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Genetic and morphological differentiation of the mangrove crab *Perisesarma guttatum* (Brachyura: Sesarmidae) along an East African latitudinal gradient

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The genetic structure and morphometric differentiation of mangrove crab *Perisesarma guttatum* populations were examined among shelf connected locations along a latitudinal gradient on the East African coast. Over 2200 specimens were sampled from 23 mangrove sites for geometric morphometrics analysis. Population genetic analyses of mitochondrial cytochrome *c* oxidase subunit I (COI) DNA sequences were used to evaluate connectivity among populations. A total of 73 haplotypes were detected, and almost no haplotypes were found in common between two highly supported phylogeographic clades: southern Mozambique (Inhaca Island and Maputo Bay) and a northern clade that included north Mozambique, Tanzania and Kenya. These two clades were identified based on the species' populations pairwise genetic differentiation and geographical location. Φ_{ST} values were considerably high between the two clades, indicating the presence of significant population genetic structure between Kenya and South Mozambique. However, each clade was composed of genetically similar populations along the latitudinal gradient, and no significant population structure was found within each clade because the Φ_{ST} values were not significant. The morphometric analysis corroborated the division into two clades (i.e. Inhaca Island/Maputo Bay and northern populations) and also detected less shape variation among populations that were few kilometres apart. The significant spatial genetic structuring between the southern and the northern populations of *P. guttatum* along the geographic gradient under study, combined with morphological differences, suggests that these populations may be considered as cryptic species.

ADDITIONAL KEYWORDS: geometric morphometrics – hydrologic phenomena – larval dispersal – mtDNA COI gene – population structure.

INTRODUCTION

Perisesarma guttatum is an omnivorous species usually associated with the root system of *Rhizophora mucronata*, which, similar to other sesarmid and grapsid crabs, plays an important role in the ecological processes related to leaf turnover (Lee, 1998). However, very little is known about the biology of most of these tropical crabs, and even their systematic classification is still not settled, despite the recent steps made to clarify phylogenetic relationships (Schubart *et al.*, 2006). Furthermore, it should be noted that *Perisesarma samawati* individuals have been considered as *P. guttatum* until 2004, when *P. samawati* was described (Gillikin & Schubart, 2004). Accordingly, the majority of the literature dealing with *P. guttatum* most likely includes both species

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(Gillikin & Schubart, 2004). *Perisesarma guttatum* is endemic to East Africa, occurring from Somalia down to the north coast of South Africa, in Madagascar and in the Red Sea (Vannini & Valmori, 1981).

Perisesarma guttatum has five zoeal stages lasting from 22–25 days, and one megalopa stage (Lago, 1993). Females release the larvae around the new and full moons and larvae migrate offshore, most likely because of intolerance to the low salinity level of mangrove waters and to optimize larval dispersal (Flores, Saraiva & Paula, 2002). Larvae in the megalopa stage reinvade the mangrove habitat selectively settling in nearshore habitats, near their respective adult populations (Paula, Dray & Queiroga, 2001). This implies specific and differential transport and the recognition of appropriate chemical or other environmental cues for settlement (Paula *et al.*, 2001). Thus, dispersal is expected to occur as a result of ocean currents and the admixture of larvae from source populations before recruitment to adult habitat. Thereby, this life history may promote high levels of gene flow and homogenization of local populations (Avise, 2004). Nevertheless, physical and biological factors that promote genetic and morphological differentiation among contiguous populations are still poorly understood (Dawson, 2001; Waters *et al.*, 2005). Prior research on crustacean decapods (Fratini & Vannini, 2002; Gopurenko & Hughes, 2002) has indicated a restriction in gene flow and an inferred lack of interpopulation dispersal, even between geographically close sites, despite the high potential for dispersal. The opposite genetic pattern, however, characterized by low levels of population differentiation as a result of high larval dispersal capacity and recent common ancestry of haplotypes, can also be found in crabs (Cassone & Boulding, 2006).

Oceanic currents can have different effects on the genetic structure of marine populations. They can be responsible for the dispersion of planktonic larvae, acting as gene-exchange corridors or, alternatively, can constitute extrinsic and invisible physical barriers to gene flow (Palumbi, 1994). Along the East African coastline, three main current systems influencing the coast can affect the dispersal potential of planktonic larvae (Lutjeharms, 2006): (1) the warm Agulhas Current, which flows southwards from Mozambique along the eastern coast of South Africa; (2) the Mozambique Current, through the Mozambique Channel, with a contribution coming from east of Madagascar, the East Madagascar Current; and (3) the Equatorial Convergence, which at the south coast of Tanzania splits, proceeding northwards and southwards along the Tanzanian and Mozambican coastlines. In particular, the Agulhas Current has a marked influence on the distribution of a number of species in the South West Indian and South Atlantic Oceans (Lutjeharms, 2006). First, certain species may have evolved adaptations to the Agulhas Current system, using it as a mean of transportation during their particular life cycles; second, the current may carry organisms such as larvae within its waters (Lutjeharms, 2006).

Several studies have combined molecular and morphological characters to analyse population structure (Jordaens, van Riel & Backeljau, 2003; Pinheiro *et al.*, 2005; Sousa *et al.*, 2007; Vasconcellos *et al.*, 2008). The combined use of genetic and morphological data allows the interpretation of patterns of variability and also the recognition (or not) of discrete groups of individuals along coastal areas. Contradictory information can arise, however, from genetic and morphological data. In particular, phenotypic plasticity, defined as the production of multiple phenotypes from a single genotype depending on environmental conditions (Miner *et al.*, 2005), can occur. As an example, Silva & Paula (2008) showed that phenotypic variation observed in the chelae of *Carcinus maenas* and *Pachygrapsus marmoratus* was not corroborated by mitochondrial (mt)DNA genetic data. Other examples of observed morphological differentiation not yet supported by molecular markers include closely-related species of brachyuran crabs (Reuschel & Schubart, 2006) and varunid crabs (Spivak & Schubart, 2003), and the UK populations of the shore crab *Carcinus maenas* (Brian *et al.*, 2006). In this latter study, the authors suggested that the pattern of morphological variability of the species was largely determined by local environmental conditions. On the other side, morphological similarity might be a misleading measurement of relatedness among specimens, as demonstrated by Schizas *et al.* (1999) for the copepod genus *Microarthridion* along the south-eastern and Gulf coasts of the USA. In this case, morphological similarities as a result of convergent evolution within similar habitat constraints masked genetic differences among these copepods. Consequently, it is important to combine morphometric and genetic analyses when comparing populations to obtain a more correct and complete view about the basis of their differentiation.

In the present study, a morphological and mtDNA survey of adult *P. guttatum* populations sampled in 23 mangrove sites along a geographical gradient is presented. Carapace morphology and genetic variation were examined among these natural populations of *P. guttatum* along the East African coast, aiming to reveal the levels of population diversity and differentiation along a latitudinal cline. The convergence of the morphological and genetic results obtained with respect to the species population structure was also analysed. Phylogeographical analyses and statistical

comparisons among populations were also made to assess possible patterns in population differentiation.

MATERIAL AND METHODS

Sampling collection

Sampling was conducted on the East African coast, between Mozambique and Kenya, along a greater than 3000 km geographical gradient, between July 2005 and September 2006. A nested sampling design was adopted along the geographical region under analysis, including six study areas (areas A, B, C, D, F, and H; Fig. 1), each one with different sampling sites (three to five replicates), and single sampling points (Fig. 1E, G). Study areas were several hundreds of kilometres apart, whereas sites within each area were from kilometres to tens of kilometres apart. The scale was thus nominally defined as 100s km and 10s km. A total of 22 sites were sampled for the genetic analysis and 23 for the morphometric analysis. Fifty males and 50 females of *P. guttatum* were collected at each site by hand during spring low tides, in the margins of mangrove creeks. Whole specimens were shipped in absolute ethanol to the laboratory. Distances among populations were measured by following coastlines and using the software package ARCVIEW, version 3.2 (Environmental Systems Research Institute).

Genetic analysis

DNA was isolated directly from pereiopods for a subset of samples. Tissues were incubated at 38 °C overnight in 500 mL of extraction buffer (100 mM Tris-HCl, 10 mM EDTA, 100 mM NaCl, 0.1% SDS) and 3 μL of 20 mg mL^{-1} proteinase K. Total genomic DNA extractions were performed using sequential phenol–chloroform extraction steps, as described by Hillis *et al.* (1996). The DNA obtained was resuspended in low TE buffer (10 mM, pH 8.0, Tris-HCl, 0.1 mM, pH 8.0, EDTA).

