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Complex genetic patterns in the mangrove wood-borer *Sphaeroma terebrans* Bate, 1866 (Isopoda, Crustacea, Sphaeromatidae) generated by shoreline topography and rafting dispersal

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ABSTRACT

A phylogeographic analysis of the widely distributed marine wood-boring isopod *Sphaeroma terebrans* Bate 1866 was carried out to test for potential genetic differentiation and geographical structure. The species spends its entire life cycle in mangrove roots and is characterized by low active dispersal ability and no early dispersal stages. As the species has a mainly circumtropical distribution, long-range dispersal mechanisms, such as rafting on floating wood, could have played important roles in the distribution of the species throughout the world. However, as high genetic diversity and very low gene flow levels have been found among very close localities, physical barriers related to changes in coastal topography could have contributed to the contemporary genetic structure. Mitochondrial (*COI* and 16S) and nuclear (Histone3) sequences were analysed from 13 populations of *S. terebrans*. High levels of genetic population differentiation ($\Phi_{ST} = 0.84$) were observed among the 13 populations analysed and they were not explainable by an isolation by distance model (Mantel test: $p > 0.13$). Four major lineages were recognized by phylogenetic and spatial genetic analyses. One lineage (Clade D), highly divergent from the others, was only found in one of the two populations from Mahè (Seychelles Is.) and it shows morphological affinities with South Asian populations. The other 12 populations are represented by three haplogroups. Clades B and C are not highly differentiated from each other, but both of them appear very distant from Clade A, probably representing a different species with significant departure from neutrality, as suggested by Mismatch distribution, Tajima's and Fu's Fs tests. Contrary to expectation, the cosmopolitian *S. terebrans* seems to comprise more than one species.

1. Introduction

Marine invertebrates often have complex life histories, such as lack of larval dispersal phases and poor adult locomotor abilities. In these organisms, a low dispersal ability is generally considered to be negatively correlated with the gene flow and positively with the genetic structure. The expectation is that organisms with much lower dispersal potential (e.g. brooders, species with non-planktonic larvae, etc.) should present a larger number of species, often cryptic ones, and higher systematic distinctiveness over similar ranges (Teske et al., 2007; Palumbi, 1994). Dispersal influences the genetic structure to an extent influenced by the life history variation and the interaction of the first life stages with both biotic and abiotic environmental factors (Sherman et al., 2008). Marine organisms, characterized by poor active dispersal abilities, can nonetheless disperse over great distances by utilizing floating objects, such as seaweed and other materials, as rafts in the water (Gutow et al., 2006; Thiel and Gutow, 2005). For intertidal species, passive dispersal movements via rafting could be the most important means of passive transport (Miranda and Thiel, 2008).

Isopoda is an order of Malacostraca including various families which have colonised all viable environments, both terrestrial and aquatic. Some biological aspects of this group differentiate it from the other Malacostraca and have important effects on the population structure. Unlike most crustaceans, which present pelagic larval stages, the females of Isopoda retain the fertilised eggs in a marsupium composed of sternal processes of the abdominal segments, the oostegites, in which the eggs develop to maturation. Isopoda include entire families of marine species, among which Sphaeromatidae, comprising the genus *Sphaeroma* Bosc, 1802. *Sphaeroma. terebrans* Bate, 1866 is a wood-borer living mainly in aerial roots of the mangrove *Rhizophora mangle* in tropical and subtropical regions (Fig. 1). Mangrove forests are very interesting intertidal ecosystems, appearing as islands of different shapes, separated from each other by sandy or rocky shores of various sizes.

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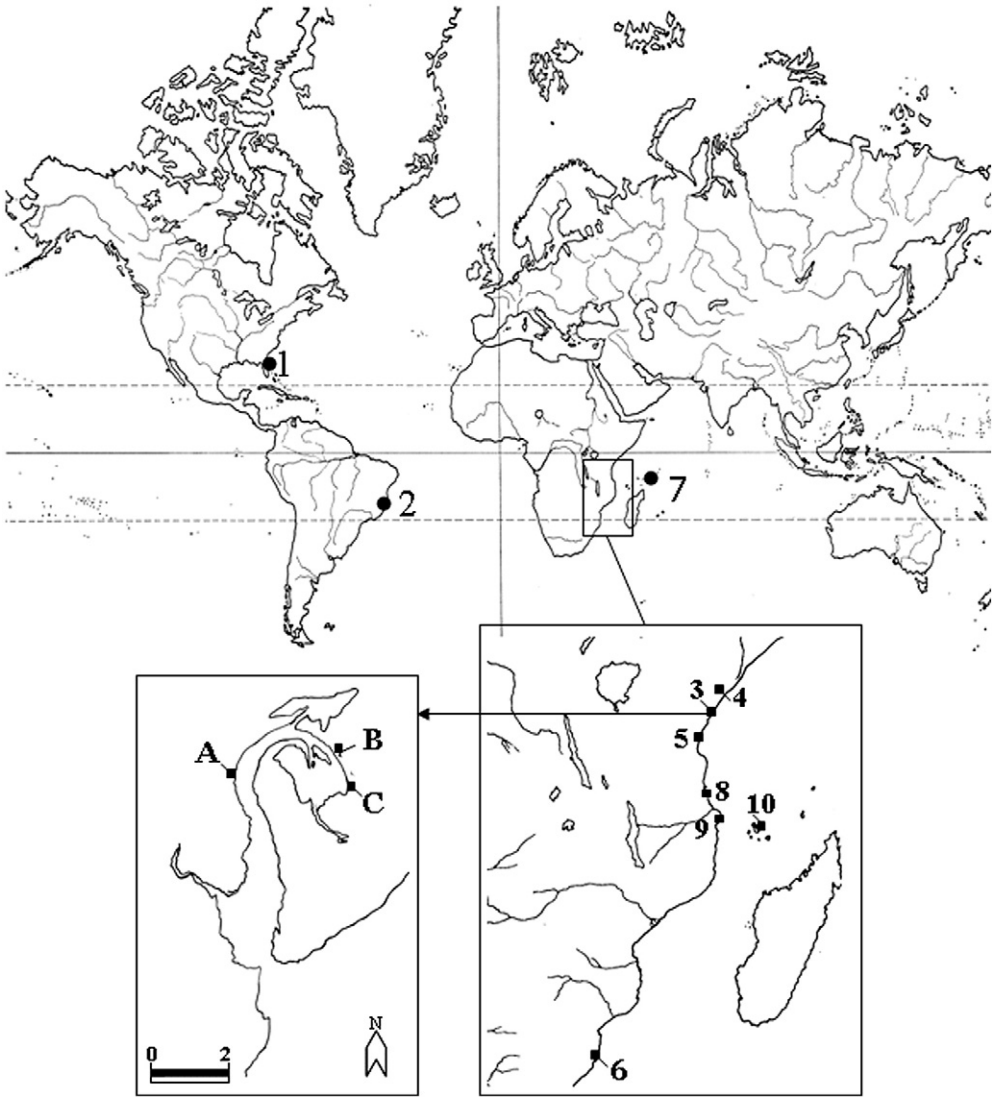

