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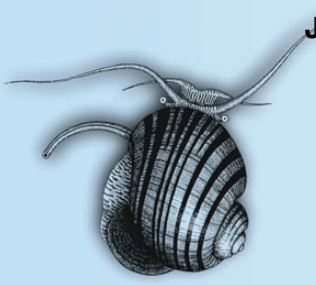

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PHYLOGENETICS OF THE GASTROPOD GENUS *NUCELLA* (NEOGASTROPODA: MURICIDAE): SPECIES IDENTITIES, TIMING OF DIVERSIFICATION AND CORRELATED PATTERNS OF LIFE-HISTORY EVOLUTION

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ABSTRACT

Despite the importance of *Nucella* as a model system in numerous fields of biology, no phylogenetic analysis of the genus, including every widely recognized species, has been conducted. We have analysed about 4,500 bp of DNA from six different genes (three mitochondrial, three nuclear) from each taxon in the genus. Our results showed western Pacific *N. heyseana* and *N. freycinetii* as distinct and distantly related, but found no evidence that *N. elongata* is distinct from *N. heyseana*. We also resolved *N. heyseana* as the closest living relative of the North Atlantic *N. lapillus* and, using the fossil record for calibration, inferred a minimum separation time between Atlantic and Pacific lineages of at least 6.2 Ma, slightly pre-dating the opening of the Bering Strait. Comparative analyses showed egg size to be evolutionarily labile, but also revealed a highly significant negative relationship between egg size and the nurse-egg-to-embryo ratio. The negative correlation indicates that evolutionary changes in egg size among species are balanced by changes in the number of nurse eggs allocated to each offspring, indicating that interspecific variation in the nurse-egg-to-embryo ratio has not been driven by divergent selection on hatching size, but may instead be a response to variation in other factors, such as parent-offspring conflict.

INTRODUCTION

Nucella Röding, 1798 is a broadly distributed, predatory marine gastropod genus whose species are found along rocky shorelines of the northern hemisphere. Most species live in the littoral zone, all lack planktonic larvae and most show extensive geographic variation in morphological, physiological and behavioural traits that appears to reflect a combination of local adaptation and phenotypic plasticity to spatially varying environmental conditions. Because of their ubiquity on rocky shores and the strong interactions they have with space-holding sessile species across a wide range of environmental conditions, species of *Nucella* have been the focus of many studies of intertidal ecology, physiology, life history evolution, shell morphology and ecotoxicology (e.g. Kitching, Muntz & Ebling, 1966; Spight, 1976b; Hughes & Elner, 1979; Pechenik, Change & Lord, 1984; Gosselin & Chia, 1994; Wootton, 1994; Navarrete & Menge, 1996; Moran & Emlet, 2001; Sorte & Hofmann, 2004). *Nucella* has been particularly important to understanding developmental ecology in marine organisms, because a wide variety of strategies for providing nutrition as yolk to developing embryos are found in the genus (e.g. Spight, 1976b; Moran & Emlet, 2001). Fully unravelling the ecological and evolutionary forces shaping interspecific differences in early development in this group, however, requires a clear understanding of the phylogenetic relationships among the species.

As for many gastropod clades, the taxonomic history of *Nucella* has had many twists and turns, primarily due to very high intraspecific variability in shell form and the tendency of workers to split (rather than lump) morphologically distinct conspecific forms (for a review, see Collins *et al.*, 1996). At present, seven North Pacific and one North Atlantic species are widely recognized. The northeastern Pacific (NEP) taxa and the amphi-Pacific *N. lima* (Gmelin, 1791) are all well defined morphologically, each distinguished by qualitatively varying shell traits (Palmer, Gayron & Woodruff, 1990; Collins *et al.*, 1996; Marko, 1998; Marko, Palmer & Vermeij, 2003; Marko, 2005). In addition to *N. lima*, there are four widely recognized species in the NEP: *N. ostrina* (Gould, 1852), *N. emarginata* (Deshayes, 1839), *N. canaliculata*

(Deshayes, 1839) and *N. lamellosa* (Gmelin, 1791). The validity of the three taxa restricted to the northwestern Pacific (NWP), *N. heyseana* (Dunker, 1882), *N. elongata* Golikov & Kusakin, 1962 and *N. freycinetii* (Deshayes, 1839) [sometimes as '*N. freycineti*' (Deshayes, 1841)] is less clear (Dall, 1915; Collins *et al.*, 1996). Unlike their northeastern relatives, the shell morphologies of the NWP taxa tend to differ more subtly and exhibit more intraspecific variation, particularly with respect to shell shape, aperture shape and external shell ornamentation (Golikov & Kusakin, 1978; Egorov, 1992; Zaslavskaya & Kolotuchina, 2003, 2005). In the Atlantic, only *N. lapillus* (Linnaeus, 1758) is widely recognized; *N. rolani* (Bogi & Nofroni, 1984), described from Vigo Bay, has been distinguished from *N. lapillus*, but genetic data from populations in Vigo Bay (e.g. Rolán *et al.*, 2004; Colson & Hughes, 2007) are not consistent with the existence of a second genetically distinct species in northwestern Spain.

No phylogenetic study to date has included representatives of all of the widely-recognized species and, among the three previous analyses, none has yielded topologically congruent trees. The most comprehensive study (Collins *et al.*, 1996), based on 778 bp of mitochondrial cytochome *b*, found support for: (1) monophyly of all four species restricted to the NEP (*N. lamellosa*, *N. canaliculata*, *N. ostrina* and *N. emarginata*); (2) a sister-group relationship between *N. freycinetii* and the lone north Atlantic taxon, *N. lapillus*; and (3) a sister-group relationship between the geologically oldest lineage, *N. lima*, and all other living species (Fig. 1A). Although this study sampled more taxa than any previous work, *N. heyseana* and *N. elongata* were not included in the molecular analysis because they were considered morphological variants of *N. freycinetii*. In contrast, a cladistic analysis based on radular morphology (Kool, 1987) suggested a close relationship between the north Atlantic *N. lapillus* and the NEP *N. lamellosa*, to the exclusion of at least one other NEP species (Fig. 1B). A third study that used a combination of mitochondrial cytochrome oxidase I (COI; 419 bp) and 12S rRNA (290 bp) sequences (Marko & Vermeij, 1999) yielded a more recently derived (but weakly supported) position for *N. lima* among the NEP taxa (Fig. 1C).

We report here a new phylogenetic analysis for the genus *Nucella* that includes all widely-accepted nominal taxa, derived from DNA sequences from three mitochondrial and three nuclear markers, a total of about 4,500 bp per species. In addition to addressing taxonomic issues and relationships of species, we used the phylogeny and the fossil record to revisit the speciation and biogeographic history of the genus, most notably the origin of the Atlantic *N. lapillus* and its relationship to the opening of the Bering Strait approximately 5.4 Mya (Vermeij, 1991; Gladenkov *et al.*, 2002; Marincovich, Barinov & Oleinik, 2002). We also used

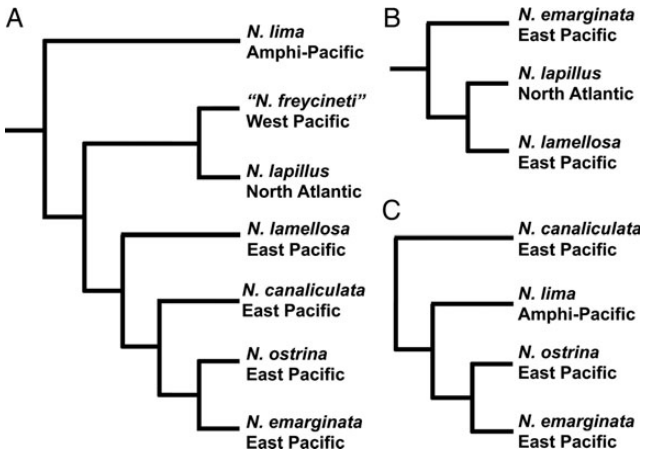

Figure 1. Previous phylogenetic hypotheses for *Nucella*. **A**. Collins *et al.* (1996). **B.** Kool (1987). **C.** Marko & Vermeij (1999).

