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RESEARCH ARTICLE

Geographical subdivision, demographic history and gene flow in two sympatric species of intertidal snails, *Nerita scabricosta* and *Nerita funiculata*, from the tropical eastern Pacific

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Abstract The patchy distribution of rocky intertidal communities in the tropical eastern Pacific (TEP) may impose severe constraints on the genetic connectivity among populations of marine invertebrates associated with this habitat. In this study, we analyzed a portion of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene in two sympatric species of marine snails, *Nerita scabricosta* and *Nerita funiculata*, common inhabitants of the rocky intertidal from the Gulf of California (Sea of Cortez) and outer Pacific coast of the southern Baja California (Baja) peninsula to northern South America, to assess genetic connectivity among populations of each species. One of our aims was to determine whether the morphological, behavioral, and ecological differences observed among populations of both species throughout their range in the TEP corresponded to population genetic differences. In addition, we were interested in elucidating the demographic history of both species. We found no evidence of genetic structure throughout the Gulf of California and outer coast of the Baja peninsula region for either species. Comparisons between Gulf of California/Baja and Panama populations, however, showed significant genetic differentiation for *N. scabricosta*, but not for *N. funiculata*. The genetic differences between Mexican and Panamanian populations of *N. scabricosta* were consistent with previously reported ecological and behavioral differences for this species between these two distant regions. However, previously reported size differences between northern and central/southern Gulf of California individuals of *N. scabricosta* do not correspond with our findings of genetic connectivity among these populations. Results from neutrality tests (Tajima's D and Fu's F_S), the mismatch distribution, and Bayesian skyline analyses suggested that both species have experienced dramatic population expansions dating to the Pleistocene.

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Introduction

Many marine invertebrates show a strict association with patchy habitats (e.g., coral reefs, hydrothermal vents, rocky intertidal zones, etc.). This specificity may impose severe constraints on the genetic connectivity among their populations because extensive habitat gaps and other potential dispersal barriers may effectively reduce gene flow (Palumbi 1994; Grosberg and Cunningham 2001). Yet these barriers may act

differentially on the members of a marine community, depending on the dispersal strategy of each species (e.g., Hurtado et al. 2004). Thus, assessing genetic connectivity among populations of a species is essential for understanding the factors that have shaped the genetic variation, which, in turn, may shed light on community dynamics and aid in the development of conservation strategies.

Owing to the patchy distribution of rocky coastline, and the presence of several associated potential barriers to dispersal, the tropical eastern Pacific (TEP) offers exceptional opportunities to study genetic connectivity of rocky intertidal invertebrates. Of special interest in this region is the 1,100 km long and 80–209 km wide Gulf of California (we use "Gulf" and "Gulf of California" interchangeably in this paper; also known as the Sea of Cortez), one of the most biodiverse and productive oceanic basins in the world (Brusca 1980; Thomson et al. 2000; Alvarez-Borrego 2002). Located in the northern portion of the TEP, and isolated from the Pacific by the Baja California (Baja) peninsula, the Gulf of California has had a dynamic geological history (Helenes and Carreño 1999) that may have contributed to population genetic differentiation of rocky intertidal invertebrates. Furthermore, the Gulf represents a critical biogeographic region that faces several conservation threats. In spite of this, population genetic patterns of rocky intertidal invertebrates have been poorly studied in the Gulf of California, and in other parts of the TEP.

Two extensive gaps devoid of rocky intertidal habitat, and composed primarily of sandy or muddy coastline interspersed with mangrove-lined lagoons, are found along the TEP (Hastings 2000). One of these, approximately 370 km in length, is located at the southern mainland part of the Gulf of California. The other, over 1,200 km in length, stretches between southern Mexico and southern Nicaragua (Fig. 1). Within the Gulf of California, most of the Baja coast is rocky, whereas the mainland Mexico coast is characterized by sandy beaches and estuaries interrupted by only short stretches of rocky coastline (Thomson et al. 2000). The Gulf itself constitutes a potential barrier to gene flow between peninsular and mainland populations of rocky intertidal marine invertebrates. In addition, the Gulf of California presents contrasting oceanographic conditions north and south of the midriff islands (Angel de la Guarda and Tiburón islands; Fig. 1), including differences in circulation patterns which sharply divide the Gulf into two well-defined geographic areas (Alvarez-Borrego 2002; Gutiérrez et al. 2004). These pronounced differences influence the composition of the marine communities within these two major areas (Soulé 1960; Walker 1960; Briggs 1974; Correa-Sandoval and Carvacho-Bravo 1992), and may also restrict gene flow between rocky intertidal invertebrate populations. Furthermore, a temperature barrier at the mouth of the Gulf of California may restrict dispersal of marine invertebrates between the Gulf and the rest of the TEP (Castro et al. 2000).

Two prosobranch gastropods of the family Neritidae, *Nerita scabricosta* Lamark and *Nerita funiculata* Menke, are common inhabitants of the rocky intertidal of the TEP. The two species overlap in most of their geographic distributions, with *N. scabricosta* reaching its southern limit in Ecuador whereas *N. funiculata* extends to Peru (Keen 1971). The northern distribution limit of both species is the Gulf of California and the outer Pacific coast of the Baja peninsula to the mid-peninsular region. Both snails are herbivores that graze on crustose algae, and although they are sympatric, they show marked differences in morphology, abundance, and microhabitat association (Garrity and Levings 1981; Levings and Garrity 1983; Bovbjerg 1984; Cushman 1989). Although adults of *N. scabricosta* are larger than those of *N. funiculata*, previous work has suggested that *N. scabricosta* is smaller in the northern Gulf of California and increases in size towards the southern Gulf (Houston 1980), a trend which we also observed in our samples. While such intraspecific size differences could be attributed to environmental factors, they may also reflect genetic differences between northern and central/southern Gulf populations.

