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RESEARCH ARTICLE

Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development

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Abstract The amount of genetic structure in marine invertebrates is often thought to be negatively correlated with larval duration. However, larval retention may increase genetic structure in species with long-lived planktonic larvae, and rafting provides a means of dispersal for species that lack a larval dispersal phase. We compared genetic structure, demographic histories and levels of gene flow of regional lineages (in most cases defined by biogeographic region) of five southern African coastal invertebrates with three main types of larval development: (1) dispersal by long-lived planktonic larvae (mudprawn *Upogebia africana* and brown mussel *Perna perna*), (2) abbreviated larval development (crown crab *Hymenosoma orbiculare*) and (3) direct development (estuarine isopod *Exosphaeroma hylecoetes* and estuarine cumacean *Iphinoe truncata*). We hypothesized that *H. orbiculare*, having abbreviated larval development, would employ a strategy of larval retention, resulting in genetic structure comparable to that of the direct developers rather than the planktonic dispersers. However, regional population structure was significantly lower in all species with planktonic larvae, including *H. orbiculare*, than in the direct developers. Moreover, nested clade analysis identified demographic histories resulting from low levels of gene flow (isolation by distance and allopatric fragmentation) in the direct developers only, and migration rates were significantly higher in all three species having planktonic larvae than in the direct developers. We conclude that the amount of genetic structure within marine biogeographic regions strongly depends on the presence or absence of free-swimming larvae. Whether such larvae are primarily exported or retained, whether they have long or short larval duration, and whether or not they are capable of active dispersal seems to have little effect on connectivity among populations.

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Introduction

In marine organisms, greater dispersal ability is generally considered to be positively correlated with levels of gene flow and negatively correlated with genetic structure (Palumbi 1994). However, a number of recent studies have challenged this paradigm. Firstly, it was found that some species with long-lived planktonic larvae are characterized by surprisingly high levels of genetic structure, because their larvae are retained locally due to a combination of behavioural and physical mechanisms (e.g. Jiang et al. 1995; Swearer et al. 1999; Barber et al. 2000; Taylor and Hellberg 2003). Secondly, some species that lack a larval dispersal

phase and have adults with poor locomotory abilities can nonetheless disperse over great distances by utilizing floating objects such as seaweeds as rafts (e.g. Johannesson 1988; Waters and Roy 2004; Teske et al. 2005).

The majority of marine invertebrates have complex life histories (Levin and Bridges 1995), but species in which the number of larval stages is reduced have been identified in wide variety of taxa. Several types of abbreviated development have been identified, of which the most extreme form is direct development, in which no free-swimming larvae exist and hatching takes place as a juvenile stage (Gore 1985; Rabalais and Gore 1985; Anger 2001). A reduction or complete lack of the larval phase is particularly common among inhabitants of freshwater, high latitudes, and deep sea environments, and has frequently been associated with an insufficient, unpredictable, or seasonally short supply of the required planktonic food source (Anger 2001 and references therein). In southern Africa, the absence of obligate marine dispersal stages may be advantageous to species occurring in estuaries. Firstly, many estuaries in arid countries become temporarily disconnected from the sea by a sand bar that forms across the estuary mouth during periods of low river flow, and recruitment of species that require access to the marine habitat to complete larval development ceases during periods of mouth closure (e.g. Wooldridge 1991, 1994, 1999). Secondly, recruitment failure may be greater in species that export their larvae to the marine habitat, as a high proportion of these may be unable to reach a suitable habitat in time to complete development (Morgan 1995; Anger 2001). Moreover, habitat complexity in the form of macrophytes tends to be greater particularly in temporarily open/closed estuaries than in permanently open estuaries and in the sea (Perissinotto et al. 2002), and this is likely to provide protection from predators (Robertson 1984; Boström and Mattila 1999; Hindell et al. 2000). The advantages of reduced levels of predation, higher food availability, and utilization of resources that are temporarily unavailable to potential competitors during closed phases may, however, be offset by the disadvantages of low levels of gene flow among populations. These include increased vulnerability to localised extinctions (Valentine and Jablonski 1986; Wares and Cunningham 2001) and slow recolonisation of habitats from which a species has been eliminated, e.g. as a result of freshwater floods or habitat degradation (Moy and Levin 1991; Evans et al. 1998; Lockyear et al. 2006).

To determine how the reduction or absence of larval stages has influenced genetic connectivity among southern African coastal invertebrate populations, we conducted phylogeographic analyses of five species with three major types of development: long-lived planktonic, abbreviated planktonic, and direct. The greatest influence of life history strategies on genetic structure tends to be at intermediate scales of ~100 km (Hellberg et al. 2002; Palumbi 2003), while on larger scales of ~1000 km, the effect of currents and environmental discontinuities may result in diversifying selection that affects all species in a region similarly, irrespective of whether or not they have planktonic larvae (e.g. Sotka et al. 2004; Teske et al. 2006). We consequently took into account recent findings that a number of southern African marine invertebrates comprise distinct evolutionary units (as identified on the basis of reciprocal monophyly and, in some cases, morphological differentiation, suggesting that these units are cryptic species) that are in most cases associated with different biogeographic provinces (e.g. Ridgway et al. 1999; Teske et al. 2006; Zardi et al. 2007, Edkins et al. 2007), by analyzing each of such units individually. We hypothesized that genetic connectivity should be greater in the species with long-lived planktonic larvae than in the direct developers. We further hypothesized that the species with abbreviated larval development would employ a strategy of larval retention, and for that reason should be characterized by genetic structure comparable to that of the direct developers.

Materials and methods

Study species and their life histories

Species with long-lived planktonic larvae

This category includes the mudprawn *Upogebia africana* (Ortmann, 1894) and the brown mussel *Perna perna* (L.). Although both species have long-lived planktonic larvae, the details of their larval development differ considerably. The estuarine *U. africana* has larvae that must complete development outside estuaries (Wooldridge 1994), and their larval development may take up to 27 days (Newman et al. 2006). The final stage (the postlarval megalopa) is able to swim actively towards suitable habitats (Shanks 1995), which allows it to recruit into estuaries. *Perna perna* Linnaeus, 1758 occurs on rocky shores and has two larval stages, a dispersal stage (the actively swimming veliger), and a recruitment stage (the pediveliger). The species has a larval period of 15–20 days (Hicks and Tunnell 1995), but it is likely that settlement can be delayed for up to three months when food availability and temperatures are low, as has been shown for other species of the family Mytilidae (e.g. Bayne 1965). The two planktonic dispersers occur along most of the coastline of South Africa and into Namibia (west coast) and Mozambique (east coast). Both comprise two mtDNA lineages (one temperate lineage on the west and south coasts and one subtropical lineage on the east coast), with distributions that overlap on the southeast coast (Teske et al. 2006; Zardi et al. 2007; Fig. 1, Table 1).

