CSIRO PUBLISHING

Marine and Freshwater Research, 2014, **65**, 59–69
http://dx.doi.org/10.1071/MF12359

Genetic homogeneity and historical expansions of the slipper lobster, *Scyllarides brasiliensis*, in the south-west Atlantic

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Abstract. Management strategies for fisheries species require understanding their connectivity and population dynamics. The Brazilian slipper lobster, *Scyllarides brasiliensis*, is one of the most commercially important slipper lobster species in South America. We investigated, for the first time, the population genetic structure and evolutionary history of this species. Analyses of sequences of the cytochrome oxidase I gene (COI) and the control region (CR) did not reveal any significant genetic structure of *S. brasiliensis* ($N = 202$) along 2700 km of the Atlantic coast (COI: $\Phi_{ST} = 0.0004$, $\Phi_{CT} = 0$–0.005, $P > 0.05$; CR: $\Phi_{ST} = 0.004$, $\Phi_{CT} = 0$–0.029, $P > 0.05$). The genetic homogeneity found suggests high levels of gene flow along the area that are possibly related to the high dispersal potential of the planktonic larvae of the species. Furthermore, the data indicate that demographic and geographical expansions of this slipper lobster population have occurred during the late and middle Pleistocene, which could be related to the fluctuating environmental conditions of that period.

Additional keywords: fisheries, genetic structure, phylogeography, Scyllaridae.

Received 25 December 2012, accepted 18 June 2013, published online 20 September 2013

Introduction

Slipper lobsters (family Scyllaridae) have received little scientific and fisheries management attention because of their lower economic importance compared with that of clawed (family Nephropidae) or spiny (family Palinuridae) lobsters (Spanier and Lavalli 2007). However, slipper lobster catches have increased over the last 10 years as a consequence of the overfishing of the Palinuridae (Groeneveld *et al.* 2006; Phillips and Melville-Smith 2006; Spanier and Lavalli 2007). This led to an initial increase in the role of slipper lobster fisheries in the economies of areas where they are fished (Australia, Brazil, Galapagos Islands, Hawaii, India, and the Mediterranean), but poor fishery management subsequently resulted in a decrease of their relative abundance (Spanier and Lavalli 2006, 2007; Oliveira *et al.* 2008; Duarte *et al.* 2010, 2011). Within the Scyllaridae, three genera, *Ibacus* (subfamily Ibacinae), *Scyllarides* (subfamily Arctidinae) and *Thenus* (subfamily Theninae) have significant fisheries because of their large body size (Spanier and Lavalli 2006). Within *Scyllarides*, the Brazilian slipper lobster, *S. brasiliensis* Rathbun, 1906, is one of the main species caught in South America, especially in the North-east region (Santos and Freitas 2002). *S. brasiliensis* can be found at 20–40 m depth in the western Atlantic, along the Brazilian coast, from 2°S (Maranhão State) to 30°S (Santa Catarina State) (Dall'Occo *et al.* 2007). The species has also been reported in Dominica, in the West Indies (Holthuis 1991).

A key factor in fisheries management is delimiting stocks (Ward 2002). The structuring and connectivity of populations is determined largely by the dispersal of individuals. The environment in which marine organisms live allows for a variety of mechanisms for dispersal among populations (Cowen and Sponaugle 2009). For most benthic invertebrates that are sessile or with limited mobility, gene exchange between populations occurs mainly during the pelagic larval phase (Thorpe *et al.* 2000) and depends on the biological characteristics of larvae and their interaction with ecological (food availability, species interactions) and physical (past and present currents, and climatic features) processes (Palumbi 2003; Shanks *et al.* 2003). Usually, species with a short planktonic larval duration tend to present higher levels of population structuring than those with long planktonic larval durations (Palumbi 2003). Nevertheless, there are exceptions. Strong population structuring can also be found in species with long planktonic larval duration, since dispersal may be affected by larval behaviour or oceanographic mechanisms that can result in high degrees of larval retention, or high selection against recruits in new environments (Palumbi 2003; Taylor and Hellberg 2003; Palero *et al.* 2008).

Achelata lobsters (spiny, slipper, and coral lobsters) have a larval phase (phyllosoma) specially adapted for long dispersal (Palero *et al.* 2008). This dispersal ability is among the greatest found in crustaceans; in some species of *Scyllarides*, the

phyllosome larvae can live in the plankton for up to 9 months (Coutures 2000; Booth *et al.* 2005; Sekiguchi *et al.* 2007). Accordingly, for some of the lobster species of *Jasus*, *Panulirus*, *Palinurus* and *Scyllarides*, no genetic differentiation is usually observed, even between populations distributed over large oceanographic distances (e.g. Tolley *et al.* 2005; Naro-Maciel *et al.* 2011; Kennington *et al.* 2013) (Table 1). However, there are exceptions in which some genetic heterogeneity has been reported (e.g. Brasher *et al.* 1992; Palero *et al.* 2008; Babbucci *et al.* 2010) (Table 1). In *Jasus verreauxi*, for example, heterogeneity among southern Australia and New Zealand populations was observed, probably due to a fall in larval survivorship while crossing the Tasman Sea (Brasher *et al.* 1992). In *Palinurus delagoae* and *P. elephas*, low genetic differentiation was found, possibly caused by the retention of some of the larvae by mesoscale oceanic processes such as eddies (Gopal *et al.* 2006; Palero *et al.* 2008; Babbucci *et al.* 2010). Similarly, strong differentiation was found between the Caribbean and south-west Atlantic populations of *Panulirus argus* (Diniz *et al.* 2005), but that diversity was later attributed to the presence of cryptic species (Tourinho *et al.* 2012). Also, in *Panulirus penicillatus*, the heterogeneity found between the eastern Pacific and the central to western Pacific was probably due to the presence of cryptic subspecies (Chow *et al.* 2011).

