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Sequence variations of the first ribosomal internal transcribed spacer of *Penaeus* species in Thailand

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Abstract

The ribosomal DNA internal transcribed spacer 1 (ITS1) was investigated in the search for additional genetic marker that is suitable for population studies of the penaeid shrimps. The sequence variations of the ITS1 were determined and found to be informative in estimating phylogenies in that they differentiate four species of penaeid shrimps, namely *Penaeus merguiensis*, *Penaeus silasi*, *Penaeus monodon* and *Penaeus semisalcatus* and the populations of *P. merguiensis* collected in the Gulf of Thailand and the Andaman Sea. The length of the ITS1 ranged from 499 to 772 bp, with a GC content of 63.30–67.37%. Four microsatellite loci are found in the ITS1 at 5′ end and the middle of region and seem to be associated with sequence divergence and size variation in *Penaeus* species. Some microsatellites were found in only one specie, $(GCGA)_4$ in *P. semisalcatus* and $(CGGA)_{4-9}$ in *P. monodon*. These microsatellite regions are considerably long enough and the level of intragenomic variation in *P. merguiensis* is less than that between different species, hence, provide a great potential use in the population studies.

Keywords: ITS1; Penaeid shrimps; *Penaeus merguiensis*; Phylogeny; rDNA

1. Introduction

Penaeid shrimps have for many centuries been considered a good source of food. Most species are found naturally in shallow, inshore, tropical and subtropical waters and many have been artificially cultured in ponds. In Thailand approximately 50 species of *Penaeus* are found (Chaitiamvong and Supongpan, 1992). The black tiger shrimp (*Penaeus monodon*) has been the most popular for aquaculture but farming of this shrimp has largely been dependent on the use of broodstock caught in the wild and this has resulted in a national shortage of good quality stock (Klinbunga et al., 1999). Another species with important commercial potential is *Penaeus merguiensis*, which is also found throughout the Indo-West Pacific region from the Persian Gulf of Thailand, Hong Kong, the Philippines, and Indonesia to New Guinea, New Caledonia and Northern Australia. *P. merguiensis* has attracted attention in aquaculture because it tolerates low water quality better than does *P. monodon*, it can be grown at high densities, larvae are easy to rear, they tolerate a wide range of salinities and temperatures, have low protein requirement, and in culture have minimal size variation. However, one of the disadvantages is the limited information on their biology and culture. In previous work, we assessed the genetic diversity within *P. merguiensis*

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stocks using the COI mitochondrial DNA marker and three nuclear loci from samples of five populations collected from the Gulf of Thailand and the Andaman Sea. The results revealed a highly significant differentiation between the Gulf of Thailand and Andaman Sea populations (F_{ST}=0.203, P<0.001), mostly due to the polymorphism of locus PvAmy (an intron of an amylase gene) (Hualkasin et al., 2003; Wanna et al., 2004). Intra-population F_{IS} values for the three loci examined were significant in the samples collected from all three populations in the Gulf of Thailand but not in those from the Andaman Sea. Therefore, it is important to obtain data from more highly variable DNA markers than the PvAmy locus in order to study the genetic divergent genotypes.

In eukaryotes, the nuclear ribosomal DNA units (rDNA) array typically consists of several hundred tandemly repeated copies of 5.8S, 18S, and 28S genes and two internally transcribed spacers, ITS1 and ITS2 (Hillis and Dixon, 1991). Nuclear rDNA sequences have become a popular marker for phylogenetic and population analyses in many organisms, with ITS1 being particularly widely used at the population and species level due to its high level of sequence variation (Cerbah et al., 1998; Harris and Crandall, 2000; Odorico and Miller, 1997; Schizas et al., 1999; Stothard et al., 1996; Vogler and DeSalle, 1994). However there have been few studies on the genetic variability of ITS1 and its applications in shrimp population studies and phylogeny. Recently, Chu et al. (2001) reported a high (18.1%) sequence divergence rate of ITS1 from 2 different populations of *Penaeus japonicus* and demonstrated a variation at the species level between *P. japonicus* and *Penaeus vannamai*. In addition, the characteristic of the ITS region of having a relatively rapid divergence rate makes it useful for solving problems of systematics and has made possible the identification of several sibling species (Hackett et al., 2000; Krüger et al., 2000; Gallego and Galián, 2001).

The goals of this study were to investigate the sequence variation of the ITS1 region in Penaeid shrimps at species and population levels. This information might be useful for future evolutionary studies and for species identification purposes.

2. Materials and methods

2.1. Samples

Four species of *Penaeus* (*P. merguiensis*, *P. silasi*, *P. monodon* and *P. semisalcatus*) collected from the Thailand peninsula were selected for this study. In order to investigate genetic variations within species, six populations of *P. merguiensis* collected from different regions were studied. A list of species, geographical regions, and abbreviation are provided in Table 1 and Fig. 1. All the ITS1 sequences obtained in this study have been deposited in GenBank under accession numbers AY331585-AY331590 and AY315657-AY315659.

2.2. DNA extraction

Genomic DNA was extracted from frozen muscle tissue of samples using the phenol/chloroform/proteinase K and ethanol precipitation method. 0.1–0.2 g of muscle tissue was ground in 600 μl of extraction buffer (10 mM Tris–HCl, pH 7.5, 100 mM EDTA, 1% SDS, 1 μg proteinase K/ml and 0.05 μg RNase/ml). The suspensions were held at 37 °C for 1 h and then at 55 °C for 2 h. DNA was extracted using two volumes of phenol/chloroform/isoamyl alcohol (25:24:1) followed by extraction with an equal volume of chloroform/isoamyl alcohol (24:1). The DNA was precipitated in cold absolute ethanol. Precipitates had excess salt removed

Table 1
Collection sites, %GC composition, and ITS1 sequence lengths of *Penaeus* species used in this study

Species	Collection site in Thailand (abbreviation)	Length of ITS1 (bp)	Average %GC
P. merguiensis	Trad (PMERTDE)	538–565	66.54
P. merguiensis	Songkhla (PMERSKE)	538–561	66.44
P. merguiensis	Suratthani (PMERSRE)	537–562	66.07
P. merguiensis	Trang (PMERTRW)	555–571	65.98
P. merguiensis	Phuket (PMERPKW)	575–577	66.15
P. merguiensis	Satun (PMERSTW)	565–581	66.18
P. silasi	Nakhonsithammarat (PSIL)	605–627	65.00
P. monodon	(PMON)	499–525	66.93
P. semisalcatus	(PSEM)	567–572	67.33
P. indicus	(PINDGB)*		
P. vannaeii	(PVANGB)*		

Asterisk denotes sequence retrieved from the GenBank.