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ORIGINAL ARTICLE

Mitonuclear discordance in genetic structure across the Atlantic/Indian Ocean biogeographical transition zone

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ABSTRACT

Aim Based on mitochondrial DNA (mtDNA) data, many organisms with ranges spanning multiple biogeographical regions exhibit genetic structure across the transition zones between these regions, while others appear to be genetically homogenous. No clear link has been found between the presence or absence of such spatial genetic discontinuities and species' dispersal potential, confounding the formulation of general predictions concerning genetic structure. The fact that discrepancies between mtDNA and nuclear markers are common across semi-permeable barriers suggests that a lack of structure could be attributable to mtDNA-specific properties of inheritance. We re-examined genetic structure in the coastal crab *Hymenosoma orbiculare*, a species that is represented by a single mtDNA lineage across the Atlantic/Indian Ocean biogeographical transition zone, by comparing mtDNA data with nuclear DNA data.

Location South Africa's cool-temperate and warm-temperate marine biogeographical provinces.

Methods DNA sequence data from the mitochondrial *COI* gene and the intron of the nuclear *ANT* gene were generated for 150 individuals of *H. orbiculare*. For each locus, we determined whether the sharing of alleles between provinces was the result of either the retention of ancestral polymorphism or of secondary contact.

Results We recovered two nuclear intron lineages whose spatial genetic structure reflects contemporary biogeographical and oceanographical conditions, indicating that the existence of a single mtDNA lineage is not a function of unexpectedly high levels of dispersal.

Main conclusions MtDNA-based genetic homogeneity is increasingly being reported in coastal organisms with ranges spanning biogeographical transition zones that define distinct evolutionary lineages in other species. Our results stress the importance of revisiting single-locus data sets by means of multilocus genetic approaches before any conclusions can be drawn about the role of biogeographical transition zones in driving genetic structure.

Keywords
Biogeographical disjunction, Brachyura, complete mitochondrial capture, diversifying selection, gene flow, incomplete lineage sorting, introgression, *Hymenosoma orbiculare*, phylogeographical break, South Africa.

INTRODUCTION

Geographical discontinuities in the composition of species assemblages are often mirrored by intraspecific genetic discontinuities, termed phylogeographical breaks, in species that transcend such disjunctions (Benzie, 1999). In coastal areas of the south-eastern and south-western United States (Pelc *et al.*, 2009), western Europe (Patarnello *et al.*, 2007), Chile

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(Brante *et al.*, 2012) and southern Africa (Teske *et al.*, 2011a), phylogeographical breaks in marine organisms have mostly been identified in areas that have strong environmental gradients or in which currents and upwelling cells may hinder along-shore dispersal. Despite evidence that genetic structure along continuous habitats with no dispersal barriers can be determined by life history (e.g. the presence or absence of long-lived planktonic larvae in sessile marine invertebrates; Ayre *et al.*, 1997), no clear link has been found between species' theoretical dispersal potential and the presence or absence of phylogeographical breaks across biogeographical disjunctions, notwithstanding that species with long-lived propagules would be expected to disperse across such disjunctions more frequently (Ayre *et al.*, 2009; Teske *et al.*, 2011a).

Many authors have explained this discrepancy by emphasizing factors other than theoretical dispersal potential, including unexpected larval dispersal distances, habitat specificity and larval retention (Neethling *et al.*, 2008; Ayre *et al.*, 2009). The possibility that the genetic markers used may not provide sufficient signal to identify genetic discontinuities is often not acknowledged, despite many recent examples of differences in levels of introgression and lineage sorting between mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) that can result in the recovery of conflicting phylogeographical patterns (Toews & Brelsford, 2012). In particular, the fact that some species comprise distinct evolutionary lineages defined by strong mtDNA-based genetic structure across biogeographical transition zones while co-occurring species appear genetically homogeneous highlights the need for a careful examination of this problem.

The South African coastline represents a suitable study area in which to test the hypothesis that the lack of genetic structure might be an mtDNA-specific artefact. The region can be divided into at least four temperature-defined marine biogeographical provinces (cool-temperate, warm-temperate, subtropical and tropical; reviewed in Teske *et al.*, 2011a), and phylogeographical breaks of various depths have been reported between all of them. Whereas many species that disperse by means of planktonic larvae are genetically structured across the disjunctions between biogeographical provinces (Teske *et al.*, 2011a), increasing numbers have been identified as having single mtDNA lineages that span two or more provinces.

