

Thesis
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MORPHOMETRIC, GENETIC AND REPRODUCTIVE
CHARACTERISTICS OF MUD CRABS (GENUS *SCYLLA*
DE HAAN, 1833) FROM SOUTHEAST ASIA

Thesis submitted to the University of Stirling
for the degree of Doctor of Philosophy

by

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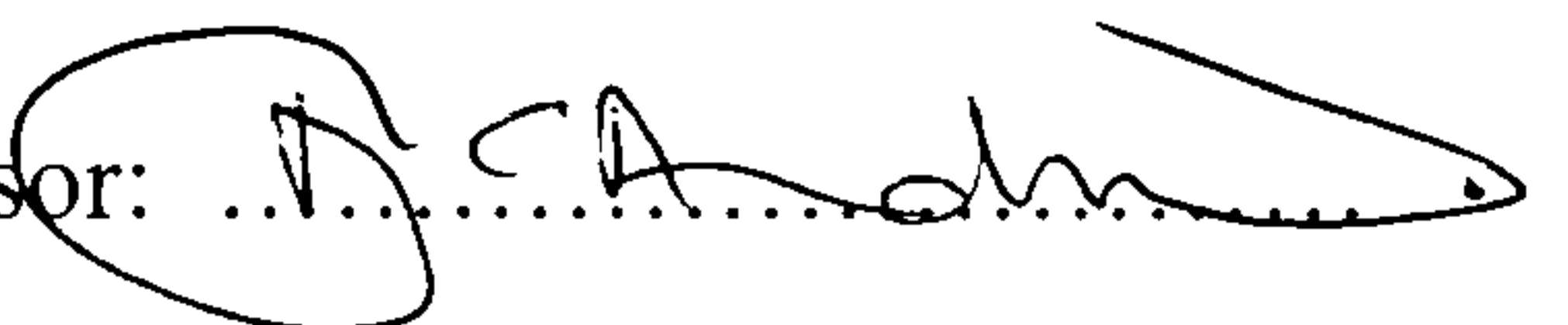
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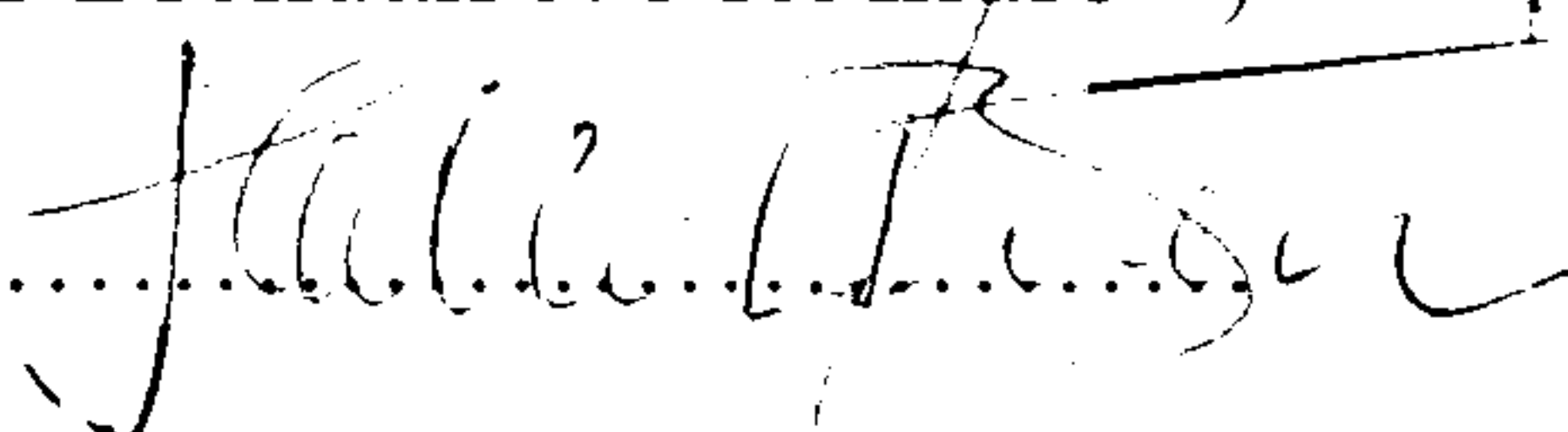
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Abstract

The edible mud crab, genus *Scylla*, is important to fisheries and aquaculture throughout the Indo Pacific region, but its taxonomic status has been confused for decades and a new classification has only recently been proposed. This project was undertaken to investigate the species status of mud crabs in Southeast Asia, with a view to deciding whether two sympatric morphs of *Scylla* found in Ban Don Bay, Surat Thani Province, Thailand, are two separate species. A further aim was to elucidate any possible pre-zygotic reproductive isolating mechanisms (RIMs) and ecological features that maintain the apparent sympatry between these two morphs.

Mud crabs were collected from a primary site (Surat Thani, Thailand) as well as from six other locations in Thailand, Vietnam, Malaysia and Bangladesh. Crab samples from the latter sites were used selectively to provide a comparison to the primary study site.

Descriptive taxonomy, multivariate morphometrics and allozyme electrophoresis were used to a) determine the number of species present within the crab samples collected; b) to ascertain which species they represent; c) to discover any geographical variation between locations sampled; d) to produce a possible phylogeny that summaries the relationship between *Scylla* species; and e) to look for pre-zygotic RIMs to explain the sympatry of the two morphs in Surat Thani.

Findings from the present study reinforce the recent revision of the taxonomy of the genus *Scylla* into four species, *S.serrata*, *S. olivacea*, *S.tranquebarica* and *S. paramamosain* and provides new information on two of the four species which are dominant within Southeast Asia, including Ban Don Bay, Surat Thani Province, *S. paramamosain* and *S. olivacea*.

Population studies showed both genetic and morphological differentiation between conspecific populations of *S. paramamosain* and *S. olivacea*, indicating stock structure for each species, although there is some disparity between morphological and genetic distances for *S. paramamosain*. This is discussed in relation to the effects of larval dispersal mechanisms and the subsequent recruitment of juvenile crabs.

Phylogenetic interpretation of both genetic and morphological characters revealed that both *S. serrata* and *S. olivacea* are the most diverged of the four *Scylla* species; however, the direction of evolution is open to interpretation and the evidence for either *S. olivacea* or *S. serrata* as the more primitive species are discussed.

Reproductive studies on the two mud crab species found in Surat Thani revealed no physical barrier to hybridization. Both species have a protracted breeding season which continues throughout the year. However, the size at first sexual maturity was significantly smaller for *S. olivacea* when compared to *S. paramamosain*. This and other potential mechanisms that may maintain these two species sympatrically are discussed.

The clarification of four *Scylla* species, and the establishment of diagnostic genetic and morphological characters that can be used to identify them, means that research can now focus on both the ecology and life history of these closely related species. Such information is needed urgently with respect to fisheries management as well as to understanding the environmental requirements of each species in order to develop their potential for aquaculture.

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Chapter One

General Introduction

A. Introduction to the genus *Scylla*

1.1 Distribution and habitat of the mud crab

Mangrove crabs or mud crabs, genus *Scylla*, are the only crabs in the family Portunidae (swimming crabs) that are characteristically found inhabiting tropical and sub-tropical mangrove swamps, although their habitat also extends to mangrove estuaries, embayments and surrounding coastal waters (MacNae, 1968. Brown, 1993).

The natural range of the mud crab extends longitudinally from the east coast of Africa to west pacific islands (Brown, 1993). The mud crab is also found in more temperate climates due to the ability of adult crabs to tolerate temperatures from 12°C to 35°C (Hill, 1979). The most northern limits of its distribution are the Southeast coast of China (Zeng and Li, 1999) and south Japan (Fuseya and Watanabe, 1996). Table 1.1 provides a summary of the countries where mud crabs are known to be part of the local mangrove fauna.

Decapod crustaceans are the dominant animal group within the mangrove forest, although the mud crab, *Scylla*, is usually only associated with the lower intertidal zone of the mangroves (Macintosh, 1982; Alongi and Sasekumar, 1992). Adult mud crabs have the ability to withstand a wide range of salinity (Davenport and Wong, 1987), with a

Table 1.1: Summary of countries where the mud crab, genus *Scylla*, is known to occur.

Region Country	Reference
North and East Africa	
South Africa	Hill, 1979; Robertson, 1996
Mozambique	Crosnier, 1962
Mauritius	Barnard, 1950*
Madagascar	Barnard, 1950*
Red Sea	Stephenson and Campbell, 1960
Central and South Asia	
Pakistan	Keenan <i>et al.</i> , 1998
India	Kathirval and Srinvasagam, 1991
Bangladesh	Ahmed, 1992; Overton and Macintosh, 1997
Sri Lanka	Jayamane, 1991
East Asia	
Thailand	Macintosh <i>et al.</i> , 1993
Malaysia	Ong, 1966
Indonesia	Holthius, 1978
Vietnam	Serene, 1952; Hong and San, 1993
China and Taiwan	Tamura, 1966*
Hong Kong	Shen, 1932
Japan	Oshiro, 1991; Fuseya and Watanabe, 1996
Philippines	Arriola, 1940; Estampador, 1949
Papua New Guinea	Quinn and Kojis, 1987
Australia	Stephenson, 1972
West Pacific	
New Zealand	Stephenson and Campbell, 1960
Hawaii	Brock, 1960*
Caroline and Mariana islands	Bablet and Cayet, 1972*
Samoa	Bablet and Cayet, 1972*
Tuamotas	Bablet and Cayet, 1972*
Solomon Islands	Brown, 1993
Fiji	Brown, 1993
Vanuatu	Brown, 1993
New Caledonia	Delathiere, 1988 *

* Cited by Brown, 1993.

tolerance limit from 2‰ to 60‰, (Hill, 1979). Thus, they can be found inhabiting a wide range of niches from the lower reaches of rivers at the limit of tidal influence to deeper subtidal areas and coastal shelf waters (Hill, 1994). At low tide, mud crabs either migrate to subtidal areas (Hill *et al.*, 1982), or alternatively burrow into the soft alluvial mud along river embankments (Zoutendyk and Bickerton, 1988). These burrows are deep (approximately 2m) and extend to below the water table, providing a cool, protected environment (Fielder and Heasman, 1978).

1.2 Life history and reproductive biology

As well as providing shelter from predation and desiccation, especially during moulting, the mangrove forest also provides a good source of food items. *Scylla* is both a scavenger and cannibalistic (Arriola, 1940). Mud crabs are opportunistic feeders, feeding primarily at night (Hill, 1976, Prasad *et al.*, 1984) on slow-moving or sessile benthic macroinvertebrates (Hill, 1976; Fielder and Heasman, 1978), including crustaceans such as grapsid and hermit crabs, and bivalve molluscs (Hill, 1979); their diet may also extend to include fish and marine snakes (Voris and Jefferies, 1995). Monitoring the movement of mud crabs has shown that they have a home range that is generally restricted to within mangrove estuaries and bays. This movement is particularly curbed between neighbouring areas when there is an area of unsuitable habitat in between (Hyland *et al.*, 1984). Juvenile and sub-adult mud crabs remain well within the mangrove zone for protection from potential predators *e.g.* monkeys, birds, and larger fish species, whereas larger adult crabs are believed to spend more time in sub-tidal areas, although the degree to which individuals move between estuarine and coastal waters is not properly understood (Hyland *et al.*, 1984). There may also be sub-populations of mud crabs living offshore, whereas other groups prefer to remain within brackish water areas. The mud crab's use of the

mangrove habitat for food and shelter, in addition to its relatively high position in the food chain, makes it a good biological indicator in assessing the status of mangrove ecosystems, including the effects of pollution (Mud crabs have been used as indicators of bioaccumulation of heavy metals in pollution studies (Narayanan *et al.*, 1987)).

As in most portunids, courtship is complex in *Scylla* (Ong, 1966). Successful mating requires the female to moult prior to intercourse. For male mud crabs to be successful in mating with a female they must protect a receptive female against other males both before and after mating has occurred, while the female exoskeleton is soft. The male transfers the sperm using two specialised pleopods (known as gonopods) which are inserted into the female's pair of genital openings and the sperm deposited in the spermathecum (Ong, 1966). The female mud crab will retain the sperm, sometimes for long periods of time, until she is ready to spawn (Du Plessis, 1971). A female produces approximately two million eggs during a single spawning and may spawn more than once in her lifetime (Brown, 1993). These eggs are retained under the female abdomen attached to the female pleopods for about 17 days where they change from orange to grey in colour prior to hatching. During this time, it is well known that these berried or "sponge-bearing" females migrate seawards to spawn (Arriola, 1940; Hill, 1994). The eggs hatch into planktonic zoea larvae and over a period of three weeks these larvae undergo five moults, during which time they are transported semi-passively by tidal currents back to the coastline. The final zoeal stage then moults to the megalopa stage. Thereafter settlement occurs on a suitable substratum (Ong, 1964). After five to 12 days the megalopa moults into the first crab stage. The time between hatching and the first crab stage is approximately one month within tropical latitudes (Ong, 1966).

It is known that juvenile crabs return to the mangrove zone, presumably for food and protection, as noted for other crustacean (shrimp) and fish species (Roberston and Duke, 1987; Vance *et al.*, 1990). However, little is known about the recruitment processes involved when young crabs enter the mangrove zone at about two centimetres carapace width (Hyland *et al.*, 1984).

Crustaceans have no bones or scales, as are used to age vertebrates, thus their average life span can only be estimated. Modern methods to age crustaceans are being developed. Concentrations of the pigment lipofuscin (also known as the “age pigment”) have been shown to correlate with the age of juvenile lobsters, *Homarus gammarus* (Donovan and Tully, 1996). However, the concentrations of lipofuscin in crustaceans can be affected by factors such as water temperature, population density and individual metabolic processes (Donovan and Tully, 1996). The lipofuscin method of aging has not been tested on mudcrabs. Hill (1975) recorded the natural mortality rate (*i.e.* on a population with no fishing mortality or recruitment) as 40% for mud crabs that are two years old and 60% for crabs during their third year. The estimated maximum life span of mud crab is approximately five years old (Ryce, 1995).

1.3 Present and potential economic importance of mud crab

1.3.1 Trading and marketing

Meat crabs for the restaurant trade dominate the mud crab market, however, there are a range of other mud crab products that are also traded. Wild caught seed crab and juveniles are traded to crab farmers to stock ponds along with recently moulted crabs for fattening. Adult crabs are sold either as meat crab (mainly males with large chelae and high muscle content), soft-shell crab (recently moulted crabs where the shell is still soft), or egg crab

(female crabs with mature ovaries) (Overton and Macintosh, 1997). Other processed, value added products include canned, frozen or chilled crabmeat (Brown, 1993).

The consumption of whole crab products is highest in those countries with Chinese communities, where the mud crab is viewed as a delicacy. These countries include Hong Kong, China, Taiwan, Malaysia, Thailand (Ferdouse, 1990, Harvey, 1990;) and Australia in cities such as Melbourne and Sydney, which have large Asian populations (Mounsey, 1989). In particular the female "egg crab", which is believed to be beneficial to human virility in Taiwan, can fetch a premium price (Chen, 1990).

The main exporting countries which meet this demand are developing countries with extensive coastlines, including India, Sri Lanka, Bangladesh, Indonesia, Vietnam, Thailand, Malaysia, Fiji, Myanmar, Papua New Guinea and the Philippines (Ferdouse, 1990; BOBP, 1991; Chong, 1993).

There is little information on the marketing of mud crab. This is primarily due to the complex market chains used to trade mud crab involving a number of middlemen and dealers. Moreover, crabs caught by local fishermen are often sold directly to distributors and the sales are rarely recorded. Even in Australia, where mud crab products are handled by the state fish marketing board, an absence of recorded sales by distributors is seen as a problem in following the market trends (Knuckey *et al.*, 1995).

The mud crabs are graded and priced according to size and sex. The morphology can also affect the price of mud crabs. In Thailand there are two phenotypes making up the mud crab fishery, (known locally as Boo Khao and Boo Dum, or black crab and white crab

respectively). The white phenotype has a higher value due to its clean appearance and larger adult size. Table 1.2 shows an example of prices in Surat Thani, Thailand, for different size, sex and morphology categories of mud crab.

An additional problem in assessing the market chain is that prices alter according to availability, which may fluctuate according to seasonal abundance of mud crab (Knuckey *et al.*, 1995). This problem of fluctuating supply could be met by production of crab through aquaculture. However, the problems of providing a hatchery produced seed supply means that presently this is not yet a reality. If this problem can be overcome then other potential markets such as the soft-shell crab and processed crabmeat markets in the

Table 1.2: Market prices for the mud crab, genus *Scylla*, in Surat Thani Province, Thailand.

Grade	Black morph		White morph		
	Buy THBkg ⁻¹	Sell THBkg ⁻¹	Buy THBkg ⁻¹	Sell THBkg ⁻¹	
Males					
	Grade A (>500g)	200	230	330	350
	Grade B (<500g)	130	160	230	270
Females					
A	Egg crab (>250g)	220	270	300	80
B	Without eggs:				
	Soft shell (>200g)	100	120	130	160
	(<200g)	70	100	60	80
	Hard shell (>200g)	130	230	(>300g) 130	170
	(<200g)	90	120	(>500g) 200	230
C	Small eggs (>300g)	160	**	(<300g) 100	125**
				(>300g) 220	250**

Source of data: Overton, unpublished data, 1996

** Female crabs with small or underdeveloped/immature ovaries are often taken to a farm and allowed to mature fully into “egg crab” in order to sell for a higher price. THB = Thai Baht, exchange rate 1USD = 24.59THB (November 1996).

