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Phylogeographic analysis reveals a deep lineage split within North Atlantic *Littorina Saxatilis*

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PHYLOGEOGRAPHIC ANALYSIS REVEALS A DEEP LINEAGE SPLIT WITHIN NORTH
ATLANTIC *LITTORINA SAXATILIS*

A thesis presented

by

Meredith M. Doellman

To

The Department of Biology

In partial fulfillment of the requirements for the degree of
Master of Science

in the field of

Biology

Northeastern University
Boston, Massachusetts
December, 2010

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ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirements
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Northeastern University, December, 2010

ABSTRACT

Phylogeographic studies provide critical insight into the evolutionary histories of model organisms; yet, to date, range-wide data are lacking for the rough periwinkle *Littorina saxatilis*, a classic example of marine sympatric speciation. I utilized mitochondrial DNA (mtDNA) sequence data to demonstrate that *L. saxatilis* is not monophyletic, but is comprised of two mtDNA lineages (I and II) that are shared with its sister-species *L. arcana* and *L. compressa*. Bayesian coalescent dating and phylogeographic patterns indicate that both *L. saxatilis* lineages (I and II) originated in the northeastern Atlantic, around the British Isles, approximately 0.64 Ma. Both lineages are now distributed broadly across the eastern, central, and western North Atlantic, and show strong phylogeographic structure among regions. The Iberian Peninsula is genetically distinct, suggesting prolonged isolation from northeastern Atlantic populations. Western Atlantic populations of *L. saxatilis* Lineages I and II both predate the last glacial maximum and have been isolated from eastern Atlantic populations since that time. The existence of two broadly distributed cryptic mtDNA lineages further complicate observed patterns of repeated incipient ecological speciation in *L. saxatilis*, because the sympatric origins of distinct ecotype pairs on northeastern Atlantic shores may be confounded by admixture of divergent lineages.

DEDICATION

To my parents, John and Maureen Doellman,
who instilled in me a love of learning.

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The path to this point has been long and winding, and there are numerous people who deserve credit for guiding me in the right direction. Above all, I am indebted to my parents, John and Maureen, without whose love, support, encouragement, advice, and occasional necessary doses of reality, I would not have arrived here today. I also thank my brother Mark and grandparents, Norbert, Rita (*in memoriam*), Robert, and Mary Lou for their unwavering love and support. I am blessed to have all of you in my life.

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Introduction

Speciation has been a central theme of evolutionary biology since its inception (Darwin 1859; Mayr 1942; Dobzhansky 1951), yet the extent to which local adaptation and differentiation contribute to speciation is still debated (Schluter 2001; Coyne & Orr 2004; Rundle & Nosil 2005; Schluter 2009; Sobel et al. 2010). Allopatric speciation, requiring geographic isolation of diverging lineages, is most common, but rare examples of sympatric speciation continue to emerge (reviewed in Coyne & Orr 2004; Bolnick & Fitzpatrick 2007). The North Atlantic rough periwinkle *Littorina saxatilis* (Olivi) represents a marine model of sympatric speciation, where local-scale ecological adaptation has produced pairs of divergent ecotypes at multiple locations in the northeastern Atlantic (Janson 1983; Johannesson et al. 1995; Hull et al. 1996; Reid 1996; Johannesson 2001; Johannesson 2003; Rolan-Alvarez 2007). Yet, despite its importance as one of the few marine examples of incipient sympatric speciation (see also Munday et al. 2004) and the recognized necessity of lineage-wide phylogenetic context for model systems of sympatric speciation (e.g., Feder et al. 2003; Bolnick & Fitzpatrick 2007), little is known about the range-wide evolutionary history of *L. saxatilis*.

The evolutionary histories of North Atlantic benthic marine species have been shaped by the trans-Arctic interchange (TAI) and subsequent, repeated glaciations throughout the Pleistocene (e.g., Vermeij 1991; Wares & Cunningham 2001; Maggs et al. 2008). The opening of a trans-Arctic migration route 5.5 million years ago (Ma) and consequent TAI (5.5 to 2.4 Ma) introduced numerous Pacific species to novel selective regimes in the North Atlantic, resulting in concordant vicariant speciation between Pacific and Atlantic lineages, across myriad taxa (Vermeij 1991; Cunningham & Collins 1998; Gladenkov et al. 2002; Vermeij 2005). The majority of participating lineages settled first on eastern Atlantic shores, attaining amphi-Atlantic

distributions, later in the Pleistocene, via east to west migration (e.g., *Nucella lapillus*; Cunningham & Collins 1998; Wares & Cunningham 2001; Vermeij 2005; Colson & Hughes 2007). In a few instances, trans-Atlantic speciation occurred following the TAI, resulting in eastern and western Atlantic sister species (e.g., *Mytilus edulis* and *M. galloprovincialis*; Cunningham & Collins 1998; Riginos & Cunningham 2005). Ensuing glacial cycles drove continued genetic differentiation, via oscillations in sea level and habitat availability, which contracted populations into limited refugia during glacial maxima, and yielded repeated range fluctuations, population subdivision, and regional extinctions (Hewitt 1996; Cunningham & Collins 1998; Wares & Cunningham 2001; Hewitt 2004; Maggs et al. 2008).

Traditionally, the Pleistocene glaciations were thought to have driven condensation of species ranges into only southern glacial refugia during glacial maxima, with range expansion and recolonization northward during interglacial periods. Thus, current phylogeographic distributions were hypothesized to follow a pattern of “northern purity” and “southern richness,” with high levels of genetic diversity and presence of “private haplotypes” (i.e., haplotypes unique to a region) indicating historic southern glacial refugia, and low diversity with high frequencies of common haplotypes appearing in recolonized northern regions (Hewitt 1996; Hewitt 2004). Interestingly, this pattern is not common among North Atlantic marine organisms (Maggs et al. 2008).

As phylogeographic data have become more plentiful, northern regions of high genetic diversity are increasingly being identified. This pattern can arise from two, easily distinguishable, historical processes: the persistence of small northern periglacial refugia (Stewart & Lister 2001; Provan & Bennett 2008) or the admixture of genetic stocks expanding from two genetically divergent glacial refugia (Petit et al. 2003; Maggs et al. 2008). Northern

periglacial refugia are denoted by the existence of “private haplotypes,” but may be characterized by low genetic diversity, if the refugial population was sufficiently small (i.e., it underwent a bottleneck). Conversely, zones of secondary contact may exhibit high diversity, but this diversity is comprised of common haplotypes.

The Pleistocene glaciations molded the genetic architecture and distribution of North Atlantic marine species, and the refugial signatures discussed above have been explored in many species (reviewed in Maggs et al. 2008). The last glacial maximum (LGM), approximately 18 Ka, has received significant attention because phylogeographic data demonstrate that its effects differed markedly between populations on eastern and western Atlantic shores (Cunningham & Collins 1998; Maggs et al. 2008). The complex coastline of the northeastern Atlantic, combined with the tempering influence of the Gulf Stream, allowed for multiple larger glacial refugia, which buffered extinctions during glacial maxima (Coyer et al. 2003; Provan et al. 2005; Colson & Hughes 2007; Hoarau et al. 2007). Greater compression of isotherms in the northwestern Atlantic, a more linear coastline, and limited rocky habitat south of the ice sheet maximum resulted in less suitable refugial habitat (Wares & Cunningham 2001; Wares 2002). A growing body of phylogeographic studies supports regional variation in historical glacial processes, evidenced by a significantly greater number of putative glacial refugia identified on eastern Atlantic shores, as compared to the western Atlantic. In a recent review Maggs and colleagues (2008) demonstrated support for seven putative eastern Atlantic refugia (two of which, the Azores, Canary Islands and northwestern Africa and the Mediterranean Sea, are not relevant to this study), but only two western Atlantic refugia.