Circulation in the southern Atlantic is complex, and offers an interesting setting to study the influence of current systems on the larval transport of marine species. In that area, the surface circulation pattern shows some dependence on predominant wind and sea depth (Stramma and England 1999). The South-Equatorial Current (SEC) flows westward across the Atlantic until it reaches the Brazilian coast, at which point (9–15°S) it splits into the Northern Brazilian Current, which flows north to Guyana and the Caribbean Sea, and the Brazil Current, which flows south along the Brazilian coast (Cirano *et al.* 2006). The South-Equatorial Current has four main branches, the south (sSEC), the central (cSEC), the north (nSEC), and the equatorial (eSEC). The Brazil Current originates from the sSEC, has several associated eddies, and flows to the region of the Subtropical Convergence (~35°S), where it breaks into two branches: one turning north, forming a recirculation cell by the Brazil Current Front, and the second continuing southward, associating with the South Atlantic subtropical gyre (Cirano *et al.* 2006) (Fig. 1). The intensity and bifurcation of the South-Equatorial Current could have an effect in the population structure of marine species living in this area, acting as a barrier to gene flow between populations of either side of this current. For example, genetic heterogeneity between populations north and south of the South-Equatorial Current has been observed in *Micropogonias furnieri* and *Holacanthus ciliares* (Puchnick-Legat and Levy 2006; Affonso and Galetti Jr 2007). Moreover, the presence of eddies and fronts could cause larval retention, affecting the dispersal potential of the species, as was observed in *Panulirus delagoae* on the south-eastern coast of Africa (Gopal *et al.* 2006).

To date, no studies have been performed on the population genetic structure of *Scyllarides brasiliensis*, one of the most commercially important slipper lobster species in South America. The aim of our study was to estimate the population structure and demographic history of *S. brasiliensis* along 2700 km of the south-west Atlantic, including samples from both sides of the South-Equatorial Current. For that, we used two mitochondrial markers: the cytochrome c oxidase I gene and the control region, which have been shown to be useful for population genetics studies in crustaceans.

Table 1. Studies of genetic structure of lobsters

Species	Region	Distance (km)[A]	Marker	Structure	Reference
Jasus edwardsii	Southern Australia and New Zealand	4600	mtDNA RFLP	No	Ovenden *et al.* (1992)
Jasus lalandii	South and west coast of southern Africa	2300	16S	Shallow[B]	Matthee *et al.* (2007)
Jasus tristani	South-east Atlantic Ocean	2000	COII	Shallow[B]	von der Heyden *et al.* (2007)
Jasus verreauxi	East coast of Australia and New Zealand	3000	mtDNA RFLP	Yes	Brasher *et al.* (1992)
Palinurus delagoae	South-eastern coast of Africa	1000	CR	Shallow[B]	Gopal *et al.* (2006)
Palinurus elephas	Eastern Atlantic Ocean	4800	COI	Shallow[B]	Palero *et al.* (2008)
	and Mediterranean Sea	5000	CR/STRS	Shallow[B]	Babbucci *et al.* (2010)
Palinurus gilchristi	Southern coast of South Africa	800	CR	No	Tolley *et al.* (2005)
Panulirus argus	Caribbean Sea	7000	Allozymes	No	Silberman *et al.* (1994)
	Northern Caribbean Sea	1500	COI/CR	No	Naro-Maciel *et al.* (2011)
Panulirus cygnus	Western Australia	700	Allozymes	No	Thompson *et al.* (1996)
	Western Australia	960	12S/STRS	No	Kennington *et al.* (2013)
Panulirus inflatus	Pacific coast of Mexico	2300	CR/12S/16S	No	García-Rodríguez and Perez-Enriquez (2008)
Panulirus interruptus	Western coast of the Baja	1300	Allozymes	Yes	Perez-Enriquez *et al.* (2001)
	California Peninsula	1300	mtDNA RFLP	No	García-Rodríguez and Perez-Enriquez (2006)
Panulirus japonicus	Eastern coast of Japan	2200	COI	No	Inoue *et al.* (2007)
Scyllarides latus	North-east Atlantic Ocean and western Mediterranean Sea	3300	STRS	No	Faria *et al.* (2013)

[A]In most studies, the distances were not indicated in the original papers, so they were estimated with the help of Google Earth.
[B]The shallow structure found in these species was indicated by the low, but significant, values of pairwise Φ_{ST} or F_{ST}. *Jasus lalandii*, Φ_{ST}: 0.056–0.084 ($P < 0.05$); *J. tristani*, Φ_{ST}: 0.09–0.13 ($P < 0.05$); *Palinurus delagoe*, Φ_{ST}: 0.023–0.033 ($P < 0.05$); *P. elephas* $\Phi_{ST} = 0.014$ ($P < 0.05$) for CR, $F_{ST} = 0.0089$ ($P < 0.05$) for STRS.

Materials and methods

Sampling

Samples of *Scyllarides brasiliensis* were collected at four sites along 2700 km of the Brazilian coast: Ceará (03°43′S, 38°32′W; $N = 34$), Rio Grande do Norte (05°47′S, 35°12′W; $N = 21$), Bahia (17°31′S, 39°11′W; $N = 75$) and Espírito Santo (21°02′S, 40°49′W; $N = 73$) (Fig. 1). Slipper lobsters were identified morphologically (Holthuis 1991) and one pereiopod or muscle tissue from each lobster was removed and preserved in absolute ethanol.