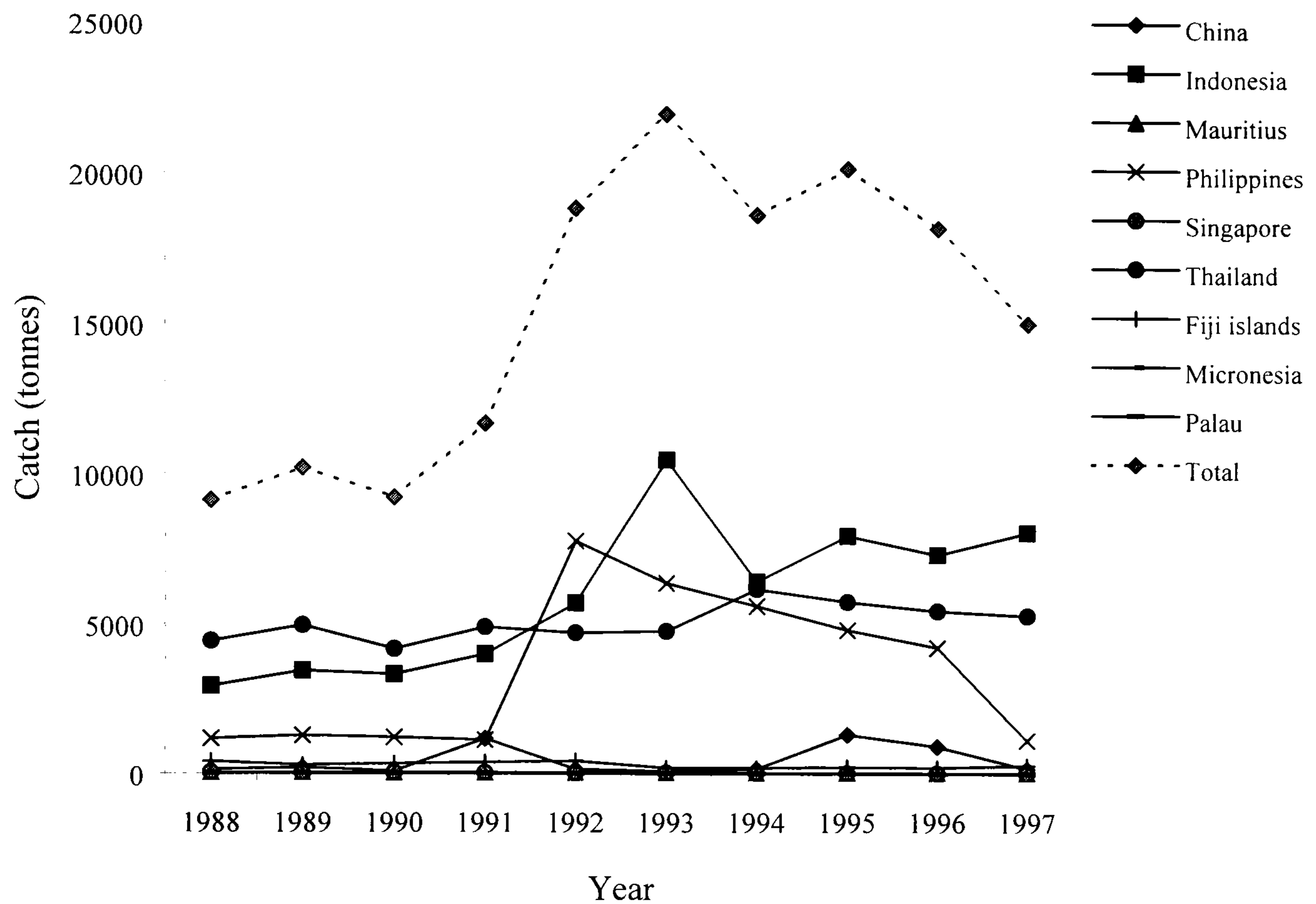
United States, Europe and Japan could be targeted for future exports. Such processed products can be worth three times more than whole crabs (Kennedy, 1985).

1.3.2 Mud crab fisheries

Presently, the market demand for mud crab is supplied predominantly from capture fisheries. The close relationship between the mud crab and the mangrove forest means that the mud crab has only been accessible to coastal fisherfolk rather than commercial fisheries (Overton and Macintosh, 1997). Mud crab fishing is artisanal, and fisherfolk use an array of different fishing tools, including collecting by hand and using simple tools such as hooks and rakes during low tide, and gill nets, seine nets, pole traps, and box traps during high tide and in subtidal areas around the mangrove fringes. This artisanal style of fishing limits the amount of crab caught on any one trip. However, the high value for mud crab means that, the numbers of people fishing both for recreation and professionally tend to be extremely high. Therefore the fishing effort can be intense within a restricted area of habitat, such as a coastal mangrove forest or a bay. For example, in North Vietnam there are reports of whole villages going crab catching in the high season (July to September) when the juvenile crabs are abundant within the mangrove forest (DRC, 1999). Children can earn USD1 – 2 per day playing truant from school to catch mud crabs (DRC, 1996).

World annual catches of mud crab peaked in 1993 at 22,030 tonnes (see figure 1.1).

However this peak in catch was short-lived, the tonnage caught after 1993 declined to 15,088 tonnes in 1997 (FAO, 1999a). One reason for the decline in mud crabs caught could be heavy exploitation. Other evidence of heavy exploitation has been recorded. For example, in Ranong, Thailand, the average size of mud crabs caught has reduced dramatically in recent years. Ten years ago (1990) 50% of female mud crab caught were immature compared to over 90% of catches in 1995 (Ryce, 1995).



Year	Capture production (tonnes)									
	China	Indonesia	Mauritius	Philippines	Singapore	Thailand	Fiji islands	Micronesia	Palau	Total
1988	125	2935	0	1178	31	4437	414	5	5	9130
1989	199	3455	0	1287	27	4965	277	5	5	10220
1990	80	3343	0	1235	38	4182	340	5	5	9228
1991	1193	4022	0	1144	33	4926	382	3	6	11709
1992	158	5729	0	7788	32	4730	435	3	6	18881
1993	84	10509	0	6401	38	4788	198	4	8	22030
1994	169	6441	0	5635	33	6194	202	4	10	18688
1995	1339	7980	22	4835	27	5776	234	5	10	20228
1996	935	7342	23	4258	19	5468	208	5	9	18267
1997	180	8100	18	1153	15	5318	290	5	9	15088

Figure 1.1: Fisheries production of mud crab, *Scylla*, from 1988 to 1997 (from FAO, 1999a).

In addition to fishing pressure, there are other factors determining the present status of mud crab stocks in Southeast Asia. Firstly, the declining quality of suitable coastal environment through the conversion of mangrove areas for large scale shrimp farming (Beveridge *et al.*, 1997 and references therein) and the clearing of mangrove forest for wood products, as well as the declining water quality from the discharge of industrial and agriculture effluents around coastal areas (Liong, 1993). Secondly, the fishing for gravid females due to their high market price means that the number of recruits to support the current crab stocks will be reduced. In Australia, legislation prevents the fishing of female mud crab in order to protect the future broodstock; however in Asia no such fishing restrictions apply, although they have been suggested (Macintosh *et al.*, 1991; Brown, 1993;). Thirdly, most of the fishing gear used is largely unselective in the sizes of crabs it catches. Therefore, a large proportion of the crabs caught are undersized and removed before they have a chance to breed. Furthermore, with the recent expansion of soft-shell crab farming in Thailand, Vietnam and Indonesia, there is an increase in the demand for small crabs. This recruitment overfishing may lead to irreversible collapse of *Scylla* stocks.

1.3.3 Mud crab culture

Coastal fisheries and aquaculture play an important role in the nutrition and economy of the Indo West Pacific region (FAO, 1999c), particularly in less developed countries within the region (Subasinge *et al.*, 1998). However, population increase and the rapid economic development in most of these developing countries is placing heavier demands on fished stocks, therefore greater emphasis has been placed on aquaculture to help meet the rising demand for fish products.

World aquaculture production has more than doubled in volume over the last ten years (15,544,640 tonnes in 1988 compared to 36,050,269 tonnes in 1997) and the Asian region accounts for over 90% of total production (FAO, 1999b). Table 1.3 shows China as the principal aquaculture producer, with 19,325,623 tonnes of aquatic products, followed by India, Japan, Indonesia and Thailand. The crustacean species cultured by the majority of

Table 1.3: List of the top 14 countries for aquaculture production, their production and its value in 1997 (FAO, 1999b).

Country	Production (metric tonnes)	Value (USD 000)
China	19,315,623	20,509,595
India	1,777,450	1,975,418
Japan	806,534	3,525,432
Indonesia	754,610	2,224,782
Thailand	575,901	1,783,038
Bangladesh	512,738	1,370,199
Vietnam	480,000	1,112,400
United States	438,331	771,183
Korea	392,427	913,141
Norway	366,281	1,043,824
Philippines	330,443	898,324
France	287,547	634,097
Chile	272,346	918,652
Taiwan	257,530	945,523
Others	2,241,653	6,842,859

these producers in the black tiger shrimp, *Peneus monodon*. However, in recent years, there has been a significant reduction in production of black tiger shrimp, particularly in Thailand, the world's biggest shrimp producing country, which witnessed a drop in production of 14.3% between 1995 and 1996 (FAO, 1999b). This decline in production was attributed to shrimp viral infections (Flegel, 1996, cited by Subasinghe *et al.*, 1998) and poor husbandry (Beveridge *et al.*, 1997). The above situation has led to strong interest in the diversification of aquaculture into other high-value species, including portunid crabs.

Mud crab is one potential candidate for larger scale aquaculture ventures, currently fetching average global prices of USD5.67 kg⁻¹ (FAO, 1999b). Unlike the other portunids, *Scylla* has the ability to withstand fluctuations in salinity and low oxygen levels as well as being able to survive out of water, thus simplifying the transportation needed, particularly during exportation (BOPB, 1991). These qualities along with its high meat content (42%-47.3% claw mass and 23.6%-36% for body muscle) make it a good candidate for aquaculture (George and Gopakumar, 1987).

Mud crab farming was first recorded in 1890 in China (Shen and Lai, 1994) and has expanded across Southeast Asia over the last 30 years (Keenan, 1999b). It is most likely that mud crab farming started in areas where the crab was fished, as a way to improve the value of the catch by holding them while they increased in weight and value (Overton and Macintosh, 1997).

Three main forms of mud crab culture are presently in practice, namely a) fattening, where marketable sized crabs are held solely to gain weight and are harvested before they moult; b) growout, where juvenile crabs are reared to marketable size; and c) soft-shell crab farming, where crabs are kept until they moult and are sold while they are still soft. Both crab fattening and growout are carried out in either a) earthen ponds (like those used for shrimp culture) (BOBP, 1991 and references therein); b) floating cages or raft systems in ponds or estuaries (Liong, 1991); or c) in fenced off areas within the mangrove forest (Say and Ikwanuddin, 1999).

Crab fattening involves a very short culture cycle (20 to 30 days) where the crabs are stocked at relatively high densities (about 15 crabs m⁻²) (Keenan, 1999b). They are fed a

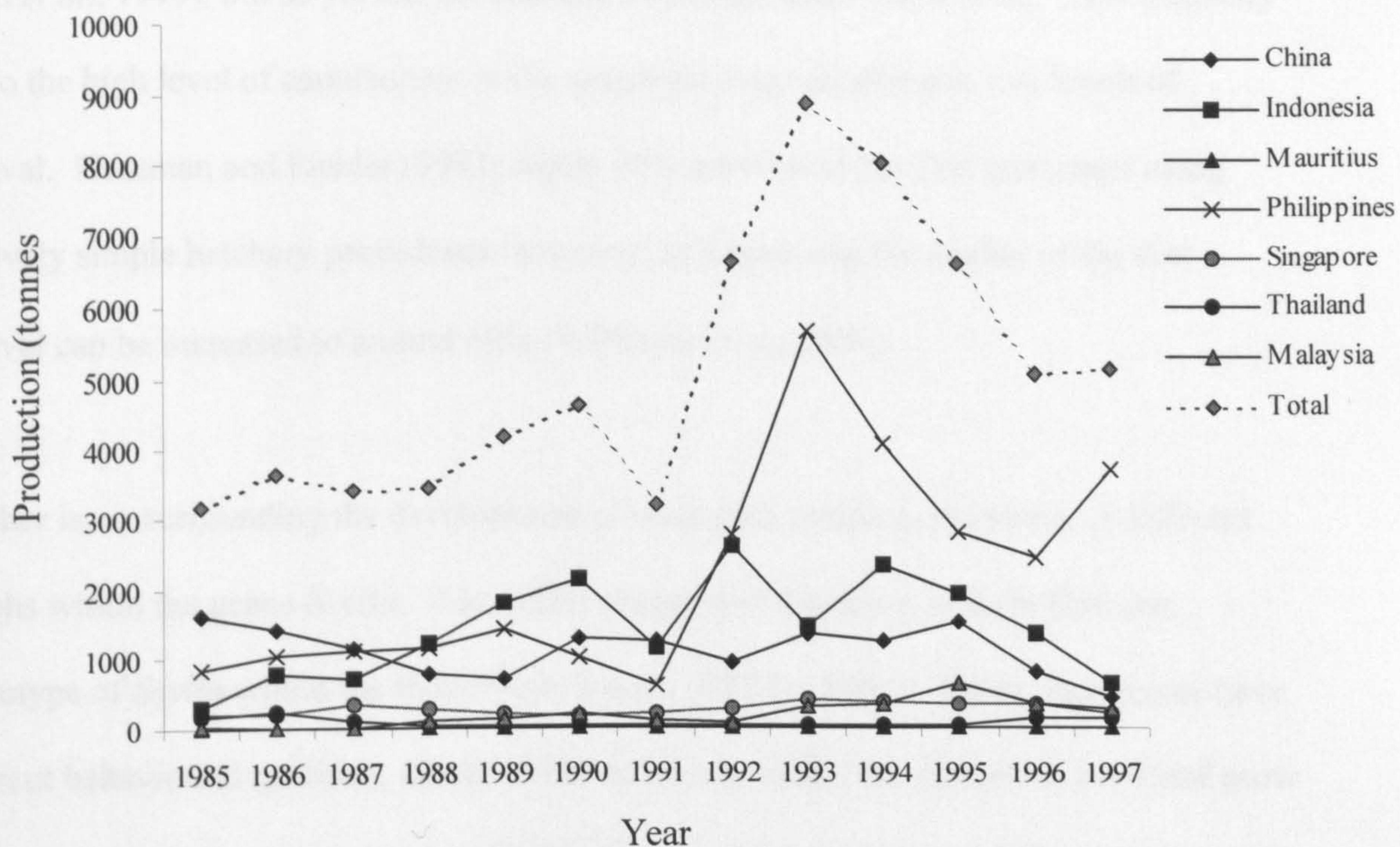
high protein diet of trashfish and molluscs (about 10% body weight per day) to increase body weight rapidly. Because the holding period is so short (within one moult period) the survival rate is usually as high as 90% (Liong, 1993). Females that have recently undergone the moult of maturity are also held while they develop ripe ovaries and can be sold as "egg crab". The short culture cycle means that there is a high turnover of stock with the minimum risk of losses due to mortality. This type of aquaculture is popular with coastal fishermen who wish to increase the value of their catch.

The other popular method of mud crab culture is the growout of mud crabs requiring juvenile crabs to moult and grow until they reach marketable size. Small crabs and juveniles are stocked (10g to 100g each). The stocking rates are usually low (1 crab m⁻² to 3 crabs m⁻²) (Keenan, 1999b). These crabs are held for four to five months until they reach marketable size (anything from 250g to 400g each). In most instances there is supplemental feeding using waste protein sources, although some of the large pond systems (up to 20 hectares), such as those found in northern Vietnam, rely on natural food supply brought into the ponds through tidal water exchange (Macintosh, 1999). Although this type of culture has lower survival rate (50% to 70%), due to cannibalism (Chen, 1990), it is still found to be a profitable venture (Agbayani *et al.*, 1990). Mud crab is also farmed in polyculture with *Gracilaria*, penaeid shrimp or milkfish in Taiwan (Chen, 1990) and the Philippines (Agbayani *et al.*, 1990; Lee and Wickins, 1992).

More recently, soft-shell mud crab culture has increased in popularity, particularly in Thailand since 1990 (Ryce, 1995) and has only very recently been introduced to Indonesia (Cholik, 1999). Small crabs (80g to 200g) are reared in individual plastic boxes within a concrete pond. They are held until they moult (which usually takes place within 45 days).

A pond of 1.5 hectares can hold up to 60,000 crabs. They are fed with trash fish daily. Once the crab has moulted, it is removed from the system, washed and frozen before being exported or sold to the local restaurant trade (Ryce, 1995). This has particular significance to overseas export, in particular to the United States. This is a very profitable venture, doubling the value of these small crabs from THB60 kg⁻¹ as hard shelled crab to THB210 kg⁻¹ as a soft-shell product (Ryce, 1995). However, due to high turnover of crabs and huge losses due to mortality and their inability to moult (about 64% of crabs stocked fail to moult), this type of aquaculture places a heavy demand on already overexploited crab stocks.