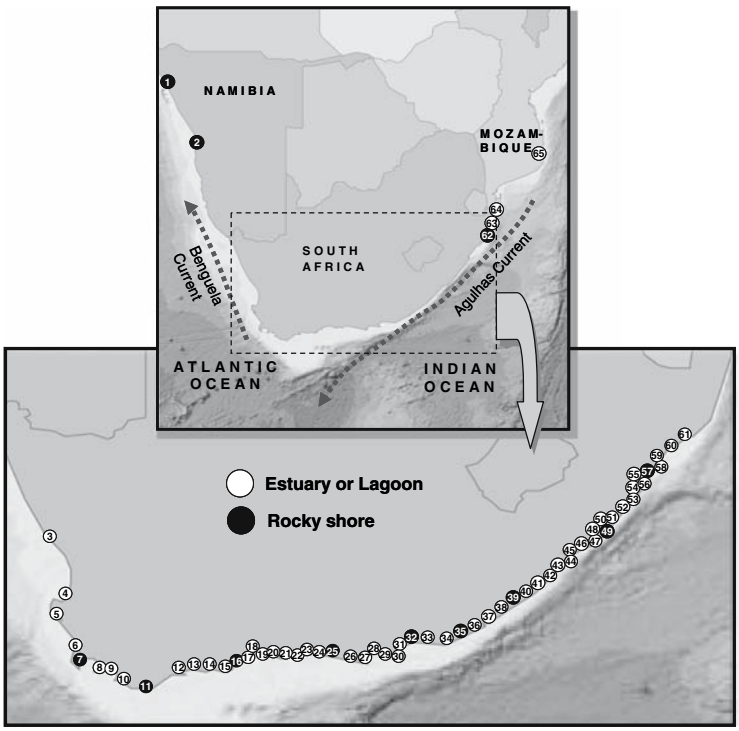

Fig. 1 Map of the sampling area showing sampling localities. Names of sites represented by numbers are listed in Table 1

Species with abbreviated larval development

The crown crab *Hymenosoma orbiculare* Desmarest, 1825 occurs primarily in estuaries, but has also been reported from the marine habitat. The megalopa (the postlarval marine dispersal stage) is absent (Guinot and Richer de Forges 1997), but *H. orbiculare* has retained the earlier planktonic larval stages (Dornelas et al. 2003). The absence of the megalopal stage should theoretically favour retention of larvae and completion of development within the estuarine environment, as the swimming abilities of the earlier larval stages (zoea) are comparatively poor (Shanks 1995). Recent morphological and genetic work identified five regional lineages of *H. orbiculare*, all of which are considered to be distinct species (Edkins et al. 2007). Two of these are found in deeper waters and are not considered here, but three are coastal and occur mostly in estuaries, with a large-bodied lineage (max. carapace width: 28 mm) occurring on the west and south coasts, and two small-bodied lineages (max. carapace width: 6.5 and 7.5 mm) occurring along the southeast and the east coasts, respectively.

Direct developers

This category includes two peracarid crustaceans that both occur in estuaries, the isopod *Exosphaeroma hylecoetes* Barnard, 1940, and the cumacean *Iphinoe truncata* Hale, 1953. *Exosphaeroma hylocoetes* is usually associated with submerged objects that may serve as rafts, such as wood or vegetation. *Iphinoe truncata*, on the other hand, is strongly associated with sandy sediments (Teske and Wooldridge 2001), which suggests that dispersal by rafting is unlikely. However, it has been suggested that because of its small size, this species is able to disperse in the marine environment as part of the plankton (Teske et al. 2006). The direct developers have smaller distribution ranges than the other three species, with *E. hylecoetes* being absent from the east coast, and *I. truncata* being absent from the southwest and west coasts. Both species comprise three main mtDNA lineages with non-overlapping ranges (Teske et al. 2006).

Sampling, DNA extraction, amplification and sequencing

Oceanographic circulation patterns, larval duration, reproductive timing and the availability of suitable habitat may show considerable variation along the range of widely distributed coastal species. Because of that, sampling of only a limited number of localities is inappropriate when studying such species (Sotka et al. 2004). We therefore decided to sample a small number of individuals at as many sites along the southern African coastline as possible, rather than obtaining large numbers of individuals from a limited number of sites. A total of 71 specimens of *U. africana*, 70 specimens of *P. perna*, 80 specimens of *H. orbiculare*, 72 specimens of *E. hylecoetes* and 82 specimens of *I. truncata* were analysed. These sample sizes are comparable to those of other recent comparative phylogeographic studies (e.g. Audzijonyte et al. 2006; Steele and Storfer 2006). Samples

Table 1 Sampling localities and number of samples of each invertebrate species collected in 63 localities in southern Africa. The majority of samples were collected in South Africa. Two sampling localities are in Namibia (Terrace Bay and Walvis Bay) and one is in Mozambique (Guíua Estuary near Inhambane). Locality numbers are used in Figs. 1 and 2

Locality no.	Locality name	*Upogebia africana*	*Perna perna*	*Hymenosoma orbiculare*	*Exosphaeroma hylecoetes*	*Iphinoe truncata*
1	Terrace Bay	–	5	–	–	–
2	Walvis Bay	–	5	4	–	–
3	Olifants	4	–	2	5	–
4	Groot Berg	3	–	3	5	–
5	Langebaan	1	–	2	–	–
6	Rietvlei	–	–	4	–	–
7	False Bay	–	–	1	–	–
8	Bot	–	–	2	2	–
9	Klein	–	–	1	5	–
10	Uilenkraals	3	–	1	8	–
11	Cape Agulhas	–	–	–	–	–
12	Breede	–	5	–	5	7
13	Duiwenhoks	4	–	–	–	1
14	Goukou	6	–	3	–	8
15	Gourits	3	–	–	3	–
16	Mossel Bay	–	5	–	–	–
17	Klein Brak	–	–	3	4	5
18	Groot Brak	1	–	–	1	3
19	Touws	–	–	–	–	2
20	Goukamma	–	–	–	–	–
21	Knysna	3	–	–	–	–
22	Piesang	–	–	–	2	–
23	Keurbooms	3	–	2	3	4
24	Groot	–	–	–	–	2
25	Tsitsikamma	–	5	–	–	3
26	Kromme	–	–	1	–	–
27	Seekoei	–	–	–	3	2
28	Kabeljous	–	–	–	–	2
29	Gamtoos	–	–	–	6	–
30	Van Stadens	–	–	–	–	4
31	Swartkops	3	–	–	–	–
32	Hougham Park	–	5	–	–	–
33	Sundays	3	–	–	–	7
34	Boknes	–	–	–	4	4
35	Kenton–on–Sea	–	5	–	–	–
36	Kowie	–	–	4	–	–
37	Great Fish	4	–	–	–	–
38	Mpekweni	–	–	–	3	3
39	Kidd's Beach	–	5	–	–	–
40	Gqunube	4	5	4	–	2
41	Cefane	–	–	–	3	–
42	Haga Haga	4	5	–	3	2
43	Qolora	–	–	8	–	–
44	Qora	2	–	–	2	–
45	Ku–Mpenzu	–	–	–	–	2
46	Mbhanyana	2	–	7	–	–