the phylogeny to trace the evolutionary history of nurse eggs in relation to egg size. Nurse eggs (also known as trophic eggs) are non- or abnormally-developing eggs consumed by offspring (a form of adelphophagy) during development (Crespi, 1992) and are found in many marine invertebrates including molluscs (Rivest, 1983; Strathmann & Strathmann, 1986; Moran & Emlet, 2001; Collin, 2004), annelids (Blake & Arnofsky, 1999; Gibson *et al.*, 2012), flatworms (Harrath *et al.*, 2009), nemerteans, kinorhynchs and echinoderms (reviewed by Levin & Bridges, 1995), along with other taxa such as sharks (Wourms, Grove & Lombardi, 1988), amphibians (Bourne *et al.*, 2001), insects (Kudo & Nakahira, 2004; Perry & Roitberg, 2005) and spiders (Perry & Roitberg, 2006). This large and diverse group of organisms all have the maternal ability to determine the fate of oocytes as nurse eggs or as normally-developing embryos; the mechanism for nurse-egg specification has been discussed for decades, but has only been explored in a handful of taxa (Gibson *et al.*, 2012).

In *Nucella*, the ratio of nurse eggs to embryos varies both within and between species, providing a unique opportunity to investigate the ecological and evolutionary correlates of this common mode of embryonic nutrition. Some species, such as *N. lamellosa*, lack nurse eggs altogether and all of the energy required for development is contained in large eggs or intracapsular fluid. In others, such as *N. ostrina*, both eggs and nurse eggs are small, with upwards of 30 nurse eggs per embryo within a capsule (LeBoeuf, 1971; Spight, 1976a); high nurse egg to embryo ratios are associated with variation in hatching size both within and among clutches, which may have an adaptive function (Lloyd & Gosselin, 2007). Here, we have used a comparative approach as a step towards understanding the evolution of nurse egg feeding in this group and potentially in others.

MATERIAL AND METHODS

Taxon sampling, DNA extraction and sequencing

New sequences from three mitochondrial and three nuclear loci (Table 1) were obtained from two specimens of each of nine nominal species of *Nucella* plus two outgroups, *Plicopurpura columellaris* (Lamarck, 1822) and *Acanthinucella spirata* (Blainville, 1832) (Table 2). South African species assigned to *Nucella* (Kilburn & Rippey, 1982) as well as the Hawaiian '*N. fuscata*' (Forbes, 1850) have been excluded from the genus based on morphological and molecular data (Dall, 1915; Vermeij, 1993; Marko & Vermeij, 1999). The only nominal species recognized in the literature that we were unable to obtain was *N. rolani* (Bogi & Nofroni, 1984), either a close relative or a morphological variant of *N. lapillus*. McLean (2006) has also suggested cryptic species within *N. canaliculata*, as is the case for *N. lima* (Cox, Zaslavskaya & Marko, 2014). Species identifications were made by PBM and NIZ. For *N. heyseana*, *N. freycinetii* and *N. elongata*, specimens were distinguished using shell characters previously described and illustrated (Golikov & Kusakin, 1962; Zaslavskaya & Kolotuchina, 2003, 2005).

DNA was extracted from foot tissue using standard proteinase K digestion followed by CTAB/chloroform extraction and precipitation with ethanol (Marko, 1998; Marko & Vermeij, 1999). COI, cytochrome *b* (Cytb), 12S rRNA, elongation factor 1-α (EF1α), internal transcribed space (ITS) and the anonymous locus (NL16) were amplified in 25-μl volumes using published protocols (Marko, 1998; Marko & Vermeij, 1999) and a combination of universal and genus-specific primers (Table 1). NL16 was isolated from a shotgun library prepared for a population study of *N. lamellosa* (McGovern *et al.*, 2010), but the priming sites were sufficiently conserved to amplify the same fragment from all species except *N. canaliculata* and the outgroups. Because thermal cycling parameters varied among taxa and loci, those instructions are available upon request from the corresponding author.

Table 1. Sequences, amplification primers, and nucleotide substitution models for *Nucella* species used in this study.

	Base pairs	Primers	References	Best-fitting model
Mitochondrial genome				
Cytochrome oxidase-1 (COI)	1176	HCOI, LCOI, COIa-H, COIf-L	Palumbi *et al.* (1991), Folmer *et al.* (1994)	GTR + G
Cytochrome b (CytB)	718	Cytb-1, Cytb-2	Collins *et al.* (1996)	GTR + G
12S rRNA (12S)	369	12SA-L, 12SK-H	Kocher *et al.* (1989), Marko & Vermeij (1999)	GTR + G
Nuclear genome				
Elongation factor 1-α (EF1α)	766–810	EF-F8, EF-R792	This study[a]	GTR + I
Internal transcribed spacers (ITS)	698–766	ITS-F, ITS-R	Heath, Hatcher & Hilbish (1996)[b]	GTR + I + G
Anonymous locus 16 (NL16)	561–570	NL16-F, NL16-R	McGovern *et al.* (2010)	HKY

[a]EF-F8: 5′-CAG ACT CGT GAA CAC GCT-3′; EF-R792: 5′-AGA CAT CCT GAA GGG GCA-3′.

[b]Includes internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2.

Amplification products were sequenced in both directions on an automated sequencer (Applied Biosystems). Each nuclear gene was initially sequenced directly from the amplicons, but then subsequently cloned and resequenced to resolve ambiguities. One clone was sequenced from each individual with the pGEM-T Easy Vector System using Electromax DH10B *E. coli* cells (Promega, Madison, WI); the cloned sequences were used in the analyses and submitted to GenBank. Sequences were edited using Sequencher v. 4.8 (Gene Codes Corporation, Ann Arbor, MI) and deposited in GenBank (Table 2).

Phylogenetic analysis

Because the only the noncoding mtDNA sequence (12S rRNA) had very few insertions and deletions, the mtDNA alignment was completed manually with SE-AL v. 2.0a11 (Rambaut, 2007). The nuclear genes all contained insertion-deletion variation among species, but these sequences were easily aligned using the default parameters in CLUSTALX v. 1.83.1 (Chenna *et al.*, 2003). We then conducted separate phylogenetic analyses for each gene followed by an analysis of the combined six-locus dataset. Trees were constructed using both cladistic and Bayesian inference with PAUP v. 4.b10 (Swofford, 2002) and MRBAYES v. 3.1.2 (Huelsenbeck & Ronquist, 2001), respectively. We constructed cladograms using heuristic searches in PAUP under the optimality criterion of unweighted parsimony (equal weighting of sites and substitution types), with stepwise addition, TBR branch swapping and zero-length branches collapsed. Robustness of clades was estimated with 5,000 bootstrap replicates, with bootstrap percentages of 70% or higher considered as indicating significant support (Hillis & Bull, 1993). Bayesian trees were built with best-fitting models for each locus/partition selected with the Akaike information criterion in ModelTest (Posada & Crandall, 1998) for each locus/partition (Table 2); in the combined six-locus Bayesian analysis, substitution model parameters were unlinked among loci. Several initial runs of MRBAYES were used to optimize the search strategy resulting in a final Markov chain Monte Carlo (MCMC) search with 4 chains of 10 million steps each, but discarding the first 20% of trees as burn-in; we used the program TRACER v. 1.5 (Rambaut & Drummond, 2007) to check that searches reached stationarity for the posterior distribution of the parameter estimates, effective sample size of the underlying posterior distribution was large enough for a reliable estimation of parameters and the average standard deviation of split frequencies converged towards zero. Posterior probabilities of 0.95 or greater were considered as indicating significant support (Leaché & Reeder, 2002).

