MORPHOLOGICAL AND MOLECULAR ANALYSIS OF *Penaeus semisulcatus* FROM THE PERSIAN GULF AND OMAN SEA

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by

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LIST OF ABBRIVATIONS

VNTR	Variable Number of Tandem Repeat
Bp	Base Pare
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleic triphosphates
EDTA	Ethylen diaminetetraacitic acid
$MgCl_2$	Magnesium Chploride
PCR	Polymerase Chain Reaction
P	Probability
SDS	Sodium Dodecyl Sulfate
ANOVA	Analysis of Variance
ASPW	Antennal Spine Width
BL	Body Length
CL	Carapace Length
DCL	Diagonal Carapace Length
ASH1	First Abdominal Somite Height
ASL1	First Abdominal Somite Length
ASW1	First Abdominal Somite Width
ASH2	Second Abdominal Somite Height
ASL2	Second Abdominal Aomite Length
ASW2	Second Abdominal Somite Width
ASH6	Sixth Abdominal Somite Height
ASL6	Sixth Abdominal Somite Length
ASW6	Sixth Abdominal Somite Width
HSW	Hepatic Spine Width

RL	Rostrum Length
TeL	Telson Length

ANALISIS MORFOLOGI DAN MOLEKUL *Penaeus semisulcatus* DARI TELUK PARSI DAN LAUT OMAN

ABSTRACK

Terdapat dua morfotip udang harimau hijau, *Penaeus semisulcatus* yang dikenalpasti di Teluk Parsi dan Laut Oman. Kedua-dua morfotip ini dibezakan melalui corak antena and garis melintang badan. Kajian ini dijalankan untuk menyiasat status taksonomi kedua-dua morfotip berdasarkan morfometrik dan analisis jujukan DNA mitokondria. Di samping itu kajian genetik populasi *P. semisulcatus* dari Teluk Parsi dijalankan untuk mambantu pemilihan induk yang sesuai dalam program pembiakbakaan spesies udang ini pada masa hadapan. Sejumlah 25 ciri diselidiki untuk kajian morfometrik. Sepuluh cirri daripadanya menunjukkan perbezaan yang signifikan antara seks tunggal dan kombinasi seks ($P < 0.05$). Analisis regresi menunjukkan korelasi antara panjang karapas dan panjang badan untuk betina dari jenis morfotip tak berjalur adalah berbeza berbanding jantan. Kajian molekul ke atas DNA mitokondria yang melibatkan gen separa 16S rRNA menunjukkan perbezaan genetik rendah secara relatif antara morfotip *P. semisulcatus* dengan 3.9 % jarak genetik. Jujukan-jujukan ini dapat membezakan kedua-dua morfotip dan memisahkan keduanya ke dalam dua klad. Sebaliknya perbezaan genetik yang dikesan melalui analisis gen COI secara konsisten adalah lebih tinggi. Perbezaan genetik yang tinggi untuk COI dengan 76 tapak perbezaan dicerap antara kedua-dua morfotip. Lapan lokus mikrosatelit baru telah dibangunkan untuk kajian struktur populasi genetik *P. semisulcatus* di dalam tiga lokasi di Teluk Parsi dan Laut Oman (Jask, Hormoz dan Kuhestak). Terdapat tiga peristiwa pengurangan heterozigositi dan pencapahan dari keseimbangan Hardy-Weinberg ditunjukkan untuk hampir semua lokus ($P < 0.001$). Namun empat lokus (lokus E2 dan B9 –

Hormoz; lokus C6 – Kuhestak; dan lokus H9 - Jask) didapati berada dalam keseimbangan Hardy-Weinberg. Analisis Micro-Checker menunjukkan kehadiran alel nol di tiga lokus mikrosatelit (B5, C6 dan C9). Perbandingan Fst berpasangan berdasarkan frekuensi alel dan genotip menunjukkan ketiga-tiga populasi dibezakan secara signifikan antara mereka (P <0.05). Nilai Fst berpasangan yang tinggi (0.106) dan nilai Nm yang rendah (2.103) tercerap antara populasi Hormoz dan Jask menandakan aliran gen yang terbatas antara kedua-dua populasi tersebut. Sebaliknya nilai Fst berpasangan yang rendah (0.016) dan niali Nm yang tinggi (15.876) tercerap antara populasi Hormoz dan Kuhestak menunjukkan aliran gen yang tinggi antara kedua-dua populasi tersebut. Dalam kajian ini ujian penugasan diuji untuk menentukan hubungan aliran gen antara ketiga-tiga populasi. Keputusan ujian penugasan menyatakan aliran gen yang tinggi antara Hormoz dan Kuhestak serta aliran gen yang terbatas antara Jask dan kedua-dua populasi Hormoz dan Kuhestak. Kajian morfometrik dan jujukan DNA mitokondria dalam kajian ini mencadangkan bahawa kedua-dua morfotip mungkin dua spesies berbeza serta kajian genetik populasi membolehkan pemilihan induk untuk program pembiakbakaan masa hadapan.

