (Fig. 3C) analyses without the four outgroup taxa. Mid-point rooting of the *Arctica islandica* network still places Haplotype X basal to all other haplotypes. Given that all other haplotypes differ by no more than 2.6% (Haplotype X is 6.8% different from its nearest neighbor, W) and all outgroup reconstructions place Haplotype X basal in the topology, we treat Haplotype X as an "outgroup" to root the remaining *A. islandica* haplotypes. Bootstrap values out of 1,000 iterations for NJ or 100 fast-stepwise heuristic iterations for likelihood are shown on the relevant topology. Resulting trees also show that the divergent Haplotype X is part of a well-corroborated *A. islandica* clade (Fig. 3A), and is genetically distinct from several other North Atlantic clam species, thus ruling out the possibility that this specimen belongs to a different, misidentified, species.

Based on the inferred rooting of the phylogenetic analyses, we detected at least three reversals in character state (positions 112, 133, and 316) within the *Arctica*

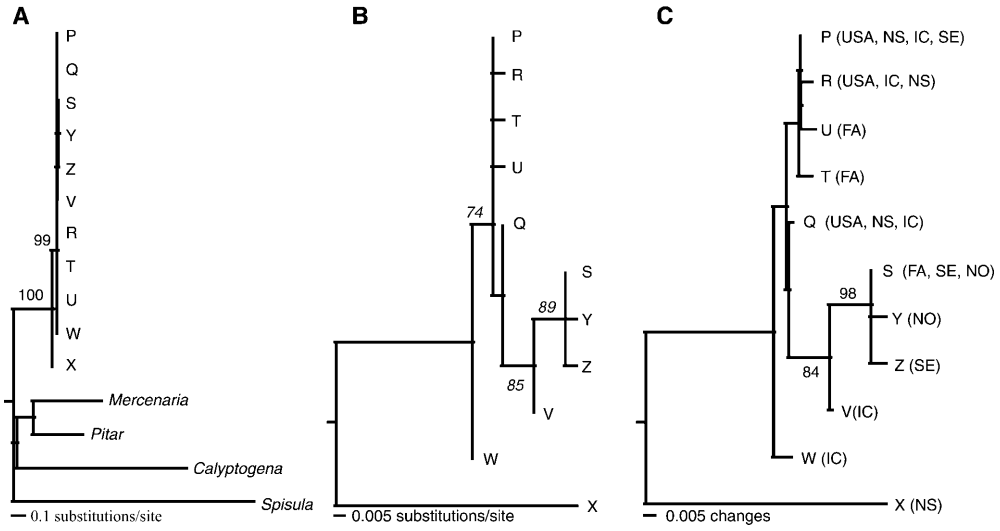

Fig. 3 *Arctica islandica*. Phylogenetic analyses of haplotypes. **A** Likelihood reconstruction employing a heuristic search (general-time-reversible model with estimated parameters) including the outgroups. **B** Likelihood reconstruction as above except outgroups have been excluded. The tree was rooted with Haplotype X. **C** Neighbor-joining (NJ) reconstruction of haplotypes based on a Log/Det model (Lake 1994; Lockhart et al. 1994). Numbers in Roman print are NJ bootstrap values, and in italic print are likelihood values (only values >60% are shown). Branch lengths are proportional to the inferred amount of nucleotide change. See "Results" for details of all reconstructions. Abbreviations are given in Table 1

islandica network. All three instances involved third position C-T changes, and we are not certain if the high ratio of third position thymines (*A. islandica*: 54.7%; for comparison *Mercenaria campechiensis*: 52.7%; *Calyptogena* sp.: 57.4%; *Pitar morrhuana*: 58.1%; *Spisula solidissima*: 48.1%) promoted the possibility of reversals. Third positions showed a very low percent composition of cytosines (*A. islandica*: 7.3%; *M. campechiensis*: 5.4%; *Calyptogena* sp.: 4.7%; *P. morrhuana*: 5.4%; except *S. solidissima*: 16.2%). Comparatively, cyt *b* in some mammalian taxa has a low percentage of guanines in third positions (e.g. Irwin et al. 1991; Matthee and Robinson 1997; Halanych et al. 1999). Such compositional bias could be due to selective pressure or mutational bias (Sueoka 1988). In mammals, guanine occupies the third position in two of the four stop codons (mitochondrial code), and it is not clear if the low third position C content observed here (all taxa but *S. solidissima*) could be due to differences in translational codes.

The AMOVA analysis, which assumed a single large group, revealed significant genetic structure among sampled populations (26.8% of observed variance) when compared to random expectations ($p < 0.001$). The AMOVA analyses considering eastern and western Atlantic populations suggested a significant genetic break between these two groups. The variance among the two groups (57.5%) was significant ($p < 0.001$) when the Iceland sample was treated as western Atlantic. In contrast, when Iceland was assigned to the eastern Atlantic group, the between-group variance was reduced (34.0%; $p < 0.016$). This suggested a western Atlantic affinity of the population in Iceland.

The ITS region was not informative because numerous specimens contained multiple alleles which confounded sequencing. Eighteen individuals from eight localities yielded identical sequences for ITS-1, but at least four distinct haplotypes for ITS-2 (for 12 individuals from six localities). The full length ITS region (including the 5.8S rDNA) in this bivalve spans 1,154 to 1,165 nucleotides, with the length variation occurring at an 11-nucleotide region near the 3′ end of ITS-2. The ITS sequences for *Arctica islandica* were deposited under GenBank Accession Numbers AF202106 to AF202109.