Divergence times

Node ages (most recent common ancestors or MRCAs) in the phylogeny were estimated with a Bayesian approach implemented in BEAST v. 1.7.5 (Drummond *et al.*, 2012), a method that assumes a relaxed uncorrelated clock with rates drawn from a lognormal distribution across branches. We used both a Yule (pure birth) process and a birth-death model for cladogenesis (in separate analyses), with unlinked (independent) substitution models for each of the six loci. Initial runs of 50 million generations were first used to optimize the scale factors of the prior function followed by five replicates runs of 100 million steps each (sampled every 1,000 steps with a discarded burn-in of 10,000 trees) with an uncorrelated lognormal distribution for the molecular clock models for each locus (Ho *et al.*, 2007). None of the nodes were constrained as monophyletic in BEAST. Convergence was assessed as described above.

To set prior distributions for nodes in the tree (as a way to calibrate rates of molecular evolution), we used information from the fossil record compiled by Collins *et al.* (1996), initially selecting five nodes. We assumed that time lags in the sequential appearance of sister-lineages in the fossil record were informative about the overall temporal pattern of speciation, such that the splitting time was more likely to be closer to the first appearance of the second species for each speciation event. For example, for node 6 (Supplementary Material Fig. S1), which is the speciation event between *N. canaliculata* and the *N. ostrina*/*N. emarginata* lineage, we used a lognormally distributed prior with a hard minimum (zero offset) of 2.5 Mya and a median age of 4.2 Mya [log(stdev): 0.5; 95% interval: 3.1–7.1 Mya], which was based on the first appearance of *N. ostrina* between 2.5 and 5.0 Mya (Addicott, 1969, 1976, 1978; Armentrout, 1981; Armentrout, Chinzer & Gladenkov, 1984). Similarly, given the first appearance of *N. emarginata* in the late Pleistocene (Marko *et al.*, 2003), we chose a lognormally distributed prior for node 7 with a hard minimum age of 0.10 Mya and a median age of 0.70 Mya [log(stdev): 0.4; 95% interval: 0.3–1.5 Mya).

The prior for node 3 was chosen based on the simultaneous appearance of *N. lamellosa* and *N. canaliculata* in the late Middle Miocene (Tortonian, 7.0–12.0 Mya) (Grant & Gale, 1931; Weaver, 1942; Addicott, 1976, 1978; Marincovich, 1983). For this node, we chose a lognormal prior with a hard-minimum of 7.0 Mya and a median age of 10.4 Mya [log(stdev): 0.3; 95% interval: 8.9–13.0 Mya). The prior for node 1 was based on the first appearance of the earliest fossils assigned to the genus (*N. tokudai*, 20.0–23.0 Mya and *N. lima* 17.0–23.0 Mya). For this node, we used a more conservative normally distributed prior to reflect greater uncertainty in the age of this node created by the very long gap in time between the first fossil *N. lima* and the first appearance of all other living species (Supplementary Material Fig. S1). The prior distribution for node 1 had a mean age of 20 Mya [log(stdev): 2.0) and a 95% interval of 16.1–23.9 Mya.

For node 2, we expected to find relatively large sequence divergences between *N. lima* and *N. freycinetii* given the ancient fossil record of *N. lima* (~20 Mya) and a Middle Miocene

Table 2. Specimens, collection sites and accession numbers for species of *Nucella* included in this study. Gene abbreviations as in Table 1.

Species, specimen	Collection site	Museum voucher[a]	COI[b]	Sequence accession numbers			ITS	NL16
				CytB	12S rRNA	EF1α		
N. emarginata, PAC 14	Pacific Grove, California	MIMB28395	KJ093770 KJ093789	KF683328	KJ093813	KF953827	KJ093836	KF953856
N. ostrina, N801	Prince Rupert, British Columbia	MIMB28393	KJ093777 KJ093794	KF683326	KJ093799	KF953830	KJ093833	KF953858
N. ostrina, N818	Campbell R., British Columbia	MIMB28394	KJ093767 KJ093784	KF683331	KJ093809	KF953835	KJ093828	KF953851
N. canaliculata, REN1	Port Renfrew, British Columbia	MIMB28396	KJ093768 KJ093779	KF683322	KJ093806	KF953842	KJ093831	–
N. canaliculata, CAT1	San Juan I., Washington	–	KJ093778 KJ093790	KF683335	KJ093811	KF953826	KJ093822	–
N. heyseana, Nh809	Kholmsk, Sakhalin I.	MIMB28387	KJ093773 KJ093786	KF683319	KJ093817	KF953834	KJ093821	KF953857
N. heyseana, Nh811	Starodubskoye, Sakhalin I.	MIMB28388	KJ093762 KJ093781	KF683324	KJ093800	KF953833	KJ093823	KF953846
N. elongata, N901	Kholmsk, Sakhalin I.	MIMB28402	KJ093769 KJ093780	KF683318	KJ093801	KF953839	KJ093826	KF953852
N. elongata, N902	Kholmsk, Sakhalin I.	MIMB28403	KJ093761 KJ093791	KF683325	KJ093814	KF953832	KJ093819	KF953845
N. lapillus, INL1	Galway, Ireland	MIMB28399	KJ093763 KJ093785	KF683327	KJ093802	KF953831	KJ093824	KF953844
N. lapillus, DMNL21	Damariscotta, Maine	MIMB28400	KJ093772 KJ093782	KF683333	KJ093807	KF953841	KJ093832	KF953853
N. lapillus, FNL4	Roscoff, France	MIMB28401	KJ093764 KJ093797	KF683330	KJ093810	KF953840	KJ093825	KF953854
N. lamellosa, N701	Prince Rupert, British Columbia	MIMB28398	KJ093771 KJ093788	KF683329	KJ093812	KF953843	KJ093818	KF953847
N. lamellosa, N716	Cordova, Alaska	MIMB28397	KJ093766 KJ093787	KF683317	KJ093815	KF953838	KJ093827	KF953855
N. lima, NI811	Juneau, Alaska	MIMB28391	KJ093775 KJ093795	KF683316	KJ093803	KF953837	KJ093830	KF953850
N. lima, N812	Juneau, Alaska	MIMB28392	KJ093760 KJ093798s	KF683323	KJ093804	KF953836	KJ093835	KF953848
N. freycinetii, N815	Kholmsk, Sakhalin I.	MIMB28389	KJ093774 KJ093796	KF683320	KJ093816	KF953829	KJ093837	KF953849
N. freycinetii, N821	Starodubskoye, Sakhalin I.	MIMB28390	KJ093759 KJ093792	KF683332	KJ093805	KF953828	KJ093834	KF953859
Acanthinucella spirata	Pacific Grove, California	–	KJ093765 KJ093793	KF683334	–	–	KJ093820	–
Plicopurpura columellaris	Panama City, Panama	–	KJ093776 KJ093783	KF683321	KJ093808	–	KJ093829	–

[a]MIMB, Museum of Marine Biology Institute of the Far East Branch of the Russian Academy of Sciences.

