

Climate-driven genetic divergence of limpets with different life histories across a southeast African marine biogeographic disjunction: different processes, same outcome

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Abstract

Genetic divergence among populations of marine broadcast spawners in the absence of past geological barriers presents an intriguing challenge to understanding speciation in the sea. To determine how differences in life history affect genetic divergence and demographic histories across incomplete dispersal barriers, we conducted a comparative phylogeographic study of three intertidal limpets (*Siphonaria* spp.) represented on either side of a biogeographic disjunction separating tropical and subtropical marine provinces in southeastern Africa. Using a combination of mitochondrial and nuclear sequence data, we identified two distinct evolutionary lineages each in both *Siphonaria concinna* (a planktonic disperser) and *S. nigerrima* (a direct developer), and panmixia in a second planktonic disperser, *S. capensis*. Although phylogeographic breaks were present in two species, how these became established differed depending on their life histories. In the direct developer, lack of gene flow following divergence, and demographic expansion from a small initial size in the species' subtropical population, point to a single colonisation event. In contrast, the evolutionary lineages of the planktonic disperser split into two genetic lineages with much larger initial population sizes and southward gene flow continued at least periodically, indicating that divergence in this species may have been driven by a combination of reduced larval dispersal and divergent selection. These findings help explain why the presence or absence of phylogeographic breaks often appears to be independent of species' dispersal potential.

Keywords: comparative phylogeography, direct developer, divergent selection, ocean current intensity, phylogeographic break, planktonic larvae

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Introduction

Phylogeographic breaks are abrupt genetic discontinuities among adjacent populations of a species. They are considered to have arisen subsequent to the establishment of a dispersal barrier that caused a formerly single evolutionary lineage of a species to diverge into two or

more lineages. Phylogeographic breaks thus provide interesting model systems for the study of evolutionary divergence and the emergence of new species. Many phylogeographic breaks in the marine realm are believed to have been initiated by former land bridges that completely isolated different components of what was initially a single population (Barber *et al.* 2006; Teske *et al.* 2007a). After the demise of the land bridges, the genetic divisions that developed during periods of vicariance persisted because the offspring of each

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regional lineage recruited primarily to the parent population (Waters *et al.* 2005; Barber *et al.* 2006). However, some marine phylogeographic breaks cannot be linked to any geological barriers, and explaining how divergence was possible without complete isolation is challenging. Such breaks are probably maintained by a combination of incomplete dispersal barriers and diversifying selection (Pelc *et al.* 2009). Incomplete barriers such as areas with high or low water temperature that separate two populations with similar habitat requirements (Avisé 1992; Waters & Roy 2004), river discharge (Ridgway *et al.* 1998), currents (Wares 2002) and even coastal dunefields (Teske *et al.* 2006; Ayre *et al.* 2009) are more commonly invoked than diversifying selection driven by regional environmental conditions that differ on either side of the break (e.g. Beheregaray & Sunnucks 2001; Teske *et al.* 2008; Jennings *et al.* 2009; Zardi *et al.* 2011). Nonetheless, the fact that many marine phylogeographic breaks are associated with steep environmental gradients (Gaylord & Gaines 2000) and that maintenance of genetic structure in regions that lack strong dispersal barriers is only possible when selection pressure is high (Wares & Pringle 2008) suggests that selection plays an important role in driving divergence.

The South African coastline presents a suitable environment for studying the effects of incomplete dispersal barriers and the potential role of divergent selection on biotic divergence (Teske *et al.* 2011a). The region has no obvious geological protrusions that could have completely isolated regional populations of species for prolonged periods of time; there were no glaciated areas during glacial maxima that would have resulted in localised extinctions; and geographic distances between suitable habitats are fairly short. Phylogeographic breaks in this region tend to be associated with discontinuities in environmental parameters (including water temperature, salinity and nutrient concentrations), while currents, upwelling cells and stretches of unsuitable habitat may represent additional incomplete barriers to gene flow (Ridgway *et al.* 1998; Teske *et al.* 2008; von der Heyden *et al.* 2008).

A particularly interesting phylogeographic break has recently been identified near Cape St Lucia in south-eastern Africa (Teske *et al.* 2009a; Fig. 1). This region is dominated by the warm, southward-flowing Agulhas Current, which is considered to be the strongest boundary current in the southern hemisphere (Quartly & Srokosz 2004). North of St Lucia, it flows in close proximity to the narrow edge of the continental shelf (Tripp 1967). To the south, the continental shelf widens to form the Natal Bight (Fig. 1), which not only deflects the current away from the coast, thus reducing its influence on coastal biotas, but also creates a persistent upwelling cell that has a strong effect on a large portion

of the bight (Lutjeharms *et al.* 2000; Meyer *et al.* 2002). The resulting drop in sea surface temperature and other changes in hydrological conditions represent a putative selection gradient for coastal organisms. Because of differences in species compositions on either side of St Lucia, this locality is considered to be the boundary between southeastern Africa's tropical and subtropical marine biogeographic provinces (Jackson 1976; Bolton *et al.* 2004; Sink *et al.* 2005). Unlike other marine biogeographic disjunctions, where no clear relationship between species' dispersal potential and the presence or absence of deep phylogeographic breaks could be identified (Cunningham & Collins 1998; Teske *et al.* 2006; Ayre *et al.* 2009), the genetic evidence so far suggests that such a relationship may exist at the St Lucia disjunction. None of the coastal invertebrates with planktonic dispersal phases studied to date have phylogeographic breaks in this region, irrespective of propagule duration (e.g. Gopal *et al.* 2006; Teske *et al.* 2006, 2009b, 2011b; Zardi *et al.* 2007). Reciprocally monophyletic lineages have so far only been identified in benthic direct developers (Teske *et al.* 2007b,c) and in a species with nonplanktonic, burrow-dwelling larvae (Teske *et al.* 2009a).

Here, we adopted a two-locus comparative approach to examine, in a spatially and temporally explicit manner, phylogeographic structure in three sympatric species of the intertidal limpet genus *Siphonaria*. Their ranges span the St Lucia biogeographic disjunction but they differ in terms of life histories (i.e. the presence or absence of planktonic larvae). To study the effects of incomplete dispersal barriers and environmental gradients on the species' phylogeographic patterns, we assessed information from demographic parameters such as population sizes, divergence times and levels of gene flow and compared differences in microhabitat preferences as potentially selective agents. Unless larval duration is very short (<1 day), it is generally not a good predictor of dispersal distance because many species with long planktonic duration can prevent being swept away from their source habitat by regulating their position in the water column (Shanks 2009). In addition, genetic structure in planktonic dispersers may be driven by environmental gradients (Banks *et al.* 2007; Wares & Pringle 2008). Nonetheless, as the location of phylogeographic breaks can differ among species with contrasting dispersal potential (Pelc *et al.* 2009), and because there is so far no evidence for genetic structure in species with planktonic dispersal phases across the St Lucia biogeographic disjunction, we hypothesised that this would be no different in species of *Siphonaria*. In contrast, in direct developers, genetic connectedness tends to decrease with increasing geographic distances between suitable habitats