Similarly, differences in the ecology and behavior of *N. scabricosta* and *N. funiculata* between the Gulf of California and Panama (Bertness et al. 1981; Garrity and Levings 1981; Levings and Garrity 1983; Cushman 1989) could correspond to population genetic differences between widely separated (up to ~5,000 km) populations of each species. In the Gulf of California, *N. scabricosta* is generally less abundant than *N. funiculata*, and it is restricted to the upper intertidal during inactivity and foraging periods, whereas *N. funiculata* inhabits both the middle and upper intertidal, commonly occurring in unprotected habitats (Cushman 1989). In Panama, *N. scabricosta* is more abundant than *N. funiculata*, and although *N. scabricosta* still occupies the upper intertidal during inactive periods, it forages over a wider vertical range that includes the middle and upper intertidal. In contrast, *N. funiculata* is restricted to crevices in the mid intertidal (Bertness et al. 1981; Garrity and Levings 1981; Levings and Garrity 1983; Cushman 1989). In addition, in the southern part of its range (i.e., Central and northern South America) *N. scabricosta* is reported to be smaller, with more globose and regularly ribbed shells (Keen 1971).

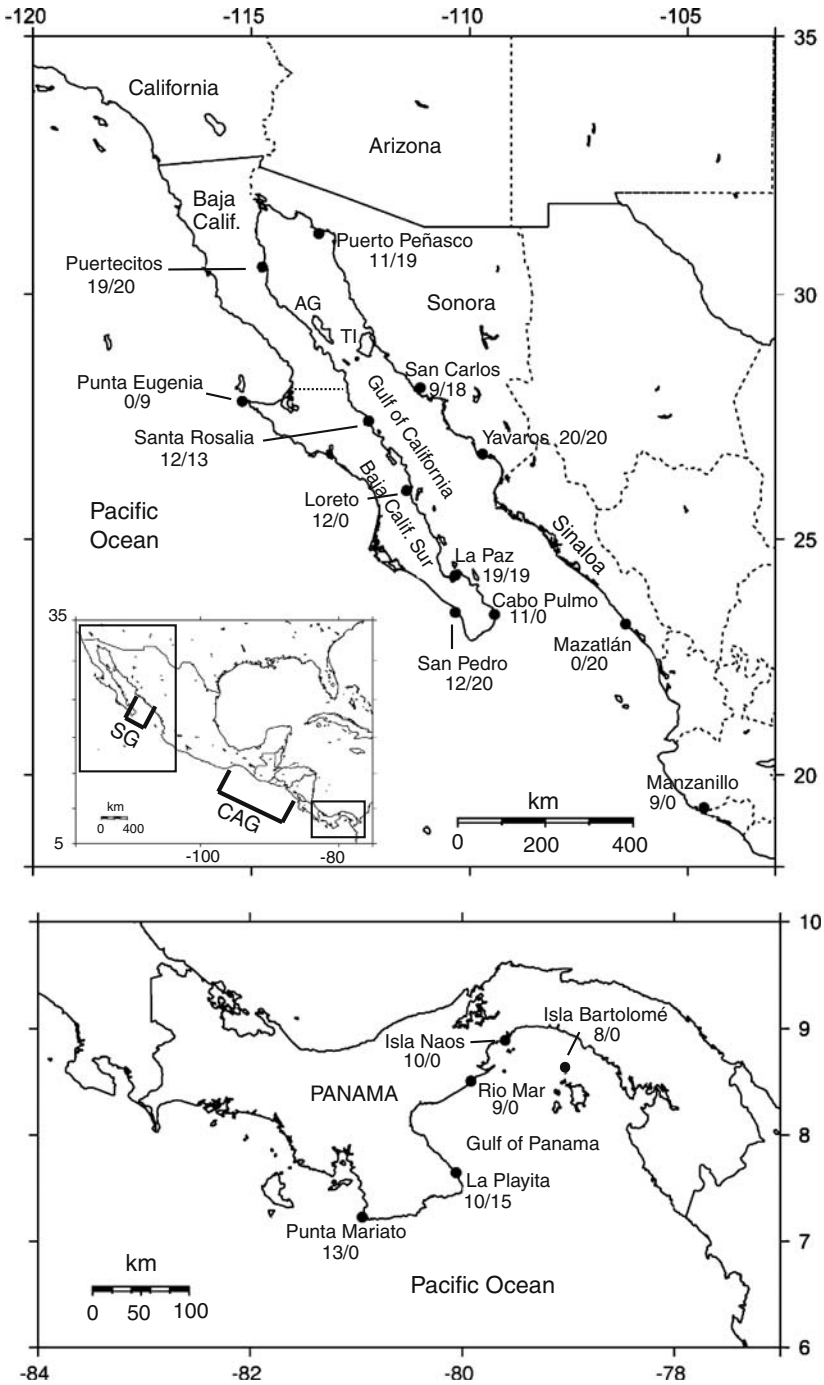

Fig. 1 Map showing collecting localities for *Nerita scabricosta* and *N. funiculata* in the tropical eastern Pacific. *Numbers* below locality names refer to sample sizes of *N. scabricosta* and *N. funiculata*, respectively, at that locality. *AG* Angel de la Guarda Island, *TI* Tiburón Island. Approximate boundaries of the two major gaps devoid of rocky intertidal habitat in the tropical eastern Pacific, the Sinaloan Gap (*SG*) and the Central American Gap (*CAG*) (Hastings 2000) are shown on the *inset*