In the eastern Atlantic, the Iberian Peninsula was a common and relatively large southern refugium for marine organisms, including the green crab *Carcinus maenas* (Roman & Palumbi

2004), the thornback ray *Raja clavata* (Chevolet et al. 2006), the littorine snails *L. obtusata* and *L. fabalis* (Kemppainen et al. 2009), and the algal species *Fucus serratus* (Coyer et al. 2003; Hoarau et al. 2007) and *Palmeria palmata* (Provan et al. 2005). Studies of algal species *F. serratus* (Hoarau et al. 2007), *P. palmata* (Provan et al. 2005), and *Ascophyllum nodosum* (Olsen et al. 2010) have also provided ample evidence for a glacial refugium in the English Channel/Brittany region, possibly in the Hurd Deep depression. Putative periglacial refugia include southwestern Ireland, for *P. palmata* (Provan et al. 2005), *F. serratus* (Hoarau et al. 2007), the gobi *Pomatochistus microps* (Gysels et al. 2004), and the bryozoan *Celleporella hyalina* (Gomez et al. 2007), as well as northern Norway or another unidentified source of boreal/North Sea haplotypes for *C. maenas* (Roman & Palumbi 2004) and *A. nodosum* (Olsen et al. 2010). Iceland or the Faroe Islands represent potential periglacial glacial refugia for *C. maenas* (Roman & Palumbi 2004), the bivalve *Arctica islandica* (Dahlgren et al. 2001), and the isopod *Idotea balthica* (Wares 2001), though this claim has spurred much debate (see Ingolfsson 1992, 2009).

In the western Atlantic, regional extinctions were common among rocky shore species because most suitable substrate was covered by the Laurentide ice sheet, and much of the current fauna, including the intertidal snails *L. obtusata* and *N. lapillus*, recolonized the region following the LGM (Ingolfsson 1992; Cunningham & Collins 1998; Wares & Cunningham 2001; Colson & Hughes 2007; Maggs et al. 2008). A limited pool of species survived the LGM in a refugium south of the ice sheet maximum (Cape Cod/Long Island region; reviewed in Wares 2002): these include *M. edulis* and *Semibalanus balanoides* (the acorn barnacle; Wares & Cunningham 2001), as well as the hermit crab *Pagurus longicarpus* (Young et al. 2002). In addition, evidence for a cryptic northern periglacial refugium in Atlantic Canada is mounting among rocky intertidal

algal species (*Chondrus crispus*, *P. palmata* and *A. nodosum*; Chopin et al. 1996; Provan et al. 2005; Olsen et al. 2010), teleost fish (*Osmerus mordax* and *Gadus morhua*; Bernatchez 1997; Bigg et al. 2008), and crustaceans (*I. balthica* and *P. longicarpus*; Wares 2001; Young et al. 2002).

Amid the extensive climatic variation of the past 5.5 My, *Littorina* (subgenus *Neritrema*) snails underwent relatively high incidences of differentiation and speciation, giving rise to five North Atlantic species, including three rough periwinkles: egg-laying *L. compressa* and *L. arcana*, and the brooding species *L. saxatilis* (Reid 1990; Vermeij 1991; Reid 1996). The brooder *L. saxatilis* has the broadest range, spanning the North Atlantic from northern Africa to the mid-Atlantic coast of North America, while both egg-laying species have narrow distributions restricted to the northeastern Atlantic coasts of Europe (e.g., France, the British Isles, and Norway; Reid 1996). *L. saxatilis* is also notable for its wide range of morphologies (Reid 1996), especially in northeastern Atlantic regions (e.g., Sweden, Spain, and Britain), where it exhibits extreme local (i.e., on the scale of meters) habitat-specific morphological differentiation (Reid 1996; Johannesson 2001; Johannesson 2003; Rolan-Alvarez 2007). In each location, pairs of divergent ecotypes display distinct morphologies (Janson 1983; Johannesson et al. 1993; Hull et al. 1996), various levels of hybridization and reproductive isolation (Hull et al. 1996; Hollander et al. 2005; Rolan-Alvarez 2007), and partial reduction of gene flow (Wilding et al. 2001; Grahame et al. 2006; Panova et al. 2006; Galindo et al. 2009).

The Swedish model of incipient speciation in *L. saxatilis* is characterized by subtle but significant morphological differentiation between ecotypes inhabiting exposed (E) and sheltered (S) environments, along a linear coastline (Janson 1983). E-ecotypes display relatively thin shells and large apertures, morphological adaptations to resist dislodgement by waves. Conversely, the

presence of mobile cobbles and high densities of crabs have selected for relatively larger, thicker shelled, smaller apertured S-ecotypes. The transition zone between habitats and thus ecotypes is wide and continuous, with a high proportion of individuals of intermediate shell shape, indicating a high incidence of interbreeding the field (Janson & Sundberg 1983). However, laboratory studies indicate assortative mating according to both size and shape (i.e., ecotype; Hollander et al. 2005) and allozyme and microsatellite analyses suggest a resultant barrier to gene flow (Johannesson & Tatarenkov 1997; Panova et al. 2006). Additionally, microsatellite data provide limited support for multiple independent sympatric divergence events between ecotypes (Panova et al. 2006).

In Britain, there is again evidence that disruptive selective pressures drive ecotype differentiation in *L. saxatilis*. The large, robust, thick-shelled, small apertured M-ecotype is found in the mid-shore cobble/boulder habitat, where frequency of shell damage from crab predation and cobble movement is high. Only meters away, H-ecotype snails, with thinner shells, higher spires, and larger apertures are found on large boulders and cliffs (Hull et al. 1996). On British shores, the transition between H and M habitats is fairly abrupt, and intermediate forms are rare (~2% of the population; Hull et al. 1996). Assortative mating is common (Hull 1998; Pickles & Grahame 1999), and when inter-ecotype mating occurs, there is a significant increase (~16%) in spontaneous abortion of embryos (Hull et al. 1996), suggesting both pre- and post-zygotic isolating barriers. Indeed, AFLP data support reduced gene flow between ecotypes on the same shore, but are equivocal on the question of *in situ* sympatric divergence, versus allopatric divergence with secondary contact (Wilding et al. 2001; Grahame et al. 2006). The existence of post-zygotic isolating mechanisms (not detected in Swedish or Spanish systems), combined with consistency in loci under selection across sites (while neutral variation shows isolation by

distance), may support a scenario of allopatric divergence with recent (post-LGM) secondary contact between ecotypes (Grahame et al. 2006).

Morphological differentiation between ecotypes is most extreme on Spanish shores, where the ribbed-banded (RB) ecotype inhabits the upper barnacle zone and the smooth-unbanded (SU) ecotype lives among *M. galloprovincialis* mussels in the wave-exposed low shore environment (Johannesson et al. 1993). The presence of a high intertidal crab, *Pachygrapsus marmoratus*, in this region drives the selection for a robust, thick-shelled, low-spired ecotype (RB) in the high intertidal, while the much smaller SU-ecotype has a large aperture, accommodating a larger foot for resistance to dislodgement by waves in the low intertidal zone. These habitats and their associated ecotypes overlap across several meters in the mid-intertidal zone, but microhabitat selection (Johannesson et al. 2000) and size-based assortative mating (Rolan-Alvarez et al. 1999) limit intermediates to 11-29% of individuals in this transition zone (Johannesson et al. 1993). Allozyme (Johannesson et al. 1993), mtDNA (Quesada et al. 2007) and microsatellite data (Galindo et al. 2009) demonstrate genetic structure based on geographic distance, not morphological variation. In addition, unlike the British system, AFLP analysis showed different loci under selection at each site, providing evidence against a single allopatric origin of ecotype differentiation (Galindo et al. 2009). Thus, the Spanish *L. saxatilis* model system likely represents multiple parallel divergence events.

Evidence of multiple incipient speciation events within regions, combined with the occurrence of similar ecological divergence across large spatial scales in the northeastern Atlantic, have established *L. saxatilis* as classic model of marine incipient sympatric speciation (Johannesson 2001; Johannesson 2003; Rolan-Alvarez 2007; see also Butlin et al. 2008). Despite these extensive localized studies of rough periwinkles in the eastern Atlantic, western Atlantic

populations remain largely unexamined, and no comprehensive evaluation of range-wide genetic variation within *L. saxatilis* exists. Here I use mtDNA sequence data and Bayesian phylogenetic analysis to reconstruct the evolutionary relationships among *L. compressa*, *L. arcana*, and a range-wide sampling of *L. saxatilis*. Using an mtDNA gene tree, I ask whether these species are genetically distinct (i.e., reciprocally monophyletic), date their origins, and define the phylogeographic structure of *L. saxatilis* populations across the North Atlantic.

