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Phylogeographic estimates of colonization of the deep Atlantic by the protobranch bivalve *Nucula atacellana*

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Abstract: The Pleistocene and post-Pleistocene evolutionary history of many North Atlantic intertidal invertebrate species is well known, but the evolutionary history of the deep North Atlantic fauna is poorly understood, specifically whether colonization of the deep North Atlantic paralleled the patterns observed in shallow water. Contemporary pan-Atlantic species distributions could result from several colonization pathways that connected different regions of the Atlantic at different times (*e.g.* Arctic, Antarctic or Panamanian pathways). To test potential colonization pathways we quantified geographic variation in nuclear and mitochondrial markers from Atlantic samples of *Nucula atacellana,* a pan-Atlantic deep-sea protobranch bivalve, using *N. profundorum* in the eastern central Pacific as an outgroup. We combined existing 16S data from North and South Atlantic populations of *N. atacellana* with new sequences of 16S, COI, and an intron of calmodulin from those populations, and newly sampled populations near Iceland. Population genetic analyses indicated a subtropical expansion via the Central American Seaway. We found no evidence for Transarctic migration to the Atlantic in *N. atacellana*, which suggests that colonization pathways may differ significantly between shallow- and deep-water fauna.

Key words: Icelandic waters, Protobranchia, population genetics, species origin, North Atlantic demographics

Introduction

The evolutionary history and recent demographics of North Atlantic benthic invertebrates has been increasingly well characterized over the past few decades for species inhabiting rocky intertidal environments of the Northwest and Northeast Atlantic (Wares 2002; Maggs *et al.* 2008; Ilves *et al.* 2010). In contrast, very little is known about the history and dynamics of deep-sea benthic invertebrates in the North Atlantic (Etter *et al.* 2011). Research on intertidal and shallow subtidal species has identified several important factors shaping current phylogeographic and species distributions. Pleistocene glaciation caused the extirpation of many

(though not all) intertidal invertebrates from the Northwest and/or Northeast Atlantic, after which recolonization occurred from refugia, adjacent populations, or the opposite side of the North Atlantic (Wares 2002; Hare and Weinberg 2004; Aboim *et al.* 2005; Kelly *et al.* 2006; Doellman *et al.* 2011; Provan and Maggs 2011; Flight *et al.* 2012; Waltari and Hickerson 2012). For some species, the history of North Atlantic populations is tied to North Pacific populations via the Arctic Ocean. The Trans-Arctic Exchange was a period of high Atlantic-Pacific connectivity after the opening of the Bering Strait in the Pliocene; this connectivity was impeded by subsequent Pleistocene glaciation, and has likely been reinvigorated in the warming following the Last Glacial Maximum (Vermeij 1991). There is genetic evidence for Atlantic-Pacific exchange via the Arctic Ocean in several taxa (vertebrates: Dodson *et al.* 2007; Laakkonen *et al.* 2013; invertebrates: Addison and Hart 2005; Govindarajan *et al.* 2005; Carmack and Wassmann 2006; Nikula *et al.* 2007; Rawson and Harper 2009).

While these forces are becoming better understood for shallow species, it is not clear whether they have played a substantial role for bathyal and abyssal species in the North Atlantic. While deep-dwelling species likely experienced smaller temperature fluctuations than their shallow counterparts, the strength of deep water formation and thermohaline circulation in the North Atlantic did change through the Pleistocene and Recent (Fichefet *et al.* 1994; Kleiven *et al.* 2003; Knies *et al.* 2007). Genetic structure consistent with expansion of North Atlantic populations farther poleward has been detected (*e.g.* Aarbakke *et al.* 2011), underscoring the role of climate change in range expansion. Because much more insight has been gained as to the factors maintaining the high diversity of the deep-sea fauna than has been gained in understanding its evolutionary origins (Rex and Etter 2010), the extent to which these changes could have affected deep-dwelling species' ranges, persistence, or connectivity largely remains an open question. A study of the deep-sea red crab *Chaceon quinquedens* (S.I. Smith, 1879) detected a spread of Atlantic populations from the subtropical Atlantic to the North Atlantic (Weinberg *et al.* 2003). Pan-Atlantic analyses of deep-sea asellote isopods detected a strong south-to-north increase in species diversity consistent with expansion northward through the Atlantic, possibly owing to the greater connectivity of the South Atlantic to neighboring ocean basins (Wilson 1998). At still larger scales, Havermans *et al.* (2013) described high genetic similarity of North Atlantic populations of *Eurythenes gryllus* (Lichtenstein, 1822) to both North Pacific and Antarctic populations, underscoring the potential for multiple colonization pathways. While these studies reveal intriguing patterns of deep-sea genetic colonization, a deeper understanding of the origin and demographics of the deep Atlantic biota is critically important to place contemporary connectivity in an evolutionary context, and to understand its connection to ongoing climate change as continued warming further opens trans-Arctic pathways.