Here, we report a re-examination of genetic structure in the crab *Hymenosoma orbiculare* Desmarest, 1823, one of the species that are apparently not structured across the disjunction between southern Africa's cool-temperate and warm-temperate provinces, by contrasting sequence data from mtDNA with data from a nuclear intron. Evidence for lineage sorting and secondary contact in the intron – coupled with the retention of ancestral polymorphism in mtDNA – would support our hypothesis that the lack of genetic structure in this species is an artefact of mtDNA-specific properties of inheritance.

MATERIALS AND METHODS

Study taxon

The crown crab, *Hymenosoma orbiculare*, is one of five southern African representatives of the genus *Hymenosoma* (Dawson & Griffiths, 2012), and is one of the most abundant coastal decapod crustaceans in the region's cool-temperate and warm-temperate biogeographical provinces. Although the species' planktonic dispersal phase is comparatively short (*c.* 20 days at 24 °C) because of abbreviated development (it has three passively dispersing zoeal stages but lacks the actively swimming megalopa stage; Papadopoulos *et al.*, 2006), the amount of gene flow between sites located within marine biogeographical regions appears to be high, as genetic structure does not follow the pattern of isolation by distance that has been reported for species with direct development (Teske *et al.*, 2007a).

Sampling and laboratory procedures

Five sampling sites were selected on the basis of distribution data for *H. orbiculare* and previously published genetic data on the ranges of evolutionary lineages of temperate coastal invertebrates (reviewed in Teske *et al.*, 2011a). Two of these are located in the cool-temperate province (sites 1 and 2, Fig. 1, Table 1), two are at the western and eastern extremes of the warm-temperate province (sites 4 and 5), and one is in the biogeographical transition zone between Cape Point and Cape Agulhas (site 3). The species has only been reported in three estuaries in the transition zone (Teske *et al.*, 2007a) and was not found in two of them during the sampling period (January 2011), possibly due to prolonged closure of the mouths of these estuaries.

We amplified portions of the mitochondrial (mtDNA) cytochrome *c* oxidase subunit I gene (*COI*) and the intron of the nuDNA adenine nucleotide transporter gene (*ANT*), as detailed in Appendix S1 (see Supporting Information). Sequences were aligned by eye in Mega 5 (Tamura *et al.*, 2011). Bayesian computational inference of the gametic phases of heterozygous *ANT* intron sequences was performed using the default parameters of the phase 2.1 algorithm (Stephens *et al.*, 2001) implemented in DnaSP 5.10.01 (Librado & Rozas, 2009). The resulting data set of intron sequences was tested for recombination using Recombination Detection Program (RDP3.44, Martin *et al.*, 2010).

Spatial genetic structure

Mitonuclear discordance resulting from the retention of ancestral polymorphism is not expected to manifest itself in predictable biogeographical patterns, because alleles that were present in the common ancestor of two regional populations should be randomly distributed between them (Funk & Omland, 2003). If alleles are primarily shared near known hybrid zones or across biogeographical disjunctions, then this