MORPHOLOGYCAL AND MOLECULAR ANALYSIS OF *Penaeus semisulcatus* FROM THE PERSIAN GULF AND OMAN SEA

ABSTRACT

There are two morphotypes of the green tiger shrimp, *Penaeus semisulcatus* recognized in the Persian Gulf and Oman Sea. The two morphotypes are distinguished by colour pattern of antenna and body transverse lines. This study is conducted to investigate the taxonomic status of the two morphotypes based on morphometrics and mitochondria DNA sequence analysis. In addition, population genetics study on *P. semisulcatus* from the Persian Gulf was performed to assist the selection of suitable broodstocks in future breeding programs. A total of 25 characters were investigated for morphometric study. Ten of these characters showed significant differentiation between single sex and combined sex ($P < 0.05$). Regression analysis showed that the correlation between the carapace length and body length of females in the non-banded morphotype, were different to those of the males. Molecular investigation of mitochondria DNA (mtDNA) using partial sequences of 16S rRNA gene showed relatively low genetic differences between the *P. semisulcatus* morphotypes with 3.9 % genetic distance. These sequences were able to distinguish between the two morphotypes, and separated them into two distinct clades. By contrast, genetic divergence detected by COI gene analysis was consistently higher. High genetic divergence for COI with 76 divergent sites was observed between the two morphotypes. Eight novel microsatellite loci were developed to study the population genetics structure of *P. semisulcatus* in three population sites in the Persian Gulf and Oman Sea (Jask, Hormoz and Kuhestak). There were incidences of heterozygosity deficiency and significant deviation from HWE showed at most loci ($P < 0.001$). However, there were four loci (loci E2 and B9

– Hormoz; loci C6 – Kuhestak; and loci H9 - Jask) found to be in the Hardy Weinberg equilibrium. Micro-Checker analysis revealed of null alleles in the three microsatellite loci (B5, C6 and C9). Pairwise Fst comparison based on allelic and genotypic frequencies indicated that the three populations were significantly differentiated from each other (P <0.05). High levels of pairwise Fst (0.106) and low levels of Nm (2.103) observed between the Hormoz and Jask populations indicated restricted gene flow between the two populations. On the other hand low levels of pairwise Fst (0.016) and high levels of Nm (15.876) observed between the Hormoz and Kuhestak populations indicated high gene flow between these populations. In this stydy, the assignment test was examined in order to find gene flow connectivity between three populations. Results from assignment test revealed high gene flow between Hormoz and Kuhestak and restricted genetic flow between Jask and both Hormoz and Kuhestak populations. Morphometric and mitochondrial DNA sequences in this study suggested that the two morphotypes might be two separate species and the population genetics study will allow the selection of broodstocks in future breeding program.

CHAPTER 1
INTRODUCTION

Shrimp is one of the world's most valuable fishery resources. Shrimp of the genus *Penaeus* constitute a diverse and abundant group of benthic taxa found in tropical and subtropical waters (Baldwin *et al*., 1998). Consequently this is one of the most important commercial shrimp in the Persian Gulf and Oman Sea.

The main species of farmed shrimp in Iran is *Penaeus indicus* although cultures of *P. semisulcatus* are increasing in Iranian coastal waters (Niamaimandi, 2006). *Penaeus semisulcatus* is being researched by the Iranian Fisheries Research Organization (IFRO) as a potential aquaculture shrimp. This species exists in two morphological types in the Persian Gulf. One morphotype is with banded antenna (the type for *P. semisulcatus*) while the other morphotype is without banded antenna. One of the main questions facing the aquaculture and brood stocking of *P. semisulcatus* is resolving the taxonomic status and genetic differentiation between the two morphotypes of *P. semisulcatus*.

Genetic definition of stocks has been a major concern towards understanding natural resources in order to ensure sustainability. Different broodstocks or seed stocks often do not show the same growth rate, disease resistance, or other important characteristics (Lester and Pante, 1992). Data on such factors and their correlations with certain genotypes are critical in developing a systematic aquaculture programme.

There have been considerable advances made in recent years, to spur the ease and utilisation of molecular markers. Such markers will assist in the development of the penaeid broodstock selection. One of the most efficient current methods to determine differentiation between and within species is by the use of mitochondrial DNA (mtDNA) and microsatellites. Baldwin *et al.* (1998) showed that sequence analyses of mtDNA to be useful in phylogenetic studies between *P. chinensis* populations in four locations (north coast of the Shandong Peninsula, west coast of Korea Peninsula, 1182 sea area (34± 450N, 124± 450E) in the Yellow Sea, and an aquaculture farm where parent shrimp were captured from wild populations at the Chinese coast of the Yellow Sea).

Lavery *et al*. (2004) used mitochondrial DNA sequences in to reconstruct the phylogeny of the *Penaeus sensu lato* genus of marine shrimp .This phylogeny was used to test the validity of hypotheses on the species groupings, in particular the subgenus/genus subdivision, and on the species evolutionary history.

Microsatellites have become increasingly utilized as genetic markers for studies of population genetics. These are co-dominant markers with high levels of polymorphism, and can generally be assumed to be selectively neutral (Jarne & Lagoda, 1996). Microsatellites have been identified from several marine organisms such as mollusks (Bierne *et al.,* 1998) and squid (Shaw, 1997), and have also been characterized in lobsters (Tam & Kornfield, 1996), and shrimps (Tassanakajon *et al*., 1998 ; Xu *et al*., 1999).

In aquaculture research, microsatellite markers are especially powerful for parentage determination (Taris *et al.,* 2005). They also are very useful for the assessment of genetic diversity in hatchery populations (Hara & Sekino, 2003). Vanavichit *et al*. (1998) used microsatellites as genetic markers in *P. monodon*, and suggested that microsatellites are suitable for shrimp genome study and broodstock management.

