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Population structure of *Litopenaeus schmitti* (Decapoda: Penaeidae) from the Brazilian coast identified using six polymorphic microsatellite loci

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Abstract

The population structure of the only *Litopenaeus* species occurring in Brazilian waters, the white shrimp *L. schmitti*, was surveyed by screening six microsatellite loci. High diversity (H_E = 0.863; average number of alleles per locus = 37.8) was found across eight geographical locations (2°S to 27°S). Estimates of overall F_{ST} (0.0060) were low but significantly different from zero ($P < 0.05$). F_{ST} pairwise estimates and AMOVA revealed a significant discontinuity around a major biogeographical boundary, near Cabo Frio, at 23°S. This separation may have been caused either by historical or on-going hydrogeographical and/or selective factors.

Keywords: Brazil, *L. setiferus*, *Penaeus*, prawn, shrimp

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Introduction

The natural populations of penaeid shrimp from the South American coast are under pressure from an intensive fishery and the introduction of cultured exotic species. Among them, the only *Litopenaeus* species occurring in Brazilian waters, the white shrimp *Litopenaeus schmitti*, may be the first to suffer consequences from the quickly spreading culture of *L. vannamei* (Paquotte *et al*. 1998; Souza 2001), because of their morphological and ecological similarity. *L. vannamei* is an eastern Pacific species of white shrimp, widely cultured in Central and South America (FAO 1997), because of its production efficiency in such systems. *L. schmitti* and *L. vannamei* are morphologically very similar, and probably have a similar ecological niche. Hybrids between *Litopenaeus* species have been successfully produced by artificial insemination (Bray *et al*. 1990), demonstrating that interspecific reproduction, however unlikely in natural conditions, is possible in this genus. Therefore, the potential for ecological interactions among the native *L. schmitti* and the exotic *L. vannamei* is high. In addition, catches of *Penaeus monodon* (from previous culture attempts) and *L. vannamei* off the coast of Brazil have already been recorded. This background highlights the urgent need for further studies that allow identification of the boundaries among the natural populations of penaeid shrimps, and the interaction of these with the oceanographic system.

L. schmitti, is distributed from Cape Catoche (21°N), in Central America, to Laguna (28°S), in southern Brazil, and is a widely exploited fisheries resource throughout all the area (Pérez-Farfante 1969). A number of biogeographical barriers exist along this distribution: the Amazon River is the boundary between the Caribbean and the Brazilian provinces of the tropical region (Floeter & Gasparini 2000), while Cabo Frio (23°S) has been recognized as the limiting boundary between the Tropical and the Warm-Temperate regions (Briggs 1974). A cold-water upwelling is present in this latter area, where the Brazil Current flows off shore under the influence of the prevalent northeastern winds and bottom topography (Rodrigues & Lorenzzetti 2001). Another hydrographic barrier potentially exists at latitudes 10–15°S, where the Southern Equatorial Current meets the geographical barrier of the South American continent, and splits into two branches. One of these branches

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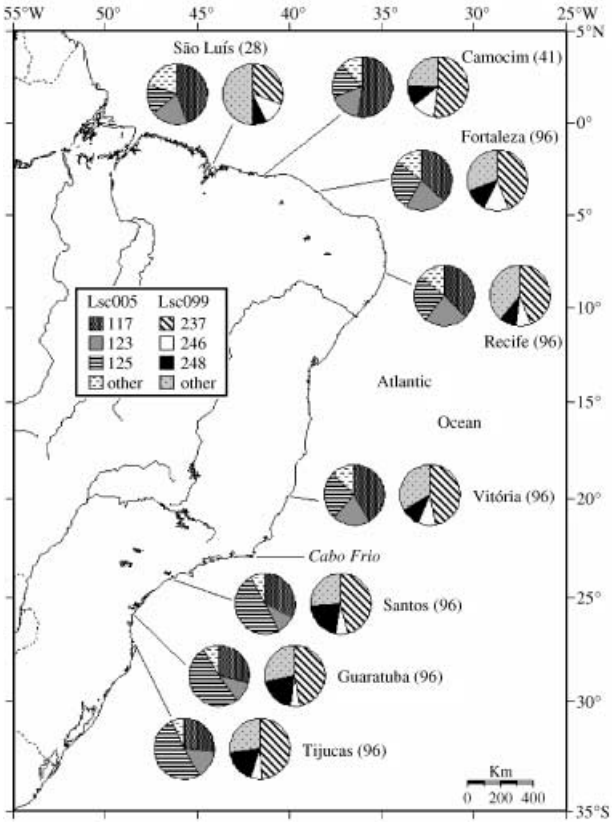

