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Genetic homogeneity in the commercial pink shrimp *Farfantepenaeus paulensis* revealed by COI barcoding gene

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ABSTRACT

The pink shrimp *Farfantepenaeus paulensis* is one of the most commercially exploited species in Brazil's South and Southeastern regions. Specific information about the status of its genetic variation is necessary to promote more effective management procedures. The genetic variation of the population of *F. paulensis* was investigated in five localities along southern and southeastern coast of Brazil. Sampling was performed with a commercial fishing boat. Total genomic DNA was extracted from abdominal muscle tissues and was used to DNA amplification by PCR. The COI gene was used as a DNA barcoding marker. The 570 bp COI gene sequences were obtained from all 45 individuals. The haplotype network showed no genetic variability among the population stocks, which was confirmed by Molecular Variance Analysis. The final alignment showed that inside species there is haplotype sharing among the sampled localities, since one haplotype is shared by 38 individuals belonging to all the five sampled regions, with no biogeographic pattern. This result is reasonable since there are no geographical barriers or habitat disjunction that might serve as a barrier to gene flow among the sampled localities. Possible reasons and consequences of the genetic homogeneity found are discussed. The results complement ecological studies concerning the offseason: since it is a single stock, the same protection strategy can be applied. However, the genetic homogeneity found in this study combined with the intensive fishery effort and the species biology can result in severe consequences for the *F. paulensis*.

1. Introduction

The pink shrimp *Farfantepenaeus paulensis* (Pérez-Farfante, 1967) is one of the most exploited species in Brazil (Paiva, 1997). It is distributed from Bahia – Brazil to Buenos Aires – Argentina, and found at 40–80 m deep (D'Incao, 1995; Costa et al., 2003). Individuals of this species, as well as individuals of the congener *Farfantepenaeus brasiliensis* (Latreille, 1917), are together known as "pink shrimps", and usually do not occur distinction between them at assessments of fishery stocks (Brisson, 1981; Chagas-Soares et al., 1995).

F. paulensis is more often captured between Ubatuba and Santos (São Paulo State), where cold waters are very close to the shore, and southward is the dominant species of pink shrimp, being the only one present in Patos Lagoon (Rio Grande do Sul State) (Paiva, 1997). The size of the individuals and the mature female ratio increases with depth, and spawning occurs in colder waters, beyond the 50 m isobaths; juveniles are usually found in estuaries or bays (Costa et al., 2008). Distribution in this species is more related to depth than to latitudes (Zenger and Agnes, 1977).

Pink shrimps represented 18% of Brazilian total production (57,344.8 t) of marine crustaceans in 2011 (IBAMA, 2011). The general shrimp production is related to pink shrimp capture, which varies in function of the artisanal fishery in Patos Lagoon, South Brazil (D'Incao et al., 2002). The state of Rio Grande do Sul (extreme south of Brazil) is the major producer, with catches exclusively performed by artisanal system, acting on the juvenile population in a very intensively way (D'Incao et al., 2002). São Paulo and Rio de Janeiro states (Southeastern Brazil) are respectively the third and fourth producers, with catches mostly performed by industrial systems and on adult stocks (Paiva, 1997).

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Genetic variations among stocks may provide direct information on the populations of a species over a geographic area of distribution (Dumont, 2008). Evaluations of population genetic structure and intraspecific genetic diversity supply data of biological and evolutionary interest and are of great value for the successful conservation or management of exploited species (McMillen-Jackson and Bert, 2004). Molecular techniques have been applied with success to support fishery issues, such as the identification of genetic variation, population structure and reproductive isolation among groups (Benzie, 2000). These techniques seems to be more reliable tools for conservation studies, helping on the identification of reproductive isolation among stocks and thus allowing the delineation fishery management units and to assess which are conservation priorities, from an evolutionary perspective (Begg et al., 1999).

Nowadays, the legal annual offseason for shrimps in southern Brazil extends from March to May (Paiva, 1997; Costa et al., 2008). The offseason was established basing on the juvenile recruitment of the pink shrimp species *F. brasiliensis* and *F. paulensis*, and ecologic studies have shown that present offseason coincides with the major recruitment of such species (Branco and Verani, 1998; Costa and Fransozo 1999). However, there are still few studies concerning the genetic variation of *F. paulensis* in South and Southeastern Brazil, making it difficult to assess if the offseason period can be applied to the entire population of the species. As example, we can cite the study of Gusmão et al. (2005) using allozymes, in which they found populations of *F. paulensis* that are genetically structured, comprising two different fishery stocks (one for South and another for Southeast Brazil).