Factors such as geographic location, marine currents, life cycle and ecological characteristics can influence the genetic distance and differentiation between populations. These have been demonstrated using microsatellites in the study of the shrimp, *Litopeneaus schmitti* that also assisted in both conservation and breeding programs alike (Maggioni *et al*., 2003). In addition microsatellites have been used to measure genetic variability of captive broodstock and wild populations of *L. vannamei* (Cruz *et al*., 2004). Furthermore, microsatellites were used to investigate genetic diversity in specific pathogen free (SPF) of *P. vannamei* in selective breeding families (Garcia *et al.,* 1994). Li *et al.* (2007) developed two microsatellite multiplex systems for black tiger shrimp *Penaeus monodon* and utilized them in genetic diversity study for two populations.

The traditional method for isolating microsatellite colonies is to create small-insert partial genomic library in a plasmid. Microsatellite enrichment has also been developed to amplify the quantity of colonies in a given library containing the microsatellite motif of interest (Nunome *et al.,* 2006).

Stock identification is essential for sustainable fisheries of marine resources. Morphometric analysis is also an influential complementary approach to genetic and environmental stock detection (Cardin, 2000). Tzeng (2004) recommended that animals with inconsequential morphometric character dissimilarities can also constitute a stock and this approach has been widely used in fishery stock differentiation studies. In recent years, morphological variability has been extensively used to examine both taxonomic and stock relationships in marine and freshwater fishes (Prado *et al.,* 2006).

The objectives of this study are:

1- To characterize two *Penaeus semisulcatus* morphotypes using morphometric indices.

2- To determine the taxonomic status of the two *P. semisulctus* morphotypes based on mitochondria (mt) DNA markers.

3- To develop microsatellite DNA loci of *P. semisulcatus*.

4- To assess the genetic diversity of *P. semisulcatus* in the Persian Gulf and Oman Sea based on the newly developed microsatellite markers

CHAPTER 2
LITERATURE REVIEW

2.1 Overview of the Persian Gulf and Oman Sea

The Persian Gulf is a semi-enclosed sea located between the Arabian Peninsula and Iran (Figure 2.1). With an area of 260,000 km^2, the Persian Gulf has a mean depth of 35 meters and a maximum depth >100m (UNEP 1999). It is separated from the Gulf of Oman and the open ocean throughout the Straits of Hormuz. The western coastline of the Persian Gulf is surrounded by desert plains, the northern coastline by the Arvand rood, and the eastern coastline by extensive mountain series. Due to the diverse environmental situations, a wide variety of marine life can be found in the Persian Gulf, including turtles, whales, dolphins and several species of fish. A lot of these animals are endemic to the Persian Gulf environment (UNEP 2002).

A series of islands are expanded along the western coast have fringing and patch coral reefs, signifying one of the most different habitats of the Persian Gulf. Seagrass beds are found along the coast of Bahrain and Qatar. Some mangrove forests (around 90 km) exist along the southern coast of Iran (UNEP 2002).

The Gulf of Oman is located in the Middle East between Iran, Oman and the United Arab Emirates. It is the opening to the Persian Gulf from the Arabian Sea and Indian Ocean, and therefore an important shipping way for the oil-producing countries in the Persian Gulf. Its maximum width is around 230 mi (370 km), and about 340 mi (545 km) long. It is joined with the Persian Gulf throughout the shallow Strait of Hormuz and generally included as a branch of the Persian Gulf, not as an arm of the Arabian Sea (Limits of Oceans and Seas 1953).

Figure 2.1. Location of Persian Gulf and Gulf of Oman

The base topography of the Persian Gulf is mainly flat and featureless, with soft sediments. It is generally deeper in the southeast, where depths of over 100 m are found, and is deepest near the opening of the Straits of Hormuz. The western part of the Persian Gulf is very shallow, with extensive intertidal areas that are less than 5 m deep and up to 5 km wide (UNEP 2002).

The main oceanic circulation pattern in the Persian Gulf is counter-clockwise and is driven by density gradients. Oceanic enters the Persian Gulf through the surface waters of the Straits of Hormuz, moves northwards along the Iranian coast, turns southwards along the western coast and exits along the bottom of the Straits as dense hyper saline water (UNEP 2002).

The two major freshwater inflows (1,456 m /s) into the Persian Gulf come from the Arvand rood River, which is a mixture of the Euphrates, Tigris and Karuan Rivers. However, due to low rainfall and high evaporation, salinity is high in the Persian Gulf in spite of its large inflow rate, and can reach up to 60 – 70 gr/l in some regions with limited water exchange. Water temperatures show high seasonal fluctuations from 15°C in winter to 35°C in summer (UNEP, 1999).

Oil drops from oil tankers and oil-related industries have contributed extensively to the deterioration of the environment. Around 25,000 oil tankers navigate in and out of the Straits of Hormuz every year, and with all this oil being pumped and transported, the waters have become heavily contaminated with oil residues. Approximately 2 million barrels of oil are spilled into the region every year from the routine discharge of dirty ballast waters and from the 800 or so offshore oil and gas platforms. The illegal discharge of crude and fuel oil by tankers has also been a major source of pollution. Although habitats such as rocky shores, sandy beaches and mangroves have shown dramatic recovery, widely distributed salt marshes are still heavily polluted and will take decades to fully recover with great effects on the marine life (UNEP 2002).