Such differences have led some authors (e.g. Vermeij 2001) to argue that these populations correspond to a distinct species, *Nerita ornata* Sowerby, or subspecies, *N. scabricosta ornata*. Molecular analyses, which could help delimit species, have not previously been conducted on *N. scabricosta*, or on *N. funiculata*.

Both *N. scabricosta* and *N. funiculata* have separate sexes, undergo copulation and internal fertilization, deposit encapsulated eggs onto rocky substrate and have free-swimming four-lobed veliger larvae that may be able to feed (Houston 1990; Brusca and Brusca 2003). Although the duration and dispersal potential of their planktonic larvae are unknown, the presence of a veliger larva, and comparisons with life history traits with several congeneric species (Lewis 1960; Waters et al. 2005), suggest the potential for long distance dispersal and, therefore, little genetic differentiation across their geographic range. Indeed, of the few studies of population genetics conducted on coastal marine animals of the Gulf of California region, several show a

relationship between larval dispersal ability and genetic differentiation (Arnaud et al. 2000; Riginos and Victor 2001; Pfeiler et al. 2005; L.A. Hurtado et al., unpublished data; but see Bernardi et al. 2003). In general, species with long-lived planktonic larvae exhibit no or little genetic structure across the Gulf of California, and thus are assumed to be good long-distance dispersers. In contrast, species with short-lived planktonic larvae or direct developers (i.e., lack a planktonic stage) show a more pronounced degree of population genetic differentiation.

In this study, we conducted population genetic analyses, using a portion of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene as a marker, to assess geographical subdivision and gene flow among populations of *N. scabricosta* and of *N. funiculata* from the Gulf of California, the Pacific coast of the Baja peninsula, and Panama. We assessed (1) whether these two sympatric snails exhibit differences in their genetic structure, and (2) whether previously reported morphological, behavioral, and ecological differences between regions, for each species, corresponded to population genetic differences. Additionally, estimates of the evolutionary demographic history of these two species were obtained using a variety of tests.

Materials and methods

Sampling

For *N. scabricosta*, 184 individuals were analyzed from eight localities in the Gulf of California, one locality from the outer Pacific coast of Baja, one mainland Mexican locality south of the Gulf (Manzanillo, Colima), and five localities in the Gulf of Panama (Fig. 1). For *N. funiculata*, 173 individuals were analyzed from seven localities in the Gulf of California, two localities from the outer coast of Baja, and one locality in the Gulf of Panama. In the field, snails were preserved on dry ice, and subsequently stored in the laboratory at −80°C.

DNA extraction and amplification

Total DNA was extracted from foot tissue using the DNeasy kit (QIAGEN Inc., Valencia, CA). A 710 bp region of the mitochondrial gene cytochrome *c* oxidase subunit I (*COI*) was amplified using primers and conditions reported in Folmer et al. (1994). PCR products were viewed by electrophoresis using 1% agarose gels. Products were sequenced in both directions on a 3700 DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were proofread and aligned with Sequencher v. 4.1 (Gene Codes Corp., Ann Arbor, MI). Assembled sequences were truncated to a fragment that lacked the primer sequences, and which contained only clear and readable nucleotides (653 bp for *N. scabricosta*; 658 bp for *N. funiculata)*. Representative *COI* sequences for both species have been deposited in GenBank (accession numbers DQ525839-DQ525841).

Population genetic analyses

ARLEQUIN version 3.1 (Excoffier et al. 2005) was used to estimate genetic diversity and conduct population genetic analyses. Haplotype diversity, h (Eq. 8.6, Nei 1987), mean number of pairwise differences, π_1 (Tajima 1983), and nucleotide diversity, π_2 (Eq. 10.6, Nei 1987) were calculated for both species. Analysis of molecular variance (AMOVA, Excoffier et al. 1992) performed in ARLEQUIN was used to test for population structure in both species. AMOVAs were conducted (1) considering each locality separately; (2) grouping the entire Gulf and outer Pacific Baja region and comparing it with Panama; and, (3) grouping the localities into Gulf mainland, Gulf Baja, outer Pacific Baja, and Panama. The calculation of significance of pairwise comparisons of F_{ST} at the 0.05 and Bonferroni corrected levels was based on 1,000 permutations of the data matrix. Estimates of the number of migrants per generation (Nm) were also obtained. Aligned DNA sequences also were imported into MEGA version 3.1 (Kumar et al. 2004) for determination of genetic distances.