Materials and Methods

Sampling and molecular analysis

I sampled 405 individuals from 32 sites (Table 1; Figure 3) across the range of *L. saxatilis* (western, central, and eastern North Atlantic). Additionally, I obtained 33 *L. arcana* and 20 *L. compressa* from the northeastern Atlantic. To confirm specimen identity in areas of sympatry, I used only females with a clearly defined brood pouch (*L. saxatilis*) or jelly gland (*L. arcana* and *L. compressa*). *L. compressa* was further distinguished by distinctive shell grooves (Reid 1996). Snails were either dissected live, frozen at -80°C, or preserved in 100% ethanol prior to extraction. Whole genomic DNA was extracted from head/foot tissue using CTAB (hexadecyltrimethylammonium bromide) extraction buffer and a standard phenol/chloroform extraction protocol (Winnepeninckx et al. 1993; Davis et al. 1994). S. Vollmer designed two new primers [LsaxMt_F, 5'-CTG ATG CCG CAA AAC TTC TT-3' and LsaxMt_R, 5'-GTC AAC TGC AAA GCC TCC TC-3'] from published sequences (Wilding et al. 1999, Quesada et al. 2007), and I amplified a 1832 bp region of the mitochondrial genome, including *NADH1*, *tRNA^{pro}*, *NADH6* and partial cytochrome *b*. PCR amplifications were obtained using Phire™ Hot Start DNA Polymerase (Finnzymes) and a thermal profile of 30 sec at 98.0°C, followed by

30 cycles of 98.0°C for 5 sec, 50.0°C for 5 sec, and 72.0°C for 1 min and 15 sec. PCR products were cleaned and sequenced in both directions using the PCR primers on ABI 3700 capillary sequencers by Agencourt Bioscience (Beverly, MA). A 633 bp sequence was obtained for *NADH1* using the forward primer. From the reverse primer, 521 bp were obtained, including 61 bp of *NADH6* and 451 bp of cytochrome *b*. I also included the 48 published sequences of Quesada and colleagues (2007;EMBL-Bank accessions AM500945-AM500965) for a total of 453 *L. saxatilis* samples (Table 1).

DNA sequence analyses

I aligned and manually edited sequences using Sequencher 4.8 ® (Gene Codes Corporation). DNA sequence variation was analyzed using DnaSP v 5.10 (Librado & Rozas 2009) by species, geographic region (Iberian Peninsula, eastern, central, and western Atlantic), and population. Calculations for lineage-specific groupings were made based on the results of the phylogenetic analyses described below. My estimates also include standard tests for deviations from neutrality, including Tajima's D, Fu and Li's D* and Fu and Li's F* (Tajima 1989; Fu & Li 1993). A maximum parsimony network was constructed with TCS 1.21 (Clement et al. 2000).

Phylogenetic analyses and divergence estimates

Phylogenetic analyses were conducted using a Bayesian approach in BEAST v. 1.5.3 (Drummond & Rambaut 2007). The best-fit model of sequence evolution was estimated as GTR+G+I using AIC criterion generated in MrModeltest 2.3 (Nylander 2004). This model was implemented in BEAST under strict clock and relaxed uncorrelated lognormal frameworks (Drummond et al. 2006), using the assumption of a constant coalescent population size parameter. Bayes Factor tests were used to evaluate differences in outcome under alternative demographic models and revealed that my data fit the molecular clock assumption (Suchard et

al. 2001). By including representative sequences from *L. fabalis* and *L. obtusata* (Quesada et al. 2007; EMBL-Bank accessions AM500966-AM500967) as outgroups and applying a prior probability distribution to the root of this tree, I was able to estimate the times of divergence [i.e., the time to most recent common ancestor (TMRCA)] for lineages of interest. The analyses were calibrated with a combination of fossil evidence and published times of divergence among members of the Atlantic *Neritrema*. With a lognormal prior, I fixed the minimum time of divergence at 2 Ma, as this is the earliest known occurrence of distinct flat (*L. fabalis*) and rough periwinkles (*L. islandica*, the extinct proposed ancestor of the *L. saxatilis* group) in the fossil record (Reid 1996). I used the previously published mean estimate for divergence among all Atlantic *Neritrema*, 2.83 Ma (Reid et al. 1996), as the mean root age and adjusted the standard deviation so that the upper bound of the 95% high probability density (HPD) approximated the opening of the Bering Strait (~5.5 Ma). I also examined lineages for signatures of population size fluctuations using the GRMF Skyride option in BEAST (Minin et al. 2008). Applying a normally distributed clock rate prior, equivalent to that estimated for the full tree, these analyses provided more conservative estimates of TMRCA than the constant population size models. All analyses were run with chain lengths of 30 million, sampling every 3000, and model performance was assessed in Tracer v. 1.5 (Rambaut & Drummond 2009). I constructed maximum clade credibility phylogenies in TreeAnnotator v. 1.5.3 (Drummond & Rambaut 2007).

Results

Genetic diversity

Analysis of the mtDNA fragment produced an alignment of 1154 bp with 125 variable sites and 113 unique haplotypes. Across the North Atlantic, *L. saxatilis* (n = 453 individuals)

haplotype diversity is high (mean \pm SE = 0.94 ± 0.01), with 95 unique haplotypes (Table 2). Populations on western (n = 197) and eastern (n = 107) Atlantic shores have equal haplotype diversities (both 0.85 ± 0.02), although a greater number of unique haplotypes were recovered from the western Atlantic (h = 45 vs. 25). The Iberian Peninsula (n = 52) shows the highest regional haplotype diversity (0.89 ± 0.02), with 14 unique haplotypes. Haplotype diversity in the central Atlantic (n = 97) is lowest, at 0.79 ± 0.04 , (h = 18). Across the eastern Atlantic, *L. arcana* (n = 33, h = 12) and *L. compressa* (n = 20, h = 9) show comparable haplotype diversities of 0.73 ± 0.08 and 0.79 ± 0.09 , respectively.

Gene tree and divergence estimates

TMRCAs estimates place the origin of the North Atlantic *Neritrema* at 2.52 Ma (95% HPD 2.01 - 3.50, Table 3). The three rough periwinkles - *L. compressa*, *L. arcana*, and *L. saxatilis* - form a well-supported monophyletic clade (Figure 1), with a TMRCAs of 0.92 Ma (0.51-1.44). None of the three species is strictly monophyletic, rather each is made up of multiple divergent mtDNA lineages, most of which are shared among species. The majority of *L. compressa* (including one *L. arcana* individual) form a basal lineage (diverged \sim 0.92 Ma). All *L. saxatilis*, the majority of *L. arcana*, and the remaining *L. compressa* are supported as a single clade with an age of 0.64 Ma (0.35 –1.00). This clade comprises four well-supported lineages: *L. saxatilis* falls into two distinct and strongly supported lineages, I and II, which diverged near the origin of the clade (\sim 0.64 Ma). Lineage I also includes *L. compressa*, whereas Lineage II contains both *L. arcana* and *L. compressa*. Two additional lineages, one composed of *L. arcana* only and the other including *L. compressa* and *L. arcana*, also fall within this clade. They diverged near the same time as Lineages I and II (\sim 0.64 Ma); however, their phylogenetic

relationships to Lineages I and II remain unresolved. All three species share one identical Lineage II haplotype (F), while a second is shared between *L. saxatilis* and *L. arcana* (Figure 2).