Table 1 continued

Locality no.	Locality name	*Upogebia africana*	*Perna perna*	*Hymenosoma orbiculare*	*Exosphaeroma hylecoetes*	*Iphinoe truncata*
47	Mngazi	–	–	8	3	–
48	Bululo	3	–	–	2	3
49	Port St. Johns	–	5	–	–	–
50	Mzimvubu	–	–	3	–	–
51	Ku–Boboyi	–	–	–	–	3
52	Mpenjati	4	–	6	–	–
53	Mzimkulu	3	–	–	–	–
54	Mtentweni	–	–	–	–	4
55	Mkomazi	3	–	–	–	–
56	Ngane	–	–	–	–	2
57	Durban	–	5	–	–	–
58	Mgeni	2	–	–	–	–
59	Tongati	–	–	–	–	1
60	Zinkwazi	–	–	–	–	4
61	Mzingazi	–	–	7	–	–
62	Mapelane	–	5	–	–	–
63	Lake Sibaya	–	–	1	–	–
64	Kosi Bay	–	5	3	–	4
65	Guíua	3	–	–	–	–
	Total	71	70	80	72	82

were collected at 23, 14, 23, 20 and 24 sampling localities, respectively (Fig. 1, Table 1).

In the case of *U. africana* and *H. orbiculare*, DNA was extracted from muscle tissue. As mtDNA of male mussels is doubly uniparentally inherited (Zouros et al. 1994), DNA of *P. perna* was extracted from the gonad tissue of female individuals. The tip of the telson was used in *E. hylecoetes*, while in the case of the small-bodied *I. truncata*, DNA was extracted from complete specimens. Genomic DNA of the crustaceans was isolated using the Chelex® extraction protocol (Walsh et al. 1991), whereas the phenol–chloroform protocol (Sambrook et al. 1989) was used for the brown mussel. A portion of the mitochondrial cytochrome oxidase c subunit I gene (mtDNA COI) was amplified using the polymerase chain reaction (PCR). Forward primer CrustCOIF (5´-TCA ACA AAT CAY AAA GAY ATT GG-3´) was used for all crustaceans in combination with a suitable suborder-specific reverse primer. Reverse primer DecapCOIR (5´-AAT TAA AAT RTA WAC TTC TGG-3´) was used for the two decapod crustaceans *U. africana* and *H. orbiculare*, and PeracCOIR (5´-TAT WCC TAC WGT RAA TAT ATG ATG-3´) was used for the peracarid crustaceans *E. hylecoetes* and *I. truncata*. The mussels were amplified with universal COI primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR reactions and sequencing were performed as described previously (Teske et al. 2006, Zardi et al. 2007).

Intraspecific phylogeny reconstructions

To identify the regional lineages that comprise each species, UPGMA trees were constructed using default parameters in PAUP* version 4.0b10 (Swofford 2002).

Within-lineage population structure

Population structure among regional lineages of coastal invertebrates was compared by calculating pairwise Φ_{ST} values among populations within lineages from distance matrices of pairwise differences using ARLEQUIN 3.1 (Excoffier et al. 2005). We selected specimens from localities within species' distribution ranges where regional lineages of most species were present, which was the case for lineages on the south coast and east coast. On the south coast, most regional lineages were present in the region between Cape Agulhas and the Gqunube Estuary (localities 11 and 40, Fig. 1). An exception was the cumacean *I. truncata*, in which specimens collected west of the Klein Brak Estuary (locality 17) and east of the Sundays Estuary (locality 33) were genetically very different from the lineage present in the remainder of the region. On the east coast, most species were present in the region between Port St. Johns and Kosi Bay (localities 48 and 63, Fig. 1). Exceptions were the isopod *E. hylecoetes*, which is absent from most of the east coast, and the mudprawn *U. africana*,

which was not found between the Mgeni and Guíua Estuaries (localities 57 and 64). As specimens from the latter estuary had COI haplotypes that were identical to those found as far south as the Haga Haga Estuary (locality 42, see "Results" section), these were included in the analyses. Sites at which only a single individual of a particular species was collected were excluded. Mean pairwise Φ_{ST} values among all populations comprising a particular regional lineage were compared by calculating 95% confidence intervals of the mean, based on 10,000 bootstrap replications generated using the program POPTOOLS 2.6.2 (Hood 2004). Mean pairwise Φ_{ST} values among lineages were considered to be significantly different from each other if their confidence intervals did not overlap.

To test for isolation by distance among populations within regional lineages, associations between matrices comprising pairwise Φ_{ST} values and corresponding pairwise geographic distances among sampling localities were tested by performing Mantel tests using MANTEL for Windows version 1.16 (Cavalcanti 2005). Values were transformed by taking the inverse of squared distances, and 50,000 permutations were specified.

Coalescent-based analyses of regional lineages

We investigated demographic histories and migration rates of regional lineages by means of coalescent-based methods (Kingman 1982), which have proved to be a powerful means of studying small samples of sequences from populations (Hudson 1990). Assuming a neutral model of evolution, the most likely pathway to the coalescent point of samples from a population is traced backwards in time. As coalescent-based methods incorporate genealogical information rather than comparing samples in a pairwise fashion, they are considered suitable for estimating population parameters even when sample sizes are small (Harding 1996), and it is thus not necessary to attempt to recover all of the haplotypes present in a particular population.