DNA extraction, amplification and sequencing

Total genomic DNA was obtained by salt extraction (Lysis buffer: 50 mM TRIS-HCl, 50 mM EDTA, 1% SDS, 50 mM NaCl; Proteinase K 20 mg mL^{-1}), followed by precipitation in isopropanol (modified from Miller *et al.* 1988). The 5′ end of the cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). The control region (CR) of the mtDNA genome was amplified using the specific internal primers RCSBE_F (ATA GCA AGA ATC AAA CTT AT) and RCSBE_R (TCA GGC ATC ATA TTT ATC). These primers were developed from sequences initially obtained using the primers CRLF and CRLR that were originally designed to amplify the CR of *Panulirus argus* (Diniz *et al.* 2005). Amplification reactions included ~10–50 ng of genomic DNA, 1 U of GoTaq Flexi DNA polymerase (Promega), 3 μL of Green GoTaq Flexi Buffer (5X), 0.2 mM of dNTPs, 2.5 mM of $MgCl_2$, 0.3 μM of each primer and 4 μg of Bovine Serum Albumin (BSA) in a final volume of 15 μL. Reactions were carried out with an initial denaturation step of 2 min at 95°C, followed by 35 cycles consisting of a denaturation step of 1 min at 92°C, an annealing step of 40 s at 46°C (LCO1490/HCO2198 and RCSBE_F/RCSBE_R), or at 40°C (CRLF/CRLR), and an extension step of 50 s at 72°C; and a final extension step of 5 min at 72°C. Forward and reverse sequences of purified PCR products were obtained using an ABI 3500 automated DNA sequencer (Applied Biosystems) (annealing temperature 64°C). Preliminary analyses indicated coamplification of multiple sequences when using universal primers for COI. This prompted us to design specific sequencing primers COISB_F (GAA CTA GGA CAG CCT GGG AGG TTG A) and COISB_R (GAG GTG TTG AGA TTA CGG TCA GT). CR sequences were obtained using the same primer pairs used for PCR amplification.

Intraspecific variability and population genetic differentiation

The sequences obtained for each gene were edited in SEQMAN II 4.0 (DNAstar Inc.). Due to the different mutation rates

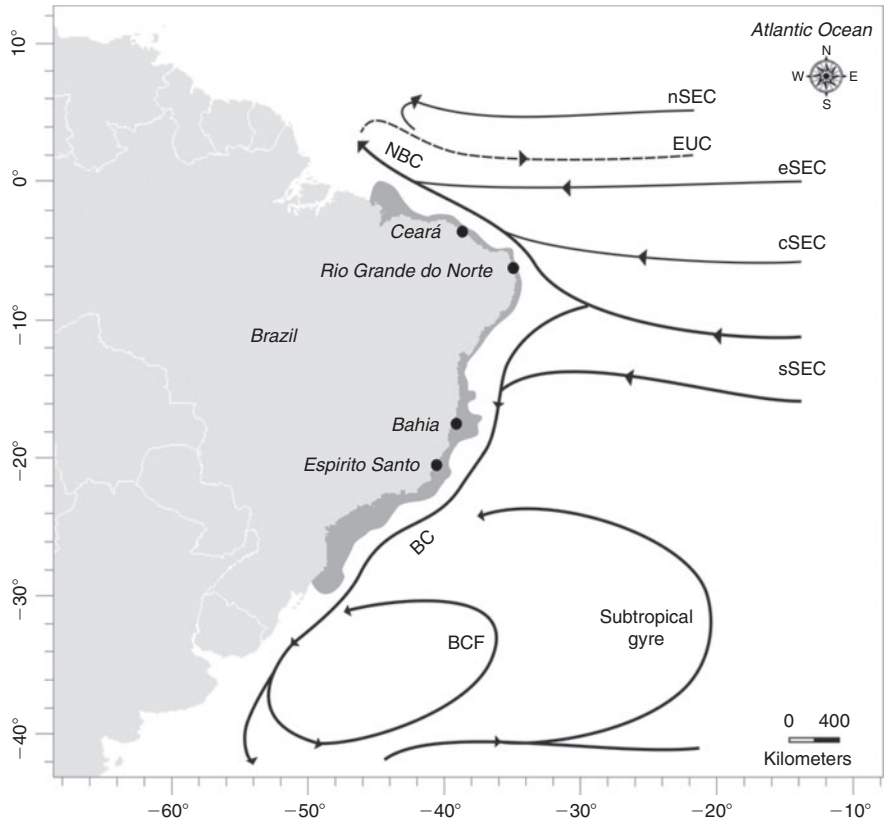

Fig. 1. Geographic distribution (dark grey area) and sampling sites (black dots) for *Scyllarides brasiliensis*. Black arrows indicate the main surface circulation pattern in the western south Atlantic. Shown are the Northern Brazilian Current (NBC); the Equatorial Undercurrent (EUC); the South-Equatorial Current (SEC), with the northern (nSEC), equatorial (eSEC), central (cSEC), and southern (sSEC) branches; the Brazil Current (BC); the Brazil Current Front (BCF), and the South Atlantic subtropical gyre (after Peterson and Stramma 1991; Stramma and England 1999).

between the COI gene and the CR, analyses were carried out separately for each region. Sequences were aligned using the CLUSTALW (Thompson *et al.* 1994) algorithm implemented in MEGA 5.0 (Tamura *et al.* 2011) and checked manually for misalignments. Gaps were not observed in the COI sequences. For CR analysis, each gap region in the alignment was conservatively replaced, without violating assumptions of independence of characters, by a single transition, regardless of gap size. All haplotype sequences were deposited in GenBank (Accession Numbers COI: JX896692 to JX896755; CR: JX896756 to JX896954).

Standard genetic diversity indices such as nucleotide (π) and haplotype (h) diversities and their variances were estimated for each locality using DNASP 5 (Librado and Rozas 2009). The number of haplotypes, polymorphic sites, transitions, and transversions were obtained using ARLEQUIN 3.11 (Excoffier *et al.* 2005). Pairwise genetic divergences between populations were estimated using F_{ST} statistics, and population structure was examined through an analysis of molecular variance (AMOVA) using ARLEQUIN. The statistical significance of estimates was assessed by 10 000 permutations. Sequential Bonferroni (Rice 1989) and False Discovery Rate (Benjamini and Hochberg 1995) corrections were used to adjust significance levels to account for multiple simultaneous tests. False Discovery Rate estimates were performed using the Excel spread sheet designed by Pike (2011). The genealogical relationships among haplotypes were assessed through a parsimony haplotype network constructed using a median-joining algorithm as implemented in the software NETWORK 4.5.1.6 (Bandelt *et al.* 1999). Due to the high diversity observed in the control region of this species (see Results), the network for that region was built by considering only the transversions. This approach aims to minimise the effect of homoplasies due to mutational saturation of transition sites, and has been used successfully in previous studies (Bowen and Grant 1997; Ball *et al.* 2007). The network for the COI gene was built by taking both types of nucleotide substitutions into account.

