A novel polymorphic mtDNA marker for population studies of the pink shrimp, *Farfantepenaeus duorarum* (Crustacea, Penaeidae)*

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Abstract

The pink shrimp, *Farfantepenaeus duorarum*, is one of the most important shrimp species commercially harvested along the Gulf and Atlantic coasts of the US. In this study we developed a mitochondrial marker suitable for population studies of the species. A 611–617 bp hypervariable portion of the AT-rich region of the mt genome was amplified and sequenced. The 617 bp long consensus sequence contained 15 polymorphic insertion/deletion sites and 165 polymorphic substitution sites. Kimura 2-parameter distances ranged from 0.00 to 0.06 with a mean of

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0.02. Among the 104 sequences, 100 haplotypes were counted if all mutations were included. If transitions were omitted, 34 haplotypes were observed. The results indicate that the hypervariable portion of the AT-rich region may be an effective marker for revealing the genetic structure of the pink shrimp off the southeast US.

The pink shrimp, *Farfantepenaeus duorarum*, is one of the most important shrimp species commercially harvested from the Gulf of Mexico and the Atlantic coast of the US. The major pink shrimp fishery occurs in the Gulf of Mexico, where it comprises up to 10% of the total US shrimp landings (SEFSC 1993). In the Bay of Campeche, where the pink shrimp is a dominant species, it comprises up to 70% of the total shrimp catch (Gracia 1995). During the last 15 years, pink shrimp landings in the Bay of Campeche have declined by about 60% (SdP 1988, Navarette et al. 1995), which suggests their stocks are depleted. Lack of knowledge of the phylogenetic relationships occurring among the population units of an exploited species may lead to the rapid erosion of its genetic structure and, locally, to the extinction of some populations (Ovenden 1990). This can be prevented by conducting a detailed analysis of a species' genetic and phylogeographic diversity and by developing and implementing an exploitation/conservation strategy (Crozier 1997).

Mitochondrial markers are known to be excellent tools for population genetic analyses (Avise 1994). A sequence of the entire AT-rich region of *F. duorarum* has already been published by Grabowski & Stuck (1999). The region is approximately 990 bp in length and lies between the genes that code for the tRNA-ile and 12S-rRNA. It is divided into three polymorphic domains separated by two conserved strains in which no intraspecific variability is observed (Grabowski & Stuck 1999).

The aim of this study was to estimate the intraspecific variability of a c. 600 bp long fragment, including one of the supposedly polymorphic domains of the AT-rich region, and its use as a potential marker for population studies of the pink shrimp.

Shrimp were collected from the Gulf of Mexico and the Atlantic coast of the US, and DNA was extracted from the tail muscle (fresh, frozen or stored in SED buffer; Amos & Hoelzel 1991; n = 104) using a CTAB-based protocol (Grabowski & Stuck 1999). A 617 bp hypervariable portion of the AT-rich region was then amplified using self-constructed primers that are rooted within the conserved domains: PDCTRL-F (5′-AgCCg ATTgg TAAAA gTTCg-3′), and PDCTRL-R (5′-ATTTC TTAAg ACCCC TCgAC-3′). PCR-amplifications were conducted in 25 μL reactions using

a 1 μL template ($< 1 - 100$ ng), 3 μM $MgCl_2$, 400 μM each dNTP (Promega, Inc.), 0.8 μM of each primer, and 2.5 U *Taq* DNA polymerase (Amersham Pharmacia and Biotech). PCR was performed in an MJ Research PTC thermal cycler and conditions were 94°C for 3 min followed by 35 cycles of 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1 min with a final elongation of 72°C for 5 min. The appropriate product was then gel-purified (Qiaquick™ Gel Extraction Kit, Qiagen, Inc.), quantified as described by Gallagher (1994), and cloned into Promega's pGEM® – T Easy Vector System utilizing blue/white selection (Sambrook et al. 1989). Plasmids containing inserts were purified with Promega's Wizard® *Plus* Minipreps DNA Purification System, and screened for inserts by EcoRI-digestion (Promega, Inc.) followed by agarose gel electrophoresis. Clones were quantified as described above and sequenced on a Li-Cor DNA 4200 DNA Automated Sequencer. Sequencing reactions were performed in an MJ Research PTC-200 thermal cycler using the SequiTherm Excell™ II Long Read DNA sequencing kit (Epicentre Technologies) and IRD-labeled M13 sequencing primers (Li-Cor, Inc.). Sequencing reaction conditions were 94°C for 2 min followed by 30 cycles of 94°C for 0.5 min, 62°C for 0.5 min, and 70°C for 0.5 min. DNA sequences were imported into OMIGA, ver. 1.1 (Oxford Molecular Ltd, Oxford England), a multiple sequence editor, and aligned with CLUSTALW (Higgins & Sharp 1988) using default settings.