Fig. 1 Location of sampling sites and allele frequencies at loci *Lsc005* and *Lsc099* across the studied area. Names identify each of the eight sampling sites (sample sizes are given in parentheses).

flows north (North Brazil Current), and the second, much weaker current, flows south close to the coastline forming the Brazil Current, which reaches 33–38°S (Peterson & Stramma 1991). The extent to which this split influences the dispersal of adult and larval marine organisms is not known.

The objective of this study was to conduct a survey on the genetic variability and population structuring of the natural populations of the white shrimp *L. schmitti* along most of the Brazilian coast. This was aimed at elucidating the influence of major hydrographic barriers on the genetic structure of this species and to obtain baseline data for fisheries and conservation management. Primer pairs for *L. setiferus* microsatellite loci (Ball *et al*. 1998) were used with success in this species but additional microsatellite markers for *L. schmitti* were also developed.

Materials and methods

White shrimp *Litopenaeus schmitti* were collected or acquired from fishermen at eight points along the Brazilian coast, during July–August 1999 (Fig. 1). After identification, a piece of tail muscle was preserved in absolute ethanol –20 °C, to reduce DNA degradation. DNA was extracted from a 1 mg piece of muscle using either a standard proteinase K–phenol/chloroform-isoamyl alcohol (PCI) protocol, based on Sambrook *et al*. (1989), or a chelating resin extraction protocol, simplified from Estoup *et al*. (1996).

In addition to using the published *L. setiferus* primers (Ball *et al*. 1998), an attempt to develop microsatellite primers for *L. schmitti* was made. The protocol used includes the construction of a genomic library enriched for $(CA)_n$ repeats according Kandpal *et al*. (1994) while the hybridization and detection procedures are based on digoxygenin-labelled probes (as described in Morgan *et al*. 1999). Plasmids were cycle sequenced with the primers T7, SP6 or both, using Perkin–Elmer sequencing kits (#4303149 or #4307175), and then read on an ABI 377 automated sequencer. Two loci were consistently polymorphic, *Lsc005* [repeat motif $(GT)_4T(GT)_{28}$; GenBank Accession no. AY135202] and *Lsc099* [repeat motif $(CA)_2T(AC)_{13}(T)_3(CT)_9$; GenBank Accession no. AY135203]. The primers for *Lsc005* amplified two fragments (see Discussion); the following analysis considers mainly the smaller of these.