Neutrality tests and demographic analyses

Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997), as implemented in ARLEQUIN, were used to test for mutation-drift equilibrium deviation in the overall sample. Most variation at the molecular level of neutral or nearly neutral sequences is the result of the equilibrium between genetic drift and mutation. When a large number of low-frequency haplotypes accumulate in a population, deviation from neutrality can be explained as resulting from selection or from demographic changes (Fu and Li 1993; Tajima 1996). Thus, putative temporal changes in population size of the overall sample were investigated through R_2 tests (Ramos-Onsins and Rozas 2002), Bayesian Skyline Plots (Drummond *et al.* 2005) and mismatch distribution analyses (Rogers and Harpending 1992). R_2 statistics and their confidence intervals were estimated by coalescence simulations using DNASP.

Bayesian Skyline Analyses were done in BEAST 1.6.2 (Drummond and Rambaut 2007). The posterior distributions of gene genealogies and population parameters were used to generate credibility intervals [highest posterior densities (HPD)] that represent both phylogenetic and coalescent uncertainty (Drummond *et al.* 2005). The nucleotide substitution models were identified through the Bayesian Information Criterion implemented in MEGA. For the COI gene, the HKY85 model and a substitution rate of 2.3% million year^{-1} (Schubart *et al.* 1998) were employed. For the CR, the substitution GTR+G+I model, with a substitution rate of 19% Myear^{-1} (McMillen-Jackson and Bert 2003), was used. The analyses assumed a strict molecular clock model, and the number of groups was set to six. For each marker, three independent MCMC analyses of 100 million generations were run by sampling every 1000th generation, with the first 10% of each run discarded as burn-in. The analyses for each gene were combined using LOGCOMBINER 1.6.2 (distributed with BEAST) and the results were summarised in piecewise-constant Bayesian Skyline Plots using TRACER 1.5 (Rambaut and Drummond 2007).

The parameters and approximate confidence intervals for the mismatch distributions were estimated using the parametric bootstrap approach implemented in ARLEQUIN under models of demographic and spatial expansion. Population growth has a strong effect on the frequency distribution of pairwise differences among all haplotypes (Rogers and Harpending 1992). Thus, populations that are stable or that experienced a sudden reduction in size present multimodal mismatch distributions, whereas the distribution appears unimodal (approximately Poisson) in populations that have passed through recent demographic or spatial expansions (possibly following a bottleneck) (Rogers and Harpending 1992; Bunje and Wirth 2008). The validity of the expansion models was evaluated using the sum of square deviations (SSD) between the observed and expected mismatch distributions and by the raggedness index (r) (Harpending 1994).

Finally, time since expansion started (t), was estimated as $t = \tau/2u$ (Rogers and Harpending 1992), where τ is the age of the demographic change in mutational units estimated in the mismatch distribution, and u is the mutation rate for the whole haplotype (Harpending 1994). The value of u was calculated as $u = 2\mu k$, where μ is the mutation rate per nucleotide and k is the length of the sequence.

Results

Intraspecific variability and population genetic differentiation

After alignment, a segment of 537 bp of the COI gene was obtained from each of the 202 *S. brasiliensis* specimens analysed. Fifty-three sites were polymorphic (56 substitutions, 43 of which were transitions). Three of these yielded non-synonymous changes. The percentage of A+T found (57.8%) was similar to that reported for lobsters of the genera *Jasus* (59.5%) and *Panulirus* (55–57%) (Ovenden *et al.* 1997; Tourinho *et al.* 2012). Of the 64 haplotypes identified, 50 were private (unique to a single locality). Seven of the 14 shared haplotypes showed a wide geographical distribution. The most common haplotype was found in 78 (38.6%) individuals from all localities. The other haplotypes differed little from the common one, resulting in a low nucleotide diversity (overall $\pi = 0.004$; range = 0.001–0.004) and moderate to high haplotype diversity (overall $h = 0.840$; range = 0.429–0.923) (Table 2).

For the control region, an aligned segment of 739 bp was obtained from each of the 201 *S. brasiliensis* individuals

Table 2. Genetic variability in *Scyllarides brasiliensis*
N, no. of specimens; N_H, no. of observed haplotypes; *h*, haplotype diversity; π, nucleotide diversity

Sampling site	Cytochrome oxidase I				Control region			
	N	N_H	*h* (s.d.)	π (s.d.)	*N*	N_H	*h* (s.d.)	π (s.d.)
Ceará (CE)	34	21	0.923 (0.037)	0.004 (0.0005)	34	34	1.000 (0.007)	0.028 (0.0009)
Rio Grande do Norte (RN)	21	6	0.429 (0.134)	0.001 (0.0004)	19	17	0.988 (0.021)	0.026 (0.001)
Bahia (BA)	75	28	0.849 (0.038)	0.004 (0.0004)	75	75	1.000 (0.002)	0.026 (0.0007)
Espírito Santo (ES)	72	33	0.870 (0.036)	0.004 (0.0004)	73	73	1.000 (0.002)	0.026 (0.0008)
All samples	202	64	0.840 (0.025)	0.004 (0.0002)	201	199	0.999 (0.0005)	0.026 (0.0004)

Table 3. Pairwise genetic distances (F_{ST}) between localities
Upper diagonal: COI gene; lower diagonal: control region. Numbers in parentheses are *P*-values. No F_{ST} value was significant ($P > 0.05$) after sequential Bonferroni and False Discovery Rate corrections. CE, Ceará; RN, Rio Grande do Norte; BA, Bahia; ES, Espírito Santo

	CE	RN	BA	ES
CE	–	0.010 (0.226)	0 (0.599)	0 (0.719)
RN	0.028 (0.011)	–	0.005 (0.307)	0.008 (0.199)
BA	0.001 (0.329)	0.020 (0.017)	–	0.001 (0.329)
ES	0 (0.740)	0.013 (0.044)	0 (0.742)	–

analysed. The alignment revealed 260 (35.4%) polymorphic sites with 315 substitutions, 247 of which were transitions. The percentage of A+T found (70.5%) was similar to that reported for the same region in lobsters of the genera *Panulirus* (73.2%) and *Palinurus* (75.4%) (Diniz *et al.* 2005; Babbucci *et al.* 2010). Levels of sequence variation (overall $h = 0.999$; range = 0.988–1.000), and nucleotide diversities (overall $\pi = 0.026$; range = 0.026–0.028) were very high (Table 2).

