Vol. 539: 153–163, 2015
doi: 10.3354/meps11516

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 12

Passive dispersal *against* an ocean current

Peter R. Teske*, Sophie Bader, Tirupathi Rao Golla

Molecular Zoology Laboratory (Aquatic Division), Department of Zoology, University of Johannesburg, Auckland Park 2006, South Africa

ABSTRACT: Boundary currents are spectacular oceanographic features whose role as the main drivers of dispersal is often taken for granted. However, numerous genetic studies of passively dispersing coastal species have challenged this idea, and have identified gene flow in the direction opposite to the currents, pointing to a role of nearshore circulation in facilitating dispersal. We explored the influence of the Agulhas Current in eastern South Africa on mitochondrial DNA gene flow in the rocky shore limpet *Siphonaria serrata*. Our study design was particularly suitable to address this issue because (1) levels of genetic structure are high, suggesting that migrants should be readily distinguishable from native individuals, and (2) the Agulhas Current flows very close to the coast in parts of the study area, and inshore dispersal in the opposite direction is unlikely. We identified a northern and a southern lineage, and although evidence for southward dispersal was particularly strong, gene flow analyses also inferred some northward dispersal. An investigation of this result revealed that the single haplotype responsible for this finding could not be clearly assigned to either lineage, and the northward dispersal scenario is thus questionable. We conclude that genetically inferred dispersal may not be biologically meaningful when lineage sorting among regional populations is incomplete and suggest that this was a problem in most previous studies investigating gene flow in southern African coastal organisms. Despite their considerable potential, genetic methods have so far contributed little towards clarifying the role of nearshore circulation in facilitating population connectivity.

KEY WORDS: Agulhas Current · Asymmetrical gene flow · Coalescent framework · Direct developer · Indian Ocean · Limpet · Phylogeography · *Siphonaria serrata* · Southern Africa

INTRODUCTION

Boundary currents are ocean currents whose flow is determined by the presence of a coastline, and it is often taken for granted that they are the main drivers of population connectivity in coastal species (e.g. Rothlisberg & Church 1994, Heath et al. 1998, Roughan et al. 2011). However, there is increasing evidence that comparatively weak ocean circulation inshore of the boundary currents also plays a significant role in dispersal, particularly when the boundary currents flow at a great distance from the coast (Teske et al. 2015). The Agulhas Current in southeastern Africa is one of the world's 5 western boundary currents, which are found off the east coasts of continents and transport warm water poleward at high velocities (Garrison 2011). It strongly influences the shelf waters of the eastern South African coastline as it follows the narrow continental shelf (Lutjeharms 2006). While direct observations indicate that the current plays an important role in dispersing the early life history stages of fishes and invertebrates (Beckley 1995, Groeneveld & Branch 2002), there are also examples of dispersal in the direction opposite to the Agulhas Current, but all are from actively dispersing species such as rock lobsters (Groeneveld & Branch 2002), sardines (Roberts et al. 2010) and dolphins (Mendez et al. 2011).

In species in which direct observations are not possible, either because the dispersing life history stages are too small to be tagged (as in planktonic dispersers) or because dispersal events are exception-

*Corresponding author: pteske101@gmail.com

ally rare (as in passively dispersing direct developers), population connectivity can be studied by means of indirect estimates. These include inferences based on larval biology (Bradbury et al. 2008), the genetic structure of adults (Palumbi 2001) and oceanography (Largier 2003, Zardi et al. 2011). While ample oceanographic data are available on the Agulhas Current, only a small fraction of studies have focused on the region's complex inshore circulation (Roberts et al. 2010, Jackson et al. 2012). Passive dispersal in a north-easterly direction may be facilitated by both wind-forced nearshore currents (Schumann 1987) and high velocity inshore counter-currents that arise temporarily at various locations along the coastline (Roberts et al. 2010). However, given the limited research conducted, it remains possible that unknown ocean circulation patterns strongly influence dispersal. In addition to uncertain oceanography, information on larval biology is insufficient to study population connectivity because dispersal cannot be reliably predicted on the basis of a species' life history (Shanks 2009, Weersing & Toonen 2009), and the arrival of propagules in a new habitat does not imply successful recruitment, e.g. because of selection against migrants (e.g. Teske et al. 2008, Papadopoulos & Teske 2014, Zardi et al. 2011, 2015). Genetic approaches should be particularly suitable to understand the relative importance of boundary currents and nearshore circulation in connecting coastal populations, because they only detect migration when recruitment was successful, and even rare dispersal events, or those facilitated by poorly understood oceanographic features, can be detected when the settlers have subsequently given rise to large numbers of offspring.

Numerous genetic studies employing analyses to study asymmetrical gene flow have challenged the dominant role of the Agulhas Current in connecting coastal populations (Table 1). However, results are inconsistent, with no clear trends in dispersal direction even in species with similar life histories. In most cases, bidirectional gene flow was inferred, but there are also examples of strictly southward dispersal that was likely facilitated by the Agulhas Current (e.g. Teske et al. 2007a, 2011a, Zardi et al. 2015), and strictly unidirectional gene flow in the opposite direction, at least in a portion of a species' range (e.g. von der Heyden et al. 2008, Bester-van der Merwe et al. 2011, Muller et al. 2012, Muteveri et al. 2015). Studies also differed in terms of how populations were defined. Some treated each site as a distinct population, irrespective whether or not these were genetically distinct, and analysed migration only between adjacent sites (e.g. von der Heyden et al. 2008, Muller et al. 2012, Chiazzari et al. 2013, Reynolds et al. 2014, Duncan et al. 2015, Muteveri et al. 2015). Others assigned sites to maximally differentiated regional groups (e.g. Macdonald et al. 2011, Teske et al. 2011a, Zardi et al. 2015), or divided them into geographically contiguous units of approximately equal size (Teske et al. 2007a). In most studies that tested for the presence of genetic structure, none was found.