Present trends in aquaculture production of mud crabs from major producing countries as recorded by the Food and Agriculture Organisation (FAO) are shown in figure 1.2. Unfortunately India, Bangladesh and Sri Lanka have not been represented in the FAO statistics although they are involved in mud crab culture. India presently produces about 2,000 tonnes of mud crab but it is believed that exports could be boosted up to 20,000 tonnes through aquaculture (Anon, 1995). Although the interest in mud crab culture has increased recently, the actual production has remained the same or, in some cases decreased in recent years (as shown in figure 1.2). This is probably due to the limited seed supply available to sustain mud crab culture. At present, the majority of seed supply is from natural resources, collected using scissor nets or beach seines (Liong, 1993). However, the natural population cannot sustain the demands of both fisheries and aquaculture, especially as aquaculture puts further demands on the stock as culture practices expand.



Year	Aquaculture production (tonnes)							Total
	China	Indonesia	Mauritius	Philippines	Singapore	Thailand	Malaysia	
1985	1617	315	1	833	213	178	24	3157
1986	1414	758	1	1034	211	223	16	3641
1987	1134	691	1	1122	330	113	5	3391
1988	784	1222	1	1136	256	32	99	3431
1989	717	1790	1	1422	205	30	145	4165
1990	1294	2111	2	1000	161	30	190	4598
1991	1241	1145	2	597	195	30	95	3210
1992	930	2605	3	2765	284	39	71	6626
1993	1350	1442	3	5653	415	25	296	8888
1994	1257	2315	5	4085	355	25	331	8042
1995	1526	1906	5	2782	342	45	623	6606
1996	797	1339	4	2440	353	132	336	5065
1997	430	630	2	3710	215	132	277	5119

Figure 1.2: Aquaculture production of mud crab, *Scylla*, from 1985 to 1997 (from FAO, 1994; 1999b).

Experimental larval rearing has been researched (Brick, 1974; Heasman and Fielder, 1983; Mann *et al.*, 1999), but as yet has not reached a commercially viable level. This is mainly due to the high level of cannibalism at the megalopa stage resulting in low levels of survival. Heasman and Fielder (1983) report 26% survival to the first crab stage using relatively simple hatchery procedures; however, by improving the quality of the diet survival can be increased to around 60% (Williams *et al.*, 1999).

Another issue surrounding the development of mud crab culture is the status of different morphs within the genus *Scylla*. It is widely recognised that there is more than one phenotype of *Scylla* within the Indo-West- Pacific (BOBP, 1991). These phenotypes have different behavioural qualities, inhabit different niches within the mangrove zone and grow to different adult sizes (Estampador, 1949a); they are also marketed at different prices (as shown in table 1.2). Until very recently, research into the aquaculture of this genus has treated these phenotypes as one species, regardless of morphology. Without the proper taxonomy of *Scylla* being revealed, comparison of research studies, extended to technical issues with crab culture, and modifications and implementations of fisheries management plans, is not possible. For example, if the growth rates are different between the different morphs then it would be beneficial to select the faster growing, larger morph for aquaculture (Keenan *et al.*, 1995).

B. Speciation in Scylla

1.4 The species concept

The question of speciation and in particular the definition of a species lies at the heart of taxonomy. The concept of species dates back to the 4th century BC, where nature was recognised as being ordered through a series of complex life forms. These were classified into groups based on their morphological similarities, which formed the basis for the term “species”. In 1749, Buffon described the term species as “ a constant succession of similar individuals that can reproduce together”. The transition, towards the idea of grouping organisms on the basis of distinctness was to become the ideology behind the Biological Species Concept (BSC) (also known as the Isolation Species Concept (ISC)) advocated by Dobzhansky (1937) and later by Mayr (1963). Species were described as “groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups” (Mayr, 1963). Avise (1994) describes this theory as being one of the most influential concepts of speciation which has retained its popularity to this day. However, several complications in applying BSC principles have arisen due to the generalising nature of the theory. Firstly, it is impossible to prove the species status between allopatric populations. Any reproductive isolating mechanisms (RIMs) that exist between geographically separated populations could simply be a “by-product” of genomic divergence following isolation (Avise, 1994). Furthermore RIMs are rarely put to the test between allopatric populations in nature, only if species live in sympatry and remain as separate identities is the BSC theory proved. Secondly, there is the issue of how much genetic exchange through the processes of geneflow and hybridization is acceptable for the status of separate biological species. Thirdly, Under BSC theory, many species may be able to interbreed and may not be derived from the same monophyletic group (*i.e.* a group containing all descendants from a common ancestor (Donoghue, 1985)) which does not

follow the modern ideas of phylogenetic reconstruction. Finally, the BSC theory is only applicable to sexually reproducing organisms, thereby excluding a vast proportion of the world's species.

Due to these limitations, there has been a drive to find alternatives to BSC. Gosling (1994) lists four main theories, while Avise (1994) suggests six that have been widely accepted as an alternative species theory to BSC. The most promising alternative has been the phylogenetic species concept (PSC) suggested by Cracraft (1983). This concept states that a species is “a monophyletic group composed of the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent”. This concept is similar to that proposed by Simpson (1951). The PSC brings in the element of time that is lacking in BSC. PSC also emphasises differentiation, whether it be as a direct result of reproductive isolation or not, which means that it can be applied to both sexual and asexual organisms (Avise, 1994). Moreover, PSC can be applied to both allopatric and sympatric populations, and to those that are living or extinct. However, one major problem with this concept is that it is difficult to decide how to interpret the threshold for speciation particularly when combined with new molecular techniques where it is possible to discover genetic uniqueness in every individual. Avise and Ball (1990) suggested a concordance of biological species and phylogenetic species concepts (concordance principles (CP)). However, as Bock (1992) pointed out, there is no single species concept that exists that can be applied to all organisms. In sexually reproducing organisms, however, the biological species concept is still the most popular theory used in making decisions on species.

1.5 Main models of speciation

Speciation is the general term for a number of different evolutionary processes that result in the production of new species. Two forms of speciation were suggested by Mayr (1963), namely, reductive speciation, where there is a reduction in the number species and additive speciation resulting in a multiplication in the numbers of species.

Reduction speciation involves the complete fusion of two formerly independent species through a complete hybridisation or intergradation event (Mayr, 1963). Hybridisation resulting in a new species is well documented in plants (Grant, 1971) however, the complete extinction of the parent species to a new hybrid is unknown. Furthermore, if the complete fusion of two species is possible, it would suggest that they are not true species at all.

Additive speciation is the general term involving any mode of speciation that adds to the diversity of living organisms. Speciation that results from a lineage splitting into two or more species is called cladogenesis (Quicke, 1993). This is the most commonly described mode of speciation in both animals and plants (Wiley, 1981). There are at least five different modes of additive speciation, specifically allopatric speciation, allo-parapatric speciation, parapatric speciation, stasipatric speciation and sympatric speciation. The two most commonly discussed models of speciation within the literature, namely allopatric and sympatric speciation, are briefly described below.

Allopatric speciation is a process created by isolation by distance where a large continuous population is broken up into smaller units by extrinsic barriers. These sub-populations then undergo changes in gene frequency as a result of evolutionary forces such as

selection, genetic drift, and mutation (these forces are described in appendix I) (Gosling, 1994). The cohesive effects of geneflow are reduced or prevented between populations due to geographic isolating barriers. In this scenario reproductive isolating mechanisms arise as an accidental “by product” of the process (Avisé, 1994).

Sympatric speciation, on the other hand, is when a new species is formed within the range of the parent population. This requires strong disruptive selection on an already polymorphic population by shifts in ecology, host, or timing of reproduction (Wiley, 1981). This type of speciation is suggested in plants (particularly those capable of self-fertilisation) (Grant, 1971) and has been recorded in some insects (Kondrashov and Mina, 1986), as shown in the disruptive selection experiments of Thoday and Gibson (Thoday and Gibson, 1962, 1970). There is some evidence to suggest this may also take place in fish species (Avisé, 1994). This type of speciation also occurs in asexual organisms or in some cases in sexually reproducing animals as a result of hybridization (Wiley, 1981).

Both allopatric and sympatric speciation events are gradual processes taking place through generations over an extended period of time. However, there is a view that speciation events may also be instantaneous, with complete speciation taking place within a few even single generations.

Instantaneous speciation, quantum speciation, saltation speciation or stasipatric speciation are terminologies for a rapid processes of speciation. This includes where a) there are major changes in chromosomal structure, such as translocation and inversions, or a change in chromosomal number; b) polyploidy in chromosomes allowing fertilisation between otherwise sterile hybrids (this is extremely common plants and rare in animals), or a

change in mating system (e.g a switch from outcrossing to self-fertilization in plants or *vice versa*). These are then reproductively isolated from the parent population and therefore constitute a new species (Avice, 1994).

Geographical separation (allopatry) was believed to be the most prominent cause of speciation by Mayr (1963), whereas White (1978) preferred the idea of chromosomal changes being the most prevalent form of speciation. It is now accepted that allopatric speciation is the most common event in animals, whereas sympatric and quantum speciation is generally confined to plants (Lynch, 1989). It is difficult to define which of these modes of speciation is most relevant to marine organisms. In particular, finding the proof of allopatry in marine species was seen as a challenge, as many researchers believed that highly fecund marine species produced larvae that had the potential to disperse over large distances (*i.e.* high population sizes with high rates of gene flow). However, Palumbi (1994) reviews many examples where allopatric speciation is possible in marine organisms, including crustaceans.

1.6 Taxonomy of *Scylla* within the Phylum Crustacea

There has been much argument on the systematics of the Crustacea in general *i.e.* whether they are part of the Phylum Arthropoda, or whether they should be seen as a separate phylum altogether (Cisne, 1982). There have also been many changes made to the classification within the Subphylum (or phylum) Crustacea. The classification of the order Decapoda is one of those areas within crustacean taxonomy that is constantly under review. It is the most diverse of all crustacean orders containing 1200 genera (approximately 10,000 species). About 85 percent of decapod species live in a marine habitat the other 11 percent are freshwater and/or terrestrial species (Bowman and Abele,

1982). Decapod crustaceans are also of interest because they contain the majority of those species with some economic importance.

Within the order Decapoda, *Scylla* falls within the infra order Brachyura (Latreille, 1831) which contains all true crab species (those crab-like groups not included are hermit crabs (Families Diogenidae and Paguridae), stone crabs (Family Lithodidae) and porcelain crabs (Family Porcellanidae)) (Ingle, 1997). It also has been concluded using modern molecular techniques that the family Dromiidae should also be removed from the Order Brachyura (Spears *et al.*, 1992).

The earliest records of brachyuran crabs are from the mid- to late Jurassic Period (as early as 195 million years B.P.). They are believed to have evolved from the palinurids (crawfishes). Brachyurans are therefore thought to be much younger in evolutionary terms than both penaeid shrimp (possibly formed as early as the late Carboniferous Period) and Caridean prawns (formed during the early Jurassic Period). However, even though brachyurans are relatively young, they express a great diversity in their morphology and ecology (Spears *et al.*, 1992).

This diversity is believed to have taken place in three waves of radiation (proposed by Guinot, 1978), of which the section Brachyrhyncha (in which *Scylla* belongs) was characterised during the Eocene Period (about 50 million years ago) and is therefore considered one of the youngest sections to be formed. Within the infraorder Brachyura, the genus *Scylla* is part of the superfamily representing the swimming crabs, Portunidae (Rafinesque, 1815). Other genera encompassed into this super family, include the blue crabs *Portunus* spp. and *Callinectes sapidus* also known as the Chesapeake bay swimming

crab (Ingle, 1997). Fossil specimens of *Scylla* from Japan (Muroka, 1976) and South Africa (Cooper and Kensley, 1991) show that this mud crab had a wide geographical spread, extending as far back as the early Pleistocene Period (1.5 to 2 million years B.P.). A more detailed classification of the genus *Scylla* is shown in figure 1.4.

1.7 Historical taxonomy of the genus *Scylla*

For the last 50 years there has been much confusion over the taxonomic nomenclature of the genus *Scylla*, and in particular whether the genus is comprised of more than one species. Forskål (1755) first named the species *Cancer serratus* from material collected from the Red Sea. Unfortunately the brevity of his description, which was published posthumously, plus the loss of the original specimen, resulted in the confusion of the taxonomy that was to follow. Fabricius (1798) also described *Scylla*, which he named *Portunus pelagicus*, collected from the Indian Ocean. Subsequently, several other species names have been proposed for the mud crab. The mud crab was eventually revised as a separate genus by De Haan (1833) and the genus name was changed to *Scylla*, presumably relating to the aggressiveness and the burrowing nature of the mud crab (in Greek mythology, “Skylia” was a sea monster that lived in a cave by the sea). From these very early descriptions the subsequent revision of the taxonomy was made by Estampador (1949a,b) who carried out an extensive study of the mud crab in the Philippines, recognising three species and one variety of mud crab. These species were based on comparative morphology, behaviour, ecology, chromosome form and the process of gamete development. Since this revision, there have been many authors both supporting (e.g. Serene, 1952) and refuting (e.g. Stephenson and Campbell, 1959) the taxonomy proposed by Estampador (this is explained in more detail in chapter three).

Phylum:	Arthropoda
Subphylum:	Crustacea
Class:	Malacostraca
Subclass:	Eumalacostraca
Superorder:	Eucarida
Order:	Decapoda Latreille, 1803
Suborder:	Pleocyemata Burkenroad, 1963
Infraorder:	Brachyura Latreille, 1803
Section:	Brachyrhyncha Borradaile, 1907
Superfamily:	Portunoidea Rafinesque, 1815
Family:	Portunidae Rafinesque, 1815
Subfamily:	Portuninae Dana, 1852
Genus:	Scylla de Haan, 1833

Figure 1.3: Classification of the genus *Scylla* (de Haan, 1833): Classification from phylum to order is similar to that proposed by Moore and McCormick, 1969. The classification from the order Decapoda follows that of Glaessner, 1969 with changes proposed by Guinot (1977) and De Saint-Laurent (1980) (extracted from Bowman and Abele, 1982 and Ingle, 1997).

However, in all cases it is accepted that more than one phenotype of *Scylla* exists in almost all locations where mud crabs occur. In the majority of cases these morphs are living in sympatry and are recognised by different names such as "black crab", "sea crab" and "golden-backed crab" depending on their general appearance or behaviour. With the increasing interest in farming mud crab the need to resolve the taxonomy has become more urgent.

1.8 Techniques used to determine species status in Crustacea

The traditional method to identify and establish species status in crustaceans has been to use comparative techniques based on external morphological features. Crustaceans lend themselves to this type of identification with well-defined body structures and spines which are extremely diverse in morphology throughout the crustacean subphylum (McLaughlin, 1982). Furthermore, colour, in particular patterning, has been extensively used to identify crustacean species (Knowlton, 1993).

However, the use of purely descriptive techniques to identify species has received much criticism due to the subjectivity of allocation of species status purely on morphological difference (Quicke, 1993). Firstly, variation in morphology of ectothermic organisms (including invertebrates) has been interpreted as morphological plasticity induced by localised environmental factors (Knowlton and Jackson, 1994). Secondly, with closely related crustacean species there is often an overlap in morphological characters between species which makes it difficult to tell them apart (Stephenson and Campbell, 1959).

Finally, it is also difficult to separate species morphology from purely functional morphology where groups of animals exhibit specialised adaptations to the environmental conditions (*e.g.* feeding structures, locomotary appendages, sensory organs) (McLaughlin,

1982). However, to date, descriptive taxonomy is still the preliminary method of species identification and description for publication in order that others working on particular taxa may know which species they are studying.

The subjectivity in using descriptive techniques to allocate species status resulted in the introduction of more objective techniques. One of these techniques is numerical taxonomy (also known as phenetics) which was popularised largely by Sneath and Sokal (1973). This technique involves the use of mathematical procedures to numerically encode morphological characters into different states and then using mathematical techniques to group individuals into taxa on the basis of their overall similarity in character states (Manly, 1994).

Multivariate techniques using morphometric data is one form of numerical taxonomy and is useful for identifying unknown samples and for distinguishing between similar taxa. Multivariate analysis allows the consideration of several related random variables simultaneously, with all variables being considered equally important unless they are weighted accordingly.

One group of multivariate techniques, known as ordination methods, plot several variables (characters) simultaneously and then locate the vectors which best describe the total variation between and within groups of individuals in a reduced number of axes.

Ordination methods, including principal components analysis and canonical variate analysis (also known as discriminant function analysis) (see Chapter Four for detailed descriptions of these techniques), have regained popularity in recent years particularly as they allow the researcher to reliably distinguish between closely related taxa on the basis of

variation in a whole range of character types (Thorpe, 1976). Among portunid crabs morphometrics have already been used successfully to distinguish planktonic stages of *Macropipus tuberculatus* from other polybiinid species in addition to providing supporting evidence that the genus *Macropipus* should be further divided into *Liocarcinus* and *Macropipus sensu stricto* (Guerao and Abello, 1999). Multivariate analysis of morphometric characters was also shown to successfully differentiate between two closely related European spider crabs, *Maja crispata* and *M. squinado* (Neumann, 1996).

However, the most common use of multivariate morphometrics for defining species boundaries in crustacean studies generally has been in conjunction with molecular techniques, comparing the degree of morphological similarity with genetic differentiation. Molecular techniques have the advantage over morphological techniques in that they allow the study of the degree of geneflow between populations therefore allowing conclusions to be made about the possibility of isolating mechanisms preventing them from interbreeding (Quicke, 1993).

