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A tropical/subtropical biogeographic disjunction in southeastern Africa separates two Evolutionarily Significant Units of an estuarine prawn

P. R. Teske · H. Winker · C. D. McQuaid · N. P. Barker

Abstract Recent phylogeographic research has indicated that biodiversity in the sea may be considerably greater than previously thought. However, the majority of phylogeographic studies on marine invertebrates have exclusively used a single locus (mitochondrial DNA), and it is questionable whether the phylogroups identified can be considered distinct species. We tested whether the mtDNA phylogroups of the southern African sandprawn *Callianassa kraussi* Stebbing (Decapoda: Thalassinidea) are also recovered using nuclear sequence data. Four mtDNA phylogroups were recovered that were each associated with one of South Africa's four major biogeographic provinces. Three of these were poorly differentiated, but the fourth (tropical) group was highly distinct. The nuclear phylogeny recovered two major clades, one present in the tropical region and the other in the remainder of South Africa. Congruence between mitochondrial and nuclear DNA indicates that the species comprises two Evolutionarily Significant Units sensu Moritz (1994). In conjunction with physiological data from *C. kraussi* and morphological, ecological and physiological data from other species, this result supports the notion that at least some of the mtDNA phylogroups of coastal invertebrates whose distributions are limited by biogeographic disjunctions can indeed be considered to be cryptic species.

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Introduction

Phylogeography is an integrative field of study that investigates spatial and temporal components of the genetic structure of populations, and which aims to explain how the observed genetic patterns became established (Avise et al. 1987; Beheregaray 2008). Phylogeographic methods are particularly useful to detect cryptic biodiversity and resolve taxonomic issues (Beheregaray and Caccone 2007), and a number of recent phylogeographic studies have indicated that biodiversity in the sea may be considerably greater than previously thought (e.g. Dawson and Jacobs 2001; Stefanni and Thorley 2003; Barber et al. 2006; Teske et al. 2006, 2007a). A serious shortcoming of the majority of such studies is that their conclusions were based on sequence data from a single locus, mitochondrial DNA (mtDNA) (Beheregaray 2008). In some cases, phylogeographic patterns identified in this way have been shown to be linked to ecological patterns, and therefore seem to indicate diversifying selection or the existence of barriers to gene flow between phylogeographic units (e.g. Rocha et al. 2005; Teske et al. 2008). However, in species with low dispersal potential, haphazard phylogeographic patterns can readily arise in the absence of any underlying environmental factors (Irwin 2002; Kuo and Avise 2005), and the validity of the patterns identified should therefore be

confirmed by additional loci. In addition, it is questionable whether or not one can speak of cryptic biodiversity when referring to mtDNA phylogroups, because the validity of a cryptic species or Evolutionary Significant Unit (ESU) should be confirmed by additional data that indicate at least partial isolation from other such units, such as morphological or behavioural differentiation, or congruent genetic differentiation at nuclear loci (Dizon et al. 1992; Moritz 1994).

The South African coastline comprises 3–4 major biogeographic provinces (Fig. 1). From west to east, these are cool-temperate, warm-temperate, subtropical and tropical, with the latter not being universally accepted. Many of the coastal species that are present in two or more of these provinces are divided into phylogroups whose distributions are limited to individual provinces (Ridgway et al. 1998; Evans et al. 2004; Teske et al. 2006, 2007a, b; Zardi et al. 2007). While such patterns seem to indicate cryptic biodiversity, this remains to be tested or confirmed by additional data. Furthermore, although there is some evidence for morphological differentiation between regional phylogroups that could indicate speciation (Ridgway et al. 1998; Teske et al. 2008), no study to date has tested whether such units could be considered as ESU based on Moritz' (1994) definition of reciprocal monophyly for mtDNA haplotypes and significant divergence of allele frequencies at nuclear loci.

In the present study, we used mitochondrial and nuclear sequence data from the sandprawn *Callianassa kraussi* (a species that is believed to have very low dispersal potential) to test whether any of the mtDNA phylogroups of this species could be considered to be ESUs.