Table 1. Direction of gene flow inferred for passively dispersing marine animals along the coast of eastern South Africa

Species	Gene flow direction	Genetic structure	Program	Reference
Acropora austera	Bidirectional (mostly southward)	Most sites	MIGRATE-N	Macdonald et al. (2011)
Acropora tenuis[a]	Bidirectional	Some sites	MIGRATE-N	Chiazzari et al. (2013)
Bullia rhodostoma	Bidirectional (mostly northward)	No	MIGRATE-N	Muteveri et al. (2015)
Caffrogobius caffer	Bidirectional	No	MIGRATE-N	Neethling et al. (2008)
Chrysoblephus puniceus[a]	Bidirectional (mostly southward)	No	MIGRATE-N	Duncan et al. (2015)
Clinus cottoides	Northward	No	MIGRATE-N	von der Heyden et al. (2008)
Exosphaeroma hylecoetes	Southward	–	IM	Teske et al. (2007a)
Haliotis midae	Bidirectional	Some sites	MIGRATE-N	Bester-van der Merwe et al. (2011)
Parechinus angulosus	Bidirectional (mostly northward)	Some sites	MIGRATE-N	Muller et al. (2012)
Perna perna	Southward	–	IM	Teske et al. (2007a)
Perna perna[a]	Southward	Yes	MIGRATE-N	Zardi et al. (2015)
Siphonaria concinna[a]	Southward	Yes	IMa	Teske et al. (2011a)
Siphonaria concinna[a]	Bidirectional	Yes	MIGRATE-N	Teske et al. (2011a)
Tetraclita serrata[a]	Bidirectional	No	MIGRATE-N	Reynolds et al. (2014)
Upogebia africana[a]	Southward	–	IM	Teske et al. (2007a)
Upogebia africana	Bidirectional	Yes	LAMARC	Teske et al. (2008)

[a]The sampled range included sites north of the region sampled in the present study

This presents a potential caveat, because alleles that are shared among recently diverged or undifferentiated 'populations' likely reflect ancestral polymorphisms rather than migration (Pinho & Hey 2010, Marko & Hart 2012). Such data sets may thus not contain sufficient signal to distinguish between native individuals and migrants.

In the present study, we illustrate this issue using genetic data from a rocky shore limpet with direct development (i.e. a species whose young hatch fully developed and in most cases remain in the parent habitat). Direct developers have very high levels of genetic population structure because dispersal events are rare, and migrants should thus be readily identifiable because they are genetically distinct from the population into which they recruited. Our results indicate that even highly significant genetic differentiation may not be sufficient to infer dispersal direction when lineage sorting is incomplete. Some haplotypes cannot be assigned to either population with much confidence, and these may be responsible for questionable estimates of gene flow.

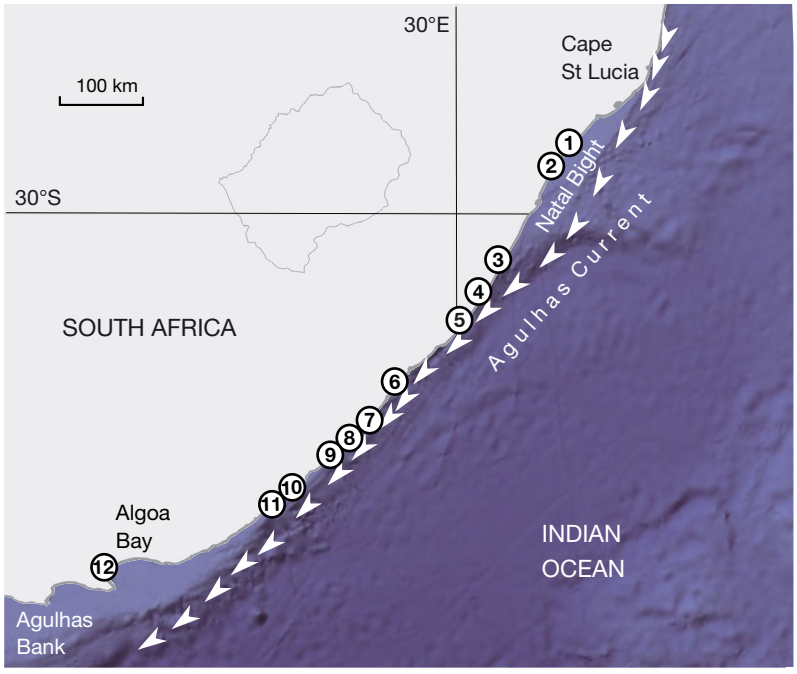

Fig. 1. The path of the Agulhas Current as it follows the edge of the continental shelf of eastern South Africa. White circles shown sampling sites at the following locations (sample sizes in brackets): 1, Nonoti River Mouth (16); 2, Shaka's Rock (39); 3, Umzumbe (35); 4, Port Edward (19); 5, Mbotyi (23); 6, Ku-Mpenzu River Mouth (10); 7, Wavecrest (34); 8, Ngongwana River Mouth (12); 9, Morgan Bay (8); 10, Cintsa (42); 11, Gqunube River Mouth (16); 12, Shark Rock, Port Elizabeth (26)

MATERIALS AND METHODS

Sampling design

The study organism, *Siphonaria serrata* (Fischer von Waldheim, 1807), is one of 6 representatives of the false limpet genus *Siphonaria* in southern Africa, and it is one of 2 species with direct development (Chambers & McQuaid 1994). This endemic species is common on rocky shores throughout the warm-temperate and subtropical marine regions of South Africa, but is absent from both the cool-temperate west coast and the tropical north-east coast (Branch et al. 2010). The fact that warm-temperate and subtropical marine communities differ considerably (Griffiths et al. 2010) and that many species have intraspecific genetic breaks across the boundary between these regions (Teske et al. 2011b) suggests that *S. serrata* may comprise at least 2 distinct regional evolutionary lineages.

Samples were collected at 12 sites along the eastern coastline of South Africa (Fig. 1). This region is particularly suitable to study the importance of boundary currents in facilitating dispersal in coastal species because the Agulhas Current flows very close to the coast as it follows the steep and narrow continental shelf. This is particularly true of the region between Sites 4 and 5 (Fig. 1), where passive dispersal in the opposite direction is unlikely (Schumann 1988, Roberts et al. 2010).