A 651-bp fragment of the mtDNA cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) using the primers developed by Folmer *et al.* (1994) (LCO1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′, HCO2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′). Each 25-μL PCR reaction included: 0.5 mM of each primer, 3.0 mM $MgCl_2$, 0.2 mM dNTPs, 2.5 μL 10 × reaction buffer, 0.25 μL of dimethyl sulphoxide, 0.2 U of *Taq* DNA polymerase (Fermentas) and 1 μL of DNA. The PCR thermal cycling conditions consisted of 35 cycles, with the profile: 94 °C denaturation for 60 s; 50 °C annealing for 60 s, and 72 °C extension for 2 min. The cycles were preceded by a 2-min denaturation step; then were finalized with an additional extension step of 2 min. All PCR products were checked for the presence of correctly sized products on 1% agarose gels. Previous to sequencing, the PCR products were purified using the Exo-Sap DNA clean-up protocol. Samples were sequenced in the forward direction using an ABI PRISM 3700 DNA analyser at Macrogen (http://www.macrogen.com). *Uca pugilator* (AF466700) and *Eriocheir japonica sinensis* (AY27430223) sequences were obtained from molecular database (http://ncbi.nlm.nih.gov/) and used as the outgroup. Sequences were aligned and edited using the software BIOEDIT, version 7.0.5.3 (Hall, 1999).

Population genetic statistics was estimated using the software DNASP, version 4.50 (Rozas *et al.*, 2003). The level of polymorphism for each population was estimated as haplotype diversity (h) and nucleotide diversity (π). Nucleotide sequence evolution models were evaluated using the likelihood-ratio tests implemented by MODELTEST (HYPHY; Posada & Crandall, 1998) (available at: http://phylemon.bioinfo.cipf.es). Akaike based model selection and hierarchical model testing were used to select the evolutionary model that best fitted the data set (Posada & Crandall, 1998). The inferred evolutionary model (GTR+I+Γ) was used to estimate pairwise genetic distances among haplotypes. Phylogenetic trees were constructed using Neighbour-joining (NJ) genetic distances and maximum parsimony (MP) methods, implemented in PAUP* (Swofford, 1998). MP analysis was performed using a heuristic search with a tree-bisection-reconnection branch-swapping algorithm and random stepwise sequence adding with ten replicates. For both NJ and MP analysis, the stability of the nodes was tested by 1000 bootstrap replicates. Median-joining networks (Bandelt, Forster & Röhl, 1995) were constructed using the software NETWORK, version 4.5.0.1 (Shareware Phylogenetic Network Software Web site; http://www.fluxus-engineering.com/sharenet.htm). The resulting network is a combination of minimum spanning trees, with median vectors (consensus sequences) added by a parsimony criterion.

An analysis of molecular variance (AMOVA) was performed using ARLEQUIN, version 3.11 (Excoffier, Laval & Schneider, 2005) to assess patterns of genetic differentiation among populations. This analysis produces estimates of variance components similar to *F*-statistics, reflecting the correlation of haplotypes at different levels of hierarchical subdivision. To assess genetic distances among populations, pairwise Φ_{ST} values were calculated using ARLEQUIN, version 3.11 (Excoffier *et al.*, 2005) and associated probability values were calculated using 10 000 permutations. Population genetic structure was also explored through the spatial analysis of molecular variance (SAMOVA) approach (Dupanloup, Schneider & Excoffier, 2002), which defines groups of populations that are geographically homogeneous and maximally

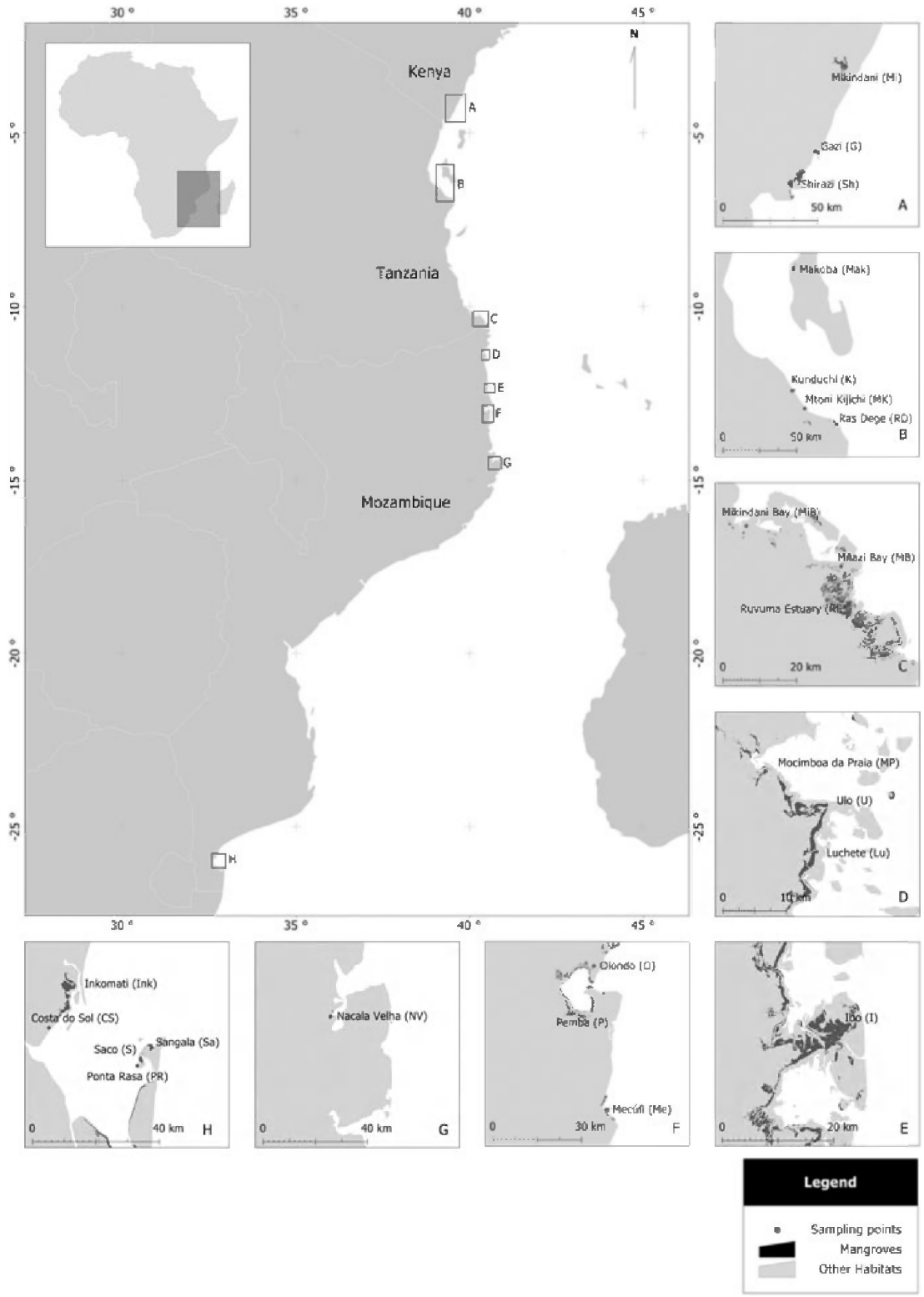

Figure 1. Sampling locations for *Perisesarma guttatum* in the East African coast. Area A: Mikindani (Mi), Gazi (G), Shirazi (Sh); Area B: Makoba (Mak), Kunduchi (K), Mtoni Kijichi (MK); Ras Dege (RD); Area C: Mikindani Bay (MiB), Mnazi Bay (MB); Ruvuma Estuary (RE); Area D: Mocimboa da Praia (MP), Ulo (U), Luchete (Lu); Point E: Ibo (I); Area F: Olondo (O), Pemba (P), Mecúfi (Me); Point G: Nacala Velha (NV); Area H: Inkomati (Ink), Costa do Sol (CS), Sangala (Sa), Saco (S), Ponta Rasa (PR).

differentiated from each other. This analysis was performed with the software SAMOVA, version 1.0 (Dupanloup *et al.*, 2002).

To assess whether genetic differentiation could be explained by geographical distance (isolation by distance), values of $F_{ST}/(1-F_{ST})$ were plotted against geographical distances for each pair of populations. The significance of correlation between genetic and geographical matrices was tested by the Mantel *Z*-test (Mantel, 1967), with 10 000 iterations, using MANTEL, version 1.18 (Cavalcanti, 2005). Tajima's *D* was calculated to assess evidence for population expansion, as well as Fu's test of neutrality (F_S). Significant negative values of Tajima's *D* and Fu's F_S are considered to comprise evidence of expanding populations. Mismatch distributions were constructed in ARLEQUIN, version 3.11. The shapes of the mismatch distributions were used to deduce whether a population has undergone population expansion (Rogers & Harpending, 1992). Populations that have been historically stable are predicted to have multimodal mismatch distributions, whereas those that have undergone a recent expansion are predicted to be unimodal (Slatkin & Hudson, 1991).