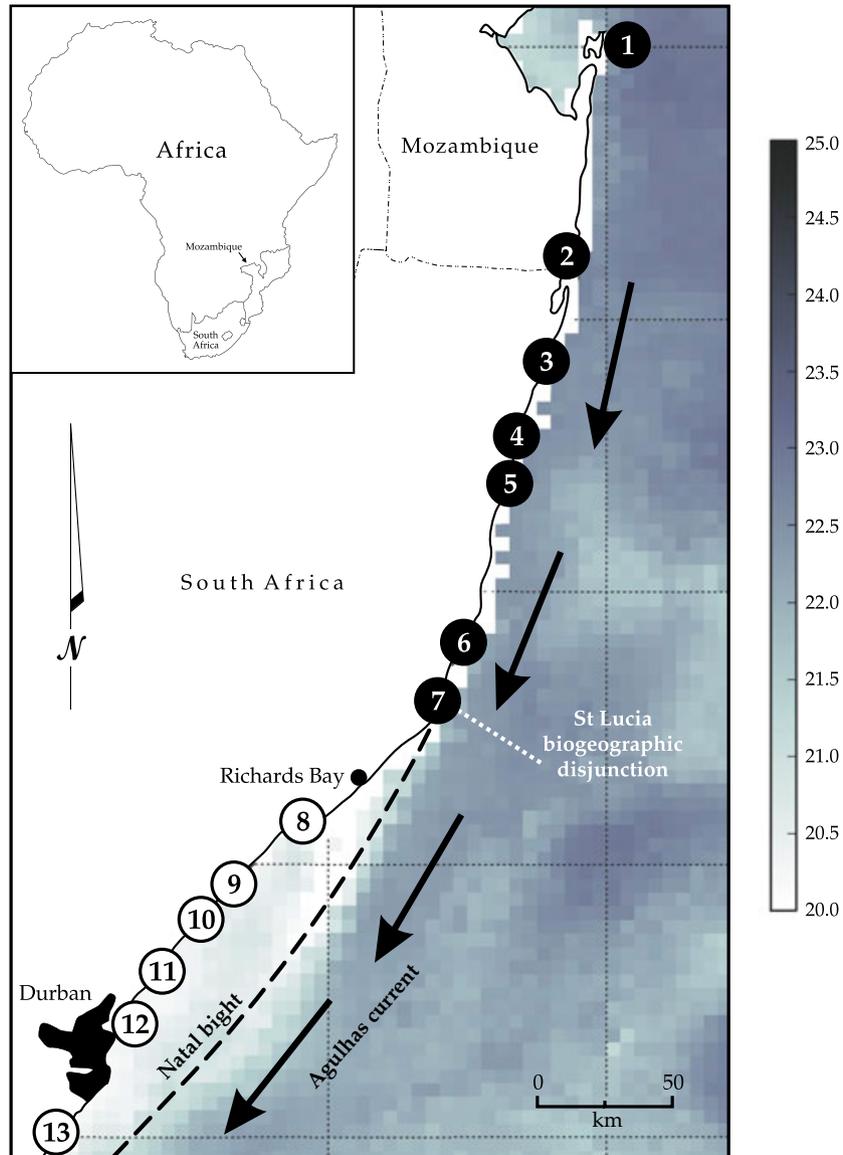


Fig. 1 A map of the northeast coast of South Africa and southern Mozambique showing sampling sites in the tropical (black circles) and subtropical (white circles) marine biogeographic provinces. Arrows indicate the core of the warm, southward-flowing Agulhas Current, and the scale bar indicates the sea surface temperature gradient. Note the lower nearshore water temperature in the Natal Bight. SST image: CSIR—Earth Observation.

(Hellberg *et al.* 2002). As long-distance dispersal is rare and sporadic in such species, regional populations are more likely to diverge from each other than in most species that have a planktonic dispersal phase (Teske *et al.* 2007c).

Materials and methods

Study taxa

The study taxa are three closely related species of the pulmonate limpet genus *Siphonaria* Sowerby, 1824 (Gas-

tro-poda: Pulmonata: Basommatophora) that occur largely in sympatry on rocky shores in subtropical and tropical regions in southeastern Africa. All three species are particularly common in the mid- to upper intertidal zone where they live attached to rocky substratum. *Siphonaria capensis* Quoy & Gaimard, 1833 and *S. concinna* Sowerby, 1824 are more widespread in southern Africa. Both species disperse by means of planktonic larvae (Chambers & McQuaid 1994a). The third species, referred to here as *S. nigerrima*, is confined to subtropical and tropical regions. This species is a direct developer, whose crawling juveniles hatch fully developed

and remain in the parent habitat. *Siphonaria nigerrima* comprises four formerly independent species described in Chambers & McQuaid (1994b): *Siphonaria nigerrima* Smith, 1903, *S. tenuicostulata* Smith, 1903, *S. annea* Tomlin, 1944 and *S. dayi* Allanson, 1959. In a study using mitochondrial and nuclear sequence data (Teske *et al.* 2007b), none of these species was recovered as a distinct monophyletic lineage. Instead, specimens collected north and south of the St Lucia biogeographic disjunction each formed a distinct monophyletic cluster. We consider this to be evidence that each of the four species actually represents a different colour morph of *S. nigerrima* (Teske *et al.* 2007b).

Sampling and laboratory procedures

A total of 361 samples were collected at 13 sites on the southeast African coast. At most of these, all three species of *Siphonaria* are present in sympatry. Seven sites are located in the tropical marine biogeographic province and six are in the subtropical province (Table 1, Fig. 1).

Samples were immediately placed into a preservation mixture containing 70% of absolute ethanol and 30% of Tris-EDTA (TE) buffer, and this medium was replaced over several days until it no longer changed colour. Small tissue samples were removed from the foot of each specimen for DNA extraction, and genomic DNA was isolated using the CTAB protocol (Doyle & Doyle 1990). A portion of the mitochondrial cytochrome oxidase subunit I gene (COI) was amplified using universal primers (Folmer *et al.* 1994), and an intron-containing region of the nuclear ATP synthase subunit β gene (ATPS β) was amplified using a forward primer

designed for *S. nigerrima* (SiphonariaATPS β : 5'-TGR ATT CCC TGA TGT TTT TGT GAG-3'; Teske *et al.* 2007b) in conjunction with a universal reverse primer (Jarman *et al.* 2002).

Polymerase chain reactions (PCR) included 2.5 μ L of 10 \times NH₄ standard reaction buffer (Promega), 6 mM (COI) or 3 mM (ATPS β) of MgCl₂, 0.16 mM of each dNTP (Bioline), 3 pmol/ μ L of each primer, 0.2–0.5 μ L of 10 mg/mL Bovine Serum Albumin, 1 U of GoTaq polymerase (Promega) and 1 μ L of DNA extraction in a total volume of 30 μ L. The PCR profile comprised an initial denaturation step (3 min at 94 °C), 35 cycles of denaturation (30 s at 94 °C), annealing (45 s at 50 °C for COI and 60 °C for ATPS β) and extension (45 s at 72 °C), followed by a final extension step (10 min at 72 °C). PCR products were either directly purified with the Wizard[®] SV Gel and PCR Clean-Up System (Promega) or were excised from a 2% agarose gel and purified using the UltraClean[™] 15 DNA Purification Kit (MO BIO Laboratories, Inc.). Samples were subsequently cycle sequenced in both directions using BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and visualised on either an ABI 3100 Genetic Analyser or a 3130xl Genetic Analyser.