At least one alternative pathway for dispersal into the Atlantic existed through the Central American Seaway (CAS), which connected the tropical Pacific and At-

lantic Oceans (via the present-day Caribbean Sea) until the rise of the Isthmus of Panama roughly 3 million years ago (MYA) (Burton *et al.* 1997; Coates *et al.* 2005). In a complex process of geological uplift and sedimentation lasting at least 10 million years (MY), the Isthmus of Panama blocked deep-water connections between the oceans (by about 12–15 MYA), eliminated the surface circumtropical current (by about 3.6 MYA), and drastically altered Atlantic circulation at all depths, notably by increasing the strength of the Gulf Stream and of North Atlantic Deep Water formation (Lessios 2008; Jackson and O'Dea 2013). While this "Great American Schism" (Lessios 2008) is usually viewed in terms of its induction of geminate species pairs on either side of the Isthmus (e.g. Lessios 1981; Dick *et al.* 2003; Mathews 2006; Quattrini *et al.* 2013), before this period gene flow and dispersal would have been facilitated between the central Pacific and central Atlantic through the CAS. Again, the evidence suggests that deep-sea species experienced cessation of gene flow earlier than did shallow water species. Finally, Pacific-Atlantic contact could have occurred via the Southern Ocean, particularly since the isolation of Antarctica and emergence of the Antarctic Circumpolar Current (ACC) between roughly 20 and 50 MYA (Scher and Martin 2006; Barker *et al.* 2007), which could have facilitated South Pacific-South Atlantic connectivity.

Among common Atlantic deep-sea taxa, protobranch bivalves stand out for three reasons. First, many groups not only survive but thrive in deep habitats, where they can be more common and/or speciose than in shallow water (Zardus *et al.* 2006; Allen 2008). Second, some species have widespread or nearly ubiquitous distributions in the Atlantic, inhabiting oceanic basins separated by thousands of kilometers (Allen 2008). Finally, emerging genetic analyses suggest that separation by depth limits deep-sea protobranch connectivity much more than does geographical distance (Zardus *et al.* 2006; Etter *et al.* 2011; Jennings *et al.* 2013). These studies have consistently found significant genetic divergence across tens to hundreds of meters of depth (and at the same depth as France and Kocher (1996) and Havermans *et al.* (2013), but low genetic divergence across thousands of kilometers of (horizontal) distance, both in the North Atlantic (Jennings *et al.* 2013) and the larger Atlantic (Zardus *et al.* 2006; Etter *et al.* 2011). The combination of widespread species distributions and high genetic connectivity across vast geographic distances suggests an unusual evolutionary history for deep-sea Atlantic protobranchs, and one potentially very different than species in adjacent shallow water habitats.

A recent high-resolution phylogeny of protobranch bivalves (Sharma *et al.* 2013) recovered a robustly supported sister-taxon relationship between the Northeastern Pacific nuculoid *Nucula profundorum* E.A. Smith, 1885 and the widespread Atlantic nuculoid *Nucula atacellana* Schenck, 1939 (formerly *Deminucula atacellana).* The species' external morphologies are extremely similar, differing mainly in that the former has an interiorly crenellated dorsal shell margin, whereas that of *N. atacellana* is smooth. Such a close relationship between species in

widely separated ocean basins is uncommon, and presents a unique opportunity to investigate patterns of the origin and spread of Atlantic populations. *Nucula profundorum* is known from the eastern margins of the Pacific, from about 16°N at 1200 m depth to about 6°S at 4000 m depth (Dall 1908); *N. atacellana* is widely distributed in the western North Atlantic and the eastern North and South Atlantic (Allen 2008). Until recently, molecular data for *N. atacellana* were limited to formalin-fixed, ethanol-preserved (FFEP) specimens from a few Atlantic locations: North American Basin, West European Basin, Argentine Basin (Zardus *et al.* 2006); however, recent cruises have obtained material from additional locations and preserved it in a manner suitable for multilocus analyses. In particular, sampling of the waters around Iceland as part of the IceAGE expedition has increased the geographic spread of *N. atacellana* specimens to cover virtually the entire north-south Atlantic axis. Furthermore, Iceland is located at a critical junction between several important ocean basins: the Greenland-Scotland Ridge (GSR) rises to approximately 600 m between Greenland and Iceland, and to approximately 500 m between Iceland and Scotland (Hansen and Østerhus 2000). The GSR defines the GIN Seas to the north, comprising the Greenland, Iceland, and Norwegian Seas, with water masses on either side of the GSR having different properties (Hansen and Østerhus 2000; Malmberg and Valdimarsson 2003). While the GSR isolates the deep basins of the Northwest and Northeast Atlantic from the deep basins of the Nordic Seas, flow over their sills potentially allows some connectivity among them (and among shallower-dwelling species). The GSR is known to act as a barrier for some North Atlantic species, but not for others (see Brix and Svavarsson 2010; Schnurr *et al.* 2014). The Reykjanes Ridge, the northernmost extension of the Mid-Atlantic Ridge, further separates waters south of Iceland into eastern and western basins. Finally, Iceland sits near the Atlantic terminus of the Transarctic Drift, representing a potential intermediate population along a North Pacific-Arctic-North Atlantic axis (Vermeij 1991). Combined with previously sampled populations, genetic analysis of *N. atacellana* and *N. profundorum* could provide valuable insights as to whether the Atlantic was colonized via the Arctic, CAS, Southern Ocean, or other pathways. Here we present genetic analysis of two mitochondrial and two nuclear loci from Atlantic populations of *N. atacellana,* using the closely related Pacific *N. profundorum* to polarize the historical spread of the former through the North Atlantic.