Morphometric analysis

Perisesarma guttatum body shape was quantified using landmark-based geometric morphometric methods (Rohlf & Marcus, 1993). A Nikon D70 digital camera with a 55-mm micro lens was used to take images of all specimens with consistent capture methods. Twelve homologous points were digitized on the carapace using the software TPSDIG, version 2.10 (Rohlf, 2006) (Fig. 2). These landmarks were chosen for their capacity to capture overall body shape. Because crustaceans have allometric growth, TPSREGR, version 1.34 (Rohlf, 2007a) was used to evaluate the role of allometry in shape variation. When detected, allometry was removed by the regression of each shape variable (the relative warps) against a measure of body size and estimating residual shape variation. To obtain shape variables, nonshape variation in the landmark coordinates was removed via generalized procrustes analysis (Rohlf & Slice, 1990). This analysis removed nonshape variation by scaling all specimens to unit size, translating them to a common location, and rotating them so that their corresponding landmarks line up as closely as possible (Kassam *et al.*, 2003). From the aligned specimens, shape variables were generated via relative warp analysis (Bookstein, 1991). These new sets of data were then treated as a set of shape variables for statistical comparisons of shape variation within and among groups. The above procedures were implemented in TPSRELW, version 1.45 (Rohlf, 2007b).

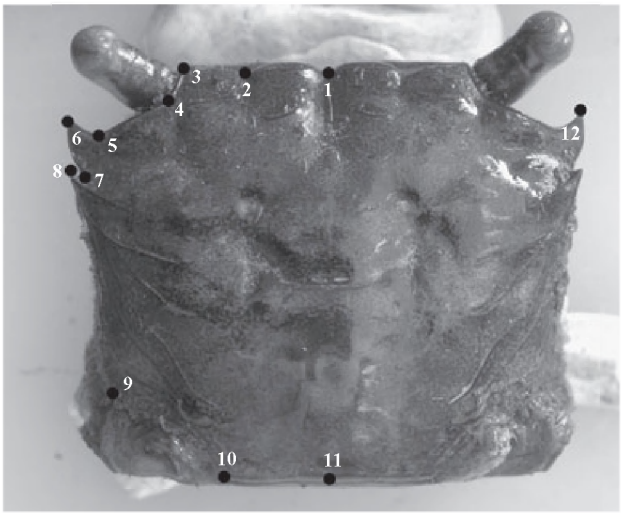

Figure 2. *Perisesarma guttatum*. Position of the 12 landmarks on the carapace.

To determine patterns of morphometric differentiation among areas, a multivariate analysis of variance (MANOVA) was performed on the relative warps scores. After assessing the degree of variation among areas, differentiation among populations and within areas was also determined, undergoing pairwise comparisons with the post-hoc Tukey's honestly significant difference (HSD) test. A canonical variance analysis was also performed on the relative warp scores to provide an ordination of all the specimens in a morphological space (Kassam *et al.*, 2003). Classification was used to determine whether or not canonical functions were effective in discriminating among groups. Shape differences were described using thin-plate spline deformation grids, generated in TPSRELW (Rohlf, 2007b). These procedures were executed in STATISTICA, version 6.0 (StatSoft Inc.).

RESULTS

Because of insufficient sampling size, probably related to preservation problems, genetic analysis was not carried out for the Inkomati population. As a result, the Costa do Sol population was included in Area H, thus forming an area constituted by the Inhaca Island and Maputo Bay populations. This same grouping pattern was maintained in the morphometric analysis, but also including Inkomati specimens in Area H (Fig. 1).

Genetic analysis

The alignment of the 651-bp sequences was straightforward, with no indels or stop codons detected upon putative translation into aminoacids. A total of 73

distinct COI haplotypes were identified for the 292 specimens assayed, and 58 positions were variable, of which 30 were parsimony informative. The average number of transitions and transversions were 3.74 and 1, respectively, and the average transition/transversion ration was 1.99. Fifty-four haplotypes (73.97%) were unique, accounting for 18.49% of the overall specimens. Haplotype 1 was almost exclusive of Area H (Fig. 1H) and accounted for 80.3% of the total specimens sampled in this area. Indeed, all haplotypes identified in the southernmost area (Fig. 1H) were restricted to this area, with the exception of haplotype 1 that was shared with Nacala Velha, a transition site (Fig. 1). Considering all individuals, the most common haplotype was haplotype 13, which represented 82 of the 292 specimens analysed and was restricted to the Mikindani to Nacala Velha areas (Fig. 1A, B, C, D, E, F, G). The distribution of haplotypes per population is summarized in Table 1.

The levels of within-population genetic diversity varied considerably among populations, with haplotype diversity (h) in the range 0.292–1.000 and nucleotide diversity (π) in the range 0.0005–0.0079. Nevertheless, two clades of populations can be defined according to the levels of within-population genetic diversity: a southern clade, comprising Ponta Rasa, Saco, Sangala (Inhaca Island, Mozambique), and Costa do Sol (Maputo Bay, Mozambique), with lower diversity levels ($h = 0.292–0.562$; $\pi = 0.0005–0.0010$); and a northern clade, from Nacala Velha (Mozambique) to Mikindani (Kenya), with higher diversity levels ($h = 0.643–1.000$; $\pi = 0.0027–0.0079$).

Concerning among-populations genetic structure, the phylogenetic clustering of COI haplotypes using both NJ and MP methods revealed identical tree topologies and levels of bootstrap support for all supported nodes (Fig. 3). Two well-supported clades were found, matching the two clades identified in the population genetic diversity analysis (southern clade and northern clade), and no significant genetic structure was found within each clade. Nacala Velha, the southernmost population in the northern clade, presented only one haplotype in common with the southern clade (haplotype 1). In another population from the northern clade, Ulo, a haplotype belonging to the southern evolutionary lineage was also observed.

The network reconstruction also revealed the existence of these two well-supported clades (Fig. 4). Clade 1 contains exclusively individuals from the southern populations, with the exception of one specimen from Nacala Velha (the southernmost population from the north clade) and one specimen from Ulo, whereas clade 2 contains exclusively individuals from the northern populations. Regarding the levels of genetic differentiation among populations the same pattern was found. Pairwise Φ_{ST} values were high (0.834–0.955) and significant ($P < 0.05$) among populations from different clades (southern and northern), and low (–0.075–0.190) and nonsignificant ($P > 0.05$) among populations from the same clade (Table 2). The same pattern was found for the average values of genetic distance among populations (Table 2): higher for between clade comparisons (2.32%–2.75%) and lower for within clade comparisons (0.05%–0.72%). Additionally, with respect to the values of genetic distance among populations within clades, the populations within the northern clade were more differentiated among each other (0.28%–0.72%), compared to the level of genetic differentiation found among the populations of the southern clade (0.05%–0.08%).

In view of the two distinct phylogeographic clades consistently supported (the southern, from Ponta Rasa to Costa do Sol, and the northern, from Nacala Velha to Mikindani) a hierarchical analysis of the genetic variation (AMOVA) was made considering this structure. In accordance, the apportioning of total variance results indicated that the large majority of the genetic variance was found between clades (85.99%), almost none among populations within clades (0.17%), and a few within populations (13.84%). Moreover, the Φ_{ST} and the Φ_{CT} values were high (0.862 and 0.860, respectively) and significant ($P < 0.05$), indicating high genetic differentiation among populations (for the total populations) and between clades, respectively, whereas the Φ_{SC} value was reduced (0.012) and nonsignificant ($P > 0.05$), indicating low genetic differentiation among populations within clades. The SAMOVA algorithm was used to investigate the hypothesis of finding population groups using not only the genetic information, but also its combination with the geographic location of those populations. A search for two significantly differentiated population clusters revealed one group consisting of the southern Mozambique populations (Ponta Rasa, Saco, Sangala, Costa do Sol), and a second group consisting of the remaining sampled populations. This analysis was performed several times by increasing the user-defined number of groups. The cluster formed by the southern Mozambique populations did not collapse with the increase in the number of groups and the northern cluster was broken progressively. Populations were not, however, grouped according to the pre-defined sampling areas. Indeed, the results of AMOVAs, considering this population structure and with an increase numbers of groups, presented low (and nonsignificant) values of Φ_{CT}.