In cases where individuals were heterozygous at the ATPS β locus, we deduced the phases using default settings for multi-allelic loci without stepwise mutation in PHASE v2.1 (Stephens *et al.* 2001), for which infiles were prepared using SEQPHASE (Flot 2010). Whenever there was more than one possible result, we selected the two alleles having the highest probability, which tended to be an order of magnitude greater than the probabilities of all other sequence pairs. Selecting alternative phases made no obvious difference in terms of the genetic

Table 1 Sampling sites (from north to south) and number of samples collected per species and site. Site numbers correspond to those used in Fig. 1

Site no.	Site name (GPS coordinates)	<i>Siphonaria concinna</i>	<i>Siphonaria nigerrima</i>	<i>Siphonaria capensis</i>
1	Ilha da Inhaca (26°00'09"S 32°54'53"E)	–	6	–
2	Ponta do Ouro (26°50'40"S 32°53'43"E)	8	12	13
3	Mabibi (27°19'57"S 32°45'06"E)	5	12	10
4	Nine Mile Beach (27°22'58"S 32°43'54"E)	5	12	4
5	Jesser Point (27°32'39"S 32°40'47"E)	5	8	10
6	Mission Rocks (28°16'41"S 32°29'11"E)	14	18	17
7	Cape St Lucia (28°31'15"S 32°24'04"E)	13	20	16
8	Lone Rock (28°54'34"S 31°56'14"E)	12	–	3
9	Zinkwazi Beach (29°16'58"S 31°26'38"E)	5	9	5
10	Blythedale Beach (29°22'27"S 31°21'03"E)	11	18	16
11	Salt Rock (29°30'17"S 31°14'18"E)	2	–	12
12	Umhlanga Rocks (29°43'40"S 31°05'20"E)	8	18	16
13	Warner Beach (30°04'43"S 30°52'23"E)	6	8	4
	Total	94	141	126

diversity of each lineage (results not shown). Obtaining good quality ATPS β sequences proved difficult in *S. capensis*. Although the aligned sequences contained several indels, length differences between sequences were small, which precluded the option of excising the two PCR products of heterozygous specimens from an agarose gel and sequencing them individually. It was also not possible to determine the two phases of each individual using the program CHAMPURU (Flot 2007) because the two bases present at a particular site could not always be identified with complete certainty, possibly because of problems associated with secondary structure or regions rich in A or T arrays. To resolve this problem, such PCR products were ligated into pCR 2.1-TOPO vectors (Invitrogen), transformed into One-Shot TOP 10 Chemically Competent Cells (Invitrogen) and plated on Luria-Bertani agar. Plasmid DNA of positive colonies from each plate was PCR amplified using M13(-20) forward and M13(-40) reverse primers, and sequenced using the reverse primer until the two phases of each individual could be discerned (8–15 colonies per individual). We selected 30 samples (15 from each province) from the complete data set and fully resolved the phases in all of them.

Sequences were aligned by eye in MEGA v5 (Tamura *et al.* 2011), and intron sequences were tested for recombination using the Recombination Detection Program (RDP3 vAlpha 44, Martin *et al.* 2010).

Identification of evolutionary lineages

Distinct evolutionary lineages were identified, and their association with marine biogeographic provinces visualised, by constructing median-joining haplotype networks in NETWORK v4516 (2009 version) (Bandelt *et al.* 1999). Sites containing indels were deleted. In one case, we constructed an intraspecific phylogeny using the neighbour-joining method (Saitou & Nei 1987) in MEGA. Evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura *et al.* 2004), and support for nodes was assessed by generating 10 000 bootstrap replications (Felsenstein 1985).

We used ARLEQUIN v3.5.1.2 (Excoffier & Lischer 2010) to estimate standard genetic diversity indices for each lineage and to test for departure from selective neutrality using Fu's F_s (Fu 1997). As tests for selective neutrality can be significant when population expansion has occurred (Fu 1997), we also calculated Harpending's raggedness index (HRI, Harpending 1994) to identify significant departures from a model of sudden demographic expansion. Significance of both Fu's F_s and HRI indicates that a locus may be under selection.

Assessment of dispersal ability

To determine whether there were differences in dispersal ability between the three species, we calculated pairwise Φ_{ST} (Michalakis & Excoffier 1996) values from distance matrices of pairwise differences among selected sites in ARLEQUIN. To exclude the impact of potential dispersal barriers other than geographic distances between sampling locations on these estimates, we only used data from the tropical province, as a reasonable number of sites and samples were available from this region for all three species (Table 1). Also, as the ATPS β intron showed very little variation in *Siphonaria nigerrima* and the data for *S. capensis* were considered questionable (see Results), we used only COI sequence data. Sites with fewer than five samples were excluded (Table 1), resulting in data sets comprising six sites for *S. concinna*, seven sites for *S. nigerrima* and five sites for *S. capensis*. Although the statistic F_{ST} (and its analogue Φ_{ST}) have been criticised for being strongly affected by within-population diversity, and F_{ST} analogues have been developed that correct for this (Hedrick 2005; Meirmans & Hedrick 2011), Φ_{ST} is nonetheless considered to be the ideal F -statistic for DNA sequence data (P. Meirmans, personal communication) because it incorporates information about the demographic history of a population (Whitlock 2011). Ninety-five per cent confidence intervals (95% CI) of mean pairwise Φ_{ST} were calculated by generating 10 000 bootstrap replications in POPTOOLS v3.2.3 (Hood 2010). Φ_{ST} values were considered to be significantly different between species if their confidence intervals did not overlap.

Divergence and gene flow

We estimated demographic parameters by sampling coalescent genealogies in a Bayesian framework. Coalescent-based methods require genetic data from randomly sampled individuals from populations and can produce accurate results even when sample sizes are low, although statistical power increases with the number of loci included (Felsenstein 2006).

The program *ima* (Hey & Nielsen 2007) was used to estimate present-day effective population sizes among sister lineages and of the ancestral population that gave rise to them, the time at which they diverged from their ancestor, bidirectional migration rates among pairs of sister lineages and the time at which migration events occurred. All runs performed in 'MCMC mode' included the following specifications: -l (number of trees saved every 100 steps) 50 000 (i.e. 5×10^6 generations); -b (burn-in, i.e. initial number of steps dis-

carded) 500 000 (i.e. 10%); $-n$ (number of heated chains) 100; $-f$ g (geometric heating scheme); and $-g1$ 0.99 $-g2$ 0.75 (terms of the geometric increment model). Suitable priors for θ_1 , θ_2 and θ_A (scaled effective population sizes of the two extant populations and their common ancestor, respectively), t (divergence time), as well as m_1 and m_2 (pairwise migration rates) were determined individually for each data set following a number of test runs. To ensure that the program was run long enough for chains to converge, we performed fifteen replicate runs using different starting seeds and reported the means of demographic parameters from the three runs with the highest effective sample sizes. Genealogies saved during the seven best runs were jointly analysed in 'Load Trees mode' to determine whether the full model fit the data significantly better than models that excluded parameters. To convert scaled model parameter estimates into demographic parameters (including effective population size N_e , divergence time T and the number of migrants per generation, M), we assumed a generation time of 1 year and specified a COI mutation rate of 1% per million years (Myr) based on marine gastropods for which calibrated fossil data are available (Cypraeidae and *Polystyra*; Meyer *et al.* 2005). This rate is intermediate between estimates of evolutionary rates for geminate marine gastropods that diverged as a result of the final closure of the Central American Seaway during the Pleistocene (Arcidae: 0.35–0.6% per Myr, Marko 2002; *Tegula*: 1.2% per Myr, Hellberg & Vacquier 1999). We also determined a mean evolutionary rate of 0.8% per Myr for the ATPS β intron by comparing percentage differences among sister lineages with those of the COI gene.

We used a second coalescent-based program, MIGRATE-N v3.2 (Beerli 2009), primarily to estimate pairwise migration rates and compare them with those obtained with IMA. Runs recorded trees every 10 steps out of a total of 50 million steps, with a burn-in of five million steps and a static heating scheme (temperatures 1, 1.5, 3, 10 000). Suitable upper bounds for θ (scaled effective population size) and m (migration rate) were set individually for each data set after a number of test runs. A recent addition to the program involves the use of Bayes factors to determine support for different scenarios of migration direction (Beerli & Palczewski 2010). These were calculated from Bézier-corrected lnML estimates of the following scenarios: bidirectional gene flow between the tropical and subtropical marine biogeographic provinces, only southward gene flow, only northward gene flow and panmixia (i.e. no differentiation among the individuals from different provinces). Model probabilities were calculated in WOLFRAMALPHA (<http://www.wolframalpha.com>). Both

IMA and MIGRATE-N were run on the CBSU computing cluster at Cornell University.

