Partial Sequence Analysis of Mitochondrial COI Gene of the Chinese Shrimp, *Fenneropenaeus Chinensis*

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Abstract Eight hundred and thirty eight base pair fragment of mitochondrial COI gene of wild and cultured populations (CP1, CP4, CP5 and CP6) of *Fenneropenaeus chinensis* was amplified and sequenced. The A, T, G and C contents of the sequence were 235 bp (28.0%), 307 bp (36.6%), 138 bp (16.5%) and 158 bp (18.9%), respectively. Furthermore, 556 bp fragment of the sequence was used to discuss the phylogenetic relationship among 14 Penaeidae species using *Alpheus armillatus* as the outgroup. From the molecular phylogenic tree constructed by neighbor-joining method, we obtained three large shrimp groups: *Farfantepenaeus*, *Litopenaeus* and *Fenneropenaeus* group. The results also indicated that there were a closer genetic relationships between *F. aztecus* and *F. paulensis*, *L. schmitti* and *L. setiferus*, *F. indicus* and *F. merguiensis*, and the genus *Farfantepenaeus* was closer to *Litopenaeus*.

Key words *Fenneropenaeus chinensis*; cytochrome oxidase subunit I (COI); DNA sequence; Penaeidae; phylogeny

1 Introduction

*Fenneropenaeus chinensis* which belongs to the family *Penaeidae* (Crustacea: Decapoda: Dendrobranchiata) is mainly distributed in Bohai Sea and Yellow Sea of China. This species is of economical importance in both fishing and aquaculture in China (Deng et al., 1990). To protect natural population from over-exploitation, artificially produced seeds have been released to enhance natural stocks since 1985. As noted by Bernatchez (1995), wildlife management strategies are contingent on an understanding of the evolutionary underpinning of contemporary biodiversity. Considering the importance of this species, genetic information is vital to the design and implementation of sound fisheries management strategies and sustainable development of aquaculture.

There have been many discussions on the population structure, phylogeny of shrimp (Baldwin et al., 1998; Benzie et al., 1993; Lester, 1979; Liu et al., 2000; Mulley and Latter, 1980; Palumbi and Benzie, 1991; Qiu, 2000; Quan et al., 2001; Tam and Chu, 1993). In this study, we sequenced a part of mitochondrial cytochrome oxidase subunit I (COI) gene to investigate genetic variations between wild and cultured populations of *Fenneropenaeus chinensis*. Furthermore, phylogenetic relationships among 14 Penaeidae species were discussed using the deposited sequences in GenBank.

2 Materials and Methods

2.1 Materials

Samples of wild *F. chinensis* population were collected from coastal waters of County Rizhao and those of the first, forth, fifth and sixth generation of cultured populations (CP1, CP4, CP5 and CP6) from Counties Jiaonan, Rizhao and Jimo in 2001 and Rizhao in 2002 respectively. Shrimp specimens were initially frozen in liquid nitrogen and subsequently stored at temperatures below -20 0C. Two individuals of each population were used for sequence analysis.

2.2 Total DNA Extraction

A small piece of muscle (about 100 mg) was ground in a glass homogenizer and incubated for 2 h at 37 0C in a 700 mL STE solution (100 mmol L^-1 NaCl, 10 mmol L^-1 EDTA, 50 mmol L^-1 Tris-HCl, pH 8.0) containing 50 mL 20% SDS and 15 mL 10 mg mL^-1 proteinase K. The DNA was isolated using a standard phenol/chloroform extraction protocol and collected by ethanol precipitation (Zhang and Ryder, 1993).

2.3 PCR Amplification

A 838 bp fragment of the 3’ end of COI gene was
amplified using primers COIF 5' - CCTGCAGGAGGAGGAYCC-3' (Palumbi and Benzie, 1991) and TL2N 5' - ATGCATATCTCATGCGCATT TAG - 3' (Quan et al., 2001). PCR amplifications were conducted in a Biometra thermocycler using an initial 3 min denaturation at 94 °C followed by 35 cycles of 1 min at 94 °C for denaturation, 1 min at 58 °C for annealing, 1 min at 72 °C for extension, and a final 10 min extension at 72 °C. In all PCR amplifications, negative controls of template-free were used to detect possible contaminations. A total of 2–3 μL of each PCR product was used for 1.5% agarose gel electrophoresis to verify the amplified fragment length with a DNA marker DL 2000 (TaKaRa Biotechnology (Dalian) Co., Ltd.), and thee visualized with ethidium bromide under ultraviolet light.

2.4 Sequencing
The amplified products were electrophoresed on a 1.5% agarose gel and purified with Waterson’s gel extraction kit. These purified products were used as the template DNA for cycle sequencing reactions performed using Dye Terminator Cycle Sequencing FS Ready Reaction Kits (Applied Biosystems), and run on an ABI 377 DNA sequencer (Perkin-Elmer Corp.). Double strands were sequenced and the primers used for sequencing were the same as those for PCR amplification.

2.5 Data Analysis
The sequences were edited and aligned by the DNASTAR software (DNASTAR, Inc.). Phylogenetic and molecular evolutionary analyses were conducted using MEGA software (Kumar et al., 2001). A 556 bp homologous segment of the 15 species were adopted for phylogenetic analysis.

3 Results and Discussion
A 838 bp fragment of COI gene was obtained from 10 individuals of five different F. chinensis populations. It was the same with the sequence in GenBank (Accession number: AF247772). No sequence variation was observed among those individuals. The A, T, G and C contents of the sequence of F. chinensis were 235 bp (28.0%), 307 bp (36.6%), 138 bp (16.5%) and 158 bp (18.9%), respectively. AT content was more than GC.

The 556 bp fragment of COI gene were analyzed to examine the phylogenetic relationship of 14 Penaeidae species (Litopenaeus stylirostris, L. vannamei, L. schmitti, L. setiferus, Farfantepenaeus subtilis, F. paulensis, F. duorarum, F. brasiliensis, F. aztecus, Fenneropenaeus chinensis, F. indicus, F. merguiensis, Marsupenaeus japonicus, Peneaus monodon). Alpheus armillatus was chosen as the outgroup.

Fig.1 shows the variable sites of COI gene sequence of the above 15 species. There is little difference of A, T, G and C contents between genus (Table 1). Genetic distances between any two of the 14 Penaeidae species were calculated based on Kimura’s 2-parameter method (Table 2). From the neighbor-joining molecular phylogenetic tree, we obtained three large shrimp groups; Farfantepenaeus, Litopenaeus and Fenneropenaeus group. The results also indicated that there was a closer genetic relationship between F. aztecus and F. paulensis, L. schmitti and L. setiferus, F. indicus and F. merguiensis, and the genus Farfantepenaeus was closer to Litopenaeus (Fig.2).

Members of the genus Peneaus represent over 90% of the cultured species worldwide. In this case, phylogenetic information can be used to guide captive breeding programs, which are intended to produce superior captive strains through hybridization of closely related species (Baldwin et al., 1998). Mitochondrial DNA has been used in fishery study with features such as its compactness, maternal inheritance and a faster evolutionary rate compared to nuclear DNA (Avise, 2000; Brown, 1983; Lu and Li, 1998). Very high genetic variations of COI gene have been observed in several