The full COI data set indicated a positive and significant trend between genetic divergence and geographic distance (multiple regression analysis: $R^2 = 0.401$; $P < 0.001$) because values of Φ_{ST} among all

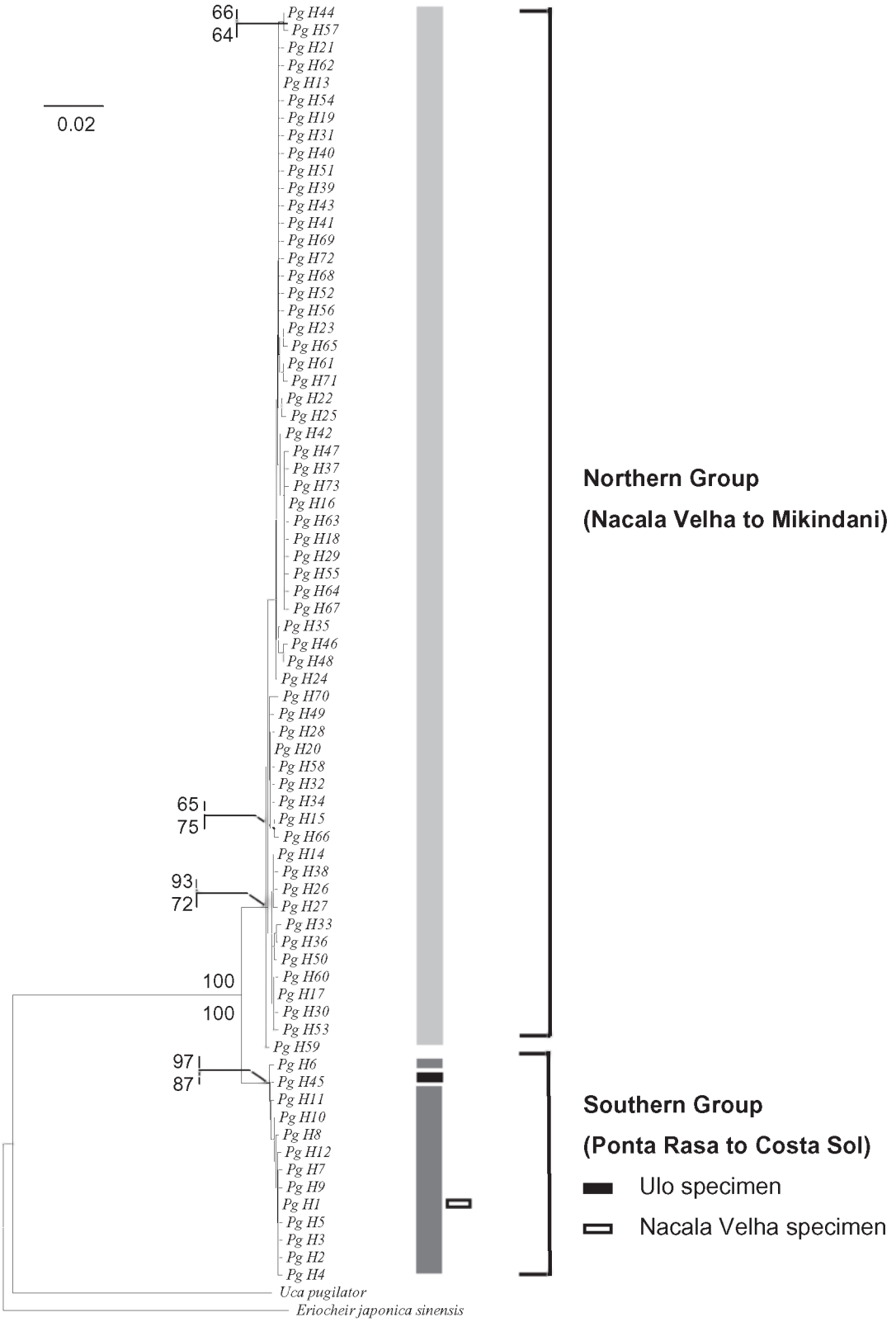

Figure 3. Phylogenetic relationships among cytochrome *c* oxidase subunit I haplotypes of the 22 populations of *Perisesarma guttatum* using Neighbour-joining (NJ) and maximum parsimony (MP) methods. Bootstrap values (NJ/MP) are, respectively, given above and under lines.

Table 1. Number of haplotypes per population for *Perisesarma guttatum* for a fragment of the cytochrome *c* oxidase subunit 1 gene mitochondrial DNA

	Localities																						
	PR	S	Sa	CS	NV	Me	P	O	I	Lu	U	MP	RE	MB	MiB	RD	MK	K	Mak	Sh	G	Mi	N° specimens
Haplotype diversity	0.371	0.562	0.331	0.292	0.778	0.873	0.874	0.801	0.86	0.643	0.867	1.00	0.905	0.89	1.00	0.933	0.917	1.00	0.795	0.667	0.833	0.8	0.772
Nucleotide diversity	0.001	0.001	0.001	0.001	0.008	0.004	0.004	0.005	0.004	0.003	0.007	0.006	0.005	0.004	0.005	0.004	0.005	0.006	0.004	0.003	0.005	0.003	0.004
Number of haplotypes	4	4	5	2	6	6	12	12	9	10	10	4	6	7	12	7	7	5	7	6	5	9	73
Number of specimens	15	17	15	19	10	11	20	22	17	8	16	10	21	10	7	10	9	5	13	12	10	15	292
H1	12	14	10	16	1																		53
H2	1																						1
H3	1																						1
H4	1			2																			3
H5			1																				1
H6			2																				2
H7			1																				1
H8			1																				1
H9		1																					1
H10		1																					1
H11		1																					1
H12				1																			1
H13					5	3	7	10	6	1	6	5	3	1	6	2	3		6	5	6	7	82
H14					1	1			1	1		1			2					2		1	10
H15					1		1	2		1				1			1						7
H16					1	3			3	1	1				2	2		1				1	15
H17					1	2	3	1	1	1	1	1	2		3		1	1	2	1	1		22
H18						1																	1
H19						1																	1
H20							1	1					2	1	1	1							7
H21							1																1
H22							1																1
H23							1																1
H24							1				1												2
H25							1		1		1		1			1					1		6
H26							1																1
H27							1																1
H28							1																1
H29								1															1
H30								1			2				1		1						4
H31								1															1
H32								1															1
H33								1															1
H34								1					1		1					1	1		5
H35								1						1		2			1	1	1	1	8

Table 1. *Continued*

	Localities																						
	PR	S	Sa	CS	NV	Me	P	O	I	Lu	U	MP	RE	MB	MiB	RD	MK	K	Mak	Sh	G	Mi	N° specimens
H36								1															1
H37									1														1
H38									2														2
H39									1														1
H40									1														1
H41												1											1
H42											1												1
H43											1												1
H44											1												1
H45											1												1
H46										1													1
H47										1													1
H48										1												1	2
H49										1							1						2
H50										1							1						2
H51															1								1
H52															1								1
H53															1								1
H54															1								1
H55															1								1
H56													1										1
H57														1									1
H58														1									1
H59														1									1
H60																1				1		1	3
H61																1							1
H62																	1						1
H63																		1					1
H64																		1					1
H65																		1					1
H66																			1				1
H67																			1				1
H68																			1				1
H69																			1				1
H70																						1	1
H71																							
H72																						1	1
H73																						1	1
Haplotype diversity	0.371	0.562	0.331	0.292	0.778	0.873	0.874	0.801	0.86	0.643	0.867	1.00	0.905	0.89	1.00	0.933	0.917	1.00	0.795	0.667	0.833	0.8	0.772
Nucleotide diversity	0.001	0.001	0.001	0.001	0.008	0.004	0.004	0.005	0.004	0.003	0.007	0.006	0.005	0.004	0.005	0.004	0.005	0.006	0.004	0.003	0.005	0.003	0.004
Number of haplotypes	4	4	5	2	6	6	12	12	9	10	10	4	6	7	12	7	7	5	7	6	5	9	73
Number of specimens	15	17	15	19	10	11	20	22	17	8	16	10	21	10	7	10	9	5	13	12	10	15	292

For the complete names and locations of the sampling sites, see Fig. 1.

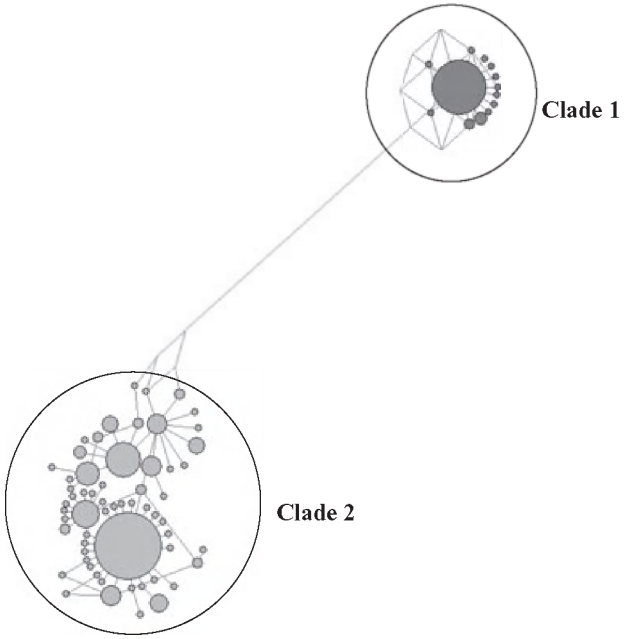

Figure 4. Network of phylogenetic relationships among all haplotypes. The area of the circle is proportional to haplotype frequency, whereas line length is proportional to the number of mutational steps.

population pairs increased with increasing geographical separation (Mantel test significant: $Z = 0.917$; $P = 0.001$) (Fig. 5). When the Mantel test was performed among the northern populations (from Nacala Velha to Mikindani), no significant relationship between Φ_{ST} and geographical separation was observed ($Z = -0.0085$; $P = 0.129$).

