1. INTRODUCTION

The term “shrimp” and “prawn” have no definite reference to any known taxonomic groups. Although the term “prawn” is sometimes applied to small species, while “prawn” is more often used for larger forms (Carpenter and Niem, 1998). In this work, we called them “prawn”.

The production of prawn in Thailand, both captured or cultured, has increased from 178,698 tons in 1989 to 362,731 tons in 1998 (Department of Fisheries, Ministry of Agriculture and Cooperative). In 1999, Thailand was the largest cultured prawn producer in the Eastern Hemisphere, accounting for 24.6 percent of total production. The value of fresh and frozen prawn export increased from 37,843 (in 1993) to 54,733 (in 2001) million baht (Customs Department, 2004). Its export value is 60-70% of all fishery products in Thailand. Among prawn product exports, the genus *Penaeus* supports the greatest value of the commercial fisheries.

In the genus *Penaeus*, there are four subgenera (*Melicertus*, *Marsupenaeus*, *Fenneropenaeus* and *Penaeus*) found in Thailand. In the subgenus *Melicertus*, three species (*P. canaliculatus*, *P. latisulcatus*, *P. longistylus*) are known. In the subgenus *Marsupenaeus*, only *P. japonicus* is found. In the subgenus *Fenneropenaeus*, four species (*P. merguiensis*, *P. indicus*, *P. silasi* and *P. penicillatus*) are found, and in the subgenus *Penaeus*, two species (*P. monodon* and *P. semisulcatus*) are found. Other species of genus *Penaeus* provides high conomic value such as subgenus *Farfantepenaeus* and subgenus *Litopenaeus.* In subgenus *Farfantepenaeus* (e.g. *P. subtilis*, *P. notialis*, *P. brasiliensis*) and subgenus *Litopenaeus* (e.g. *P. vannamei* and

P. setiferus) are found in the Gulf of Mexico (Gusmao et al., 2000). The highest prawn quantity and value export species is *P. monodon*, with *P. merguiensis* the second most valuable species (Trang Fishery Office, 2002).

P. merguiensis (banana prawn) is native to the Indo-Pacific region, and is raised extensively throughout Southeast Asia. It is a promising alternative species for aquaculture in Thailand. *P. merguiensis* can be conveniently developed in the future to avoid the reliance on wild-caught broodstock for supplying juveniles needed to sustain production (Daud, 1995). It is distributed in the Indo-West Pacific from the Arabian Sea to the South China Sea and Fiji (Carpenter and Niem, 1998). Because of its local species in the Indo-West Pacific, few competitors export this species.

To date, the largest export markets for Thai prawn are the United States and Japan, and the demand is increasing yearly. Japan particularly prefers *P. merguiensis* . Taiwan and Australia also export *P. merguiensis* to Japan at a better cost than *P. monodon* (in the same size) therefore *P. merguiensis* is interesting (Thai Shrimp Newspaper, 2003).

In Southeast Asia and the Pacific Ocean, there are four species of white prawn- *P. merguiensis*, *P. indicus*, *P. silasi* and *P. penicillatus* (Carpenter and Niem, 1998). The white prawn *P. merguiensis* De Man, *P. indicus* H. Milne Edwards and *P. penicillatus* Alcock are three very similar species. Hall (1956) commented " although adults demonstrating the features typical of the respective species may be identified fairly easily, there are many cases in which features of all three species may be exhibited by a single individual". Hall (1956) said " their outer appearance is so

greatly different, especially as regards the shape of the rostrum that everyone will consider them as different species".

Taxonomic difficulties among these closely related species, *P. merguiensis*, *P. silasi*, *P. indicus* and *P. penicillatus*, have been reported (Carpenter and Niem, 1998). The shapes of the male petasma and female thelycum are very important taxonomic characters in several genera of penaeid prawns. However, as the petasma and thelycum are not fully developed in juveniles, a positive identification of juvenile specimens is often difficult (Dall et al., 1990; Carpenter and Niem, 1998). Based primarily on morphology, only adult males of *P. merguiensis*, *P. silasi*, *P. indicus* and *P. penicillatus* can be differentiated using the 3rd maxilliped (Carpenter and Niem, 1998). However, the correct species-level identification of individuals by using the molecular marker is an important study for marine invertebrate fisheries (Daud, 1995).

When morphological and morphometric study were inadequate to separate the white prawn species clearly, molecular techniques were used, starting with allozyme analysis.

The allozyme analysis is a viable tool for analyzing genetic variation. The ability to detect variation at a single locus coding for allozymes can be used to clarify the problems of ambiguous morphological data. Allozymes markers have been reported at an interspecific level, differentiating between closely related species, especially during the juvenile stages (Lavery and Staples, 1990; Ward and Grewe, 1995; Pendrey et al., 1999). The general fisheries scientific community has come to regard allozyme electrophoresis as available tool for research and management.

Beside the protein marker as allozyme, the DNA markers are also widely used to species identification.

DNA markers in animals are derived from the nucleus and organelles. Chromosomal DNA, often referred to as nuclear DNA, is one a source of DNA in the cells. Nuclear markers with biparental inheritance (one male and one female), like the highly variable microsatellites, provide a more complete picture of population subdivision and can be used to assess genetic relationships among closely related populations as well as to identify single individuals by multilocus genotyping. Another source of DNA in the animal cells is mitochondrial DNA (mtDNA), which is carried in the mitochondria present in the cytoplasm of most cells and, in particular, the female germ cells. Few mitochondria are present in the male germ cells which have very little cytoplasm. Consequently they are inherited almost exclusively through the female or maternal line. Mitochondrial DNA is particularly well suited to taxonomic and systematics studies for many reasons including ease of isolation, the possibility of using small sections, and a high mutation rate. It evolves faster than nuclear DNA (Brown et al., 1982). In addition, the different regions of the mitochondrial genome evolve at different rates (Saccone et al., 1991) allowing suitable regions to be chosen for the question under study. Here we begin our study with mitochondrial DNA.

Because the PCR technique is widely applied, mitochondrial universal primers have been developed from conserved sequences flanking those of interest to phylogenetic/ evolutionary and population studies. Such primers allow the

amplification of the same region from a diverse range of taxa facilitating parallel research studies in new organisms (Simon et al., 1994).

Mitochondrial ribosomal RNA genes (12S and 16S rRNA) have been studies greatly because of their critical role in protein assembly (Brimacombe et al., 1990, Noller et al, 1990), universal occurrence, sequence and structure conservation, and abundance. 12S and 16S rRNA genes have been used for phylogenetic analyses of a wide range of species, including insect and crustacean (Moritz et al., 1987; Kocher et al., 1989; Machado et al., 1993; Xiong and Kocher, 1993).

Another gene in mitochondrial genome, Cytochrome oxidase subunit I, was also reported high genetic diversity in prawn (Baldwin et al., 1998, Gusmão et al., 2000) and broadly used in phylogenetic study of a wide range species (Funk, 1999; Gasser et al., 1999; Zhao et al., 2002; Hebert et al., 2003).

The objectives of this study were to examine species identification and the relationships of the banana prawn (*P. merguiensis*) and the closely related species *P. silasi* and *P. indicus*, collected from eight locations on both coasts of Thailand, by using morphology and morphometric measurements to compare with molecular markers (isozyme pattern and mitochondrial DNA partial sequencing analyses of 12S rRNA, 16S rRNA and COI gene). Finally, the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of the COI gene to obtain the species specific marker and geographic distribution between both coasts of Thailand, which will be useful for the identification of larvae and prawn fisheries.