Specimens were stored in ethanol, which was completely replaced over several days until it no longer changed colour. DNA was extracted from foot tissue using the cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle 1990), and a portion of the mitochondrial DNA cytochrome oxidase c subunit I gene (hereafter COI) was amplified using primers LCO1490 and HCO2198 (Folmer et al. 1994). Polymerase chain reactions (PCR) were performed in volumes of 20 µl containing 3 mM $MgCl_2$, 2 µl of 10 × PCR Buffer (Promega), 20 mM of dNTP mixture (Sigma-Aldrich), 0.4 µl of each primer (10 mM), 0.24 µl of bovine serum albumin, 0.5 U of Super-

Therm Taq polymerase (Separation Scientific SA), and 2 to 5 µl of DNA template (~1 to 10 ng μl^{-1}). The cycling profile included an initial denaturation step (3 min at 94°C), 35 to 40 cycles of denaturation (30 s at 94°C), annealing (45 s at 50°C) and extension (45 s at 94°C), and a final extension step (15 min at 94°C). PCR products were purified using the Qiagen QIAquick kit, amplified using BigDye v. 3.1 (Applied Biosystems) and sequenced in both directions on an ABI3730 DNA Analyzer (Applied Biosystems).

Data analyses

Sequences were aligned and edited using the program MEGA v. 6 (Tamura et al. 2013). Genetic structure among pairs of sites was determined by calculating Φ_{ST} values (Excoffier et al. 1992) in Arlequin v. 3.5 (Excoffier & Lischer 2010). To account for multiple comparisons, the false discovery rate method of Benjamini & Yekutieli (2001) was applied. The spatial analysis of molecular variance implemented in SAMOVA v. 1.0 (Dupanloup et al. 2002) was used to identify regional groups of sites that could be treated as distinct populations in subsequent analyses of gene flow. For each number of pre-specified groups, SAMOVA searches for combinations of sites that are maximally differentiated from each other. We ran the program for 2, 3 and 4 groups of sites, and in each case specified 100 simulated annealing processes.

Estimation of gene flow

Asymmetrical gene flow was estimated using 3 different programs. Two of these (MIGRATE-N and IMa2) estimate demographic parameters for predefined populations under a structured-coalescent framework (Beerli & Felsenstein 2001). The third (BEAST) illustrates migration by means of a phylogenetic tree.

MIGRATE-N

The program MIGRATE-N jointly estimates effective population sizes and migration rates among populations. We used MIGRATE-N v. 3.6.8 (Beerli & Felsenstein 2001) to analyse these parameters using sites that were assigned to 2 major regions on the basis of the SAMOVA analyses (see 'Results'). Demographic parameters were recorded every 100 steps out of a total of 10^6 steps, with the first 10^5 steps discarded as burn-in. A static heating scheme with 4 temperatures (1, 1.5, 3 and 10^6) was specified, and suitable upper bounds for population size, θ (minimum: 0, maximum: 0.1, mean: 0.01) and migration rate, *m* (minimum: 0, maximum: 1000, mean: 100) were set after a number of test runs. To ensure consistency of results, each run was repeated with the same settings as above, but with the total number of steps and the burn-in being an order of magnitude greater.

IMa2

The program IMa2 (Hey 2010) implements the isolation-with-migration model of Nielsen & Wakeley (2001). In addition to estimating effective population sizes and migration rates, IMa2 also calculates the divergence time between pre-defined populations, and it provides simple estimates of trends in population size changes by also estimating the effective population sizes of ancestral populations.

The program was run with 150 heated chains and a geometric heating scheme, with the terms of the geometric increment model being ha = 0.96 and hb = 0.9. Suitable priors for demographic parameters were determined after a number of test runs and were set to: divergence time (*t*) = 1.5, effective size of Population 1 (θ_0) = 50, size of Population 2 (θ_1) = 250, size of the shared ancestral population (θ_2) = 500, migration into Population 1 from Population 2 (*m*0 > 1) = 2 and migration into Population 2 from Population 1 (*m*1 > 0) = 2. For each data set, we performed at least 5 independent runs that only differed in terms of their starting seeds, and combined the results of those with effective sample size values greater than 1000 (indicating proper mixing of chains) in 'Load-Trees' mode. To convert scaled model parameter estimates into demographic parameters (including effective female population size N_{ef}, divergence time *T* in 10^3 yr, and the number of migrants per generation, N_{ef}m), a generation time of 1 yr and a conservative COI mutation rate of 1% per 10^6 yr were specified (Meyer et al. 2005, Teske et al. 2011a).

BEAST

The discrete phylogeographic approach (Lemey et al. 2009) implemented in BEAST (Bouckaert et al. 2014) infers the ancestral history of a sample of DNA sequences, and in that way reconstructs what demographic processes have resulted in contemporary genetic patterns (Bloomquist et al. 2010). Inference of

the most likely geographic location of ancestral nodes was used to estimate gene flow. When sampled sequences are in a different location than that inferred for the ancestral clade within which they are nested, then this indicates that they are migrants.

We constructed a maximum clade credibility (MCC) tree in BEAST v. 2.2.1 (Bouckaert et al. 2014), with geographic location states inferred for each node. The specified settings were (1) site model: Hasegawa-Kishino-Yano (HKY) model of nucleotide substitution (Hasegawa et al. 1985), i.e. the model implemented in BEAST that is most similar to the T92+I model (Tamura 1992) for the *S. serrata* data selected using the Bayesian Inference Criterion (Schwarz 1978) in MEGA, with empirical nucleotide frequencies, and both kappa and the substitution rate being estimated; (2) clock model: strict clock, with a mutation rate of 0.5% per site per 10^6 yr, corresponding to a conservative divergence rate of 1% per 10^6 yr for marine invertebrates (e.g. Meyer et al. 2005); and (3) coalescent constant population set as prior. Default settings were used for all other priors. Location within a particular marine biogeographic province (see 'Results') was specified as a discrete trait. The Markov-chain Monte Carlo was run for 5×10^7 generations, with trees stored every 10^4 generations. Convergence was assessed using Tracer v. 1.6. Of the resulting trees, 10% were discarded as burn-in, and MCC trees were drawn in FigTree v.1.4.2.

Gene genealogy reconstructions

As a means of critically assessing how the above gene flow analyses identified migrants, we reconstructed intraspecific genealogical relationships by constructing an outgroup-rooted phylogenetic tree and a haplotype network.