Materials and methods

Sampling and specimens. — Specimens of *N. atacellana* were collected from 12 Atlantic cruises covering five decades of research (Table 1, Fig. 1); older FFEP collections were from 10 cruises to the North American, West European, and Argentine Basins as described in detail in Zardus *et al.* (2006). The newest material

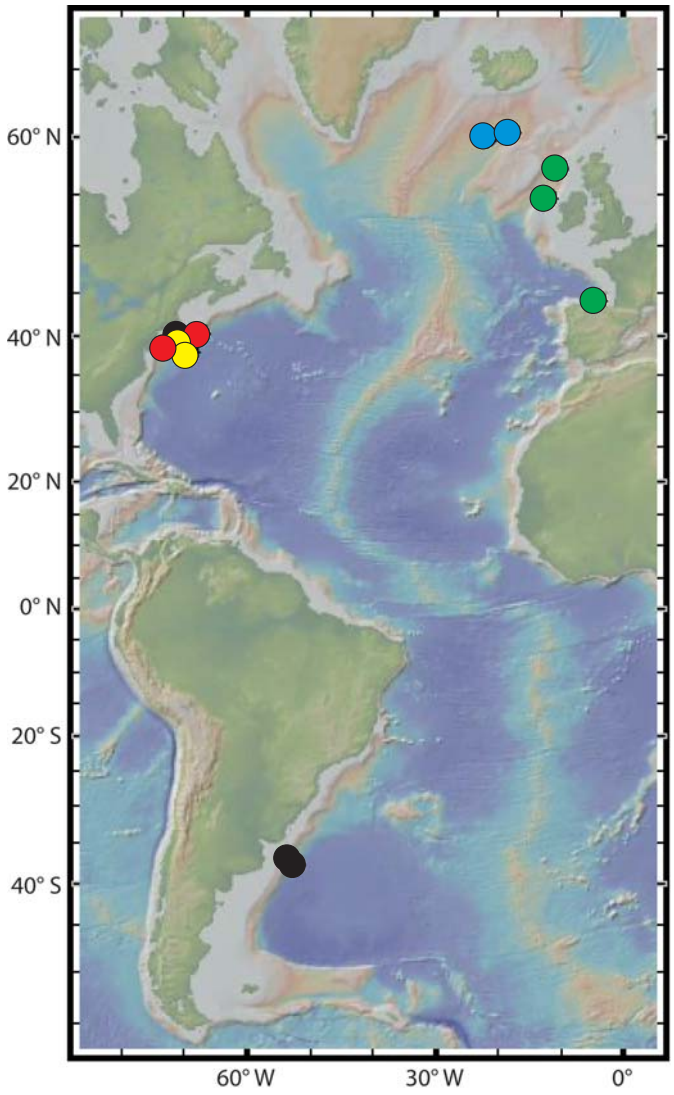

Fig. 1. Cruise regions in the Argentine Basin (black), North American Basin (red: Hessler/Sanders cruise; yellow: *Endeavor* cruise), West European Basin (green), and Iceland Basin (blue) from which specimens of *Nucula atacellana* were obtained.

was collected on two research cruises: one on board the R/V *Endeavor* in 2008 to the same North American Basin (NAB) region originally sampled by Hessler and Sanders (1967), and one on board the R/V *Meteor* in 2010 to the waters around Iceland. On these last two cruises, samples obtained with epibenthic sleds were quickly sieved at cold (4°C) temperatures, and specimens either flash-frozen or preserved in pre-chilled (-20°C) 95% ethanol. For clarity, we refer to the 1960s *N. atacellana* from the NAB as "NAB", and the 2008 *Endeavor* NAB material as

Table 1

Sampling information for *Nucula atacellana* and *Nucula profundorum.*

Nucula atacellana									
Group /Basin	Station	Lat	Lon	Depth (m)	Year	Sequences			
						s-16S	l-16S	COI	CAL[A]
ARG: n=30	245	-36.928	-53.007	2707	1971	8 GB			
	256	-37.667	-52.317	3910	1971	18 GB			
	259	-37.222	-52.75	3310	1971	4 GB			
WEB: n=6	DS87	44.087	-4.323	1913	1974	1 GB			
	ES197	57.35	-10.483	2200	1981	1 GB			
	ES283	54.65	-12.25	2946	1985	3 GB			
	ES289	57.317	-10.417	2190	1985	1 GB			
NAB: n=94	62	39.433	-70.55	2496	1964	8 GB			
	73	39.775	-70.222	1400	1964	6 GB			
	77	38.012	-69.267	3806	1965	17 GB			
	85	37.987	-69.437	3834	1965	8 GB			
	“83” (87)	39.812	-70.68	1102	1965	11 GB			
	103	39.727	-70.623	2022	1966	17 GB			
	115	39.653	-70.408	2040	1966	6 GB			
	209	39.793	-70.832	1500	1969	14 GB			
	340	38.235	-70.338	3310	1973	4 GB			
	MMSM3	38.614	-72.856	2055	1985	2 GB			
	MMSM8	40.172	-67.623	2180	1985	1 GB			
ENN: n=42	4	39.781	-70.709	1600	2008	2; KJ950184 –KJ950185	2; KJ950244 –KJ950245	2[B]	2[B]
	5	39.759	-70.713	1900	2008	4; KJ950186 –KJ950189	4; KJ950246 –KJ950249	4[B]	4[B]
	6a	39.637	-70.503	2200	2008	19; KJ950190 –KJ950208	1; KJ950250	1[B]	1[B]
	7a	39.45	-70.467	2500	2008	4; KJ950209 –KJ950212	3; KJ950251 –KJ950253	2[B]	2[B]
	9a	39.24	-70.399	2700	2008	3; KJ950213 –KJ950215	3; KJ950254 –KJ950256	3[B]	3[B]
	10	39.037	-70.781	2800	2008	2; KJ950216 –KJ950217	2; KJ950257 –KJ950258	2[B]	2[B]
	14a	38.295	-70.494	3300	2008	1; KJ950218	1; KJ950259	1[B]	1[B]
	17a	38.133	-70.317	3500	2008	3; KJ950219 –KJ950221	3; KJ950260 –KJ950262	3[B]	3[B]
	18/a	38.093	-69.708	3800	2008	4; KJ950222 –KJ950225	4; KJ950263 –KJ950266	4[B]	4[B]
ICE: n=16	963	60.045	-21.492	2746	2011	16; KJ950226 –KJ950241	2; KJ950267 –KJ950268	2; KJ950271 –KJ950272	2; KJ950277 –KJ950280
	979	60.348	-18.142	2568	2011	1; KJ950242	1; KJ950269	1; KJ950273	1; KJ950275 –KJ950276
Nucula profundorum									
Group /Basin	Station	Lat (N)	Lon (E)	Depth (m)	Year	Sequences			
						s-16S	l-16S	COI	CAL[A]
NE Pac: n=1	6	32.902	-117.766	1045	2012	1; KJ950243	1; KJ950270	1; KJ950274	1; KJ950281 –KJ950282