[b]Two portions of COI were amplified separately (see Material and Methods).

appearance of the *N. freycinetii* lineage with *N. freycinetii saitoi* Hatai & Kotaka, 1959 in the fossil record at 13.7–13.8 Mya (Amano, Vermeij & Narita, 1993; Collins *et al.*, 1996). However, the sequence data from living *N. f. freycinetii* were completely inconsistent with this interpretation of the fossil record, with only 4% COI divergence between *N. lima* and *N. f. freycinetii*; even with a relatively slow divergence rate (e.g. 1% per Myr), COI should have been >13% divergent between *N. lima* and *N. f. freycinetii* if *N. f. saitoi* and *N. f. freycinetii* are sister taxa. Because *N. f. freycinetii* appears much later in the fossil record than *N. f. saitoi*, we decided to assign a prior to node 2 using the first appearance of *N. f. freycinetii* in the Pliocene (Collins *et al.*, 1996). Therefore, the prior distribution for node 2 had a hard minimum of 2 Mya, a median age of 4.0 Mya [log(stdev): 0.4] and a 95% interval of 2.9–6.4 Mya. Given the taxonomic uncertainties, we repeated our analyses with no prior on node 2.

Node 4 received no prior distribution given that *N. heyseana* is not known from the fossil record. We also initially placed no prior on node 5, the split between the Atlantic *N. lapillus* and the Pacific *N. heyseana*, which potentially corresponded to the opening of the Bering Strait between 5.3 and 5.5 Mya (Marincovich & Gladenkov, 1999; Gladenkov *et al.*, 2002; Marincovich *et al.*, 2002; Gladenkov, 2003). For comparison, we also conducted an additional BEAST analysis with the addition of a normally-distributed but relatively narrow prior on node 5 with a mean age of 5.4 Mya (stdev: 0.1) and with a 95% confidence interval of 5.2–5.6 Mya. Normally-distributed priors are justified for biogeographically-based calibrations in which the population split may have happened either prior to or after the age of the biogeographic event (Ho, 2007).

Life-history traits

Mean values for egg volume, embryo counts and nurse-egg counts were obtained and calculated from published data for all species except *N. heyseana* and *N. f. freycinetii* (Table 3). Sample sizes and standard deviations were not available for most of the data. Data for *N. heyseana* were collected from Kholmsk, Sakhalin Island (36 capsules from Kholmsk), Vanino, Toki Bight (7 capsules) and Vostok Bay (30 capsules); for *N. freycinetii* capsules were analysed from Magadan, Tau'skaya Inlet (33 capsules). For the comparative analyses, egg volumes were log-transformed and the nurse-egg-to-embryo ratios were converted to relative proportions by dividing each species' ratio by the largest of the ratios; we then arcsine-transformed the relative proportions.

Comparative analyses

We used the subset of analyses collectively referred to as CONTINUOUS within the program BayesTraits v. 2.0 (Pagel & Meade, 2006) to test hypotheses about evolutionary change in egg volume and the nurse-egg-to-embryo ratio. CONTINUOUS implements a phylogenetically-controlled generalized least-squares model for the analysis of comparative data in which the phylogeny is converted into a variance-covariance matrix representing the shared evolutionary path between the species (Pagel, 1999; Pagel, Meade & Barker, 2004). The default or null constant variance random walk model in CONTINUOUS has only one parameter, alpha (α), which describes the instantaneous variance in trait evolution (Pagel, 1999).

We iteratively compared this default model with more complex models (with additional parameters) to find the best-fitting model of character state change for egg size and the nurse-egg-to-embryo ratio. First, we compared two variants of the default constant variance random walk model ('Model A') in CONTINUOUS, one in which trait co-variation was allowed to be non-zero (correlated evolution of traits) and another in which trait co-variation was fixed at zero (uncorrelated evolution). We then compared the best-fitting of these two models (correlated or uncorrelated evolution) with a directionally constrained model ('Model B') in which evolutionary change is limited to one direction in both traits. Lastly, we tested if the addition of three other branch-length scaling parameters improved the fit of the model to the data. These three were (1) lambda (λ), which indicates how well the tree topology predicts the pattern of evolution of traits; (2) delta (δ), which describes how the evolutionary rate in traits is distributed across the tree from the root to tips; and (3) kappa (κ), which can be used to test whether trait evolution is consistent with either a gradual mode of change or a punctuated pattern of change.

Each Bayesian analysis in CONTINUOUS was conducted with a sample of 10,000 trees from MRBAYES and consisted of MCMC searches of 500,000,000 steps, a sampling period of 1,000, a burn-in of 50,000,000 steps, uniform priors (range: −100.00 to 100.00) and an iteratively adjusted average parameter acceptance rate between 20 and 40%. For each comparison of different models of trait evolution, we calculated Bayes factors (BF) from the logarithm of the harmonic mean of the likelihoods from each MCMC search as $2(\log_e[\text{harmonic mean(better model)}] - \log_e[\text{harmonic mean(worse model)}])$; all of the likelihoods reported are $\log_e$ harmonic means of the likelihoods sampled in each search. We interpreted the significance of BF using the guidelines of Kass & Raferty (1995).

Once the best-fitting models were chosen through BF comparison of alternative models for pairs of traits, we inferred ancestral states for egg volume and the nurse-egg-to-embryo ratio with Bayesian MCMC in CONTINUOUS by first creating a posterior distribution of model and scaling parameters selected for traits as described above and then using those posteriors to estimate the ancestral states for each node in the phylogeny. We also inferred ancestral states using weighted squared-change

Table 3. Mean life-history characteristics of *Nucella* species.

Species	Egg volume	No. of embryos /capsule	No. of nurse eggs /capsule	Nurse-egg-to-embryo ratio	Sources
N. emarginata	388	14	530	37	1,2,3
N. ostrina	377	17	575	33	2,4,5,6,7
N. canaliculata	1011	26	17	0.7	3,4,8,9
N. heyseana	733	46	44	1.1	This study
N. lapillus	381	34	627	18	1,10,11,12
N. lamellosa	1305	52	0	0	5,7,12,13
N. lima	503	34	666	20	5
N. freycinetii	1596	27	0	0	This study

[1]Collins *et al.* (1996); [2]LeBoeuf (1971); [3]Houston (1971); [4]Spight (1976a); [5] Spight (1976b); [6]Emlen (1966); [7]Seavy (1977); [8]Rivest (1981); [9]Spight (1977); [10]Fioroni (1966); [11]Costello and Henley (1971); [12]Pechenik *et al.* (1984); [13]Lyons & Spight (1973).

parsimony in MESQUITE (Maddison & Maddison, 2002), a simpler method that estimates ancestral states by minimizing the squares of the difference between ancestor and descendant. Although parsimony approaches take phylogenetic relationships into account, they are not robust to violations of assumptions of constant rate of evolution or equal probability of change in either direction and do not take phylogenetic uncertainty into account (Pagel, 1999; Oakley & Cunningham, 2000; Webster & Purvis, 2002; Pedersen, Holyoak & Newton, 2007). Nevertheless, parsimony methods have fewer assumptions and do not involve any iterative fitting of models of character state change to phylogenies. Because *N. heyseana* and *N. elongata* were not distinct from each other in our trees and because most life-history characteristics of the two nominal species are highly similar, we conducted the comparative analyses of life history characters without the nominal taxon *N. elongata*.