A better understanding of the structure of these populations, including the role of fisheries in the context of human and environmental impacts, is necessary for the development of conservation policies and restoration in the marine system (Blaber et al., 2000). In this context, the pink shrimp *F. paulensis* represents one of the most exploited fishery stocks in Brazil's South and Southeastern regions. Specific information about the status of its genetic variation is necessary to promote more effective management procedures. Considering the great economic importance of pink shrimp fishery in Brazil and the consequent and visible decrease in its populations, we aimed to analyze the genetic variation of *F. paulensis* in five localities along southern and southeastern coast of Brazil.

2. Material and methods

2.1. Sampling

Sampling of fresh specimens was performed at three localities along São Paulo state, Brazil: north region (Ubatuba, 23°26′S, 45°04′W), central region (Santos, 23°57′S, 46°19′W) and south region (Cananéia, 25°1′S, 47°55′W) (Fig. 1). Geographic coordinates related to the sampled points were recorded using a GPS (Global positioning system).

Sampling was performed on February and August 2012, with a commercial fishing boat equipped with *otter-trawl* and *double-rig* nets (mesh size 18 mm and 20 mm, respectively) and mouth opening of 5 m, which was hauled for 30 min. The biological material was stored in ice, identified according to specific keys (Costa et al., 2003), preserved in 75–90% ethyl alcohol and deposited in the Crustacean Collection of the Department of Biology, Faculty of Philosophy, Science and Letters at Ribeirão Preto, University of São Paulo (CCDB/FFCLRP/USP), Brazil. Complementary specimens from other two localities (Rio Grande do Sul and Rio de Janeiro states) and previously cataloged in the CCDB collection were analyzed (Fig. 1).

2.2. Molecular analysis

Total genomic DNA was obtained from the abdominal muscle tissues of individuals. This procedure has the advantage of being performed from small amounts of biological tissue. Obtaining and manipulation of genetic material are in accordance with SISBIO license for sampling and genetic analysis of decapods, (CGEN No. 11777-1, Issue Date: 09/16/2007 to FLM). The COI gene has been frequently used with success to analyze phylogenetic relationships in many marine crustaceans, including species of *Penaeus* and other Decapoda (Quan et al., 2001). This gene was chosen for molecular analysis due to its wide variability in evolutionary rates (Moritz et al., 1987), allowing to verify the occurrence of inter-population variation within species, as already done in other studies with shrimps (Gusmão et al., 2000; Vergamini et al., 2011; Terossi and Mantelatto, 2012; Rossi and Mantelatto, 2013). The COI gene is also used as a "barcode" marker for most living animal (Hebert et al., 2003).

The protocol for extraction of specimens was based on the work of Mantelatto et al. (2007, 2009). Muscle tissues extracted from each individual were placed in lysis buffer and proteinase K (500 μg/mL) and then incubated for 24 h at 55 °C. After dry bath prior to centrifugation, 200 μL NH_4OAc (7.5 M) was added in each sample; after this, 600 μL of cooled isopropanol was added, so decanting the DNA. After 48 h of samples cooled at 20 °C, the resulting pellet was washed with 15 μL of 70% EtOH, centrifuged, freeze-dried in an Eppendorf Concentrator 5301® and resuspended in 20 μL of TE buffer. The concentration of the extracted DNA from each sample was measured in a Nanodrop spectrophotometer 2000®.

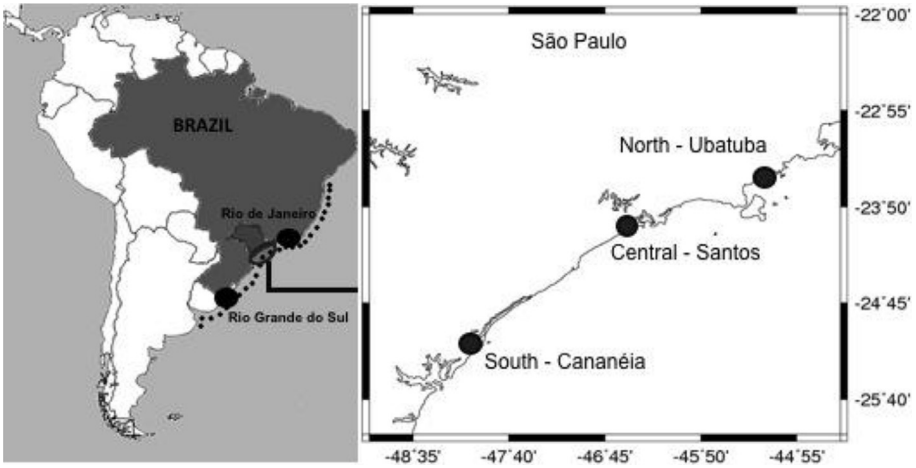

Fig. 1. Map showing the five sampled regions of the present study. Small dots indicate the known distribution of *F. paulensis*.