The phylogenetic tree was reconstructed using a maximum-likelihood analysis in MEGA. In addition to including a single representative of each COI haplotype, the data set also included a single sequence each of the Australian congeners *Siphonaria diemenensis* and *S. zelandica* (Colgan & da Costa 2013) as outgroup taxa. A test analysis using all published sequences from the genus revealed that these were the species most closely related to *S. serrata*, even more closely than the other southern African species *S. capensis*, *S. concinna* and *S. nigerrima* (Teske et al. 2011a). The HKY model (including a gamma distribution parameter of 0.24) was selected using the Bayesian Information Criterion in MEGA. Specified settings included partial deletion with a site coverage cut-off of 95%, nearest-neighbour-interchange as the maximum likelihood heuristic tree inference method, a neighbour-joining tree as the initial tree, and the branch swap filter set to 'very strong'. Support for nodes was based on 1000 bootstrap replications (Felsenstein 1985).

The haplotype network was constructed using the reduced median algorithm in Network v. 4.613 (Bandelt et al. 1999). Haplotype networks are particularly useful to infer genealogical relationships among sequences when levels of divergence between populations are low. Phylogenetic trees assume that ancestral haplotypes are extinct, but these are often not only still present in a population, but can even be the most abundant haplotypes (Crandall & Templeton 1993), and networks are suitable to identify them. Furthermore, networks do not assume that genealogical information is strictly bifurcating (Templeton et al. 1992).

RESULTS

A total of 280 COI sequences were generated, which were 486 bp in length, and 45 unique haplotypes were recovered. All sequences were submitted to GenBank (accession numbers KT710202 to KT710481). Levels of genetic structure were high among most pairs of sites (Table 2).

The SAMOVA for 2 groups (Table 3) assigned sites to clusters that were in most cases spatially contiguous, with a northern and a southern group whose boundary is located between Sites 4 and 5. An exception was Site 9, which clustered among the northern sites, potentially because it comprised a large number of migrants from the north. When more than 2 groups were specified, southern Sites 6 and 9 were recovered as being distinct, again a likely result of a comparatively large number of migrants from the north being present at these sites, both of which had small samples sizes. Given that Φ_{CT} was largest for the 2-group arrangement, we analysed gene flow between the northern (Sites 1 to 4) and southern (Sites 5 to 12) region in subsequent analyses, and interpreted the outlier sites as containing a particularly large proportion of migrants.

Estimation of gene flow

All 3 approaches to estimate gene flow identified bidirectional dispersal (Fig. 2; Figs. S1a & S2a in the Supplement at www.int-res.com/articles/suppl/m539p153_supp.pdf), although the IMa2 estimates

Table 2. Genetic structure (Φ_{ST} values) among pairs of sites based on COI sequence data of *Siphonaria serrata*. Site locations are shown in Fig. 1. *p < 0.05 (corrected: p < 0.016), **p < 0.01 (corrected: p < 0.003)

	Sites										
	1	2	3	4	5	6	7	8	9	10	11
2	0.02										
3	0.09	0.04									
4	0.27**	0.21**	0.08								
5	0.55**	0.56**	0.50**	0.37**							
6	0.75**	0.75**	0.71**	0.56**	0.14						
7	0.65**	0.66**	0.62**	0.50**	0.03	0.03					
8	0.81**	0.77**	0.73**	0.60**	0.06	0.02	0.00				
9	0.10	0.05	0.00	0.03	0.28*	0.48**	0.45**	0.55**			
10	0.92**	0.87**	0.86**	0.81**	0.30**	0.31**	0.15**	0.09	0.82**		
11	0.68**	0.68**	0.64**	0.49**	0.04	0.00	0.00	0.00	0.42**	0.22*	
12	0.90**	0.86**	0.84**	0.78**	0.35**	0.38**	0.26**	0.28**	0.78**	0.36**	0.31**

Table 3. Results of SAMOVA analyses on COI sequences of *Siphonaria serrata* from 12 sites in south-eastern Africa

No. of groups	Grouping	Φ_{CT}	p
2	(1,2,3,4,9) (5,6,7,8,10,11,12)	0.65	<0.01
3	(1,2,3,4) (5,6,7,8,10,11,12) (9)	0.64	<0.01
4	(1,2,3,4) (5,7,8,10,11,12) (6) (9)	0.62	<0.01

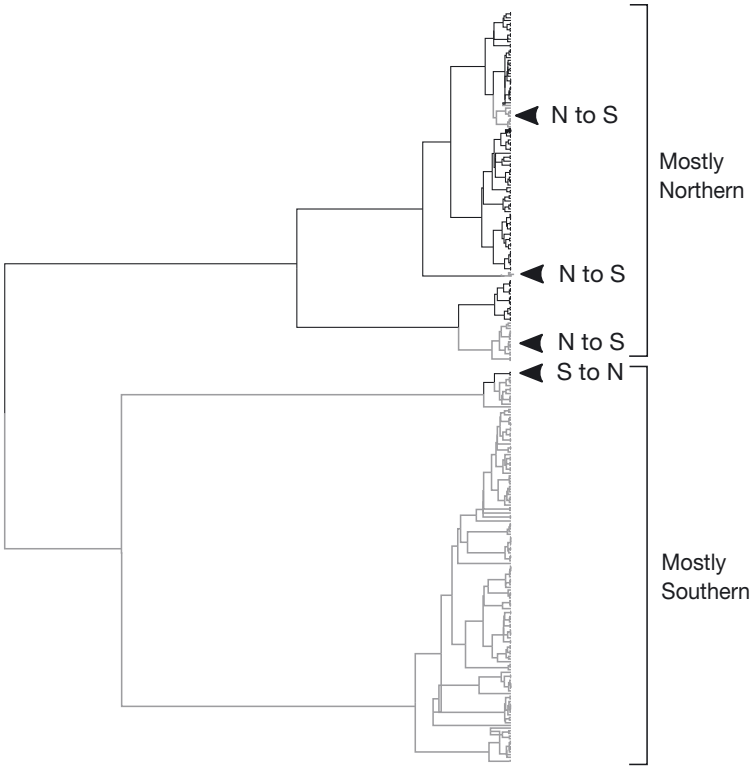

Fig. 2. A maximum clade-credibility tree constructed from COI sequences of *Siphonaria serrata* from eastern South Africa. Black indicates origin in the northern sampling range (Sites 1 to 4) while grey indicates origin in the south (Sites 5 to 12). Arrows indicate migration events (N = north, S = south)

were ambiguous because neither was significantly different from zero (Fig. S2a). The actual migrants could only be identified with BEAST. The MCC tree recovered 2 major lineages, each of which largely comprised individuals from a particular region (Fig. 2). Several clades of the northern lineage included individuals collected in the southern region, indicating multiple southward dispersal events. In only one instance did individuals collected in the northern region cluster within the southern lineage (2 individuals), indicating northward dispersal.