With respect to genetic techniques, allozyme electrophoresis has proved to be a very popular and dependable technique in delineation of biological species (Richardson *et al.* 1986); in particular its ability to directly determine genetic relationships within and among populations resulting in the understanding of the extent of population differentiation and speciation (Keenan and Shaklee, 1985). Allozyme electrophoresis is the procedure for separating allozymes (one or more variants of an enzyme coded by different alleles at the same gene locus) in a supporting medium on the basis of their electric charge (Richardson *et al.*, 1986) (a more detailed explanation of allozyme electrophoresis is described in Chapter Five). Samples of a genus from one locality can be tested where it is expected that

there is more than one species (*i.e.* sympatric species) or to decide whether two or more geographically isolated populations belong to the same species (conspecific populations) or different species (allopatric species).

Following the biological species concept, a single fixed allelic difference between populations of sexually reproducing species is adequate to recognise two populations evolving independently, warranting recognition as two species, as it indicates no hybridisation between species is taking place (Richardson *et al.*, 1986; Thorpe and Sole-Cava, 1994). In most situations, there are morphological, behavioural or ecological differences between populations to differentiate between the species. Therefore allozyme electrophoresis only confirms genetically what is already suspected.

However, in the situation of potential cryptic species allozyme electrophoresis may be a powerful tool for species identification where morphology fails to distinguish between species (Richardson *et al.*, 1986). Moreover, as allozyme electrophoresis is cost-effective compared to other genetic techniques, it is perfect for a wide scale study using a large number of individuals from each site (Murphy *et al.*, 1996).

In crustacean taxonomic studies, the combined use of multivariate morphometrics and allozyme electrophoresis has been successful in defining species boundaries for allopatric populations (*e.g.* freshwater amphipods, genus *Paramelita*: Stewart, 1992;) and sympatric populations (*e.g.* alphaeid shrimp, genus *Synalpheus*: Duffy, 1996). Congruence in the results using different techniques provide convincing evidence for distinct species (Quicke, 1993). Equally any discordance between morphological and genetic results may be

particularly informative, indicating that the rate at which morphological and genetic divergence is taking place is not uniform.

In the present study, all three techniques of species identification described above were used in a comparative way in order to ascertain the status of species within the genus *Scylla* in South and Southeast Asia. Moreover, this study attempts to elucidate what, if any, pre-zygotic reproductive isolating mechanisms are present that can explain the persistence of sympatry between the phenotypes of *Scylla* observed throughout the southeast Asian region.

In this particular study, the primary objective is to elucidate the species within the genus *Scylla*. However, morphological and genetic characters used to determine the species status of *Scylla* can also be used to ascertain the evolutionary history (phylogeny) of this genus that has resulted in the present species observed (Wiley, 1981; Swofford *et al.*, 1996). A range of methods have been devised to reconstruct phylogenetic trees from morphological and allozyme data. The details of these methods are described in more detail in Chapter Six.

C. Objectives

1.9 General objectives

The overall objectives of this study were:

- 1) To investigate the species status of mud crabs of the genus *Scylla* in south and southeast Asia, based on their meristic, morphometric, genetic and reproductive characteristics.

- 2) To study the possible pre-zygotic reproductive isolating mechanisms and ecological features that permit the apparent sympatry between the two phenotypes of *Scylla* found in Ban Don Bay, Surat Thani Province, Thailand.

Ban Don Bay, Surat Thani, on the Gulf of Thailand coast, was selected as principal study site, because two phenotypes of *Scylla* are present in approximately equal numbers.

Collections of *Scylla* from two other sites in Thailand and from Vietnam, Bangladesh and Sarawak were used for comparison with the genetic and morphometric information obtained from Surat Thani.

1.10 Specific objectives

The specific objectives of this study were:

- a) To elucidate the common characters used in previous taxonomic descriptions of *Scylla* and assess the nomenclature for the present species identified in terms of the published descriptions, and in relation to fresh specimens and museum collections examined during this study.
- b) To compare different populations of *Scylla* collected from seven sites in south and southeast Asia (Surat Thani, Ranong and Chantaburi (Thailand); Thai Binh, and Can Gio (Vietnam); Paikgasir (Bangladesh); Sematan (Sarawak)) using discriminant function analysis to see whether the populations can be separated by their phenotype, and to identify those morphometric characters that are responsible for any discrimination between “morphs”.

- c) To classify using discriminant function analysis the groups collected in the present study by comparison with museum specimens from the Zoological Museum, University of Copenhagen.
- d) For the two morphs sampled among the selected study sites, using pairwise Mahalanobis D^2 distances to elucidate any pattern of geographical variation.
- e) To use allozyme electrophoresis to elucidate the species status of mud crabs of the genus *Scylla*, in particular whether the two phenotypes of *Scylla* found in Surat Thani, Thailand, are two species and to understand the pattern of geographical variation for the two phenotypes collected from selected study sites.
- g) To investigate the phylogeny of the genus *Scylla* using a) Unweighted pair group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973); b) Distance Wagner (Farris, 1972); c) Maximum likelihood (Felsenstein, 1981); and d) Wagner parsimony (Kluge and Farris, 1969) approaches for allele frequency data.
- h) By coding morphological characters and using a Wagner parsimony approach to produce a dendrogram to compare with those phylogenetic trees produced using gene frequency data.
- i) To compare male genital morphology for structures that may prevent cross mating between the two apparently sympatric phenotypes of *Scylla* in Surat Thani.

- j) To study the degree of sexual maturity within female populations of *Scylla* over eighteen months to observe any variations in the breeding patterns between the two morphs found in Surat Thani. This may indicate a species divergence in reproductive strategy.

- k) To estimate the size at maturity for female mud crabs of the two sympatric phenotypes of *Scylla* from Surat Thani using a) minimum size at maturity, b) breakpoint analysis and c) probit analysis.

Chapter Two

Description of study sites and general methodology

2.1 Choice of study sites

The geographical area chosen for the present study covered Southeast Asia (Malaysia, Thailand, Vietnam) plus Bangladesh. Within these four countries seven discrete coastal locations were chosen, namely Surat Thani, Southeast Thailand; Ranong, Northwest Thailand; Chanthaburi, eastern Thailand; Thai Binh, northern Vietnam; Can Gio, southern Vietnam; Sematan, Sarawak; and Paikgasir, Bangladesh. Figure 2.1 shows the locations of all seven study sites. These sites were chosen for a) their degree of geographical separation; b) their consistency in the availability of crabs (as they are all good crab fishing sites); and c) their good local infrastructure and technical support (including local expertise) to aid the collection and transportation of mud crabs samples.

The study sites range over approximately 20° of latitude and 20° of longitude; all locations experience a tropical climate except for Paikgasir, Bangladesh and Thai Binh, Vietnam, which are situated at more northerly latitudes and therefore experience a semi-tropical/warm temperate climate (refer to table 2.1 which summarises the habitat of the seven study locations). Paikgasir was the most northerly and westerly location (22°72'N; 89°36'E) and Sematan (1°51'N; 109°47'E) situated in Sarawak the most southerly and easterly location within the region sampled. Five of the seven locations are situated on the eastern side of the Thai/Malaysian peninsula within the influence of the South China Sea and the Gulf of Thailand. The remaining two locations are situated on the western

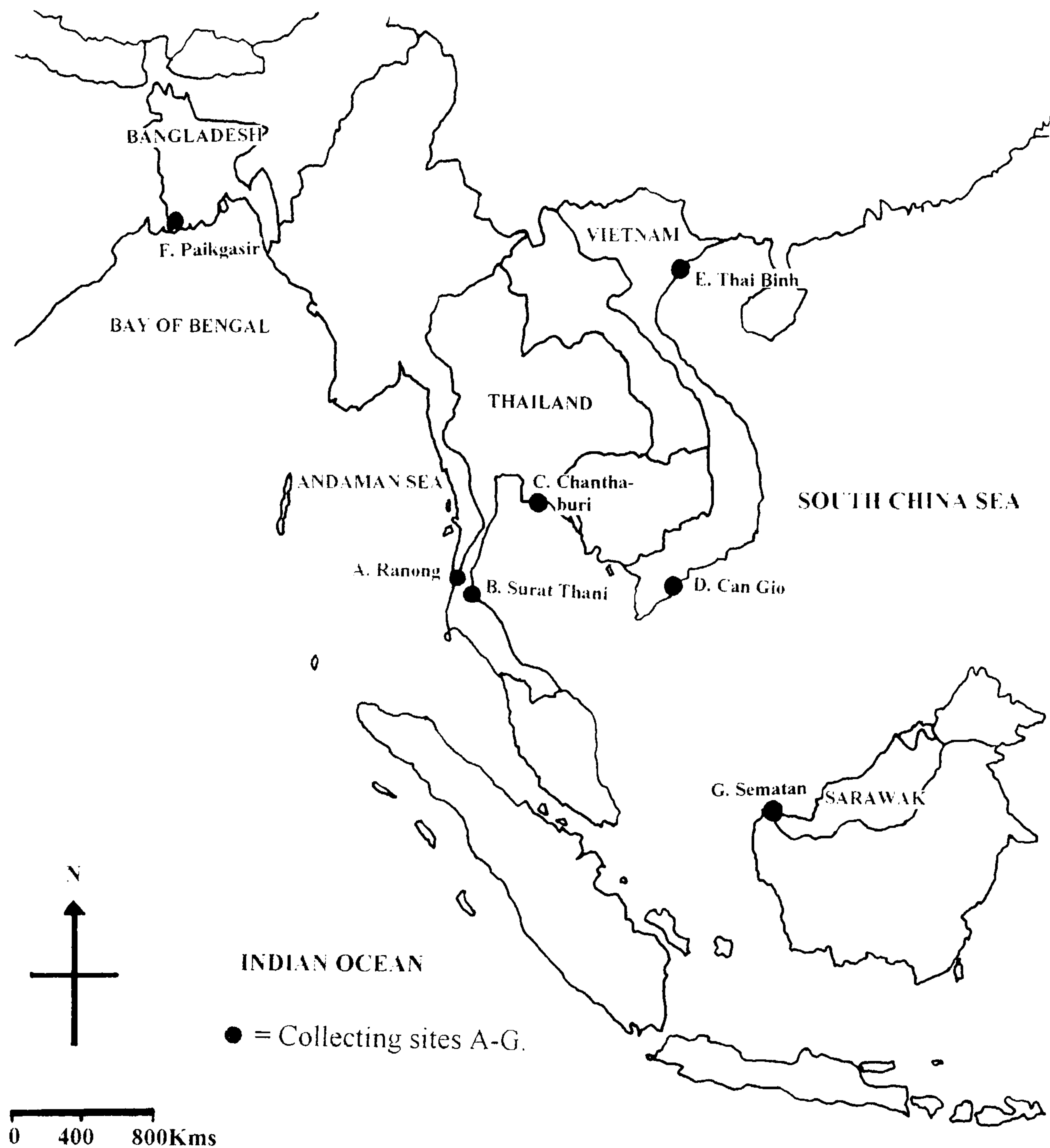


Figure 2.1: Locations of mud crab (genus *Scylla*) collecting sites in Southern and Southeast Asia.

Table 2.1: Summary of the habitat of the seven study sites used for the collection of mud crabs (genus *Scylla*).

Parameter	LOCATION						
	Surat Thani	Ranong	Chanthaburi	Thai Binh	Can Gio	Sematan	Paikgasir
Location	9°02' - 9°25'N 99°08' - 99°27'E	9°21' - 10°42'N 98°24' - 98°56'E	12°30' - 12°40'N 101°50' - 102°08'E	20°30'N; 106°34'E	10°22'N; 106°46'E	1°51'N; 109°47'E	22°72'N; 89°36'E
Climate	Tropical	Tropical	Tropical	Subtropical	Tropical	Equatorial and Tropical	Subtropical
Temperature mean range	27.7°C 26.5 - 35°C	28°C 25 - 34°C	28°C nd	24°C 6 - 38°C	27°C 25.5 - 29°C	26°C 25.8 - 27.1°C	25°C 19 - 29°C
Rainfall	100mm month ⁻¹	33mm month ⁻¹	nd	nd	100mm month ⁻¹	nd	nd
min	nd	nd	9.5mm month ⁻¹	nd	nd	100mm month ⁻¹	nd
max	500mm month ⁻¹	50mm month ⁻¹	55.6mm month ⁻¹	50mm day ⁻¹	400mm month ⁻¹	920mm month ⁻¹	nd
Monsoon season	NE: Sep - Nov*	NE: Nov - Feb SW: May - Oct.	nd	Jul - Aug	NE: Nov - April SW: April - Oct	Dry: April - Sept Wet: Oct - Feb	May - Oct
Soil type	Muddy soils, clayey texture. Intertidal acid sulphate soils.	Laterite. High silt loading in riverbanks	nd	Acid sulphate soils. Wide mudflats (12m wide) composed of alluvium	Sulphatic clay or mud.	Podzols. Sandy mineral soils along the river system. Acid sulphate.	Alluvium and loamy clay soil. Very high alluvial deposition along river system.
Tidal range (MLWS - MHWS)	1.30m - 1.89m	2.5m	1.68m	2.6m - 3.9m	3.3m - 4.1m	1.2m - 3.7m	nd
Vegetation type	Coastline still fringed with some mangrove forest. Species include: <i>Exocaria agallocha</i> , <i>Avicennia</i> spp., <i>Sonneratia</i> spp., <i>Rhizophora</i> spp., <i>Bruguiera</i> spp., <i>Ceriops tagal</i> , <i>Acrostichum aureum</i> and <i>Nypa fruticans</i> .	Coastline has some original mangrove forest and some replanted areas. Species include: <i>Avicennia alba</i> , <i>Bruguiera</i> spp., <i>Ceriops tagal</i> , and <i>Rhizophora</i> spp.	Coastline still fringed with some mangrove forest. Species include: <i>Rhizophora</i> spp., <i>Nypa fruticans</i> , <i>Avicennia</i> spp., <i>Bruguiera</i> spp., <i>Ceriops</i> spp., <i>Lumnitzera</i> spp., <i>Xylocarpus</i> spp.	Allmost all of the mangrove forest is replanted. <i>Kandelia candel</i> is the main species planted. Mangrove area has been made a BIOSPHERE reserve (UNESCO).	Coastline has some original mangrove forest and some replanted areas. Reforested areas are planted with <i>Rhizophora apiculata</i>	Coastline still has vast areas of natural mangrove forest. Predominant species include: <i>Avicennia</i> spp., <i>Sonneratia</i> spp., <i>Rhizophora</i> spp., <i>Ceriops tagal</i> , <i>Exocaria</i> spp., <i>Xylocarpus granatum</i> , <i>Bruguiera cylindrica</i> , <i>Oncosperma tigillaria</i> , <i>Nypa fruticans</i>	Coastline still has vast areas of natural mangrove forest. Predominant species include: <i>Heritiera jomes</i> , <i>Nypa fruticans</i> , <i>Sonneratia apetala</i> , <i>Ceriops decandra</i> , <i>Avicennia officinalis</i> , <i>Exocaria agallocha</i>
Coastal land use	Agriculture and fisheries.	Fishing, charcoal, production, aquaculture.	Fruit growing, heavy industry, fishing, aquaculture	Salt, sedge, rice, agriculture, fishing, aquaculture.	Salt, rice, agriculture, fishing, aquaculture.	Oil palm, rice, timber, rubber, agriculture, fisheries, aquaculture.	Jute, tea, rice and other agricultural crops.

NE = Northeast; SW = Southwest; nd = no data

* Months are abbreviated e.g. Feb = February

coastline of the peninsula and are influenced by the Bay of Bengal and the Andaman Sea. All the project sites are located in large coastal river deltas, which are fringed by mangrove forests and mudflats; the natural coastal habitat of the mud crab, *Scylla*. Moreover, all locations have a history of artisanal crab fishing, ensuring that all the mud crabs sampled were from the local area.

The author previously studied mud crab at four of the locations, namely Surat Thani, Thailand; Ranong, Thailand; Can Gio, Vietnam; and Sematan, Malaysia (Overton, 1994; Overton *et al.*, 1996). The other three sites (Thai Binh, Vietnam; Chanthaburi, Thailand and Paikgasir, Bangladesh) were also included for those reasons listed on the previous page. In summary, all the chosen locations had good logistical support and provided the opportunity to sample mud crabs from known local habitats.

2.2 Description of Study sites

Although all collection sites are climatically similar, there is considerable difference in their degree of coastal rural development. Primarily, development has been at the expense of converting mangrove or former mangrove forest areas to other land uses. Surat Thani and Chanthaburi still have fringes of mangrove forest, but much of the coastal area has been developed for aquaculture and, in particular, shrimp ponds. Can Gio and Ranong both have some original forest, plus areas of replanted forest. Both these sites have experienced relatively less rural development than Surat Thani and Chanthaburi and as a result both sites have recently been made biosphere reserves under the UNESCO Man and Biosphere Programme (MAB) (UNESCO, 1996b; 1998).

Almost all of the mangrove forest in Thai Binh has been replanted; most of the original forest was cut to provide fuel and as foundation material for sea dyke construction (Macintosh, 1996). The sites in Sarawak and Bangladesh, on the other hand, still have vast areas of natural mangrove, although both sites are now threatened by logging activities. There is still relatively little rural development in the Sarawak and Bangladesh sites compared to the other study locations.

2.2.1 Primary study site (Surat Thani)

2.2.1.1 Location

Surat Thani Province is situated on the Southeast coast of the Thai peninsula along the western Gulf of Thailand. Ban Don Bay (9°02' to 9°25'N; 99°08' to 99°27'E) is the main coastal feature within Surat Thani Province; it extends from Chaiya District in the north to Donsak in the east (see figure 2.2). The coastline area is approximately 156km² and the width of the shoreline varies from 10km to 18km. Ban Don Bay is influenced by the catchment area of the Tapee River, which covers 5200m², forming a single deltaic plain around the bay (Kositratana, 1988).