Materials and methods

Study species

Callianassa kraussi is commonly found in sheltered marine habitats and estuaries throughout much of southern Africa,
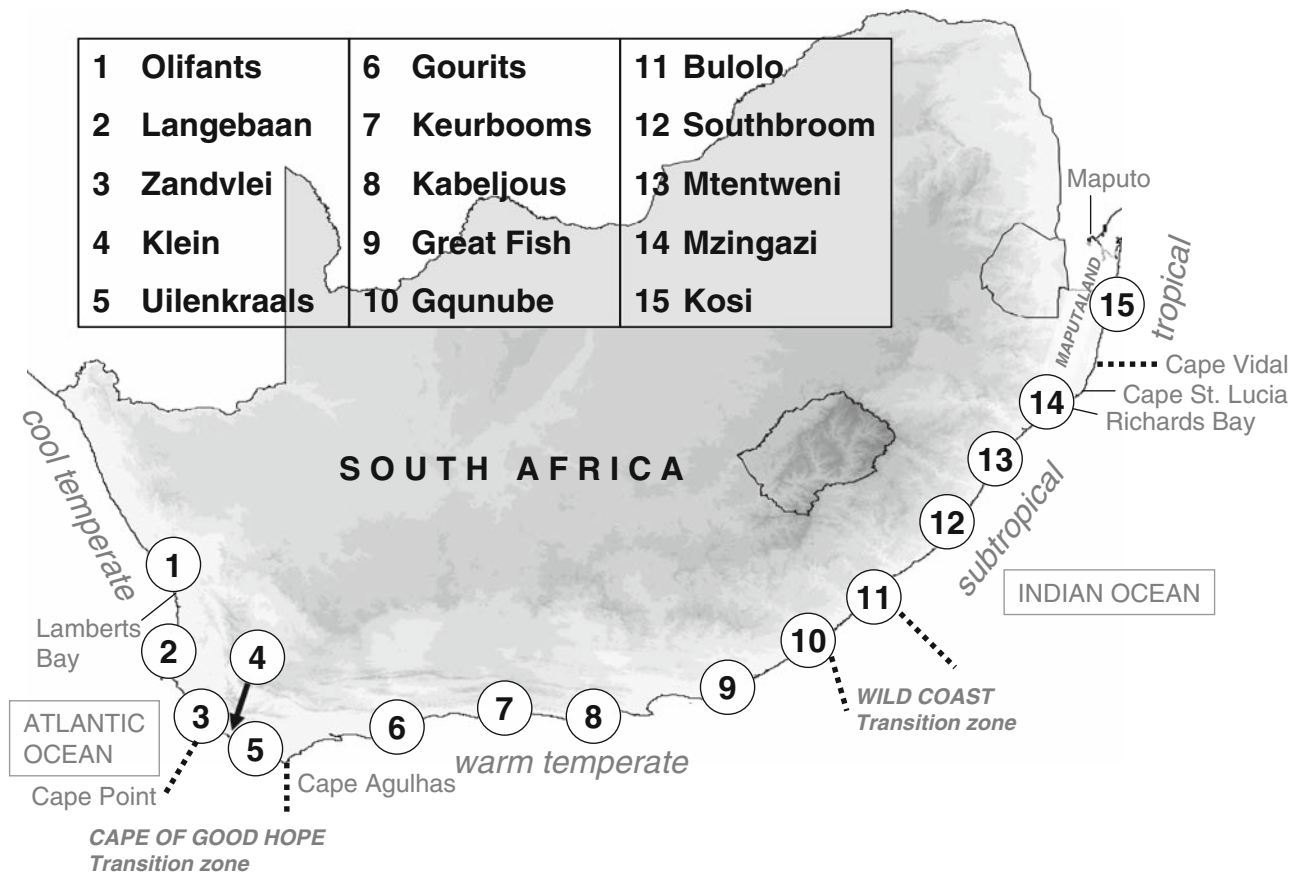

Fig. 1 A map of South Africa showing sampling localities, marine biogeographic provinces and various localities mentioned in the text. The approximate positions of boundaries between the region's four main marine biogeographic provinces (cool-temperate, warm-temperate, subtropical and tropical) are indicated by *dotted lines*. Specimens of the sandprawn *Callianassa kraussi* were collected in 14 estuaries/lagoons throughout the region, and a single specimen of an unidentified congener (referred to as *Callianassa* sp. in Fig. 2) was collected in the Olifants Estuary

where it is often the dominant macroinvertebrate species (Day 1981). This prawn has an unusually abbreviated larval development that is limited to only the first two zoeal stages (Forbes 1973). These larvae are non-planktonic and remain in the parent burrow, and the post-larvae then excavate their own burrows in the wall of the parent burrow. The juvenile burrows eventually become disconnected from the parent burrows and acquire an independent opening with the surface. The absence of a long-lived planktonic dispersal stage suggests that genetic connectivity between populations is likely to be very low, and that any phylogeographic patterns identified could therefore have arisen without the structuring influence of dispersal barriers. Despite their apparently low dispersal potential, sandprawns tend to be well represented in most South African estuaries, and they have a wide distribution range that spans all four of South Africa's biogeographic provinces and extends into Mozambique on the east coast (Forbes 1973).