Unlike the MCC tree, the maximum-likelihood tree recovered the clade that pointed to northward dispersal basal relative to a clade comprising northern and southern lineages, rather than nested within the southern lineage (Fig. 3), so this clade cannot be clearly grouped with either lineage. The haplotype network (Fig. 4) supports the notion that it could equally well have originated in the north, as it was equally differentiated from both the ancestral (i.e. most common) northern haplotype and from the ancestral southern haplotype. Moreover, the other haplotype of the clade, which was only found in the south, is the younger of the two (i.e. separated from the network's ancestral haplotypes by even more nucleotide substitutions than the haplotype present in both regions).

To determine whether the problematic clade was responsible for the inference of bidirectional gene flow in the MIGRATE-N and IMa2 analyses, we also ran these programs without the 14 individuals having one of the 2 haplotypes in this clade. Both programs inferred southward gene flow for the reduced data set (Figs. S1b & S2b).

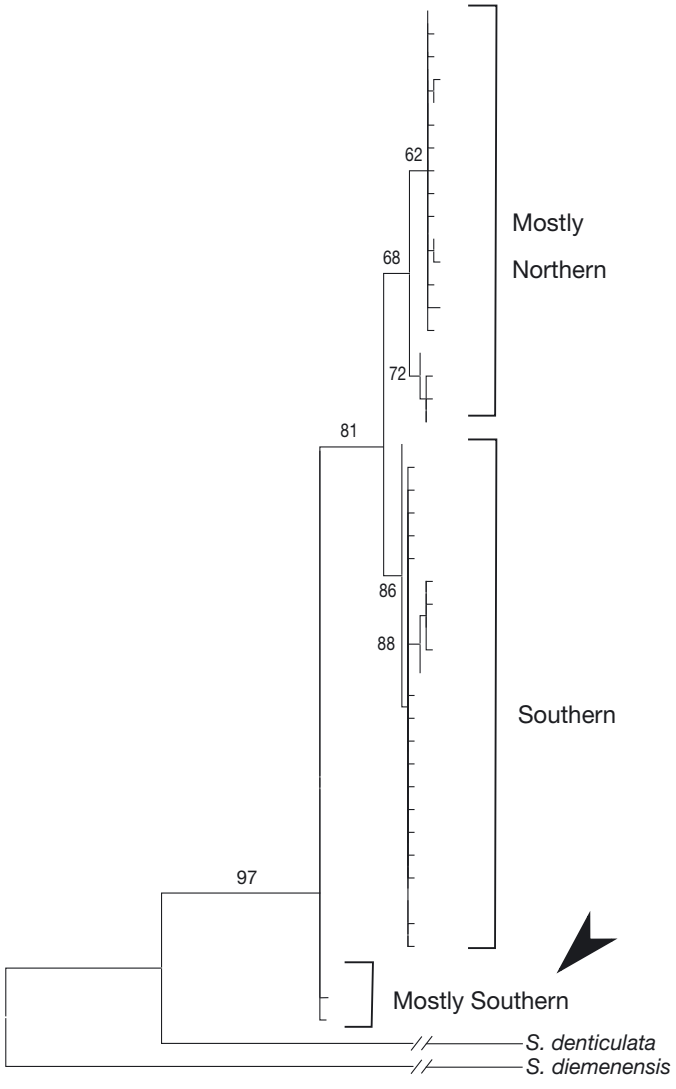

Fig. 3. A maximum-likelihood phylogenetic tree constructed from 45 unique COI haplotypes of *Siphonaria serrata*, with 2 closely related Australian congeners used as outgroup taxa. The arrow points at a clade comprising 2 haplotypes whose ancestral lineage was inferred as being of southern origin in the MCC tree (Fig. 2). Bootstrap values >50% are shown next to some nodes

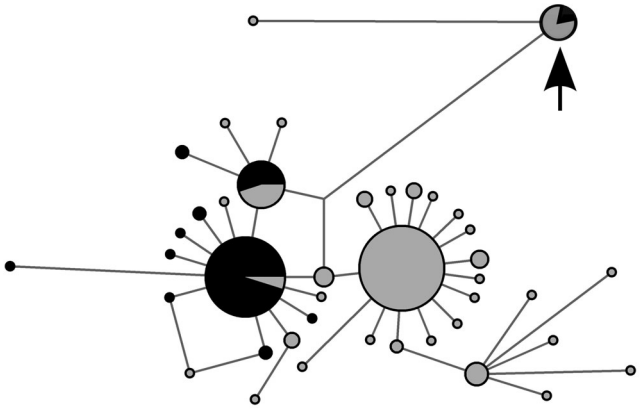

Fig. 4. A haplotype network of *Siphonaria serrata* COI haplotypes. Locations are indicated using colours, with black indicating northern sites (1 to 4) and grey indicating southern sites (5 to 12). The size of circles representing haplotypes is proportional to their frequency, with the smallest circles representing a single individual, and the shortest connections between haplotypes representing a single nucleotide difference. The arrow indicates the only haplotype found in both regions that was more common in the south (all other haplotypes present in both regions were more common in the north)

DISCUSSION

In this study, we assessed the dispersal direction of the direct developer *Siphonaria serrata* in eastern South Africa, a region whose coastline is strongly influenced by the southward-flowing Agulhas Current. All 3 methods of gene flow inference identified bidirectional gene flow, with more southward than northward dispersal. Graphical methods challenged the interpretation of the single haplotype responsible for the inference of northward migration as originating from the south. Instead, this haplotype is part of a somewhat distinct cluster that cannot be clearly assigned to either of the species' main regional evolutionary lineages with much confidence. It is equally possible that it was originally present in the north and subsequently established itself in the south, where it is presently more common (at least in our samples). These results indicate that one cannot blindly rely on the results of coalescent-based estimates of gene flow, and that a careful examination of the data using multiple complementary approaches is required to address the issue whether or not passive dispersal against a boundary current is biologically meaningful.