Demographic analyses

ARLEQUIN was used to conduct Tajima's (1989) D and Fu's (1997) F_S tests, and calculate the corresponding P values, and to examine mismatch distributions of DNA sequences for each species (Harpending 1994; Schneider and Excoffier 1999). Tajima's D and Fu's F_S statistics are very powerful for detecting departures from population size equilibrium caused by population expansions or bottlenecks (Tajima 1996; Aris-Brosou and Excoffier 1996; Fu 1997; Ray et al. 2003). Multimodal mismatch distributions of pairwise differences between haplotypes are expected for stationary populations, whereas unimodal distributions are expected for populations that have experienced recent demographic expansions (Harpending 1994). For all demographic analyses, data for *N. scabricosta* from Panama and Mexico were separated in two groups and analyzed separately owing to the population structure observed

between the two geographic areas; all populations of *N. funiculata* analyzed were genetically homogeneous and thus were analyzed as a single group (see second subheading of Results).

The demographic histories of *N. scabricosta* and *N. funiculata* also were inferred from Bayesian skyline analyses implemented in BEAST version 1.2 (Drummond et al. 2005). The Bayesian skyline analysis utilizes Markov chain Monte Carlo (MCMC) sampling of sequence data to estimate a posterior distribution of effective population size through time (Drummond et al. 2005). Analysis of the *COI* data set with Modeltest 3.06 (Posada and Crandall 1998) indicated that the model of nucleotide substitution that best fit our data using the Akaike Information Criterion was GTR + I + G. Bayesian skyline analyses were run under these conditions using four gamma categories. In the first set of runs, the mean mutation rate per site per generation was set at 1.15×10^{-8}. We arrived at this rate by assuming (1) an average pairwise sequence divergence rate of ~2.3% per million years (Knowlton et al. 1993; Brower 1994; Hellberg and Vacquier 1999) and (2) a generation time of 1 year. To take into account the possibility of a faster *COI* molecular clock in *Nerita* (Waters et al. 2005; M. Frey, unpublished data), we also conducted runs employing mutation rates based on divergence rates of 3.5% (*N. funiculata*) and 4.0% (*N. scabricosta*) per million years. The number of grouped intervals (m) was set to ten. Ten million iterations of the MCMC chains were run, sampling every 1,000 iterations; the first one million chains were discarded as burnin. The Bayesian skyline plots were generated with the program Tracer version 1.2.1. (Drummond et al. 2005).

Results

Genetic diversity

All *COI* nucleotide sequences were translated to amino acid sequences and no terminal codons or indels were found among the sequences of either species. Thus, we are confident to have amplified and sequenced the mitochondrial *COI* gene and not a nuclear pseudogene. Similarly, no evidence for heteroplasmy, which has been reported in some marine bivalves (Zouros et al. 1994; Passamonti and Scali 2001), was observed in any of the individuals.

A total of 78 haplotypes was found for the 653 bp fragment in the 184 *N. scabricosta* individuals examined, of which 60 were observed in single individuals, eight were shared by two individuals, five by three individuals, one by four individuals, one by six individuals, one by fourteen individuals, and one by twenty individuals; the most common haplotype was observed in 49 individuals. A total of 125 different haplotypes was found for 173 individuals of *N. funiculata* examined (658 bp), of which 98 were observed in single individuals, 19 were shared by two individuals, five by three individuals, and two by four individuals; the most common haplotype was observed in 14 individuals. All substitutions observed for *N. funiculata* were synonymous changes; five replacement substitutions were observed in *N. scabricosta*. In addition, a single fixed amino acid difference was found in the *COI* protein segment between the two species. Genetic diversity was higher in *N. funiculata* than in *N. scabricosta* (Table 1).

Population genetic analyses

Values for F_{ST} and number of migrants per generation (Nm) suggest that *N. scabricosta* exists as a large genetically uniform population across all Mexican localities sampled (Table 2). The only exception was a significant F_{ST} value at the 0.05 level for the pairwise comparison between populations from La Paz and Santa Rosalia on the Gulf Baja coast. This value, however, was not significant at the Bonferroni-corrected level. All pairwise F_{ST} comparisons among localities in Panama resulted in a zero value, suggesting no genetic differentiation across the Panama region sampled. In contrast, eight of ten pairwise F_{ST} comparisons between Mexican and Panamanian localities were significant at the Bonferroni-corrected level, and the remaining two were significant at the 0.05 level, indicating high genetic differentiation and reduced gene flow between these two regions (for space constraints, all Panama localities were pooled in Table 2). The AMOVA results obtained by grouping the Gulf and outer Pacific

Table 1 *Nerita* spp. Summary of mitochondrial *COI* diversity (±SD)

Species	N	L	K	h	k	π_1	π_2
N. scabricosta	184	653	78	0.912 ± 0.016	81	3.079 ± 1.608	0.005 ± 0.003
N. funiculata	173	658	125	0.991 ± 0.003	105	5.589 ± 2.697	0.008 ± 0.004

N number of individuals, L sequence length, K number of haplotypes, h haplotype diversity, k number of polymorphic sites, π_1 mean number of pairwise differences, π_2 nucleotide diversity

Table 2 *Nerita scabricosta*. Values for F_{ST} (above the diagonal) and number of migrants per generation (Nm; below the diagonal)

	PN	SP	PU	SR	LO	LP	CP	PP	SC	YA	MA
PN		***0.14***	***0.21***	***0.23***	***0.18***	***0.15***	**0.15**	***0.19***	**0.13**	***0.15***	***0.21***
SP	3.08		0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PU	1.93	*		0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
SR	1.63	7.99	*		0.00	**0.11**	0.06	0.07	0.01	0.02	0.00
LO	2.34	*	*	*		0.03	0.00	0.00	0.00	0.00	0.00
LP	2.80	*	23.95	4.02	16.23		0.00	0.00	0.00	0.00	0.03
CP	2.84	*	*	8.30	*	*		0.00	0.00	0.00	0.00
PP	2.17	*	*	6.39	*	*	*		0.00	0.00	0.00
SC	3.21	*	*	51.10	*	*	*	*		0.00	0.00
YA	2.77	*	*	24.70	*	*	*	*	*		0.00
MA	1.85	*	*	*	*	29.24	*	*	*	*	