Nested clade phylogeographical analysis was used to differentiate among various evolutionary scenarios that may have resulted in present-day phylogeographic patterns evident in regional lineages of the southern African coastal invertebrates. Ninety-five percent plausible sets for linkages of haplotypes within regional lineages were estimated using the program TCS 1.21 (Clement et al. 2000), which implements the method described in Templeton et al. (1992). Hierarchically nested clades were identified using the nesting rules described in Templeton et al. (1987, 1992), and interior and tip haplotypes within each nested clade were identified based on the criteria in Crandall and Templeton (1993) and Castelloe and Templeton (1994). The program GEODIS 2.5 (Posada et al. 2000) was used to identify significant associations between nested clades and geography, using a categorical permutation contingency analysis (100,000 permutations). This was followed by calculation of the average geographical distance of the haplotypes in that clade from the geographical center of the clade (D_c) and from the geographical center of all clades at the next nesting level (D_n), as well as the difference between tip clades and interior clades for D_c and D_n for clades where departure from panmixia was identified. The use of geographical centers of clades rather than comparisons of populations suggests that this method is well suited to our sampling approach of including a large number of sites, but few specimens from each site. As dispersal along the coastline of southern Africa essentially takes place in one dimension, we specified along-coast distances between sampling localities, rather than each locality's geographical coordinates. The most likely phylogeographic scenario explaining the observed phylogeographic patterns was inferred using the latest version of the inference key for the nested haplotype tree analysis of geographic distances (Templeton 2004; latest version posted on November 11, 2005).

Ongoing migration rates within regional lineages were estimated using the program IM (Hey and Nielsen 2004), which simultaneously estimates pairwise migration rates between two groups of populations, as well as their time of divergence and effective population sizes. We used the same regional groupings of populations as in the analyses of population structure (i.e. south coast lineages and east coast lineages), but divided each of these into two geographic units that spanned approximately the same amount of coastline. Exploratory analyses showed that in some cases, effective sample size values (a measure of how well the program's Markov chains are mixing) were low and posterior probability plots were difficult to interpret when too many model parameters were incorporated into the analyses. To keep the procedure as simple as possible, we forced effective population sizes to be equal for both groups of populations, which is reasonable given that they were drawn from the same regional lineage and comprised approximately the same number of populations. The HKY model (Hasegawa et al. 1985) and an inheritance scalar of 0.25 for mitochondrial DNA were specified, and the following search strategy was used: −b500000 –l 0.5 –n20 –k20 –fg –g10.01 –g22 –j4. Suitable upper bounds for effective population size (–*q*1), divergence time (–*t*) and migration rates (–*m*1 and –*m*2) were established independently for each data-set after a number of exploratory runs. To ensure consistency of results, ten independent runs with random starting seeds and at least 1.5 million genealogical steps were performed for each data-set. Final estimates of migration rates were calculated based on the means of the three runs with the highest effective sample sizes. Migration rates were converted to the effective number of female migrants per generation (i.e. those that

establish themselves in new populations and pass their genes on to the next generation) by multiplying the mean migration rate parameter m by the mean effective population size parameter θ.

Results

Sequences generated

Partial COI sequences obtained for *U. africana*, *P. perna*, *H. orbiculare*, *E. hylecoetes* and *I. truncata* were 642, 400, 598, 616, and 590 bp in length, respectively. In combination with previously published sequences (Teske et al. 2006, Zardi et al. 2007), a total of 52, 27, 34, 44, and 56 unique haplotypes were recovered for these species, respectively. All sequences were submitted to GenBank (*U. africana*: DQ070321–DQ070362 and DQ351379–DQ351388; *P. perna*: DQ351427–351476 [note that 70 samples were randomly selected from this larger data-set to obtain a compatible sample size]; *H. orbiculare*: DQ351389–DQ351423; *E. hylecoetes*: DQ070257–DQ070280 and DQ351342–DQ351361; *I. truncata*: DQ070286–DQ070320 and DQ351362–DQ351378).

Intraspecific phylogeny reconstructions

For all five species, intraspecific monophyletic units could be identified that were confined to specific portions of each species' distribution range (Fig. 2). The numbers of distinct regional lineages identified ranged from two in the planktonic dispersers *U. africana* and *P. perna* (Fig. 2a and b) to three in the remaining species. A limited amount of overlap among regional lineages was found in the two planktonic dispersers, whereas the ranges of the other species were strictly segregated.

Within-lineage population structure

Mean pairwise Φ_{ST} values among all populations comprising a particular regional lineage (or a portion thereof, see "Materials and methods" section) were not significant for any of the nine lineages of southern African coastal invertebrates (Table 2), indicating a lack of genetic structure in any of these (although particularly in the case of the direct developers, this could also be an artifact of the small number of specimens per locality). However, comparisons of the magnitude of Φ_{ST} revealed that it was significantly lower in all six regional lineages of species with planktonic larvae (including *H. orbiculare*) than in the regional lineages of the direct developers, as 95% confidence intervals did not overlap. Hence, while there was no significant structure in any of the regional lineages, the amount of genetic structure was significantly lower in the planktonic dispersers. Moreover, significant isolation by distance was found in all three lineages of the direct developers, but in none of the lineages of planktonic dispersers (Table 2).

Coalescent-based analyses of regional lineages

The number of individual haplotype networks constructed for regional lineages of each species corresponded to the number of intraspecific monophyletic units identified using phylogeny reconstructions (Fig. 3), except that the westernmost lineage of *I. truncata* was recovered as two regionally distinct networks (Fig. 3k and l). In all cases, the number of steps connecting haplotypes of different regional lineages of a particular species exceeded the cut-off point beyond