Historical trends in effective population size were investigated using the Extended Bayesian Skyline Plot (Heled & Drummond 2008). This method can identify departures from the neutral coalescent model (Kingman 1982) in single genetic lineages through evolutionary time and can incorporate sequence data from multiple loci. We specified 250 million generations (with a burn-in of 10%) in BEAST v1.6.1 (Drummond & Rambaut 2007), unlinked the partitions in cases where data from both loci were used (only COI sequences were used for *S. capensis*), and specified empirical base frequencies and a strict clock model. The rate for COI was fixed to 1% per Myr, whereas the rate of 0.8% per Myr for ATPS β was specified as a prior. The best-fitting evolutionary model for each data set was identified using FINDMODEL (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>), an online program that uses MODELTEST script (Posada & Crandall 1998) and constructs a starting tree using the program WEIGHBOR (Bruno *et al.* 2000). We repeated each run three times to ensure that the program was run for a sufficiently long period of time to allow searches to converge on similar values. The results were checked using the program TRACER v1.5 (Rambaut & Drummond 2009) to ensure that effective sample sizes for all demographic statistics were above 200 for each run. If they were not, then we increased the number of generations to 500 million.

Results

Identification of evolutionary lineages

The final length of the COI sequences was 408 bp in *Siphonaria concinna*, 516 bp in *S. nigerrima* and 507 bp in *S. capensis*. Haplotypes of both *S. concinna* and *S. nigerrima* could be assigned to distinct lineages (Fig. 2a, c and d), with phylogeographic breaks associated with the St Lucia marine biogeographic disjunction, whereas those of *S. capensis* were recovered as a single lineage (Fig. 2f). No haplotypes were shared between marine biogeographic provinces in *S. nigerrima*, while a mixture of both lineages was identified at most subtropical sites in *S. concinna* (Lineage 1 was found throughout the region sampled, whereas Lineage 2 was not found north of site 8).

Trimmed and aligned ATPS β intron sequence lengths were 227 bp for *S. concinna*, 230 bp for *S. nigerrima* and 277 bp for *S. capensis*. Heterozygous individuals were identified in the two planktonic dispersers, but not in the direct developer *S. nigerrima*. The two distinct genetic lineages of *S. concinna* and *S. nigerrima* identified using the COI sequence data were also recovered

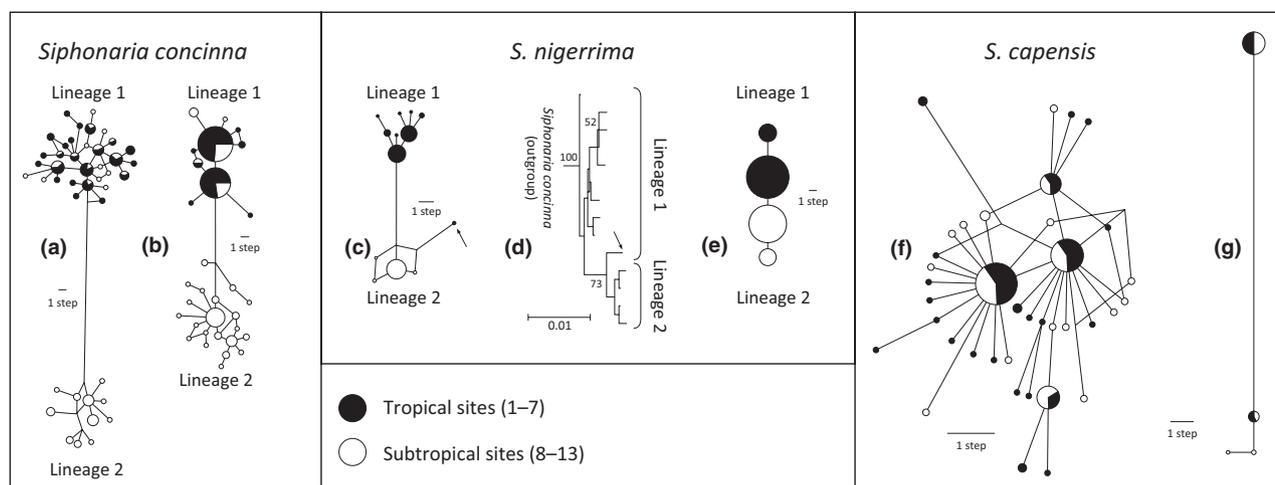


Fig. 2 Evolutionary lineages of three limpets of the genus *Siphonaria* in southeast Africa, and their association with the region's tropical and subtropical marine biogeographic provinces. Genealogies were reconstructed using COI sequences (a, c, d and f) and ATP5 β sequences (b, e and g). The arrows in c and d point to a rare tropical COI haplotype that is closely associated with the subtropical lineage (Lineage 2) of *S. nigerrima*.

using nuclear intron data (Fig. 2b, e). Four alleles were recovered in *S. capensis*, of which two were common and two rare. Nine out of 30 samples needed to be cloned, and each included a combination of the two more common alleles. As was the case for the COI sequences, the intron alleles of *S. capensis* showed no obvious association with marine biogeographic provinces. No evidence for recombination was found in the intron sequences of any of the three species. All unique sequences generated in this study were submitted to GenBank (accession numbers JN603047–JN603179) and are indicated for each individual in Table S1 (Supporting information).

A rare COI haplotype of *S. nigerrima* from the tropical province was placed in a derived position in the haplotype network relative to the cluster of subtropical haplotypes (Fig. 2c), suggesting some northward dispersal after the two lineages diverged. However, such a placement is attributable to the fact that haplotype frequency is interpreted as being positively correlated with haplotype age in network reconstructions (e.g. Castellote & Templeton 1994). In a phylogenetic reconstruction (Fig. 2d), this haplotype was instead recovered in a sister taxon relationship with the subtropical cluster and is thus more likely to belong to the tropical lineage. The nonderived position of the rare tropical haplotype is further supported by the fact that the two individuals having it also had the most common ATP5 β allele found in the tropical province. Recent migrants from the subtropical province would be expected to have a subtropical allele.

Genetic diversity indices based on COI sequence data were generally higher for the planktonic dispersers than

for the direct developer, particularly in the case of the subtropical lineage of *S. nigerrima*, which had only four haplotypes (Table 2). The ATP5 β intron was highly variable in *S. concinna* but had relatively low signal in *S. capensis* and *S. nigerrima*. Fu's F_s was significantly different from zero in four cases. However, the fact that the sudden expansion model was not rejected by the corresponding estimates for Harpending's raggedness index suggests that the reason for the significant tests for selective neutrality was population expansion rather than background selection. While COI data tended to be more variable than ATP5 β intron data in all three species, the number of steps between haplotypes was greater among COI haplotypes than among intron alleles in *S. concinna* and *S. nigerrima* only. In *S. capensis*, COI haplotypes differed by up to six steps, whereas ATP5 β alleles differed by up to 12. Also, while no indels were found in the data sets of *S. concinna* and *S. nigerrima*, there were eight indels in that of *S. capensis*, a possible indication that two different versions of the intron might be present in this species. This, and the low information content and sample size of the ATP5 β sequences, prompted us to estimate demographic parameters for this species using COI data only.