RESULTS

Molecular phylogeny

Individual loci. Bayesian phylogenetic trees were consistent across loci (Fig. 2) with respect to well-supported nodes (cladistic trees for individual loci were similar to the Bayesian trees and are therefore not shown). Trees for all six loci shared the three sister-groupings of (1) *N. lima* with *N. freycinetii*, (2) *N. heyseana* with *N.*

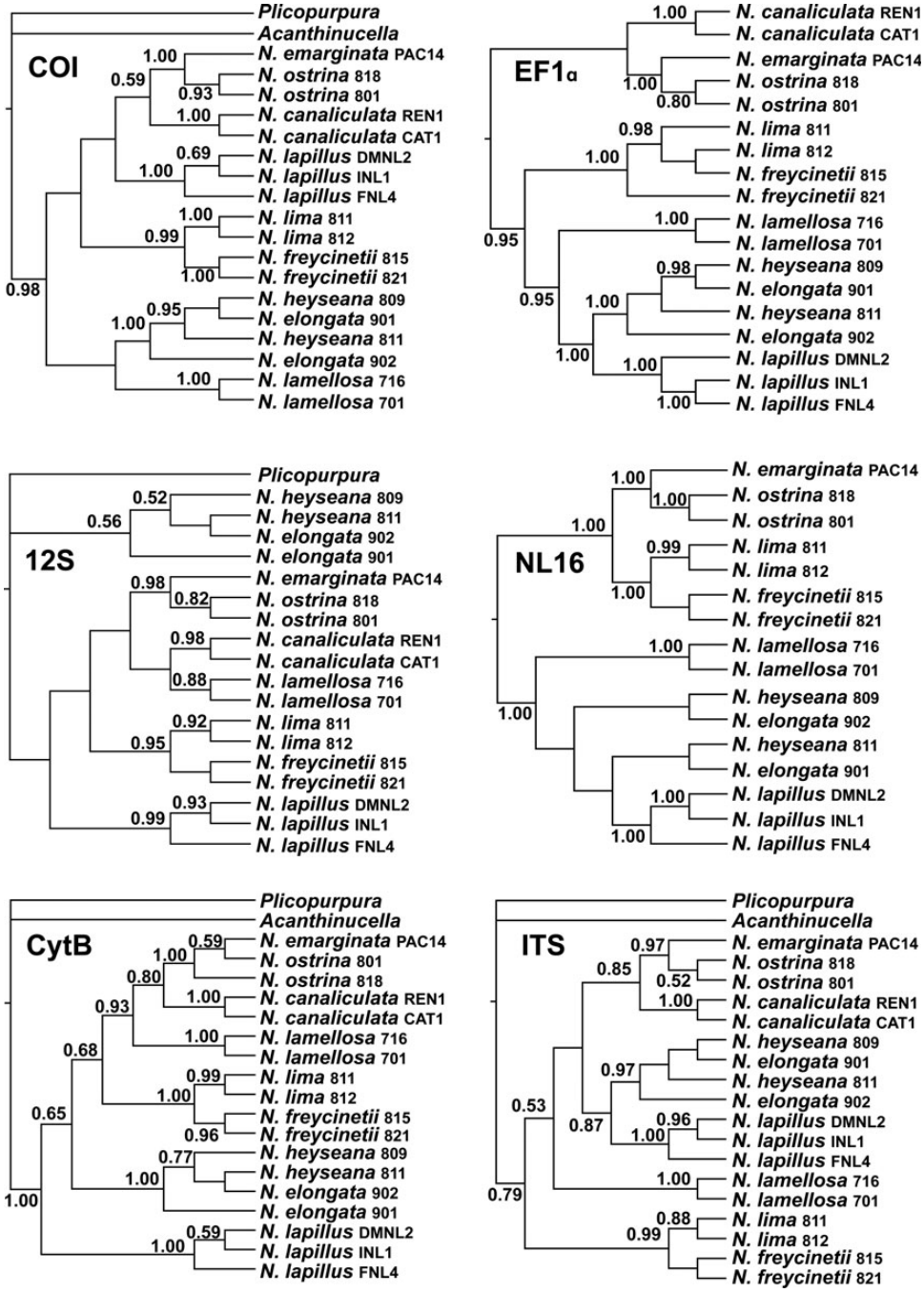

Figure 2. Bayesian phylogenies of *Nucella* based on sequences of three mtDNA (CO1, 12S and CytB) and three nDNA (EF1α, NL16 and ITS) genes. Numbers at nodes are posterior probabilities.

elongata, and (3) *N. emarginata* with *N. ostrina*. A major difference between the mtDNA trees and the nDNA trees was the position of *N. lapillus*: although each mtDNA tree had a different placement of this species (none of which were well supported), all three nDNA loci had *N. lapillus* as the sister lineage to the *N. heyseana*/*N. elongata* clade; EF1α provided the strongest support (posterior probability PP = 1.0) for this relationship. Sequence divergences between *N. elongata* and *N. heyseana* were very small across all loci (COI showed less than 1% divergence) and the two sequences obtained from each of these two species were not reciprocally monophyletic for any locus.

Trees for four of six loci (COI, CytB, EF1α and ITS) also contained a NEP clade consisting of *N. emarginata*, *N. ostrina* and *N. canaliculata* (Fig. 2). As with the analysis of Collins *et al.* (1996), CytB sequences provided support (PP = 0.93) for the inclusion of the fourth NEP species, *N. lamellosa*, in this clade (Fig. 2). However, trees for three loci (COI, EF1α and NL16) each had *N. lamellosa* as more closely related to NWP taxa; a fourth locus (ITS) also placed *N. lamellosa* as distantly related to the other NEP species. Two of the nDNA loci (EF1α and NL16) strongly supported *N. lamellosa* (PP = 0.95–1.0) as the sister-lineage to a clade consisting of *N. lapillus* and *N. heyseana*/*N. elongata*.

Concatenated dataset. The Bayesian analysis of all six loci (~4,500 bp) resulted in a tree with three clades: (1) *N. lapillus*/*N. heyseana*/*N. elongata*/*N. lamellosa*; (2) *N. emarginata*/*N. ostrina*/*N. canaliculata*; and (3) *N. freycinetii*/*N. lima* (Fig. 3). A sister-group relationship between the first two clades received weak support from the Bayesian analysis (PP = 0.68) and no support from the bootstrapped cladistic analysis (bootstrap percentage BS = 50%), as did the monophyly of the *N. lapillus*/*N. heyseana*/*N. elongata*/*N. lamellosa* clade (PP = 0.76; BS = 50%). Excluding cytochrome *b* third codon positions produced trees with slightly stronger support in the Bayesian analysis for the position of *N. lamellosa* (PP = 0.82), but this same node received even less support from bootstrapping in the cladistic analysis (BS < 50%). Sister-species relationships between *N. lapillus* and *N. heyseana* and between *N. lima* and *N. freycinetii* were each well supported, as was monophyly of the three morphologically similar NEP species, *N. ostrina*, *N. emarginata* and *N. canaliculata* (Fig. 3).

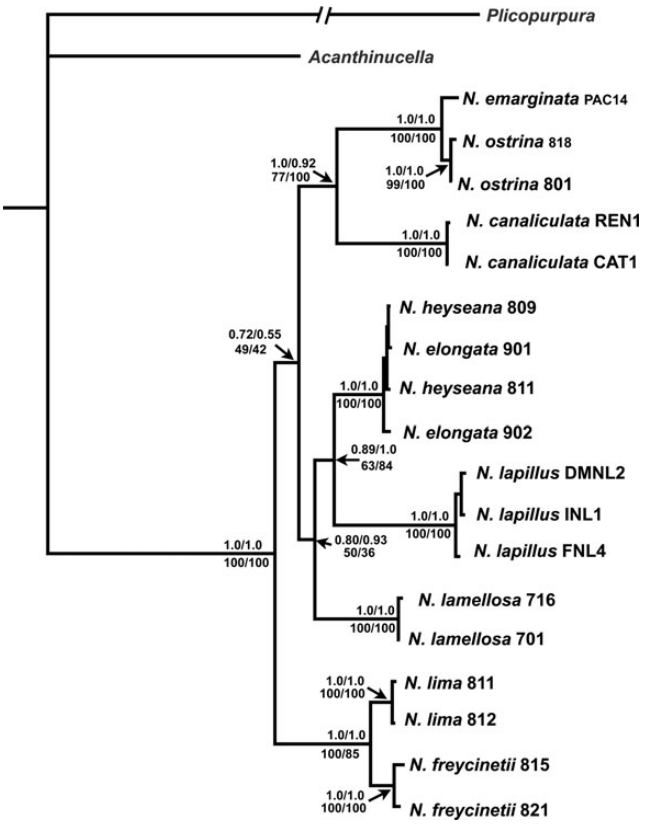

Figure 3. Bayesian phylogeny of *Nucella* based on the combined mtDNA and nDNA gene sequences. Numbers on top are posterior probabilities from the Bayesian analysis and numbers below nodes are bootstrap percentages from the cladistic analysis. Numbers on the left are from analyses including all nucleotide positions and numbers on the right are from analyses excluding mitochondrial third codon positions.