2.2.1.2 Climate

The climate is intermediate between an equatorial and tropical monsoonal one. The dry season is usually from February to March. The rainfall is generally moderate (100mm month⁻¹) until the Northeast monsoon in September when it increases up to 500mm in November. Temperatures remain constant, the average range is from 26.5°C to 27.7°C, the coolest time being in the wettest months (29°C) and the hottest in April (33°C to 35°C) (Bunpapong and Paw, 1988).

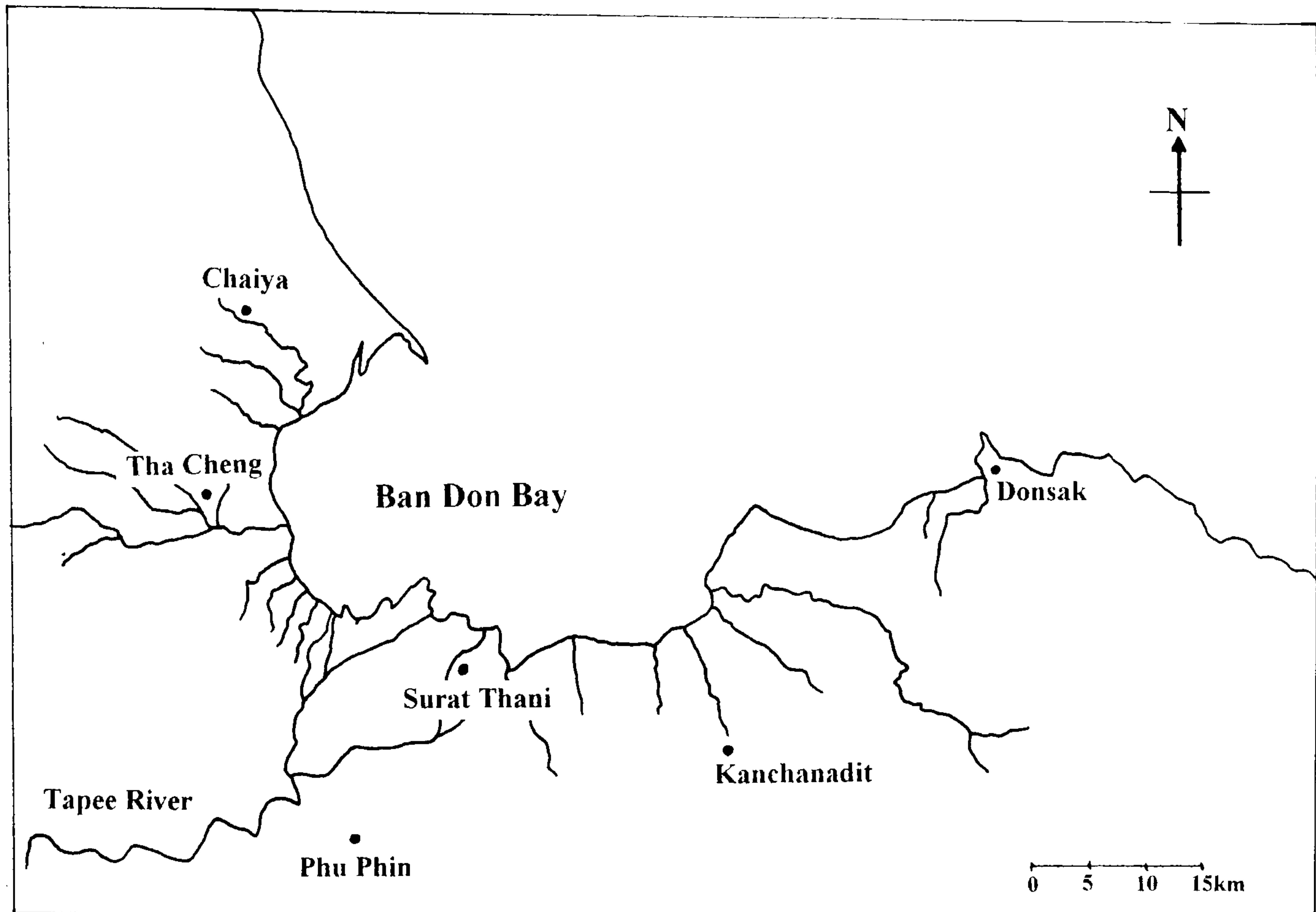


Figure 2.2: Map of Ban Don Bay, Surat Thani Province, Southern Thailand (adapted from Rattanachote and Dangwatanakul, 1993).

2.2.1.3 Soil type

The coastal zone is composed of mud flats and tidal areas. The mud flats are sub tidal and composed of dark grey muddy soils with a clayey texture. The intertidal zone is composed of acid sulphate or potentially acid sulphate soils. These soils are grey with yellow or brown mottling and are also clayey in texture (Hungspreugs and Limpsaichol, 1988; Rattakul, 1995).

2.2.1.4 Hydrographic features

The tidal regime in Ban Don Bay is predominantly diurnal with a maximum spring tide high of 1.89m above mean sea level and low water of 1.30m below mean sea level. The average estuarine current velocity is 2.04km h^{-1} with a mean tidal range of 0.32m (Paw *et al.*, 1988). Strong waves do build up with the Northeast monsoon along the East Coast of Thailand; however, Ban Don Bay is shallow and well sheltered and is not affected by wave action.

2.2.1.5 Vegetation type

There are about 2,200ha of natural mangrove left in Surat Thani Province (Charupatt, 1993). There are eight dominant species of mangrove present in Ban Don Bay. *Exocaria agallocha* and *Avecinnia* spp. occur on the seaward mangrove fringe. Further inland there is a mixture of *Sonneratia* spp., *Rhizophora apiculata*, *Rhizophora mucronata*, *Xylocarpus* spp., *Bruguiera* spp. and *Ceriops tagal*. Along the Tapee River, *Sonneratia caseolaris* dominates with some *Nypa fruticans* and *Acrostichum aureum* also present (Aksornkae and Euimmoh, 1988).

2.2.1.6 Land use

The majority of the coastal human population living around Ban Don Bay are involved in agriculture (mainly rubber, oil palm, coconut, rice, coffee and orchard plantations) and/or fisheries. Up until the 1970s, most households were involved in rice cultivation. This changed as saltwater intrusion salinised the soils and the rice paddy was abandoned. These abandoned areas were subsequently converted for coastal aquaculture. Aquaculture has now become an important source of income in Ban Don Bay. Areas converted for shrimp ponds have extended from abandoned rice fields to areas converted from mangrove forest (Rattakul, 1995). In 1993 alone, 25.2ha of former mangrove forest was converted into shrimp ponds (Rattakul, 1995).

2.2.1.7 Fisheries and aquaculture

The main fished commercial aquatic species landed in Surat Thani include shrimp (mainly *Penaeus merguensis*), crab (*Scylla* spp., *Portunus* spp.), whiting (*Sillago* spp.), sardines (*Dussumiera* spp.), herring (*Clupeoides* spp.), mackerel (*Scomber* spp.), anchovies (*Stolephorus* and *Thryssa* spp.), gizzard shad (*Anadontostoma chacunda*) and croaker (*Johnius*, *Otolithoides* and *Therapon* spp.), plus squid (*Loligo edulis*), cuttlefish (*Sepia esculenta*), octopus (*Octopus* spp.) and other molluscan species. In 1993, total value of fisheries production of 35,686 tonnes in Surat Thani was worth THB122,869,000 (USD4,914,760)(Department of Fisheries, Thailand, cited by Rattakul, 1995).

Coastal aquaculture has existed in Ban Don Bay since 1976. However the dramatic decline in catch per unit effort (CPUE) in the Gulf of Thailand in the early 1980s led the Department of Fisheries, Thailand to promote coastal aquaculture in the province with funds received from the World Bank and Asian Development Bank (Haemaprasit and Paw,

1988). The forms of coastal aquaculture promoted within Ban Don Bay have included shrimp culture (mainly *Penaeus monodon*), finfish culture and shellfish culture. Shrimp farms now cover about 42,892 rai (6,863 ha), producing 21,226 tonnes of shrimp of which 95% are tiger shrimp (*Penaeus monodon*). Finfish culture in Surat Thani is concentrated on Seabass (*Lates calcarifer*) and grouper (*Epinephelus malabaricus*). In 1996 27 tonnes and 5 tonnes were produced respectively. Since the early 1980s oysters have been one of the major aquaculture products from Surat Thani. The cultured species include *Saccostrea commercialis*, *S. lugubris* and *Crassostrea belcheri*. In 1996, 710 farms produced 10,782 tonnes of oysters (Department of Fisheries, Thailand, 1981-1998). The green mussel (*Perna viridis*), short-necked clam (*Paphia undulata*) and the blood cockle (*Anadara granosa*) are also important products from Surat Thani (Haemaprasit and Paw, 1988). Mud crab culture has existed in Surat Thani for at least 30 years, taking place on a small scale within coastal villages. Crab culture has traditionally been in the form of fattening, or growout of juvenile crabs caught locally by fishermen. Surat Thani, and in particular Kanchanadit District, is regarded as the main area for crab farming in Thailand (Harvey, 1990) due to its relatively long history of mud crab fishing and pond culture. In recognition of this importance, in 1992 the Bay of Bengal Programme, in collaboration with the Department of Fisheries of Thailand, ran a workshop on mud crab biology and culture in Surat Thani (BOBP, 1992).

2.2.1.8 Reasons for choice of location

Surat Thani was chosen as the primary study site for several reasons. Firstly, there are two clear phenotypes of *Scylla* which live apparently sympatrically in Ban Don Bay. They are known locally as white crab and black crab (in Thai “boo khao” and “boo dum” respectively). Secondly, these two phenotypes are caught locally in roughly equal numbers

all year round, making this location particularly suitable for regular, long-term sampling. Thirdly, as noted above, Surat Thani has a history of mud crab fishing, and more recently of crab farming, therefore there is good local knowledge about *Scylla*. Finally, technical support was available from the Surat Thani Brackishwater Coastal Aquaculture Research Centre, which is run by the Department of Fisheries and is located in Kanchandit District, Surat Thani Province. The main research interests at the research centre include both oyster culture and the growout phase of mud crab culture. Thus the centre had both facilities and expertise to assist with the present study. Samples of mud crab of both morphs were collected from local fishermen through a dealer based in Surat Thani Town.

2.2.2 Secondary study sites (within Thailand)

In Thailand, sites in Ranong and Chanthaburi Provinces were also sampled. Like Surat Thani, these are important locations for the mud crab research conducted by the Department of Fisheries, Thailand.

2.2.2.1 Ranong

2.2.2.1.1 Location

Ranong Province is situated 600km Southwest of Bangkok; it is the border province between Myanmar and southern Thailand on the Andaman Sea coast (9° 21' to 10° 42' N, 98° 24' to 98° 56' E) (Spalding *et al.*, 1997). Surrounded by a mountain range, Ranong has been isolated from the full extent of development experienced by the rest of Thailand until fairly recently (1997) when an airport was opened in Ranong.

2.2.2.1.2 Climate

Ranong has a tropical climate with mean air temperatures of 25°C to 28°C, the highest temperatures reaching 34°C. Ranong Province also experiences the highest rainfall in Thailand, approximately 4m annually, with a maximum of 6m (data provided by Meteorological Department of Thailand, 1966-1995). Ranong experiences two monsoon seasons; the Northeast monsoon takes place from November to February and the Southwest monsoon from May to October (Ryce, 1995).

2.2.2.1.3 Hydrographic features

The tidal range is approximately 2.5m in the mouth of the Klong Ngao estuary, but this can be as high as 4m (Spalding *et al.*, 1997). Even with high rainfall, the coastal and estuarine waters surrounding the mangrove has a high estuarine salinity, averaging approximately 32‰ (Wolanski and Wattayakorn, 1990).

2.2.2.1.4 Vegetation type

Ranong Province contains the largest remaining area of mangrove forest in Thailand; this covers 22,600ha of a large delta plain formed by silt deposited by the Kra Buri River. This mangrove area is bisected by many smaller interconnecting tributaries and canals, the largest being the Ngao estuary (“Klong Ngao” in Thai). Klong Ngao supports approximately 1150ha of mangrove forest (Wolanski and Wattayakorn, 1990; Ryce, 1995). The Ranong mangrove forest is composed of mainly evergreen species, including *Avicennia alba*, *Bruguiera cylindrica*, *B. parviflora*, *Ceriops tagal*, *Rhizophora apiculata* and *Rhizophora mucronata* (Spalding *et al.*, 1997).

2.2.2.1.5 Land use

The Ranong mangrove area has been exploited heavily, mainly for wood used in charcoal production and house construction, while some sites were exploited for tin mining until the mid 1980s (Macintosh *et al.*, 1993). The mangroves are also important for both commercial and artisanal fishing. The collection of aquatic products, including mud crab, is important for food and a source of extra income for small fishing communities who still live within the mangroves. Recently, a large area of the Ranong mangrove system has been designated as a biosphere reserve and the mangrove forest reclassified as protected area (UNESCO, 1996b).

2.2.2.1.6 Fisheries and aquaculture

Although crab fishing is still a significant income-generating activity for many coastal families in Ranong (Macintosh, 1998), the numbers of crab caught has declined in recent years. Ryce (1995) has quoted 65 tonnes of mud crab sold annually by the only village dealer in Klong Ngao. This is 40% less than the 109 tonnes sold in 1990 from the same location (Macintosh *et al.*, 1993).

In addition to fishing, aquaculture has become an important industry in Ranong. Various aquaculture activities, including pond culture of crab and shrimp, caged fish farming and oyster and mussel culture (Macintosh *et al.*, 1993) have become significant in the local economy. Annual shrimp production is presently estimated around 12,562 tonnes (Department of Fisheries, Thailand, 1981-1998). Oyster farming produces about 10 tonnes annually. The Department of Fisheries has promoted cage farming of grouper and sea bass. In 1996, 179 tonnes and 5 tonnes of sea-bass and grouper were produced respectively. In addition to the traditional fattening of recently moulted crabs, soft-shell

crab culture has also become an important industry (Macintosh, 1998), since it was established in Ranong in 1989 (Pripanapong and Tongdee, 1998). As Ranong is one of the few remaining places in Thailand where the supply of wild seed crab is still abundant, the province has become one of the main suppliers of mud crabs to other aquaculture ventures in southern Thailand.

2.2.2.1.7 Reasons for choice of location

In addition to the research conducted by the Department of Fisheries, Ranong featured as the main study site for an European Union (EU) project on “Environmental Assessment of Mangrove Reforestation as a Means of Improving Coastal Protection, Stability and Fisheries Production” (Macintosh, 1998). The mud crab fishery was studied as part of this EU project, and later (1995-1999) in a project funded by DANCED (Danish/SE Asian Collaboration in Tropical Coastal Ecosystems Research and Training Project).

Under the EU project, crab samples were collected from Had Sai Khao, which is located in Muang district of Ranong Province (see figure 2.3). Had Sai Khao is a traditional fishing village, where mud crab is one of the dominant aquatic species captured and traded. The mud crabs were bought from a dealer in Ngao village. Local fishermen who sold to this dealer were interviewed to ensure that the crabs were from the local area.

Three colour morphs of mud crab are present in Ngao Estuary, locally they are called white crab (boo khao), black crab (boo dum) and green crab (boo kiew). The black morph (boo dum), is the most prevalent *Scylla* morph in the Ngao Estuary although the other two morphs (boo kiew and boo khao) are caught on rare occasions. As a result only the black morph was collected from Ranong Province in the present study.

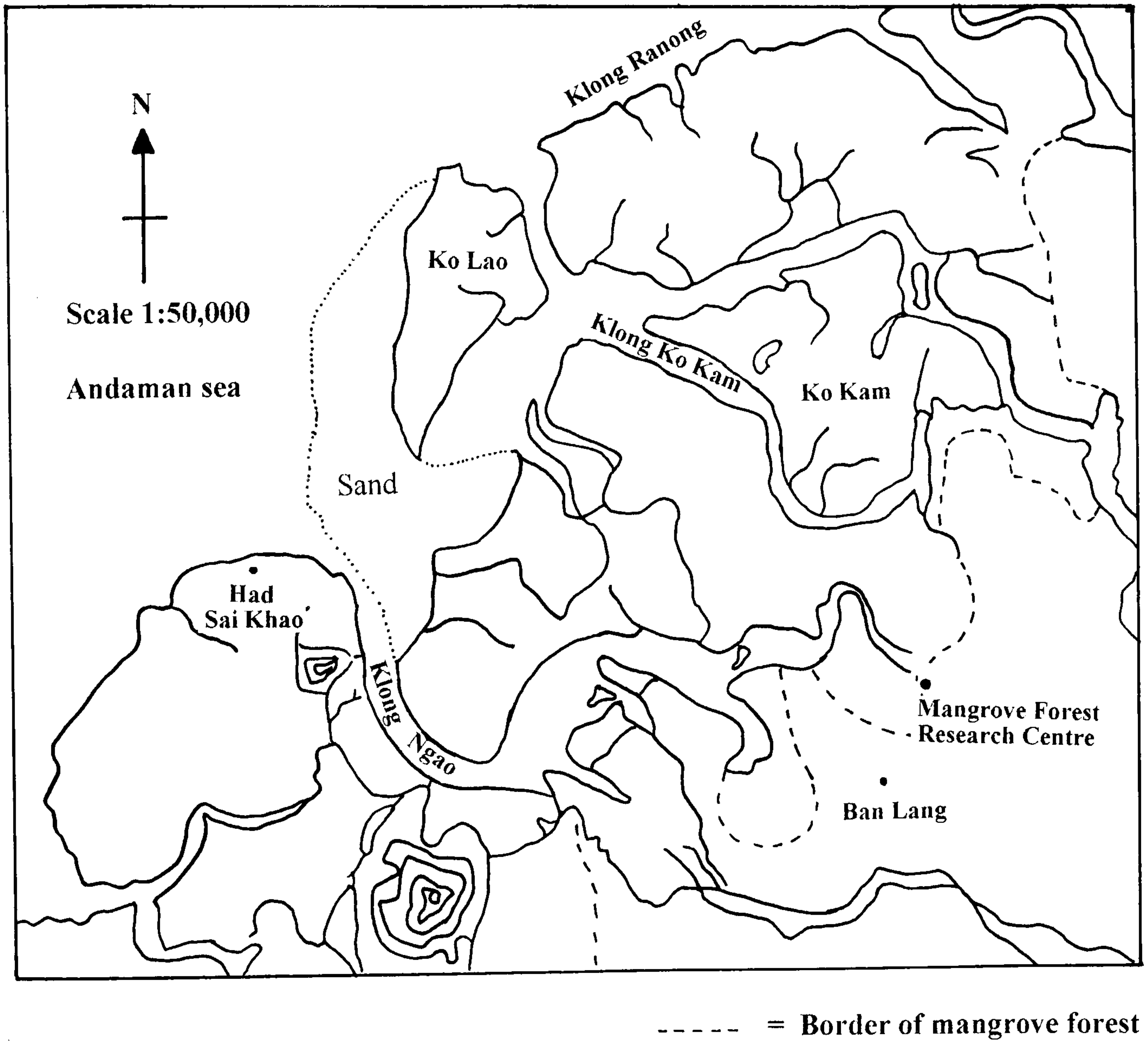


Figure 2.3: Map of Klong Ngao, Ranong Province, Southern Thailand (adapted from Ryce, 1995).