Assessment of dispersal ability

As expected on the basis of the species' life histories, mean pairwise Φ_{ST} values among sites in the tropical province were significantly greater in the direct developer *Siphonaria nigerrima* ($\Phi_{ST} = 0.30$, 95% CI = 0.22–0.39) than in the planktonic dispersers, and they were of

Table 2 Number of individual allele sequences generated (N), number of haplotypes recovered (H), haplotype diversity ($h \pm SD$), nucleotide diversity ($\pi \pm SD$), Fu's F_s and Harpending's raggedness index (HRI) of each evolutionary lineage of two southern African species of *Siphonaria*

Species	Lineage	Locus	N	H	h	π	F_s	HRI
<i>Siphonaria concinna</i>	1	COI	70	32	0.955 ± 0.008	0.007 ± 0.004	-26.46**	0.03
		ATPS β	140	10	0.604 ± 0.026	0.004 ± 0.003	-4.62	0.13**
	2	COI	24	11	0.910 ± 0.030	0.007 ± 0.004	-4.14	0.09
		ATPS β	48	21	0.884 ± 0.026	0.014 ± 0.008	-20.84**	0.01
<i>S. nigerrima</i>	1	COI	88	9	0.652 ± 0.030	0.002 ± 0.001	-2.98	0.14**
		ATPS β	176	2	0.196 ± 0.043	0.003 ± 0.002	2.92	0.73
	2	COI	53	4	0.146 ± 0.065	0.0004 ± 0.0005	-3.16**	0.56
		ATPS β	106	2	0.144 ± 0.051	0.0006 ± 0.0010	-0.00	0.53
<i>S. capensis</i>	COI	126	38	0.843 ± 0.024	0.004 ± 0.003	-27.57**	0.04	
	ATPS β	60	4	0.409 ± 0.069	0.045 ± 0.023	23.48	0.47	

* $P < 0.05$ or $P < 0.02$, which corresponds to $P < 0.05$ in the case of Fu's F_s (Fu 1997), ** $P < 0.01$.

similar magnitude in *S. concinna* ($\Phi_{ST} = 0.07$, 95% CI = 0.03–0.10) and *S. capensis* ($\Phi_{ST} = 0.05$, 95% CI = 0.02–0.08).

Divergence and gene flow

Effective population sizes of genetic lineages estimated under the isolation-with-migration model (iMA) were typically larger for *Siphonaria concinna* than for *S. nigerrima*, and the population size of Lineage 1 of *S. nigerrima* was an order of magnitude greater than that of Lineage 2 (Table 3). Divergence time estimates of the two evolutionary lineages of both species fell into the Late Pleistocene (~10–650 thousand years ago). Migration among regions was only found in *S. concinna*. It was of a very low magnitude, strictly southwards and occurred ~104 (ATPS β data) – 166 (COI data) thousand years ago (Fig. 3d). Likelihood curves for present population sizes and migration rates based on COI sequences of *S. capensis* did not have distinct peaks. Although the divergence time estimate was significantly different from zero, this may be an indication that this data set violates the isolation-with-migration model's assumption of at least partial isolation among lineages.

A log-likelihood ratio analysis of nested models for the *S. concinna* data set (combined COI and ATPS β data), based on 36 000 genealogies from seven independent iMA runs, indicated that a model that excluded northward gene flow was the only nested model that fit the data better than the full model ($P = 0.96$, Table 4).

In contrast to the result from the iMA analyses, a model of bidirectional gene flow in *S. concinna* was more strongly supported in the MIGRATE-N analyses than strictly southward dispersal (Table 5), although the estimate for northward dispersal was not significantly different from zero. Estimates of migration rates were

higher than those obtained with iMA, but still of a very low magnitude, namely 1.4×10^{-3} (97.5% confidence intervals: 0.8×10^{-3} – 2.1×10^{-3}) individuals per generation for southward dispersal and 1.4×10^{-4} (0.0 – 3.7×10^{-4}) individuals for northward dispersal. Standard deviations of mean lnML values are low (Table 5), which indicates that the program was run long enough for different runs to consistently arrive at similar estimates. As Lineage 2 of *S. concinna* is not present in the tropical province, we consider the results from the iMA analysis to be more reliable. An estimate of divergence time from MIGRATE-N (not shown) indicates that some gene flow was contemporary, but this feature of the program is experimental and there is no publication that deals with it yet. While inferences about gene flow and other demographic parameters made in iMA are quite robust even when the isolation-with-migration model is violated (Strasburg & Rieseberg 2010), the method for estimating migration time is considered to be unreliable (Strasburg & Rieseberg 2011). Hence, southward gene flow in *S. concinna* could well be a contemporary process. Although levels of gene flow between the provinces must be high in *S. capensis*, the likelihood curve of at least one demographic parameter was consistently difficult to interpret because it had no distinct peak and no clearly defined upper limit. As for the iMA analyses, we consider this to be an indication that samples from the two provinces are not sufficiently different for estimating gene flow in this species.

Extended Bayesian Skyline Plots (Fig. 4) were constructed using the following evolutionary models for each partition: *S. concinna* (Lineage 1): COI: HKY (Hasegawa *et al.* 1985) + Γ , ATPS β : TrN (Tamura & Nei 1993) + Γ ; *S. concinna* (Lineage 2): COI: HKY + Γ , ATPS β : GTR (Rodriguez *et al.* 1990) + Γ ; *S. nigerrima* (Lineage 1): HKY for both COI and ATPS β ; *S. nigerrima*

Table 3 Demographic parameters of the southern African limpets *Siphonaria concinna* and *S. nigerrima* (two evolutionary lineages each) and 95% confidence limits estimated under the isolation-with-migration model (iwa)

Species	N_1	N_2	N_A	T	M_1	M_2
<i>Siphonaria concinna</i>	0.9 (± 0.03) (0.7–1.5)	2.5 (± 0.04) (1.8–5.7)	1.2 (± 0.02) (0.8–7.2)	0.36 (± 0.01) (0.06–3.87)	0.00 (± 0.00) (0.00– 1.20×10^{-6})	3.1×10^{-6} (± 0.08) (6.0×10^{-7} – 2.0×10^{-5})
<i>S. nigerrima</i>	0.2 (± 0.00) (0.1–0.6)	0.03 (± 0.00) (0.01–0.15)	0.09 (± 0.02) (0.02–1.36)	0.26 (± 0.01) (0.21–0.45)	–	–
<i>S. capensis</i>	?	?	0.25 (± 0.04) (0.14–1.10)	0.07 (± 0.01) (0.02–0.08)	?	?

Parameters include effective population sizes of samples from evolutionary lineages 1 and 2 (N_1 and N_2) and population sizes of ancestral populations (N_A) in million individuals, time at which lineages diverged (T) in million years, and number of migrants into the tropical province (M_1) and into the subtropical province (M_2) per generation. Values reported are the means from three runs (\pm SD) with the highest effective sample sizes and the means of upper and lower confidence limits. Migration rate parameters were set to zero in *S. nigerrima* because of the two lineages' complete geographic isolation. Question marks indicate that likelihood curves had no distinct peaks.

(Lineage 2): COI: TrN, ATP5 β : HKY; and *S. capensis* (COI only): GTR + Γ . The time to the most recent common ancestor of the extant haplotypes of two evolutionary lineages, namely Lineage 1 of *S. concinna* and Lineage 1 of *S. nigerrima* (Fig. 4), is much longer ago than that of their sister lineages, a possible indication of a more ancient origin. The population sizes of both lineages were stable for a long period of time and then increased towards the present. At the time of coalescence of the extant haplotypes of Lineage 2 of *S. concinna*, this population was about half as large as that of Lineage 1 ($\sim 360\,000$ and $\sim 700\,000$ individuals, respectively). In contrast, Lineage 2 of *S. nigerrima* originated much more recently than its sister lineage and initially had a very small population size (~ 6000 individuals) that expanded rapidly towards the present. The single lineage of *S. capensis* also appears to be relatively young ($\sim 400\,000$ years), but presently, it has the largest population size of the five lineages investigated. As no more than two loci were used, 95% confidence intervals are very large for all five plots and were omitted in Fig. 4 to avoid losing detail. For example, the median estimate of 5000 individuals for Lineage 2 of *S. nigerrima* at its origin has a confidence interval ranging from 8×10^{-10} to $\sim 110\,000$ individuals.