2.2 Shrimp fishery in the Persian Gulf

Fisheries have an ancient history in the Persian Gulf. The best shrimp resources are found on the Iranian side of the Persian Gulf and it was there that trade shrimp fishing started from 1959. Shrimp fishing in Saudi Arabia started around 1963, Bahrain from 1966 followed by Qatar in 1969. In Iran, landings reached 9600 tonnes in 1964-65 and in Kuwait 3335 tonnes in 1966-67, while landings from Saudi Arabia and Bahrain peaked at 7400 tonnes in 1973-74. The period from 1959-69 was a remarkable phase in the industrial shrimp fisheries in Persian Gulf, characterised by high catch-rates, increasing landings and the appearance of new companies (Khorshidian, 2002).

Trawl fishery for penaeid prawns started along the mid-northern shoreline of the Persian Gulf in the 1960s where the Bushehr waters became one the main areas of harvest. The green tiger prawn, (*P. semisulcatus*) which is the focus of this study is one of the most important species in the Persian Gulf. Many fishermen vessels from Bushehr are actively involved in the shrimp fishery (Niamaimandi, 2006). Bushehr fishing area is the main catch area for non banded morphotype. The length of fishing season is flexible. The opening time of a fishing season is determined by pre-season surveys estimating abundance. The catch volume of the shrimp permitted for a fishing season and the determination of the end of fishing season are based on CPUE trends (kg/boat-day) during the fishing season and/or a final catch rate (Niamaimandi, 2006).

There are three major considerations and reasons why the management of the shrimp fishery is important:

- Biological: High mortality and short life duration need a suitable framework of harvesting the shrimp for maximising incomes. High inter-annual variations in the stock and being an easy prey in the food chain would also influence the higher trophic levels.

- Economical: Controlling based on stock assessment reduces the doubts in investment due to non-predictable stock fluctuations. High domestic demand and good export market make shrimp fishery industry important to improve the economy and subsequently quality of life for the people in the Persian Gulf area.

- Social: The finfish fishery almost stops in summer due to the migration of fishes to colder waters. The shrimp season continued about six weeks in the summer when there is low activity in the fish sector. Shrimp is also a main source of protein and valuable in food nutrition. It is tasty and easy to prepare (Khorshidian, 2000).

2.2.1 Target species and by-catch

The main target shrimp species in Bushehr is the *P. semisulcatus* (non banded morphotype) (around 80%), and fishing mostly takes place during daytime. However, additional income is also generated from by-catch. The by-catch is grouped into three major groups: small adult fish, juveniles, and large adults, according to the Iranian Fisheries Company (Shilat). The large fish include about 8-10% of the whole catch by weight while the other by-catch species, 60-65%. Shrimp contain the remainder of the catch. *Penaeus semisulcatus* is the main species in the northern part of the Persian Gulf, while *P. merguensis* (Banana shrimp) is the major species in the southern part (Hormoz Straits) (Khorshidian, 2000).

2.3 Biology and ecology of the penaeid shrimp

The world catch of shrimp in 2000 was recorded at over three million tonnes. From 1970 to 2000, Penaeidae on average include 42.2% of the world catch of shrimps (FAO 2000). Penaeid shrimps have for many centuries been considered a useful resource of food. Many species live in shallow, inshore tropical and subtropical waters and a lot of have been bred and cultured in ponds. Around 125 species are recognized from the broad west Indo-Pacific region (Dall *et al.,* 1990). Penaeid shrimps are fast growing and short lived, typically with a life cycle of about one year.

2.3.1 Biology of penaeid shrimp

2.3.1.1 Life cycle

Penaeidea shrimps have four types of life cycles, defined by the habitats of each life stage. The life cycle goes through several stages of development: from fertilized egg to nauplius, zoea to mysis, post larva to juveniles and finally juveniles to adult. According to Dall *et al.* (1990) the life history patterns of Penaeid shrimp can be classified into four different categories.

-Type 1: All periods of the life history are estuarine and the eggs may not be entirely demersal. Some or all of these species may move into sheltered inshore waters to spawn.

-Type 2: The post larvae migrate to estuarine nursery grounds. As the juveniles mature, they migrate from the estuaries; some species spawn comparatively closer to the inshore, while others shift into deeper waters of the continental shelf for spawning. Some species may have pelagic eggs.

-Type 3: The postlarvae migrate to shallow inshore waters, frequently with seagrass and algal beds with high salinity; offshore migrations are like to those of Type 2. Some species in the group have pelagic eggs.

-Type 4: The full life cycle is in offshore waters. Species maybe do not have a benthic phase (specified by a separate cycle). Others are deep-water species with benthic juveniles and adults; eggs of these two sub-groups are possibly pelagic.

Penaeus semisulcatus has a Type 3 life cycle (Figure 2.2). They spawns in offshore and the larvae develop in that area but the juveniles grow in sea grass or algal beds inshore and in the lower reaches of estuaries (Jackson *et al.*, 2001). Dall *et al.* (1990) proposed three models to explain life history of shrimps namely equatorial, tropical/sub-tropical and moderate. Due to its geographical area, *P. semisulcatus* in the Persian Gulf may exhibit the tropical/sub-tropical pattern.