Lack of population differentiation

Coalescent-based analyses of gene flow are often applied to different sites inhabited by what should in fact be considered one and the same population (e.g. Bester-van der Merwe et al. 2011, Duncan et al. 2015, Muteveri et al. 2015). The inferred bidirectional gene flow may in many cases be explained by the sharing of alleles due to a lack of differentiation. Interestingly, there is little information on what level of differentiation is required to identify migrants by means of genetical methods, an exception being the program BayesAss+ (Wilson & Rannala 2003), for which

an F_{ST} value ≥0.05 is recommended (Faubet et al. 2007). To address the issue of whether or not dispersal can occur against a boundary current, it seems prudent to only study regional populations that are not only genetically differentiated, but in which lineage sorting is complete.

The data set generated for *S. serrata* in the present study, which is based on a single locus and can thus be readily interpreted without the use of sophisticated coalescent-based software, is a borderline example suitable to illustrate this point. Lineage sorting is clearly evident between the 2 regional lineages, but it is not yet complete. There are presently 2 DNA sequence-based studies from eastern South Africa in which lineage sorting was complete, and both reject the idea of passive dispersal against the Agulhas Current (Fig. 1). In both cases, simple haplotype networks clearly depict the direction of gene flow, and challenge the results of coalescent-based analyses. Using the program LAMARC v. 2.02 (Kuhner 2006), Teske et al. (2008) inferred that mudprawns exchanged migrants in both directions, but a haplotype network of the species' completely distinct subtropical and warm-temperate lineages shows that the subtropical lineage was absent from the 2 northernmost sites. This surprising discrepancy was subsequently explained by the fact that all versions of LAMARC prior to version 2.1.8 produced a composite of information about the direction of gene flow, which often reversed the direction of asymmetrical gene flow (M. Kuhner pers. comm.). Similarly, haplotype networks in Teske et al. (2011a) showed that the subtropical lineage of the limpet *S. concinna* was absent from the tropical province, whereas the tropical lineage was present in both provinces. This result was rejected by MIGRATE-N analysis (which inferred bidirectional gene flow), but confirmed by IMa2 analysis (which inferred strictly southward gene flow).

Choice of genetic markers

While mtDNA continues to be the most commonly used genetic marker in ecological and evolutionary studies (Beheregaray 2008), polymorphic microsatellites are becoming increasingly popular (Selkoe & Toonen 2006). Theoretically, they should be more suitable to study gene flow than is mtDNA, because their much greater information content increases the likelihood that regional evolutionary lineages are genetically distinct, and migrants readily identifiable. While microsatellites have recently proven useful to study the relative importance of boundary currents and nearshore circulation in southern Africa (Zardi et al. 2015) and temperate Australia (Teske et al. 2015), numerous studies have identified mtDNA-based genetic breaks that were not present in the microsatellite data (e.g. Monsen & Blouin 2003, Larmuseau et al. 2010). We therefore believe that mtDNA will continue to be useful to study gene flow direction, provided it is used appropriately.

While the shortcomings of mtDNA-only data sets are well documented (Ballard & Whitlock 2004), the issue of incomplete lineage sorting clearly also affects other marker types, and the inclusion of nuclear markers in the data set per se does not present a universal solution to this problem. For example, von der Heyden et al. (2008), Muller et al. (2012) and Reynolds et al. (2014) used a combination of mtDNA and nuclear sequence data (introns or ITS), while Bester-van der Merwe et al. (2011) and Duncan et al. (2015) used polymorphic microsatellites, and all inferred bidirectional gene flow among mostly non-differentiated populations. One area where microsatellites are superior is in determining the timing of migration, so as to distinguish historical gene flow from near-contemporary migration, but this depends on the software used. Strasburg & Rieseberg (2011) considered the migration time estimates produced by IMa (an earlier version of IMa2) to be unreliable, and we have refrained from going into detail about these and the divergence time estimates primarily because of the present controversy whether or not time-dependent dating should be applied to sequence data (Ho et al. 2011, Emerson & Hickerson 2015). Analyses based on DNA sequences are typically used to infer demographic events that occurred during the Pleistocene or earlier (Mmonwa et al. 2015). The Agulhas Current weakened considerably during glacial phases (Hutson 1980), and it is likely that northward dispersal was particularly common during that time. Hence, even if northward gene flow was inferred between 2 completely distinct evolutionary lineages, it would not necessarily reflect the impact of contemporary oceanography. Questionable dating is not an issue in assignment tests for microsatellite data, such as BayesAss+ (Wilson & Rannala 2003), which identifies migrants that arrived in a population no more than 2 generations ago. Such tests are clearly more suitable than the coalescent-based or tree-based approaches used in the present study to confirm that migration was facilitated by contemporary oceanographic conditions, rather than the very different conditions during previous glacial and interglacial phases.

The Agulhas Current as a dispersal barrier

The genetic discontinuity identified in *S. serrata* coincides with a biogeographic disjunction between South Africa's warm-temperate marine biogeographic province and the subtropical province (Emanuel et al. 1992). The entire coastline between this point and Algoa Bay (Fig. 1) is often considered a transition zone (Teske et al. 2011b). Phylogeographic breaks may be present anywhere in this region, and the ranges of the geminate sister lineages often overlap (e.g. Teske et al. 2007b, 2008, von der Heyden 2009, Mmonwa et al. 2015). Although Langrangian particle simulations indicate that northward dispersal close to the coast is possible in the southern portion of the transition zone (Assis et al. 2015), there is so far no evidence that this can displace passively dispersing coastal organisms beyond the phylogeographic break of *S. serrata*, which is located in the far north of the transition zone. The Agulhas Current flows particularly close to the coast from about Port Edward (Site 4) southwards, accelerates, and causes nearshore currents to flow parallel to it (Schumann 1988). The retention of passively dispersing species is only possible when these remain in the nearshore area, where some small-scale northward displacement is facilitated by wind-driven currents, but it is unlikely that this drives northward migrations over greater distances. There is also no evidence for temporary counter-currents that might facilitate northward dispersal in the area around Port Edward, although such a reversal of surface flow has been reported just south of Mbotyi (Site 5) (Roberts et al. 2010). The location of the phylogeographic break is thus explained by contemporary oceanography, and suggests that the strong influence of the Agulhas Current on the coast presents a significant barrier to the northward migration of passively dispersing coastal species.