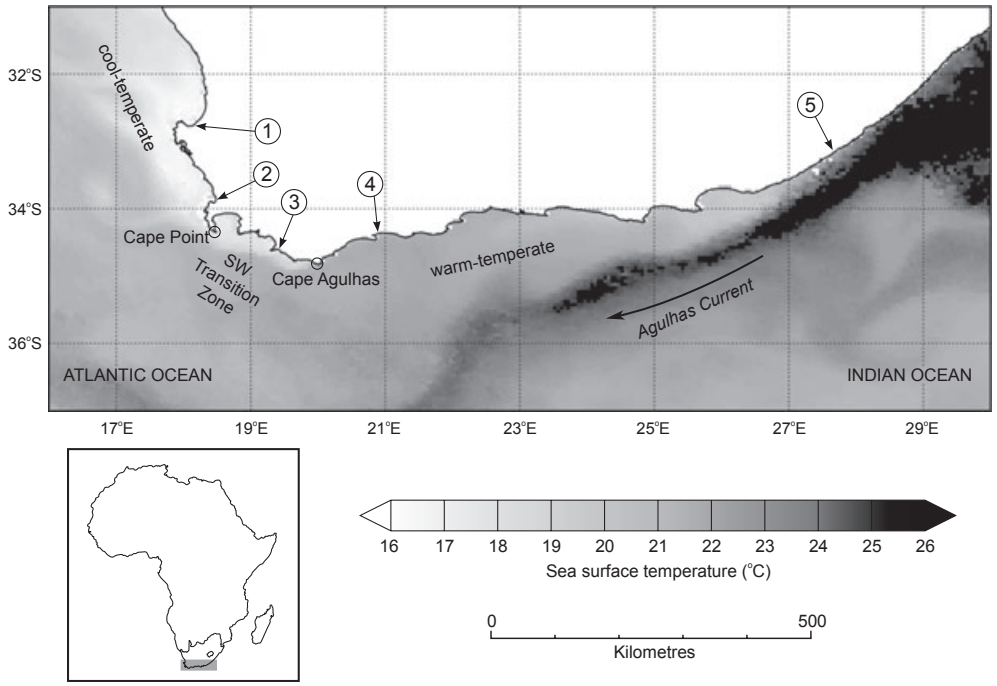

Figure 1 A map of the southern portion of South Africa showing the region's two temperate marine biogeographical provinces (the cool-temperate west coast and the warm-temperate south coast), and the location of five sites at which samples of *Hymenosoma orbiculare* were collected. The area between Cape Agulhas and Cape Point is often considered a transition zone between the two provinces. No additional evolutionary lineages were found in the unsampled region between sites 4 and 5, or north of site 1 (seven additional sites; Teske *et al.*, 2007a). Sea surface temperatures were calculated as an average from five days in February (austral summer) 2007. The core of the Agulhas Current is darkest, and the light areas west of Cape Agulhas represent cold-water upwelling. Sea surface temperature image: CSIR–Earth Observation (SW, south-west).

Table 1 Sites at which specimens of the southern African crab *Hymenosoma orbiculare* were collected.

Site no.	Site name	Location	Sample size
1	Great Berg	32°46′10″ S; 18°08′44″ E	30
2	Rietvlei	33°53′25″ S; 18°29′00″ E	30
3	Uilenkraals	34°36′23″ S; 19°24′33″ E	30
4	Breede	34°24′26″ S; 20°50′53″ E	30
5	Gqunube	32°55′59″ S; 28°01′59″ E	30

Site numbers correspond to those used in Fig. 1. For each sample, one *COI* sequence and two *ANT* sequences were generated.

would be evidence for secondary contact with partial introgressive hybridization. We constructed median-joining haplotype networks in Network 4600 (Bandelt *et al.*, 1999) in order to determine whether the *COI* and *ANT* data exhibited genetic patterns that could be linked to the region's biogeography. We also used an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) as implemented in GenoDive 2.0b20 (Meirmans & van Tienderen, 2004) to test whether a hypothesis in which populations were assigned to groups associated with marine biogeographical provinces (i.e. warm-temperate, cool-temperate and transition zone) was more strongly supported than one that assumed no genetic structure. Significance was tested using 1000 permutations. The same program was used to test for structure among individual sites by calculating pairwise Φ_{ST} values from distance matrices of pairwise differences among sites. To determine whether a combination of sites that was not concordant with the region's biogeography explained genetic structure for the *COI* data better than the groupings specified for AMOVA, we used the spatial analysis of molecular variance in samova 1.0 (Dupanloup *et al.*, 2002), a method that aims to define groups of samples that are maximally differentiated from each other. We specified a range of groupings (K = 2–4) and 100 simulated annealing processes.

For the *ANT* data, we also investigated whether any regional lineages are able to interbreed, and, if so, whether most of the introgression occurred near the biogeographical transition zone, indicating that sharing of alleles between regions was unlikely to be the result of the retention of ancestral polymorphism. Locus-specific hybrid indices were estimated using the maximum-likelihood-based method described in Buerkle (2005) and implemented in GenoDive. This approach requires that, in addition to the putative hybrid site, a reference site and an alternative site are also specified. Hybrid indices close to 1 indicate that an individual has a high affinity to the reference site and values close to 0 indicate affinity to the alternative site. We considered individuals with intermediate hybrid indices (i.e. whose 95% confidence intervals did not include 1 or 0) to be heterozygous for the two *ANT* lineages.