2.2.2.2 Chanthaburi

2.2.2.2.1 Location

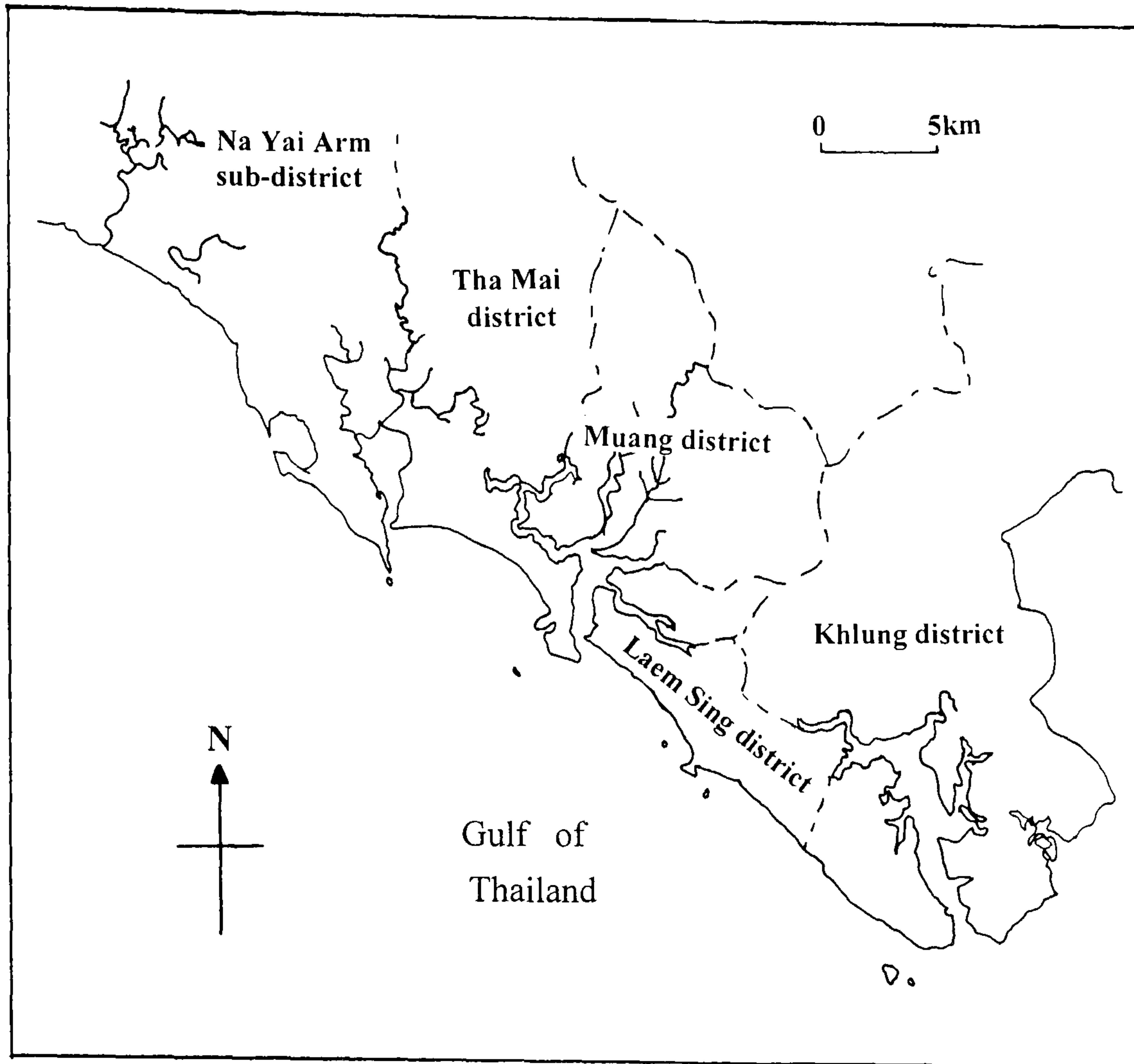
Chanthaburi Province is located on the eastern side of the Gulf of Thailand, 25km west of Bangkok and only 40km from the border with Cambodia (12°30' to 12°40'N; 101°50' to 102°08'E). Chanthaburi has 250km of coastline and is recognised for its large estuarine system, which previously supported a substantial area of mangrove forest. The dominant sea currents flow from east to west along the coastline. The coastal zone has four large inlets, namely Kung Kraben Bay, Ao Ko Nok Estuary, Chanthaburi Estuary, and Welu Estuary (Marsden, 1994) (see Figure 2.4).

2.2.2.2.2. Climate

The Climate in Chanthaburi is tropical with an average air temperature of 28°C. The rainy season is from mid May to October. The rainfall is heaviest in June (55.6mm) and lightest in December (9.5mm) (Marsden, 1994). The tidal range is approximately one metre with a maximum of 1.68m (Hydrographic Department, 1997).

2.2.2.2.3 Vegetation type

The area of mangrove forest in Chanthaburi Province declined greatly from 14,500ha in 1986 to 2,400ha in 1991. This was due mainly to the rush to invest in coastal shrimp farming in the late 1980s. The mangroves in Chanthaburi are composed of *Rhizophora apiculata*, *Rhizophora mucronata* and *Nypa fruticans* near the seashore, *Avicennia* spp. and *Bruguiera* spp, followed by *Ceriops* spp., *Lumnitzera* sp. and sometimes *Xylocarpus* spp. occur near the landward fringe (Rattakul, 1995).



----- District border

Figure 2.4: Map of the coastal districts in Chanthaburi Province, East Thailand.

2.2.2.2.4 Land use

Even though there is little heavy industry and the population density is low, much of the former mangrove area has been converted into shrimp ponds, coconut plantations or for urban development (Rattakul, 1995). Agriculture, especially fruit growing, contributes a large percentage of the total income generation in Chanthaburi.

2.2.2.2.5 Fisheries and aquaculture

Shrimp farming is also one of the dominant activities in the area. First introduced in 1975, and promoted by the Department of Fisheries in 1982, shrimp ponds covered an area of 24,903ha of coastal land by 1991. In 1994, Chanthaburi was the largest shrimp producing province in Thailand (Marsden, 1994). Presently, 36,344 tonnes of shrimp are produced annually (Department of Fisheries, Thailand, 1981-1998). The oyster *Crassostrea* has also been farmed in Chanthaburi within the King's project in Kung Kraben Bay, but the problems of air exposure has resulted in poor production. Approximately 2,216 tonnes of oysters were produced in Chanthaburi Province in 1996. Chanthaburi is also one of the main locations in Thailand for crab culture (Harvey, 1990). Mangrove associated fisheries also provide a valuable income. In 1977, USD3, 000 worth of aquatic products were collected on average from a km² of mangrove area within Klung District.

Chanthaburi Coastal Aquaculture Development Centre has been conducting research on *Scylla* for many years, in particular hatchery research for mud crab seed production (Srimukda, 1995). The research programme has two main objectives: a) to determine the best facilities to mature female crabs from the post copulatory stage to the spawning stage; and b) to study the hatchery production of stage four crabs (juveniles) for stocking in

growout ponds. This Department of Fisheries research centre is also researching the hatchery production of finfish and shrimp in addition to mud crab.

2.2.2.2.6 Reasons for choice of location

Chanthaburi was chosen as a site because a) it is one of the more important coastal provinces in Thailand with regards to crab fishing and culture; and b) it provided an intermediate location between Surat Thani, Southern Thailand and the two locations in Vietnam.

A visit to Chanthaburi Coastal Aquaculture Development Centre was arranged in November 1996. Three morphs of *Scylla* are identified in Chanthaburi. Those known locally as black crab and white crab are found in roughly equal abundance. The third morph (known as green crab or “boo kiew”) represents less than 2% of the total number of crabs caught around Chanthaburi. Unfortunately, due to the lack of availability of the other two morphs, only one morph (white crab) could be collected for this study. The crabs were bought from a dealer who only deals in fished crab. Interviews were carried out with fishermen, as well as the crab dealer, to confirm that the crabs were local.

2.2.2 Secondary study sites (other locations)

2.2.3.1 Thai Binh

2.2.3.1.1 Location

Thai Binh Province (province number 19) is situated in the southern part of the Red River Delta (20°30’N; 106°34’E). The coastline is composed of a lower mud flat, mangroves, upper mudflats and small sand islands located within the river mouths. Rivers surround three sides of the province, the remaining side forming the coastline to the Gulf of Tongkin

(see figure 2.5). The coast of Thai Binh Province is divided into two districts (Thai Thuy and Nam Dinh) by the Tra Ly River (Tri, 1997).

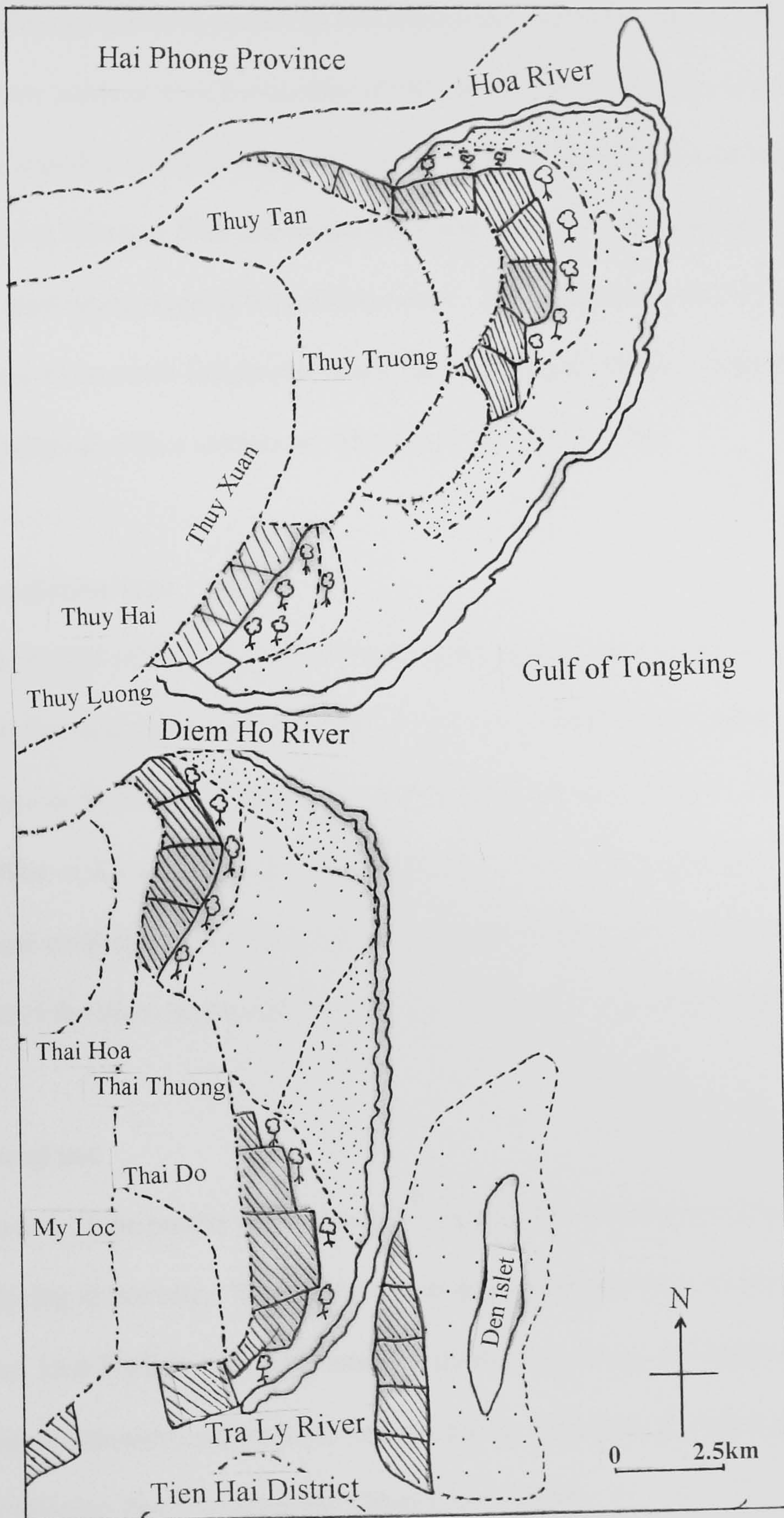
The study site within Thai Thuy District, Thai Binh Province, results from the merger of two former districts (Thai Ninh and Thai Anh) in 1969. Its 31km of coastline is protected by a seadyke (national Dyke number 7) from tropical monsoons striking the northern coast of the Gulf of Tongking.

2.2.3.1.2 Climate

Thai Binh experiences a subtropical climate with a mean monthly air temperature of 23°C to 24°C. From September to March the temperature drops below 20°C. The extremes of air temperature range from 5°C to 6°C in January to 37°C to 38°C in July (Tri, 1997). The coastal areas of Thai Binh Province are regularly affected by typhoon storms, the most vulnerable time being from July to October. Strong winds can gust up to 144 – 180km hr⁻¹. These strong winds are accompanied by heavy rainfall which can be up to 500mm hr⁻¹ (Tri, 1997). Rainfall at other times during the rainy season (May to October) averages 50mm day⁻¹.

2.2.3.1.3 Soil type

Acid sulphate soils are common in this province. The salty acidic soil means that rice yields are low. Thus other income-generating activities, such as salt production, are vital to the local economy. Thai Binh experiences high accretion rates of alluvium and sand along its coastline; deposited by the Red River as it enters the sea (Macintosh, 1996). Wide mudflats (in some cases up to 12km) may be covered with alluvial deposits up to 0.5m thick (Danish Red Cross/ Vietnam Red Cross, 1994).



Key

- | | | |
|----------|---------------------|-------------------|
| Mud flat | Sand/alluvium | Aquaculture ponds |
| Sea dyke | Mangrove plantation | |

Figure 2.5: Map of Thai Thuy District, Thai Binh Province, North Vietnam. (adapted from Danish Red Cross, 1994).

2.2.3.1.4 Hydrographic features

The coastal salinities can drop as low as 9‰ during the monsoon season when large flows of freshwater are washed from the mouths of the Red River towards the coastal deltaic provinces (Danish Red Cross/Vietnam Red Cross, 1994). Because the average elevation of the land is only 0.70m to 1.20m above sea level, any damage to the river dyke system results in the land being flooded with saline water. This commonly occurs during the typhoon season when wave height can reach 5m (Macintosh, 1996). Tides in the Gulf of Tongking are diurnal with a maximum tidal range of 2.6m to 3.9m.

2.2.3.1.5 Vegetation type

In 1994, with the aid of the Danish Red Cross, a programme was set up to involve poor families from five communes in Thai Thuy District in replanting (and later managing) a mangrove zone in front of the seadykes on the newly formed mud flats. This involved planting 2000ha of *Kandelia candel* over three years. This is the only significant coastal mangrove now existing in Thai Thuy District. The trees are presently five years old, and management of the trees, to prevent cutting, has so far been successful (Macintosh, 1999).

2.2.3.1.6 Land use

Thai Binh is one of the poorer provinces in Vietnam, with a high percentage of the population living in poverty. The main income-generating activities in the coastal communes of Thai Binh Province include salt production, sedge production, rice paddy (in the less saline communes), agriculture (onions, garlic, tobacco), fishing and, more recently, aquaculture (Danish Red Cross/Vietnam Red Cross, 1994).

2.2.3.1.7 Fisheries and aquaculture

After the successful replanting of mangrove forest, a new economically valuable resource has been realised both in terms of forest protection against storm damage and the increase in aquatic products that the mangroves support. In one commune (Thuy Hai Commune), over half the coastal population in are involved in fisheries and aquaculture; these activities contribute 60% to 80% of their total income (Macintosh, 1999).