Divergence times

Divergence times under the birth-death model showed a radiation of most of the extant lineages of *Nucella* over a relatively short period of time in the Middle Miocene (15–9 Mya), with two additional speciation events at *c.* 1 and 3 Mya, that produced the sister-pair of *N. emarginata* and *N. ostrina* and the sister-pair of *N. lima* and *N. freycinetii*, respectively (Fig. 4). The posterior for the divergence time between *N. heyseana* and *N. lapillus* (which potentially corresponds to the trans-Arctic interchange) was unexpectedly old, with a mean of *c.* 8.6 Mya and a 95% highest posterior density interval of 6.4–11.0 Mya (Fig. 4). Rates of sequence evolution corresponded to divergence rates of 1.3, 1.9 and 0.3% per Myr for the mtDNA (COI, CytB and 12S, respectively) and 0.6, 0.2 and 0.8% per Myr for the nDNA (ITS, EF1α and NL16, respectively). As expected, all nodes were slightly older under the Yule model of speciation (Morlon, Potts & Plotkin, 2010), by roughly 0.1–0.9 Myr (not shown). For example, the divergence time between *N. lapillus* and the *N. heyseana*/*N. elongata* lineage had a mean of *c.* 9.8 Mya and a 95% highest posterior density interval of 7.8–12.6 Mya.

The additional analysis that included a prior distribution for node 5 (birth-death model) that corresponded to the opening of

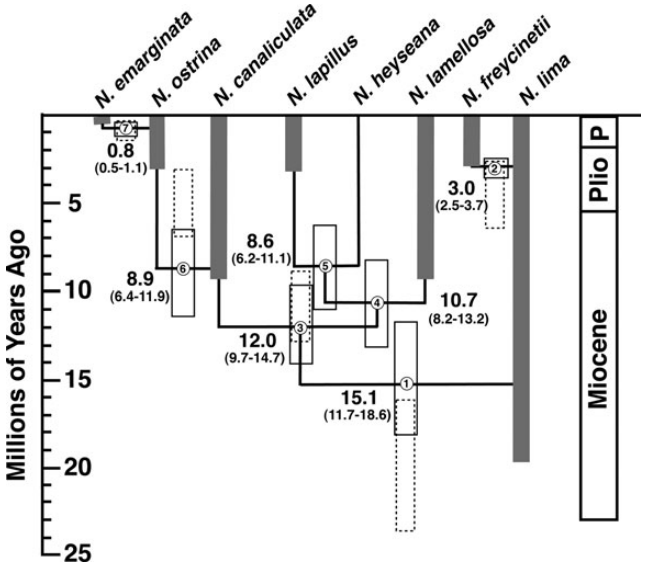

Figure 4. Bayesian chronogram for *Nucella* under the birth-death model without a prior on the age of node 5, which potentially corresponds to the opening of the Bering Strait. Closed vertical grey bars show the fossil record of each species, open boxes show the 95% highest posterior density interval for each node in the three and dashed boxes show the 95% confidence intervals for priors for nodes' ages. Numbers beside nodes are splitting times with 95% posterior densities in parentheses.

the Bering Strait about 5.4 Mya resulted in only a slightly compressed distribution of nodes, with younger dates (by ~1–2 Myr) for deeper nodes in the tree (Supplementary Material Fig. S2). An analysis that had no prior for node 2 (birth-death model) produced qualitatively similar results, and indicated an even younger split between *N. lima* and *N. freycinetii* of 1.8 Mya (Supplementary Material Fig. S3).

Life history evolution

Viewed in the context of the phylogeny, a wide range of maternal provisioning of embryos has evolved in *Nucella*, including: (1) large eggs with no nurse eggs; (2) intermediate-sized eggs with few nurse eggs per embryo; and (3) small eggs with many nurse eggs per embryo (Fig. 5). Across the phylogeny, egg size and the nurse-egg-to-embryo ratio were highly correlated: the CONTINUOUS analysis revealed that the default constant variance random walk model with negatively correlated trait change ($-\ln L = -6.417$) was a better fit to the data than the same model lacking correlated trait change ($-\ln L = -10.814$); a comparison between the harmonic means of likelihood scores recorded during each MCMC search indicated that the model with correlated trait change received strong support from Bayes factors (BF = 8.794). There was no support for a directionally-constrained model with correlated trait change ($-\ln L = -9.970$) and the addition of additional branch length scaling parameters to the constant variance random walk model with correlated trait change ($-\ln L = -6.417$) showed no support for models containing each of λ ($-\ln L = -7.034$), κ ($-\ln L = -7.209$) and δ ($-\ln L = -6.829$). Therefore, these three parameters were not included in the model used to reconstruct ancestral states.

The Bayesian ancestral states (Fig. 5) showed that since separation from a common ancestor, some lineages have had large increases in egg volume and corresponding decreases in the number of nurse eggs per embryo (*N. lamellosa*, *N. freycinetii* and *N. canaliculata*) whereas other lineages showed decreases in egg volume and increases in the number of nurse eggs per embryo (*N. emarginata* plus *N. ostrina*, *N. lapillus* and *N. lima*). The reconstructions also showed two independent losses of nurse eggs (*N. lamellosa* and *N. freycineti*) and a near loss in two other lineages (*N. canaliculata* and *N. heyseana*). Parsimony ancestral states were nearly identical to the Bayesian estimates and therefore are not shown.

DISCUSSION

Phylogeny and validity of the NWP taxa

The inclusion of all widely recognized species and about 4,500 bp of sequence data resolved several long-standing uncertainties about the identities and relationships of species of *Nucella*. We confirmed that *N. freycinetii* and *N. heyseana* are distinct species, but also showed that they are distantly related: *N. freycinetii* has recently diverged from *N. lima* (rather than being sister to *N. lapillus*), whereas the *N. heyseana* lineage (including the nominal *N. elongata*) is the sister-lineage to the lone Atlantic species, *N. lapillus*. This outcome is consistent with anatomical and life history differences as well as large allozyme divergence between populations of *N. heyseana* and *N. freycinetii* in allopatry and sympatry (Zaslavskaya & Kolotuchina, 2003, 2005; Kartavsev *et al.*, 2006; Mae, Kanno & Kijima, 2013). Our trees also showed that specimens previously referred to as '*N. freycineti*' (Collins *et al.*, 1996) were *N. heyseana*, which we verified by including the CytB sequence for '*N. freycineti*' (NFU69713) in our analysis (not shown); Collins *et al.*'s image of '*N. freycineti*' also corresponds to Dunker's type specimen for *N. heyseana* (Zaslavskaya & Kolotuchina, 2003).