Fig. 1. Distribution of *Sphaeroma terebrans,* represented as red lines. Numbers, as indicated in Table 1, correspond to the sampling sites. In detail, number 3, Mida Creek, is represented by the three sampling sites A, Sita, B, Dabaso, and C, Mida.

The discontinuous distribution of mangroves, often limited to very narrow creeks, represents an interesting natural model in which the dispersal abilities of the species inhabiting these ecosystems can be investigated in relation to their reproductive strategies and the influence of physical barriers.

The burrows in the aerial roots made by *S. terebrans* constitute a shelter and a reproductive habitat which are seldom abandoned. Males leave the burrows after copulation, while females remain inside the burrow even after the offspring are released from the brood pouch. The mother spends most of the time in the hole, blocking the entrance with her telson and creating a flow of water with her pleopods to oxygenate the environment and provide a food supply. The protective behaviour of the female is therefore crucial for the survival of the offspring (Messana et al., 1994; Messana, 2004; Thiel, 1999, 2001). This species, whose complete life cycle probably occurs within the same mangrove wood, has thus far been considered a unique and cosmopolitan species (Harrison and Holdich, 1984). Previous molecular results (Baratti et al., 2005), suggesting the existence of a cryptic species-complex in *S. terebrans,* encouraged us to investigate more populations and to explore the roles of different factors in structuring the species. The main objective of this study was to understand the influence of dispersal abilities and habitat disjunction on the genetic structure of *S. terebrans* and to examine the hypothesis of low gene flow among populations using genetic data. Two mitochondrial genes (Cytochrome oxidase I and 16S) and one nuclear gene (Histone 3) were analysed in Indian and Atlantic Ocean populations of *S. terebrans* to investigate the effects of dispersal patterns, coastal topography and sea currents in modelling the genetic structure of this taxon. *Sphaeroma* taxa are also present in single holes bored in the pneumatophora of another mangrove tree (*Sonneratia alba*). Since some slight morphological differences were found in individuals boring roots of the two different mangrove trees (*S. alba* and *R. mangle*), in this study we included a mangrove ecosystem (Mahè Island, Seychelles Archipelago) where both tree species host *Sphaeroma* populations.

2. Material and methods

2.1. Samples

Tissue samples were collected from 205 individuals from 12 mangrove ecosystems (Table 1, Fig. 1). In the table we indicated SEY-Rhizophora and SEY-Sonneratia as the two populations from Mahè

Table 1
Sampling sites, codes and their geographic coordinates. Numbers as in Fig. 1.

Country	No.	Sampling site	Codes	Geographic coordinates	
				Latitude	Longitude
America					
Florida	1	Indian Lagoon River	FLO	28°40′21.15″N	80°46′20.21″W
Brazil	2	Juqueriquerè	JUQ	23°46′53.08″S	45°38′27.68″W
Africa					
Kenya	3a	Sita	KWS	3°20′41.99″S	39°58′33.86″E
	3b	Dabaso	KWD	3°21′25.83″S	39°58′23.65″E
	3c	Mida	KWM	3°20′02.18″S	39°57′17.52″E
	4	Lamu	KLI	2°16′23.21″S	40°53′26.90″E
	5	Gazi	KGB	4°25′32.49″S	39°30′57.32″E
Mozambique	6	Inhaca Island	INH	25°59′18.78″S	32°55′18.07″E
Seychelles	7	Cape Ternay	SEY-Rhizophora	4°40′36.25″S	55°22′45.33″E
			SEY-Sonneratia	4°38′42.00″S	55°22′48.06″E
Zanzibar Is.	8	Kisakasaka	ZANZ A–B	6°15′37.13″S	39°19′44.21″E
Tanzania	9	Ras Dege	TANZ	6°52′00″ S	39°28′ 60.80″ E
Comoros	10	Ouriveni, Moroni Island	COM	11°22′13.55″S	43°46′47.39″E

Island boring *Rhizophora* and *Sonneratia* mangrove trees respectively. The isopods were individually preserved in absolute ethanol. DNAs were obtained from pereiopods and extracted using the Salting Out procedure (Miller et al., 1988).

2.2. Molecular analyses

The cytochrome oxidase I gene (*COI*) was amplified for all sampled individuals. The COI data set consisted of a new set of sequences (asterisks in Table 2) and some previously published sequences (Baratti et al., 2005). Two other genes, one mitochondrial (16S rRNA) and one nuclear (histone H3), were amplified for one individual randomly chosen from each sampling locality, except for Zanzibar island. This sampling locality showed evidence for two different sympatric taxa based on COI analysis (see Results), so two individuals randomly chosen belonging to the two different clades (A and B in Table 1) were analysed for 16S and H3 genes. One specimen of *Sphaeroma* cf *serratum* from Sardinia Island (Italy) was used as the outgroup taxon for all amplifications.