Discussion

The St Lucia biogeographic disjunction represents a dispersal barrier between South Africa's tropical and subtropical marine biogeographic provinces, two coastal regions that differ only slightly in terms of species composition (Bolton *et al.* 2004; Sink *et al.* 2005). We identified phylogeographic breaks in this region in both the direct developer *S. nigerrima* and the planktonic disperser *S. concinna*, but not in the planktonic disperser *S. capensis*. This confirms the notion that the presence or absence of phylogeographic breaks in codistributed species need not be strictly linked to theoretical dispersal potential (Cunningham & Collins 1998; Teske *et al.* 2007c; Neethling *et al.* 2008; Ayre *et al.* 2009). However, the mechanism leading to the evolution of phylogeographic breaks may not be identical for species with high and low dispersal potential. By taking the limpet species' demographic histories and information about past climatic conditions and changes in surface circulation of the Western Indian Ocean into consideration, we are in a position to explore what factors may have contributed towards the creation of phylogeographic breaks in some members of this group. The processes that drove genetic divergence across the St Lucia disjunction are likely to have been similar to those that resulted in phylogeographic breaks in other coastal regions where populations of planktonic dis-

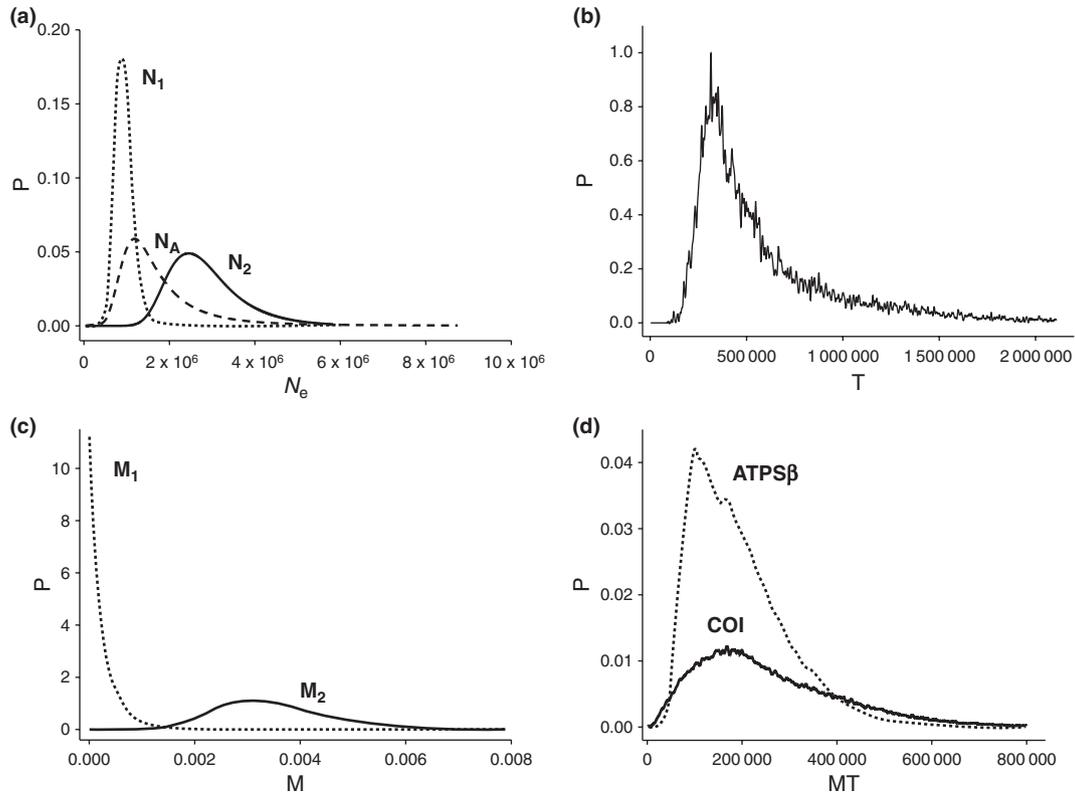


Fig. 3 Examples of likelihood plots from *IMA* analyses based on combined data from the COI and ATPS β loci of *Siphonaria concinna*; (a) effective population sizes of Lineage 1 (N_1), Lineage 2 (N_2) and the ancestral population prior to genetic divergence (N_A); (b) time in years at which lineages 1 and 2 diverged; (c) number of migrants per thousand generations into the tropical province (M_1) and into the subtropical province (M_2), and (d) time in years at which migration occurred (MT) shown for each locus (P = posterior probability).

Table 4 Results of the model selection feature in *IMA* based on sequence data of *Siphonaria concinna*

Model	d.f.	2LLR	<i>P</i> -value
$\theta_1, \theta_2, \theta_A, m_1 = m_2$	1	11.866	<0.01
$\theta_1, \theta_2, \theta_A, m_1, m_2 = 0$	1	176.776	<0.01
$\theta_1, \theta_2, \theta_A, m_1 = 0, m_2$	1	0.003	0.96
$\theta_1, \theta_2, \theta_A, m_1 = m_2 = 0$	2	200.956	<0.01
$\theta_1 = \theta_2, \theta_A, m_1, m_2$	1	7.958	<0.01
$\theta_1 = \theta_2 = \theta_A, m_1, m_2$	2	8.376	<0.05
$\theta_1 = \theta_2, \theta_A, m_1 = m_2$	2	26.420	<0.01
$\theta_1 = \theta_2, \theta_A, m_1 = m_2 = 0$	3	261.874	<0.01
$\theta_1 = \theta_2 = \theta_A, m_1 = m_2$	3	29.700	<0.01

2LLR = log-likelihood ratio statistic, d.f. = degrees of freedom for each model. *P*-values for the 2LLR statistic were calculated using the χ^2 calculator available at <http://www.stat.tamu.edu/~west/applets/chisqdemo.html>

persers are unlikely to ever have been completely isolated, such as Florida (Avice 1992) and southern California (Dawson 2001). Although several studies on such systems have suggested that divergent selection was

important in driving genetic divergence (Sotka *et al.* 2004; Jennings *et al.* 2009; Pelc *et al.* 2009), these studies have primarily invoked reduced gene flow through the effects of incomplete dispersal barriers. We present a model in which selection is crucial to explaining how genetic differentiation among populations of species with high dispersal potential can evolve in the face of ongoing gene flow.

Range expansion into new habitats as a precursor to divergence

Although *Siphonaria concinna* and *S. nigerrima* have congruent phylogeographic breaks across the St Lucia biogeographic disjunction, differences in dispersal potential suggest that the mechanisms leading to the evolution of these breaks must have differed between the two species.

Siphonaria nigerrima likely originated in the tropical province. The time to the most recent common ancestor of the extant haplotypes of its tropical lineage (Lineage 1) is 16 times that of its subtropical lineage (Lineage 2).