CONCLUSION

Genetic markers can be a powerful tool to study the impact of ocean currents on the dispersal of coastal species. While microsatellites are in most cases indispensable to studying gene flow in high-dispersal species in which levels of genetic structure are very low (Coleman et al. 2013, Teske et al. 2015, Zardi et al. 2015), we believe that mtDNA sequences will continue to provide useful information on dispersal in species that are highly structured, provided they are only used in cases where shared ancestral polymorphism between regional sister lineages can be ruled out. We concede that genetic methods have at this stage contributed little to understanding southern Africa's oceanography, but their correct application, in conjunction with new methods, should remedy this situation in the near future.

Acknowledgements. We are grateful to Nadine Strydom, Anusha Rajkaran and Isabelle Papadopoulos for providing samples of *S. serrata*. Two anonymous reviewers are gratefully acknowledged for their comments on an earlier version of this manuscript. This study was funded by the PADI Foundation (Grant No. 10981 awarded to P.R.T.) and the University of Johannesburg (Spectrum). T.R.G. gratefully acknowledges the NRF (CSUR Grant No. 87702) and the University of Johannesburg's Global Excellence and Stature (GES) program for financial support.

LITERATURE CITED

Assis J, Zupan M, Nicastro KR, Zardi GI, McQuaid CD, Serrão EA (2015) Oceanographic conditions limit the spread of a marine invader along southern African shores. PLoS ONE 10:e0128124

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. Mol Ecol 13:729–744

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Beckley LE (1995) The Agulhas Current ecosystem with particular reference to dispersal of fish larvae. In: Okemwa E, Ntiba MJ, Sherman K (eds) Status and future of large marine ecosystems of the Indian Ocean. A report of an international symposium and workshop. IUCN, Gland, p 255–276

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98:4563–4568

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. Mol Ecol 17:3754–3774

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Ann Stat 29:1165–1188

Bester-van der Merwe AE, Roodt-Wilding R, Volckaert FA, D'Amato ME (2011) Historical isolation and hydrodynamically constrained gene flow in declining populations of the South-African abalone, *Haliotis midae*. Conserv Genet 12:543–555

Bloomquist EW, Lemey P, Suchard MA (2010) Three roads diverged? Routes to phylogeographic inference. Trends Ecol Evol 25:626–632

Bouckaert R, Heled J, Kühnert D, Vaughan T and others (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput Biol 10:4:e1003537

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE (2008) Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc R Soc B 275:1803–1809

Branch GM, Griffiths CL, Branch ML, Beckley LE (2010) Two oceans: a guide to the marine life of Southern Africa. Struik Nature, Cape Town

Chambers RJ, McQuaid CD (1994) A review of larval development in the intertidal limpet genus *Siphonaria* (Gastropoda: Pulmonata). J Molluscan Stud 60:415–423

Chiazzari B, Macdonald A, Schleyer M (2013) High-latitude connectivity of the scleractinian coral *Acropora tenuis* in the south-western Indian Ocean, identified using nuclear intron and mitochondrial sequence data. Afr J Mar Sci 35:233–241

Coleman MA, Feng M, Roughan M, Cetina-Heredia P, Connell SD (2013) Temperate shelf water dispersal by Australian boundary currents: implications for population connectivity. Limnol Oceanogr Fluids Environ 3: 295–309

Colgan DJ, da Costa P (2013) Possible drivers of biodiversity generation in the *Siphonaria* of southeastern Australia. Mar Biodiv 43:73–85

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134: 959–969

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Duncan M, James N, Fennessy ST, Mutombene RJ, Mwale M (2015) Genetic structure and consequences of stock exploitation of *Chrysoblephus puniceus*, a commercially important sparid in the South West Indian Ocean. Fish Res 164:64–72

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. Mol Ecol 11:2571–2581

Emanuel BP, Bustamante RH, Branch GM, Eekhout S, Odendaal FJ (1992) A zoogeographic and functional approach to the selection of marine reserves on the west coast of South Africa. S Afr J Mar Sci 12:341–354

Emerson BC, Hickerson MJ (2015) Lack of support for the time-dependent molecular evolution hypothesis. Mol Ecol 24:702–709

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10: 564–567

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Faubet P, Waples RS, Gaggiotti OE (2007) Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. Molec Ecol 16:1149–1166

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Garrison T (2009) Essentials of oceanography. Brooks/Cole Cengage Learning, Belmont, CA

Griffiths CL, Robinson TB, Lange L, Mead A (2010) Marine biodiversity in South Africa: an evaluation of current states of knowledge. PLoS One 5:e12008

Groeneveld JC, Branch GM (2002) Long-distance migration of South African deep-water rock lobster *Palinurus gilchristi*. Mar Ecol Prog Ser 232:225–238

Hasegawa M, Kishino H, Yano TA (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174

Heath M, Zenitani H, Watanabe Y, Kimura R, Ishida M (1998) Modelling the dispersal of larval Japanese sardine, *Sardinops melanostictus*, by the Kuroshio Current in 1993 and 1994. Fish Oceanogr 7:335–346

Hey J (2010) Isolation with migration models for more than two populations. Mol Biol Evol 27:905–920

Ho SY, Lanfear R, Bromham L, Phillips MJ, Soubrier J, Rodrigo AG, Cooper A (2011) Time-dependent rates of molecular evolution. Mol Ecol 20:3087–3101

Hutson WH (1980) The Agulhas Current during the late Pleistocene: analysis of modern faunal analogs. Science 207:64–66

Jackson JM, Rainville L, Roberts MJ, McQuaid CD, Lutjeharms JRE (2012) Mesoscale bio-physical interactions between the Agulhas Current and the Agulhas Bank, South Africa. Cont Shelf Res 49:10–24

Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22:768–770

Largier JL (2003) Considerations in estimating larval dispersal distances from oceanographic data. Ecol Appl 13: 71–S89

Larmuseau MHD, Raeymaekers JAM, Hellemans B, Van Houdt JKJ, Volckaert FAM (2010) Mito-nuclear discordance in the degree of population differentiation in a marine goby. Heredity 105:532–542

Lemey P, Rambaut A, Drummond AJ, Suchard MA (2009) Bayesian phylogeography finds its roots. PLOS Comput Biol 5:e1000520

Lutjeharms JRE (2006) The Agulhas Current. Springer, Heidelberg

Macdonald AH, Schleyer MH, Lamb JM (2011) *Acropora austera* connectivity in the south-western Indian Ocean assessed using nuclear intron sequence data. Mar Biol 158:613–621