Significant pairwise F_{ST} values ($P < 0.05$) are indicated in bold and significance after Bonferroni correction for multiple comparisons ($P < 0.00091$) is shown in bold italics. * = Nm is undefined and approaches panmixia

Locality abbreviations: *PN* Panama (five localities combined), *SP* San Pedro, *PU* Puertecitos, *SR* Santa Rosalía, *LO* Loreto, *LP* La Paz, *CP* Cabo Pulmo, *PP* Puerto Peñasco, *SC* San Carlos, *YA* Yavaros, *MA* Manzanillo

Baja vs. Panamanian localities were consistent with these findings; differences between the two groups accounted for ~20% of the total genetic variance observed, while genetic variance within each group explained 0% of the total variance, and ~80% of the genetic variation was attributable to genetic differentiation within localities relative to the total sample. The mean value for both uncorrected *p* distance and Kimura's (1980) 2-parameter (K2P) genetic distance between the Mexican and Panamanian populations of *N. scabricosta* was 0.005 (mean within population values were 0.003 and 0.004, respectively); corresponding mean distances between *N. scabricosta* and *N. funiculata* were 0.130 and 0.147, respectively.

The F_{ST} and Nm results for *N. funiculata* (Table 3) suggest that this species constitutes a large panmictic population across the entire area sampled. F_{ST} values were small and non-significant at the 0.05 level in all comparisons. The AMOVA results were consistent with a large genetically undifferentiated population for this species across the range examined. Grouping the localities into four groups (i.e., Gulf mainland, Gulf Baja, outer Pacific Baja, and Panama) showed that genetic differences among the groups or among localities within the groups explain 0% of the total variance, whereas 100% of the genetic variation was attributable to the genetic differentiation within localities relative to the total sample.

Demographic analyses

Values for Tajima's *D* and Fu's F_S were negative and significant (Table 4) for pooled localities of *N. funiculata*, and for the Mexican and Panamanian populations of *N. scabricosta*, consistent with a population expansion for both species. Similarly, mismatch distributions of pairwise differences between haplotypes for both species had a typical unimodal shape of populations that have experienced a sudden population expansion (Fig. 2). The sum of square deviations (*SSD*) and

Table 3 *Nerita funiculata*. Values for F_{ST} (above the diagonal) and Nm (below the diagonal)

	PN	PE	SP	PU	SR	LP	PP	SC	YA	MZ
PN		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PE	*		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SP	*	*		0.00	0.00	0.00	0.00	0.00	0.00	0.00
PU	*	*	*		0.02	0.00	0.00	0.00	0.00	0.00
SR	*	*	*	31.08		0.01	0.00	0.00	0.04	0.02
LP	*	*	*	*	48.77		0.00	0.00	0.01	0.00
PP	*	*	*	*	2350.46	*		0.00	0.00	0.00
SC	*	*	*	*	*	*	*		0.02	0.01
YA	*	*	*	*	12.45	46.92	*	24.78		0.00
MZ	*	*	*	*	28.69	*	*	255.38	*	

None of the pairwise F_{ST} values were significant ($P > 0.05$). * = Nm is undefined and approaches panmixia

Locality abbreviations: *PN* Panama, *PE* Punta Eugenia, *SP* San Pedro, *PU* Puertecitos, *SR* Santa Rosalía, *LP* La Paz, *PP* Puerto Peñasco, *SC* San Carlos, *YA* Yavaros, *MZ* Mazatlán

Table 4 *Nerita* spp. Results of neutrality tests (Tajima's *D* and Fu's F_S) and the mismatch distribution for *N. funiculata* and the separate Mexican and Panamanian populations of *N. scabricosta*

Species/ group	Neutrality tests		Mismatch distribution				
	Tajima's *D*	Fu's F_S	τ	θ_0	θ_1	*SSD*	rg
N. scabricosta							
México	**−2.19**	**−26.60**	4.42	0.12	4.91	0.005	0.014
Panama	**−2.42**	**−26.54**	1.77	0.94	22.46	0.003	0.029
N. funiculata	**−2.17**	**−25.05**	5.88	0.01	>1000	0.001	0.013

Mismatch distribution parameters: τ, time to the population expansion (=2*ut*, where *u* is the mutation rate for the entire gene segment and *t* is the number of generations since the expansion); *SSD* sum of square deviations; $\theta_0 = 2uN_0$ and $\theta_1 = 2uN_1$, where N_0 and N_1 are the population sizes before and after the expansion; *rg* raggedness statistic (Harpending 1994). Significant values ($P < 0.05$) are in boldface type

raggedness statistic (*rg*) were low and not statistically significant for *N. funiculata* and the two groups of *N. scabricosta* (Table 4), supporting the sudden-expansion model (Harpending 1994; Schneider and Excoffier 1999). For the above tests, pooling all localities for *N. scabricosta* yielded the same conclusions.