No population differentiation was observed using any of the analytical methods with either marker. The overall Φ_{ST} (COI: 0.0004; CR: 0.004) and the pairwise F_{ST} values (COI: 0–0.010; CR: 0–0.028) were low and not significant after sequential Bonferroni and False Discovery Rate corrections ($P > 0.05$) (Table 3). Similarly, the analyses of molecular variance showed that nearly 100% of the variation occurred within localities in all scenarios of population structure simulated (COI: $\Phi_{CT} = 0.001$–0.005, $P > 0.05$; CR: $\Phi_{CT} = 0.001$–0.029, $P > 0.05$). Finally, the parsimony median-joining networks were also consistent with very little population differentiation among groups of the four geographic areas. The networks showed a star-like shape, in which most of the unique haplotypes were closely related to the common central haplotype (Fig. 2).

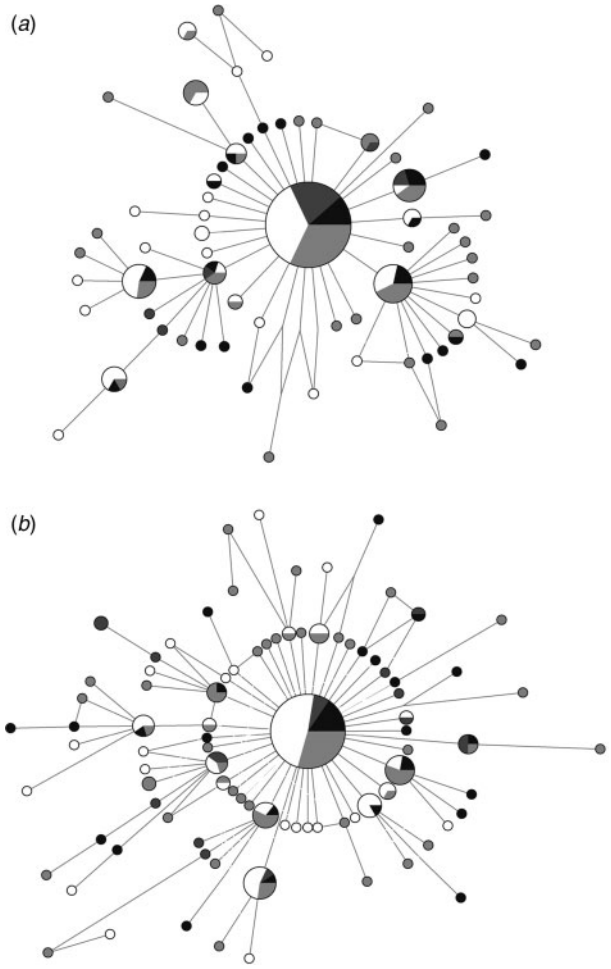

Fig. 2. Parsimony median-joining networks for the (*a*) COI gene and (*b*) control region of *Scyllarides brasiliensis*. The network of control region sequences was constructed using only the transversions. Sizes of the circles are proportional to the frequency of each haplotype. Line lengths are relative to the number of mutations between haplotypes (shortest lines = 1 mutation). Black circles correspond to Ceará samples; dark grey to Rio Grande do Norte; white to Bahia; and light grey to Espírito Santo.

Neutrality tests and demographic inferences

The Tajima's D (COI: −2.334, $P < 0.001$; CR: −1.770, $P < 0.005$) and Fu's Fs (COI: −27.315, $P < 0.001$; CR: −23.720, $P < 0.001$) tests indicate a significant deviation from neutrality for both markers in the overall sample. The deviation is related to a large number of rare haplotypes and can be explained by either selection or demographic factors. Considering the likely neutrality of the variation in the CR and in COI (only three amino acid substitutions), the significant negative values of D and Fs observed in *S. brasiliensis* are more probably due to population expansion (Aris-Brosou and Excoffier 1996). Significant values were obtained for the R_2 statistics (COI: 0.082, $P < 0.001$; CR: 0.161, $P < 0.001$). The

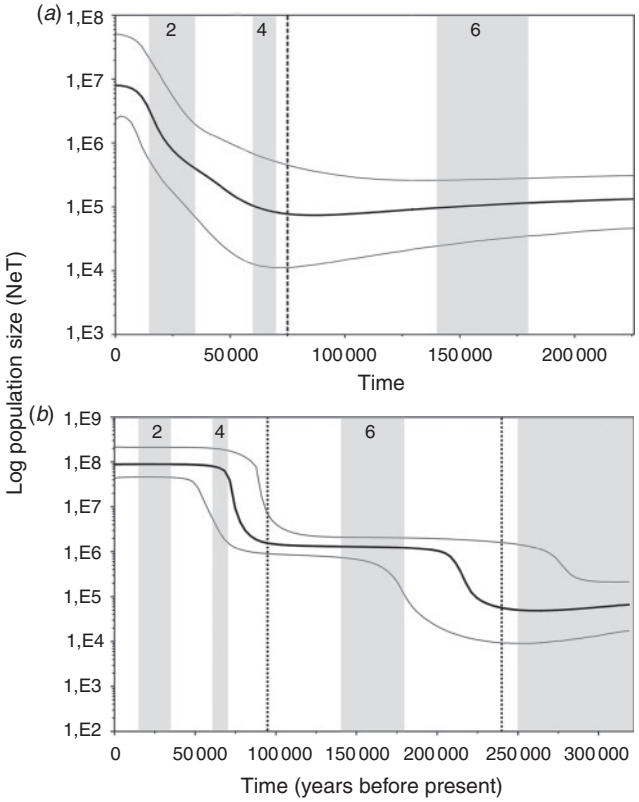

Fig. 3. Bayesian Skyline Plots for the (*a*) COI gene and (*b*) control region of *Scyllarides brasiliensis*. The maximum time is the upper 95% HPD of the root height. The median estimate (black solid line) and 95% HPD limits (grey solid line) are indicated. Dotted lines correspond to the approximate onset of the expansion events. The main glaciations over the last 250 000 years are shaded in grey: MIS6 (180–140 000 years ago), MIS4 (70–60 000 years ago) and MIS2 (35–15 000 years ago) (Gibbard and Cohen 2009).

