Vol. 319: 191–198, 2006 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published August 18

Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the southwestern Indian Ocean

K. Gopal[1], K. A. Tolley[1,3], J. C. Groeneveld[2], C. A. Matthee[1,*]

[1]Evolutionary Genomics Group, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa

[2]Marine and Coastal Management, Private Bag X2, Roggebay 8012, Cape Town, South Africa

[3]South African National Biodiversity Institute, Kirstenbosch Research Centre, Private Bag X7, Claremont 7735, Cape Town, South Africa

ABSTRACT: The spiny lobster *Palinurus delagoae* inhabits the deep shelf waters and oceanic ridges of the southwestern Indian Ocean, where it supports commercial fisheries. To investigate population structure and the physical processes that may have influenced female gene flow in this species, a portion of the mitochondrial DNA (mtDNA) control region (547 base pairs) was sequenced for 285 lobsters from the southeastern coast of Africa (6 sites) and 49 lobsters from Walters Shoals, a submerged seamount on the Madagascar Ridge. Lobsters from these 2 areas shared no haplotypes and differed by at least 27 mutational steps. An analysis of molecular variance showed significant genetic partitioning, and pairwise comparisons suggested that lobsters from Walters Shoals are distinct from those of other sampling areas. There was shallow genetic partitioning between 4 southern sites (South Africa) and 2 northern sites (Mozambique), suggesting 2 Management Units along the African coast. A mismatch distribution and Fu's *F*-test indicated fairly recent demographic changes within populations, consistent with that found in the sister species *P. gilchristi*. Female gene flow along the African coast may be propagated by larval dispersal in the Mozambique and Agulhas Currents and countercurrent migrations by benthic juveniles along the shelf, but the mtDNA data strongly suggest that larvae at Walters Shoals have been, or are currently still retained by other oceanographic processes. The magnitude of mtDNA divergence among lobsters from the southeastern coast of Africa and those at Walters Shoals, together with the absence of any shared haplotypes between these regions strongly suggest that these 2 taxa might in fact represent distinct species.

KEY WORDS: *Palinurus delagoae* · Walters Shoals · mtDNA control region · Population structure · Demographic expansion

INTRODUCTION

The spiny lobster *Palinurus delagoae* inhabits the upper slope and deep shelf waters (150 to 600 m depth) of the southwestern Indian Ocean. It is found in temperate waters (12 to 18°C) along the African coast from eastern South Africa (30° S) to central Mozambique (17° S) (Fig. 1). Geographically isolated populations occur on the narrow southeastern shelf of Madagascar (Berry & Plante 1973) and at Walters Shoals (33° 9′ to 16′ S, 44° 49′ to 56′ E; Fig. 1). The latter is the shallowest seamount on the Madagascar Ridge, reaching 15 m below the sea surface and located roughly 400 nautical miles (n miles) south of Madagascar (the nearest land mass) and 600 n miles east of South Africa. Along the African coast, *P. delagoae* has a long history of exploitation as a targeted species of trap-fisheries (Groeneveld 2000, Palha de Sousa 2001) and as a minor bycatch in multi-species crustacean trawl fisheries (Fennessy & Groeneveld 1997). Fishing off south-

*Corresponding author. Email: cam@sun.ac.za

eastern Madagascar has been exploratory (Roullot 1988), and high seas vessels report occasional catches from Walters Shoals (J. C. Groeneveld pers. obs.).

Palinurus delagoae is a large (up to 4 kg), long-lived and slow-growing species, and has been the subject of many fisheries biological studies summarized in Groeneveld et al. (2006). It is found on a wide variety of substrates, ranging from steep rock to areas of organically rich mud or sand substrata with coral fragments. The species exhibits ontogenetic, long-shore and reproductive migrations. Pueruli (a transparent larval stage) settle at >600 m depth and then gradually migrate shorewards to adult habitats at depths of 150 to 350 m (Cockcroft et al. 1995). Juveniles along the African coast make long-shore migrations up to 500 km, against the southwesterly flowing Agulhas Current (Groeneveld 2002). Egg-bearing females aggregate in shallower depths in summer, moving deeper after spawning in fall and winter (Koyama 1971, Kondritskiy 1976). These migrations affect the position of larval release relative to ocean currents, and may therefore have far reaching implications for larval dispersal and gene flow.