Coalescent-based analyses

The isolation-with-migration model (Nielsen & Wakeley, 2001) can be used to distinguish between two competing demographic models that explain the sharing of alleles between evolutionary lineages. The 'isolation model' assumes recent divergence with complete isolation, and attributes the sharing of alleles between populations to incomplete lineage sorting. The 'migration model', on the other hand, assumes that divergence took place longer ago, that lineage sorting is complete, and that alleles are shared because of post-divergence gene flow. We used the program IMa2 (Hey, 2010) to distinguish between these two scenarios by jointly estimating the effective population sizes of the two extant province-associated populations (θ_0 and θ_1) and their common ancestor (θ_2), the time at which they diverged (t) and the migration rates between them ($m_{0>1}$ and $m_{1>0}$), as detailed in Appendix S1. We excluded samples from site 3 (transition zone) as this site could not be clearly grouped with either biogeographical province (see Results). Non-zero estimates for migration rates and divergence time were considered to support the migration model.

RESULTS

COI and *ANT* sequences were 456 bp and 215 bp long, respectively. The *COI* gene had 49 variable sites (11%), of which six were in first codon positions and 43 in third codon positions. None of these resulted in different amino acid sequences between individuals when translated using the genetic code for invertebrate mtDNA. The *ANT* intron data had 34 variable sites (16%). No evidence for recombination was found for the intron. All sequences have been submitted to GenBank (accession numbers KF279697–KF280146).

Spatial genetic structure

The haplotype networks of both markers were characterized by dominant haplotypes that had given rise to large numbers of derived haplotypes (so-called 'star phylogenies'), which is an indication of population expansion (Castelloe & Templeton, 1994; Fig. 2). However, whereas the *COI* sequences had a single dominant haplotype that was present at all five sampled sites and was particularly common in the cool-temperate province, the network constructed from *ANT* intron sequences had two dominant haplotypes. One of these was absent from the warm-temperate province, as were all of the derived haplotypes differing from it by up to four nucleotide substitutions. The other dominant haplotype was particularly common in the warm-temperate province and in the transition zone, and most of the rare haplotypes derived from it were absent from the cool-temperate province.

Several of the pairwise Φ_{ST} values among sampling sites estimated from *COI* were significant (Table S1 in Appendix S2), but only the intron data showed a clear correlation between genetic structure and the region's biogeography. For

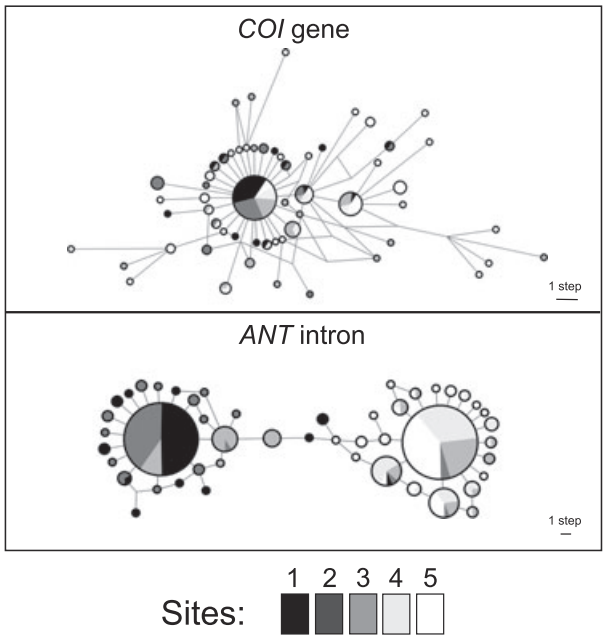

Figure 2 Median-joining haplotype networks constructed from mitochondrial *COI* and nuclear *ANT* intron sequences of *Hymenosoma orbiculare* from South Africa. Site numbers correspond to those in Fig. 1 and Table 1.

the latter, all Φ_{ST} values were significant, except those between sites 1 and 2 (cool-temperate province) and between sites 4 and 5 (warm-temperate province). AMOVA (Table 2) revealed that the only grouping that was significantly different from the expectations of genetic homogeneity was one in which the *ANT* sequences were grouped into a cool-temperate group (sites 1 and 2), a warm-temperate group (sites 4 and 5) and a transition zone group (site 3). All Φ_{CT} values for *COI* data were non-significant and not significantly greater than zero, indicating that this marker does not show any regional genetic structure. This conclusion is also supported by the results from the SAMOVA analyses (Table S2 in Appendix S2), where none of the groupings that maximized Φ_{CT} when 2, 3 and 4 groups were specified had significant Φ_{CT} values.