Table 5 Natural logarithms of Bézier-corrected marginal likelihoods (lnML) and Bayes factors of evolutionary lineages of the limpet *Siphonaria concinna* represented in the tropical and subtropical marine biogeographic provinces. Each lnML value is reported as the mean (\pm SD) from three runs

Dispersal direction	Mean lnML (\pm SD)	ln Bayes factor	Model choice	Model probability
Bidirectional	-2216.54 (\pm 0.86)	0.00	1 (best)	0.998
Northward	-2234.47 (\pm 2.03)	-17.92	3	0.000
Southward	-2223.00 (\pm 0.92)	-6.46	2	0.002
Panmixia	-2271.12 (\pm 3.37)	-54.47	4	0.000

Bayes factors were calculated by subtracting the Bézier-corrected lnML of the model with the least negative lnML value (bidirectional dispersal) from the lnML of each the other models (northward dispersal, southward dispersal and panmixia). Values smaller than -2 suggest preference for a bidirectional dispersal scenario, whereas values >2 would indicate support for alternative dispersal directions or panmixia (Kass & Raftery 1995). *Siphonaria nigerrima* and *S. capensis* were not analysed because in the former, the two regional lineages identified shared no haplotypes and in the latter, a single lineage was found.

The population in the subtropical province has low genetic diversity and a smaller effective population size, and no evidence for gene flow between the provinces was found. *Siphonaria nigerrima* occurs only in the northern portion of the subtropical province, even though there is ample rocky shore habitat to the south of its southern distribution limit, and it was also absent from two sites in the northern subtropical province (Table 1, Fig. 1). This suggests that the subtropical lineage of *S. nigerrima* was founded by small numbers of individuals from the north, that it was subsequently effectively isolated from its source population and that it has not fully established itself in the subtropical province, perhaps because of its low dispersal potential or because it is not well adapted to tolerate cooler environmental conditions in this habitat.

The extant haplotypes of Lineage 1 of *S. concinna* (which is present in both provinces) coalesce later than those of Lineage 2 (which is confined to the subtropical province), possibly indicating that this species also originated in the tropical province and established itself in the subtropical province more recently. A southward range expansion of *S. concinna* into what is today the subtropical province may have occurred during one of several interglacial phases during the late Pleistocene, when intensification of the warm Agulhas Current increased sea surface temperatures throughout much of southern Africa (Crowley & North 1991; Flores *et al.* 1999).

Divergence driven by climate oscillations

Evidence that lineages associated with a specific marine biogeographic province gave rise to new lineages that became established in a different province indicates range expansion or colonisation during one climatic phase, followed by divergence during a subsequent climatic phase. The mechanism driving divergence in the two species of *Siphonaria* may have been analogous

to that reported for the southern African snail *Nassarius kraussianus*, a rare example of a coastal species of which fossils from the last glacial phase have been found. This snail underwent a range expansion from the subtropical province to the region's temperate provinces during the previous interglacial (Teske *et al.* 2007d, 2011a), approximately 116–127 thousand years ago (Kaspar *et al.* 2005). Unlike other warm-water species whose fossil records indicate simultaneous range expansions (Tankard 1975), the range of *N. kraussianus* did not contract during the subsequent glacial interval (shells of this species were used as ornaments by humans during this time, d'Errico *et al.* 2005), suggesting that adaptation to cooler water temperatures must have occurred. The populations associated with the different provinces eventually evolved into two distinct genetic lineages that are today associated with the temperate and subtropical/tropical provinces, respectively.

Although the fossil record for subtropical, and particularly tropical, southern African marine molluscs is comparatively poor, reconstructions of sea surface temperatures during glacial and interglacial phases indicate that the ranges of these species would have shifted substantially in response to climate oscillations. During the Last Glacial Maximum, sea surface temperatures in the study area were approximately 4 °C lower (Prell *et al.* 1980), a difference that is comparable to the temperature difference between the tropical and subtropical provinces. The Agulhas Current was significantly weaker during the summer months and may have been completely replaced by cooler waters during the winter months, allowing subtropical species assemblages to dominate what is today the southern portion of the tropical province (Hutson 1980). A possible scenario explaining divergence in *S. concinna* as a result of climate oscillations is illustrated in Fig. 5. The species was originally absent from the subtropical province (Fig. 5a). Following a southward

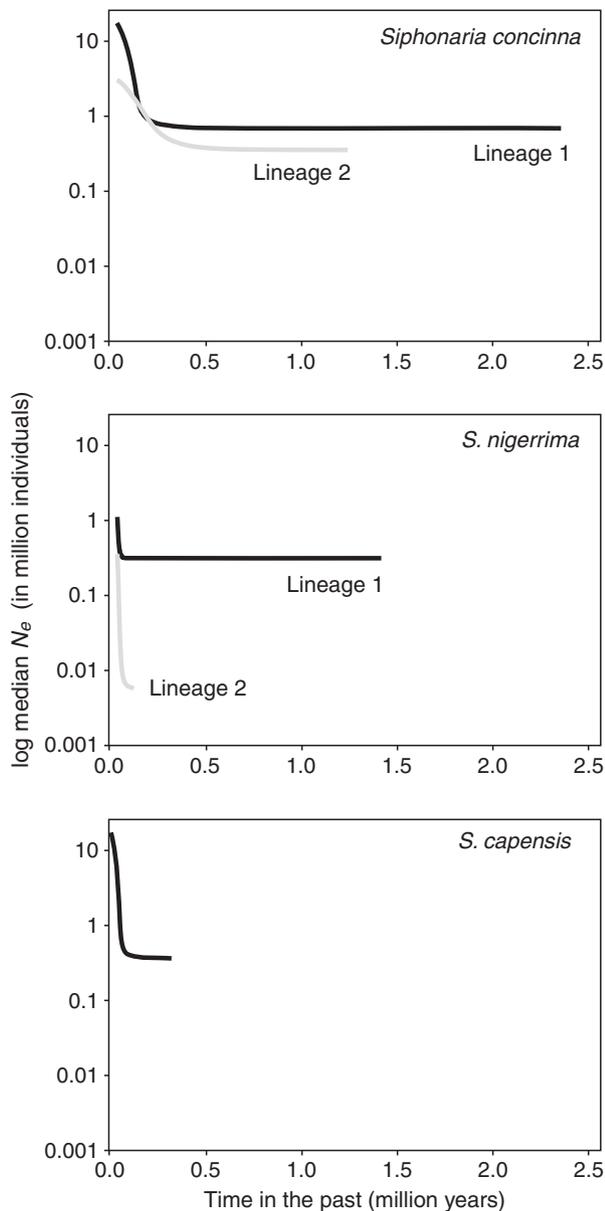


Fig. 4 Extended Bayesian Skyline Plots depicting changes in effective population size as a function of time in five evolutionary lineages of the limpet genus *Siphonaria*.

range expansion during an interglacial phase into what is today the southern portion of the tropical province (Fig. 5b), the population in the south of the species' range may have undergone a selective sweep when conditions again became subtropical during the subsequent glacial phase, resulting in the evolution of two lineages (Fig. 5c). Intensified flow of the Agulhas Current during subsequent interglacials would have facilitated southward dispersal. Much of what is today the subtropical province may initially have been a hybrid zone (Fig. 5d), but low gene flow and selection against the small number of migrants from the tropi-

cal province during one or more subsequent glacial phases (Fig. 5e) would have completed the process of evolutionary divergence. The fact that no hybrids of the two lineages of *S. concinna* were found indicates that, despite southward gene flow during subsequent interglacials (Fig. 5f), the two lineages are today reproductively isolated. Under the scenario described above, one would not expect new lineages to arise during every climate oscillation. Once a lineage has adapted to environmental conditions in a particular biogeographic province, new arrivals from the sister lineage would be unlikely to establish themselves because they would compete for the same ecological niche.

Our data can only provide rough estimates of the times at which divergence and population expansions occurred, as demographic estimates were based on only two loci so that confidence intervals were very wide. Our estimate for the time to the most recent common ancestor of Lineage 2 of *S. concinna* considerably predates the estimate for the divergence of the species' two lineages, but even if our assumption that Lineage 1 is older and gave rise to Lineage 2 is correct, the time to the most recent ancestor of Lineage 2 does not indicate when this split occurred. Unlike the result from *IMa*, this estimate does not incorporate information on ancestral polymorphism and postdivergence gene flow (Arbogast *et al.* 2002).