Sample acquisition and laboratory analyses

Fifty-nine specimens of *Callianassa kraussi* were collected at 14 sites that span the species' South African distribution range (Fig. 1). Sample sizes ranged from 1 to 17 (Table 1). Genomic DNA was extracted using the Chelex extraction method (Walsh et al. 1991). Sample sizes were increased to 26 for selected populations in one analysis (see 'Data analyses'). For the outgroup in 'phylogenetic analyses' we also collected samples of an unidentified congener in the Olifants Estuary (Fig. 1) that is referred to here as *Callianassa* sp. A portion of the COI gene was sequenced to identify phylogeographic patterns, as previous studies showed that this marker provides good resolution at this scale (Teske et al. 2007a, b; Zardi et al. 2007). The COI gene was amplified using forward primer CrustCOIF (5′-TCA ACA AAT CAY AAA GAY ATT GG-3′) and reverse primer Perac COIR (5′-TAT WCC TAC WGT RAA TAT ATG ATG-3′; Teske et al. 2006). Two additional markers were amplified for a subsample of the individuals collected, namely a portion of the 16S rDNA marker using primers 16SarL (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16SbrH (5′-CGG GTC TGA ACT CAG ATC ACG T-3′; Palumbi 1996), and a portion of the 18S rDNA marker using primers 5′-EM (5′-TYC CTG GTT GAT YYT GCC AG-3′) and 18S-1262R (5′-GGT GGT GCA TGG CCG TY-3′; Weekers et al. 1994; Samraoui et al. 2003). Each 25 µl PCR reaction contained 0.16 µM of each dNTP, 2.5 µl of $10 \times NH_4$ reaction buffer, 3 pmol µl^{-1} of each primer, 6 mM (COI) or 3 mM (16S and 18S rDNA) of $MgCl_2$, 0.1 µl of BIOTAQ™ DNA Polymerase (5 units µl^{-1}, Bioline) and 1 µl of genomic DNA. The PCR profile consisted of an initial denaturation step (3 min at 94°C), 35 cycles of denaturation (30 s at 94°C), annealing [45 s at 48°C (COI

and 16S rDNA) or 60°C (18S rDNA)] and elongation (45 s at 72°C), and a final extension step (10 min at 72°C). PCR products were purified using the Wizard® SV Gel and PCR Clean-Up system (Promega), cycle sequenced using Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and analysed on an ABI 3100 genetic analyser.

Sequence alignment

The COI sequences did not contain any indels and could therefore be readily aligned by eye in MEGA version 4.0 (Tamura et al. 2007). 16S and 18S rDNA sequences were aligned using default settings of the CLUSTALW (Higgins et al. 1994) alignment option in MEGA. After removal of ambiguous bases from the sequences' 5′ and 3′ ends, final sequence lengths were 570 bp (COI), 405 bp (16S rDNA) and 828 bp (18S rDNA).

Phylogenetic analyses

For each of the three molecular markers amplified, intraspecific phylogenetic relationships among regional lineages of *Callianassa kraussi* were reconstructed using the neighbour-joining method (Saitou and Nei 1987). We constructed these trees in MEGA using maximum composite likelihoods (Tamura et al. 2004) of Tamura–Nei distances (Tamura and Nei 1993). Support for nodes was assessed by means of 10,000 bootstrap replications. The outgroup comprised sequences of the unidentified *Callianassa* sp. from the Olifants Estuary, as well as sequences of other prawns downloaded from GenBank (Table 1; Fig. 2).

Nested clade phylogeographic analysis

The program ANECA version 1.1 (Automated Nested Clade Analysis; Panchal 2007) was used to infer phylogeographic scenarios that were likely to have been responsible for departures from panmixia among groups of COI haplotypes. ANECA implements the programs TCS (construction of a statistical parsimony haplotype network; Templeton et al. 1992; Clement et al. 2000) and GEODIS (nesting of clades and calculation of the *Dn* and *Dc* statistics; Templeton and Sing 1993; Posada et al. 2000), and automates the inference process using the latest version of the GEODIS inference key. Due to considerable genetic differentiation between two of the lineages, we increased the cut-off point for the maximum number of steps between haplotypes until all haplotypes were included in a single network.

Test for isolation-by-distance

To investigate the sandprawns' dispersal ability, we tested for isolation-by-distance (IBD) among four populations

Table 1 Collection localities and GenBank accessions of samples of the southern African estuarine sandprawn *Callianassa kraussi* and several outgroup species used in this study

Species	Collection locality and coordinates	GenBank accession numbers		
		COI	16S rDNA	18S rDNA
Callianassa kraussi	Langebaan 33°3′S 17°57′E	FJ644710-13	FJ644863-64	FJ644894
	Zandvlei 34°6′S 18°28′E	FJ644714-17	FJ644865-66	FJ644895
	Klein 34°25′S 19°18′E	FJ644718-20	FJ644867	FJ644896
	Uilenkraals 34°36′S 19°24′E	FJ644721-23	FJ644868-69	FJ644897
	Gourits 34°20′S 21°53′E	FJ644724-26 (FJ644727-49)	FJ644870	FJ644898
	Keurbooms 34°2′S 23°23′E	FJ644750-52 (FJ644753-75)	FJ644871-72	FJ644899
	Kabeljous 34°0′S 24°56′E	FJ644776-77 (FJ644778-801)	FJ644873-74	FJ644900
	Great Fish 33°36′S 26°54′E	FJ644802-04 (FJ644805-27)	FJ644875-76	FJ644901
	Gqunube 32°55′S 28°1′E	FJ644828-29	FJ644877-78	FJ644902
	Bulolo 31°39′S 29°31′E	FJ644830	–	FJ644903
	Southbroom 30°56′S 30°18′E	FJ644831-34	FJ644879	FJ644904
	Mtentweni 30°42′S 30°28′E	FJ644835-38	FJ644880	FJ644905
	Mzingazi 28°48′S 32°5′E	FJ644839-44	FJ644881-82	FJ644906-07
	Kosi 27°0′S 23°50′E	FJ644845-61	FJ644883-92	FJ644908-17
Callianassa sp.	Olifants 33°40′S 21°43′E	FJ644862	FJ644893	FJ644918
C. subterranea			DQ079706[a]	DQ079740[a]
Callichirus major		AF436025[b]		
Munida proto		AY350986[c]		