We amplified a 498 bp COI fragment using the primers mtd10 5′-T TGA TTT TTT GGT CAT CCA GAA GT-3′ of Roehrdanz (1993) and Florence 5′-C CTA AAA AAT GTT GAG GGA A-3′ (Baratti et al., 2005). For 16S, we amplified a 529 bp fragment with the primers 16SL2 (5′-TGCCTGTTTAT-CAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCAGATCACACGT-3′) (Schubart et al., 2006). A 302 bp fragment of the nuclear histone H3 gene was also successfully amplified with the primers 5′-ATGGCTCGTAC-CAAGCAGACVGC-3′ and 5′-ATATCCTTRGGCATRATRGTGAC-3′ (Colgan et al., 1998).

We followed PCR protocols as in Baratti et al. (2005) for *COI* and as in Baratti et al. (2004) for 16S. For histone 3, we followed the protocol of Colgan et al. (1998). The PCR products were electrophoresed and purified using the ExoSAP-IT kit (Amersham Biosciences, Uppsala, Sweden). Purified DNA was quantified with a spectrophotometer, sequenced with an ABI sequencing kit (Big Dye Terminator Cycle Sequencing v. 2.0-ABI PRISM, Applied Biosystems, Foster City, U.S.A.) and then analysed with an ABI Prism 310 automated sequencer. Sequences used in this study have been deposited in GenBank under accession numbers reported in Table 1.

Sequence chromatograms were visualised with CHROMAS software ver. 1.45 (Technelysium Pty. Ltd.). The sequences (manually corrected) were analysed with ProSeq v 2.9 Beta (http://helios.bto.ed.ac.uk/evolgen/filatov/proseq.html) and aligned using CLUSTALX v. 1.81 (Thompson et al., 1997). In order to determine if sequences were nuclear (numts, Song et al., 2008; Buhay, 2009) or mitochondrial copies, the following steps were followed. First, sequence chromatograms were checked for double signals. Thereafter, alignments were analysed looking at frame-shift mutations, stop codons and variability at the three codon positions. Finally, corrected sequences were compared to GenBank ones.

2.3. Data analysis

Uncorrected p-distances (p) were obtained by MEGA v. 3.1 (Kumar et al., 2004). Nucleotide diversity (π) and haplotype diversity (H) were calculated according to Nei (1987), using the program DnaSP 4.10 (Rozas et al., 2003). The number of haplotypes were calculated with ARLEQUIN software vers. 3.5.1.2 (Excoffier and Lischer, 2010). Chi-squared tests (implemented in PAUP* 4.0b10; Swofford, 2001) were used to test the hypothesis of homogeneity of base frequencies among sequences.

The likelihood mapping method (Strimmer and von Haeseler, 1997) was executed with TREE-PUZZLE (Schmidt and von Haeseler, 2007) to test the a priori phylogenetic signal in the mtDNA portions studied. Phylogenetic congruence among *COI*, 16S and H3 data partitions was also performed using the partition-homogeneity test implemented in PAUP* (Swofford, 2001). Testing of the evolutionary model that best fits our data was conducted with MODELTEST 3.04 (Posada and Crandall, 1998), based on a likelihood ratio test. Different models of nucleotide substitutions were fitted to each data set and the combined data set. For *COI*, the TRN model was the best one selected (Tamura and Nei, 1993), while for 16S and H3 the HKY model was selected (Hasegawa et al., 1985). Both models were corrected for rate heterogeneity among sites with a Gamma distribution (G, Yang, 1993). For *COI* + 16S + H3, the HKY + I + G model was selected. We carried out a phylogenetic reconstruction by Maximum Parsimony (MP, Kluge and Farris, 1969) using PAUP, performed with ACCTRAN optimization and tree bisection TBR branch swapping, considering all characters as unordered and equally weighted, gaps treated as fifth state. A strict consensus tree was calculated when more than one tree resulted. Neighbour-Joining (NJ) (Saitou and Nei, 1987) analysis was performed using PAUP, with all the parameter values estimated by MODELTEST. Non-parametric bootstrapping with heuristic searches of 2000 replicates for MP and NJ was used to assess confidences of branches in MP and NJ. A Bayesian analysis (BI) was also performed with MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001), with clade support assessed by posterior probabilities and using the substitution model selected by MODELTEST. Four Markov chains, one heated and three cold, were allowed to run for 2 million generations using random starting trees. Trees were sampled every 100 generations, yielding 8000 samples of the Markov chain after a "burn-in" of 2000 generations. MP, NJ and BI analyses were performed for *COI*, 16S and H3 sequences separately and in a combined analysis (total evidence approach) for the taxa sequenced for both genes.

Table 2
Populations used in this analysis, with their GenBank Accession numbers for the analysed gene portions. First row: locality name and abbreviations; second row: N = number of specimens; first column: haplotypes. Last column: CL, haplotype memberships to *COI* clades. Asterisks indicate populations whose *COI* sequences were obtained from a previous study (Baratti et al., 2005).