A – Numbers reflect number of individuals; GenBank Accessions list each allele separately
B – Sequences from Jennings et al. 2013
GB – Sequences obtained by downloading haplotypes from GenBank according to Zardus et al. 2006

"ENN". The *N. profundorum* specimen was collected on a cruise on board the R/V *Melville* in 2012 to the eastern North Pacific.

Genetic analyses. — To confirm close the phylogenetic relationship between *N. atacellana* and *N. profundorum,* a phylogenetic tree was estimated using seven nuculoid species available from Sharma *et al.* (2013), plus the *N. profundorum* specimen and representatives of all major genetic lineages in *N. atacellana* (see below and Jennings *et al.* 2013). Only the mitochondrial small subunit rRNA (16S) and cytochrome oxidase *c* subunit I (COI) markers could be used for this tree, which was computed in BEAST v1.8.0 (Drummond *et al.* 2012). The COI marker was partitioned into codons; these partitions and 16S were each given independent GTR models. Each locus was modeled with a relaxed lognormal uncorrelated clock (Drummond *et al.* 2006), and a birth-death prior for the tree. One Markov chain was run for 10 million steps, sampling every 1000 steps, with operators tuned according to the BEAST manual. Convergence to the posterior was assessed after the run in Tracer 1.5 by verifying that all estimated sample sizes (ESS) were sufficiently large (as per the BEAST and Tracer manuals), and the appropriate burnin selected. The consensus tree was made in TreeAnnotator v1.7.5 and edited in FigTree v1.3.1 (tree.bio.ed.ac.uk/software/figtree).

Loci were selected to complement the work of Zardus *et al.* (2006) on FFEP *N. atacellana,* in which a short 196 base pair (bp) portion of 16S was sequenced. These 16S haplotypes were obtained from GenBank for the NAB, West European Basin (WEB), and Argentine Basin (ARG) populations (AF029093–AF029104; DQ269458–DQ269466) and used to reconstruct population sequence sets according to the haplotype frequencies of Zardus *et al.* (2006). The short 16S fragment (s-16S) was also amplified from 42 ENN and 16 ICE individuals using primers and protocols in Zardus *et al.* (2006). For *N. profundorum* and a subset of 23 ENN and 3 ICE specimens, the universal 16SarL/16SbrH primers of Palumbi *et al.* (1991) were used to amplify a longer 515 bp fragment of 16S (l-16S) that contains the s-16S region. A 652bp portion of COI was amplified from these specimens using the universal LCO1490/HCO2198 primers of Folmer *et al.* (1994) and PCR conditions described in Jennings *et al.* (2013). A nuclear marker was also sequenced for this subset of individuals. The first intron of the calmodulin gene (CAL) was amplified using the CAL1/CAL2 primers of Duda and Palumbi (1999), and the C1 PCR protocol described in Jennings and Etter (2011).

Successful PCR reactions were sequenced by Agencourt, Inc. (a Beckman-Coulter company, Beverly MA); chromatograms were edited in Sequencher 5.0.1 (Gene Codes Corp., Ann Arbor, MI). For each locus, alignments were made using the CLUSTAL algorithm (Larkin *et al.* 2007) in BioEdit with default parameters and were checked by hand. For introns, heterozygous positions were detected in the chromatograms using Sequencher and haplotypes were reconstructed by comparing against an in-house database of previously haplotyped and phased *N. atacellana* individuals (Jennings *et al.* 2013). When needed, new

nuclear sequences were phased as described in Jennings *et al.* (2013). New sequences generated in this work were deposited in GenBank under Accessions KJ950184–KJ950282 (Table 1).

Using the s-16S data, Zardus *et al.* (2006) detected significant geographic differentiation between the North (NAB, WEB) and South (ARG) Atlantic samples of *N atacellana*, and further differentiation within the NAB by depth (shallower vs. deeper than ~3000m); in a five-locus analysis of the ENN material, Jennings *et al.* (2013) detected significant bathymetric population structure exactly in accordance with the separation detected by Zardus *et al.* (2006). Because there is genetic differentiation between shallow and deep groups in the Northwest Atlantic but not between the NAB and ENN collection cruises, specimens were pooled among these cruises and separated geographically into shallow vs. deep groups. Using these genetically distinct locations (ARG, NAB/ENN shallow, NAB/ENN deep, ICE, WEB), a haplotype network was computed for the s-16S fragment of all *N. atacellana* individuals using TCS 1.21 (Clement *et al.* 2000). Gaps were treated as a 5th base, and the connection limit was set at the default 95%. As an exploratory tool, networks were constructed with and without *N. profundorum*.