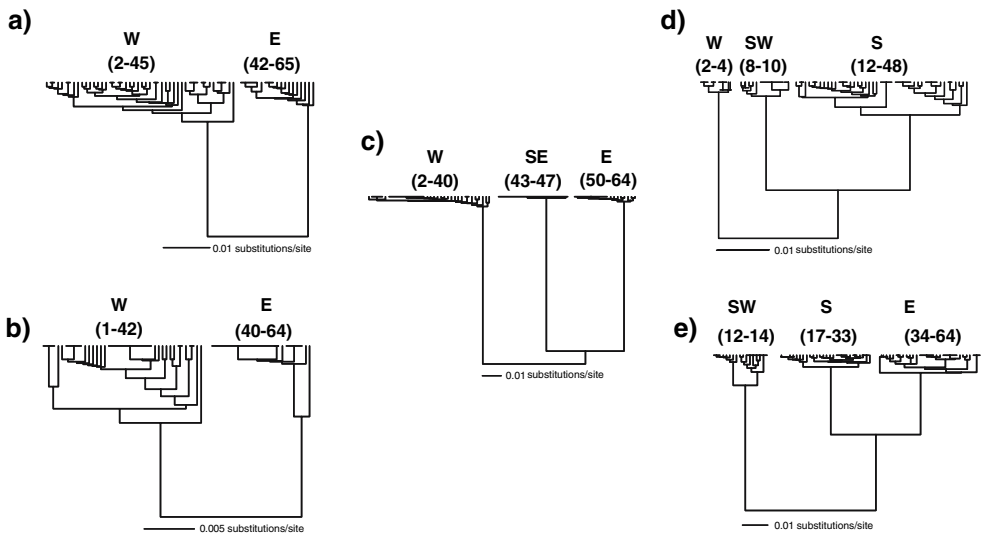

Fig. 2 UPGMA trees constructed from COI sequences of five southern African coastal invertebrate species: **a** *Upogebia africana*; **b** *Perna perna*; **c** *Hymenosoma orbiculare*; **d** *Exosphaeroma hylecoetes*; **e** *Iphinoe truncata*. Regional lineages are identified by the following letters: W: western; SW: southwestern; S: southern; SE: southeastern; E: eastern. Sampling locality numbers, which correspond to those in Table 1 and Fig. 1, indicate the distribution ranges of each regional lineage

Table 2 Genetic structure within regional lineages of five southern Africa coastal invertebrate species with three different life history strategies. In order to directly compare regional lineages of species whose distribution ranges are not congruent, localities were included from portions of the south coast and east coast where most species were represented

Life history	Species	Region	Localities	*N*	Mean Φ_{ST}	95% C.I.	Mean *P*	IBD *r*	IBD *t*	IBD *P*
Planktonic	*Upogebia africana*	South coast	13–40	45	−0.02	−0.08–0.04	0.56	−0.01	−0.02	0.49
		East coast	47–64	15	0.18	0.06–0.31	0.44	−0.43	−1.53	0.13
	Perna perna	South coast	11–40	21	0.03	−0.01–0.08	0.63	0.27	2.60	0.88
		East coast	48–63	6	0.03	0.02–0.50	0.51	−0.44	−1.11	0.13
Abbreviated	*Hymenosoma orbiculare*	South coast	14–40	10	0.02	−0.08–0.16	0.65	0.84	2.60	0.99
		East coast	49–63	6	0.14	−0.03–0.32	0.41	−0.42	−1.05	0.15
Direct	*Exosphaeroma hylecoetes*	South coast	12–38	36	0.53	0.46–0.60	0.11	−0.33	−1.93	0.03
	Iphinoe truncata	South coast	17–40	28	0.70	0.64–0.72	0.15	−0.43	−2.21	0.01
		East coast	47–63	15	0.71	0.58–0.82	0.11	−0.77	−2.50	0.01

The sampling localities that were included are indicated by locality numbers; these correspond to the ones in Table 1. Ninety−five percent confidence intervals of mean pairwise Φ_{ST} values among populations comprising regional lineages were based on 100,000 bootstrap replications. Results of Mantel tests on matrices of pairwise Φ_{ST} and pairwise geographic distance are preceded by isolation-by-distance (IBD). *N*: number of pairwise comparisons; IBD *r*: Pearson product-moment correlation; IBD *t*: Mantel *t* test statistic; IBD *P*: *P*-value of isolation-by-distance analysis

which the probability of a particular connection being true was below 95%. For simplicity, only those nested clades for which significant departures from panmixia were identified are indicated. In the case of the two species with long-lived planktonic larvae, contiguous range expansion was inferred as the evolutionary scenario shaping genetic structure (Table 3). This range expansion had mostly taken place in lower level clades into areas already occupied by the regional lineage of the particular species. However, in the western lineage of *H. orbiculare* (Fig. 3e), a significant departure from panmixia was identified at the highest nesting level. The basal haplotype of this lineage was only found as far east as the Goukou Estuary (locality 14), but several derived haplotypes were present as far east as the Gqunube Estuary (locality 40), a pattern that indicates contiguous range expansion. In contrast to the three species with planktonic larvae, demographic scenarios inferred for the direct developers were mostly the result of low levels of gene flow, and included restricted gene flow with isolation by distance and allopatric fragmentation.

Analyses of gene flow using the program IM revealed very different asymmetric migration rates between south coast and east coast (Fig. 4; see Fig. 5 for examples of posterior probability plots). On the south coast, bidirectional gene flow was identified in the three planktonic dispersers, whereas the direct developers were characterized by regional geographic units that did not exchange migrants (Fig. 4a). In all four species present on the east coast, on the other hand, only southward gene flow was identified (Fig. 4b). The amount of such gene flow differed considerably between the planktonic dispersers and the direct developer *Iphinoe truncata*, with gene flow in the latter being only a fraction of that of the planktonic dispersers.

Discussion

Phylogeographic implications of life histories

Species with long-lived planktonic larvae

Regional lineages of species with long-lived planktonic larvae were characterized by high migration rates and panmixia at higher nesting levels. Although departures from the expectations of panmixia were found at lower level nesting clades, caution may be warranted when interpreting the importance of such results, as they are based on a small number of segregating sites and small sample sizes. Dispersal by means of long-lived planktonic larvae has long been considered to be advantageous to ensure connectivity between populations (Scheltema 1975; Caley et al. 1996). Wooldridge (1994) found that the mudprawn *U. africana* employs a larval export strategy, with newly-hatched larvae being flushed into the ocean during nocturnal ebbing tides. Physiological work by Paula et al. (2001) supported this finding by determining that the optimum salinity for the development of the larvae is near seawater, with the first zoeal stage having a slightly wider tolerance range to lower salinities typical of the estuarine habitat than the subsequent stages. Wooldridge (1999) suggested that mudprawn larvae may employ mechanisms that allow them to remain in the vicinity of their parent habitat to avoid dispersal away from suitable habitat. The low levels of genetic structure found within the two regional lineages of *U. africana* suggest that if such a strategy is indeed employed, then the number of larvae that disperse away from their parent habitat is nonetheless sufficiently large to prevent genetic divergence among lineages within regions. Unlike the actively

Table 3 Results of nested clade analyses for regional lineages of five southern African coastal invertebrate species. Results of non-significant tests have been omitted