Sampling sites	KWD*	KWS*	KWM	COM	ZANZ A + B	FLO*	JUQ*	KGB*	KLI	INH	TANZ	SEY-Sonneratia	SEY-Rhizophora	COI	16S	Histone3	CL
N	21	16	15	1	19	15	15	18	21	16	17	15	16				
DABASO1	6	2												AF453223	EF460853	FJ656800	A
DABASO2	1													AF447859			A
DABASO3	1		1											AF453224			A
DABASO4	1													AF453225			A
DABASO5	2													AF453228			A
DABASO6	1													AF453226			A
DABASO7	1													AF453227			A
DABASO8	5	5											1	AF453229			A
DABASO9	1													AF453230			A
DABASO10	1													AF453233			A
DABASO11	1	3												AF453231			A
SITA1		1	9										4	AF453234			A
SITA2		1												AF447835			A
SITA3		3												AF453236			A
SITA4		1												AF453237			A
MIDA1			1											EU449129			A
MIDA2			1											EU449130			A
MIDA3			1											EU449131			A
MIDA4			1											EU449133			A
MIDA5			1											EU449134			A
COMORE1				1										FJ656810	FJ656809	FJ656798	A
ZANZ1					6									AF453232	EF460852	FJ656807	A
ZANZ2					3									AF453238	EF460854	FJ656804	B
ZANZ3					1									AF453239			A
ZANZ4					1									AF453241			A
ZANZ6					1									AF453242			B
ZANZ7					1									AY247973			B
ZANZ8					2									AY247974			B
ZANZ9					1									AY247975			B
ZANZ10					1									AY247976			B
ZANZ11					2									AY247977			A
FLORIDA1						11								AY247978	EF460858	FJ656802	C
FLORIDA2						1								AY247979			C
FLORIDA3						2								AY247980			C
FLORIDA4						1								AY247981			C
BRAZIL1							4							DQ911474	EF460859	FJ656803	C
BRAZIL2							4							DQ911476			C
BRAZIL3							1							DQ911477			C
BRAZIL4							3							DQ911478			C
BRAZIL5							1							DQ911479			C
BRAZIL6							1							EU449124			C
BRAZIL7							1							EU449125			C
GAZI1								1						AY247982	EF460855	FJ656795	B
GAZI2								12						AY247983			B
GAZI3								1						AY247984			B
GAZI4								1						AY247985			B
GAZI5								1						AY247786			B
GAZI16								1						EU449123			B
LAMU1									3					DQ911480			B
LAMU2									5					DQ911481			B
LAMU3									3					DQ911482			B
LAMU4									3					DQ911483			B
LAMU5									1					DQ911484	EF460857	FJ656806	B
LAMU6									1					DQ911485			B
LAMU7									1					DQ911486			B
LAMU8									1					DQ911487			B
LAMU9								1	1					DQ911488			B
LAMU10									1					DQ911489			B
LAMU11									1	1				DQ911490			B
MOZ1										1				DQ911491			B
MOZ2										1				DQ911492	FJ666124	FJ656805	B
MOZ3										1				DQ911493			B
MOZ4										1				DQ911494			B
MOZ5										1				DQ911495			B
MOZ6										2				DQ911496			B
MOZ7										1				DQ911497			B
MOZ8										1				DQ911498			B
MOZ68										1				DQ911499			B
MOZ9										1				DQ911500			B
MOZ10										1				DQ911501			B
MOZ11										1				DQ911502			B
MOZ12										2				EU224318			B

Table 2 (*continued*)

Sampling sites	KWD*	KWS*	KWM	COM	ZANZ A + B	FLO*	JUQ*	KGB*	KLI	INH	TANZ	SEY-Sonneratia	SEY-Rhizophora	COI	16S	Histone3	CL
N	21	16	15	1	19	15	15	18	21	16	17	15	16				
TANZ1											1			DQ911503	EF445550	FJ656796	B
TANZ2											2			DQ911504			B
TANZ3											1			DQ911505			B
TANZ4											1			DQ911506			B
TANZ5											1			DQ911507			B
TANZ6											6			DQ911508			B
TANZ7											1			DQ911509			B
TANZ8											2			EU224319			B
TANZ9											1			EU224320			B
TANZ10											1			EU224321			B
SEYB1												1		EU224317			D
SEYB2												2		DQ911475			D
SEYB3												1		DQ911510			D
SEYB4												2		DQ911511			D
SEYB5												1		DQ911512	EF460856	FJ656797	D
SEYB6												1		DQ911513			D
SEYB7												2		DQ911514			D
SEYB8												1		DQ911515			D
SEYB9												1		EU224325			D
SEYB10												2		EU449127			D
SEYB11												1		EU224323			D
SEYM1													1	DQ911516			A
SEYM2													2	EU224322			A
SEYM3													1	AF453240			A
SEYM4													1	EU224324			A
SEYM5													1	EU224326	EF460860	FJ656801	A
SEYM6													1	EU339189			A
SEYM7													1	EU339190			A
SEYM8													1	EU339192			A
SEYM9													1	EU339193			A
SEYM10													1	EU449128			A

Tajima's test of neutrality (Tajima, 1989), comparing the average number of pairwise nucleotide differences (k) among haplotypes in a sample (M) expected from the number of segregating sites (K), was used to infer the population history. A population that has undergone population expansion may result in the rejection of the null hypothesis of neutrality. Alternatively, Mismatch Distribution analyses were used to evaluate possible events of population expansion and decline (Rogers, 1995). A population that has experienced a rapid expansion or bottleneck in the recent past shows a unimodal pattern. Fu's *Fs* represents the probability of observing a similar or a higher number of haplotypes in a random neutral population given the observed value of theta. In populations that have undergone recent expansion, large negative values of *Fs* are expected. All demographic analyses were computed in DNAsp 4.10 (Rozas et al., 2003).

Analyses of molecular variance (AMOVA; Excoffier and Lischer, 2010) and spatial analyses of molecular variation (SAMOVA; Dupanloup et al., 2002) were computed using the *COI* data set with ARLEQUIN and SAMOVA softwares respectively. We estimated the potential number of populations using SAMOVA, clustering geographically homogeneous populations into a user-defined number of groups (K) so that the proportion of total genetic variance observed between groups (Φ_{CT} index) was maximized. We conducted the analysis for 2–4 potential populations. The AMOVA was carried out at three hierarchical levels: among groups (Fct), among all populations (Φst) and among populations within groups (Φsc). ARLEQUIN was also used to calculate the pairwise ΦST with Tamura & Nei (TrN) distances and gamma correction and their significance (10,000 permutations).