COI sequences in brackets were used to test for isolation-by-distance only

[a] Porter et al. 2005

[b] Morrison et al. 2002

[c] Machordom and Macpherson 2004

from the warm-temperate marine biogeographic province (localities 6–9, Fig. 1). For this analysis, additional COI sequences were generated for a total of 26 samples per site (shown in brackets in Table 1). The reason why not all COI sequences were included was that the structuring effects of currents and/or environmental gradients associated with biogeographic barriers affect most coastal invertebrates in southern Africa similarly, irrespective of their dispersal potential (Teske et al. 2007a). Inappropriate pooling of such genetically distinct regional units (as was done in e.g. York et al. 2008) results in significant IBD being identified even in species that exhibit panmixia within marine bio-

geographic provinces (Teske et al. 2008). The warm-temperate province is particularly suitable to test for IBD because there are no oceanographic features that are likely to act as barriers, and current flow along the coast can take place in both easterly and westerly directions (Teske et al. 2007a; von der Heyden et al. 2008). We used the ISOLATION BY DISTANCE WEB SERVER version 3.15 (Jensen et al. 2005) to conduct a Mantel test (1967) to test for correlations between a genetic distance matrix containing uncorrected pairwise linearised Φ_{ST} values (Slatkin 1995) and a geographic distance matrix containing shortest along-coast distances in km, and specified 10,000 randomisations to test
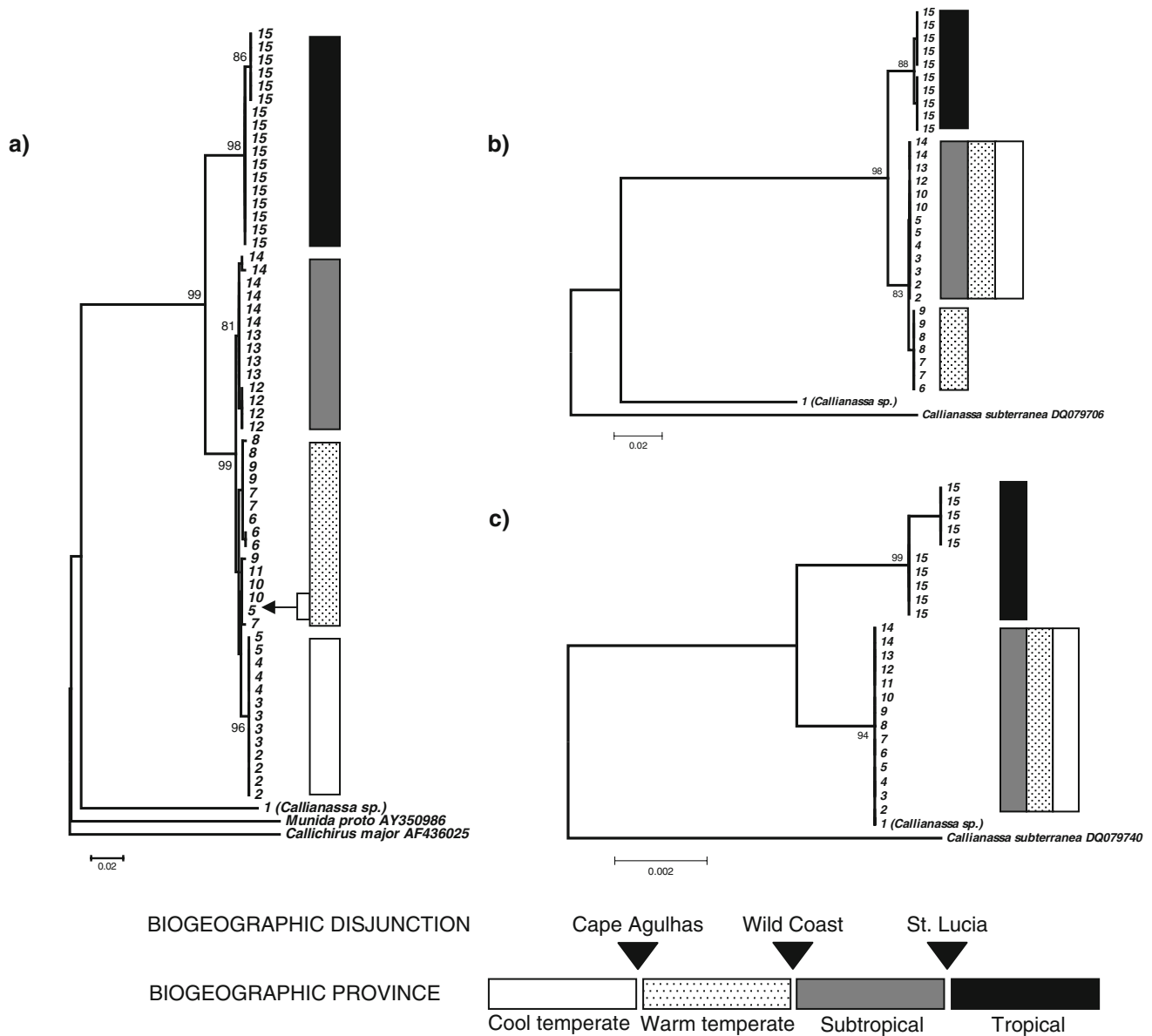
Fig. 2 Intraspecific phylogenetic relationships among regional lineages of the sandprawn *Callianassa kraussi* based on three molecular markers: **a** cytochrome oxidase subunit 1 (COI), **b** 16S rDNA and **c** 18S rDNA. In each case, a neighbour-joining tree was reconstructed using maximum composite likelihoods. Nodal support (>75%) from 10,000 bootstrap replications is shown on the left of some clades. Association of haplotypes with marine biogeographic provinces is indicated by *shaded bars*, which are explained in the legend. The *arrow* in **a** indicates a specimen having a haplotype typical of the warm-temperate province that was found in the cool-temperate province