In the marine environment, widespread larval dispersal can result in limited or no genetic structure, as has been observed for a number of species (e.g. *Jasus edwardsii*, Ovenden et al. 1992; *Nephrops norvegicus*, Stamatis et al. 2004; *Farfantepenaeus duorarum*, McMillen-Jackson & Bert 2004). Larval dispersal routes of *Palinurus delagoae* and its congener *P. gilchristi* (from the southern coast of South Africa) remain unknown, although a recent population genetic study of *P. gilchristi* showed a well-mixed gene pool among individuals from across its distribution (Tolley et al. 2005). Despite the apparent 'free' mixing of larvae found in some species, fragmented gene pools have been recorded in several species of rock and spiny lobsters (*Jasus* [*Sagmariasus*] *verreauxi,* Brasher et al. 1992; *Panulirus argus,* Sarver et al. 1998; *Panulirus interruptus*, Perez-Enriquez et al. 2001).

At least 2 factors suggest that *Palinurus delagoae* might exhibit spatial genetic structure across its distribution. First, in some instances breeding individuals are separated by large distances and deep intervening waters, and this distribution pattern exposes larvae to 2 different ocean current systems, i.e. the Mozambique and Agulhas Currents along the African coast and the East Madagascar Current off Madagascar (Lutjeharms 1988). Secondly, *P. delagoae* exhibits morphological variation across its range, with specimens from Madagascar (and Walters Shoals, J. C. Groeneveld pers. obs.) more spinose than those along the African coast (Berry & Plante 1973). Even the African shelf individuals are distinctly variable, as highlighted by Barnard (1926) in the initial description of the species as 2 varieties, *natalensis* from eastern South Africa and *delagoae* from Mozambique. A later revision of the genus in the southwestern Indian Ocean synonymised the 2 varieties and raised them to specific rank (*P. delagoae*). The Madagascan population was also included in the latter species, but placed with uncertainty (Berry & Plante 1973).

The aim of this study was to determine whether genetic structure could be detected for *Palinurus delagoae* by sequencing the hypervariable region I of the mitochondrial DNA (mtDNA) control region. Inferences regarding the contemporary genetic population structure, combined with information on life-history of the species and the oceanographic history of the southwestern Indian Ocean, are used to infer larval distribution patterns and recent evolutionary history. Given the commercial importance of this species, the genetic data could be essential for directing future management decisions.

MATERIALS AND METHODS

Tissue samples were obtained from 334 individuals captured by traps from 4 sampling sites along the east coast of KwaZulu-Natal, South Africa (KZN-1–4), 2 sites along the Mozambique coast (Inhaca and Boa Paz) and 1 site at Walters Shoals on the Madagascar Ridge (Fig. 1). Total genomic DNA was extracted from leg tissue stored in 96% ethanol, using a standard digestion buffer containing 5% Chelex^{TM} 100 (Bio-Rad Laboratories) and 10 to 15 µl Proteinase-K (10 mg ml^{-1}). Digestions were performed for 1 h. The digest was subsequently spun down, the supernatant removed, and used directly in PCR. A portion of the control region was amplified in a 25 µl reaction volume containing 5 µl of the supernatant with the genomic DNA, 0.25 µM of each primer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 1 X Buffer (50 mM KCl, 10 mM Tris-HCl, pH 9) and 0.25 unit Super-Therm Taq DNA polymerase (Southern Cross Biotechnology). The primers used for the amplification (L13473 and H14306) were initially designed to amplify the control region of *Palinurus gilchristi* (Tolley et al. 2005). The PCR thermal profile used was 95°C for 1 min, followed by 35 cycles of 35 s at 95°C, 30 s at 50°C and 1 min at 72°C, with a final extension at 72°C for 30 s. An aliquot of the PCR product was electrophoresed on a 1% agarose gel containing ethidium bromide, and visualised by ultraviolet light. PCR products were cycle sequenced using a fluorescently labeled dye-terminator kit (ABI), purified with Sephadex spin columns and analysed on an Applied Biosystems 3100 genetic analyser. MacClade v4.0 (Maddison & Maddison 1992) was used to align sequences manually and to identify haplotypes. All