The correlation between geographical distances and genetic distances was tested with the Mantel test (Mantel, 1967) using NTSYS v.2.2 (Rohlf, 2005) on the whole species and on the different clades independently (on clades A, B and B + C separately; clade D was excluded from this test since it comprised only one population).

3. Results

3.1. Data analysis

Sequences have been deposited in the GenBank data base (accession numbers are listed in Table 1). Since insertions/deletions, stop codons or differences in variation rates were not detected along the sequences, we excluded the amplifications of nuclear copies of the gene fragments analysed in this study.

The *COI* gene fragment translates into 166 amino acids. Of the 498 base pairs, 194 were polymorphic (total number of mutations: 287), whereas 158 were parsimony informative. The different populations showed 104 haplotypes (including 35 haplotypes of a previous study, Baratti et al., 2005) (Table 2). The sequences of all populations showed a *COI* haplotype diversity (H) (Table 3) ranging from 0.46 (Florida) to 0.98 (Mozambique), with the exclusion of Comoros for which only one specimen was available. The nucleotide diversity (π) ranged from 0.002 in the Gazi Bay and Florida populations to 0.113 in Zanzibar Island.

After alignment, the 16S gene portions were 455 bp in length, with 183 variable sites, among which 93 were parsimony informative. The H3 region showed 35 variable sites and only 5 parsimony informative sites.

3.2. Phylogenetic analyses

The phylogenetic relationships among all 104 unique *COI* haplotypes resulting from the MP, NJ and BI analyses showed high topological similarity among the trees. All three methods yielded the same four clades: clade A including the Kenyan populations of Mida Creek (Dabaso, Sita and Mida), some individuals collected at Zanzibar, Seychelles (SEY-Rhizophora), Comoros Islands, two specimens from Lamu and Mozambique; clade B consisted of the populations from the

Table 3
Estimates of genetic diversity and neutrality tests. Haplotype diversity (H), nucleotide diversity (π), clade membership, results of Mismatch Analysis (SSD: sum of square deviations; r: Harpending's raggedness index) for *COI* sequence data set.

						Mismatch analysis	
"Clade A"		H	π	Tajima's D	Fu's Fs	SSD	r
Dabaso		0.87	0.004				
Sita		0.85	0.003				
Mida		0.65	0.012				
Sey-Rhizophora		0.94	0.011				
Comoros		0	0				
Zanzibar-A		0.86	0.113				
	Global			−2.003	−3.2	0.020	0.118
"Clade B"							
Zanzibar-B		0.75	0.112				
Lamu		0.9	0.056				
Mozambique		0.98	0.044				
Tanzania		0.87	0.007				
Gazi		0.56	0.002				
	Global			−0.61	0.261	0.033	0.113
"Clade C"							
Florida		0.46	0.002				
Brazil		0.85	0.005				
	Global			1.08	0.471	0.023	0.137
"Clade D"							
SEY-Sonneratia		0.97	0.046	−0.95	−0.8	0.025	0.082

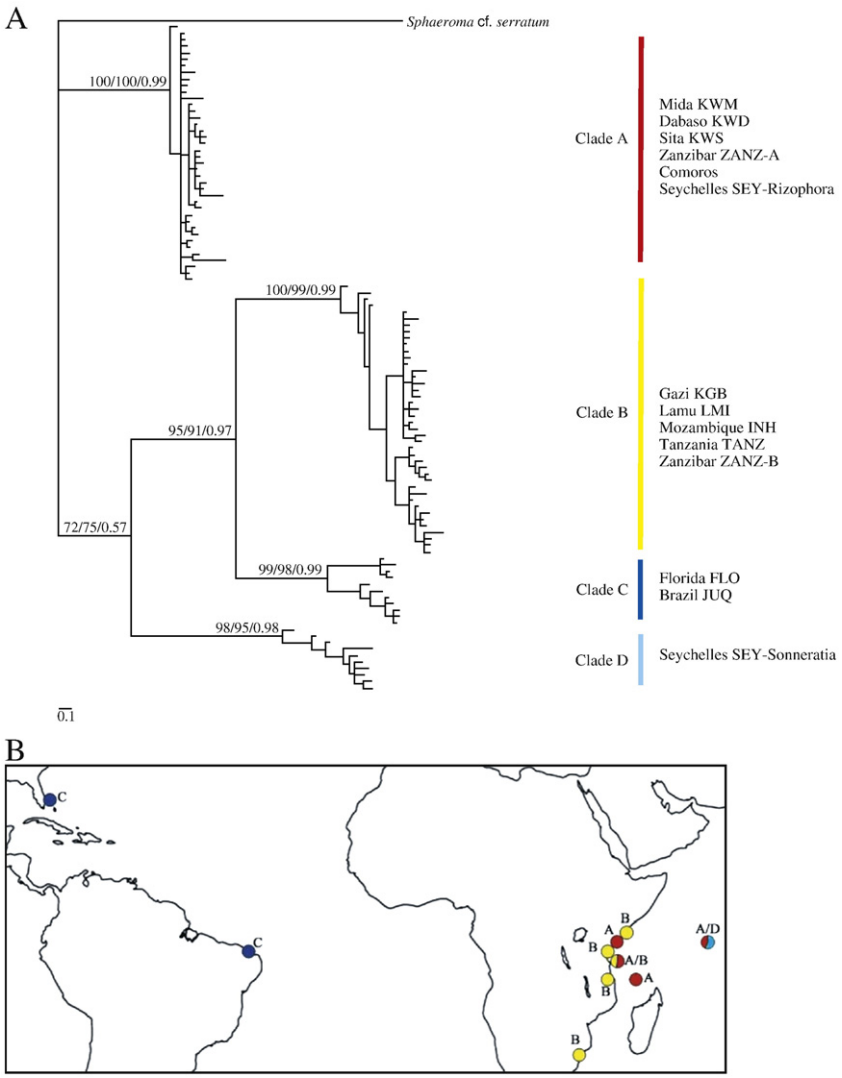

Fig. 2. A) Consensus of proposed phylogenetic relationships obtained with *COI* data. The consensus diagrams summarize the results of the three reconstruction methods, Bayesian posterior probability, MP and NJ bootstrap values at the nodes. B) Distribution of clades (represented as pie charts with haplogroup frequencies coloured as in the tree) for each of the sampling sites. Abbreviations as in Table 2.