The conclusion that both *N. scabricosta* and *N. funiculata* have experienced dramatic population expansions in the TEP was also supported by Bayesian skyline analyses (Fig. 3). Employing a molecular clock calibration of 4.0% pairwise sequence divergence for the *COI* gene in *N. scabricosta* resulted in a expansion that dated to approximately 50,000 years ago for the pooled Mexican populations, and to about 100,000 years ago for the Panamanian populations. For *N. funiculata*, pooling all populations from the TEP, and applying a molecular clock calibration of 3.5% sequence divergence per million years, yielded an expansion dating to about 150,000 years ago. When a more conventional molecular clock calibration of 2.3% pairwise sequence divergence per million years was used, the expansions dated to about 200,000 years ago for both *N. funiculata* and the Panamanian population of *N. scabricosta*, and to about 70,000 years ago for *N. scabricosta* from Mexico (not shown).

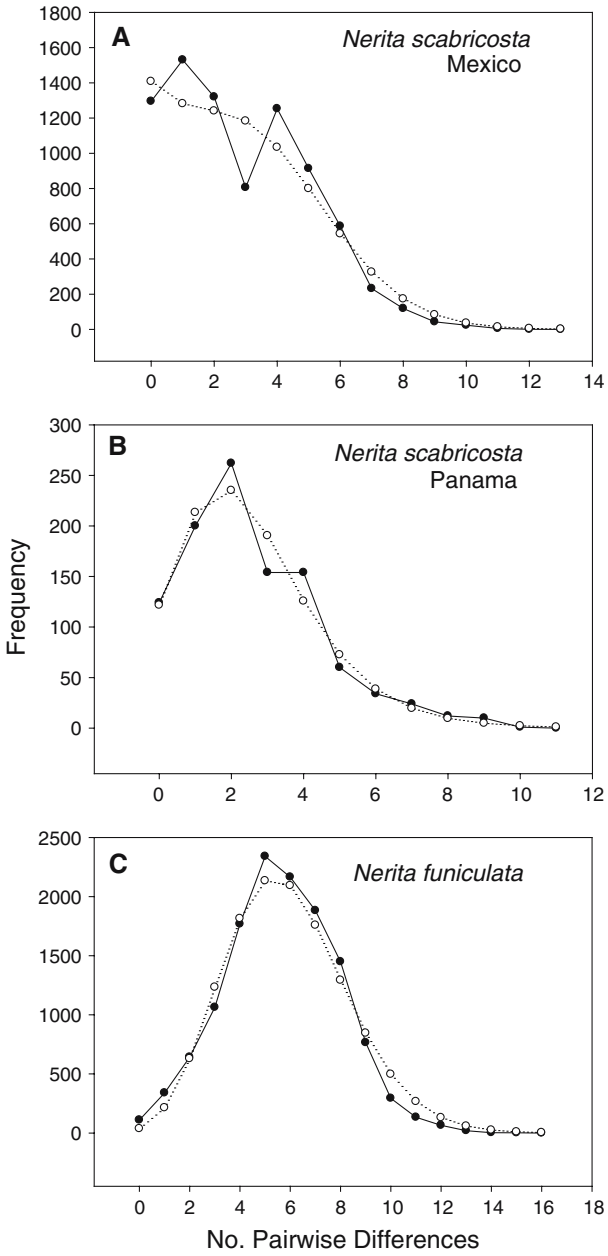

Fig. 2 *Nerita* spp. Mismatch distributions of pairwise differences among haplotypes of *N. scabricosta* (**a** and **b**) and *N. funiculata* (**c**) from the tropical eastern Pacific. *Solid circles* represent observed values; *open circles* represent expected values under the sudden-expansion model

Discussion

Our results show that despite the presence of several factors that could potentially cause population genetic differentiation of rocky intertidal invertebrates in the Gulf of California and the Pacific coast of the Baja peninsula, populations of the rocky intertidal snails *N. scabricosta* and *N. funiculata* are essentially panmictic in this region. For *N. scabricosta*, however, we found significant genetic differences between the Mexican and Panamanian localities. In contrast, no evidence was found for restricted gene flow between Mexican and Panamanian populations of *N. funiculata*. Therefore, *N. funiculata* appears to exist as a large panmictic population across coastal areas of the TEP.

The observation that these two congeneric rocky intertidal snails differ in their population genetic structure was surprising given that they overlap almost