Bayesian Skyline Plots detected a clear increase in effective population size (Fig. 3). Similarly, the mismatch distributions were compatible with models of demographic and spatial expansion (P(SSD) and P(r) >0.05 for COI and CR under both models) (Fig. 4).

The age of the demographic expansion (τ) in mutational units estimated in the mismatch distributions was 2.178 (95% CI = 1.123–3.076) for the COI gene and 20.084 (95% CI = 18.770–20.305) for the control region. For spatial expansion, τ was 2.071 (95% CI = 1.924–2.841) for the COI gene and 20.085 (95% CI = 17.850–20.252) for the control region. The demographic and spatial expansions started at ~88 000 (95% CI = 45 000–124 000) years ago for the COI gene, similar to 71 000 (95% CI = 66 000–72 000) years ago estimated for the control region. These estimates were supported by the Bayesian Skyline Plots, which indicate that the overall population has experienced a period of relatively constant population size, followed by an evident expansion that started ~75 000 years ago (95% HPD = 65 000–100 000) for the COI gene and 95 000 years ago (95% HPD = 85 000–115 000) for the control region (Fig. 3). Furthermore, an older expansion event around 240 000 years ago (95% HPD = 230 000–290 000), was also hypothesised for the control region (Fig. 3).

Discussion

This study is the first to investigate the genetic population structure of a *Scyllarides* species in the west Atlantic. *S. brasiliensis* presented very high levels of genetic variation, homogeneously distributed along 2700 km of the South American coast. Bayesian reconstructions of historical demography revealed two events of population expansion: an older one, which started ~240 000 years ago, and a younger expansion that started ~95–70 000 years ago.

Molecular diversity and population genetic differentiation

The high genetic variability observed in *S. brasiliensis* (COI: $h = 0.840$, $\pi = 0.004$; CR: $h = 0.999$, $\pi = 0.026$) seems to be typical of decapods, since similar results have been reported for lobsters and shrimp using the same mitochondrial markers (e.g. McMillen-Jackson and Bert 2004; Naro-Maciel *et al.* 2011).

Analyses of the two mitochondrial markers did not reveal any significant genetic structure for *S. brasiliensis* along the South American coast, suggesting extensive gene flow among localities. The lack of differentiation between populations of *S. brasiliensis* could be explained on the basis of biological traits that determine the dispersal potential. Population homogeneity is generally expected in marine species with high fecundities and long planktonic phases (Palumbi 2003; Cowen and Sponaugle 2009). In the south-west Atlantic slipper lobster, *Scyllarides deceptor*, adult females can spawn up to ~190 000 eggs (Oliveira *et al.* 2008), and in some *Scyllarides* species the phyllosome larvae can live in the plankton for up to 9 months (Booth *et al.* 2005). Panmixia has recently been reported for the slipper lobster *Scyllarides latus* across 3300 km in the north-eastern Atlantic and Mediterranean Sea (Faria *et al.* 2013). Likewise, high levels of genetic homogeneity have been observed between geographically distant populations of other lobster species. In *Panulirus japonicus*, for example, no significant genetic differentiation was found (COI sequences) across ~2200 km of the eastern coast of Japan (Inoue *et al.* 2007). Similarly, no significant differences were observed for the control region of mtDNA among populations of *Panulirus inflatus* along over 2000 km of the Pacific coast of Mexico (García-Rodríguez and Perez-Enriquez 2008) or among *Palinurus gilchristi* populations along ~800 km of the southern coast of South Africa (Tolley *et al.* 2005). Other examples of genetic homogeneity in lobster populations can be found in the literature (Table 1). In most cases, ocean currents and the long larval phase are considered the primary bases for the similarities, and panmixia has been partially attributed to mixing during the planktonic phase (e.g. Tolley *et al.* 2005; García-Rodríguez and Perez-Enriquez 2006).

Similarly high levels of population homogeneity have also been reported in several fish species and invertebrates with planktonic larval phases along the south-west Atlantic coast (e.g. with *Lutjanus purpureus*: Gomes *et al.* 2012; *Scomberomorus cavalla*: Santa-Brígida *et al.* 2007; *Ocyurus chrysurus*: Vasconcellos *et al.* 2008; *Farfantepenaeus brasiliensis* and *Litopenaeus schmitti*: Gusmão *et al.* 2005; *Ucides cordatus*: Oliveira-Neto *et al.* 2007; *Cardisoma guanhumi*: Oliveira-Neto *et al.* 2008).

The lack of population structuring observed in *S. brasiliensis* contrasts with the restricted gene flow reported for