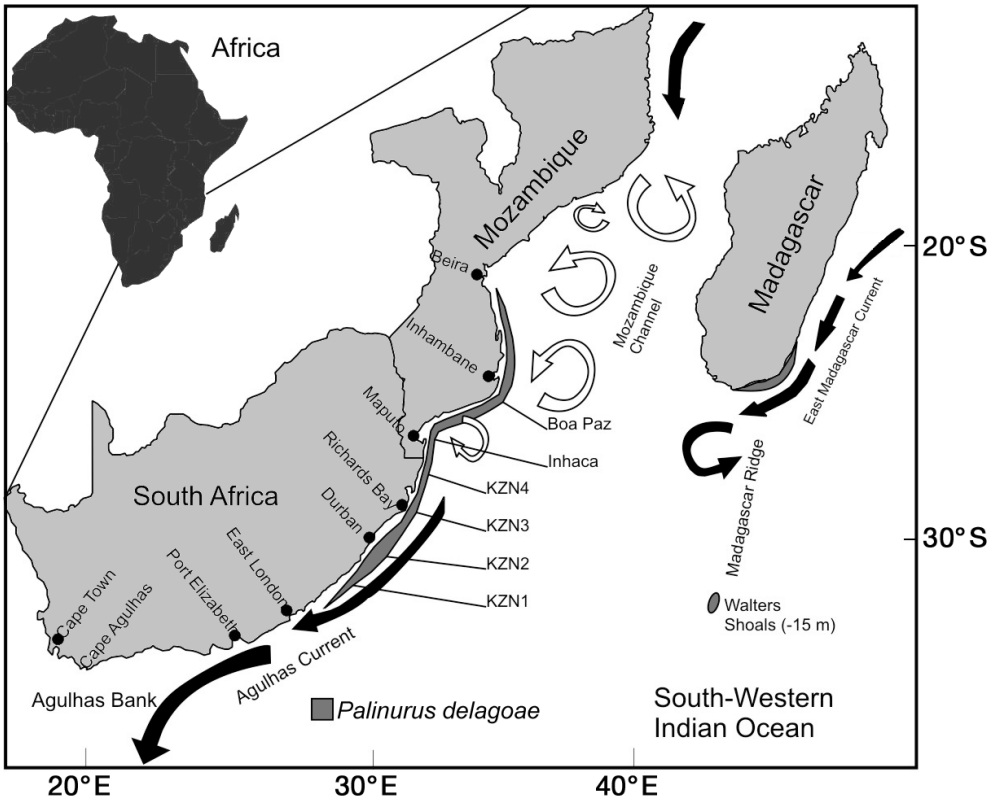

Fig. 1. *Palinurus delagoae.* Distribution in the south-western Indian Ocean, with the 7 sampling sites indicated (northern: Boa Paz and Inhaca; southern: KZN-1–4; Walters Shoals). White arrows indicate anti-cyclonic eddies and black arrows indicate the present understanding of the major sea current systems of the region after Lutjeharms (1988) and Ridderinkhof & De Ruijter (2003)

haplotype sequences have been submitted to GenBank (DQ269501 to DQ269660).