Lineage II, with a TMRCA of 0.45 Ma (0.23 – 0.73, Table 3), is older and more diverse (Figure 2) than Lineage I, with a TMRCA of 0.32 Ma (0.15 – 0.53). Within these lineages there is further geographic subdivision: western Atlantic haplotypes (haplogroups A and J) fall into well-supported clades within Lineages I and II, respectively, as do Iberian Peninsula haplotypes (haplogroups D and H). Remaining haplotypes are shared broadly across central and northeastern Atlantic populations.

Phylogeography

The ranges of both *L. saxatilis* lineages (I and II) overlap broadly across the North Atlantic, but geographic subdivision is evident and patterns of overlap differ among regions (eastern, central, and western) and between basal and derived haplotypes (Figure 2; Figure 3). Basal haplotypes of both Lineages I (haplogroup B) and II (haplogroup F) are present across the northeastern and central Atlantic, in Britain, the Faroe Islands, and Iceland. Lineage I basal haplotypes (haplogroup B) are also found in France and Greenland, whereas Ireland and Sweden contain basal Lineage II haplotypes (haplogroup F).

Derived Lineage I and II haplotypes are also present across the central and northeastern Atlantic. Haplogroup B (Lineage I) has a northern distribution, across Ireland, the Faroe Islands, Iceland, and Greenland, whereas haplogroup E (Lineage I) is found primarily on North Sea shores (France and Sweden), but also in Ireland, Iceland, and Greenland. Lineage II derived haplogroup I is spread among France, Ireland, and the Faroe Islands, whereas the divergent haplotype G is localized to southwestern Britain.

All four haplotypes recovered from Greenland are also common across the northeastern (main haplotypes C, B, E) or northwestern (J) Atlantic (i.e., there are no private haplotypes). The same three northeastern Atlantic haplotypes (C, B, E) are also found in Iceland, along with two private haplotypes (in haplogroups C and F).

At the southeastern edge of our sampling range, the Iberian Peninsula contains derived haplotypes from both Lineages I (haplogroup D) and II (haplogroup H). The diversity within H is much deeper than other haplogroups, with a TMRCA of 0.28 Ma (0.13 – 0.48; Table 3). Unlike the rest of the eastern Atlantic, thirteen out of fourteen Iberian Peninsula haplotypes are exclusive to this region (i.e., private haplotypes); the fourteenth Iberian haplotype (D) was also recovered from the Faroe Islands. The ranges of Lineages I and II do not overlap on the Iberian Peninsula: Lineage I (haplogroup D) is found on Spain's Biscay Coast, while Lineage II (haplogroup H) is restricted to the Atlantic coast.

The western Atlantic is populated with derived haplotypes from both Lineages I and II, and all haplotypes but one (J, also present in Greenland) are unique to this region. The range of Lineage I (haplogroup A), extends from south of Cape Cod throughout the southern Gulf of Maine (GOM), whereas Lineage II (haplogroup J) spans the southern and northern GOM. Although their ranges overlap throughout the southern GOM (Figure 3 inset), haplogroups A and J are more closely related to central and eastern Atlantic haplotypes than they are to each other (Figure 2). Western Atlantic haplotypes from Lineage I (haplogroup A), which are found predominantly in southern populations in the western Atlantic, show greater diversity than those from Lineage II (haplogroup J).

Skyride analysis

GMRF Skyride analyses show no extreme fluctuations in population size over time and Bayes Factor comparisons reveal that demographic histories do not differ significantly from a constant population size model. We present the data for the western Atlantic only (Figure 4) because inferred past population dynamics for Lineages I and II show similar patterns across the North Atlantic. Lineage I (haplogroup A) shows a consistently higher estimate of effective population size over time and TMRCA estimates are slightly older, using both the constant population size model (0.19 Ma, 0.07 – 0.35) and the more conservative Skyride model (0.06 Ma, 0.03 – 0.10). Lineage II (haplogroup J) has younger, though overlapping, estimates of TMRCA (0.14 Ma, 0.05 – 0.26, and 0.03 Ma, 0.01 – 0.06, respectively).

Discussion

Gene tree

The mtDNA gene tree revealed surprising diversity and a complex evolutionary history for the three North Atlantic rough periwinkles - *L. compressa*, *L. arcana*, and *L. saxatilis*. Cryptic genetic diversity is present in each species, with *L. compressa* and *L. arcana* (the two egg-laying species) each found in four lineages, while *L. saxatilis* (the brooder) is present in two. These results uphold basic relationships within the accepted *Littorina* phylogeny (Reid 1996), although speciation events appear to be more recent than previously estimated (Reid et al. 1996). The most basal lineage, predominantly composed of *L. compressa* and exclusive to egg-laying species, diverged ~ 0.92 Ma, supporting egg-laying as an ancestral characteristic (Reid 1996). Wilding et al. (2000a) reported that this oldest/basal lineage was found only in *L. compressa*; however, my data show that *L. arcana* also possesses this basal lineage. Divergence of this early

egg-laying lineage coincides with the middle Pleistocene transition (1.25 – 0.70 Ma), a period of intensification in glacial activity (Clark et al. 2006), as well as with the estimated time of divergence between flat periwinkles *L. obtusata* and *L. fabalis* (Tatarenkov 1995).

Extensive lineage sharing among the three species has been previously documented (Small & Gosling 2000; Wilding et al. 2000a, b) and is expected in relatively young species groups (Avice 2000). Although *L. saxatilis* employs a brooding life history strategy unique among *Littorina* (Reid 1996), it shares two identical haplotypes with *L. arcana* and one with *L. compressa*. Because *L. saxatilis* and *L. arcana* have been shown to hybridize (though *L. compressa* does not, Warwick et al. 1990; Mikhailova et al. 2009), it is possible that haplotype sharing is due to current or recent introgressive hybridization. However, sharing due to ancestral variation is more likely (see also Wilding et al. 2000a,b) because (1) the shared haplotype (F) is basal, (2) no shared haplotypes are observed at sites where the species were sampled together (Table 1), and (3) a more complete complement of possible haplotype diversity is not shared across the sympatric ranges of the three species (unlike *L. obtusata* and *L. fabalis*; Kemppainen et al. 2009).

My data indicate that *L. saxatilis* and its brooding life history strategy arose around 0.64 Ma. At approximately the same time, *L. saxatilis* diverged into two distinct lineages that are also about the same age as two of the three exclusively egg-laying lineages (Figure 1). Because the ranges of both egg-laying (i.e., ancestral) rough periwinkles (*L. arcana* and *L. compressa*) are limited to the northeastern Atlantic (Reid 1996) and the lineage split within *L. saxatilis* occurred near the time of species origin, it seems most likely that both lineages arose in the northeastern Atlantic. In addition, the basal diversity in *L. saxatilis* in both Lineages I (haplogroup B) and II (haplogroup F), some of which is also shared with the egg-laying species, is centered in this

region around the British Isles (Figure 2; Figure 3). Phylogeographic patterns suggest that, following divergence, both *L. saxatilis* lineages radiated outward, reaching their current ampho-Atlantic distributions. Both nucleotide diversity and TMRCA indicate that range expansion may have occurred approximately 100 Ky earlier for Lineage II (~ 0.45 Ma) than Lineage I (~ 0.32 Ma).

Phylogeography

Phylogeographic differences in both distributions of basal and derived haplotypes and patterns of lineage overlap across the range of surveyed *L. saxatilis* populations (Iberian Peninsula, northeastern, central, and western Atlantic) likely result from regional variation in historical glacial processes. The Iberian Peninsula displays unique genetic patterns, including independent ranges of Lineages I and II, a high frequency of private haplotypes, relatively high haplotype diversity and population subdivision with isolation by distance (see Quesada et al. 2007), which indicate relative population isolation and stability (Lessa et al. 2003). Contrary to previous hypotheses (Quesada et al. 2007), my data indicate that the Iberian Peninsula was colonized (from the northeastern Atlantic) at least twice, once by each lineage (I & II). Bayesian estimates suggest that most *L. saxatilis* populations on the Iberian Peninsula have been isolated from remaining *L. saxatilis* since Lineage II colonized the Atlantic coast of the Iberian Peninsula approximately 0.28 Ma. For other taxa, the Bay of Biscay has also served as an isolating barrier (e.g., *L. obtusata* and *L. fabalis*, Kemppainen et al. 2009; *Ascophyllum nodosum*, Olsen et al. 2010) and prevented the recolonization of the northeastern Atlantic by Iberian Peninsula genetic stocks during interglacial periods (e.g., *Fucus serratus*, Hoarau et al. 2007).