To extend this analysis, the *N. profundorum* sequences were used in BEAST (v. 1.8.0 and v. 2.0, see below) to polarize phylogeographic reconstructions of Atlantic *N. atacellana* populations. Two datasets were compiled for BEAST: the s-16S fragment from all specimens *(N. profundorum* + ARG, NAB, ENN, WEB, ICE), and a three-locus dataset with l-16S, COI, and CAL from the recently collected specimens *(N. profundorum* + ENN, ICE). For each locus in both datasets, the HKY (Hasegawa, Kishino and Yano) model of mutation was selected (because there were not enough data to obtain good estimates in more parameter-rich models), with four gamma-distributed categories of rate variation and estimated equilibrium nucleotide frequencies. Each partition was modeled with a relaxed uncorrelated lognormal clock; the mean COI rate was set to 1 and mean rates for all other loci were estimated relative to it. The starting tree calculated via UPGMA, and default parameters for all priors were used, except for the relative mean rates of loci other than COI, each of which was assigned a standard normal prior.

For the first analysis in BEAST 1.8.0, only the s-16S dataset was used, and a partition for geographic location was created. The discrete phylogeography model of Lemey *et al.* (2009) was employed to reconstruct geographic history at internal nodes, using a Yule prior for the tree. One Markov chain was run for 50 million steps, sampling every 5000 steps, with operators tuned according to the BEAST manual. Convergence and tree annotation were conducted as above, with tree branches colored by reconstructed ancestral location, and probabilities of occurrence for all locations (calculated as 95% highest posterior densities (HPD) in BEAST) given at the nodes. None of the populations was constrained to be monophyletic in these analyses; trees were edited in FigTree v1.3.1.

For the second analysis in BEAST 2.0.2, the 3-locus dataset was used in an application of starBEAST (Heled and Drummond 2010). A genealogy was constructed by nesting the three independent locus trees inside a "species tree" of individuals, and geographic locations were estimated on the genealogy using the discrete phylogeography model described above. All parameters and priors were assigned as above, except that the genealogy was assigned a coalescent prior with constant population size. This analysis was run for 50 million steps and analyzed as described above.

Results and discussion

The nuculoid phylogenetic tree (Fig. 2) showed a highly supported sister-taxon relationship between *N. atacellana* and *N. profundorum* (Bayesian posterior probability of 1.0), with these species clustered with *Ennucula granulosa*

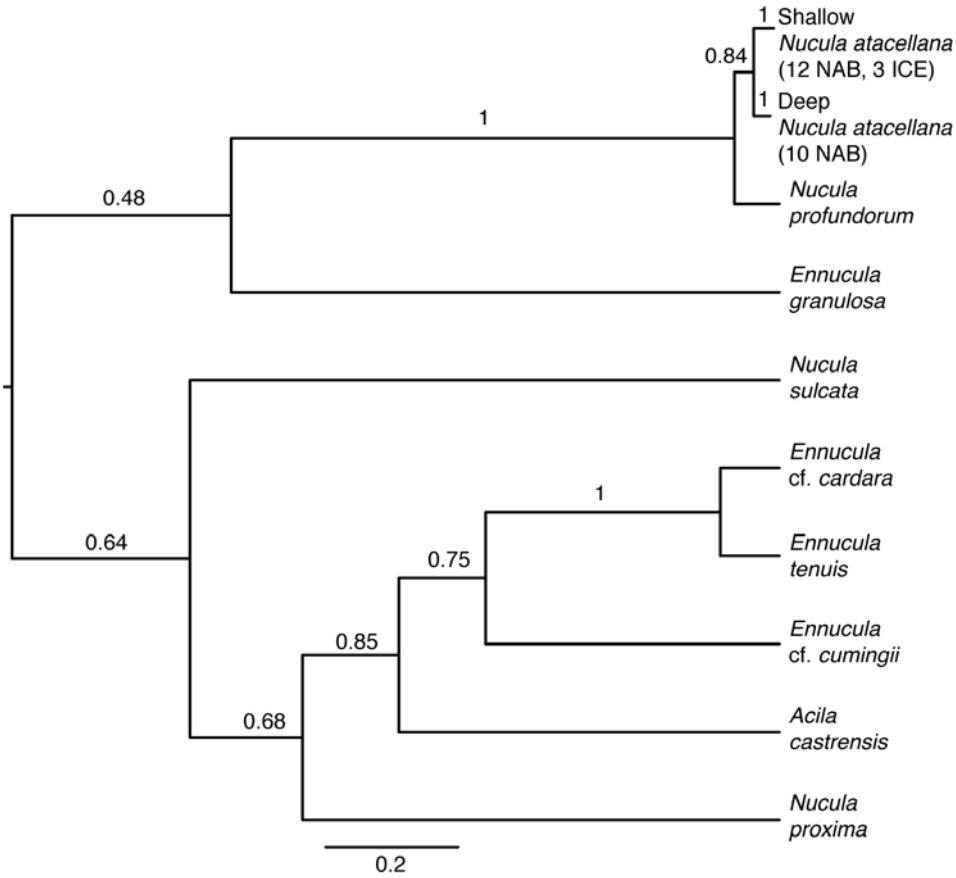

Fig. 2. Bayesian phylogenetic tree of nuculoids. Numbers on branches indicate posterior probabilities.