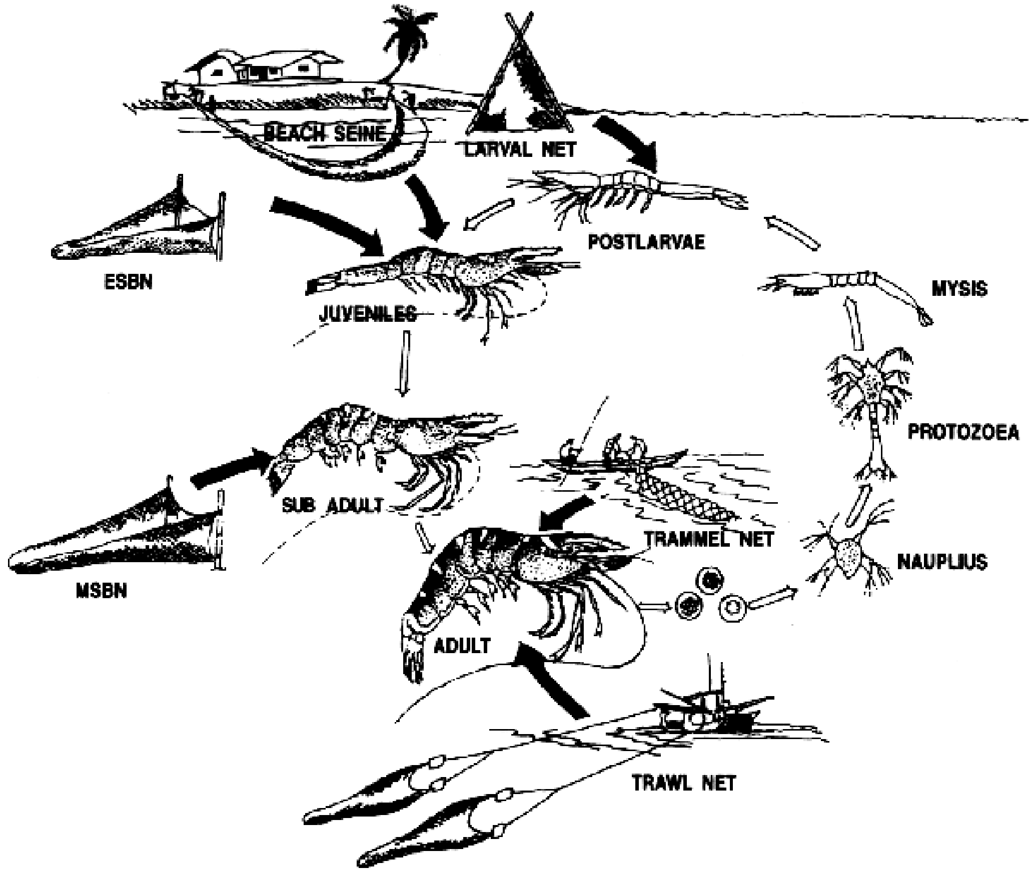

Figure 2.2. General life cycle of penaeid shrimp which are targeted by different fisheries (modified from Khan & Latif ,1995)

2.3.1.2 Migration

The penaeids often live in different habitats in different stages of the life history. Thus, they have to migrate between these habitats to complete their life cycle. Four types of migrations are found (1) larval and post larval migration from spawning to nursery ground, (2) juvenile migration out of the nursery area, (3) adult migration, usually to deeper waters offshore and (4) spawning migration in several species (Dall *et al.*, 1990).

Generally penaeids species spawn in offshore, while the juveniles occupy estuarine or inshore habitats (Types 2 and 3). Vertical migration throughout the pelagic larval phase, together with transport by water currents, is the main method that takes post larvae to their nursery areas. The larval stage of penaeids is moderately short, around three weeks. During this period, the larvae may move more than 100 km among the offshore spawning ground and the inshore nursery habitats (Jackson *et al.,* 2001). Lonergan (1994) found that the post-larvae of *P. semisulcatus* in the Gulf of Carpentaria (Australia) settled in seagrass beds along the coastal and estuarine habitats. The juvenile nursery habitats can differ according to species. Their investigation showed that *P. semisulcatus* juvenile were mainly inhabited in sea grass and algae beds of shallower waters in 200 m of the inter-tidal zone. Therefore, only the seagrass beds in shallow waters seems to be important in settlement and nursery areas for tiger prawns in the Gulf of Carpentaria (Khorshidian, 2002).

One important area which can be considered a good nursery habitat for shrimp larvae is the mangrove forest. Iranian mangrove forests are extended in the northern part of the Persian Gulf and Oman Sea. The areas of Iranian mangrove forests are approximately 10700 ha which maximum area of 67.5 km^2, extended between Khamir Port and northwest of Qeshm Island, and minimum area of 0.01 km^2 in Bardestan estuary (Zahed *et al,.* 2010).

Mangrove forests are nursery and feeding ground for fishes and many species of epifauna and also nesting and breeding or reproduction place for sea and seashore birds (Biagi & Nisbet, 2006). Within Iranian mangrove forests, fishing and shrimp culture activities are carried out and these forests can be considered for ecotourism. In addition to being nursery to numerous estuarine organisms, mangrove forests also protect coastal area and shoreline from severe waves, sea storm and erosion (Zahed *et al.,* 2010).

After the nursery period, the juveniles migrate to offshore, to deeper waters, with probable movement to the shore (Dall *et al.,* 1990). Juveniles of *P. semisulcatus* were found generally at a depth of less than 20 m while the dispersion of subadults extended more (Somers, 1987). The migration from a habitat to another needs the shrimps to respond either to some internal physiological cue associated with size, or to some transformation in their environment, or both (Dall *et al.,* 1990). For instance, *P. semisulcatus* has been found to migrate mainly during the full and new moon (Khorshidian, 2002).