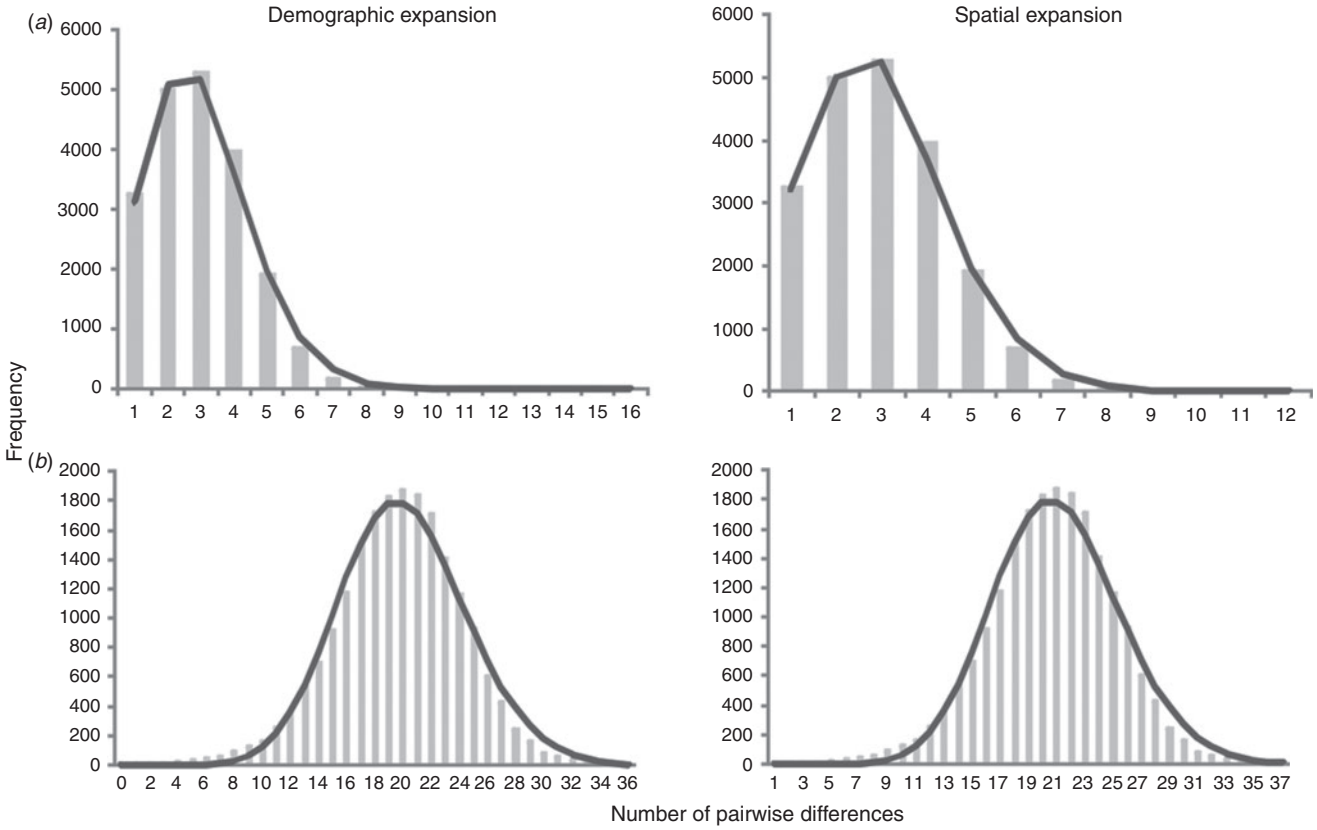

Fig. 4. Mismatch distribution for the (*a*) COI gene and (*b*) control region of *Scyllarides brasiliensis* under the models of demographic and spatial expansion. Grey bars show the observed frequency distribution for the number of pairwise differences among all individuals sampled. The solid lines show the expected distribution under each population expansion model.

Micropogonias furnieri and *Holacanthus ciliares* from the same area (Puchnick-Legat and Levy 2006; Affonso and Galetti Jr 2007), indicating that the bifurcation of the South-Equatorial Current can have different effects on the genetic structure of marine species, not acting as a barrier to gene flow between localities for *S. brasiliensis* (Fig. 1). This different phylogeographic pattern could be explained by ecological and biological features of the species. For example, the eggs and larvae of *Micropogonias furnieri* are subject to estuarine retention, decreasing their dispersal potential (Puchnick-Legat and Levy 2006). The presence of eddies and fronts in the south-west Atlantic also seems not to affect the retention of *S. brasiliensis* larvae, as observed in *Palinurus elephas* and *P. delagoae* (Gopal *et al.* 2006; Palero *et al.* 2008; Babbucci *et al.* 2010). Climate phenomena and oceanographic processes that vary seasonally and yearly are clearly important for larval drift, and partly explain the genetic homogenisation of lobster populations (García-Rodríguez and Perez-Enriquez 2008). For example, the intensification of mesoscale ocean processes associated with 'El Niño' events seems to promote larval transport and, hence, connectivity between lobster populations in the tropical Atlantic (Rudorff *et al.* 2009). Larval dispersal patterns and larval development for many lobster species, including *S. brasiliensis*, remain poorly understood, but the circulation and the oceanographic processes in the southern Atlantic coupled with the long larval period of slipper lobsters could be an important mechanism for dispersal and larval mixing, allowing gene flow among *S. brasiliensis* localities along the South American coast.

Demographic history

The lack of population structuring observed in *S. brasiliensis* could be a result of contemporary gene flow, but it may also reflect historical processes. The star-like haplotype networks obtained (Fig. 2) indicate that a population expansion occurred during the recent history of the species, where the common and widespread haplotype is probably the ancestral condition from which the rare haplotypes were recently derived by point mutations (Slatkin and Hudsont 1991). This result has also been found for other lobsters (e.g. Gopal *et al.* 2006; García-Rodríguez and Perez-Enriquez 2008; Babbucci *et al.* 2010).

During the Pleistocene (~2.6 million to 10 000 years ago), climatic fluctuations of the glacial–interglacial cycles (Imbrie *et al.* 1992) influenced the demographic history and distribution of several marine species (e.g. Lavery *et al.* 1996; Maggs *et al.* 2008; Babbucci *et al.* 2010; Fernández *et al.* 2011). These climatic oscillations produced changes in sea levels, temperatures, current patterns, upwelling intensity, and coastal habitats (Rohling *et al.* 1998; Lambeck *et al.* 2002). The advance and retreat of the ice sheets through multiple glacial cycles, the most recent of which was the last glacial maximum (LGM) ~23 000–18 000 years ago, had a major impact on the present-day species distribution (Provan and Bennett 2008).