Populations from Inhaca Island (Ponta Rasa, Saco and Sangala) and from Ulo had negative values for Tajima's D that deviated significantly from zero (Table 3). None of the other populations had a significant value of Tajima's D and Mecufi and Luchete populations had positive and not statistically significant values. Large significant negative values of F_S were observed for several populations: the Inhaca Island populations, Costa do Sol, Pemba, Olondo, Ibo, Luchete, Mnazi Bay, Mikindani Bay, and Mikindani (Table 3). Only Nacala Velha had a positive value. The results of the Fu's tests were consistent with data from Tajima's D-tests.

Six separate assemblages of mismatch distributions were constructed: southern Mozambique (Area H), Area F, Area D, Area C, Area B, and Kenya (Area A) (Fig. 6). Mismatch distributions of southern Mozambique showed a smooth and unimodal curve, suggesting that a sudden population expansion had occurred. The remaining assemblages generated uneven distributions, which is consistent with a lack of population expansion.

Morphometric analysis

Shape analysis showed carapace differences among populations and among groups. Analyses performed on males revealed that the first relative warp (RW1) explained 46.71%, the second (RW2) 17.5%, and the third (RW3) 8.03%, explaining 72.24% of the variance in total. The first axis revealed shape variation in the rostrum and on the distance between landmarks 3 and 6, corresponding to a rounding of the region defined by landmarks 3, 4, 5, and 6. RW2 explained shape variation in the lateral-posterior zone of the carapace, and RW3 accounted differences as a result of variation in the carapace width. The same variation pattern was observed in females, with the first three axes explaining 41.64%, 16.36%, and 13.33%, respectively.

MANOVA revealed significant differences among the sampling areas (males: Wilks' $\lambda = 0.367$, $F = 10.171$; $P < 0.0001$; females: Wilks' $\lambda = 0.295$, $F = 12.091$; $P < 0.0001$), being well discriminated. Figure 7 shows the distribution of individuals along the first two canonical axes and, at the top and along the plots, splines relative to the non-uniform component (the overall set of relative warps) are visualized. It can be seen that the medium shape from Area H was more concave, and the distance between the lateral spines and the rostrum is smaller. These differences were clearer in the males.

Considering the sampling areas, for males, correct classification was 31.3% for Area A, 53% for Area B, 60.8% for Area C, 41.2% for Area D, 41.3% for Area F and, finally, 71% for Area H. The overall rate of individuals correctly classified into areas was 49.8%, which is better than random assignment (tau statistics = 47.4%). When analysing each population separately, 40.2% of specimens were correctly classified into groups. Nevertheless, individuals that were not correctly classified were grouped in the contiguous populations and not in a different area. A similar pattern of correct classification was observed in females (Area A, 30.8%; Area B, 48.5%; Area C, 54.1%; Area D, 52.6%; Area F, 33.6%; Area H, 78.1%). In this case, correct classification accounted for 49.6% when considering the overall analysis, which is slightly better than chance (tau statistics = 48.8%).

The comparisons made among areas, obtained with the post-hoc Tukey's HSD test, showed that Area H was significantly different from the other areas, when the shape variables considered comprised the first three relative warps. The comparisons made between the Areas A, B, C, D, and F resulted in the absence of significant differences between the Areas that were closer together. Thus, the shape variation explained by the RW1 presented significant differences between Areas D–F and Area A, and RW2 presented significant differences between Area D and Areas A–B.

Table 2. ΦST and genetic distance among populations

	PR	Sa	S	CS	NV	ME	P	O	I	MP	U	LU	MIB	RE	MB	RD	MK	K	MAK	G	SH	MI
PR		0.08%	0.07%	0.05%	2.35%	2.68%	2.62%	2.57%	2.75%	2.65%	2.54%	2.48%	2.62%	2.53%	2.49%	2.65%	2.53%	2.70%	2.68%	2.67%	2.56%	2.69%
Sa	0.018*		0.08%	0.08%	2.33%	2.64%	2.59%	2.54%	2.71%	2.62%	2.51%	2.44%	2.58%	2.51%	2.45%	2.63%	2.51%	2.68%	2.65%	2.63%	2.53%	2.66%
S	0.001*	0.014*		0.06%	2.32%	2.62%	2.58%	2.53%	2.70%	2.60%	2.50%	2.43%	2.57%	2.49%	2.44%	2.62%	2.40%	2.66%	2.64%	2.62%	2.52%	2.65%
CS	–0.024*	0.037*	0.018*		2.34%	2.65%	2.60%	2.57%	2.73%	2.63%	2.53%	2.45%	2.60%	2.53%	2.48%	2.64%	2.52%	2.69%	2.68%	2.66%	2.55%	2.67%
NV	0.840	0.834	0.853	0.864		0.61%	0.59%	0.60%	0.62%	0.54%	0.67%	0.66%	0.60%	0.58%	0.63%	0.60%	0.62%	0.71%	0.59%	0.55%	0.61%	0.59%
ME	0.920	0.914	0.925	0.932	–0.017*		0.44%	0.43%	0.42%	0.38%	0.53%	0.48%	0.43%	0.43%	0.50%	0.42%	0.49%	0.48%	0.42%	0.39%	0.46%	0.40%
P	0.898	0.889	0.902	0.907	–0.025*	0.018*		0.42%	0.44%	0.35%	0.52%	0.51%	0.42%	0.41%	0.48%	0.43%	0.45%	0.55%	0.42%	0.38%	0.43%	0.41%
O	0.885	0.883	0.890	0.897	–0.034*	0.006*	–0.020*		0.46%	0.36%	0.55%	0.50%	0.44%	0.42%	0.47%	0.44%	0.46%	0.54%	0.43%	0.39%	0.45%	0.42%
I	0.911	0.901	0.915	0.921	0.014*	–0.022*	0.031*	0.044*		0.35%	0.51%	0.52%	0.46%	0.43%	0.51%	0.41%	0.51%	0.50%	0.41%	0.35%	0.49%	0.39%
MP	0.947	0.938	0.952	0.955	–0.039*	0.035*	–0.031*	–0.001*	–0.001*		0.45%	0.47%	0.39%	0.37%	0.45%	0.35%	0.40%	0.51%	0.33%	0.29%	0.39%	0.32%
U	0.856	0.847	0.864	0.871	–0.059*	–0.024*	–0.013*	–0.013*	–0.021*	–0.046*		0.60%	0.55%	0.53%	0.60%	0.52%	0.57%	0.63%	0.52%	0.46%	0.57%	0.50%
LU	0.891	0.881	0.898	0.904	–0.037*	0.000*	0.022*	–0.012*	0.083	0.101*	0.011*		0.51%	0.49%	0.53%	0.51%	0.51%	0.56%	0.52%	0.49%	0.50%	0.51%
MIB	0.890	0.882	0.894	0.898	–0.021*	–0.025*	–0.018*	–0.025*	0.033*	0.007*	–0.003*	–0.008*		0.44%	0.49%	0.45%	0.45%	0.55%	0.44%	0.42%	0.43%	0.44%
RE	0.917	0.904	0.920	0.925	–0.051*	0.010*	–0.058*	–0.043*	0.050*	0.026*	–0.019*	–0.032*	–0.043*		0.47%	0.44%	0.44%	0.56%	0.43%	0.39%	0.42%	0.43%
MB	0.914	0.902	0.922	0.927	–0.052*	0.055*	0.009*	–0.035*	0.115	0.092*	0.008*	–0.042*	0.007*	–0.053*		0.50%	0.48%	0.60%	0.49%	0.45%	0.49%	0.49%
RD	0.919	0.909	0.921	0.929	–0.027*	–0.031*	0.004*	–0.009*	–0.044*	–0.015*	–0.046*	0.015*	0.013*	0.010*	0.034*		0.50%	0.53%	0.43%	0.38%	0.48%	0.37%
MK	0.912	0.899	0.918	0.921	–0.055*	0.026*	–0.042*	–0.042*	0.085*	0.023*	–0.006*	–0.056*	–0.041*	–0.075*	–0.055*	0.036*		0.61%	0.47%	0.44%	0.46%	0.46%
K	0.930	0.919	0.937	0.943	0.010*	–0.042*	0.133*	0.085*	0.040*	0.190*	0.020*	–0.007*	0.072*	0.104*	0.087*	0.002*	0.103*		0.54%	0.52%	0.60%	0.50%
MAK	0.918	0.907	0.925	0.927	–0.029*	–0.006*	–0.016*	–0.016*	–0.013*	–0.064*	–0.036*	0.054*	0.007*	0.014*	0.041*	–0.048*	0.024*	0.088*		0.35%	0.46%	0.35%
G	0.936	0.928	0.942	0.946	–0.014*	0.044*	–0.014*	0.004*	–0.018*	–0.071*	–0.041*	0.099*	0.036*	0.02*	0.084*	–0.053*	0.060*	0.161*	–0.057*		0.42%	0.33%
SH	0.907	0.898	0.912	0.920	–0.016*	0.052*	–0.016*	–0.009*	0.114	0.048*	0.030*	–0.017*	–0.023*	–0.056*	–0.009*	0.068*	–0.073*	0.150*	0.057*	0.083*		0.47%
MI	0.921	0.913	0.928	0.930	0.015*	0.027*	0.040*	0.033*	–0.005*	–0.023*	–0.014*	0.104	0.067*	0.082*	0.118*	–0.062*	0.099*	0.102*	–0.037*	–0.044*	0.127	