for significance. We also tested for significant genetic structure among the four warm-temperate populations by estimating pairwise Φ_{ST} values (uncorrected p-distances, 10,000 permutations) using ARLEQUIN version 3.11 (Excoffier et al. 2005).

Results

A total of 16 COI haplotypes, four 16S rDNA haplotypes and three 18S rDNA alleles of *Callianassa kraussi* were

recovered, as well as one sequence per marker for the unidentified *Callianassa* sp. from the Olifants River.

Phylogenetic analyses

In the case of the COI sequence data, genetic discontinuities that coincided with the boundaries between marine biogeographic provinces were identified at three localities (Fig. 2a). The first of these phylogeographic breaks coincided with the Cape Agulhas boundary (southwest coast, Fig. 1), which separates the cool-temperate west coast from

the warm-temperate south coast. A second break was identified along the Wild Coast (southeast coast), which is a transition zone between the warm-temperate south coast and the subtropical southern east coast. Differentiation between haplotypes associated with the three regions separated by these two phylogeographic breaks was minimal, and phylogeographic patterns poorly developed. The subtropical and cool-temperate lineages were reciprocally monophyletic, but the latter was nested among haplotypes from the warm-temperate province. In one case, a haplotype typical of the warm-temperate province was even found in the easternmost locality in the cool-temperate province (Uilenkraals, locality 5). A third phylogeographic break separated haplotypes of specimens collected at Kosi Bay (located in the northern portion of the east coast) from those in the remainder of the country. COI haplotypes from Kosi Bay were recovered as a distinct phylogenetic lineage with high bootstrap support.

This third phylogeographic break was also clearly evident in phylogenies reconstructed using sequences of the more slowly-evolving 16S rDNA and 18S rDNA markers (Fig. 2b, c, respectively), both of which recovered the samples from Kosi Bay and those in the remainder of the country as distinct and reciprocally monophyletic clades. Samples collected south of Kosi Bay had two 16S rDNA haplotypes (one of which was only found in populations from the western portion of the south coast), while all of them shared the same 18S rDNA allele. This same allele was also present in the outgroup taxon *Callianassa* sp. that was collected on the west coast just beyond of the northern–western distribution limit of *C. kraussi*.

Nested clade phylogeographic analysis

Significant departures from panmixia were identified in five cases (Fig. 3). Restricted gene flow with IBD (inference chain: 1-2-3-4 NO) was inferred in the case of two haplotypes from the subtropical biogeographic province (clade 1-4, localities 12 vs. localities 13 and 14) and a 2-step clade that comprised haplotypes from the cool-temperate and warm-temperate provinces (clade 2-1, localities 2, 3, 4 and 5 vs. localities 5, 7, 9, 10 and 11). Allopatric fragmentation (inference chain: 1-19 NO) was inferred for clade 3-1 [warm-temperate localities (clade 2-2) vs. a mixture of warm and cool-temperate localities (clade 2-1)], clade 4-1 [temperate (clade 3-1) vs. subtropical localities (clade 3-3)] and for the entire cladogram [the tropical lineage (clade 4-2) vs. all other sites (clade 4-1)].

Test for isolation-by-distance

Most pairwise Φ_{ST} values among the four populations from the warm-temperate biogeographic province were signifi-

cant ($\Phi_{ST} = 0.05$–0.17, $P < 0.05$). A single exception was the comparison between populations 7 and 9 ($\Phi_{ST} = 0.01$, $P = 0.24$). A Mantel test for isolation-by-distance based on Slatkin's linearized Φ_{ST} values showed no significant correlation between genetic and geographic distance ($r = 0.24$, $P = 0.29$).