Species	Lineage	Clade	Sub-clades	D_c	D_n	Inference chain	Interpretation
Upogebia africana	West	2-4	1-11 (I)	S	S	1-2-11-12-NO	Contiguous range expansion from the southwest to southeast coast
		4-1	3-1 (T)	S	–	1-2-11-12-NO	Contiguous range expansion from south coast to west coast
			3-2 (I)	S	–		
	East	Total	2-1 (T)	L	L	1-2-11-12-NO	Contiguous range expansion along the east coast
			2-2 (I)	S	S		
			I–T	S	S		
Perna perna	West	2-2	1-9 (T)	L	L	1-2-11-12-NO	Contiguous range expansion (South Africa to Namibia)
		2-3	1-4 (I)	S	S	1-2-11-12-NO	Contiguous range expansion (South Africa to Namibia)
			1-7 (T)	–	L		
Hymenosoma orbiculare	West	Total	3-1 (I)	S	S	1-2-11-12-NO	Contiguous range expansion in an eastward direction
			3-2 (T)	L	L		
Exosphaeroma hylecoetes	South	2-1	1-6 (T)	S	L	1-2-3-5-15-NO	Past fragmentation*
			I–T	–	S		
		2-2	1-3 (I)	–	L	1-2-3-4-NO	Restricted gene flow with isolation by distance
			1-7 (T)	S	S		
			I–T	L	L		
		3-2	I–T	L	–	1-2-3-4-NO	Restricted gene flow with isolation by distance
		3-3	2-5 (I)	–	L	1-19-20-2-3-4-9-NO	Allopatric fragmentation
			2-6 (T)	S	S		
			I–T	–	L		
		Total	3-1 (T)	S	–	1-2-11-12-13-14-21-NO	Insufficient evidence to discriminate between long-distance movements and the combined effects of gradual movement during past range expansion and fragmentation**
			3-2 (T)	S	L		
			3-3 (I)	S	–		
Iphinoe Truncata	South	2-3	1-6 (T)	–	L	1-19-NO	Allopatric fragmentation between localities 23/24 and locality 26
			1-7 (I)	S	S		
			I–T	–	S		
		2-4	1-9 (I)	–	L	1-19-NO	Allopatric fragmentation between locality 17 and localities 19/20***
			1-10 (T)	S	–		
			I–T	L	–		

Table 3 continued

Species	Lineage	Clade	Sub-clades	D_c	D_n	Inference chain	Interpretation
	East	2-5	1-8	S	S	1-19-20-2-I/T status cannot be determined	Inconclusive outcome
			I–T	S	S	1-19-20-2-11-12-13-14-YES	Sampling design inadequate to discriminate between contiguous range expansion, long-distance colonization, and past fragmentation
		3-1	2-1 (T)	S	L		
			2-2 (T)	S	–		
			2-6 (I)	S	–		
			I–T	–	S		
		Total	3-1 (T)	–	L	1-2-3-4-NO	Restricted gene flow with isolation by distance
			3-2 (T)	S	S		
			3-3 (I)	S	–		

Abbreviations used are: T = Tip clade, I = Interior clade, D_c = Within-clade distance, D_n = Nested clade distance, I–T = Distance between interior and tip clades, S = Significantly small distance, L = Significantly large distance, – = Distance not significant

* Supported by a larger than average number of steps between clades

** The choice of clade 3–3 as being internal is questionable. The ranges of clades 3–2 and 3–3 overlap and are separated from the range occupied by clade 3–1 by a number of localities from which *Exosphaeroma hylocoetes* was absent, suggesting that allopatric fragmentation is a likely scenario for these two major regional lineages

*** *Iphinoe truncata* was not found in the Groot Brak estuary (locality 18). This interpretation was based on the assumption that if it is present, then the haplotypes of this population are likely to be very similar to those in the adjacent Klein Brak estuary (locality 17), as was the case in *E. hylecoetes*

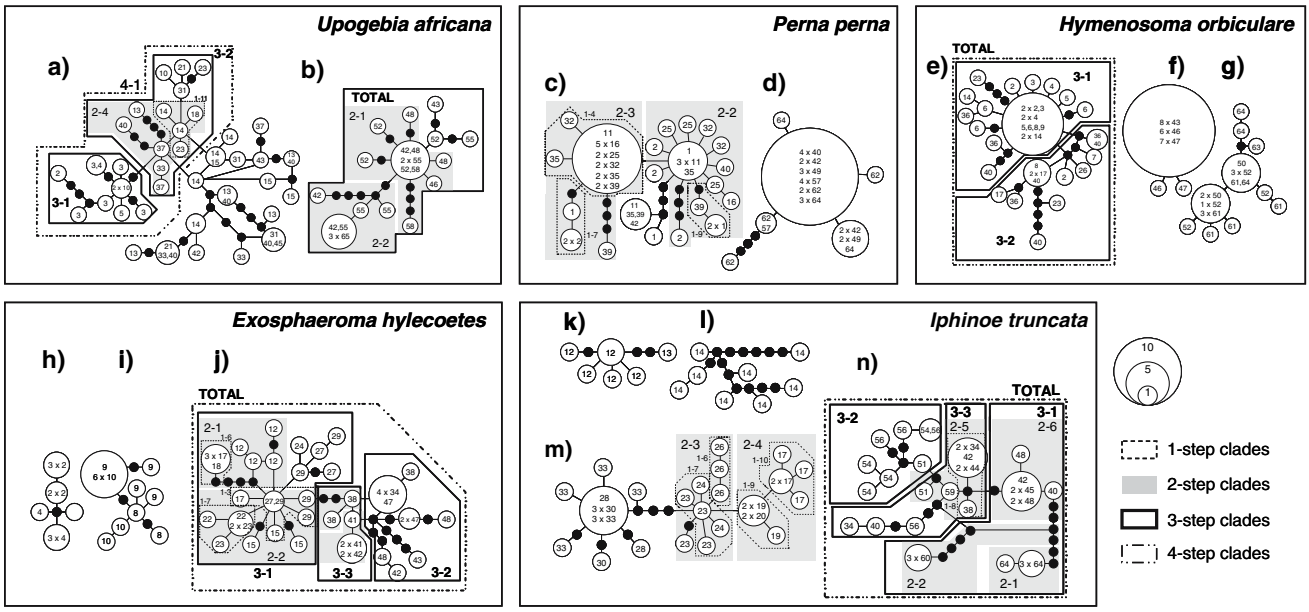

Fig. 3 Haplotype networks of regional lineages of five southern African coastal invertebrates. Letters denote the following lineages: *Upogebia africana*: **a** western lineage; **b** eastern lineage; *Perna perna*: **c** western lineage; **d** eastern lineage; *Hymenomsoma orbiculare*: **e** western lineage; **f** southeastern lineage; **g** eastern lineage; *Exosphaeroma hylecoetes*: **h** western lineage; **i** southwestern lineage; **j** southern lineage; *Iphinoe truncata*: **k** Breede and Duiwenhoks estuaries; **l** Goukou Estuary; **m** southern lineage; **n** eastern lineage. Numbers within white circles indicate at which sampling localities a particular haplotype was found, including its frequency (see Table 1 for localities represented by numbers). If a haplotype was found more than once at a particular locality, this is indicated by a number indicating its frequency followed by "×" and the number of the sampling locality. Black circles are interior node haplotypes not present in the samples. For simplicity, only nested clades for which significant relationships between genetic and geographic distances were found are indicated. Numbers of these correspond to those used in Table 3. Symbols in the right bottom corner indicate approximate sizes of circles for different haplotype frequencies and outlines used to denote clades at different nesting levels