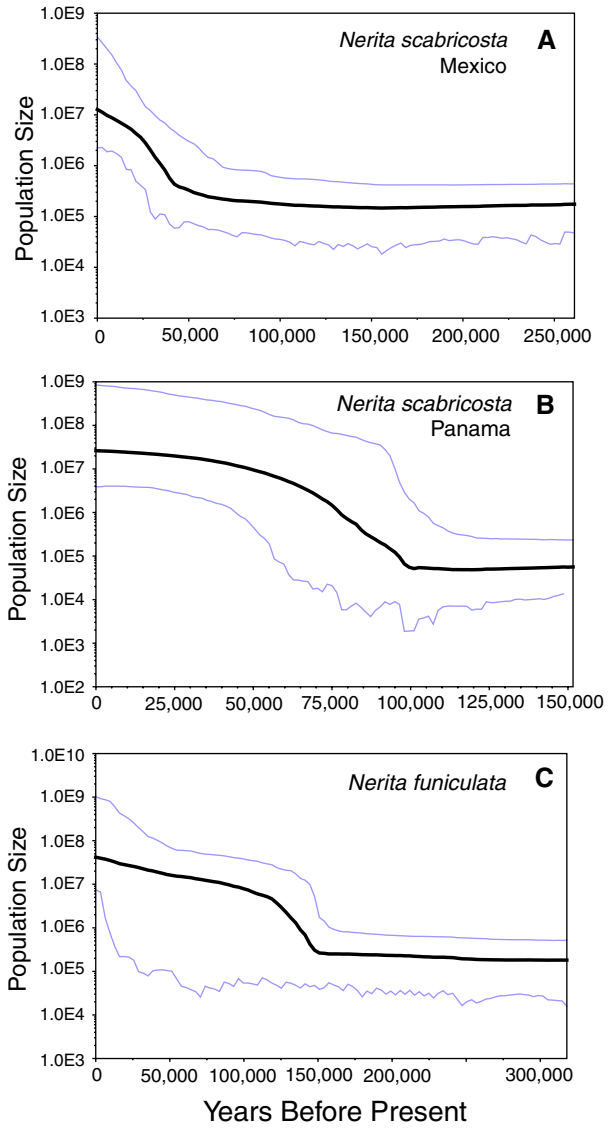

Fig. 3 *Nerita* spp. Bayesian skyline plots showing changes in effective population sizes over time in *N. scabricosta* (**a** and **b**) and *N. funiculata* (**c**). Time estimates along the *x*-axis were obtained by assuming an average pairwise sequence divergence rate of 4.0% per million years for *N. scabricosta* and 3.5% per million years for *N. funiculata*. Population size on the *y*-axis is given on a logarithmic scale. The *thick solid line* represents the mean estimate of population size; the *thin solid lines* show the 95% *HPD* (highest posterior density) intervals. Data for *N. scabricosta* from Panama ($N = 50$) and Mexico ($N = 134$) were analyzed separately owing to evidence for structure among populations from the two geographic regions. For *N. funiculata*, data from all localities were combined ($N = 173$). Note that different time scales are used in the three figures

completely in their geographical and ecological distributions. This discrepancy may be related to differences in the longevity of their larvae and/or the vertical strata at which their larvae are transported. In *N. funiculata*, the high levels of gene flow between the Gulf of California and Panama take place despite (1) the large distance (~ 5,000 km) separating these areas, (2) the extensive gap in rocky intertidal habitat between southern Mexico and southern Nicaragua (over 1,200 km), and (3) the potential dispersal barriers posed by oceanographic currents encountered between the two areas. If the larvae of *N. funiculata* live longer than the larvae of *N. scabricosta*, then this may explain the genetic structure differences between the two species. The larvae of *N. scabricosta* must live long enough, however, to allow effective mixing across the Gulf of California and Pacific Baja peninsula. Alternatively, larvae of *N. scabricosta* may travel mainly in a vertical range in which surface equatorial currents and counter-currents deflect them from the coast, whereas larvae of *N. funiculata* may use currents at deeper strata that maintain connectivity between Mexican and Panamanian localities. Nonetheless, long-distance dispersal across thousands of kilometers is not unusual for species with pelagic larvae, as has been observed in several species that remain genetically uniform even at whole ocean and inter-ocean scales (Palumbi 1994; Lessios et al. 2003; Hurtado et al. 2003, 2004), including *N. atramentosa* from the central and western Pacific (Waters et al. 2005).

Reported size differences between northern and central/southern Gulf of California individuals of *N. scabricosta* (Houston 1980) do not correspond with our findings of genetic connectivity among these populations. Thus, size differences probably reflect different phenotypic responses to environmental differences between the two main Gulf regions. Significant genetic differences found between Mexican and Panamanian populations of *N. scabricosta*, however, are consistent with previously reported ecological, behavioral, and morphological differences for this species between these two distant regions (Keen 1971; Garrity and Levings 1981; Levings and Garrity 1983; Cushman 1989). Whether the populations of *N. scabricosta* from Mexico and Panama represent two different species, or just two genetically differentiated populations, is unclear from our data. The genetic distance between the two populations was very small (~0.5%), suggesting that they are conspecific. But if speciation has occurred then insufficient time has passed for a complete lineage sorting of the two species (i.e., two reciprocally monophyletic lineages, each representing one species, are not observed). Of the 50 individuals examined from Panama, 27 (54%) have unique haplotypes and 16 (32%) harbored the most common haplotype observed in this region, which was observed in only four out of 134 individuals examined from Mexican localities. Two other haplotypes were also shared between individuals from Panama and Mexico. One was observed in a

single individual from Panama and two from Mexico, and the other in a single individual of each region. In contrast, the proportion of individuals with unique haplotypes in Mexican localities was lower (20.1%), and the most common haplotype in this region was found in 44% of the individuals, but it was not observed in any of the individuals examined from Panama.