Polymerase chain reactions (PCRs) were performed on 0.2 mL 96-well microtitre plates on a Hybaid 'Touch Down' thermocycler. The 10 μL reactions contained 1× Qiagen proprietary buffer [Tris–HCl, KCl $(NH4)_2SO_4$, 1.5 mM $MgCl_2$]; 1× Qiagen 'Q solution'; 200 μM each dNTP; 0.5 μM unlabelled primer; 0.5 μM fluoro-labelled primer; 1 μ Qiagen *Taq* DNA polymerase; 10–50 ng template DNA and chemical water (Sigma). Primer sequences and 5'-labelling were as follows: (Pse primers from Ball *et al*. 1998): *Pse002* (CTGAAATACAACCACTTTGC and HEX-CGGGATTCGTGCTTGAGGG), *Pse004* (NED-GATCACG-TGACTCTGCAAAG and CGTTCAGATTGTCAACTTC-GCG), *Pse017* (6-FAM-GATCTCGCTCATCGCTTCAAGC and TTGTGAAAATCGTAAGCGCTGTC), *Pse028* (HEX-GATCCTTCTAGCTAAATGGG and GATCGAAGGTAA-ACTTTATTATC), *Pse035* (NED-CACGTGAGGGACAA-GAGCATTG and CTTTCATACTCACGCTAACATTTG), *Pse036* (6-FAM-GACTTTGTATTTTCATAAACGCTG and CGCTATATTTCGCAGTAAGGCTAC), *Lsc005* (TGTTATTCCAGATTCCTTGCTC and 6-FAM-GCAGT-GAAACAGAAGCGAAG), s*Lsc099* (AATACGTTGCAA-AGGGGAGA and NED-ACCGCGGCTGTAATCTGTAA). PCR cycling conditions comprised a initial denaturing step of 2 min at 95 °C; followed by five cycles of 30 s at 95 °C, 1 min at 50 °C, 45 s at 72 °C; followed by 25 cycles of 30 s at 94 °C, 1 min at 50 °C, 45 s at 72 °C; followed by a final elongation and dATP tailing step of 30 min at 72 °C. Genotyping was conducted using multiplex electrophoresis on an ABI 377 automated sequencer running GENESCAN 3.1 software (Applied Biosystems). PCR products were diluted 6–28 times before screening on the sequencer. Sample sizes are shown in Fig. 1.

Table 1 Genetic variability and structuring indices across eight *Litopenaeus schmitti* microsatellite loci. *n*, sample size; *A*, number of observed alleles; H_E, estimated heterozygosity; H_O, observed heterozygosity (in parenthesis 95% confidence intervals)

Locus	*n*	Size range	*L. setiferus* size range*	*A*	H_E	H_O	F_{ST}†
Pse002	406	92	88	01	0.000	0.000	—
Pse004	626	143–257	160–210	50	0.933(0.928–0.939)	0.930(0.897–0.956)	−0.00051
Pse017	622	110–272	120–140	70	0.952(0.940–0.956)	0.944(0.925–0.963)	0.00229
Pse028	632	164–246	136–264	34	0.918(0.912–0.926)	0.891(0.854–0.921)	0.00038
Pse035	356	206–426	180–250	—	—	—	—
Pse036	632	111–173	105–161	28	0.916(0.907–0.924)	0.915(0.884–0.930)	0.00057
Lsc005	634	109–133	—	13	0.710(0.652–0.729)	0.662(0.613–0.720)	0.03562
Lsc099	631	205–286	—	32	0.751(0.711–0.799)	0.759(0.689–0.858)	0.00396

*Ball *et al*. (1998).
†Estimated through GDA (Lewis & Zaykin 2001).

Descriptive statistics, Fisher's exact tests for Hardy–Weinberg and linkage disequilibrium, and estimations of Wright's (1951) fixations indexes were performed using GDA (Lewis & Zaykin 2001). Molecular analysis of variance, pairwise F_{ST} estimations and Mantel tests for correlation between a geographical distances and F_{ST} pairwise matrixes were performed using ARLEQUIN 2.000 (Schneider *et al*. 2000). Exact probability tests for gene and genotypic differentiation were conducted using GENEPOP 3.3 (Raymond & Rousset 1995). Neighbour-joining trees were constructed from F_{ST} pairwise estimates using the program NEIGHBOUR from PHYLIP, Version 3.57c (Felsenstein 1995). Trees were visualized and drawn using TREEVIEW (Page 1996). The possibility of recent population size fluctuations was tested using the program BOTTLENECK (Piry *et al*. 1999), which compares the Hardy–Weinberg equilibrium heterozygosity (H_E) and the expected heterozygosity under mutation–drift equilibrium (H_{eq}). Reductions in effective population size cause $H_E > H_{eq}$, because alleles are lost faster than heterozygosity (Cornuet & Luikart 1997).