Below diagonal: pairwise ΦST values among populations; above diagonal: the average (%) pairwise differences among populations (haplotype frequencies and GTR+I+Γ genetic distance).
*No significant pairwise Φ_{ST} values ($P > 0.05$).

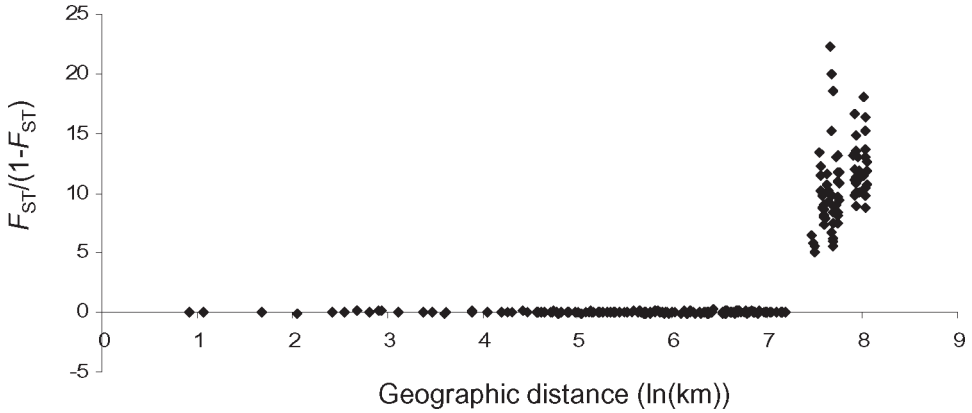

Figure 5. Isolation by distance in *Perisesarma guttatum* samples. The divergence estimates are plotted versus geographic distance for each pair of populations.

Table 3. Parameters for the mismatch distribution for the *Perisesarma guttatum* mitochondrial DNA populations

	Parameters			Goodness-of-fit tests				Tajima's *D* test		Fu's test	
	θ_0	θ_1	τ	SSD	P_{SSD}	Ragged.	$P_{Ragged.}$	*D*	*P*	F_S	*P*
PR	0	99999.0	0.479	0.0402	0.75	0.181	0.57	–1685	0.017*	–2.369	0.003*
S	0	3.6	2.980	0.2272	0.15	0.212	0.22	–1.706	0.025*	–2.526	0.001*
Sa	0	99999.0	0.811	0.0171	0.28	0.161	0.35	–1.518	0.043*	–2.677	0.002*
CS	0	0.433	3.000	0.0077	0.50	0.255	0.59	–1.120	0.183	–1.152	0.04*
NV	0	5.596	4.096	0.0726	0.29	0.163	0.29	–1.273	0.085	0.461	0.57
Me	0.004	10.332	3.705	0.0131	0.66	0.037	0.94	0.573	0.724	–0.768	0.294
P	0	8.105	4.090	0.0305	0.22	0.087	0.27	–0.646	0.302	–5.674	0.004*
O	0	6.663	4.658	0.0295	0.29	0.054	0.48	–0.661	0.259	–4.831	0.012*
I	0.002	8.967	3.332	0.0022	0.93	0.016	1.00	–0.403	0.365	–2.796	0.042*
Lu	0	99999.0	3.951	0.0203	0.32	0.069	0.32	0.165	0.594	–5.021	0.000*
U	0	99999.0	0.496	0.2738	0.01	0.041	1.00	–1.592	0.039*	–2.403	0.118
MP	0.004	2.231	4.814	0.0491	0.46	0.133	0.71	–0.335	0.419	–0.073	0.443
RE	0.009	8.54	4.016	0.0155	0.62	0.500	0.79	–0.009	0.515	–0.963	0.26
MB	1.619	99999.0	2.453	0.0257	0.45	0.077	0.74	–0.354	0.372	–4.010	0.001*
MiB	0.007	11.543	3.967	0.0094	0.44	0.031	0.75	–0.417	0.380	–5.021	0.009*
RD	0.016	101.25	3.051	0.0088	0.65	0.053	0.62	–0.726	0.273	–2.092	0.077
MK	0	37.148	4.227	0.0629	0.13	0.161	0.19	–0.047	0.482	–2.300	0.061
K	0	61.875	5.102	0.1003	0.15	0.240	0.48	–0.855	0.269	–1.805	0.052
Mak	0	4.519	5.215	0.0304	0.47	0.069	0.73	–1.089	0.137	–1.522	0.142
Sh	0	16.592	4.389	0.0277	0.19	0.088	0.37	–0.035	0.514	–1.405	0.176
G	0	2.642	5.074	0.0492	0.44	0.169	0.43	–1.161	0.144	0.528	0.312
Mi	0.563	5.133	2.531	0.0111	0.63	0.042	0.74	–0.977	0.177	–3.901	0.002*

Tajima's *D* and Fu's F_S test values and their statistical significance are also presented. θ_0 and θ_1, pre-expansion and post-expansion populations size; τ, time in number of generations elapsed since the sudden expansion episode; SSD, sum of squared deviations; Ragged, raggedness index; P_{SSD} and P_{Ragged}, probability that expected mismatch distributions (1000 bootstrap replicates) have significantly larger than observed mismatch distributions.
*Statistically significant ($P < 0.05$).

MANOVA also revealed significant differences between the groups north and south (males: Wilks' $\lambda = 0.606$, $F = 9.001$; $P < 0.0001$; females: Wilks' $\lambda = 0.898$, $F = 4.965$; $P < 0.0001$). According to the discriminant analysis, 68.6% of the males and 63.3% of the females were correctly classified as belonging to the southern group, and 98.9% of both sexes were correctly classified as specimens of the northern group.