In the northeastern Atlantic, from the Brittany Peninsula to the Faroe Islands, the phylogeographic pattern is the most complex and thus difficult to interpret. It has been

hypothesized that the geographic complexity of this region's shoreline and repeated glacial disturbance due to ice cover and sea-level change resulted in many glacial refugia, including the English Channel/ Hurd Deep, southwestern Ireland, the Faroe Islands, and northern Norway (reviewed in Maggs et al. 2008). The broad distributions of Lineage I and II basal haplogroups (B and F, respectively) suggest survival in multiple glacial refugia throughout the Pleistocene. Lineage I haplogroup C, which is distributed across the Faroe Islands, Iceland, Greenland, and southwestern Ireland, likely diverged in a more northern refugium, perhaps the Faroe Islands, but may also have survived in southwestern Ireland. Lineage II haplogroup I shows a similar pattern whereas lineage II haplogroup E may have recolonized the North Sea from a different northern refugium (e.g., northern Norway or a deep trench south of Norway, see Maggs et al. 2008). Localization of haplotype G to southwestern Britain supports a marine refugium in the Hurd Deep, during the LGM (Provan et al. 2005; Olsen et al. 2010), while the extent of native diversity in the Faroe Islands (nine private haplotypes) provides evidence that this area did indeed serve as a marine refugium during the LGM (Dahlgren et al. 2000; Wares 2001; Roman & Palumbi 2004; but see Ingolfsson 1992, 2009).

My data suggest that the two central Atlantic (Iceland and Greenland) populations of *L. saxatilis* have experienced the highest levels of glacial disturbance, likely including local extinction. Similar to many marine species (Ingolfsson 1992; Wares & Cunningham 2001), low haplotype diversity in this region indicates that *L. saxatilis* recolonized recently, likely following the LGM, and phylogeographic patterns show that recolonization proceeded predominantly from the northeastern Atlantic. A subset of the variation found in the Faroe Islands, Ireland, and the North Sea (haplotypes C, B, E), were found in both Iceland and Greenland. The presence of two private haplotypes in Iceland, while Greenland lacked private haplotypes, indicates that

recolonization likely occurred via east to west stepping-stone migration. Interestingly, Greenland also appears to have been recolonized from northwestern Atlantic populations (haplotype J).

For the western Atlantic, my data suggest three key findings: (1) this region was colonized by *L. saxatilis* in two separate trans-Atlantic migration events, (2) contrary to previous hypotheses (Reid 1996; Blakeslee & Byers 2008), western Atlantic *L. saxatilis* survived the LGM and (3) Lineages I and II survived in separate southern and northern glacial refugia, respectively. Lack of trans-Atlantic haplotype sharing in either lineage demonstrates that neither Lineage I nor II colonized the western Atlantic following the LGM (Wares & Cunningham 2001). TMRCA and haplotype diversity indicate that Lineage I (haplogroup A) arrived on western Atlantic shores prior to Lineage II (haplogroup J), possibly as long as 0.35 Ma, but likely 0.19 Ma during the second-to-last interglacial period. TMRCA indicates that lineage II (haplogroup J) may have arrived during the last interglacial. However, as haplogroup J is more distantly related to the rest of Lineage II than haplogroup A is to Lineage I, haplogroup J may have been isolated from eastern Atlantic Lineage II earlier than estimated. Haplogroup A appears to have survived the LGM south of the Laurentide ice sheet (e.g., Wares & Cunningham 2001; Wares 2002), and Skyride analysis indicates that effective population size remained relatively high throughout the LGM (Figure 4). Haplogroup J likely survived in a northern location, providing additional evidence for a periglacial refugium in Atlantic Canada (Chopin et al. 1996; Wares 2002; Provan et al. 2005; Bigg et al. 2008; Maggs et al. 2008; Olsen et al. 2010). Since the termination of the LGM (~ 18 Ka) both populations appear to have expanded, and now share a zone of secondary contact in the southern GOM (see Maggs et al. 2008). It is likely that the brooding life-history strategy (Johannesson 1988) and flexible habitat requirements (Reid 1996) allowed *L. saxatilis* to survive drastic climatic change and rocky habitat limitation associated

with the LGM, whereas many rocky intertidal organisms suffered local extinction (e.g., *L. obtusata* and *Nucella lapillus*; Cunningham & Collins 1998; Wares & Cunningham 2001; Colson & Hughes 2007). Lack of extreme population fluctuations in either lineage over this time period (Figure 4) indicates that the LGM did not severely impact genetic diversity, but instead drove the distribution of genetic variation (e.g., Olsen et al. 2010) in western Atlantic *L. saxatilis*.

Implications for the speciation model

The strength of the *L. saxatilis* sympatric speciation model lies in the evidence for multiple independent origins of divergence within and across regions (Johannesson 2001); but until now, the species-wide phylogeny required to demonstrate this (Johannesson 2003) was lacking. In light of the demonstrated cryptic mtDNA diversity and role of allopatric genetic divergence (e.g., Pleistocene glaciations) within *L. saxatilis* lineages, Spanish, British, and Swedish speciation models must be carefully re-examined. On Spanish shores (Iberian Peninsula), similar sympatric divergence is hypothesized in both Lineages I and II, which have independent ranges (Biscay and Atlantic coasts, respectively) and little gene flow between them (Quesada et al. 2007). Given the long-term stability and population differentiation on the Iberian Peninsula, the likelihood of divergence in allopatry due to glacial disturbances is low. Thus, my data suggest at least two independent sympatric divergence events, in lineages separated by ~0.64 My of mtDNA divergence, and support the Spanish *L. saxatilis* model of sympatric speciation (see also Rolan-Alvarez et al. 2004; Quesada et al. 2007; Rolan-Alvarez 2007; Galindo et al. 2009).

Sympatric divergence is less certain in Swedish and British model systems (see also Grahame et al. 2006). Lower sea-level and ice cover rendered the North Sea uninhabitable for marine organisms during the LGM, and the shores of Sweden and eastern Britain were

recolonized less than 10 Ka (Panova et al. 2006; Hoarau et al. 2007). Thus, I cannot rule out the possibility that allopatric morphological divergence between Lineages I and II took place within separate glacial refugia, and recolonization led to secondary contact in Sweden (where we found both I and II) and Britain (where Lineage I may have been missed in our samples). Indeed, the degree of intrinsic post-zygotic reproductive isolation observed between ecotypes in Britain (Hull et al. 1996) is greater than expected for divergence of only 10 Ky. If morphological divergence arose with lineage divergence in allopatry, it would be difficult to demonstrate. One would not expect a one-to-one correspondence to remain between mtDNA lineage and ecotype because there is evidence of on-going, though restricted, nuclear gene flow between *L. saxatilis* ecotypes (Wilding et al. 2001; Grahame et al. 2006; Panova et al. 2006) and mtDNA is known to cross species boundaries readily (reviewed in Chan & Levin 2005).

Future work should examine the link between mtDNA lineage and nuclear gene exchange (i.e., AFLP, SNP, or microsatellites) to elucidate the importance of historic allopatric divergence across the range of this model organism. Britain, Sweden, and the region between Spanish Atlantic and Biscay coasts will be of particular interest in defining the relative roles of and/or interaction between sympatric and allopatric processes. In addition, a focus on western Atlantic *L. saxatilis* is needed. Significant spatial and temporal separation of Lineages I and II, with recent (< 20 Ka) secondary contact in the southern GOM, make this the ideal region for detailed examination of interactions between lineages. Identification of reduced nuclear gene flow or reproductive isolation between mtDNA lineages in this region could lead to redefinition of the species *L. saxatilis*.