Discussion

Cryptic divergence across biogeographic disjunctions

The present study represents the first genetic assessment of whether or not mtDNA phylogroups of coastal invertebrates in southern Africa can be considered to be ESUs. A phylogeographic break associated with a tropical/subtropical biogeographic disjunction was identified in the sandprawn *Callianassa kraussi* using both mitochondrial and nuclear sequence data and in both cases, the lineages identified were reciprocally monophyletic. This indicates that mtDNA phylogroups of coastal invertebrates whose distributions are limited by biogeographic disjunctions are unlikely to have arisen randomly. Instead, the structuring effects of dispersal barriers between the marine biogeographic provinces may have resulted in allopatric fragmentation. It is even possible that environmental conditions characteristic of each province (e.g. temperature) may have exerted some measure of diversifying selection on the corresponding phylogroups, a process that may result in speciation in different units of a species even if they do not occur in complete isolation (Diekmann et al. 2004; Rundle and Nosil 2005).

This hypothesis is supported by physiological experiments on the osmoregulatory abilities of sandprawns from the warm temperate and subtropical provinces. At salinities below those of seawater, hemolymph osmolality of warm temperate prawns is lower than that of the subtropical prawns (Forbes 1974; Cerff 1986). At very low salinities (~3 ppt), hemolymph osmolality in the warm temperate prawns decreases sharply (Forbes 1974), but decreases only slightly in the subtropical prawns (Cerff 1986; Newman et al., unpublished data). The ability of the subtropical sandprawns to osmoregulate more efficiently at low salinities may be an adaptation to salinity in subtropical temporarily open/closed estuaries generally being lower than in temperate estuaries due to a combination of more rainfall and less seawater input (reviewed in Harrison 2004). Morphological and physiological data from other invertebrates species further support the existence of cryptic species that are associated with individual South African marine biogeographic provinces. Ridgway et al. (1998) considered representatives of the South African limpet *Patella granularis* on either side of the Wild Coast biogeographic

Fig. 3 A TCS haplotype network of COI haplotypes from the cool-temperate, warm-temperate, subtropical and tropical biogeographic provinces. *Shading* of each haplotype indicates in which marine biogeographic province it was found (see Fig. 2), and *small black dots* are interior node haplotypes not present in the samples. Nested clades for which significant departures from panmixia were identified are indicated, and phylogeographic scenarios inferred for these are mentioned in the text
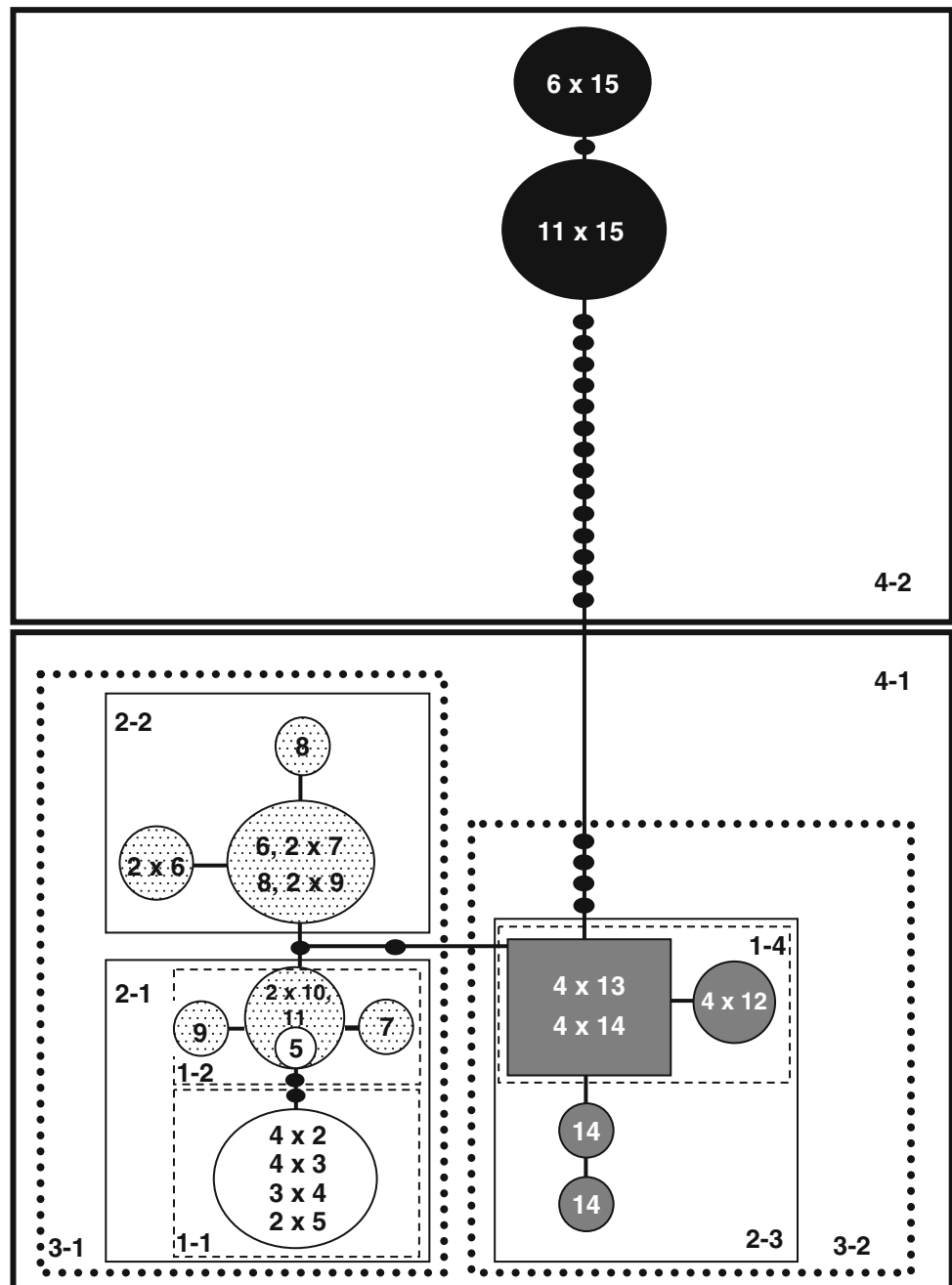
disjunction to be sufficiently differentiated genetically (based on allozyme data) and morphologically to consider them distinct species. In the mudprawn *Upogebia africana*, mtDNA divergence and morphological differentiation across the same disjunction could additionally be linked to physiological differentiation: larvae of the subtropical lineage of this species cannot complete development at or below 14°C (Teske et al. 2008), whereas completion of larval development is possible at such low temperatures in the species' warm-temperate lineage. Similarly, adults of the subtropical lineage of the marine mussel *Perna perna* survive heat and desiccation stress better than their temperate counterparts (G.I. Zardi and K. Nicastro, unpublished data). In summary, the genetic, ecological, morphological and physiological data available to date indicate both neutral genetic differentiation and adaptive differentiation of the mtDNA phylogroups of southern African coastal invertebrates.