Haplotype diversity (h) and nucleotide diversity (π) were calculated using the software Arlequin v2.0 (Schneider et al. 2000). Relationships among haplotypes were investigated using parsimony median-joining networks and the program Network v4.1 (Bandelt et al. 1999). Pairwise analyses of molecular variance (AMOVA) were run in Arlequin v2.0 where levels of variation (Φ_{ST}) among and within the 7 sampling sites were estimated. The Tamura-Nei model of nucleotide substitution was used and the α value of the gamma distribution was estimated using maximum likelihood in PAUP* v4.0b10 (Swofford 2002). The significance of the resultant Φ_{ST} statistics were tested with 10 000 permutations. A spatial analysis of molecular variance (SAMOVA) was performed to further test for population structure (Dupanloup et al. 2002). Isolation by distance, or the relationship between genetic and geographic distance, was investigated using the Mantel test (Mantel for Windows v1.11 available at: http://life.bio.sunysb.edu/morph/). Fu's F_S-test (Fu 1997) was used to test for mutation-drift equilibrium. Populations that have recently undergone a demographic change (such as expansion) are expected to be out of mutation-drift equilibrium and a significant negative value would be obtained (Fu 1997, Schneider et al. 2000). The possibility of demographic change was also investigated using mismatch distributions (Harpending et al. 1998, Schneider & Excoffier 1999). The timing of putative expansions were approximated using the estimates of the parameter τ generated in Arlequin v2.0. The equation $T = \tau/2u$ was used to estimate the time since demographic change (T), where τ is the age of the demographic change in mutational units estimated in the mismatch distribution, and u is the sum of the per-nucleotide mutation rate per generation in the region sequenced (Rogers & Harpending 1992). A 4 yr generation time for *Palinurus delagoae* was assumed. In the absence of a calibrated clock for lobster control region and to present a range of reasonable values, 2 mutation rates (μ) were used to estimate u (μ = 5 and 15%; per million years; Wilson et al. 1985). Thus, the estimate of u varied according to the range of mutational rates applied (u_1 at 5% = 0.0000547 and u_2 at 15% = 0.000164).

RESULTS

Analyses of 547 base pairs of 334 individuals resulted in 160 haplotypes of which 74% were unique (35% of all had unique haplotypes; Appendix 1, available at: www.int-res.com/articles/suppl/