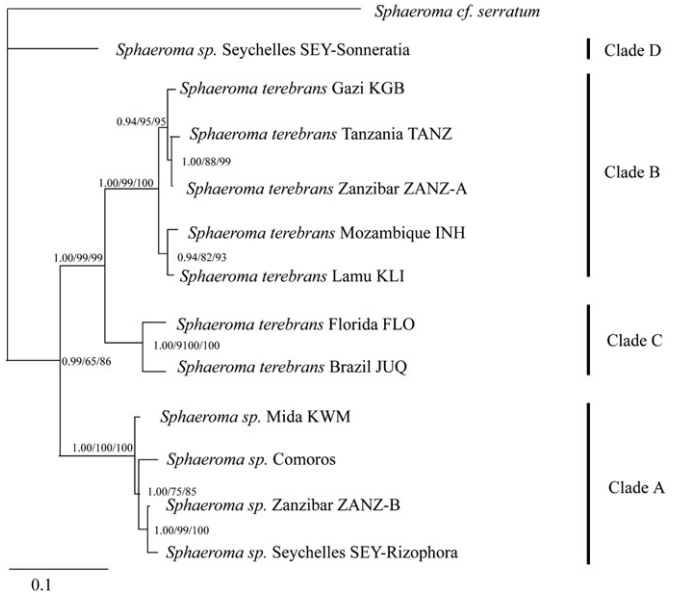

Fig. 3. Bayesian phylogenetic analysis obtained with combined (*COI*, 16S and H3) data. The consensus diagrams summarize the results of the three reconstruction methods, with Bayesian posterior probability, MP and NJ bootstrap values at the nodes. Abbreviations as in Table 2.

African coast (Kenya: Lamu, Gazi; Tanzania: Ras Dege and some specimens from Zanzibar Island; Mozambique); clade C with American populations (Brazil and Florida) and clade D with the specimens from Seychelles (SEY-Sonneratia) (Fig. 2). The tree obtained only considering the 1st and 2nd positions did agree with the other *COI* topologies.

Phylogenetic reconstruction performed for *COI*, 16S and H3 (only NJ, see ahead) separately produced the same trees. The total evidence approach (*COI*, 16S and H3 together, Fig. 3) confirmed the clades obtained with the genes considered separately, but with a different position of clades A and D in comparison with *COI* topology. In Fig. 3 the clade closer to *S. terebrans* clades B and C is clade A. We found a low nucleotide divergence among H3 sequences. The BI and MP analyses did not produce resolved trees. However, the NJ tree (Fig. 4) showed no differentiation at the intra-haplogroup level (excluding SEY-Rhizophora in Clade A), but the clades were differentiated as in Fig. 3. H3 sequences presented the largest divergence between SEY-Rhizophora and SEY-Sonneratia (p = 3%), which live in the same locality but different species of mangrove trees.

3.3. Mismatch, Tajima's and Fu's test results

The results of neutrality tests and demographic parameters of the *S. terebrans COI* data set and its phylogroups are shown in Table 3. Fu's *Fs* and Tajima's test statistic indicated a significant departure from neutrality (excess of low-frequency haplotypes) only in the sequence data of "A" group, as expected when a population is under selection or expansion. Unimodal distributions were also observed only in the same group. These results showed non-significant differences, as predicted by the growth expansion model (measured by the sum of squared deviation; $p>0.05$). Our analyses supported the idea that "A" group had experienced rapid expansion (R and SSD with $p>0.05$, Table 4).

3.4. Population genetic structure

The SAMOVA analyses revealed that the highest value among-group genetic differentiation ($Fct = 0.60$) was related to the three pooled groups (clades A, B + C and D). Haplotype sharing was not observed among regions belonging to different clades but was observed within the same group of localities belonging to the same clade. Hierarchical analysis of mtDNA diversity using AMOVA showed that most of the total variation was among groups (61%; $Fct = 0.60$), 24% among populations of the same group ($Fsc = 0.62$) and the remaining 15% of variation was detected within the populations. Among all populations, the value was very high (0.84). The Indian ocean populations were found to be highly structured ($\Phi st = 0.85$; $p<0.05$). indicating that, even along the coast, the levels of gene flow of this species are limited. However, some of the western Indian Ocean coastal populations had Φst mean values in the 'moderate' range of Wright (1978) genetic differentiation values (0.06–0.15), indicating a reasonable level of gene flow among them (Table 4). This is the case of Sita (KWS), Dabaso (KWD) and Mida (KWM), or between Gazi Bay (KWG) and Zanzibar (ZANZB) (Table 4).

However, the Mantel test applied to Φst and geographical distances was never significant, suggesting that an isolation by distance model is not supported statistically (whole species: $r2 = 0.56$, $P = 0.43$; clade A: $r2 = 0.38$; $p = 0.65$; clade B: $r2 = 0.59$; $p = 0.156$; group B + C: $r2 = 0.21$, $p = 0.131$).