Discussion

The cyt *b* sequence data provided resolution of recent history and genetic structure of ocean quahog populations. Based on the presence of shared haplotypes and AMOVA analyses, Icelandic populations were more genetically similar to western that eastern Atlantic populations. The phylogenetic reconstructions did reveal some historical structure, namely a clade comprised of some eastern Atlantic haplotypes (S, V, Y, Z), which was well supported by bootstrap analysis. More sampling in the northeast Atlantic/southern Arctic Ocean is needed to determine the geographic distribution of this "eastern Atlantic" haplotypic clade. Interestingly, a nermertine parasite (*Malacobdella grossa*) is common in Scandinavian populations of *Arctica islandica* (authors' personal observations), but despite extensive sampling of western Atlantic populations it has only been reported once (Jones 1979). Neither the oceanic expanse between Iceland and Nova Scotia nor the circulation around Cape Cod seem to disrupt gene flow.

The haplotype diversity of *Arctica islandica*, a boreal subtidal bivalve, yields an interesting contrast to other boreal and arctic intertidal mollusks, sea urchins, and crustaceans that have been the subject of molecular

phylogeographic studies (e.g. Johannesson 1988; Palumbi and Kessing 1991; Collins et al. 1996; Reid et al. 1996; Caporale et al. 1997; reviewed by Cunningham and Collins 1998) and paleontological analyses (e.g. Bousfield and Thomas 1975; Stanley 1986; Vermeij 1989a,b, 1991a, b; Strasser 1999). Whereas present-day distributions of some intertidal species have been shaped by glaciation events, the *A. islandica* present-day genetic diversity was apparently influenced by the recent Holocene climatic optimum (warm period). Two observations, in particular, support this conclusion. First, ocean quahog populations show greater diversity (haplotypes per individuals sampled) in northern localities. The northern range of *A. islandica* was considerably larger during the most recent climatic optimum at 8,400 to 4,900 years BP (Fig. 1; Funder and Weidick 1991). This may have maintained or promoted higher genetic diversity through a greater habitat area and larger population sizes.

Second, the lack of unique haplotypes, as is the case with *Arctica islandica* in USA waters, suggests a recent bottleneck or founder event (Nei et al. 1975). During the climatic optimum, summer sea surface temperatures reached at least 18 °C from Nova Scotia south (except for a pocket of cooler water in the Gulf of Maine; Bousfield and Thomas 1975). Even though *A. islandica* adults display "submergence" in warmer waters (Franz and Merrill 1980a), studies on swimming behavior predict a depth range of larval occurrence at 0 to 35 m with an avoidance of temperatures exceeding 20 °C (Mann and Wolf 1983). Because larvae have the best chances for survival to metamorphosis at 10 to 12 °C (Landers 1976; see also Lutz et al. 1982), it is likely that successful recruitment south of Cape Cod did not occur during the optimum. In addition to high temperatures, habitat area in the south may have been greatly reduced. Sea level, which was rebounding from previous glaciation, was lower than today, with only parts of the continental shelf submerged (Emery and Garrison 1967; Bousfield and Thomas 1975; Franz and Merrill 1980b). These arguments suggest that during this climatic optimum, *A. islandica* south of Cape Cod may have gone extinct, and then repopulated the Mid-Atlantic Bight as temperatures declined and sea level continued to rise following the optimum (ca. 6,000 years BP; Bousfield and Thomas 1975; Franz and Merrill 1980b). A similar situation probably occurred in the Mediterranean, where populations existed from 31,500 to 9,800 years BP (Froget et al. 1972). Unlike the western Atlantic, Mediterranean populations apparently were never reestablished after this optimum.

If the climatic optimum (and the confounding influence of sea-level change) was more important to the genetic structure of present-day populations than glaciation, we predict that yet unsampled northern populations of *Arctica islandica* (e.g. the White Sea and northern Norway) will have comparatively high haplotypic diversity. In contrast, if population structure had been shaped by extinctions induced by glaciation events, as in intertidal species (Ingólfsson 1992; reviewed by Cunningham and Collins 1998), then we would expect reduced haplotypic diversity in the north due to local extinctions and bottleneck events. The pattern observed thus far in *A. islandica* is most consistent with the climatic optimum hypothesis. This pattern may also be present in other boreal subtidal organisms.

The cyt *b* data also seem to suggest that dispersal occurs predominately from the western Atlantic to the eastern Atlantic, and not vice versa. Of the four western Atlantic haplotypes detected, three were found in Iceland and one in Sweden. The two unique Faroe Island haplotypes are phylogenetically most closely related to the most common American haplotype, P (each one nucleotide substitution removed from P, Figs. 2 and 3). In contrast, only three of the ten haplotypes found in the eastern Atlantic and Iceland were present in western Atlantic waters. This postulated directionality of dispersal also conforms to expectations based on the easterly flow of the Gulf Stream. Larvae released from Faroe Island populations would be carried to the European mainland, but near Iceland it is conceivable that larvae would either be retained or swept into colder northern waters by eddies.

The observed size difference between individuals in Maine and those of Georges Bank and south of Cape Cod suggested that the Maine population may be genetically distinct. Neither the cyt *b* data nor the growth studies by Kraus et al. (1992) support this notion. The lack of geographic structure in cyt *b* indicates that a single population of *Arctica islandica* exists on the continental shelf of the eastern USA. However, if the Mid-Atlantic Bight population is historically young (recolonized ~6,000 years BP), then subpopulations may exist in USA waters that share identical cyt *b* haplotypes only because they have not been separated long enough for genetic differentiation to have occurred. Studies employing molecular markers with greater resolving power (e.g. AFLPs and microsatellites) could determine whether the observed pattern of genetic homogeneity in this region is a result of recent coancestry or contemporaneous gene flow.

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