Results

Of the eight scored loci (Table 1), *Pse002* proved to be monomorphic as recorded by Ball *et al*. (1998) for *Litopenaeus setiferus*, and *Pse035* did not produce interpretable results. The remaining four *L. setiferus* loci and two *L. schmitti* loci showed consistent amplification and were highly polymorphic (Table 1). Exact test analysis with 3200 permutations detected no significant departure from Hardy–Weinberg equilibrium or linkage among loci, after a Bonferroni sequential test (Rice 1989), for $\alpha = 0.05$.

Overall F_{ST} (0.0060) was significantly different from zero (95% CI 0.0004–0.0169, after 10 000 bootstraps). However, values were variable across loci (Table 1). F_{ST} pairwise estimates were always different from zero ($P < 0.001$ from 16 000 permutations) when comparing sites south of 23°S (Tijucas, Guaratuba, Santos) with those north of that latitude (Vitória, Recife, Fortaleza, Camocim, São Luís). Estimates of number of migrants per generation among populations were high, ranging from a few dozen (e.g. Tijucas-Camocim) to infinity (e.g. Vitória-Recife). A neighbour-joining tree obtained from the pairwise estimates of F_{ST} is shown on Fig. 2. Mantel tests among F_{ST} pairwise estimates and a matrix of geographical distances indicated a positive correlation between distance and isolation ($0.01 > P > 0.001$), however, the significance of the test was likely driven by the genetic gap between the southern and northern populations (Fig. 2).

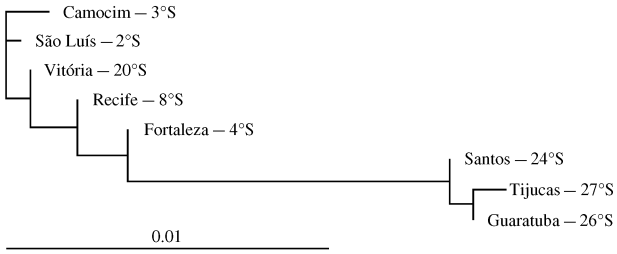

Fig. 2 Unrooted neighbour-joining tree of the sampled populations, based in F_{ST} pairwise estimates.

When considering a north–south structuring in an AMOVA with 16 000 permutations, 98.9% of the variation was found within populations and 0% among populations within groups, whereas 1.1% was attributed to variation between the southern and northern groups. However small, the variation among groups was significant ($0.01 < P < 0.05$). When the loci were considered separately, *Lsc005* was found to be responsible for 77.7% of the total variance among groups, followed by *Lsc099*, which accounts for 9.7%. Figure 1 illustrates the allele frequencies of the most common alleles at these loci across the studied area.

No differences in heterozygosities were observed among the southern (mean $H_E = 0.85$; $s = 0.05$) and northern (mean $H_E = 0.87$; $s = 0.04$) groups. Regarding the comparison between H_E and H_{eq}, no significant heterozygosity excess

was observed for any of the loci, across the eight sample sites, when considering either a two-phase mutation model or a stepwise mutation model. Therefore, the studied data set does not provide evidence of significant size fluctuations in a recent past (Cornuet & Luikart 1997).

Finally, most of the differentiation between the northern and southern population groups can be attributed to locus *Lsc005*. This locus is located in a minisatellite containing two microsatellite regions. One of the primers has two annealing sites in the sequence, therefore the PCR product presents two fragments of distinct size, the bigger containing the smaller. The number of different alleles observed for the small product was 13, whereas for the large product 21 alleles were observed. However homoplasy is very likely, as both microsatellite regions may mutate independently. After combining the information from the large and small products, considering only the 259 individuals homozygous for either product, 37 different alleles were observed. When that subsample was compared using exact probability tests for gene and genotypic homogeneity, the same highly significant differentiation among southern and northern populations was observed.