My work highlights the critical importance of placing focused model systems into a broad phylogeographic perspective. Studies of phylogeography are increasingly identifying

cryptic species (e.g., Dawson et al. 2005; Jolly et al. 2005; Gomez et al. 2007; Griffiths et al. 2010), which requires revised views of species and speciation in marine environments. Given the substantial emphasis on *L. saxatilis* as a model system for sympatric speciation, more comprehensive studies are sorely needed. I have demonstrated that the genetic background of *L. saxatilis* is much more complex than previously understood. More importantly, I have shown that speciation may be ongoing at more than one scale in the *L. saxatilis* system.

Table 1. Sample collection information, including site, coordinates, sample size (n) and number of haplotypes (h).

Species	Collection site	Coordinates	n	h
<i>L. saxatilis</i>				
Western Atlantic				
	Greenport, New York	41° 06' N, 72° 21' W	15	2
	Jamestown, Rhode Island	41° 26' N, 71° 23' W	15	4
	Nahant, Massachusetts	42° 25' N, 70° 54' W	40	11
	Wells, Maine	43° 20' N, 70° 32' W	15	8
	Pemaquid, Maine	43° 50' N, 69° 30' W	20	11
	Chamberlain, Maine	43° 53' N, 69° 28' W	21	7
	South Lubec, Maine	44° 47' N, 67° 00' W	29	4
	Quoddy Head, Maine	44° 49' N, 66° 56' W	15	3
	Dipper Harbor, New Brunswick	45° 05' N, 66° 25' W	27	7
Central Atlantic				
	Kulusuk, Greenland	65° 34' N, 37° 10' W	13	4
	Akureyri, Iceland	65° 48' N, 18° 03' W	6	2
	Hafnir, Iceland	63° 55' N, 22° 41' W	13	3
	Vik, Iceland	63° 24' N, 19° 01' W	8	3
	Bour, Faroe Islands	62° 05' N, 07° 23' W	10	3
	Sandavagur, Faroe Islands	62° 02' N, 07° 08' W	18	5
	Suduroy, Faroe Islands	61° 33' N, 06° 47' W	7	3
	Arnafjordur, Faroe Islands	62° 15' N, 06° 31' W	10	3
	Vidareidi, Faroe Islands	62° 21' N, 06° 32' W	12	5
Eastern Atlantic				
	Tjarno, Sweden	58° 52' N, 11° 06' W	8	3
	Aran Islands, Ireland	53° 06' N, 09° 40' W	7	3
	Galway Bay, Ireland	53° 12' N, 09° 17' W	15	8
	Nynian's Cave, Scotland	54° 36' N, 04° 52' W	9	3
	Thornwick Bay, England	54° 08' N, 00° 07' W	9	2
	Lizard Point, England	49° 57' N, 05° 11' W	4	2
	Golden Cap, England	50° 21' N, 04° 21' W	7	1
	Portland Bill, England	50° 30' N, 02° 27' W	2	2
	Mumbles, Wales	51° 47' N, 05° 06' W	10	6
	West Dale Bay, Wales	51° 33' N, 04° 18' W	15	5
	Cap Griz-Nez, France	50° 51' N, 01° 34' W	8	3
	Cote de Granit Rose, France	48° 50' N, 03° 28' W	3	1
	Point St Mathieu, France	48° 19' N, 04° 46' W	7	3

Species	Collection site	Coordinates	n	h
<i>L. saxatilis</i>				
	Iberian Peninsula			
	Areolonga, Spain	43° 43' N, 07° 29' W	12	3
	Roncudo, Spain	43° 18' N, 08° 46' W	12	4
	Ons, Spain	42° 20' N, 08° 55' W	12	4
	Cies, Spain	42° 12' N, 08° 53' W	12	5
	Vigo, Spain	42° 05' N, 08° 53' W	4	1
<i>L. arcana</i>				
	Thornwick Bay, England	54° 08' N, 00° 07' W	5	2
	Lizard Point, England	49° 57' N, 05° 11' W	3	2
	Great Castle Head, Wales	51° 47' N, 05° 06' W	5	2
	West Dale Bay, Wales	51° 33' N, 04° 18' W	7	4
	Cote de Granit Rose, France	48° 50' N, 03° 28' W	7	4
	Point St Mathieu, France	48° 19' N, 04° 46' W	6	2
<i>L. compressa</i>				
	Aran Islands, Ireland	53° 06' N, 09° 40' W	9	1
	Lizard Point, England	49° 57' N, 05° 11' W	4	2
	Black Rock, Wales	51° 33' N, 04° 18' W	5	4
	Point St Mathieu, France	48° 19' N, 04° 46' W	2	2

Table 2. Summary statistics for a 1154bp segment of mtDNA in North Atlantic rough periwinkles, including sample size (n) and number of mtDNA haplotypes (h). Haplotype diversity and nucleotide diversity are listed as $H \pm SD$ and $\pi \pm SD$, respectively. For tests of departure from neutral expectations, values in bold indicate significance at $p < 0.05$.

Species	Region/Clade	n	h	Haplotype diversity (H)	Nucleotide diversity (π)	Theta (Θ)	Tajima D	Fu & Li D*	Fu & Li F*
<i>L. saxatilis</i>		453	95	0.94 \pm 0.01	0.0054 \pm 0.0001	15.09	-1.71	-6.62	-4.96
	Lineage I	258	56	0.91 \pm 0.01	0.0022 \pm 0.0001	9.79	-2.17	-7.11	-5.84
	Lineage II	195	39	0.86 \pm 0.02	0.0045 \pm 0.0002	8.89	-1.26	-3.16	-2.80
	Western Atlantic	197	45	0.85 \pm 0.02	0.0045 \pm 0.0001	8.54	-1.16	-5.53	-4.35
	CC south	30	6	0.64 \pm 0.06	0.0007 \pm 0.0001	1.51	-1.26	-2.66	-2.61
	GOM south	96	30	0.87 \pm 0.02	0.0037 \pm 0.0003	7.40	-1.34	-3.98	-3.52
	GOM north	71	12	0.52 \pm 0.07	0.0006 \pm 0.0001	2.28	-1.97	-3.53	-3.55
	Central Atlantic	97	18	0.79 \pm 0.04	0.0031 \pm 0.0004	5.44	-1.02	0.17	-0.35
	Greenland	13	4	0.53 \pm 0.15	0.0026 \pm 0.0010	3.87	-0.86	0.07	-0.20
	Iceland	27	5	0.76 \pm 0.05	0.0025 \pm 0.0005	3.11	-0.21	1.47	1.12
	Faroe Islands	57	14	0.83 \pm 0.04	0.0034 \pm 0.0005	4.77	-0.58	-0.05	-0.29
	Eastern Atlantic	107	25	0.85 \pm 0.02	0.0045 \pm 0.0002	5.20	-0.61	-2.60	-2.17
	Sweden	8	3	0.71 \pm 0.12	0.0043 \pm 0.0007	3.47	2.16	1.49	1.82
	Ireland	22	9	0.84 \pm 0.06	0.0051 \pm 0.0005	5.49	0.23	-0.35	-0.20
	Britain	59	14	0.76 \pm 0.04	0.0038 \pm 0.0002	4.52	-0.07	-2.02	-1.58
	France	18	6	0.76 \pm 0.07	0.0030 \pm 0.0007	4.52	-0.99	-1.88	-1.88
	Iberian Peninsula	52	14	0.89 \pm 0.02	0.0057 \pm 0.0005	6.42	0.05	-0.97	-0.72
	Biscay coast	12	3	0.32 \pm 0.16	0.0006 \pm 0.0004	1.32	-1.75	-2.11	-2.29
	Atlantic coast	40	11	0.86 \pm 0.03	0.0036 \pm 0.0004	4.23	-0.08	-0.10	-0.82
<i>L. arcana</i>		33	12	0.73 \pm 0.08	0.0050 \pm 0.0008	7.39	-0.80	-1.23	-1.29
<i>L. compressa</i>		20	9	0.79 \pm 0.09	0.0076 \pm 0.0012	8.17	0.27	0.69	0.66

Table 3. Estimated node ages and time to most recent common ancestor (TMRCA) in millions of years (mean, median, 95% high probability density).