Table 2

Locus-by-locus p-distances within *Nucula atacellana* and between *Nucula atacellana* and *Nucula profundorum,* computed with pairwise deletion of missing data. Values shown are mean p-distance ± standard error.

Locus	Within *Nucula atacellana*	Between species
16S	0.025 ± 0.007	0.047 ± 0.015
COI	0.027 ± 0.004	0.097 ± 0.011
CAL	0.022 ± 0.005	0.027 ± 0.008

(Verrill, 1884), and the other nuculoids (*Ennucula* cf. *cardara*, *Ennucula tenuis* (Montagu, 1808), *Ennucula* cf. *cumingii*, *Acila castrensis* (Hinds, 1843), *Nucula proxima* Say, 1822) in a separate clade (though with low support). Also highly supported was a previously detected separation of shallow (<2700 m) vs. deep (>2800 m) populations of *N. atacellana* in the Argentine and North American Basins. This tree suggests that, despite the high similarity of the two species examined here, they are indeed separate species.

Sequences of *N. profundorum* were highly similar to those of *N. atacellana* at all loci; the mean p-distances between *N. profundorum* and *N. atacellana* at 16S and COI were higher than the mean p-distance within *N. atacellana*; at CAL the means were similar (Table 2). In a recent analysis of nuclear intron diversity, such high similarity was rarely seen among congeners (Jennings and Etter 2011), implying that these two *Nucula* species are very closely related and probably represent sister species. The haplotype network revealed a star-like topology centered on a dominant haplotype shared among all locations, with a longer side-chain of haplotypes found either only in the Argentine Basin or shared among the deep Argentine and North American Basins (Fig. 3). This network placed *N. profundorum,* closest to (five steps from) the most abundant deep haplotype. Using the rough heuristic method of Castelloe and Templeton (1994), the central shared haplotype was most likely the ancestral one.

Bayesian phylogeographic analysis of the large dataset (s-16S from all populations) indicated genetic separation among some ocean basins (Fig. 4), recovering a significant separation of [most ARG individuals and deep (>2800 m) NAB+ENN individuals] from [ICE, WEB, five ARG individuals, and shallow NAB+ENN (<2700 m) individuals], consistent with previous findings (Zardus *et al.* 2006). Within this shallow clade, haplotypes were most numerous in the Northwest Atlantic, with clades of ICE, WEB, and ARG haplotypes mixed in among shallow NAB+ENN haplotypes. Bayesian posterior probabilities of geographic location traced the oldest lineages to the ARG and deep NAB+ENN lineages with high support and little transitioning among locations; the younger clade was centered on the shallow Northwest Atlantic with more frequent transitions to ICE, WEB, and ARG. Considering the most likely history of geographic spread, the geographic sequence ARG + (deep NAB) → shallow NAB → ICE →WEB was recovered as the

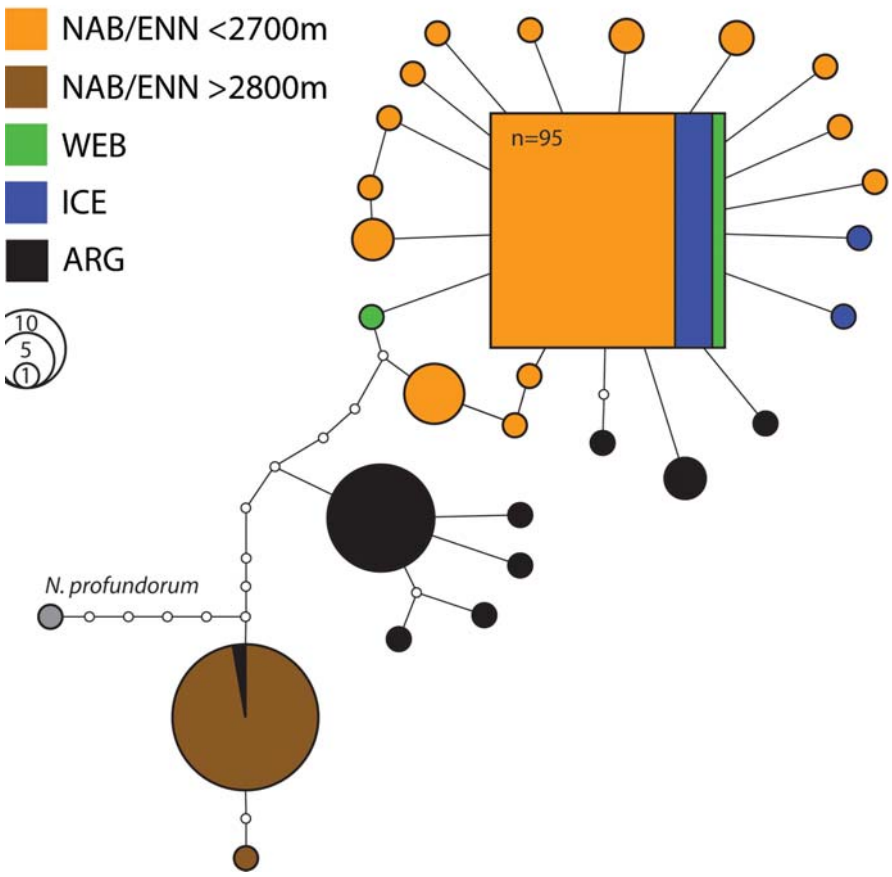

Fig. 3. Haplotype network for s-16S dataset and all geographic locations. Shapes represent different haplotypes with size indicating the number of individuals detected possessing that haplotype; lines represent single base pair differences between haplotypes, regardless of length. Small open circles represent unsampled haplotypes required to complete the network. The large square is the most likely ancestral haplotype using the rough heuristic of Castelloe and Templeton (1994).

best tree topology; however, the sequence ICE → WEB received moderately low support (PP = 0.4). Although more restricted in sampling locations, the multilocus phylogeographic reconstruction supported this reconstruction (Fig. 5), with deep and shallow NAB lineages in separate clades, and two recent incursions to Icelandic waters from the shallow NAB clade.