Tests for the *ANT* intron-specific hybrid status of individuals of *H. orbiculare* revealed that more than half of the individuals in the biogeographical transition zone (site 3) had a combination of typical cool-temperate and warm-temperate *ANT* alleles (values of the hybrid index were mostly close to 0.5; Table 3). Such individuals were comparatively rare outside of the transition zone (3–13%), and the two sites at the extremes of the sampled range (sites 1 and 5) had no individuals with two alleles representative of the other province.

Coalescent-based analyses

The IMa2 analyses clearly supported the migration model for the *ANT* data, with a divergence time estimate that was

Table 2 Results of AMOVA analyses of *COI* and *ANT* intron sequences of *Hymenosoma orbiculare*, with geographically contiguous sites assigned to two or three groups.

Locus	Grouping	*F*-statistic	Percentage of variation	Value	95% confidence interval
COI	(1 + 2 + 3) (4 + 5)	Φ_{IS}	90	1.00**	1.00–1.00
		Φ_{SC}	6	0.06**	0.02–0.09
		Φ_{CT}	4	0.04	0.00–0.07
	(1 + 2) (3 + 4 + 5)	Φ_{IS}	91	1.00**	1.00–1.00
		Φ_{SC}	6	0.07**	0.01–0.10
		Φ_{CT}	3	0.03	0.00–0.05
	(1 + 2) (3) (4 + 5)	Φ_{IS}	92	1.00**	1.00–1.00
		Φ_{SC}	7	0.03**	0.01–0.01
		Φ_{CT}	1	0.01	0.00–0.03
ANT	(1 + 2 + 3) (4 + 5)	Φ_{IT}	21	0.78	0.71–0.84
		Φ_{IS}	7	0.25**	0.14–0.40
		Φ_{SC}	16	0.36**	0.26–0.44
		Φ_{CT}	55	0.55	0.47–0.60
	(1 + 2) (3 + 4 + 5)	Φ_{IT}	20	0.80	0.72–0.85
		Φ_{IS}	7	0.25**	0.14–0.40
		Φ_{SC}	7	0.20**	0.13–0.24
		Φ_{CT}	67	0.67	0.58–0.71
	(1 + 2) (3) (4 + 5)	Φ_{IT}	24	0.76	0.67–0.82
		Φ_{IS}	8	0.25**	0.13–0.40
		Φ_{SC}	0	0.00	0.00–0.01
		Φ_{CT}	68	0.68**	0.59–0.73

F-statistics reflect the following hierarchical levels: Φ_{IT}, within individuals; Φ_{IS}, among individuals within populations; Φ_{SC}, among populations within groups; Φ_{CT}, among groups of populations.
**$P < 0.01$.

Table 3 Percentage of individuals of *Hymenosoma orbiculare* from selected sites having high, intermediate or low hybrid indices based on sequence data from the *ANT* intron; (a) site 3 treated as a putative hybrid site; and (b) sites on the west coast and (c) sites on the south coast treated as hybrid sites.

	Sites			Hybrid index at the putative hybrid site		
	Putative hybrid site	Reference site(s)	Alternative site(s)	High (%)	Intermediate (hybrids) (%)	Low (%)
a	SW (3)	W (1 + 2)	S (4 + 5)	13	57	30
b	1	2	S (4 + 5)	93	7	0
	2	1	S (4 + 5)	80	13	7
c	4	5	W (1 + 2)	97	3	0
	5	4	W (1 + 2)	90	10	0

Numbers of sites correspond to those in Fig. 1; SW, site 3, located in the transition zone on the south-west coast; W, sites on the cool-temperate west coast; S, sites on the warm-temperate south coast. Hybrid indices were categorized as being either high (95% confidence intervals include 1; i.e. individuals at the putative hybrid site are grouped with the reference site), low (confidence intervals include 0; individuals at the putative hybrid site are grouped with the alternative site) or intermediate (no preference for either reference or alternative site due to hybridization of typical alleles from the cool-temperate and warm-temperate provinces).

significantly greater than estimates obtained from randomized data sets (Fig. 3a, Table S3 in Appendix S2), and non-zero unidirectional gene flow from the warm-temperate to the cool-temperate province (Fig. 3b). The results were not as straightforward for the *COI* data. Although divergence time was also significantly greater than zero, the values of t for randomly reshuffled data sets were nearly identical to the t of the original data (Fig. 3a, Table S3). This may be an indication that non-zero estimates of t can result from random haplotype differences between populations rather than true divergence, and the assumption that IMa2 is more suitable than other coalescent genealogy samplers when populations have diverged very recently (Kuhner, 2009) may not hold. Although a model of bidirectional gene flow was identified for the *COI* data (Fig. 3b), neither migration rate estimate was significantly different from zero (Table S3).