We originally attempted direct-sequencing using M13- and T7/SP6-tailed primers (Li-Cor, Inc.) in PCR reactions but were unable to obtain reliable sequences owing to the high AT content of the region (up to 82%); cloning was therefore necessary. To increase the yield of target-DNA in PCR reactions, we incorporated T7/SP6-tails on the primers described above.

All amplified sequences along with the information on their geographic origins are deposited for public access in the GenBank Nucleotide Sequence Database (accession numbers AF328297-AF328400). The amplified fragment was 611–617 bp long. The 617 bp long consensus sequence contained 15 polymorphic sites displaying insertions/deletions and 165 polymorphic sites displaying substitutions, 24 transversions and 157 transitions. Kimura 2-parameter distances, estimated with Arlequin 1.1 (Schneider et al. 1997), ranged from 0.00 to 0.06 with a mean of 0.02. In an interspecific comparison with the corresponding region of *Farfantepenaeus notialis,* the Kimura 2-parameter distance was approximately 0.07. Among the 104 sequences, 100 haplotypes were counted if all mutations were included. If transitions were omitted, 34 haplotypes were observed. The results indicate that this hypervariable portion of the AT-rich region is an effective marker

for revealing the phylogeographic structure of the pink shrimp off the southeast US.

The primers described above were used in an attempt to amplify the corresponding region from two different penaeid shrimps that are commercially fished in the same regions: the brown shrimp (*Farfantepenaeus aztecus*) and the white shrimp (*Litopenaeus setiferus*). No amplification was observed. Further, in a comparison against sequences deposited at the National Center for Biotechnology Information (NCBI) using NCBI's BLAST WWW Server (Basic Local Alignment Search Tool; Altschul et al. 1990), it was found that the primer sequence was 100% identical with the corresponding regions of *F. notialis*, another very important commercial species.

In conclusion, we have identified a region of DNA in the pink shrimp, *F. duorarum*, that contains sufficient polymorphism to be a suitable marker for studying its genetic variability and population structure. We have also determined that the primers designed for this study may be effective in a similar study of *F. notialis.*

References

Amos B., Hoelzel A. R., 1991, *Long-term preservation of whale skin for DNA analysis*, Rep. Int. Whal. Comm., Spec. Iss. No. 13, 99–103.

Altschul S. F, Gish W., Miller W., Myers E. W. & Lipman D. J., 1990, *Basic local alignment search tool*, J. Mol. Biol., 215, 403–410.

Avise J. C., 1994, *Molecular markers, natural history and evolution*, Chapman and Hall, New York, 511 pp.

Crozier R. H., 1997, *Preserving the information content of species: Genetic diversity, phylogeny, and conservation worth*, Ann. Rev. Ecol. Syst., 28, 243 –68.

Grabowski M., Stuck K. C., 1999, *Structure and intraspecific variability of the control region mtDNA in the pink shrimp, Farfantepenaeus duorarum (Decapoda, Penaeidae)*, [in:] *Crustaceans and the biodiversity crisis*, Vol. I, pp. 333–344, F. R. Schram & J. C. von Vaupel Klein (eds.), Brill Acad. Publ., Leiden.

Gracia A., 1995, *Impact of artisanal fishery on production of the pink shrimp Penaeus (Farfantepenaeus) duorarum Burkenroad, 1939*, Cien. Mar., 21 (3), 342–359.

Higgins D. G., Sharp P. M., 1988, *Clustal: a package for performing multiple sequence alignment on a microcomputer*, Gene, 73, 237–244.

Navarette A., Garduno H., Gracia A., 1995, *La pesqueria de camarón en alta mar: golfo de México y Caribe mexicano*, [in:] *Pesquerias relevantes de México*, Tomo I, pp. 37–71, Instituto Nacional de la Pesca, Mexico.

Ovenden J. R., 1990, *Mitochondrial DNA and marine stock assessment: A review*, Austr. J. Mar. Fresh. Res., 41 (6), 835–53.

Sambrook J., Fritsch E. J., Maniatis T., 1989, *Molecular cloning. A laboratory manual*, 2nd edn., Cold Spring Harbor Lab. Press, Cold Spring Harbor, New York.

Secretaria de Pesca (SdP), 1988, *Annuario estadistico de pesca 1986*, Direction Gral de Informática, Estadistica y Documentación, Secretaria de Pesca, Mexico.

Schneider S., Kueffer J. M., Roessli D., Excoffier L., 1997, *Arlequin version 1.1: a software for population genetic data analysis*, Genetics and Biometry Lab., Univ. Geneva (Switzerland).

Southeast Fisheries Science Center (SEFSC), 1993, *Status of fishery resources of the southeastern United States for 1992*, NOAA Tech. Memo. No. NMFS-SEFSC-326, 1–89.