Although our CytB tree (Fig. 2) placed *N. lamellosa* as the sister taxon to the NEP trio of *N. emarginata*, *N. ostrina* and *N. canaliculata* (as did the CytB tree of Collins *et al.*, 1996), most of

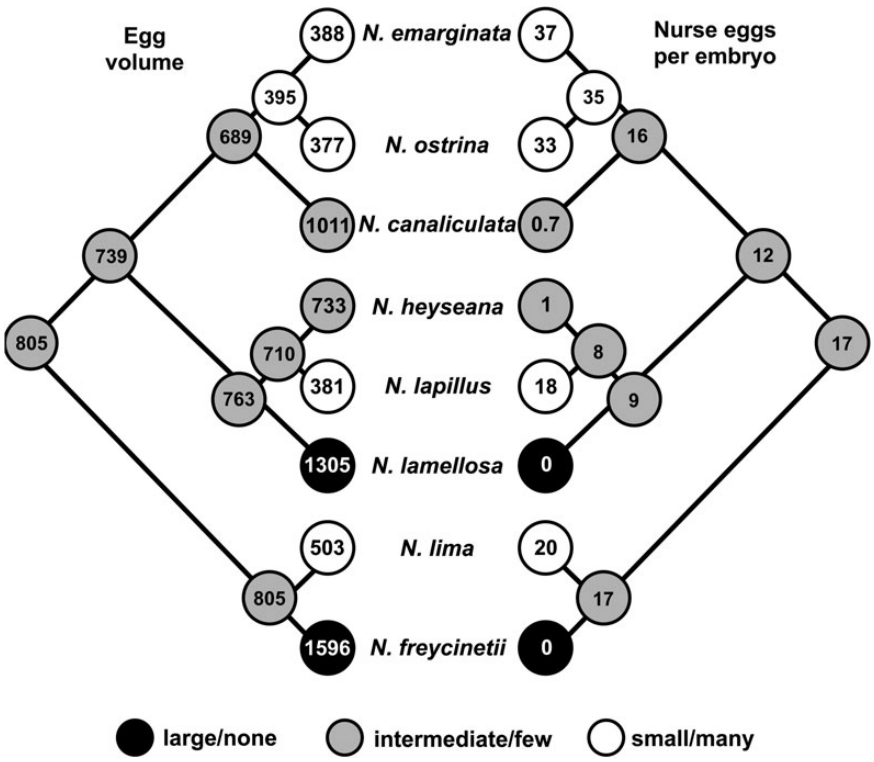

Figure 5. Bayesian ancestral states for egg volume and the nurse-egg-to-embryo ratio in *Nucella*.

the loci, as well as the concatenated dataset, indicated that *N. lamellosa* probably shares a more recent common ancestor with the NWP taxa and the Atlantic *N. lapillus*. The potentially erroneous position of *N. lamellosa* based on CytB could be a consequence of the relatively rapid radiation of most lineages of *Nucella* in the late Middle Miocene combined with the relatively high rate of divergence at CytB. Third positions of CytB are completely saturated after 5 Myr (see Collins *et al.*, 1996: fig. 3), but relatively conserved first and second codon positions provide few synapomorphies. Although the exclusion of third positions from CytB sequences (Supplementary Material Fig. S4) results in a tree that is consistent (with respect to *N. lamellosa*) with most of the other loci (particularly the nuclear genes), no single analysis could provide unequivocal support for the phylogenetic position of *N. lamellosa*. The position of *N. lamellosa* in most of our trees was, however, consistent with Kool's (1987) morphological analysis that indicated that *N. lamellosa* shares a more recent common ancestor with *N. lapillus* than with any of the other NEP species.

Timing of diversification

Most of the splitting times between extant lineages of *Nucella* are concentrated within the late Middle Miocene, 15–9 Mya (Fig. 4), suggesting an accumulation rate of at least one extant species per Myr. The start of the *Nucella* radiation coincided with the beginning of the Miocene Climatic Transition (MCT) at 14 Mya, a major shift in global climate towards the overall cooler temperatures of today (Savin *et al.*, 1985; Woodruff & Savin, 1985; Flower & Kennett, 1993; Zachos *et al.*, 2001; Holbourn *et al.*, 2005; Kawagata, Hayward & Kuhnt, 2007; Johnson, Hayward & Holbourn, 2011). The MCT likely played a role in the diversification of *Nucella*; cool climates have been causally linked to speciation in the North Pacific through the restriction of northern, amphi-Pacific ancestral taxa to eastern and western refugia (Vermeij, 1989; Cox *et al.*, 2014) as well as regional vicariance in the NWP caused by the separation of the Sea of Japan and the East China Sea into isolated basins by lowered sea level during glacial climates of the Pleistocene (Zenkevitch, 1963; Briggs, 1974).

With no prior distribution on the age of node 5, the 95% highest posterior density interval for the divergence time between Atlantic *N. lapillus* and Pacific *N. heyseana* (6.4–11.0 Mya) was similar to the date (7–8 Mya) inferred by Collins *et al.* (1996) using CytB, significantly exceeding the age of the opening of the Bering Strait (5.3–5.5 Mya), given that the lower bound on the 95% highest posterior density interval for the divergence time between *N. lapillus* and *N. heyseana* exceeds the date for the opening of the Bering Strait. However, we do not view this result as necessarily inconsistent with the fossil record of the trans-Arctic interchange given that the opening of the Bering Strait is based on the first fossil appearances of trans-Arctic taxa in both the Pacific and the Atlantic (Gladenkov *et al.*, 2002). For the earliest participants in the exchange, molecular phylogenetic divergence times between trans-Arctic taxa may be older than suggested by the fossil record if the splits between Pacific and Atlantic taxa happened before the arrival of a trans-Arctic taxon was captured in the fossil record.

That said, the BEAST analysis revealed a large discrepancy between a late Middle Miocene molecular clock divergence (~9 Mya) and a middle Pliocene appearance of *N. lapillus* in the North Atlantic (1.8–3.4 Mya; Glibert, 1959, 1963; Richards, 1962; Cambridge & Kitching, 1982; Collins *et al.*, 1996). We cannot rule out the possibility that *N. lapillus* separated from *N. heyseana* within the North Pacific prior to the opening of the Bering Strait, even though *N. lapillus* is not known from the fossil record of the North Pacific. The relatively old molecular split between *N. lapillus* and *N. heyseana* may also indicate that other Pacific taxa that appeared to have arrived in the North Atlantic in the 'second wave' of trans-Arctic taxa at 3.6 Mya (Collins *et al.*, 1996; Marincovich *et al.*, 2002) could have been in the Atlantic in the late Miocene (Zaslavskaya, Sergievsky & Tatarenkov, 1992; Grant *et al.*, 1984; Grant, 1986; Grant & Stahl, 1988), but eluded fossil preservation in the North Atlantic. Although misidentifications of fossils and erroneous ages assigned to fossils are both potential sources of error in our analysis, we think that our molecular dates are accurate given that the sequence divergence rates inferred with BEAST (for COI) were very similar to those inferred with fossils for other gastropods (Reid, Rumbak & Thomas, 1996) and bivalves (Marko, 2002).

Evolutionary trends

The phylogeny showed that some ecological and morphological traits in *Nucella* are highly evolutionarily labile, whereas others are tightly correlated with phylogeny. For example, our results illustrated convergence in shell shape between the distantly related *N. freycinetii* and *N. heyseana*, whose similarity in form has led some to consider them as conspecific (Collins *et al.*, 1996). In contrast, some morphological and ecological characteristics vary with phylogeny. For example, large apertural teeth and axial varices (see data in Collins *et al.*, 1996), traits that reduce vulnerability to low-shore and subtidal predators, as well as to dislodgement by predators in subtidal habitats (Palmer, 1977; Carefoot & Donovan, 1995; Donovan, Danko & Carefoot, 1999; Savazzi & Sasaki, 2004), are found only in *N. lamellosa*, *N. heyseana* and *N. lapillus*, three species that make up a specialized clade of subtidal-capable species. Because *N. lima* and *N. freycinetii* are also low-shore species, specialization to high shore environments appears to be a derived state in the NEP clade of *N. canaliculata*, *N. emarginata* and *N. ostrina* (see also Collins *et al.*, 1996).