DISCUSSION

We explored whether discrepancies between expected and observed genetic connectivity across the Atlantic/Indian

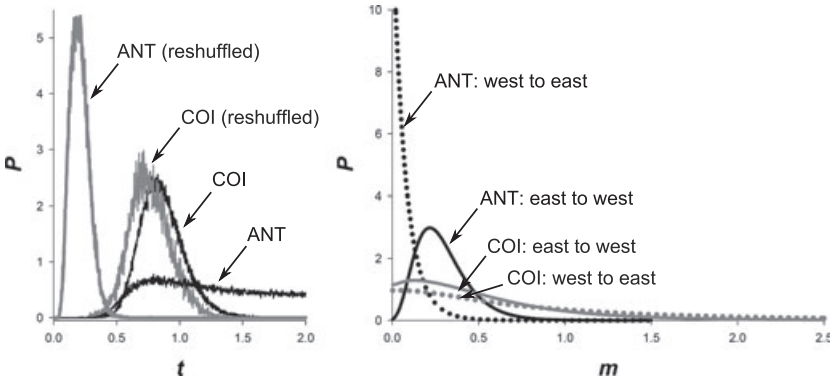

Figure 3 Likelihood plots of (a) divergence time and (b) migration rates, generated by IMa2 from sequence data of *Hymenosoma orbiculare* in South Africa. For divergence time, plots for four different data sets are shown: *ANT* intron and *COI* sequences assigned to a cool-temperate and a warm-temperate population; *ANT* (reshuffled) and *COI* (reshuffled): *ANT* and *COI* sequences randomly reshuffled between populations. The curve for *ANT* did not return to zero after having reached its peak. Plots of migration rates depict gene flow between cool-temperate (west coast) and warm-temperate (south coast) populations using the two observed data sets. *P*, posterior probability; *t*, scaled divergence time; *m*, scaled migration rate.

Ocean transition zone could be an artefact of mtDNA data providing insufficient signal in single-locus data sets to detect genetic divergence. Genealogies reconstructed from mitochondrial *COI* sequences and nuclear *ANT* intron sequences of the coastal crab *Hymenosoma orbiculare* were strikingly different, with the former recovering a single lineage and the latter two lineages.

Several lines of evidence suggest that the biogeography-linked genetic structure identified for *ANT* is the result of this marker having completed lineage sorting. The fact that individuals with *ANT* alleles from both lineages were particularly common near the biogeographical transition zone between southern Africa's temperate provinces, and the support for the 'migration model' (i.e. post-divergence gene flow) based on coalescent modelling, indicate that the sharing of alleles between populations is the result of secondary contact rather than the retention of ancestral polymorphism. In contrast, no meaningful pattern of spatial genetic structure was found for the mtDNA *COI* data, and estimates of gene flow were not significantly different from zero, suggesting that these data may not be sufficiently informative to estimate migration rates, because the two regional populations are genetically too similar. There are two potential explanations for the existence of a single *COI* lineage: complete mitochondrial capture and incomplete lineage sorting. The two are difficult to disentangle, because in both cases the sharing of alleles between regional populations reflects the retention of ancestral polymorphism rather than secondary contact. While mitochondrial capture is more likely because of the higher rate of lineage sorting of the mitochondrial genome (Funk & Omland, 2003), the possibility that lineage sorting of the less variable *ANT* intron is a result of linkage to a gene that is under divergent selection between southern Africa's temperate provinces cannot be ruled out without generating data from additional nuDNA loci or by conducting transplantation experiments.