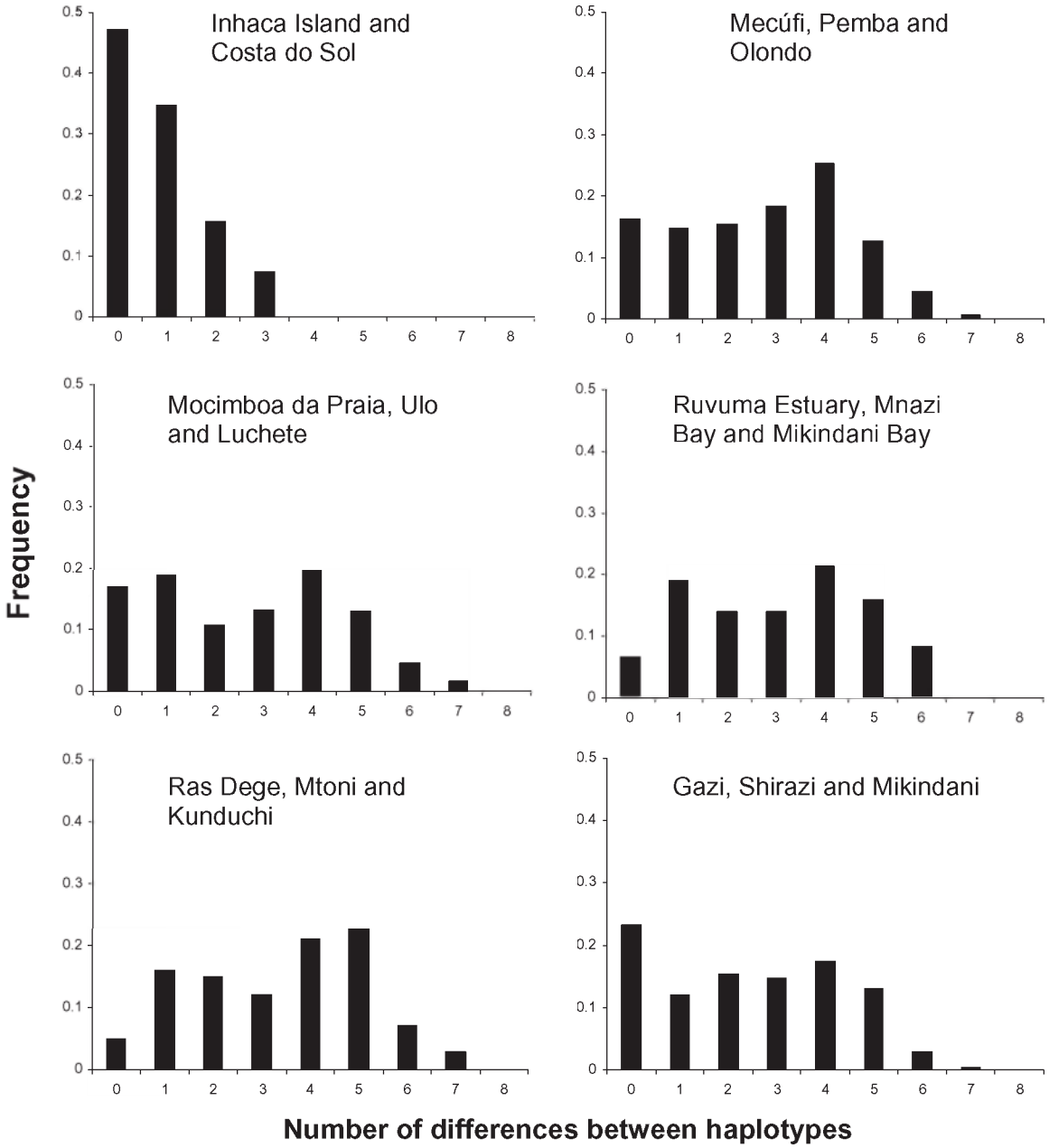

Figure 6. Frequency distributions of the number of pairwise nucleotide differences (mismatch distributions) between *Perisesarma guttatum* mitochondrial haplotypes.

In the analysis of differentiation among populations, Maputo Bay populations (Costa do Sol and Inkomati) and the Inhaca Island populations (Saco, Ponta Rasa, and Sangala), the southern group, was revealed to be very different from the remainder for both males and females. These differences in carapace shapes were assessed through a post-hoc Tukey's HSD test and were statistically significant. In general, the areas sampled along the coast showed significant differences among them, whereas the locations sampled within each area did not present significant differences among each other. As an example, the area defined by Maputo Bay and Inhaca Island populations is statistically different from the area defined by Pemba, Olondo, and Mecufi. However, Sangala, Ponta Rasa, Saco, Costa do Sol, and Inkomati did not show differences among them, and Pemba, Olondo and Mecufi also did not show differences among them. However two exceptions were registered: within Area D, significant differences

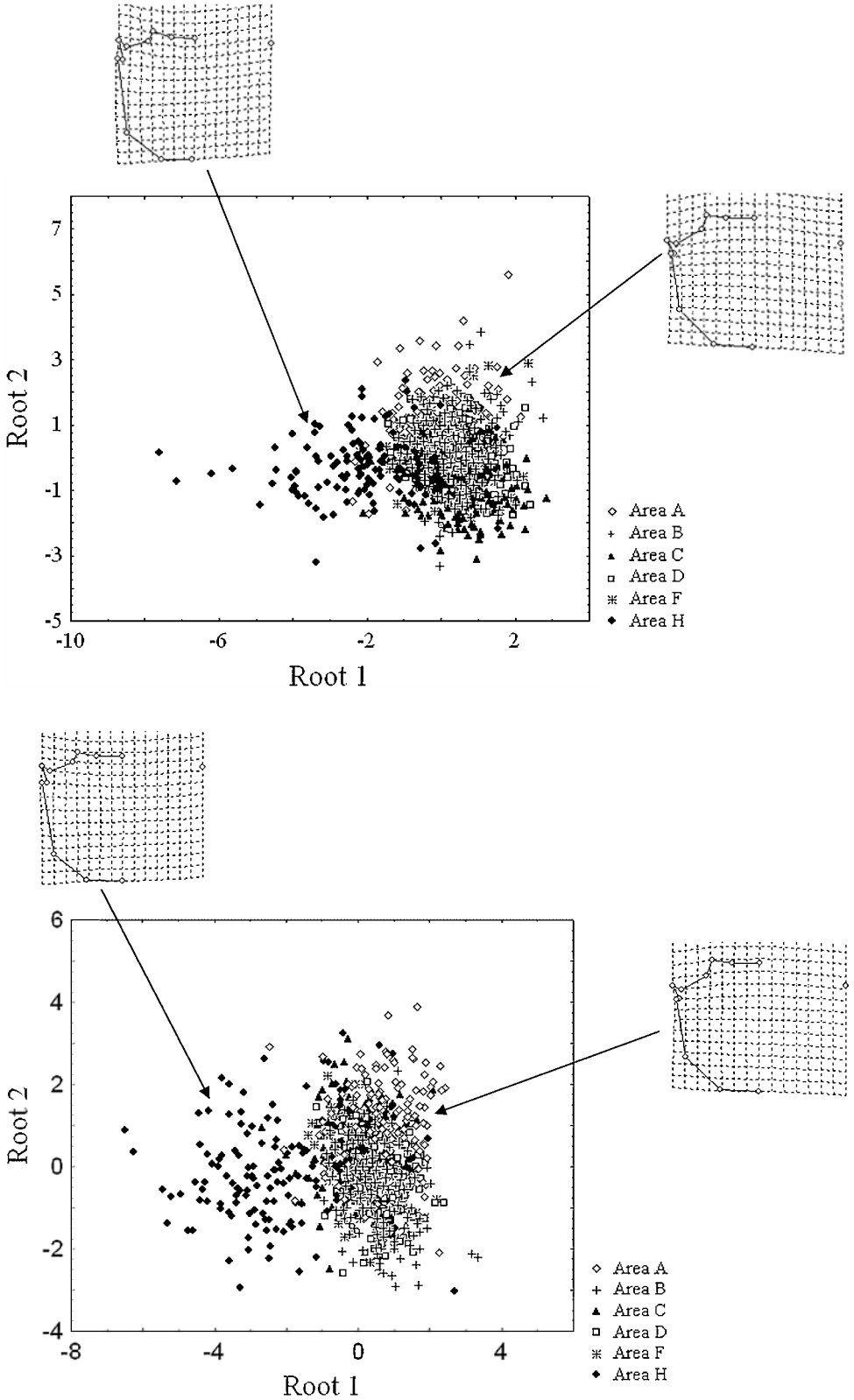

Figure 7. Ordination of all crab specimens along the first two canonical axes together with spline grids showing shape deformation for *Perisesarma guttatum* (upper: males; lower: females). Specimens are labelled according to their sampling Area (Areas A, B, C, D, F, and H).

between Mocimboa da Praia and Luchete were found (Tukey's HSD test, $P = 0.027$) and, within Area B, the same significant differences were observed between Ras Dege and Mtoni Kijichi (Tukey's HSD test, $P = 0.0026$).

DISCUSSION

The assessment of the population differentiation of the mangrove crab *P. guttatum* through the combined analysis of genetic and morphometric data was congruent. Analysis of mitochondrial DNA variation (using Φ-statistics, phylogenetic trees, and SAMOVA) and the analysis of carapace shape variation (using geometric morphometrics) revealed two highly supported phylogeographic clades: the southern Mozambique clade (Inhaca Island and Maputo Bay) and the northern clade that included north Mozambique, Tanzania, and Kenya. However, at this stage, it is unclear whether a clinal variation separates both clades or, instead, a major break occurs. Logistical constraints precluded this issue being addressed during the present study.