In summary, we consider periodic strengthening and weakening of the Agulhas Current, and the associated shifts in the position of the tropical/subtropical marine biogeographic disjunction, as the most probable mechanism driving divergence and creating phylogeographic structure in species with high dispersal potential along the southeast African coast.

The evolution of the phylogeographic break in *S. nigerrima* may also have been linked to climate oscillations and associated shifts in the location of the biogeographic disjunction. However, this species' low dispersal ability, and the fact that it is not present throughout much of the subtropical province, suggest that selection against migrants was probably less important in driving divergence. A single long-distance colonisation event (Fig. 5h), and the subsequent isolation of the resulting two populations because of the existence of long stretches of sandy beach that represents habitat unsuitable for intertidal rocky shore limpets (Fig. 5i,j), would have been sufficient to complete this process.

The potential roles of gene flow and divergent selection

Hypotheses that explain the evolution of phylogeographic breaks in areas where populations of marine

organisms with high dispersal potential have not been completely isolated for long periods of time have invoked either incomplete dispersal barriers or the structuring effects of environmental discontinuities (Pelc *et al.* 2009; Teske *et al.* 2011a). Below, we discuss the potential role of these two mechanisms to explain why a phylogeographic break between tropical and subtropical populations was identified in one planktonic disperser, *S. concinna*, but not the other, *S. capensis*.

Larval dispersal. The propagule duration of siphonariid veliger larvae differs considerably among species. Some larvae remain pelagic for only a few days (Olivier & Penchaszadeh 1968), while others settle after several weeks (Creese 1980). A much shorter larval dispersal phase in *S. concinna*, coupled with various factors that have at least temporarily reduced gene flow between the regions (e.g. weakening of the Agulhas Current during glacial phases, loss of recruitment because of advection of larvae away from the coast, upwelling in the northern portion of the Natal Bight and larval retention in the nearshore area) may have resulted in both divergence by drift, and in regional adaptations becoming fixed. The fact that the eggs of *S. concinna* are larger than those of *S. capensis* (Chambers & McQuaid 1994a) indicates that the larvae of the former are likely to settle more quickly so that the potential for long-distance dispersal is reduced.

Divergent selection. Although localised barriers to dispersal such as upwelling cells and currents are often invoked to explain how phylogeographic breaks are maintained (e.g. Wares 2002; Waters & Roy 2004), not all marine biogeographic disjunctions are as well-

defined as the disjunction near Cape St Lucia. Phylogeographic breaks associated with biogeographic transition zones, in which the ranges of distinct evolutionary lineages overlap over vast areas and are thus unlikely to be strongly affected by localised barriers, have been identified in southern California (Seapy & Littler 1980), Florida (Briggs 1974) and elsewhere in southern Africa (Teske *et al.* 2007c, 2008; Zardi *et al.* 2007). The highly porous nature of many dispersal barriers, and the evidence for adaptive differentiation linked to environmental gradients in sister lineages of several planktonic dispersers found in adjacent southern African marine biogeographic provinces (Teske *et al.* 2008, 2009a, Zardi *et al.* 2011), support the idea that selection against immigrants from adjacent provinces is important in maintaining distinct regional genetic lineages. For example, unlike the larvae of the temperate lineage of the prawn *Upogebia africana*, those of the species' subtropical lineage cannot complete development at temperatures typical of the warm-temperate province during winter (Teske *et al.* 2008), and adults of the subtropical lineage of the mussel *Perna perna* can tolerate desiccation stress and sand inundation better than those of their temperate sister taxon (Zardi *et al.* 2011).

Slightly different habitat requirements between *S. concinna* and *S. capensis* could have affected the recruitment success of immigrants from the other province. *Siphonaria capensis* is particularly common in rock pools, whereas *S. concinna* is more prevalent on horizontal rocks (Gray & Hodgson 1997). The latter is thus more likely to be affected by heat and desiccation stress, selective forces that differ significantly between the tropical and subtropical provinces.

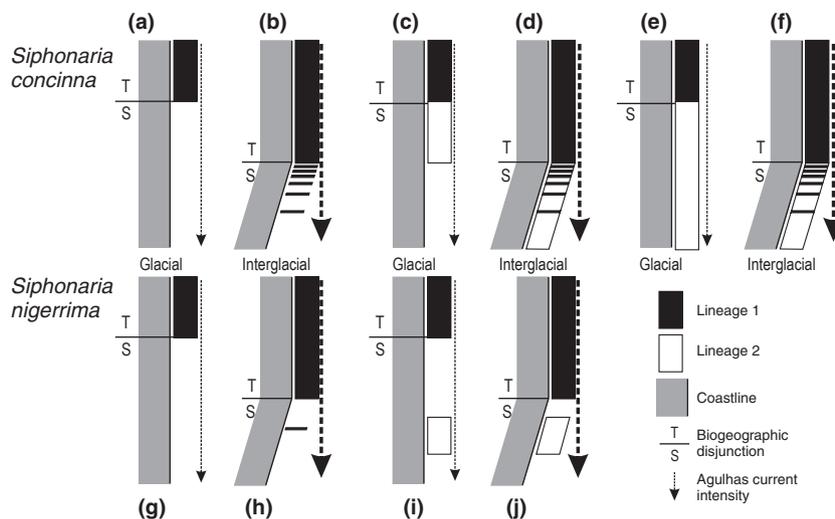


Fig. 5 Scenarios explaining how phylogeographic breaks in the planktonic disperser *Siphonaria concinna* and the direct developer *S. nigerrima* may have evolved. It is assumed that during glacial maxima, the coastline followed what is today the edge of the Natal Bight (T = tropical province, S = subtropical province).

Lack of genetic structure in recently expanded populations

Although there are some reasonable hypotheses explaining the presence of a phylogeographic break in *Siphonaria concinna* and the lack of structure in *S. capensis*, it is important to note that the two species should perhaps not be directly compared because they have different demographic histories. Panmixia is often interpreted as an indication of high gene flow (e.g. Nóbrega *et al.* 2004; Neethling *et al.* 2008; Ayre *et al.* 2009), but genetic homogeneity can also arise as a result of a recent range expansion or a colonisation event (Cunningham & Collins 1998). In species whose haplotype networks show clear signatures of recent demographic expansion (i.e. a star phylogeny), genetic differentiation across marine biogeographic disjunctions may not yet be detectable using DNA sequence data from a small number of loci. Indeed, the Extended Bayesian Skyline Plots indicate that, while *S. concinna* has long been represented in both subtropical and tropical southeastern Africa, *S. capensis* is likely to be a comparatively recent addition to the rocky shore fauna of this region. A recent range expansion may also explain why the ascidian *Pyura herdmani* (a poor disperser whose larvae remain in the water column for less than a day) does not have a phylogeographic break across the St Lucia marine biogeographic disjunction (Teske *et al.* 2011b). The absence of phylogeographic breaks identified in all planktonic dispersers studied thus far, except *S. concinna*, may thus be an artefact of the genetic markers used having insufficient power to detect departures from panmixia. We conclude that lack of genetic structure across marine biogeographic disjunctions in southern Africa and elsewhere may require re-analysis using neutral genetic markers more suitable to study recent demographic events (i.e. microsatellites), in conjunction with physiological experiments or genomic approaches for detecting signatures of adaptive differentiation.

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Data accessibility

DNA sequences: GenBank accession JN603047–JN603179. Final DNA sequence assembly uploaded as online supplemental material.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 GenBank accession numbers of individual specimens of three southern African limpets of the genus *Siphonaria*.

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