Congruence between biogeography and nuclear DNA structure

The genealogy of the *ANT* intron reflects the contemporary biogeography of coastal south-western Africa; the province-specific genetic structure is similar to that found in numerous other coastal species (Teske *et al.*, 2011a) and shows a strong link with water temperature. Data on the physical and chemical characteristics of estuaries in south-western Africa confirm that the water temperatures in cool-temperate estuaries tend to be lower than in those of the warm-temperate province (Harrison, 2004), and organisms on the west coast are also much more likely to be affected by cold water upwelling (Demarcq *et al.*, 2003). In addition, the unidirectional migration rate from the warm-temperate south coast to the cool-temperate west coast inferred using IMa2 is supported by the region's oceanography, as the dispersal of planktonic larvae between the region's coasts occurs primarily via surface water that drifts in a westward direction west of Cape Agulhas, with the highest influx occurring during summer (Shannon & Chapman, 1983). The chances of larvae originating from the south coast establishing themselves on the west coast are, however, reduced by summer upwelling, which moves them away from the coast (Lutjeharms & Meeuwis, 1987). Large-scale colonization is common during warm temperature anomalies, but the fact that populations of warm-temperate species only persist in sheltered habitats on the west coast (Branch, 1984) suggests that they are selected against by colder water temperatures. The decreasing abundance of alleles associated with the warm-temperate lineage of *H. orbiculare* from the transition zone westwards resembles a pattern found in a southern African mudprawn across the region's subtropical/warm-temperate biogeographical disjunction. Physiological experiments showed that the ability of the larvae of this mudprawn's warm-water population to complete development was reduced when they were

exposed to the lower temperatures typical of the warm-temperate province during winter (Teske *et al.*, 2008). Given that marine biogeography is believed to be shaped primarily by water temperature (Murawski, 1993) and is thus a direct result of species' thermal tolerance ranges (Pörtner *et al.*, 2007), it is likely that diversifying selection plays a central role in maintaining the Atlantic/Indian Ocean phylogeographical break in south-western Africa.

Implications of the lack of mtDNA-based genetic structure

Mitonuclear discordance is increasingly being reported as the number of multilocus data sets increases; cases in which both markers are represented by the same number of evolutionary lineages, but with significant differences in the geographical extent of introgression, are particularly common (Toews & Brelsford, 2012). The present study reports a rare type of mitonuclear discordance – the lack of mtDNA-based genetic structure as a possible consequence of complete mitochondrial capture. The phenomenon may be under-reported in the literature, as it is difficult to detect because there are no surviving examples of the extinct mtDNA lineage (Irwin *et al.*, 2009).

Genetic homogeneity across biogeographical transition zones is not uncommon (Kelly & Palumbi, 2010), and ongoing barcoding initiatives are likely to uncover many more examples of such patterns. In southern Africa, approximately half of the species present across the Atlantic/Indian Ocean transition zone that have been studied to date are not genetically structured (Table 4). A clear trend that has emerged is that all of these have relatively high dispersal potential in having at least a short planktonic larval dispersal phase. While there are also examples of planktonic dispersers being genetically structured, not a single case of genetic homogeneity has been reported for low-dispersal species with direct development, pointing to a link between lack of genetic structure and high dispersal potential that needs to be explored further. *Hymenosoma orbiculare* is so far the only species in which a mitonuclear discrepancy in genetic structure was found, and it may be that the *ANT* intron is unusual in this species, possibly as a consequence of selection. However, nuclear data have been generated for very few other species, and at this stage it is not possible to draw any firm conclusions from this. Several of the species in Table 4 may be good candidates for studying the prevalence of mitonuclear discordance more systematically.

The possibility that a lack of genetic structure can be a locus-specific artefact has received little attention in the literature on marine phylogeographical studies. This may be a consequence of the dominance of studies that have

Table 4 Southern African coastal animals that are not genetically structured across the Atlantic/Indian Ocean biogeographical disjunction on the basis of mtDNA data.