Discussion

Following a generalized trend in biological sciences, DNA-based markers have been used with increasing frequency, in recent years, to study the diversity of natural populations of penaeid shrimps. This is mainly because these markers tend to show high variability and reproducibility. Benzie (2000) reviewed most of the work on the genetic diversity of penaeid prawns and concluded that estimates of variability from DNA-based markers showed much higher levels of diversity in natural populations than those inferred from allozymes. However, Benzie (2000) also highlighted that the data from DNA-based markers tended to confirm observations based on allozyme data as, for example, with *Penaeus monodon* around Australia (Brooker *et al*. 2000).

The population structure observed in the our study did not appear to be related to the separation of the Southern Equatorial Current at 10–15°S, as Vitória (20°S) seems to be more closely related to the northern group of populations. As can be seen from the map in Fig. 1 this location is much closer to Santos, the nearest southern location, than to Recife, the nearest to the north. However, the differentiation between southern and northern population groups did coincide with the well-known biogeographical boundary at Cabo Frio (23°S). There are three possible explanations for this: (i) the upwelling at 23°S represents a physical barrier to dispersal; (ii) different environmental conditions, associated with localized upwelling excerpt some form of selection on loci closely linked to the microsatellites screened in populations of *Litopenaeus schmitti*; (iii) the observed genetic differentiation results from the existence of past barriers to gene flow. Allozyme work on *Farfantepenaeus brasiliensis* across the same area found some interesting variability, but the overall differences among southern and northern populations were not significant (Gusmão *et al*. 2000).

When populations within the northern and southern groups were tested for isolation by distance, through Mantel tests, no significant values were observed. Put together with a lack of any significant genetic differentiation between northern populations this suggests that the western boundary currents along Brazil may be acting to homogenize population structure along the coast or are having little influence in terms of isolating the local populations. *L. schmitti* can clearly traverse the area in which the Southern Equatorial Current diverges into northern and southern branches, probably through active adult-mediated dispersal.

Work on *P. monodon* population genetics based on microsatellites has found significant genetic differences among populations in Australia (pairwise F_{ST} estimates 0.000–0.026, Brooker *et al*. 2000), Thailand (F_{ST} = 0.001–0.012, Supungul *et al*. 2000) and the Philippines (F_{ST} = –0.001 to 0.013, Xu *et al*. 2001). Recent allozyme data have also revealed *P. monodon* structuring across the Indonesian archipelago (Sugama *et al*. 2002). New molecular data are progressively uncovering high levels of population structuring across relatively short distances for other *Penaeus* species as well (Aubert & Lightner 2000; Garcia-Machado *et al*. 2001). Taken together with results from this study it would seem that population structure in penaeid shrimps may be influenced by environmental factors that occur across relatively small geographical scales and which are associated with coastal waters. These maybe related to larger scale biogeographical barriers recognized in other taxa and which have been suggested as influencing population structure in penaeid shrimps previously (Dall *et al*. 1990; Benzie 2000).

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References

Aubert H, Lightner DV (2000) Identification of genetic populations of the Pacific blue shrimp *Penaeus stylirostris* of the Gulf of California, Mexico. *Marine Biology*, **137**, 875–885.

Ball AO, Leonard S, Chapman RW (1998) Characterization of $(GT)_n$ microsatellites from native white shrimp (*Penaeus setiferus*). *Molecular Ecology*, **7**, 1251–1253.

Benzie JAH (2000) Population genetic structure in penaeid prawns. *Aquaculture Research*, **31**, 95–119.

Bray WA, Lawrence AL, Lester LJ, Smith LL (1990) Hybridization of *Penaeus setiferus* (Linnaeus, 1767) and *Penaeus schmitti* Burkenroad, 1936 (Decapoda). *Journal of Crustacean Biology*, **10**, 278–283.

Briggs JC (1974) *Marine Zoogeography*. McGraw-Hill, New York.

Brooker AL, Benzie JAH, Blair D, Versini JJ (2000) Population structure of the giant prawn *Penaeus monodon* in Australian waters, determined using microsatellite markers. *Marine Biology*, **136**, 149–157.