The mismatch analysis suggests that both demographic and spatial expansion of *S. brasiliensis* occurred at ~95 000–70 000 years ago during an interglacial episode of Late Pleistocene related to the marine isotope Stage (MIS) 5 (Selivanov 1992; Gibbard and Cohen 2009). Additionally, the Bayesian Skyline Plot for the control region detected that this population appears to have experienced an older demographic expansion followed by a longer period of relatively constant population size (Fig. 3). The onset of this older expansion event occurred ~240 000 years ago during an interglacial episode in the Middle Pleistocene related to MIS 7 (Selivanov 1992; Gibbard and Cohen 2009). The more recent main glaciations (MIS 2, 4 and 6) appear to have had little impact on population size, given that the population showed nearly constant growth or continuous exponential growth during those periods (Fig. 3). Moreover, given its long population history, pre-LGM coalescence times indicate that *S. brasiliensis* was not affected by LGM. This trend was also observed in some species of the Mollusca, Arthropoda, Echinodermata and Chordata (Cárdenas *et al.* 2009; Marko *et al.* 2010; Ibañez *et al.* 2012). Due to the uncertainty in mutation rates and previous calculation errors in applying the formula of Rogers and Harpending ($t = \tau/2u$) (Schenekar and Weiss 2011), it is difficult to compare the date of demographic events between lobster studies. However, it is feasible that the demographic expansions for *S. brasiliensis* and *Panulirus argus*, both distributed in the west Atlantic, occurred probably during the same period, and that expansions of *Palinurus elephas*, *P. delagoe*, *P. elephas*, *P. gilchristi*, and *Panulirus inflatus* were more recent, since the τ value reported for *P. argus* was similar to that found for *S. brasiliensis*, but larger than those reported for other species (Table 4).

Palaeontological and palynological records, and a large body of biogeographical data, suggest that most of the biota, particularly temperate species, persisted through glacial periods in lower-latitude refugia where climatic conditions were less extreme. They then recolonised other areas when the climate became warmer (Hewitt 1999; Provan and Bennett 2008). Populations inside the refugia thus have a longer demographic history with higher levels of genetic diversity than populations in areas that were recolonised from those refugia (Maggs *et al.* 2008; Provan and Bennett 2008). This scenario of 'recolonisation from different refugial areas' is not apparent for *S. brasiliensis* given the genetic homogeneity observed along the South America coast.

The similarity between the times estimated for expansion events (demographic and spatial expansion) and the genetic homogeneity suggests that demographic expansion may have occurred simultaneously along the complete range of distribution and was thus associated with a geographical (spatial) expansion. A similar demographic pattern was also observed in the gastropod *Concholepas concholepas* (Cárdenas *et al.* 2009) and in the fish *Larimichthys polyactis* (Wu *et al.* 2012). The long-distance dispersal during expansion helps preserve high levels of diversity (Fayard *et al.* 2009). It is possible that the high dispersal potential of planktonic larvae helps this preservation. The patterns that shape the genetic structure of species are complex; nevertheless, the relationship between expansion events and interglacial periods during the late and middle Pleistocene suggests that this species is able to increase its distribution and population size during periods of suitable climate ('habitat tracking': Provan and Bennett 2008). The lack of population structure, coupled with recent population expansion, has been observed in several marine crustaceans (e.g. McMillen-Jackson and Bert 2003, 2004; García-Rodríguez and Perez-Enriquez 2008). Nonetheless, further studies of comparative phylogeography and demography are necessary to clarify how the glacial–interglacial cycles during the Pleistocene influenced the demographic history of species in the south-west Atlantic.

The high levels of genetic variation observed indicate that the recent strong exploitation of *S. brasiliensis* has not had time to produce any detectable effects on the genetic make-up of its population. Furthermore, the genetic homogeneity observed along 2700 km of south-west Atlantic coast suggests extensive gene flow promoted by the high dispersal potential of its larvae. This discovery has important implications for the conservation of this slipper lobster, since it indicates that the whole Brazilian coast could be treated as a single management unit for fishery purposes.

Table 4. Demographic parameters for lobster species
CI, confidence interval of τ (tau)

Species	Region	Marker	τ (tau)	CI		Reference
Palinurus elephas	Eastern Atlantic Ocean and Mediterranean Sea	COI	0.807	–	–	Palero *et al.* (2008)
Panulirus argus	Northern Caribbean Sea, Lineage 1	COI	3.047	2.660	3.459	Naro-Maciel *et al.* (2011)
	Northern Caribbean Sea, Lineage 2	COI	4.400	1.770	6.611	
Scyllarides brasiliensis	South-west Atlantic	COI	2.178	1.123	3.076	This study
Palinurus delagoae	Southern coast	CR	3.490	1.440	9.960	Gopal *et al.* (2006)
	Northern coast	CR	4.200	2.100	7.520	
	Walters Shoals	CR	2.990	1.360	3.860	
Palinurus elephas	Eastern Atlantic Ocean	CR	4.880	1.968	9.100	Babbucci *et al.* (2010)
	Mediterranean Sea	CR	6.015	3.094	9.238	
Palinurus gilchristi	Southern coast of South Africa	CR	1.700	0.956	1.991	Tolley *et al.* (2005)
Panulirus inflatus	Pacific coast of Mexico	CR	8.367–11.166	–	–	García-Rodríguez and Perez-Enriquez (2008)
Scyllarides brasiliensis	South-west Atlantic	CR	20.084	18.770	20.305	This study

Acknowledgements

The authors thank C. Schrago by invaluable support during the first stages in the Bayesian Skyline Analysis, B. A. Rodríguez for the supporting calculations in the GFAM computer cluster and D. Almeida, F. Azevedo, E. Araujo, L. Barbosa, P. Coelho Filho, P. Cruz, H. Cunha, M.C.O. Matos, N. Leite Jr., S. Lima, F. Magalhães, R. Monteiro, P. Paiva, A. Reis, S. Ribeiro, E. Rios, and E. Routledge for help with sample collections. The authors thank the two anonymous reviewers for providing valuable comments and suggestions. Financial support for this work was provided by Brazilian Ministries for Fisheries and Aquaculture (MPA) and Science, Technology and Innovation (MCTI), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). This work is part of the thesis in Genetics of the first author at the Federal University of Rio de Janeiro (UFRJ).

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