Higher taxon	Species	Larval dispersal	Adult dispersal	nuDNA structure	Reference
Ascidiacea	*Pyura herdmani**,‡	Yes	No	No (intron)	Teske *et al.* (2011b)
Ascidiacea	*Pyura stolonifera**,‡	Yes	No	No (intron)	Teske *et al.* (2011b)
Crustacea	*Hymenosoma orbiculare*	Yes	Low	Yes (intron)	(this study)
Crustacea	*Jasus lalandii*	Yes	Moderate	—	Matthee *et al.* (2007)
Crustacea	*Tetraclita serrata*	Yes	No	No (ITS)	Reynolds (2011)
Mammalia	*Arctocephalus pusillus pusillus*	No	High	—	Matthee *et al.* (2006)
Mollusca	*Afrolittorina africana*	Yes	Low	—	Matumba (2013)
Mollusca	*Afrolittorina knysnaensis*	Yes	Low	—	Matumba (2013)
Mollusca	*Cymbula miniata*	Yes	Low	—	Mmonwa (unpubl. data)**
Mollusca	*Cymbula oculus*	Yes	Low	—	Mmonwa (unpubl. data)**
Mollusca	*Mytilus galloprovincialis*†	Yes	No	—	Zardi *et al.* (2007)
Mollusca	*Nassarius kraussianus*	Yes	Low	—	Teske *et al.* (2007b)
Mollusca	*Octopus vulgaris**,†,§	Yes	Moderate	—	Teske *et al.* (2007c)
Mollusca	*Scutellastra argenvillei*	Yes	Low	—	Mmonwa (unpubl. data)**
Mollusca	*Scutellastra barbara*	Yes	Low	—	Mmonwa (unpubl. data)**
Mollusca	*Scutellastra cochlear*	Yes	Low	—	Mmonwa (unpubl. data)**
Mollusca	*Tricolia africana*/*Tricolia capensis**,¶	Yes	Low	—	Nangammbi (2010)
Mollusca	*Tricolia bicarinata*/*Tricolia insignis*/*Tricolia kraussi**,¶	Yes	Low	—	Nangammbi (2010)
Pisces	*Caffrogobius caffer*	Yes	Low	—	Neethling *et al.* (2008)
Pisces	*Chrysoblephus laticeps*§	Yes	Moderate	No (STRs)	Teske *et al.* (2010)

*Low sample size; lack of structure based on phylogenetic monophyly rather than *F*-statistics.
†Introduced or potentially non-native species.
‡Very short larval dispersal phase (< 1 day).
§Also occurs offshore or at greater depths.
¶No genetic differences found between multiple species.
nuDNA, nuclear DNA; ITS, internal transcribed spacer; STRs, short tandem repeats (microsatellites).
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exclusively employed mtDNA data. Most studies that have failed to find genetic structure across marine biogeographical disjunctions have attributed this to unexpected discrepancies between theoretical and realized dispersal. For example, Ayre *et al.* (2009) concluded that a lack of structure in two low-dispersal species across the Bass Strait in south-eastern Australia, combined with the presence of phylogeographical breaks in species with planktonic dispersal phases, was most likely to be a result of the former having less specific habitat requirements. Other factors that have been proposed as explanations include: increased dispersal success either by means of very high reproductive output and extended larval dispersal phases (Neethling *et al.*, 2008; Groeneveld *et al.*, 2012) or by association with strong currents (Silva *et al.*, 2010); a recent range extension where the leading edge has not yet diversified genetically (Tolley *et al.*, 2005); reduced impact of climate-driven divergence by association with deeper-water habitats (Fernández *et al.*, 2013); and anthropogenically mediated transport of sessile adults (Nóbrega *et al.*, 2004). While these hypotheses are reasonable, genetic homogeneity may in some cases reflect technical rather than biological issues, and the most effective way of separating these competing hypotheses will be to take a multilocus genetic approach.

CONCLUSIONS

The relationship between biogeography and phylogeography is important for understanding both the distribution and the evolution of species, but the fact that no clear relationship has been identified between the presence or absence of phylogeographical breaks and species' dispersal potential has precluded the formulation of predictions concerning the geographical patterns of genetic structure (Dawson, 2012). Based on nuclear intron data, we identified a clear biogeography-linked pattern of genetic structure in a species that was thought to be genetically homogenous across the Atlantic/Indian Ocean biogeographical disjunction on the basis of mtDNA data. Such locus-specific artefacts may account for at least some of the many examples of apparent 'panmixia' across strong environmental gradients that define distinct sister lineages in other organisms. This suggests that these barriers do affect species with similar life-history traits in a similar way, but that some data sets do not adequately reflect contemporary environmental conditions. Our two-locus data set is not suitable to identify the mechanism responsible for the observed mitonuclear discordance unambiguously, but it clearly shows that marine phylogeographical research can be expected to benefit greatly from placing more emphasis on identifying loci that are informative at the evolutionary level of interest. As such, the present work has laid the foundation for future studies on the relative importance of mitochondrial introgression and selection in coastal southern Africa, a region that seems particularly suited to study such phenomena because of its rich marine biodiversity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary methods.
Appendix S2 Supplementary results.

BIOSKETCH

The authors share an interest in uncovering the evolutionary processes that have contributed towards shaping contemporary biogeographical patterns.

Author contributions: P.R.T. and I.P. conceived the study, collected the samples and generated the sequence data; P.R.T. analysed the data and led the writing; C.D.M. and L.B.B. contributed to the writing; N.P.B. and C.D.M provided logistical support.

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