Gulland (1984) described on a probable latitudinal gradient in migratory behaviour of penaeid shrimps. Within the Persian Gulf, tagging experiments in Kuwait, have shown that adult migration is normally restricted. The maximum-distance was recorded as 85 km, however, most captures were of much shorter distance and no marked shrimps were reported from other countries (van Zalinge, 1984).

2.3.1.3 Spawning

All Type 3 species seems to spawn on the continental shelf area, generally under 100 m depth. The larvae from demersal eggs are able to migrate into the upper layers. Although Type 3 typically exhibits offshore spawning, inshore migration prior to spawning has also been observed in *P. semisulcatus* (Khorshidian, 2002). Dall *et al*. (1990) reported that the life history dynamics differ significantly between and within species, maybe in response to differing environmental situations.

2.3.1.4 Growth

A typical report of growth for the whole life cycle has not been published for any penaeid species. However, the penaeids may conform to the typical crustacean growth model of S-shaped growth in different life history stages. Most approximations of growth refer to the later life history stages including the change in growth rate with size (Dall *et al.,* 1990).

2.3.1.5 Seasonality

A regular bimodal seasonal dispersion in the abundance of juveniles was known throughout six-year research on this species in Australia but the size of catches was different from year to year (Jackson *et al.,* 2001). The reproductive activity of *P. semisulcatus* in Australia may have two peaks, in September and during January to March. They spawn in deep waters around 40 to 70 m. It has a wide spawning season and broad geographic diversity; so the larvae of *P. semisulcatus* can tolerate a wide range of temperature (21°C to 30°C) and salinity (28 to 35 ppt). The larvae possibly move more than 100 km between the offshore spawning grounds and the inshore nursery habitats (Jackson *et al.,* 2001).

2.3.1.6 Maturity

The carapace length (CL) at first maturity of females is 29 mm (Crocos, 1987). Egg production is seasonal, with a main spawning peak from August to September and also a minor scale in February. February peak occurred only in a special area in the spawning grounds. The essential pattern of spawning for *P. semisulcatus* is when some individuals of a group breed at about six months of age, but the major spawning usually occurs at the age of 10-12 months.

The spawning pattern, in the Persian Gulf, as estimated by the difference in percentage of mature females and also by post larval incidence, shows two peaks for each year, one in spring and the other one is takes place in autumn. These peaks are different in relative importance from year to year, although the spring peak was the more important compared to the autumn peak. Age at spawning was 12 months for the majority of the shrimps. Regularly spawning take places near shore on muddy sand bottoms (Khorshidian 2002).

2.4 Taxonomy of *Penaeus semisulcatus* (De Hoan, 1844).

The morphological appearance of *P. semisulcatus* is shown in Figure 2.3.

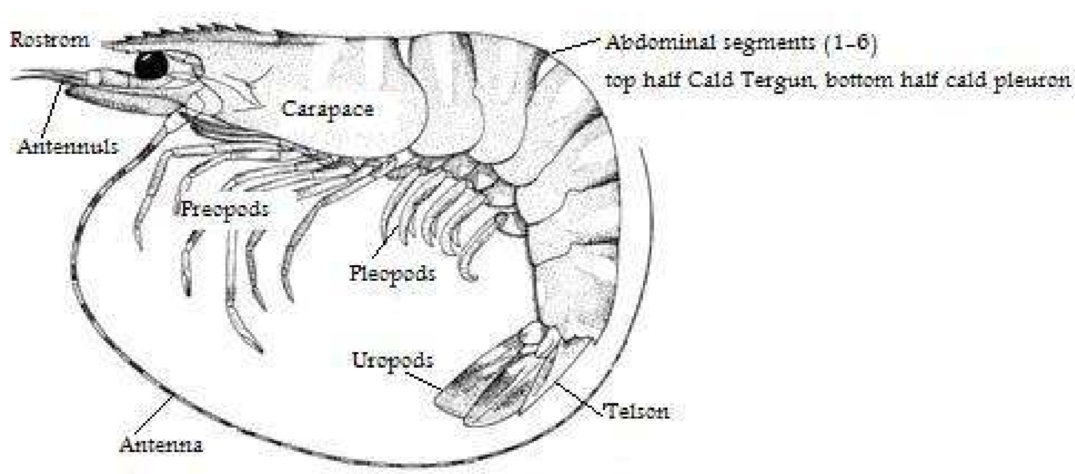

Figure 2.3. Morphology of *Penaeus semisulcatus* – the banded morphotype (Holthuis, 1980).

Clasification of *Penaeus semisulcatus* is as follows (Holthuis, 1980):

Phylum: Arthropoda

Class: Crustacea

Series: Eumalacostraca

Superorder: Eucarida

Order: Decapoda

Suborder: Natantia

Infraorder: Penaeidea

Superfamily: Penaeoidea

Family: Penaeidae

Genus: *Penaeus*

Subgenus: Penaeus

Species: *Penaeus semisulcatus*

2.4.1 Description of *P. semisulcatus* (Holthuis, 1980)

2.4.1.1 Distinctive anatomical characters

The carapace of *P. semisulcatus* is smooth. The rostrum is armed with 5 to 8 teeth on dorsal, and 2 to 4 teeth on the ventral margin; adrostral crest and groove extending beyond epigastria tooth. The post rostral crest almost reaches the posterior margin of the carapace, with a distinct median groove. The gastrofrontal crest is absent; antennal crest very prominent, ending above posterior third of the hepatic crest (Carina). The gastro-orbital crest extends over posterior 2/3 of the distance between the hepatic spine and the orbital margin. The hepatic crest is straight, sloping anteroventrally; fifth pereopod with exopod (Figure 2.5 and 2.6).