4. Discussion

Dispersal mechanisms alone cannot explain the genetic structure of many marine invertebrates, but several factors (such as physical barriers, vicariant events and sea circulations) can combine to produce the current population structure of a species (Hayes and Karl, 2009; Ayre et al., 2009). Hence, contrary to expectations of panmixia in the sea

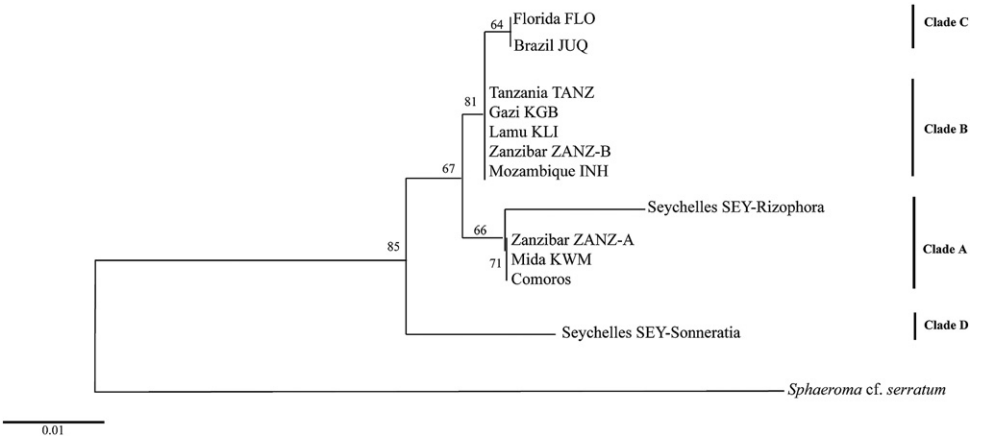

Fig. 4. Neighbour joining tree based on the Histone 3 sequences with bootstrapping values at branch nodes obtained after 2000 replicates. Abbreviations as in Table 2.

Table 4
Φst among populations.*No significant pairwise values (P>0.05).

	KWD	KWS	KWM	COM	ZANZ-A	ZANZ-B	FLO	JUQ	KGB	KLI	INH	TANZ	SEY-Rhizophora
KWS	0.11*												
KWM	0.12*	0.05*											
COM	0.78	0.82	0.21										
ZANZ A	0.19	0.13	0.04	0.11									
ZANZ B	0.83	0.81	0.73	0.57	0.58								
FLO	0.98	0.98	0.92	0.98	0.89	0.80							
JUQ	0.96	0.96	0.90	0.95	0.86	0.78	0.92						
KGB	0.87	0.86	0.80	0.76	0.72	0.05	0.84	0.82					
KLI	0.83	0.82	0.77	0.70	0.68	0.16	0.77	0.76	0.23				
INH	0.89	0.88	0.82	0.79	0.74	0.25	0.85	0.84	0.31	0.12			
TANZ	0.96	0.97	0.91	0.96	0.87	0.19	0.96	0.94	0.22	0.34	0.46		
Sey-Rizophora	0.36	0.29	0.10	0.42	0.11	0.76	0.96	0.93	0.83	0.79	0.85	0.94	
Sey-Sonneratia	0.93	0.92	0.86	0.87	0.82	0.76	0.93	0.91	0.81	0.77	0.82	0.91	0.89

environment, complicated fine-scale population structures can be found (Teske et al., 2007; Banks et al., 2007; Sherman et al., 2008). Some discontinuities in biocoenosis distributions have already been described, particularly in the western Indian Ocean area, even for species with planktonic larval stages (Duke et al., 2002; Silva et al., 2010).

The geographical distribution of *S. terebrans* far exceeds the distances that could potentially be covered by its dispersal potential. Passive transport evidently provides an efficient means of long-distance dispersal, which depends on the stochastic dispersion of juveniles or adults by rafting. In contrast, some sympatric or very close cryptic species coexist in the western Indian Ocean, suggesting that historical or ecological barriers played an important role in the phylogeographic disjunction events. Although levels of genetic divergence do not represent an absolute evaluation of taxonomic status on account of their taxon-related nature, the genetic divergences found among populations of different clades (A vs B + C = 21%; A, B + C vs D = 22%) can be compared with those described in the literature for crustaceans (Baratti et al., 2005; Lefebure et al., 2006). For a wide range of Crustacea, a divergence range of 16% has been indicated to differentiate two different species using *COI* (Lefebure et al., 2006). Therefore, the percentage of nucleotide divergence found among populations belonging to different clades is largely over the limit for different species belonging to the same genus. In contrast, Clades B and C present a nucleotide divergence (p = 10%) corresponding to the known level of distance between geographically distant populations (Baratti et al., 2005; Lefebure et al., 2006).

Panmixia in the examined *S. terebrans* populations is strongly rejected by the phylogenetic analysis, since genetic variation is partitioned into well-defined clades and shared haplotypes have a tendency to occur within very narrow geographical boundaries (in the same creek, as at Mida Creek: Sita, Mida and Dabaso). AMOVA revealed high levels of genetic structuring among coastal and insular populations, with the major percentage of genetic variance and absence of gene flow found among populations belonging to the different clades A, B and D (significant Φst values, Table 4). Most pairwise Φst values were significantly different from zero (Table 4). The lowest levels of differentiation (Φst non-significant or <0.15) were observed either between sites within the same creek (Mida, Sita and Dabaso) or between islands with a high potential of migrant exchanges (Zanzibar and Seychelles) and other close localities (Table 4). AMOVA also assigned most of the *COI* variation of the populations to differences among groups. This clearly shows that populations comprising the three clades (A, B + C and D) are evolving independently and they probably represent three distinct species (Figs. 2, 3). The hypothesis of different cryptic species is supported by the sympatry of the haplotypes belonging to different mitochondrial and nuclear haplogroups (mangrove ecosystems of Seychelles and Zanzibar Islands, Figs. 3, 4). This hypothesis is also strengthened by the lower genetic divergence between very distant populations (for example Zanzibar and American populations; p = 14%) than that observed between the two sympatric ones inhabiting the same island (p = 19%).