	TMRCA (Ma)			Node age (Ma)		
	Mean	Median	95% HPD	Mean	Median	95% HPD
<i>Neritrema</i>	2.52	2.36	2.01-3.50	2.51	2.35	2.02-3.44
Rough periwinkles	0.92	0.87	0.51-1.44	0.92	0.87	0.50-1.41
<i>L. compressa</i>	0.92	0.87	0.50-1.44	0.92	0.87	0.50-1.41
<i>L. arcana</i>	0.92	0.87	0.50-1.44	0.92	0.87	0.50-1.41
<i>L. saxatilis</i>	0.62	0.59	0.32-0.98	0.64	0.61	0.35-1.00
Lineage I	0.32	0.30	0.15-0.53	0.32	0.30	0.15-0.53
Lineage II	0.45	0.43	0.23-0.73	0.45	0.43	0.23-0.71
Lineage I, western	0.19	0.17	0.07-0.35	0.17	0.15	0.07-0.28
Lineage II western	0.14	0.13	0.05-0.26	0.15	0.13	0.05-0.27

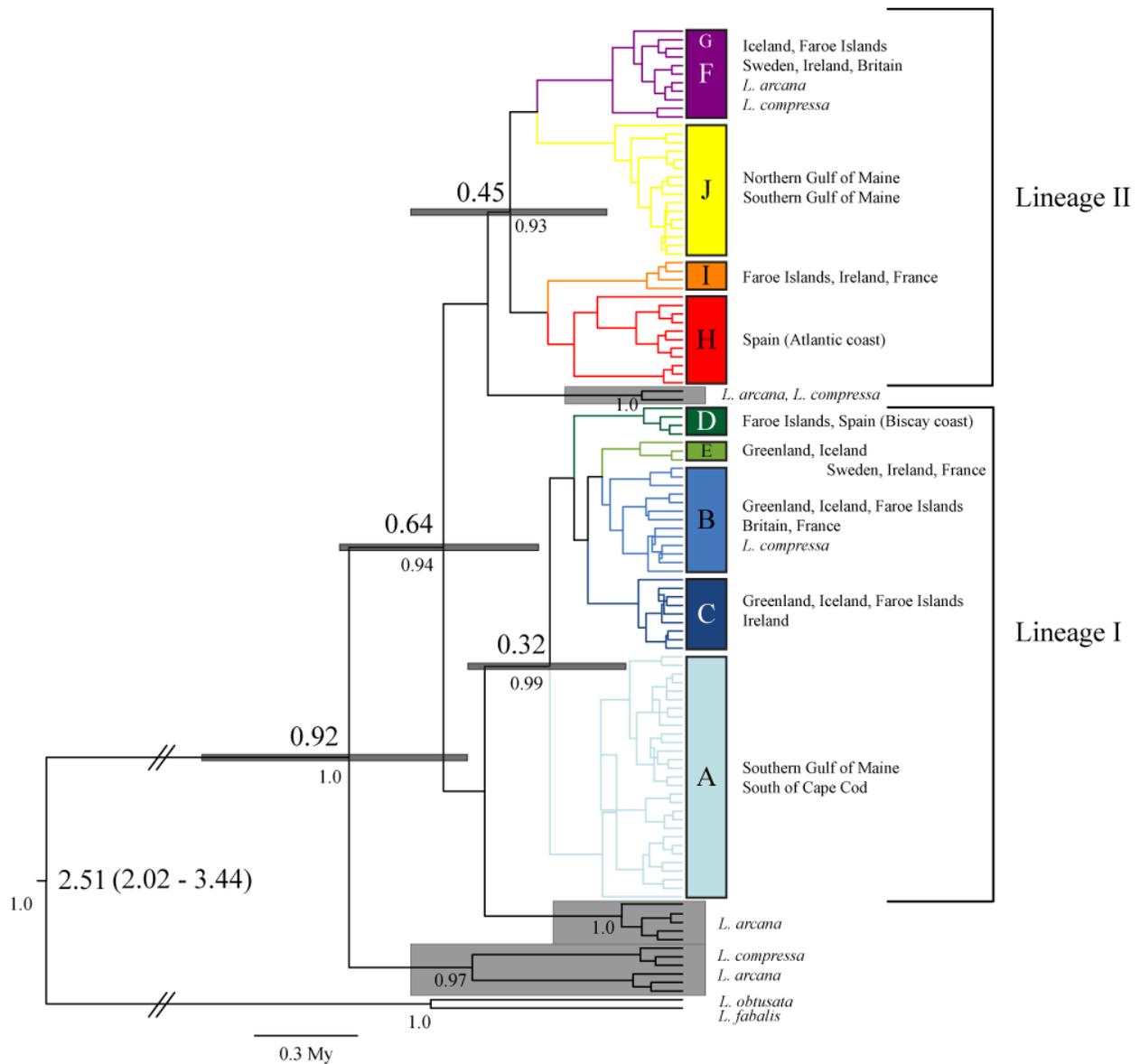


Figure 1. Bayesian phylogeny of rough periwinkles based on mtDNA. A maximum clade credibility tree was obtained from BEAST analysis. Values above and below each node indicate mean node age and posterior probability, respectively. Gray error bars indicate 95% high probability densities for node ages. Posterior probabilities less than 80% are not shown. All colored clades are supported with posterior probabilities greater than 80%, except for B and C, whose positions within Lineage I are not resolved. Colors correspond to Figure 2. Regions containing each clade are listed to the right of the figure. Gray boxes surround clades not containing *L. saxatilis*. For simplicity, this tree was created using unique haplotypes only; however, values included refer to analyses using the full data set (topologies were identical).