swimming megalopa of *U. africana*, the larvae of mytilid mussels such as *P. perna* are not capable of active dispersal. The spreading of the European mussel *Mytilus galloprovincialis* along the South African coast provided an opportunity to study dispersal distances in this region (these are likely to be similar in the indigenous *P. perna*). McQuaid and Phillips (2000) monitored changes in mussel distributions and found that their larvae are dispersed like passive particles. Although wind-driven dispersal of up to 97 km per year was possible, frequent wind reversals during the dispersal phase resulted in 90% of recruits being recovered within less than 5 km of the parent population after a period of 4 years. Dispersal of mussel larvae is thus strongly influenced by hydrological conditions. Estimates of genetic structure and levels of gene flow were similar to those of *U. africana*, suggesting that the possibility of active larval dispersal in the mudprawn does not seem to influence connectivity between populations strongly.

Species with abbreviated larval development

Larval retention within estuaries is considered to be an evolutionarily advanced strategy (Anger 2001), which is limited to a comparatively small number of species in which the larvae are physiologically adapted to tolerate the osmotic and thermal stresses characteristic of the estuarine habitat. The larvae of *H. orbiculare* can tolerate a wider range of salinity and temperature than most other estuarine decapod species in southern Africa (Papadopoulos et al. 2006). Considering that most (>70%) estuaries along the southern African coastline close seasonally or occasionally (Whitfield 2004), and that such estuaries are able to support greater densities of macrobenthic species than permanently open estuaries (Teske and Wooldridge 2001), wide physiological tolerance ranges and completion of larval development within estuaries are advantageous strategies in this region. Each of the zoeal stages of *H. orbiculare* has been recorded in numerous estuaries along the South African coastline, including both permanently open estuaries (Broekhuysen 1955; Wooldridge and Callahan 2000) and temporarily open/closed systems (Newman, unpubl. data). In addition, long-term data on the density and distribution of crown crab larvae in the permanently open Gamtoos Estuary on the southeast coast (site 29) suggest that these are retained and complete their development within the estuarine environment (Newman, unpubl. data). In contrast, Papadopoulos et al. (2002) found no evidence for the retention of larval *H. orbiculare* in the Mlalazi Estuary on the northeast coast (also a permanently open estuary). The first zoeal stage was exported out of the estuary, and none of the

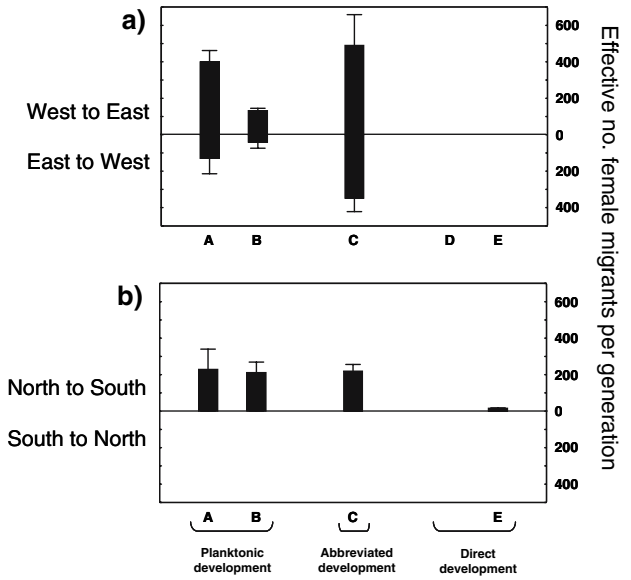

Fig. 4 Estimates of asymmetrical gene flow (effective number of female migrations per generation) within regional lineages of five southern African coastal invertebrates along **a** South Africa's south coast and **b** east coast estimated using IM. Bars depict mean values based on three replicates scaled to neutral evolutionary rate and whiskers are standard deviations. Populations were selected from regions where most species were present. **a** = *Upogebia africana* (**a** sampling localities 13–23 and 27–40; **b** 48–53 and 55–65); **b** = *Perna perna* (**a** 11–25 and 32–40; **b** 49–57 and 62–65); **c** = *Hymenosoma orbiculare* (**a** 14–26 and 36–40; **b** 50–52 and 61–64); **d** = *Exosphaeroma hylecoetes* (**a** 12–23 and 27–38); **e** = *Iphinoe truncata* (**a** 17–26 and 28–33; **b** 48–54 and 56–64)

subsequent larval stages were recorded within the estuary. However, the latter study was conducted over two periods of 3 weeks, and more extensive sampling is necessary to confirm that larval retention does not occur there.

Demographic histories, levels of population structure and amount of gene flow in *H. orbiculare* were similar to those of the species with an extended marine dispersal phase, and very different from those of the two direct developers. Although we cannot exclude the possibility that the three lineages of *H. orbiculare* have slightly different life histories, our results indicate that the occasional export of zoea larvae, and passive dispersal into estuaries, may be sufficient to maintain high levels of gene flow. Hence, although this species is less well equipped to migrate among estuaries than the mudprawn (which has a megalopa), it nonetheless experiences sufficient gene flow to prevent genetic divergence among populations within regional lineages.

Direct developers

Population genetic theory suggests that even a small amount of migration between populations will prevent genetic divergence by drift (Lewontin 1974; Slatkin 1985; Mills and Allendorf 1996). Hence, if high levels of genetic subdivision are found in marine populations, then very little gene flow has taken place. The isopod *E. hylecoetes* should theoretically be successful at colonizing new habitats, because rafting provides a mechanism in which a large number of individuals arrive simultaneously to found a new population (Paulay and Meyer 2002). However, the amount of genetic structuring identified for this species was similar to that of the non-rafting *I. truncata*.