Given these results, both Trans-Arctic exchange from the Pacific to Iceland, and ancient ancestry of Icelandic *N. atacellana* seem highly unlikely. Instead, an origin in the low-latitude Atlantic seems more probable given the samples attained thus far. In this scenario, infiltration of the tropical Atlantic could have occurred via the Central American Seaway, before the rise of the Isthmus of Panama approximately 3 million years ago (Burton *et al.* 1997; Coates *et al.* 2005). This barrier to gene flow caused speciation in several marine invertebrate

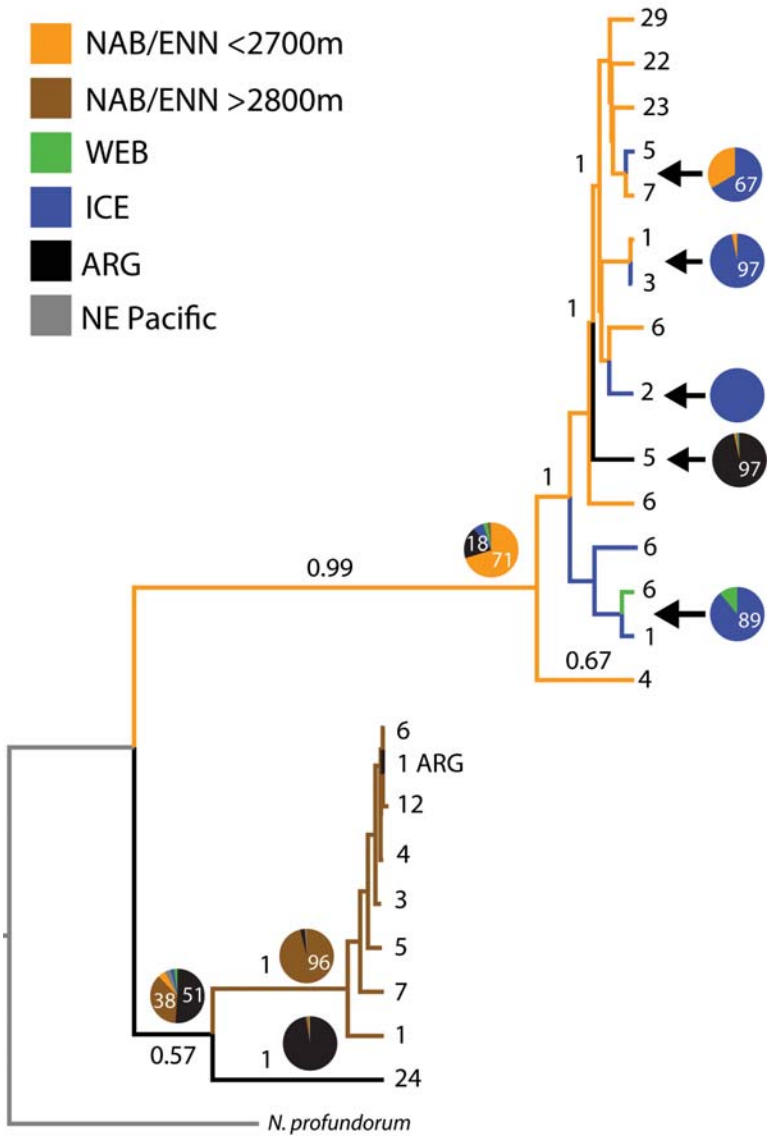

Fig. 4. Bayesian phylogeographic analysis for the s-16S dataset, rooted a posteriori with *N. profundorum.* Nodes containing multiple individuals all from a single geographic location have been collapsed for clarity with counts of individuals at the tips. Branch shading depicts the geographic location receiving the highest support, with 95% highest posterior densities (HPDs) for all locations represented by labeled pie graphs at nodes. Branch labels are posterior probabilities. Full, uncollapsed tree is given in Supplementary Online Material (fig. 1) available at http://www.degruyter.com/view/j/popore.2014.35.issue-2/popore-2014-0017/suppl/popore-2014-0017_suppl.pdf

groups, including echinoderms, crustaceans, and mollusks (Lessios 2008). Furthermore, *N. atacellana* does not seem to occur north of the GSR (Allen 2008, pers. obs.), which may mean that some geographic or environmental factor ex-