Marko PB, Hart MW (2012) Retrospective coalescent methods and the reconstruction of metapopulation histories in the sea. Evol Ecol 26:291–315

Mendez M, Subramaniam A, Collins T, Minton G and others (2011) Molecular ecology meets remote sensing: environmental drivers to population structure of humpback dolphins in the Western Indian Ocean. Heredity 107: 349–361

Meyer CP, Geller JB, Paulay G (2005) Fine scale endemism on coral reefs: archipelagic differentiation in turbinid gastropods. Evolution 59:13–125

Mmonwa KL, Teske PR, McQuaid CD, Barker NP (2015) Historical demography of southern African patellid limpets: congruence of population expansions, but not phylogeography. Afr J Mar Sci 37:11–21

Monsen KJ, Blouin MS (2003) Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. Mol Ecol 12:3275–3286

Muller CM, von der Heyden S, Bowie RC, Matthee CA (2012) Oceanic circulation, local upwelling and palaeoclimatic changes linked to the phylogeography of the Cape sea urchin *Parechinus angulosus*. Mar Ecol Prog Ser 468:203–215

Muteveri T, Matthee CA, Bowie RCK, von der Heyden S (2015) High population connectivity and Pleistocene range expansion in the direct-developing plough shell *Bullia rhodostoma* along the South African coast. Afr J Mar Sci 37:21–31

Neethling M, Matthee CA, Bowie RCK, von der Heyden S (2008) Evidence for panmixia despite barriers to gene

flow in the southern African endemic, *Caffrogobius caffer* (Teleostei: Gobiidae). BMC Evol Biol 8:325

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158:885–896

Palumbi SR (2001) The ecology of marine protected areas. In: Bertness M, Gaines SD, Hay ME (eds) Marine ecology: the new synthesis. Sinauer, Sunderland, MA, p 509–530

Papadopoulos I, Teske PR (2014) Larval development reflects biogeography in two formerly synonymised southern African coastal crabs. Afr J Aquat Sci 39: 347–350

Pinho C, Hey J (2010) Divergence with gene flow: models and data. Annu Rev Ecol Evol Syst 41:215–230

Reynolds TV, Matthee CA, von der Heyden S (2014) The influence of Pleistocene climatic changes and ocean currents on the phylogeography of the Southern African barnacle, *Tetraclita serrata* (Thoracica; Cirripedia). PLoS ONE 9:e102115

Roberts MJ, van der Lingen CD, Whittle C, Van den Berg M (2010) Shelf currents, lee-trapped and transient eddies on the inshore boundary of the Agulhas Current, South Africa: their relevance to the KwaZulu-Natal sardine run. Afr J Mar Sci 32:423–447

Rothlisberg PC, Church JA (1994) Processes controlling the larval dispersal and postlarval recruitment of Penaeid prawns. In: Sammarco PW, Heron ML (eds) The biophysics of marine larval dispersal. American Geophysical Union, Washington, DC, p 235–252

Roughan M, Macdonald HS, Baird ME, Glasby TM (2011) Modelling coastal connectivity in a Western Boundary Current: seasonal and inter-annual variability. Deep-Sea Res II 58:628–644

Schumann EH (1987) The coastal ocean off the east coast of South Africa. Trans R Soc S Afr 46:215–229

Schumann EH 1988. Physical oceanography off Natal. In: Schumann EH (ed.) Coastal ocean studies off Natal, South Africa. Lecture notes on coastal and estuarine studies. Springer, New York, NY, p 101–130

Schwarz G (1978) Estimating the dimension of a model. Ann Stat 6:461–464

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Shanks AL (2009) Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373–385

Strasburg JL, Rieseberg LH (2011) Interpreting the estimated timing of migration events between hybridizing species. Mol Ecol 20:2353–2366

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Mol Biol Evol 9:678–687

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619–633

Teske PR, Papadopoulos I, Zardi GI, McQuaid CD, Edkins MT, Griffiths CL, Barker NP (2007a) Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. Mar Biol 152:697–711

Teske PR, Papadopoulos I, McQuaid CD, Newman BK, Barker NP (2007b) Climate change, genetics or human choice: Why were the shells of mankind's earliest ornament larger in the Pleistocene than in the Holocene? PLoS ONE 2:e614

Teske PR, Papadopoulos I, Newman BK, Dworschak PC, McQuaid CD, Barker NP (2008) Oceanic dispersal barriers, adaptation and larval retention: an interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn. BMC Evol Biol 8:341

Teske PR, Papadopoulos I, Mmonwa KL, Matumba T, McQuaid CD, Barker NP, Beheregaray LB (2011a) Climate-driven genetic divergence of limpets with different life histories across a southeast African marine biogeographic disjunction: different processes, same outcome. Mol Ecol 20:5025–5041

Teske PR, von der Heyden S, McQuaid CD, Barker NP (2011b) A review of marine phylogeography in southern Africa. S Afr J Sci 107:43–53

Teske PR, Sandoval-Castillo J, van Sebille E, Water J, Beheregaray LB (2015) On-shelf larval retention limits population connectivity in a coastal broadcast spawner. Mar Ecol Prog Ser 532:1–12

von der Heyden S (2009) Why do we need to integrate population genetics into South African marine protected area planning? Afr J Mar Sci 31:263–269

von der Heyden S, Prochazka Ki M, Bowie RC (2008) Significant population structure and asymmetric gene flow patterns amidst expanding populations of *Clinus cottoides* (Perciformes, Clinidae): application of molecular data to marine conservation planning in South Africa. Mol Ecol 17:4812–4826

Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163: 1177–1191

Zardi GI, Nicastro KR, McQuaid CD, Hancke L, Helmuth B (2011) The combination of selection and dispersal helps explain genetic structure in intertidal mussels. Oecologia 165:947–958

Zardi GI, Nicastro K, McQuaid CD, Castilho R, Costa J, Serrão EA, Pearson GA (2015) Intraspecific genetic lineages of a marine mussel show behavioural divergence and spatial segregation over a tropical/subtropical biogeographic transition. BMC Evol Biol 15:100

Editorial responsibility: Karen Miller, Hobart, TAS, Australia

Submitted: June 1, 2015; Accepted: October 7, 2015
Proofs received from author(s): October 22, 2015