Dispersal potential of *Callianassa kraussi*

Significant genetic divergence among local populations of coastal invertebrates (as identified among four *C. kraussi* populations from the warm-temperate biogeographic

province) can arise even in species that have long-lived planktonic larvae and that should therefore have high dispersal potential (e.g. Banks et al. 2007; Matthee et al. 2007; Nicastro et al. 2008). However, within biogeographic regions (i.e. in the absence of barriers to gene flow), genetic lineages of such species are not subdivided into monophyletic smaller-scale regional units, and they do not show a pattern of isolation-by-distance (IBD; Matthee et al. 2007; Teske et al. 2007a). Tests for IBD can therefore be considered a useful way to distinguish passive dispersers with high dispersal potential from low-dispersal species such as benthic direct developers (Teske et al. 2007a), whose phylogeographic patterns may have arisen in the absence of any barriers to gene flow.

The results from two different phylogeographic analyses performed for *C. kraussi* (NCPA and a Mantel test) seem contradictory, with the NCPA identifying restricted gene flow with IBD in two instances and allopatric fragmentation in three instances, and the Mantel test for correlations between genetic and geographic distance matrices being non-significant. Recently, NCPA has been criticised for being biased towards false positives, in particular inferences of restricted gene flow or contiguous range expansion under simulated scenarios of panmixia (Panchal and Beaumont 2007). We nonetheless consider the method to be a useful means of exploring one's data, and concur with a recent comment by Garrick et al. (2008) that rather than abandoning NCPA altogether, the inferences should be compared with the results of complementary analyses. In the case of *C. kraussi*, significant departures from panmixia indicating little or no gene flow can be readily explained. First, the populations comprising clade 2-1 span a biogeographic disjunction, and the close proximity of populations separated by a barrier to gene flow in the form of an environmental discontinuity therefore resulted in an inference of IBD (the restricted gene flow component of the inference, on the other hand, is quite acceptable). Inferences of allopatric fragmentation at higher nesting levels also involve samples collected on either side of biogeographic disjunctions. The only significant departure from panmixia that does not involve a dispersal barrier is the inference of restricted gene flow in the case of the subtropical clade 1-4 (locality 12 vs. localities 13 and 14).

NPCA can yield significant results at lower level nested clades (which are based on very few sequences) that are often meaningless at higher level clades or when sample sizes are increased. For example, Teske et al. (2007a) found inferences of range expansion between two regions at lower level nested clades, but at higher levels, no significant departure from panmixia was found among all of the representatives of the regions. It is therefore likely that additional haplotypes will be recovered in population 12 if sample sizes at that locality were increased.