Analysis of mitochondrial DNA sequence data revealed that *P. guttatum* populations along the East African coast were characterized by high haplotypic diversity, close genetic similarities among haplotypes (limited nucleotide diversity), and by the presence of many rare haplotypes within sampling localities. The described genetic pattern can be interpreted as an effect of high maternal effective population size (Lewontin, 1974; Lavery, Moritz & Fielder, 1996), by an increasing mutation rate, or by a combination of both. This diversity pattern was also observed in other studies of marine invertebrate species with high reproductive potential, such as the study by Fratini & Vannini (2002) regarding the portunid mangrove crab *Scylla serrata* and the study by Cassone & Boulding (2006) regarding the grapsid crab *Pachygrapsus crassipes*. Furthermore, the low nucleotide diversities observed may be a result of the relatively short existence of haplotypes, with newly-created haplotypes going extinct after obtaining only a few additional base pair differences (Cassone & Boulding, 2006). Population bottlenecks followed by demographic expansions might also contribute to this pattern of genetic diversity. Indeed, the unimodal mismatch distributions observed for southern Mozambique populations, the star-like shape of the network within each clade, and the statistically significant negative values of Tajima's *D* and Fu's *F* are also consistent with the hypothesis of a recent population expansion subsequent to a bottleneck. Benzie *et al.* (2002) obtained similar results in a study on mtDNA variation in Indo-Pacific populations of the giant tiger prawn *Penaeus monodon*, with the south-east African peripheral populations displaying patterns indicative of a relatively recent bottleneck. According to these authors, the fall in sea level in the Pleistocene is likely to have removed shelf habitat suitable for *P. monodon* in southern Africa, and these would have been reinvaded by populations further north after the latest major sea level rise (Forbes *et al.*, 1999). Similar results were obtained by Kochzius & Nuryanto (2008) for the giant clam *Tridacna crocea*, where neutrality tests and mismatch distribution also indicated demographic expansion. These authors suggested a similar explanation for the population size changes, with the reduction of habitat and subsequent population bottlenecks during sea level low stands, whereas the availability of new habitats after the rise of the sea level at the end of the last glacial enabled recolonization and growth of the reduced populations. A similar event might also have occurred with *P. guttatum*, with losses of suitable habitats and sequent colonization of new areas. This migration pattern would explain the typical results of population expansion achieved.

The degree of population genetic structure found in *P. guttatum* suggests that the individuals sampled along the coast represent two distinct clades. The two regional clades were identified based on *P. guttatum* populations pairwise genetic differentiation and geographical location, with each clade being composed of genetically similar populations along a latitudinal gardient. In addition, Φ_{ST} values (Hartl & Clark, 1997) were considerably high and significantly greater than random distributions of sequences among the two clades, supporting, along with the molecular phylogram attained, the presence of significant population genetic structure along the East African coast, between Kenya and South Mozambique. However, no significant population structure was found within each clade because the Φ_{ST} values were small (–0.076–0.190) and not significant ($P > 0.05$).

Furthermore, AMOVA revealed high levels of genetic structuring among south Mozambique and northern populations, with almost 86% of the total genetic variance being found between these two clades. Indeed, almost no haplotypes were shared between the two clades. These results suggested a reduced gene flow between the two clades, which is probably explained by the currents in the region. Hydrographic features, such as current gyres, eddies, and countercurrents, can promote the isolation of local marine invertebrate populations, as is the case for the Brittany and English Channel populations of the polychaete *Pectinaria koreni* (Jolly *et al.*, 2005) and for the North of France populations of the stalked barnacle *Pollicipes pollicipes* (Quinteiro, Rodríguez-Castro & Rey-Méndez, 2007). Somewhere between

Maputo and Durban, the warm southward Agulhas Current (Lutjeharms, 2006) might be responsible for the dispersion of the larvae originated in Inhaca Island and Maputo Bay southwards. Thus, it should be expected that populations from further south would be similar to those sampled at Inhaca Island and Costa do Sol. However, because the north coast of South Africa is the southern geographic limit of *P. guttatum*, it was not possible to capture sufficient specimens to achieve a statistically valid sampling number for this southernmost region. Nevertheless, other studies (Gibbons, Barange & Hutchings, 1995; Gibbons & Thibault-Botha, 2002) have shown, for euphausiid and siphonophore species, a tight agreement between the circulation components of the Agulhas current system and species biogeography. Furthermore, the hydrography of Maputo Bay and adjacent neritic waters has demonstrated a complex dynamics, which included countercurrents and eddies derived from the Mozambique Current (Lutjeharms & da Silva, 1988). These eddies and northward inshore countercurrents may contribute to the retention of larvae in areas adjacent to Maputo Bay and restrict offshore dispersal, as suggested by Paula *et al.* (2001) for a set of brachyuran megalopae. Accordingly, this retention of larvae could prevent gene flow, and thereby explain the observed differences between northern and southern clades. Moreover, observations made by Sætre & da Silva (1984) in the Mozambique Channel showed a flow southward along the Mozambique coastline, and also an anti-cyclonic circulation in the northern clade of the channel. Although the circulation in this channel as a whole is not yet fully understood (Lutjeharms, 2006), the anti-cyclonic circulation pattern should constitute the major part of the flow through the Mozambique Channel, allowing an almost random dispersion of the larval planktonic phases and contributing to the homogenization of populations along the coast. Accordingly, the apparent limited gene flow between the two geographic clades and the similarities observed among populations within clades supported the hypothesis of both homogenization and isolation roles of the hydrographic dynamics along the eastern African coast.

The southernmost population in the northernclade, Nacala Velha, had one haplotype in common with the southern clade, and Ulo (another population from the northern clade) also had a haplotype belonging to the southern evolutionary lineage. One possible explanation for these results would be the migration of individuals between the two clades, which could be achieved by rafting of adults or egg masses (Johannesson, 1988) or, most likely in this species, by dispersive larvae (Crisp, 1978). However, considering that these two specimens did not show any atypical shape pattern for their population, but rather possess the typical carapace shape of the northern clade, hybridization events could also explain these results. In any case, the direction of these migratory and/or hybridization events appears to be, preferentially, from the southern clade to the northern clade because no haplotypes from the northern evolutionary lineage were observed in the southern populations. Finally, the genetic versus morphological pattern observed might also indicate that the morphological differentiation found can be a plastic response to habitat-specific selective pressures and not a direct cause of genetic differences. Nevertheless, a larger number of specimens with the same genetic profile as these two specimens from Nacala Velha and Ulo should be analysed.

The main morphometric differences observed concerned the rostrum shape and the distance between the rostrum and the first lateral spine and, despite a clear differentiation in mtDNA COI sequences between northern and southern clades, differences in morphological characters were not so obvious. Nevertheless, statistically significant differences among areas were found, especially between area H (southern clade) and the remaining areas (northern clade), in line with the pattern of differentiation between north and south. Furthermore, not only the southern populations of Inhaca Island and Maputo Bay (Costa do Sol and Inkomati) were found to be similar to each other, but also differences among populations within the northern clade did not show a clear pattern of differentiation according to geographic distance, with populations that were several hundreds of kilometres apart not showing statistically significant morphometric shape variation. Thus, corroborating the pattern of within geographic clade homogeneity, the lack of shape differentiation among populations may indicate that similar ecological pressures are acting, leading to the development of similar morphological characters. These ecological pressures can have diverse origins, such as foraging, defence, habitat availability, mating, and food acquisition (Smith, 2004). However, two exceptions between two populations in areas B and D were seen. These local shape variations can be explained by shape plasticity, as already demonstrated in previous studies performed with crab species (Rosenberg, 1997; Rufino, Abelló & Yule, 2004; Silva & Paula, 2008). This phenotypic plasticity can be the result of specific ecological conditions, as suggested by Monteiro, Bordin & Reis (2000). Indeed, the same environmental factors that justify the lack of shape differentiation among populations, may also contribute to local shape variation, if acting in different directions.

In the present study, two population clades were thus define (southern clade and northern clade), with all pairwise genetic distances in the range 2.32–

2.75%. In the study by Hebert *et al.* (2003) regarding biological identifications through DNA barcodes, divergence values between species were ordinarily greater than 3%, with the exception of some congeneric species pairs that were genetically distinct but showed low divergence (0.6–2.0%), suggesting their recent origin. Considering these COI-based identification system estimations and the statistically significant but not obvious morphological differences, we suggest the presence of a species complex for these *P. guttatum* populations, with the existence of two cryptic species. Recent investigations suggest that marine biodiversity may be much higher than earlier estimates, and an important hidden source of diversity in marine systems comprises the phenomenon of cryptic species complexes (Mathews, 2006). Many cryptic species have been detected in the marine environment, such as in the species *Xiphopenaeus kroyeri* and *Xiphopenaeus riveti* (Gusmão *et al.*, 2006) and in the genus *Alpheus* (Mathews, 2006). In summary, it appears that, despite the potential for high gene flow in marine species that have larval stages, genetic panmixia cannot be assumed. Indeed, the currents that may allow gene flow between populations can also act as barriers to it, even when the distribution appears to be continuous. This was particularly evident along the geographic gradient under study, where the significant spatial genetic structure observed between the southern and the northern populations of *P. guttatum*, combined with morphological differences, suggests that these populations may be considered as cryptic species.

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