The Mismatch distribution analysis, Fu's *Fs* and Tajima's tests showed that haplogroups B and C represent populations at equilibrium, with Fu's *Fs* and Tajima's tests not significant and a multimodal Mismatch distribution (Table 3). In contrast, the significance of Fu's *Fs* and Tajima's tests (p<0.05) and the unimodal mismatch distribution suggest that some non-neutral processes occurred for haplogroup A. In the apparent absence of dispersal barriers today, the explanation for the present distribution of the flora and fauna of mangrove communities must be based on past barriers. Sea level fluctuations, delineating the actual shoreline topography, have affected the East African coast, with possible changes in the shape of some coastal biotopes such as creeks and their opening to the sea (Kairu and Nyandwi, 2000). These geomorphological changes could have affected the exchanges between the fauna and flora of the creek ecosystems and the open ocean.

The continental and insular coastal ecosystems in the western Indian ocean analysed in this study are characterized by highly diversified environments, with rocky or sandy open shores alternating with closed embayments. The latter are often connected with the open sea through very narrow openings, as in the case of Mida Creek (Fig. 1), which could have undergone entrance closure and a subsequent very reduced re-opening of the mouth, characterized by an interruption of the inflow inside the creek.

Substrate transport is a fundamental factor in structuring the phylogeography of isopods. Mangroves are viviparous, rather than generating dormant resting seeds like most flowering plants. Mangroves disperse propagules via water, with varying degrees of viviparity or embryonic development while the propagule is attached to the parent tree. Molecular studies of mangrove trees support the hypothesis of long-distance dispersal by propagules. Previous studies found high gene flow levels among populations of *R. mangle* separated from each other by hundreds of kilometres (Arbela´ez-Cortis et al., 2007). Moreover, *Rhizophora* propagules survive long-distance dispersal better than those of other mangrove trees such as *Avicennia* (Triest, 2008). Isopods probably do not follow the same dispersal mechanisms as their host because they do not bore into the propagules. However, they might follow the same current routes since they probably disperse via floating broken mangrove roots or wood. Mangrove tree roots, hosting wood-boring isopod communities, probably contribute to the rafting of the isopods, since the broken pieces of wood can be swept away during the tidal sea currents and transported for long distances. The transport of floating wood is difficult via sea water inflow currents entering creeks with a very narrow mouth. In Mida Creek, the cutting of mangroves for wood utilization is very substantial compared with other coastal localities (house construction, fuel and boat building, Dahdouh-Guebas and Mathenge, 2000). Although this phenomenon may be fairly recent, the large amount of waste mangrove pieces left by humans among the mangroves may contribute to the dispersal into the sea of rafted isopods.

Clade A may represent a cryptic species generated by past isolation of a *S. terebrans* population that remained isolated for a long time. The new species probably colonised open sites in the western Indian ocean (Seychelles, Comoros, Zanzibar, Lamu and Mozambique) via the strong outflow current, often cohabiting with *S. terebrans* (Zanzibar, Lamu and Mozambique). As explained in a previous paper (Baratti et al., 2005), the very strong outlet currents at the creek mouth during the ebb tide could eject floating pieces of mangrove into the Somali Current (SC: flowing south–north in summer and north–south in winter), which could then transport the wood for several kilometres. While it is very difficult for floating wood to pass exactly through the mouth of a creek when the tide rises and water enters the creek, the catching capacity of an island at the crossroads of two opposite currents might be much higher. Zanzibar, Seychelles and Comoros Islands are situated almost exactly where, in winter, the SC converges with the East African Coastal Current (EACC) and deviates eastward to become the South East Counter Current (SECC) (Schott and McCreary, 2001).

Clades B and C probably represent the typical *S. terebrans*, whose present global distribution originated from large-scale transport by ocean currents via rafting. This kind of transport has been invoked for many marine invertebrates with early non-planktonic stages and low active dispersal abilities. Rafting is also important in structuring established coastal communities (Muhlin et al., 2008). Clade D represents a pseudocryptic species, not characterized by morphological variations at diagnostic characters commonly used to discriminate *Sphaeroma* taxa at the specific level (Harrison and Holdich, 1984). However, a difference in the shape and length of the VII pereiopod, as detected in some populations from the Philippines (Messana, personal observations) and Hinchinbrook Island (Harrison and Holdich, 1984), was observed in the population SEY-Sonneratia (Messana G., personal observation).

The actual genetic structure of *S. terebrans* is also more complicated: some populations very close to each other show a level of genetic differentiation not explainable by passive dispersal via rafting. The 13 populations analysed in this study are grouped into four clades, with no (clades A, B, C Fig. 2) or very slight morphological differences (clade D) distinguishing them (Filippelli MT. personal observation). For various reasons, isopods have historically suffered from high levels of taxonomic and systematic confusion, especially at the species level. Much of this confusion results from species descriptions based on morphological characters that fail to distinguish the different species found here.

The high genetic differentiation levels detected among the different haplogroups suggest insufficient morphological investigations (pseudocryptic species) or divergence related to life history or physical characteristics of the ecosystem (Knowlton, 1993). The lack of strong morphological differences in non-sympatric populations should prevent us from assigning systematic significance to these taxa.

Our study investigated two mitochondrial markers and only one nuclear marker. We believe that a large nuclear multilocus assay is needed to account for male gene flow and population differentiation. Some polymorphic microsatellites have been characterized for this species (Baratti et al., 2009) and these markers will be suitable to infer parentage, relatedness and phylogeographic patterns in this taxon, which includes some cryptic species. Although the analysis of mtDNA sequence data demonstrates that levels of population structure and population differentiation are related to the mode of development in *S. terebrans*, this pattern has still not been strongly demonstrated on a genetic basis. *S. terebrans* lives in burrows bored in the mangrove wood and roots, where mating and the reproductive cycle take place, with males either leaving or staying in the burrows after copulation, while females always remain inside the burrow even after the offspring are released. The study of the family burrow dynamics and the population genetic structure could shed light into the dispersal mechanism of this species.

BM coordinated the study, carried out the molecular and statistical analyses, provided specimens and drafted the manuscript. FM carried out the molecular and statistical analyses. MG provided specimens for the study, carried out the morphological analysis and helped with the manuscript. All authors read and approved the final manuscript.

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