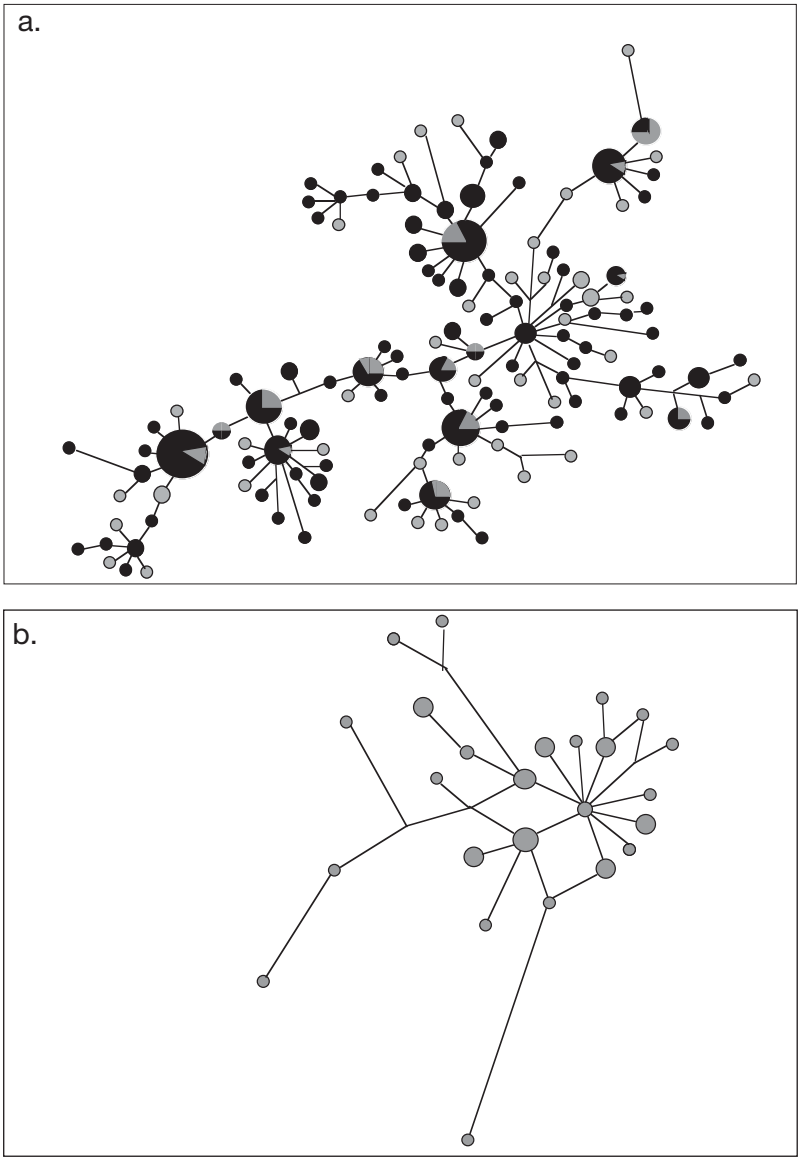

Fig. 2. *Palinurus delagoae.* Median-joining network from (a) the African coast and (b) Walters Shoals. Sizes of the circles are proportional to the frequency in which each haplotype occurs, and the lengths of the branches are proportional to the number of base changes between haplotypes. The shortest branches indicate 1 base change. (a) For each haplotype, the frequencies in the northern population (Boa Paz and Inhaca) are shown in grey, and for the southern population (KZN-1–4) in black

m319p191_app.pdf). Most haplotypes differed by a single site change from each other. There were no shared haplotypes between lobsters from Walters Shoals and the African coast, and at least 27 mutational steps separate these 2 geographic regions. For network construction, the haplotypes from these 2 assemblages could not be linked with more than 95% confidence, so networks were constructed separately for African and Walters Shoals sites. A large number of haplotypes were shared among the African sampling sites and the network contained many reticulations (alternate equally likely solutions). Due to the large number of reticulations, only 1 of the 54 possible shortest solutions to the network is displayed (Fig. 2a). For Walters Shoals, only 3 possible shortest solutions were obtained, and these are displayed as a single network (Fig. 2b).

Total π for all sites, including Walters Shoals, was 0.022 ± 0.011 and was lower within the individual sampling sites, ranging from 0.006 ± 0.004 for Walters Shoals to 0.009 ± 0.006 for Boa Paz (Table 1). Average *h* for all the sites combined was high (0.983 ± 0.002) and varied only slightly when sampling sites were considered separately (Table 1).

The Φ_{ST} value across all 7 sites showed significant genetic partitioning (Φ_{ST} = 0.69, $p < 0.001$), and pairwise comparisons among sampling sites showed the largest differences between the African coast and Walters Shoals (Table 2). The Φ_{ST} estimated among the 6 African sites (excluding Walters Shoals) was substantially lower (Φ_{ST} = 0.01, $p < 0.05$), although some pairwise comparisons were significant between KZN-1–4 and the Mozambique (Boa Paz and Inhaca) sampling sites (Table 2). This was supported by the SAMOVA, which suggested the African coast is structured into northern (Boa Paz and Inhaca) and southern (KZN-1–4) populations. Although pairwise comparisons of some sites within each of these 'populations' also showed significant differences, the divergence between these 2 geographic groups is greater than if each site is considered a separate population (Table 3). The Fu's *F*-test and mismatch distributions were run separately for Walters Shoals, the combined KZN samples (southern population) and combined Boa Paz and Inhaca samples (northern population). Fu's F_S-test was significant for each of the comparisons, suggesting a recent demographic change in each population of this species (Table 4). The mismatch distributions either point to a population expansion in each area or alternatively a selective sweep as indicated by the strong expansion waves (Fig. 3). The Mantel test showed no significant isolation by distance regardless of whether Walters Shoals

Table 1. *Palinurus delagoae*. Sampling localities from 6 regions off southeastern Africa and 1 from Walters Shoals. Sample sizes (n), sampling dates (mo/yr), haplotype diversity (*h*) and nucleotide diversity (π) with 95% confidence intervals are shown

Sampling area	n	Sampling date	*h* (95% CI)	π (95% CI)
KZN-1	50	5/2004	0.957 ± 0.015	0.008 ± 0.005
KZN-2	77	1 & 3/2004	0.977 ± 0.008	0.009 ± 0.005
KZN-3	38	7/2004	0.966 ± 0.015	0.008 ± 0.004
KZN-4	46	9/2004	0.980 ± 0.010	0.007 ± 0.004
Inhaca	35	2/2005	0.988 ± 0.010	0.009 ± 0.005
Boa Paz	39	2/2005	0.999 ± 0.006	0.009 ± 0.006
Walters Shoals	49	11/2004	0.960 ± 0.012	0.006 ± 0.004
Total	334		0.983 ± 0.002	0.022 ± 0.011

was included ($r^2 = 0.91$; not significant, ns) or excluded ($r^2 = 0.23$; ns).