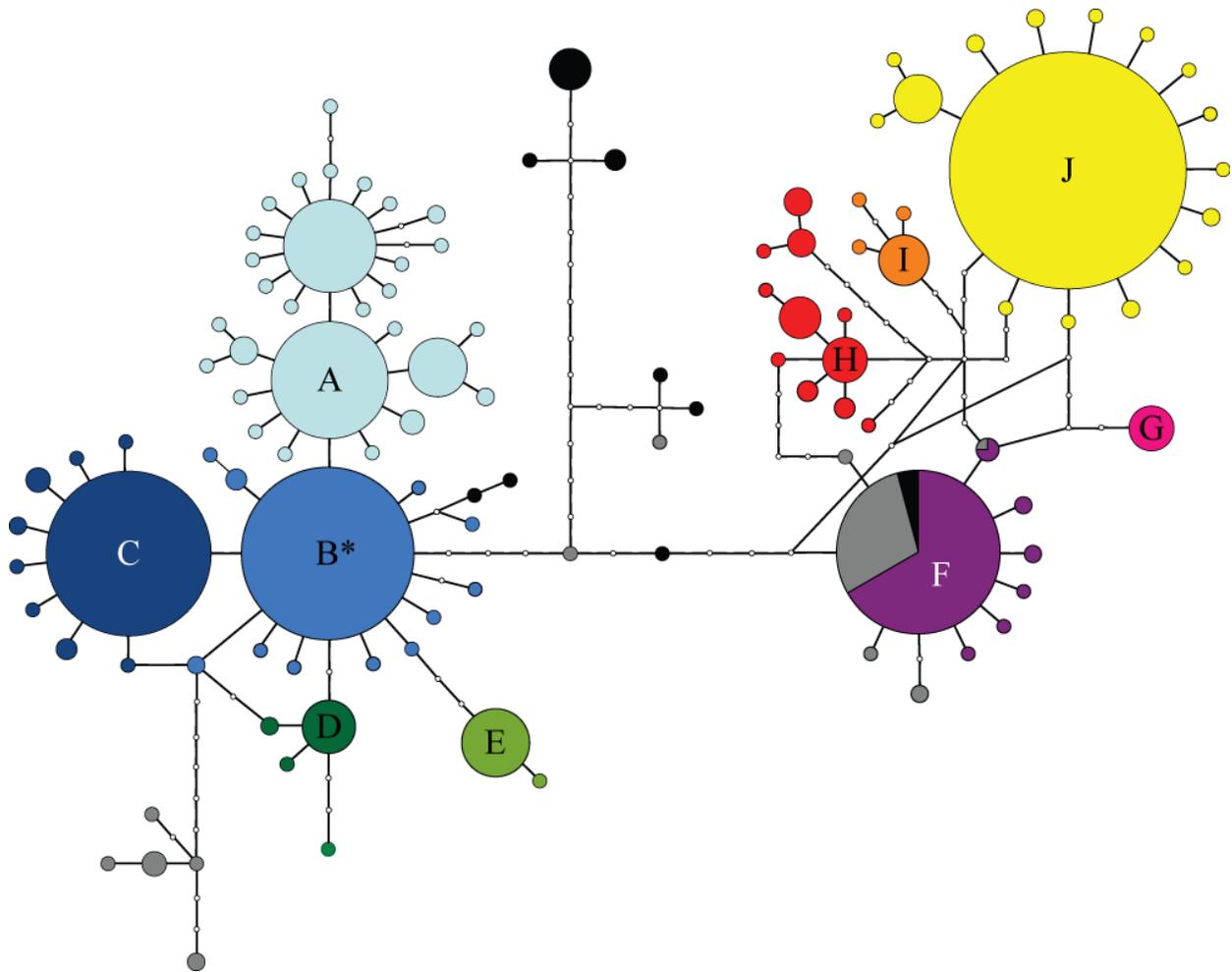


Figure 2. Maximum parsimony network for rough periwinkles. Lines indicate 95% connection limit and the asterisk denotes the ML root haplotype. Gray coloring indicates *L. arcana*; black coloring indicates *L. compressa*. All colored mtDNA haplotypes are *L. saxatilis* and letters indicate haplogroups referred to in the text. Circle size is proportional to number of individuals, with the smallest circles representing one and the largest representing 66. Small open circles denote putative intermediate haplotypes not recovered in our sampling.

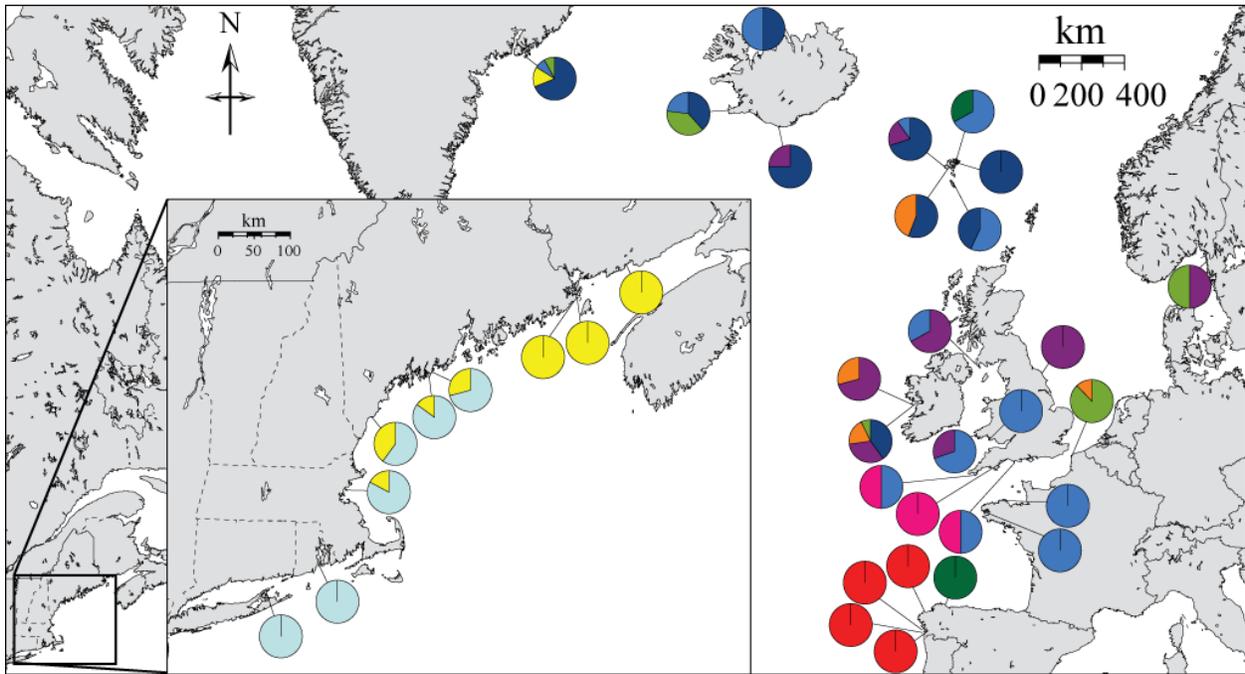


Figure 3. Haplotype frequencies for *L. saxatilis* collected at each site (Table 1). Colors correspond to the haplotype network in Figure 2.

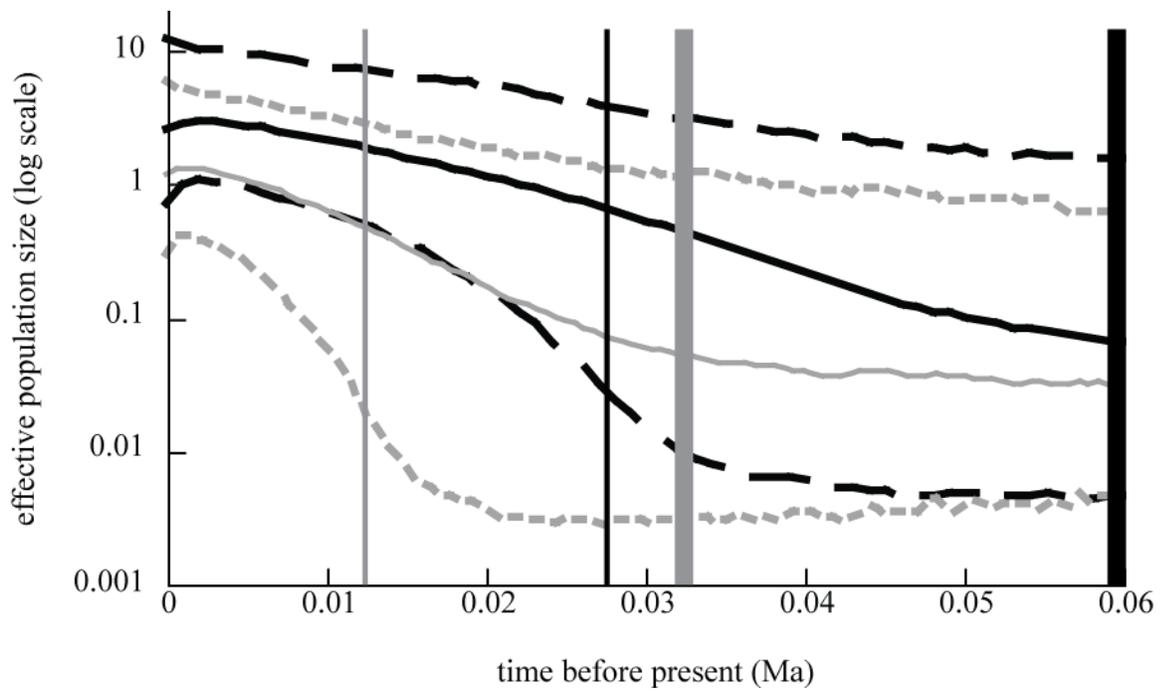


Figure 4. GMRF Bayesian Skyride plot of effective population size over time for Lineages I and II on western Atlantic shores. Lineage I (haplogroup A) is shown in black and Lineage II (haplogroup J) is shown in gray. Solid and dashed traces represent the median and 95% HPD values for effective population size. Thick vertical lines indicate mean TMRCA, while thin vertical lines show the lower 95% HPD TMRCA, as estimated under a population growth model.

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