Cornuet JM, Luikart G (1997) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Dall W, Hill BJ, Rothlisberg PC, Staples DJ (1990) *The Biology of Penaeidae*. *Advances in Marine Biology*, **Vol. 27** (ed. Blaxter JHS, series ed. Southward AJ) p. 489. Academic Press, London.

Estoup A, Largiadèr CR, Perrot E, Chourrout D (1996) Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology*, **5** (4), 295–298.

FAO (1997) Review of the state of world aquaculture. *FAO Fisheries Circular*, *no. 886, Rev 1*. FAO, Rome.

Felsenstein J (1995) PHYLIP *(Phylogeny Inference Package)*, Version 3.57c. Distributed by the author. Available at http://evolution.genetics.washington.edu/phylip.html

Floeter SR, Gasparini JL (2000) The southwestern Atlantic reef fish fauna: composition and zoogeographic patterns. *Journal of Fish Biology*, **56**, 1099–1114.

Garcia-Machado E, Robainas A, Espinosa G *et al*. (2001) Allozyme and mitochondrial DNA variation in Cuban populations of the shrimp *Farfantepenaeus notialis* (Crustacea: Decapoda). *Marine Biology*, **138**, 701–707.

Gusmão JF, Lazoski C, Solé-Cava AM (2000) A new species of *Penaeus* (Crustacea: Penaeidae) revealed by allozyme and cytochrome oxidase I analyses. *Marine Biology*, **137**, 435–446.

Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region specific markers. *Proceedings of the National Academy of Sciences of the USA*, **91**, 88–92.

Lewis PO, Zaykin D (2001) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data*, Version 1.0 (d16c). Free program distributed by the authors over the Internet. Available from: http://lewis.eeb.uconn.edu/lewishome/software.html

Morgan T, Rogers AD, Ivengar A (1999) Novel microsatellite markers for the European oyster *Ostrea edulis*. *Molecular Ecology*, **9**, 489–504.

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, **12**, 357–358.

Paquotte P, Chim L, Martin JLM, Lemos E, Stern M, Tosta G (1998) Intensive culture of shrimp *Penaeus vannamei* in floating cages: zootechnical, economic and environmental aspects. *Aquaculture*, **164**, 151–166.

Perez-Farfante I (1969) Western Atlantic shrimp of genus *Penaeus*. *Fishery Bulletin*, **67**, 461–591.

Peterson RG, Stramma L (1991) Upper level circulation in the South Atlantic Ocean. *Progress in Oceanography*, **26**, 1–73.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in effective population size from allele frequency data. *Journal of Heredity*, **90**, 502–503.

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rodrigues RR, Lorenzzetti JA (2001) A numerical study of the effects of bottom topography and coastline geometry on the Southeast Brazilian coastal upwelling. *Continental Shelf Research*, **21**, 371–394.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Press, New York.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN *Ver 2 000: a Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Souza J (2001) Desempenho da Pesca e da Aqüicultura. *Síntese Anual Da Agricultura de Santa Catarina Instituto CEPA/SC*, pp. 150–153.

Sugama K, Haryanti Benzie JAH, Ballment E (2002) Genetic variation and population structure of the giant tiger prawn, *Penaeus monodon*, in Indonesia. *Aquaculture*, **205**, 37–48.

Supungul P, Sootanan P, Klinbunga S, Kamonrat W, Jarayabhand P, Tassanakajon A (2000) Microsatellite polymorphism and the population structure of the black tiger shrimp (*Penaeus monodon*) in Thailand. *Marine Biotechnology*, **2**, 339–347.

Wright S (1951) The genetic structure of populations. *Annals of Eugenics*, **15**, 323–354.

Xu ZK, Primavera JH, de la Pena LD, Pettit P, Belak J, Alcivar-Warren A (2001) Genetic diversity of wild and cultured black tiger shrimp (*Penaeus monodon*) in the Philippines using microsatellites. *Aquaculture*, **199**, 13–40.

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