Estimates of τ differed only slightly according to population, all with overlapping 95% confidence intervals (CI) (Table 4). The τ values and their 95% CI were used to provide a rough date of the age since the population expansions, as were the range of values for *u*. Estimates based on the lower mutation rate (5% per million years) would suggest population expansions occurred prior to the last glacial maximum (LGM), between ca. 30 000 to 40 000 yr ago (Table 4). The higher mutation rate would suggest more recent expansions after the LGM, on the order of ca. 9000 to 13 000 yr ago.

Table 2. *Palinurus delagoae*. Pairwise level of variation (Φ_{ST}) values between the 7 sampling areas (bottom of matrix) and significance levels (top of matrix). ns = not significant

Sampling area	1	2	3	4	5	6	7
KZN-1	–	ns	ns	ns	< 0.05	< 0.05	<0.001
KZN-2	0.004	–	ns	ns	< 0.05	< 0.05	<0.001
KZN-3	0.000	0.010	–	ns	ns	ns	<0.001
KZN-4	0.000	0.007	0.000	–	ns	< 0.05	<0.001
Inhaca	0.027	0.025	0.000	0.020	–	ns	<0.001
Boa Paz	0.033	0.023	0.010	0.030	0.000	–	<0.001
Walters Shoals	0.895	0.889	0.900	0.900	0.891	0.883	–

Table 3. *Palinurus delagoae*. Results of SAMOVA showing the *F*-values for the sampling areas (F_{SC} = variance among populations within groups; F_{ST} = variance among haplotypes among groups; F_{CT} = variance among groups). Number of hierarchical groups for the sampling areas in each group is shown. Partitioning of variance among groups (F_{CT}) is highest when there are 2 hierarchical groups (shown in bold)

No. of groups	Sampling areas	F_{SC}	F_{ST}	F_{CT}
2	Boa Paz + Inhaca KZN-2 + KZN-1 + KZN-4 + KZN-3	0.001	0.025	**0.024**
3	Boa Paz + Inhaca KZN-2 KZN-1 + KZN-4 + KZN-3	–0.004	0.017	0.021
4	Boa Paz Inhaca KZN-2 KZN-1 + KZN-4 + KZN-3	–0.004	0.016	0.020
5	Boa Paz + Inhaca KZN-3 KZN-2 KZN-4 KZN-1	–0.004	0.013	0.017

DISCUSSION

The mtDNA results suggest that there is shallow, but significant, genetic partitioning among *Palinurus delagoae* populations from the southern (KZN-1–4) and northern (Inhaca and Boa Paz) sites of the African coast. This partitioning supports earlier morphological studies that suggested the occurrence of 2 populations along the African coast, i.e. var. *natalensis* from South Africa and var. *delagoae* from Mozambique (Barnard 1926, Berry & Plante 1973). Given the genetic and morphological differences, they should at least be considered as distinct management units (Moritz 1994). The results further indicate that the spiny lobsters at Walters Shoals are genetically distinct from the populations on the African continental shelf, and these populations might in fact represent distinct species (see below). No specimens were available from the narrow southeastern shelf of Madagascar, and that population could therefore not be placed with certainty.

The mismatch distributions and Fu's *F*-test suggest that all 3 populations have undergone recent population expansions and, although highly speculative (due to uncertainty in mutation rates), the expansions can be dated back to between ca. 9000 and 40 000 yr (Table 4). At the faster mutation rate of 15% per million years, it would appear that *Palinurus delagoae* underwent a very recent population expansion (ca. 9000 to 13 000 yr ago), possibly resulting from climatic changes since the LGM. If the mutation rate is slower (5% per million years) the date is pushed back substantially, pre-dating the height of the LGM. If the more recent date is accepted then a similar process to that hypothesized for