In addition to fishing, pond aquaculture has become more popular in recent years as a way of significantly increasing income. The aquatic products farmed include shrimp, crabs, fish, molluscs and seaweed. In 1993, the total production of brackishwater aquaculture products in Thai Binh was 751 tonnes. Although only 16 tonnes of this was crab production, it is considerably more valuable than fish and seaweed, and comparable to shrimp in terms of price and marketability within Vietnam. Although there is a lack of quantitative data on crab production in Thai Binh over the last four to six years, there has been a perceived increase in production. The apparent rise in crab production is attributed largely to the restoration of the mangrove habitat acting as a nursery for juvenile fish, shrimp and crabs (Macintosh, 1996; 1999).

2.2.3.1.8 Reason for choice of location

Thai Binh was visited as part of a review of the coastal environmental preservation project in Thai Binh Province. The project (implemented by the Danish Red Cross) involved the replanting of mangrove trees to protect the seadykes against storm damage, thus reducing the damage to coastal villages (Danish Red Cross/Vietnam Red Cross, 1994). The project reviews (Macintosh, 1996; 1999) have provided a detailed account of crab fishing and aquaculture practices within Thai Binh Province, where mud crab culture has preceded

shrimp farming in the area as a significant income-generating activity. The close proximity of Thai Binh to Hanoi and even to China, makes transportation and marketing of mud crabs relatively easy and highly profitable.

The collection was made in Thuy Hai Commune in Thai Thuy District. Only one mud crab phenotype was discovered in Thai Binh Province. This phenotype resembled the white crab collected from Thailand. The crabs were purchased from the only crab dealer in Thuy Hai Commune who buys mud crabs directly from fishermen and hand collectors of aquatic products. From interviewing him, it was confirmed that the crabs obtained were from Thuy Hai Commune.

2.2.3.2 Can Gio

2.2.3.2.1 Location

Can Gio is a suburban district 65km south of Ho Chi Minh City (formerly Saigon) within the Mekong Delta (10°22'N, 106°46'E) (see figure 2.6). The Mekong Delta flows into the South China Sea. Can Gio experiences irregular semi-diurnal tides with high tidal amplitudes (3.3m to 4.1m).

2.2.3.2.2 Climate

The climate is typically tropical with a pronounced dry season from November to April (Northeast monsoon) and wet season from mid April to late October (Southwest monsoon).

The temperature is similar all year round with mean monthly temperatures of 25.5°C to 29°C. The monsoons bring strong winds, in some cases up to 3.9km hr⁻¹. The rainfall is moderate with a monthly mean of 100mm, peaking in September with 300mm to 400mm (Nam and My, 1992).

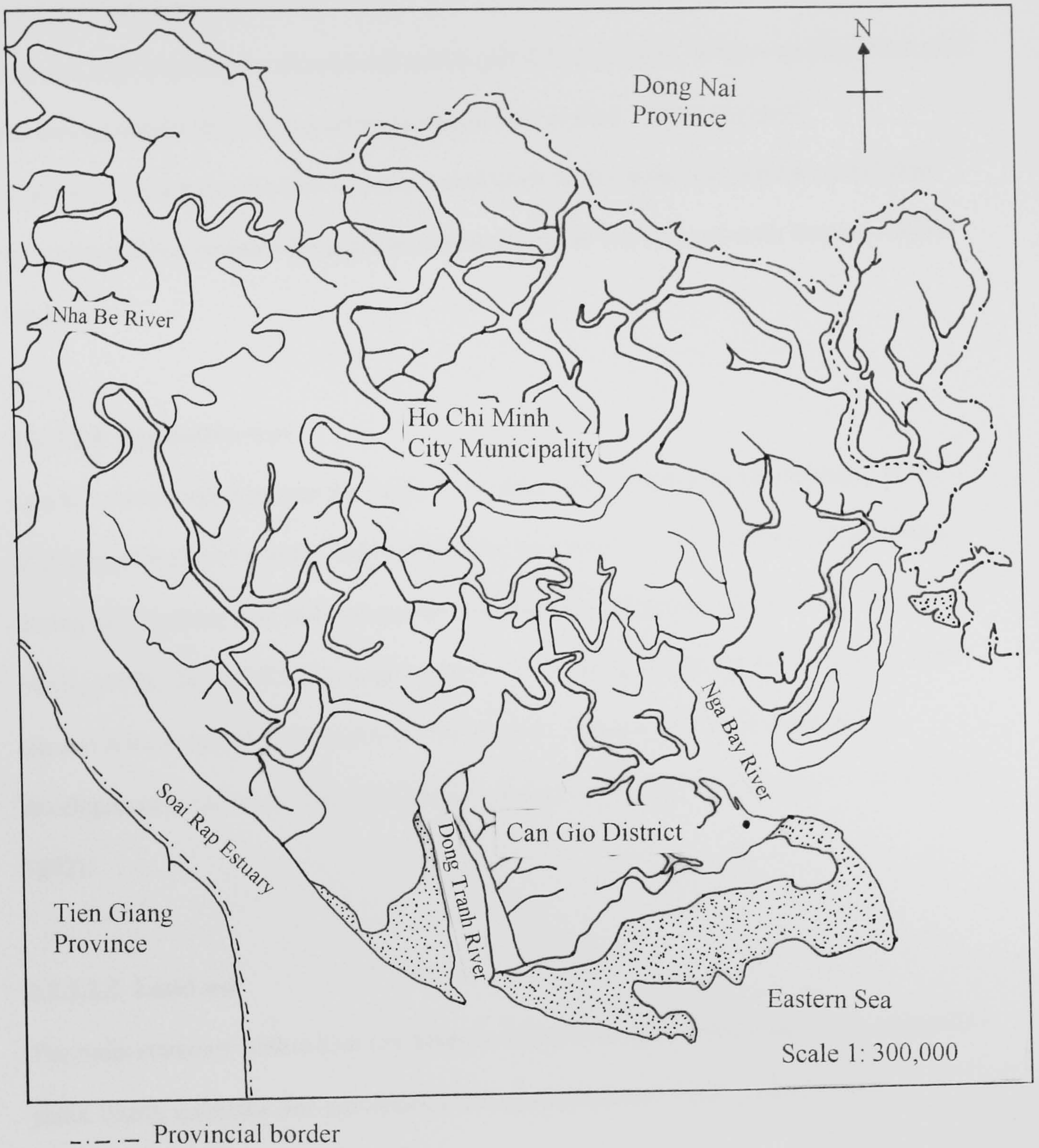


Figure 2.6: Map of Can Gio District, South Vietnam (adapted from UNESCO-MAB, 1998).

2.2.3.2.3 Soil type

The soil type is generally alluvial and acidic (pH 4.5 to pH 6.5). Where land has been used for shrimp ponds, the soil is a saline sulphatic clay or mud. There are large quantities of sulphites present; when exposed to air these cause acid sulphate conditions, which has been a problem for aquaculture development (shrimp and crab farming) in this location.

2.2.3.2.4 Vegetation type

Can Gio forest park contains the majority of mangroves within Can Gio District. Over 40,000ha of mangrove forest were completely destroyed by a combination of defoliants during the Vietnam War and subsequent over exploitation of the coastal area (UNESCO-MAB, 1998). Since 1978, a mangrove reforestation programme has been initiated. This aims to reduce coastal and riverbank erosion and to support the socio-economic development in the area. The main species planted is *Rhizophora apiculata* (Nam and My, 1992).

2.2.3.2.5 Land use

The main economy within Can Gio District consists of agricultural crops (rice, vegetables, yams, beans, coconuts and pineapples), salt production and finally fisheries and aquaculture (UNESCO-MAB, 1998).

2.2.3.2.6 Fisheries and aquaculture

There are over 150 mangrove-associated aquatic species recorded within the Can Gio forest reserve. These include seabass (*Lates calcarifer*), mullet (*Mugil affinis*), catfish (*Pangasius* spp.) shrimps (*Acetes*, *Penaeus* spp. and *Metapenaeus* spp.) and mud crab

(*Scylla* spp.). The main fisheries are for shrimp, grouper, seabass, squid and crab. In 1991 10,514 tonnes of fish and shrimp were caught in Can Gio District (Nam and My, 1992)

In terms of coastal aquaculture, shrimp farming is the most significant component. In 1991 there were 360 ha of intensive shrimp ponds and 3,640 ha of traditionally grown shrimp (Nam and My, 1992). However, there has been a decline in shrimp farming recorded in recent years (UNESCO, 1998) after some government backed shrimp farming projects failed due to technical difficulties.

In Can Gio District, crab culture takes place in ponds and floating cages. In 1991, 140 households raised soft-shelled crabs and 78 households raised fattened crabs and egg crabs (Nam and My, 1993).

2.2.3.2.7 Reasons for choice of location

Can Gio featured as the other main study site for the EU project “Environmental Assessment of Mangrove Reforestation as a Means of Improving Coastal Protection, Stability and Fisheries Production” (Macintosh, 1998). Therefore it was convenient to arrange a visit under this project. In addition, Can Gio is well positioned between Thai Binh, located in North Vietnam and the east coast locations in Thailand.

The crabs were purchased from a primary dealer located at the border between Nga Be and Can Gio districts, along the Nga Be River. It was confirmed that he had bought the mud crabs directly from local fishermen.

Although Serene (1952) describes four morphotypes of *Scylla* from the Mekong Delta, only one phenotype was found in Can Gio. This phenotype is similar to the white morph present in Thailand.

2.2.3.3 Sematan

2.2.3.3.1 Location

Sematan is a small coastal village, located in Lundu District, Kuching Division, at the western tip of Sarawak (Malaysia), near the border with Kalimantan on the Island of Borneo (1°51'N; 109°47'E) (see figure 2.7). Sematan is approximately 109 km west of the capital, Kuching, Sematan.

2.2.3.3.2 Climate

Sematan experiences a climate that borders between equatorial and tropical; hot and humid with high rainfall. Monthly air temperatures are fairly consistent throughout the year, with average temperatures ranging from 25.8°C to 27.1°C (Tropical Coastal Ecosystems Project (TCEP), unpublished). The dry season is from April to September and the wet season from October to February although there is rain all year round. The highest average total monthly rainfall in Sematan is in January (920mm), the lowest is in May (100mm) (Sematan weather station 1987 to 1998). The relative humidity is reasonably high, ranging from 40% to 98% saturation. The annual mean surface wind speed between 1995 and 1998 was low (3.24km hr⁻¹). The maximum wind speed recorded for these three years was 54.72km hr⁻¹ (recorded in November) with an extreme wind speed of 75.6km hr⁻¹ (TCEP, unpublished).

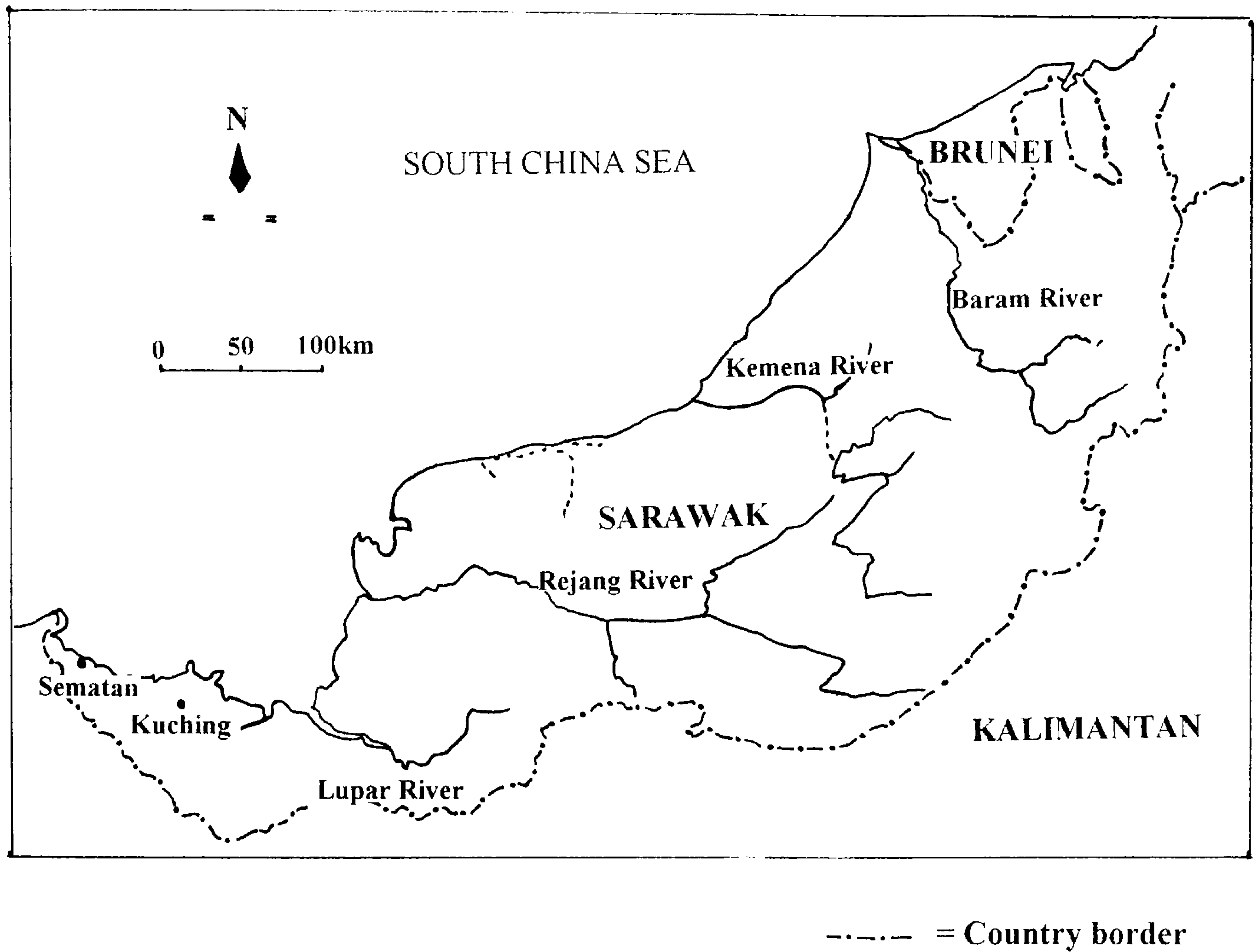


Figure 2.7: Location of Sematan, Sarawak, East Malaysia. (adapted from Lohor, 1993).

2.2.3.3.3 Soil type

Podzols represent the dominant soil group in Sematan. Organic soils are found in the west of Sematan, adjacent to the Sungai Samunsan tributaries. Sandy mineral soils (also known as arenaceous soils) are also found in this area and along the coastline (that which is found along the coast is marine of origin). There is also a build up of alluvial soils that are accreting within the river system. Alluvial soils and gley soils are also found along the coast. These gley soils are mottled with iron deposits. Clay-like sulphitic soils are found along the riverbanks, close to the river mouth (TCEP, unpublished).

2.2.3.3.4 Hydrological features

The main river within the study site is Sungai Sematan (the Sematan River). This river is fed by the Sungai Sebako and Sungai Serayan in the southwest. The Sungai Sematan is 2km long and flows northwesterly, forming the Sungai Sematan estuary which runs into the South China Sea.

The tidal range at Kuala Sematan is from 1.2m to 3.7m. At Palau Lakai (the nearest standard reference port) the lowest tide is 0.0m and the highest tide is recorded at 5.7m, the mean sea level recorded at 3.1m (1999 hourly tide tables (Sarawak) and navigational aids list).

2.2.3.3.5 Vegetation type

Twenty six percent of the total mangroves in Malaysia are situated in Sarawak (approximately 45,700km²) (Spalding *et al.*, 1997), of which 9,930km² is part of the forest reserves concentrated around the delta areas of the Sarawak, Rajan and Trusan-Lawas rivers. Sematan mangroves cover an area of about 1,735 hectares (Lohore, 1993) much of

which is in pristine condition. The rest of the forested area is a mixture of peat swamp, heath and dipterocarp forest. Mangrove species include *Avicennia* and *Sonneratia* communities on the seaward fringe along with *Rhizophora apiculata*, *R. mucronata*, *Ceriops tagal*, *Exoccaria*, *Xylocarpus granatum* and *Bruguiera cylindrica* forest. In some areas nypa palms (*Nypa fruticans*) form the dominant species along with *Oncosperma tigillaria*, especially up river where there is a greater freshwater influence. Nypa palms make up 17.8% of Sarawak's total mangrove forest (Spalding *et al.*, 1997). This forest is being exploited, with two logging companies now operating in the mangrove forest (TCEP, unpublished).

2.2.3.3.6 Land use

Sematan produces oil palm, rice, timber, black pepper, rubber, coconuts, pineapples, citrus fruits, sago, cocoa, coffee and aquatic products from mainly coastal fisheries and aquaculture. Data for Sarawak fish landings in 1981 showed that a third of the total fish caught comprised of mangrove associated species (Kam 1985; cited by Lohore, 1993).

2.2.3.3.7 Fisheries and aquaculture

In 1991, the total landings from coastal fishing amounted to 33,562 metric tonnes. Fishing is a full time activity for about 300 families within Sematan although many more fish on a part time basis. Because the weather often prevents smaller fishing boats going out to sea, the fishermen on average only fish for six months of the year in total, the remainder of their income derived from other income generating activities.

Over 48 marine species were landed in Lundu district in 1997. These include jewfish, mackerel, herring, and marine catfish. Other species caught are the blue swimming crab

(from June to August), big shrimp (from March to May), squid (April to August) and jellyfish (February to April). Seabass is also a popular fish species associated with mangrove estuaries and lagoons.