A non-significant Mantel test, and the fact that (with one exception) significant departure from panmixia indicating limited dispersal was inferred only when comparing populations associated with different biogeographic provinces, indicates that genetic connectivity among *C. kraussi* populations is higher than expected on the basis of the species' abbreviated larval development. Significant genetic structure between most of the warm-temperate populations suggests some local self-recruitment (as has also been shown for species with very high dispersal potential; Banks et al. 2007; Matthee et al. 2007; Nicastro et al. 2008), but gene flow seems to be sufficiently high to prevent the formation of haphazard genetic patterns that are not linked to any obvious environmental features, as found in benthic direct developers (Teske et al. 2007a). Even though larval stages, postlarvae and adults of *C. kraussi*, are rarely found outside their burrows, movement within estuaries has been reported. Forbes (1978) noted migrations of postlarvae, and Vorsatz (2000) not only observed movement of adults from one region of an estuary to another to escape unfavourably low salinities, but also found postlarvae in midwater plankton tows during a period of strong tides. Forbes (1978) hypothesised that the early postlarval stages may have taken over the role of dispersal that in other thalassinid prawns is carried out by planktonic larvae, and the genetic data from the present study support this idea.

The St. Lucia biogeographic disjunction

The region near St. Lucia (southern Maputaland, Fig. 1) is characterised by gradients in coastal environmental conditions that in combination may represent a barrier to the dispersal of coastal organisms. The continental shelf widens, the influence of the warm southward-flowing Agulhas Current is reduced, and the coastal environment is dominated by a persistent upwelling cell (Schumann 1988). There is also a marked difference in water turbidity because the region south of St. Lucia receives run-off from several large rivers (Flemming and Hay 1988). Furthermore, the scarcity of suitable habitats between Kosi Bay and Richards Bay (Fig. 1) may limit the amount of gene flow between populations of some coastal invertebrates. *Callianassa kraussi*, for example, is not represented in any of the river-dominated estuaries or coastal lakes in this region (Bruton 1980; Hart 1980).

Southern Maputaland has been identified as a transition zone between tropical Indo-Pacific and subtropical southeast African biota (Stuckenberg 1969). Groups of coastal organisms that exhibit changeovers in species composition in this region include intertidal fauna (Jackson 1976; Bolton et al. 2001) and benthic seaweeds (Bolton et al. 2004). Based on abundance data of rocky intertidal invertebrates and seaweeds, Sink et al. (2005) concluded that

the exact location of the biogeographic disjunction between the tropical and subtropical regions is at Cape Vidal (Fig. 1). Although distinct phylogeographic patterns have previously been identified on the southwest coast and on the southeast coast (Ridgway et al. 1998; Evans et al. 2004; Teske et al. 2007a, b), none of the planktonic dispersers that were collected north and south of St. Lucia had a phylogeographic break in this region (the crab *Hymenosoma orbiculare*, Edkins et al. 2007; the mudprawn *Upogebia africana*, Teske et al. 2007a; and the mussel *Perna perna*, Zardi et al. 2007). Based on a dataset with a larger samples size, shallow population structuring was however found in southeastern Africa in the spiny lobster *Palinurus delagoae* (Gopal et al. 2006), although this genetic discontinuity did not coincide with the St. Lucia biogeographic disjunction. Populations north and south of this region shared haplotypes, and the most significant genetic grouping was found between South African populations that included samples from north and south of the disjunction versus populations from Mozambique. In direct developers, on the other hand, there was some indication of more clear-cut genetic discontinuities. First, samples of the estuarine cumacean *Iphinoe truncata* from the Kosi system were monophyletic, but poorly differentiated from other populations from the east and southeast coast (Teske et al. 2007a). Secondly, rocky shore limpets of the genus *Siphonaria* from north of Cape Vidal were genetically distinct from samples collected south of this region (Teske et al. 2007c). These groups were also poorly differentiated, and there was a large sampling gap between northern and southern sites, so that the presence of genetically intermediate forms in the unsampled region cannot be ruled out. The results of the present study on the sandprawn *C. kraussi* thus present the first clear evidence for a phylogeographic break associated with the biogeographic disjunction at St. Lucia. Although sandprawns seem to have greater dispersal potential than expected, the fact that such a deep phylogeographic break could arise in this species, while only shallow genetic structuring was identified in a species with an extended larval dispersal phase (Gopal et al. 2006), highlights the limitations of the sandprawn's potential dispersal by means of postlarvae. Hence, while no IBD was found on the south coast (a region with an abundance of suitable habitat), the scarcity of suitable habitat in Maputaland could have played a role in the evolution of the phylogeographic break, in addition to environmental discontinuities. The extreme northeast of South Africa and southern Mozambique have been poorly studied because of logistical difficulties in reaching sampling sites (Bolton et al. 2004). This study highlights the importance of incorporating samples from this region when studying cryptic biodiversity in southern Africa.

Conclusion

The *Callianassa kraussi* lineage present north of the St. Lucia biogeographic disjunction shows considerable genetic differentiation from the lineages in the remainder of the country. That this is evident even at the level of the slowly-evolving 18S rDNA suggests that this distinct evolutionary significant unit may represent a new, tropical species that remains to be formally described. In addition to morphological, ecological and physiological evidences from other species (e.g. Ridgway et al. 1998; Rocha et al. 2005; Teske et al. 2008), this result supports the notion that mtDNA phylogroups of species that have reasonably high dispersal potential (i.e. whose regional lineages are not affected by IBD), and whose distributions closely match the ranges of biogeographic provinces, are likely to be distinct ESUs or even cryptic species.

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