Table 4. *Palinurus delagoae*. Results of the F_S-test (with p-values) from 3 populations defined by SAMOVA (southern = KZN-1–4; northern = Inhaca and Boa Paz; Walters Shoals), and estimates of nucleotide diversity (τ, 95% CI) from the mismatch distribution. Estimated times since expansion (T) for each population are shown (rounded to nearest 100 yr) for the range of mutation rates (5 and 15%) and the ranges of τ

	F_S	τ (95% CI)	T (5%)	T (15%)
Southern coast	–25.42	3.49	32 000	10 600
	(p < 0.001)	(1.44–9.96)	(13 200–91 400)	(4400–30 400)
Northern coast	–25.44	4.20	38 532	12 800
	(p < 0.001)	(2.10–7.52)	(19 300–69 000)	(6400–22 900)
Walters Shoals	–16.71	2.99	27 430	9100
	(p < 0.001)	(1.36–3.86)	(12 500–35 400)	(4100–11 800)

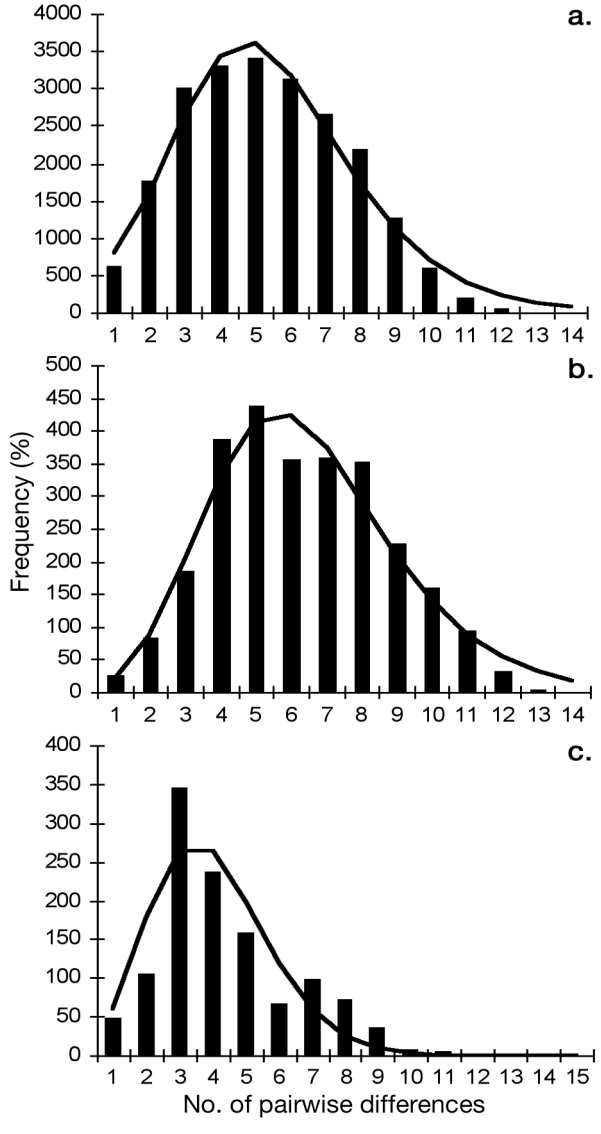

Fig. 3. *Palinurus delagoae.* Distribution of pairwise differences between individuals for the mtDNA control region for (a) the southern population (KZN-1–4), (b) northern population (Inhaca and Boa Paz), and (c) Walters Shoals. Solid line: expected distribution given a recent demographic change

the congener *P. gilchristi* could explain the expansion, i.e. previously exposed continental shelf became inundated after the end of the LGM to provide a new and larger habitat for lobster populations to expand into (Tolley et al. 2005). This may also have been the case at Walters Shoals, because it became inundated only within the present interglacial period (Anderson et al. 1988). An alternative hypothesis for the patterns obtained by the mismatch distributions is that they might indicate a selective sweep. However, the expansion events of the 2 disjunct populations displayed overlapping time estimates (although with large SE) and this tends to reduce the likelihood of the selective sweep hypothesis.