The petasma (in males) with distomedian projections reaches as far as the costae. The free border of the ventral costae is unarmed or minutely serrated near the apex while the outer surface of the lateral lobes is minutely tuberculate. The thelycum (in females) has lateral plates, their median margins forming tumid lips. The anterior process has raised edges, delimiting a depressed area. Posterior process is convex, partly inserted between the lateral plates (Holthuis, 1980) (Figure 2.4).

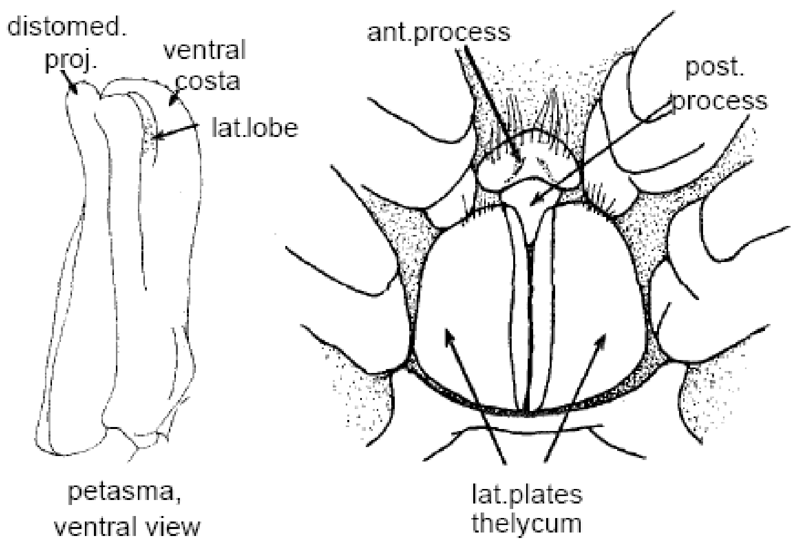

Figure 2.4. Anatomical characters of reproductive system in penaeidea family shrimp (Holthuis, 1980).

2.4.1.2 Colour variation

The body is pale brown, sometimes greenish. The carapace is often with 2 yellow-cream dorsal transverse bands. The abdomen is brown-grey with pale yellow dorsal transverse bands. The antenna is banded white and brown. The pereopods and pleopods are dull red to brown grey, with white, sometimes bluish, specks. The proximal part of the uropods is yellowish, while the distal part is brown-bluish to brown greenish. The proximal part of the uropods is yellowish while the distal part is brown-bluish to brown greenish. The tips are bluish or reddish. The fringe of setae usually brown-red. Juveniles are creamish, with irregular mottled brown bands on all abdominal segments (Holthuis, 1980).

2.4.1.3 Distinguishing characters of sympatric species

Penaeus monodon

For *P. monodon* the adrostral crest and groove reach at most as far as the epigastric tooth compared to *P. semisulcatus* where they extend further than the epigastric tooth. The hepatic crest (carina) is horizontal but sloping anteroventrally in *P. semisulcatus*. The fifth pereopod is without exopod compared to *P. semisulcatus* which has an exopod in *P. semisulcatus*. Furthermore, there are also differences in colouration (Figure 2.5).

***Penaeus (Fenneropenaeus*) species**

The hepatic crest is not clear in Penaeus (Fenneropenaeus) species. The colour of the body is white to pinkish and not banded. Adrostral crest and long groove, extending near to the posterior margin of the carapace in the other species of *Penaeus*. The gastrofrontal crest is clear (absent in *P. semisulcatus*). The distal part of uropods is generally blue. Maximum total length males in males 18 cm and in femeles 23 cm (Holthuis, 1980).

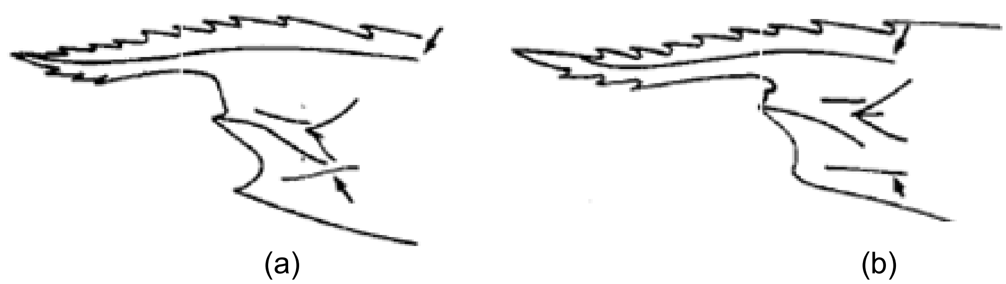

Figure 2.5. Anterior part of carapace in (a) *P. semisulcatus* and (b) *P. monodon* (adapted from Holthuis, 1980).