Both direct developers were characterized by geographically disjunct clusters of haplotypes, but little structure was found within these clusters. Hence, despite the fact that Φ_{ST} values were higher than in the planktonic dispersers (indicating more genetic structure), they were nonetheless non-significant. Genetic connectedness between populations of direct developers tends to be reasonably high among adjacent populations, but decreases with increasing geographic distance (Hellberg et al. 2002), a trend that was confirmed by the fact that significant isolation by distance was only found in the direct developers investigated in this study. Hence, at local scales, there is little difference in terms of recolonisation potential between planktonic dispersers and direct developers. Nested clade analyses revealed that at higher nesting levels, regional lineages of the direct developers showed signatures of isolation by distance and even allopatric fragmentation, and no effective gene flow was identified between the two major geographic units on the south coast. The fact that dispersal in the direct developers is sporadic and the number of individuals that colonize new habitats is likely to be low compared to the number of propagules released into the sea by species with larval dispersal, indicates that regional populations even of highly abundant direct developers will genetically diverge over time. (e.g. Hellberg 1994; Marko 1998; Edmands 2001; Sponer and Roy 2002). The lack of detectable migration among south coast lineages of the direct developers does not necessarily mean that migration does not take place at all, but can be explained in several other ways: (1) migration rates are too low to be detected with the level of sampling employed in this study; (2) although there is gene flow among lineages, the low number of new alleles is quickly eliminated by genetic drift and (3) the regional lineages are in fact distinct cryptic species, so new alleles entering a neighbouring lineage will not establish themselves.

Overall, phylogeographic structure in the direct developers is characterized by very low levels of gene flow and because of this, the potential for genetic divergence in the absence of an absolute barrier is likely to be high. While rafting may facilitate the establishment of new, outlying populations (Johannesson 1988), it seems to occur too rarely to prevent genetic divergence by drift and possibly even speciation among regional lineages of *E. hylecoetes*.

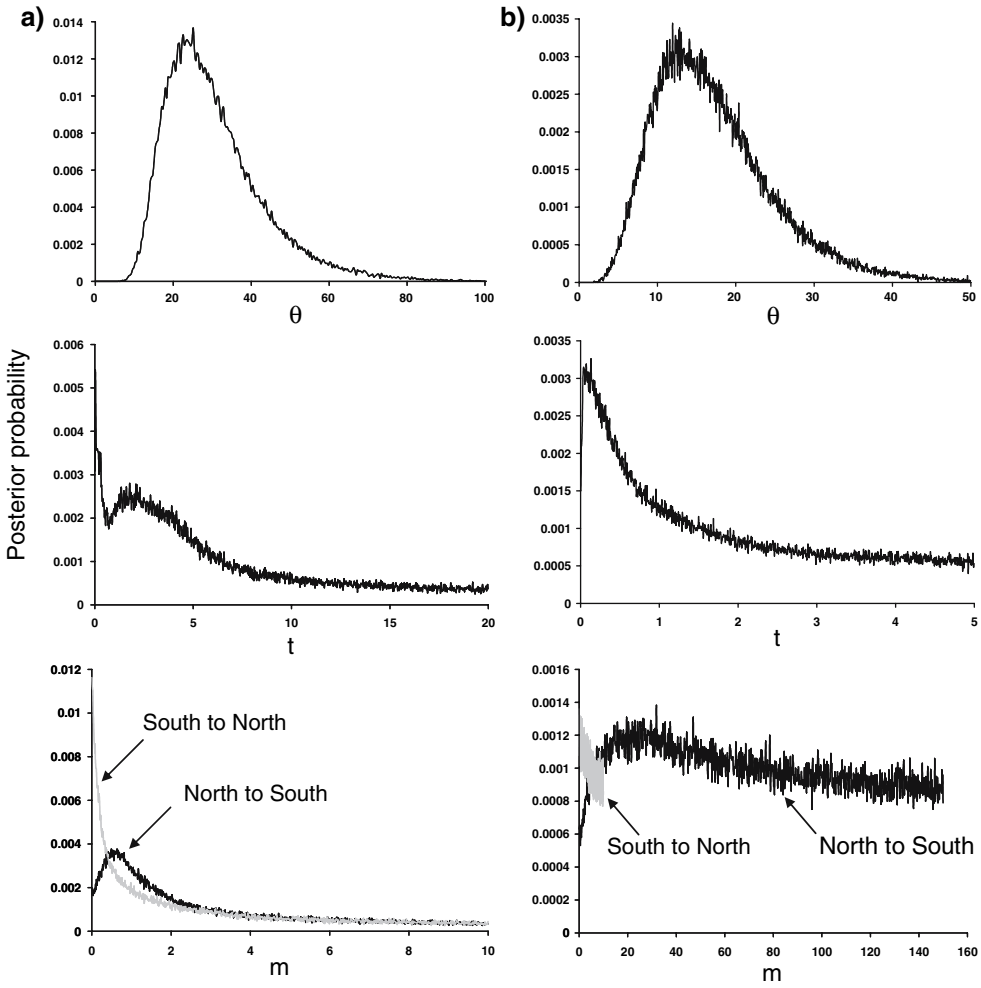

Fig. 5 Examples of IM results showing posterior probability plots of the parameters θ (population size), t (divergence time) and m (migration rate; all scaled to neutral evolutionary rate) of the eastern lineages of **a** the cumacean *Iphinoe truncata* and **b** the crab *Hymenosoma orbiculare*

Conclusion

Investigations of large-scale phylogeographic patterns along the southern African coastline revealed that coastal invertebrate species may be divided into two to three major lineages that in most species are associated with marine biogeographic provinces (e.g. Ridgway et al. 1999; Evans et al. 2004; Teske et al. 2006; Zardi et al. 2007). The number of major lineages and their geographic ranges are not strongly affected by life history strategy because of the structuring effects of currents and environmental discontinuities that may result in diversifying selection. However, our results indicate that within these regional lineages, the effects of life history strategies on phylogeographic parameters may differ considerably. Direct developers showed low levels of gene flow and evidence of regional phylogeographic fragmentation, while the presence of planktonic larvae was found to prevent genetic divergence within regional lineages. However, larval duration, larval swimming ability and larval retention strategies had little effect on connectivity between populations of planktonic dispersers. Even though estimates of dispersal distance tend to be concordant with the dispersal abilities of marine organisms (Kinlan and Gaines 2003), our findings contribute to the growing evidence that the advantages of planktonic dispersal (including decreased fluctuations in population sizes, high recolonisation rates, low extinction rates and low genetic structure) may be obtained irrespective of larval duration (Palmer and Strathmann 1981; Eckert 2003). This implies that there may be no trade-offs for the advantages afforded by larval retention and/or abbreviated larval development.

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