In contrast to our findings with *N. scabricosta*, previously reported differences in the behavior of *N. funiculata* between the Gulf of California and Panama do not correspond to any population genetic differentiation. Thus, these differences likely reflect different behavioral responses to ecological and/or environmental changes between the two regions. While *N. funiculata* is more abundant than *N. scabricosta* in the Gulf of California, the inverse occurs in Panama. In the Gulf of California, *N. funiculata* inhabits the middle and upper intertidal zone, often occurring in unprotected habitats. In Panama, however, *N. funiculata* is restricted to middle intertidal crevices, and this constraint may explain the lower density of this species in Panama. Levings and Garrity (1983) found evidence that *N. funiculata* from Panamanian localities remain most of the time hiding in crevices as a mechanism to avoid fish and crab predation. Indeed, this snail grazes only within a short distance (~50 cm) of the occupied crevice, and over short periods, showing extremely high fidelity of return (96%) to a particular crevice (Levings and Garrity 1983). These observations suggest that predation pressure for this snail is generally much lower in the Gulf of California. Interspecific competition with *N. scabricosta* may be another factor constraining the distribution and density of *N. funiculata* in Panama. Compared to the Gulf of California, *N. scabricosta* forages over a wider vertical range in Panama, which may indicate increased competition for food between these two sympatric *Nerita* species. This may be a consequence of differences in food availability between the highly productive Gulf of California and the more oligotrophic waters of the TEP in Panama. In Panama, the larger individuals of *N. scabricosta* graze lower in the intertidal, overlapping with *N. funiculata*. Certainly, the larger size and mobility may allow *N. scabricosta* to out-compete the smaller *N. funiculata*. Indeed, the presence of *N. scabricosta* in rocky zones in Panama has a dramatic impact on the rocky intertidal community from the splash zone to the mid-intertidal zone, reducing abundance and composition of crustose algae, barnacle settlement, and density and size structure of other co-occurring herbivorous snails such as *Littorina* (Levings and Garrity 1983).

Similar to the species of *Nerita* studied here, other marine animals with long-lived larvae, and therefore increased potential for long-distance larval dispersal, show no genetic differentiation across the Gulf of California. These include the portunid crab *Callinectes bellicosus* (Pfeiler et al. 2005), the rocky reef blennioid fish *Ophioblennius steindachneri* (Riginos and Victor 2001), and the pearl oyster *Pinctada mazatlanica* (Arnaud et al. 2000). This latter species, however, shows substantial genetic differentiation between populations from the Gulf of California and Panama, similar to our results for *N. scabricosta*. Within the Gulf of California, effective genetic mixing among populations of each of these species apparently prevents any local genetic differentiation. Indeed, simulations of surface circulation of the Gulf using numerical models show that circulation patterns effectively mix particles across the whole basin, although this process appears to take several weeks and is highly asymmetric (Gutiérrez et al. 2004).

Yet several marine animal species do exhibit genetic differentiation across the Gulf of California, or between the Gulf and the Pacific Baja peninsula. Isolation by distance, pelagic larval duration and larval distribution patterns appear to explain the genetic structure across Gulf localities of the rocky reef blennioid fishes *Axoclinus nigricaudus* and *Malacoctenus hubbsi* (Riginos and Nachman 2001; Riginos and Victor 2001), and the penaeid shrimps *Penaeus* (= *Litopenaeus*) *stylirostris* and *Penaeus* (= *Farfantepenaeus*) *californiensis* (Aubert and Lightner 2000; de la Rosa-Vélez et al. 2000). Also, circulation patterns in the Gulf appear to affect dispersal and recruitment of the blue shrimp *Litopenaeus stylirostris* (Calderón-Aguilera et al. 2003). Furthermore, the lack of a planktonic stage may explain the extremely high genetic differentiation among populations of the rocky intertidal isopod *Ligia occidentalis* (L.A Hurtado et al., unpublished data). Similarly, the Baja peninsula has served as a barrier to gene flow between Gulf and Pacific Baja peninsula populations of eight fish species with disjunct populations in this region (Terry et al. 2000; Huang and Bernardi 2001; Stepien et al. 2001; Bernardi et al. 2003). No genetic differentiation was observed, however, in four other fish species with analogous disjunct populations (Bernardi et al. 2003). Finally, large genetic differentiation between a Gulf mainland and a Pacific Baja peninsula locality has been reported for the guitarfish *Rhinobatos productus* (Sandoval-Castillo et al. 2004).

Our results suggest that historically *N. funiculata* has had on average a larger effective population size than *N. scabricosta*, as indicated by the greater number of haplotypes and pairwise sequence differences observed for this species. Also, results from neutrality tests,

mismatch distributions, and Bayesian skyline analyses all suggest that both *N. scabricosta* and *N. funiculata* have experienced dramatic population expansions in the TEP dating to the Pleistocene. Although dates for the beginning of the expansions are only approximations based on uncertain molecular clock calibrations, our results suggest that populations of *N. scabricosta* from Panama and Mexico have had distinct demographic histories, with the estimated expansion (based on the faster molecular clock) of the Panama population dating to ~100,000 years ago compared to ~50,000 years ago for the Mexico population. The estimated date of the population expansion of *N. scabricosta* from Mexico based on Bayesian skyline analysis is similar to that reported for the swimming crab *C. bellicosus* from the Gulf of California (~67,000 years ago) which was derived from mismatch analysis of *COI* sequences (Pfeiler et al. 2005). Both *Nerita* and *Callinectes* possess pelagic larval stages which may have been subjected to similar environmental conditions during this period that favored the dramatic increases in population sizes seen for these species. We are currently analyzing gene sequences of other intertidal marine invertebrates with pelagic larval stages from the TEP to ascertain whether those species restricted to the Gulf of California also carry the genetic signature of a Pleistocene population expansion that corresponds to that seen in Gulf populations of *N. scabricosta* and *C. bellicosus*.

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