The two life history traits we focused on, egg size and the ratio of nurse eggs to embryos within egg capsules (Fig. 5), were also highly labile in the phylogeny and were strongly inversely correlated. Among adelphophagic taxa such as *Nucella*, an adaptive response to selection on provisioning of yolk to embryos could be achieved through either a change in egg yolk mass (estimated by volume), a change in the number of nurse eggs, or both. One long-standing hypothesis about the functional origin of nurse-egg feeding is that evolutionary changes in egg volume may be constrained by substantial changes that might be co-required in other morphological and physiological traits (such as larger reproductive structures, larger female body size, or substantial changes to oogenetic processes) (reviewed by Perry & Roitberg, 2006); nurse eggs may represent an alternative maternal provisioning mechanism that can more readily modulate offspring size in response to spatial and temporal environmental heterogeneity. Studies of intraspecific variation in gastropods are consistent with the idea that nurse egg provisioning varies in an adaptive manner within species, because egg capsules with more nurse eggs have larger hatchlings (Spight, 1976a, b; Gallardo, 1979; Rivest, 1983; Hadfield, 1989; Chaparro *et al.*, 1999) and larger hatchlings show increased survival in more stressful environments (Spight, 1976a, b; Moran & Emlet, 2001). In *N. ostrina*, nurse-egg-to-embryo ratios (and total yolk volume allocated per viable embryo) are positively correlated with habitat wave energy (Gosselin & Rehak, 2007; Lloyd & Gosselin, 2007), a pattern that could have adaptive value if wave action increases the risk of starvation by hindering the foraging of hatchlings. Wave exposed environments may also be more temporally variable than wave-protected shores, a condition that may favour the evolution of nurse eggs when mothers

cannot change egg size by plasticity (Noriyuki, Kawatsu & Osawa, 2012).

However, while there is compelling evidence for adaptive variation in the nurse-egg-to-embryo ratio within species of *Nucella*, our results are not consistent with the idea that, among species, this ratio is more labile than egg volume as a means of provisioning embryos with yolk; egg volume doubled in some lineages (*N. lamellosa* and *N. freycinetii*) and showed similarly large reductions in others (*N. lapillus* and *N. ostrina*/*N. emarginata*). Our phylogeny also showed that egg size can evolve very rapidly, as illustrated by the doubling in egg volume in *N. freycinetii* in as little as 2 Myr (Figs 4, 5). This particular change in egg volume is larger than changes seen in planktotrophic species that experienced rapid and large shifts in oceanic productivity over similar time scales (Moran, 2004). Because egg size in *Nucella* was strongly negatively correlated with the nurse-egg-to-embryo ratio, we suggest that, among species, changes in egg volume are balanced by changes in the number of nurse eggs allocated to each offspring.

The negative correlation between these two maternal allocation strategies indicates that, unlike what appears to occur within species, interspecific variation in the nurse-egg-to-embryo ratio is not driven by divergent selection on hatching size. Variation in the nurse-egg-to-embryo ratio is clearly not related to hatching size across species; for example, one of the largest phylogenetic contrasts in maternal provisioning strategies (*N. emarginata*/*N. ostrina vs N. canaliculata*) corresponds to only an 8% difference in hatching size (Spight, 1976a), which is greatly surpassed by intraspecific variation in hatching size (67% differences) attributed to intraspecific variation in the nurse-egg-to-embryo ratio in *N. ostrina* (Lloyd & Gosselin, 2007). Moreover, the large phylogenetic contrast between *N. emarginata*/*N. ostrina* and *N. canaliculata* occurs between lineages that often live in the same habitat, further suggesting that yolk allocation into either egg size or nurse eggs among species may represent a tradeoff that has adaptive value in some way that does not involve selection on hatching size.

Another long-standing hypothesis about nurse eggs is that they may evolve under selection for rapid development, because large amounts of intracellular yolk may impede and slow cell cleavages; packaging yolk into nurse eggs (to be consumed by older embryos) may permit more rapid cell cleavage during early development (Gallardo, 1979). While we lack complete information about the duration of intracapsular development for most species, NEP species that have widely varying nurse egg allocation strategies (*N. emarginata*, small eggs and many nurse eggs; *N. canaliculata*, large eggs and very few nurse eggs; *N. lamellosa*, large eggs and no nurse eggs) have similar times to hatching (3–5, 3–5 and 4.5 months, respectively; Strathmann, 1987; see also Collin, 2004 for a similar pattern in the gastropod genus *Crepidula*). While this observation does not rule out selection for rapid development as a factor in the early evolution of nurse eggs, it does demonstrate that, for the living species, nurse eggs do not play a major role in determining development times.

Because our data suggest that yolk-packaging strategies are not well explained by either constraints on the evolution of egg size or selection for rapid development, we are left with a puzzling question: what explains the large differences in nurse-egg-to-embryo ratios (and egg size) among species of *Nucella*? An alternative to the two classical hypotheses described above is that the wide variation in nurse-egg-to-embryo ratio among species may reflect the evolutionary problem of parent-offspring conflict played out through intracapsular cannibalism (Crespi, 1992; Perry & Roitberg, 2006; Kamel, Grosberg & Marshall, 2010), a phenomenon that has been anecdotally reported in *Nucella* (Largen, 1967; Gosselin & Chia, 1994). All cannibalization of siblings entails a loss of inclusive fitness for individuals that consume capsule-mates, but some sibling cannibalism may still be advantageous for an encapsulated larva or juvenile because individual offspring are always more closely related to themselves than to full-sibs or their mother (Trivers, 1974). In *Nucella*, females can store sperm from multiple males for months (Strathmann, 1987) and mating strategies vary among species, potentially resulting in interspecific variation in the degree of relatedness among capsule-mates, and thus in how much fitness is lost by cannibalistic larvae and their mothers. In species where capsule-mates are more likely to be full siblings, selection may favour a yolk-provisioning strategy with a high nurse-egg-to-embryo ratio to decrease the likelihood that one viable embryo will encounter and consume another viable embryo. The fitness reduction incurred by cannibals and their mothers would be lower in species where capsule-mates are more likely to be half-sibs (Crespi, 1992), so these species might be more likely to provision yolk to embryos through the production of large eggs. We do not have enough data on mating systems to look for a correlation between intracapsular relatedness and the nurse-egg-to-embryo ratio among species, but what is known about mating strategies is consistent with the parent-offspring conflict hypothesis. The species (*N. lamellosa*) with the largest reproductive aggregations (tens to hundreds of individuals) lacks nurse eggs altogether; other species that mate in pairs or in small aggregations (*N. emarginata* and *N. ostrina*) have the largest nurse-egg-to-embryo ratios in the genus (Strathmann, 1987).

CONCLUSIONS

This is the first phylogenetic study of *Nucella* to include all of the nominal North Pacific taxa and, although not all relationships within the genus were resolved, our data and analyses demonstrated the validity of NWP *N. heyseana* and *N. freycinetii* as distantly related taxa, revealed that extant lineages diversified during the Miocene Climate Transition, and confirmed that *Nucella* was likely one of the earliest participants in the trans-Arctic interchange. A strong negative correlation between egg size and the nurse-egg-to-embryo ratio in *Nucella* suggests that changes in egg volume among species are balanced by changes in the number of nurse eggs allocated to each embryo and that interspecific variation in the nurse-egg-to-embryo ratio is probably not driven by divergent selection on hatching size. Variation in these life-history traits may instead be an evolutionary response to differences in mating systems, intracapsular relatedness and parent offspring conflict. Analyses of intracapsular relatedness among species with different mating systems may shed additional light on the high diversity of offspring provisioning strategies in *Nucella* and other gastropods with encapsulated development.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *Journal of Molluscan Studies* online.

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