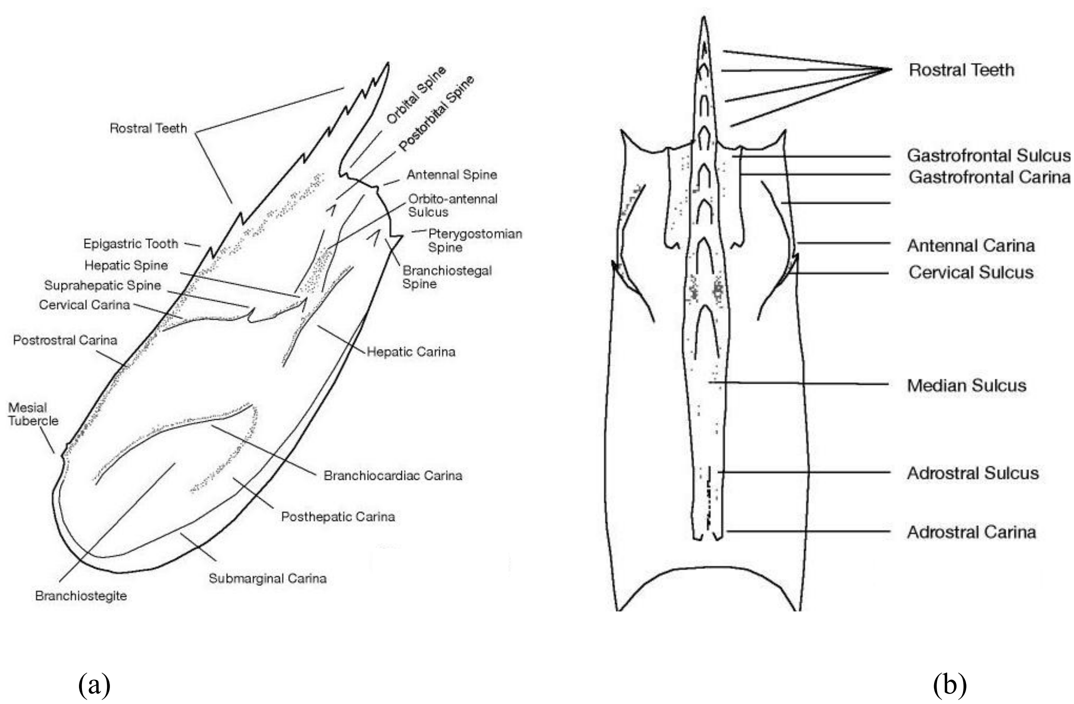

Figure 2.6. Carapace (a) Lateral view (b) Dorsal view (adapted from Holthuis, 1980).

2.4.1.4 Geographical distribution

Penaeus semisulcatus is distributed from south and east Africa to India and Sri Lanka, including the Red Sea, the Persian Gulf and western Madagascar. Its distribution further extends north as far as Korea, Japan, the Philippines and south in New Guinea and northern Australia; the species has also entered the eastern Mediterranean through the Suez Canal (Holthuis, 1980) (Figure 2.7).

Figure 2.7. Global geographical distribution of *P. semisulcatus* (Holthuis, 1980).

Penaeus semisulcatus live in the continental shelf from the seashore to depth of about 130m, but is most abundant in water less than 60 m deep over mud, sandy-mud or sandy-grit (Holthuis, 1980).

2.5 Application of morphometric characters in shrimp population studies

Morphometric characters are quantitative measurements of lengths between defined anatomical characters. They are extensively used in order to differentiate between fishery stocks (Avsar, 1994) and species. Morphometric variation can be influenced by various factors that include stress, food or reproductive cycle (Chu *et al*., 1995).

Morphometric characteristics have been analysed in order to differentiate stocks of *Penaeus* species such as *P. stylirostris* (Aubert & Lightner, 2000) and *P. japonicus* (Tzeng & Yeh, 1999). They have also been utilized in statistical taxonomic analysis of shrimp in order to investigate differentiation among members of the genus *Metapenaeus* for example *M. bennettae* and *M. dalli* (Salini & Moore, 1985). Two morphotypes of *P. subtilis* have been recognized based on morphometric differentiations (Perez farfante, 1969). However, one of them was recently suggested as a new species based on molecular examinations (Gusmao *et al*., 2000).

In penaeid shrimps, difference in the length-weight connections among sexes, species, and throughout different seasons, and locations, have been described for both natural and cultured populations (Primavera *et al.,* 1998). Seasonal changes in the length-weight relationship of wild shrimp populations have been correlated to seasonal variations in reproductive biology, where mature shrimps have higher weight/length fraction than immature ones (Chu *et al*., 1995).

Many studies have been conducted to elucidate evolutionary relationships in *Penaeus* species. Recent research indicated that *Penaeus* relationships based on morphological parameters can be misleading (Tsoi *et al*., 2005). Palumbi and Benzie (1991) showed relatively high genetic differentiation between two morphologically and ecologically similar species belonging to the kuruma shrimp, *Litopenaeus japonicas*. Although it can be easily bred, most hatcheries still depend on wild populations as the source of broodstock for generating larvae of higher value. (Benzie, 1998). Thus, determination of the source of wild broodstock is very important.

The fact that we can witness large genetic distances among morphologically similar species might lead us to conclude that this is a recurring pattern within the family Penaeida. In these situations, molecular genetics can be a valuable tool for reexamination of the systematic relationships of Penaeid shrimp (Baldwin *et al.,* 1998).

As organisms are essentially multidimensional, multivariate approaches are essential to obtain a precise assessment of morphological variation between various taxa. According to Grandgean *et al.* (1997), morphometric investigations in crustaceans based on multivariate analysis have been directed mostly to studies of growth patterns and determination of sexual maturity. Morphometric variation between stocks could supply a basis for stock structure, and would be applicable in order to investigate short-term, environmentally induced variation (Tzeng, 2004)