Although the genetic difference observed between the African and Walters Shoals populations could have arisen due to long-term isolation (no mixing of adult/ larval individuals), it is equally plausible that there was a past founder event at Walters Shoals followed by the complete absence of gene flow. There is a deep oceanic trench between Walters Shoals and the African shelf, which precludes genetic exchange through adult migrations. Some contemporary larval exchange between the populations may be possible, although it is unlikely, given the geographic distance between the sites and the prevailing ocean currents (Fig. 1). Drift tracks of weather buoys in the southwestern Indian Ocean have shown that waters from the East Madagascar Current reach the Agulhas Current only irregularly, and that the East Madagascar Current proper retroflects into the central Indian Ocean on passing the southern tip of Madagascar (Lutjeharms 1988). Additionally, larvae originating from the African shelf are unlikely to cross the Mozambique Channel, because the Agulhas Current will probably entrain them southwestwards along the African coast, and away from Walters Shoals. The genetic structure detected in our study supports the hypothesis that larval dispersal across the Mozambique Channel is limited (Berry & Plante 1973), but more importantly the lack of shared mtDNA haplotypes and large Φ_{ST} values suggest that there has been prolonged historical isolation between the African shelf populations and that of Walters Shoals. Given the data at hand, it seems reasonable to suggest that the 2 taxa are in fact distinct biological species that differ genetically and morphologically (Berry & Plante 1973, J. C. Groeneveld pers. obs.). Consequently, in addition to the present oceanic currents that may form a barrier to gene flow, the taxa might also be isolated based on pre- and/or postzygotic barriers to gene flow.

The SAMOVA suggested that sampling sites along the African coast could be partitioned into northern (Inhaca and Boa Paz) and southern (KZN-1–4) populations, thus placing the geographic boundary between the 2 populations in the vicinity of the interface between the Mozambique and Agulhas Currents. Some genetic exchange between the 2 systems is likely because benthic juvenile migrations from KZN to Inhaca have been shown from tag-recapture data (Groeneveld 2002), and water masses from the Mozambique Current feed into the Agulhas Current (Ridderinkhof et al. 2001). This doubtlessly transports larvae across the interface. We propose that some larvae are retained in the slow-moving anti-cyclonic eddies moving southwards along the Mozambique shelf-edge (Ridderinkhof et al. 2001, De Ruiter et al. 2002), through a combination of life-history attributes and behaviour. Factors that would enhance larval retention are: (1) release of larvae inshore of strong currents (observed in *Palinurus delagoae* females that move inshore to 150 to 160 m depth to spawn; Koyama 1971); (2) a shortened larval drifting phase to reduce over-dispersal and loss (shown for a congeneric, *P. elephas*; Kittaka et al. 2001); and (3) the ability of larvae to swim and position themselves in the water column to take advantage of eddies and counter currents (shown for *Jasus edwardsii*; Chiswell & Booth 1999). We suggest similar mechanisms retain a proportion of the *P. delagoae* larvae in eddies of the Mozambique Current, upstream of the southern KZN-1–4 population, thus giving rise to the shallow genetic partitioning between the 2 populations. *P. gilchristi* larvae off southern South Africa appear to be likewise retained inshore of the Agulhas Current, where downstream larval drift is redressed by return migrations of juveniles (Groeneveld & Branch 2002).

In summary, it appears that despite the potential for high gene flow in marine species that have larval stages carried by oceanic currents, genetic panmixia cannot be assumed. In fact, the currents themselves may serve to reduce gene flow between regions even when the distribution seems continuous. This study calls into question the taxonomic status of the Walters Shoals population; its geographic isolation and the lack of shared haplotypes with the African populations suggest that it may be a separate species. In addition, the significant spatial genetic structuring between the southern and northern populations of *Palinurus delagoae* along the African coast, combined with morphological differences, suggest that these populations may be considered as separate Management Units.

Acknowledgements. The authors are indebted to Marine and Coastal Management in Cape Town, and to N. du Plooy from Capfish for collecting the lobster samples for this study. N. Solomons assisted in the laboratory. M. Cherry provided editorial comments. K.G. was supported by the NRF and SABI funding through grants to C.A.M. (GUN 2053617).

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Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: December 20, 2005; Accepted: March 15, 2006
Proofs received from author(s): July 19, 2006