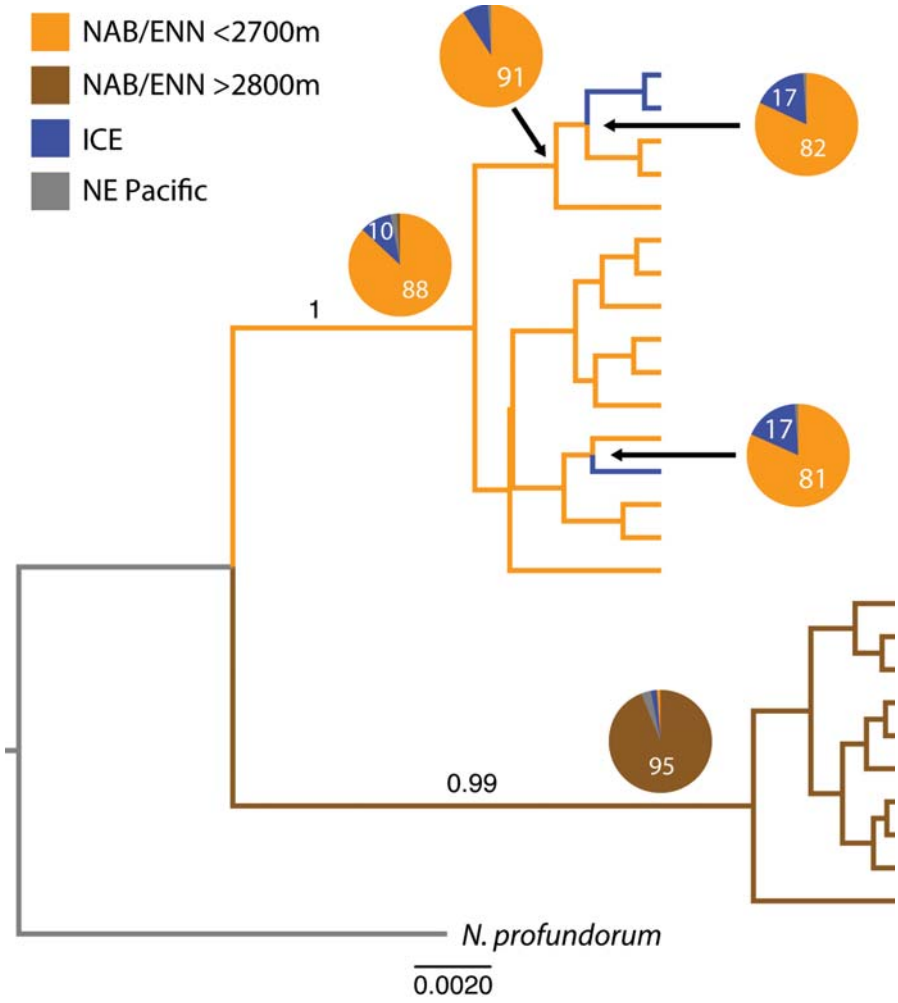

Fig. 5. Bayesian phylogeographic analysis for the three-locus dataset, rooted *a posteriori* with *N. profundorum.* Branch shading, pie graphs, and branch labels are as in Fig. 4.

cludes it from the Atlantic sub-Arctic region and makes trans-Arctic exchange unlikely. The GIF Ridge does seem to coincide with species' boundaries for many deep-sea isopods (Brix and Svavarsson 2010). If a mid-Atlantic origin of *N. atacellana* proves to be correct, it would have been followed by a northward and southward spread, and eastward expansion from the North Atlantic to the West European Basin. While there was no significant separation of Icelandic specimens from NAB + ENN specimens, indicating that the Reykjanes Ridge does not significantly restrict east-west gene flow of *N. atacellana*, polarity was implied by the derived placement of West European lineages relative to ICE lineages, repeated NAB → ICE transitions, one ICE → WEB transition, and no evidence of westward transitions.

Genetic studies of Atlantic deep-sea fauna are few, and tend to focus on Pleistocene glacial dynamics of North Atlantic species, and interactions with the Southern Ocean for South Atlantic species; little research exists more broadly on the routes of pan-Atlantic colonization from other oceans for deep-sea species. Nonetheless, a preliminary picture can be drawn as a guide for developing hypotheses. In a comparative study of 45 (globally) widely-distributed peracarids, Brandt *et al.* (2012) reported the highest Atlantic-Pacific similarity in the North (24 species); fewer species (18) found in both northern basins of both oceans and at least one southern basin of one ocean, and the fewest (7 species) found in all four basins. While species' distributions are affected by far more than colonization routes over evolutionary time (and, in the deep-sea, are especially confounded by incomplete sampling and cryptic species), this bias may imply that Atlantic colonization is more likely through the Arctic than by other routes. To this point, Malyutina and Brandt (2007) found just two of 45 species in the isopod family Munnopsidae in both the South Atlantic and South Pacific, although presumed Southern Ocean origins of two genera of stalked crinoids in the North Atlantic (Eléaume *et al.* 2012) point to the possibility of the southern route. In contrast, Brix and Svavarsson (2010) found that many Atlantic deep-sea isopod species boundaries coincide with the GIF Ridge, implying that, while Nordic Sea and Arctic Ocean faunas may reflect Transarctic exchange, fewer species extend farther south into the Atlantic proper across the GIF. In keeping with this idea, Atlantic colonization from the Pacific via the Central American Seaway with no evidence of Transarctic exchange has been established for several deep-sea coral species (Thoma *et al.* 2009; Herrera *et al.* 2012; Pante *et al.* 2012) and a clade of the mysid genus *Pseudomma* (Meland 2004); a similar pattern was found in a shallow-water bryozoan (Schwaninger 2008).

Given the limited sampling of *N. profundorum* and the difficulty of obtaining nuclear DNA sequences of sufficient size from FFEP material, it was not possible to fully resolve the details of demographic spread of *N. atacellana* through the Atlantic. However, given the indirect connection of all Icelandic (and West European) individuals to the Pacific, the conclusion that Iceland is not the ancestral Atlantic population seems reasonable. While more thorough sampling of *N. atacellana* is required to fully elucidate the most likely pathway, Transarctic exchange seems highly unlikely for this species.

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