In recent years aquaculture has been promoted in Sarawak. Shrimp farming is now prevalent along the coastal zone. The production for 1997 was reported to be 42.06 metric tonnes valued at 1,300,000 Malaysian Ringgit (USD 448,276). There are also three projects to promote cage culture of the seabass, *Lates calcarifer*. Two projects are based in Sungai Sebako, the third is located in Kampung Tamaga Melayu. The Sematan Fisheries Research Station is currently carrying out a sea bass hatchery project. The culture of the blood cockle, *Anadara granosa*, is also taking place on a small scale on the mudflats in Kuching division (Lohore, 1993).

Mud crab culture is relatively new in Sarawak where the pond culture of crabs started in the 1980's; crab ponds still exist, but are not that popular. One of the reasons for this is that there is a shortage of land available to build ponds. In 1992, the Inland Fisheries Division of the Department of Agriculture introduced pen culture of mud crab in former logged mangrove areas in Sematan (Say and Ikwanuddin, 1999).

Small areas of mangrove (9m x 18m) are fenced off using wood or Nypa palm (*Oncosperma tigillaria*). A ditch is dug inside the perimeter of the fence to hold water when it is low tide. This more environmentally friendly pilot project to culture mud crab was designed to assist local fishermen in improving their income. The success of this project has led to similar projects being set up in other districts within Sarawak (Say and Ikwanuddin, 1999).

There is also a fisheries research station based at Sematan. The main body of their research involves the artificial breeding and hatchery production of mud crab seed.

2.2.3.3.8 Reasons for choice of location

Sematan was an additional site used within the EU project on “Environmental Assessment of Mangrove Forestation as a Means of Improving Coastal Protection, Stability and Fisheries Production” (Macintosh, 1998). This location was also one of the selected study sites for the project funded by DANCED (Danish/SE Asian Collaboration in Tropical Coastal Ecosystems Research and Training Project). Sematan was visited as part of the EU project. The considerable distance between Sarawak and the Thai/Malay peninsula provided a good easterly site for comparison of the effect of latitude on the forms of *Scylla* in Malaysia.

The local fishermen in Sematan recognise three phenotypes of *Scylla*. These are known locally as “ketam nypa” (nypa crab), “ketam bakau” (mangrove crab) and “ketam pasir” (sand crab). Only the most abundant morph, ketam bakau, which is recognised by its red claws was collected during this trip. Its morphology is similar to that of the black crab (boo dum) in Thailand.

The crabs were collected from two sources. Some of them were obtained by the author, members of the Inland Fisheries Division of the Department of Agriculture and local villagers. These crabs were fished from the mangroves surrounding Sematan using baited traps set at high tide and collected at the following low tide. The remaining crabs were collected from intertidal fenced pens, stocked with locally fished crabs.

2.2.3.4 Paikgasir

2.2.3.4.1 Location

Paikgasir is situated within Kulna District, southeast Bangladesh, close to the border with India (22°72'N; 89°36'E). Paikgasir lies within the broad delta formed by the Padma and Jamuna rivers, which join to form the Megna River (see figure 2.8). This delta is one of the largest in the world, stretching 200km from east to west and entering from the north into the Bay of Bengal.

2.2.3.4.2 Climate

Paikgasir experiences a subtropical monsoon climate where 80% of the rainfall falls between May and mid October. Mean annual rainfall is approximately 1,500mm (Spalding *et al.*, 1997). The air temperatures are warm but seasonal. The coldest temperatures are recorded in January (19°C), rising to average 29°C in May. The Bangladesh coastline is particularly susceptible to cyclones, tornadoes, thunderstorms and floods. Maximum wind speeds recorded in Bangladesh have reached 235km hour⁻¹ (Haider *et al.*, 1991).

2.2.3.4.3 Soil type

The soil is composed predominantly of alluvium and loamy clay soil on the surface with a neutral pH (7-8). Every year 2.4 billion tonnes of sediment is transported by the major rivers of Bangladesh. This has a profound effect on the geomorphology of the flood plains and coastal region (Khan *et al.*, 1994).

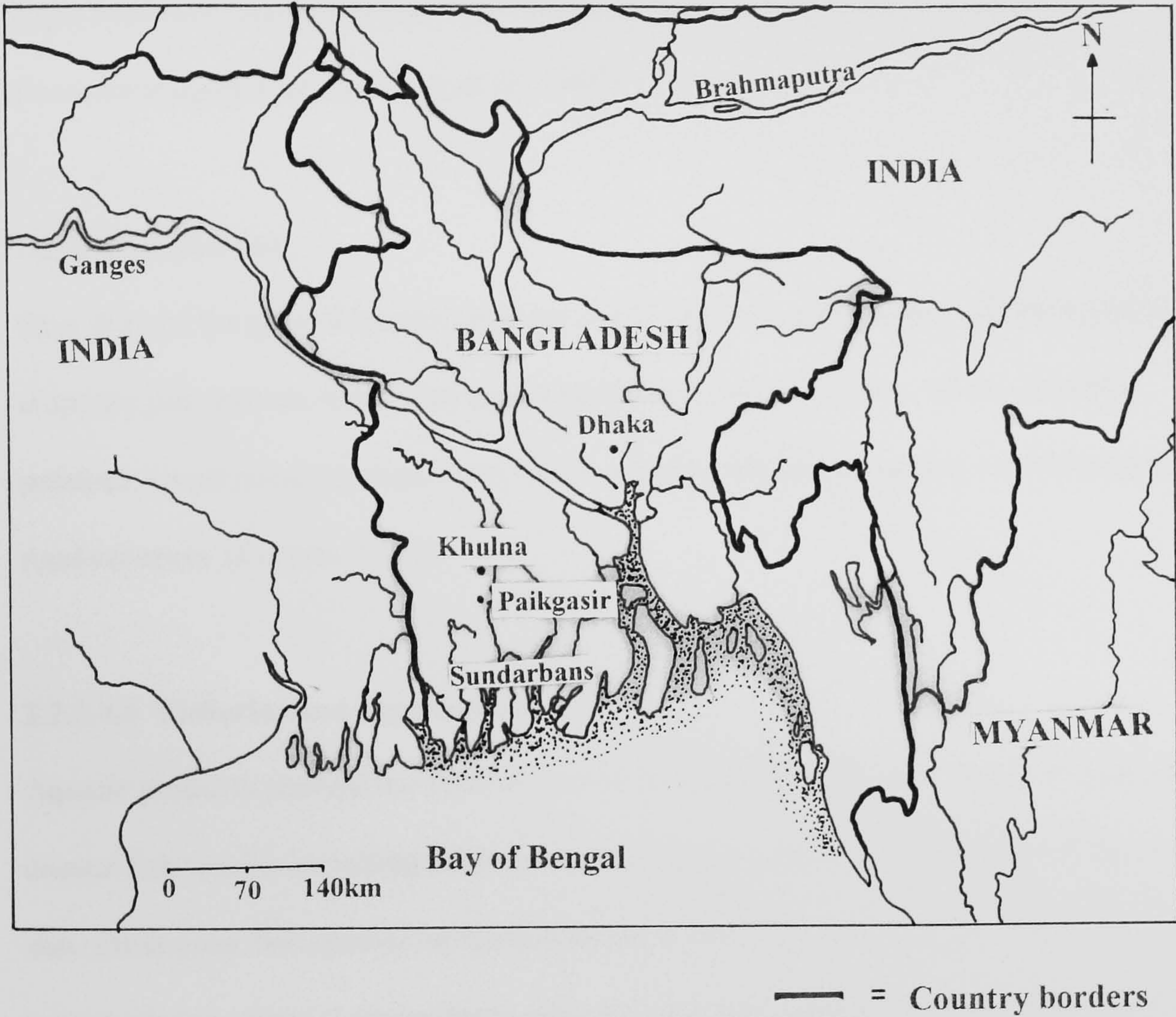


Figure 2.8: Location of Paikgasir, Khulna Division, Bangladesh (adapted from Encarta, 1997).

2.2.3.4.4 Vegetation type

Paikgasir is located in the Sundarbans, the largest single expanse of mangrove forest remaining in the World, extending from the Southwest corner of Bangladesh across the border into West Bengal, India. The main mangrove species include *Heritiera fomes*, *Nypa fruticans*, *Sonneratia apetala*, *Ceriops decandra*, *Avicennia officinalis* and *Excoecaria agallocha* (Spalding *et al.*, 1997).

2.2.3.4.5 Land use

Over 40% of the gross domestic produce is derived from agriculture. The principal cash crops are jute and tea, with other agricultural crops, including rice, wheat, oilseeds, potatoes, sweet potatoes, sugarcane, bananas, mangoes and pineapples contributing to the rural economy (Encarta, 1997).

2.2.3.4.6 Fisheries and aquaculture

Aquatic products provide the chief source of protein in Bangladesh. Small riverine and coastal fish species including *Hilsa* (a clupeid species) are an important part of the local diet. Freshwater fish species have often made up the majority of the protein consumed, but with declining inland fisheries more emphasis has been placed on coastal fish species, including the mud crab, as other possible sources of protein (Ahmed, 1992).

Paikgasir is an important distribution centre for crabs caught within the Sundarbans. Mud crab is an incidental crop caught when harvesting the shrimp ponds. Paddy fields are flooded in the summer months (March to August) to form vast shallow ponds called “gers”. These gers can be as large as 40 hectares each. Tiger shrimp (*Penaeus monodon*) is the main species cultured, though other local shrimp are also farmed. There are about

200 of these ponds in Paikgasir. Juvenile crabs find their way into the ponds from the surrounding habitat and feed off the shrimp. Up to 20kg of crab are caught per person per day from the ponds (Overton and Macintosh, 1997).

Within the Southwest region of Bangladesh, about 1200 tonnes of mud crabs were caught in 1991 (Ahmed, 1992). As the crabs can reach a high price, they are transported from Paikgasir to Dhaka where two major crab centres export to Thailand, Malaysia and Taiwan (Overton and Macintosh, 1997). As the majority of the Bangladesh population are muslim, they do not eat amphibious species such as the mud crab, thus the mud crab is exported to other countries where there is a greater demand. This trading has only been successful since the beginning of the 1990s. Techniques for culturing crab are still to be developed in Bangladesh (Khan and Alam, 1992). Due to lack of expertise and financial constraints, many attempts to date to culture mud crabs have not been successful.

2.2.3.4.7 Reason for choice of location

A visit was made to Paikgasir with support from the ODA (now DFID) Fisheries Resource Unit (BAFRU) based in Dhaka, Bangladesh. Paikgasir provides an additional location on the western side of the Thai/Malay peninsular. Only one morph of *Scylla* was available in Paikgasir. This phenotype resembled the black morph collected in Thailand. The crabs were collected in conjunction with local fishermen using hand nets from the large shrimp gers surrounding the village.

2.3 Collection of mud crab samples

Mud crabs were sampled from catches obtained using local fishing methods. Thus the mud crabs sampled represented the exploited part of the local crab population. Crab samples were collected either by going on crab collecting trips with local people, or by obtaining the samples from unsorted catches from local fishermen using a primary dealer to make the transaction. The fishermen were interviewed to confirm the location of their catches and the types of fishing gear used.

The majority of the crabs were caught using box traps (a netted collapsible trap, which is popular in the majority of locations sampled), but other methods such as gill nets, pole traps, and crab hooks were also used. Popular fishing sites varied from the coastal mangrove fringes to the estuarine creeks surrounding the locations chosen.

Care was taken to avoid crabs from crab ponds, which may have been imported for pond stocking from neighbouring provinces, or in some cases from even further afield. Large fish markets were also avoided due to the uncertainty of the source of mud crabs being sold there.

2.3.1 Numbers of mud crab collected

The numbers of crabs collected and their year of collection are summarised in table 2.2. This table also shows that not all the crabs collected were used in all three methods employed to determine species status of the genus *Scylla*. Surat Thani was the primary study site; therefore samples of the two morphologies present in Surat Thani were collected on four occasions. The material collected from other locations was used

Table 2.2: Summary of mud crab collections from 1994 to 1997 for the study of the genus *Scylla* from South and Southeast Asia.

Country	Location and morph	Number of individuals and year of collection												Total	
		Descriptive taxonomy		Morphometrics		Allozyme electrophoresis		Reproductive isolating mechanisms		Reproductive isolating mechanisms		Total			
		Male	Female	Male	Female	Male	Female	Male (gonopod morphology)	Female (sexual maturity)	Male	Female	Male	Female	Male	Female
Thailand	Surat Thani	Black	72	88	1994 16	1996 28	1995 19	1995 25	5	835	Male	Female	96	96	
					1996 28	1996 30	1996 32	1996 34					Female	948	
					1997 28	1997 30									
		White	73	81	1994 28	1996 29	1995 15	1995 25	6	780	Male	Female	94	94	
					1996 24	1996 26	1996 20	1996 27					Female	886	
					1997 21	1997 26									
Vietnam	Ranong	Black	32	12	1994 26	Nc	1996 32	1996 12	Na	Na	Male	Female	58	58	
														12	
	Chanthaburi	White	22	13	1996 22	1996 13	1996 12	1996 12	Na	Na	Male	Female	22	22	
	Thai Binh	White	27	27	1996 27	1996 27	1996 13	1996 29	Na	Na	Male	Female	40	40	
Malaysia	Can Gio	White	30	Nc	1994 30	Nc	Nc	Nc	Na	Na	Male	Female	30	30	
Bangladesh	Sematan	Black	25	16	1994 25	Nc	Nc	Nc	Na	Na	Male	Female	25	25	
	Paikgasir	White	29	21	1995 29	1995 21	Nc	Nc	Na	Na	Male	Female	29	29	

Nc no collection

Na not applicable

selectively to provide additional information for comparison with the two sympatric morphs collected from Surat Thani.

The ideal situation would have been for all locations to be included in all aspects of the study. In most cases the morphological data (including morphometric and descriptive data) were obtained *in situ* and therefore all locations sampled are represented. However limitations in the transportation and storage of animals made it difficult to represent some locations in the allozyme electrophoresis.

The aim was to collect approximately 30 crabs of each sex from each location. However, the numbers of individuals collected depended on their availability. In the case of allozyme electrophoresis, the problems of transporting large numbers of crabs back to the laboratory also meant that in some cases the number of specimens available was less than 30 crabs per sex and/or location.

All sampled female crabs and selected male specimens from the primary study site, Surat Thani, were also used to provide information on the reproductive characteristics of the two morphs found living sympatrically in this location.

In addition to the samples collected for this study, *Scylla* specimens were obtained from a concurrent investigation conducted by Dr C.P. Keenan at Bribie Island Aquaculture Research Centre, Queensland, Australia. Six crabs labelled as *Scylla serrata*, six crabs labelled as *Scylla tranquebarica* and five crabs labelled as *Scylla olivacea* from the nomenclature proposed by Keenan *et al.* (1998) were obtained for comparison with the

Scylla morphs collected in the present study and to aid in the construction of a phylogenetic tree for the genus *Scylla*.

2.3.2 Size range of crabs collected

The size ranges of crabs collected in this study are listed in table 2.3, and show that mainly adult crabs were used, with a size range of 73.2mm to 145mm (internal carapace width).

The primary reason for this is that juvenile mud crabs are much harder to capture than the adults, especially within the mangrove habitat (S. Moser pers.com.). Another reason the size selectivity of the fishing gear used to capture mud crabs in Asia. The box trap is the popular fishing method in most of the sites sampled. The entrance to the box trap restricts the size of crabs that can enter the trap. The entrance is too small for very large crabs to enter the trap and is too difficult for juvenile and small adult crabs which would have to climb up the trap and force their way in through the trap entrance.

It is also possible that the mud crab fishermen primarily sort any small crabs at sea, or at home for their own ponds; thus the smaller crabs are already removed from the “unsorted” catches presented for sale.

Table 2.3: Size range (using internal carapace width) of mud crabs collected from locations within Southern and Southeast Asia.

Location	Size of mud crabs sampled (mm)		
	Mean	Minimum	Maximum
Surat Thani: Black	102.2	86.3	130.3
Surat Thani: White	109.1	72.3	145.6
Ranong	95.6	87.0	104.9
Chanthaburi	108.1	93.3	135.9
Thai Binh	88.8	73.2	101.1
Can Gio	93.3	82.2	102.5
Sematan	97.1	82.5	114.4
Paikgasir	95.4	80.9	117.4

2.4 Selection criteria

In general adult crabs were selected as they were readily available from fished catches, showed all the adult characteristics for descriptive taxonomy, and made it easy to remove tissues for allozyme electrophoresis.

Only healthy crabs were selected. This meant that they were free of any injury, highly receptive to physical stimuli and all their limbs were present. Recently moulted, or soft-shelled crabs, were rejected due to their increased risk of mortality during transportation to the laboratory.

Those crabs with regenerated limbs, especially the chelae, were also rejected, or were used only for electrophoresis, as the resulting morphometric data would have distorted the results of the multivariate analysis undertaken. Moreover, crabs with missing or damaged appendages also survive less well during transportation (Overton, 1994).

2.5 Data collection

For the majority of the study, morphological data (including meristic and morphometric data) were collected on location. For allozyme electrophoresis the mud crabs were transported to the laboratory for processing. Individually tied crabs were transported alive in cardboard boxes. As *Scylla* can live out of water for up to three days, it is unnecessary to transport them in water.

The allozyme electrophoresis was carried out at the Institute of Aquaculture, University of Stirling, Scotland for the first year and then in the Department of Ecology and Genetics, University of Aarhus, Denmark for years two and three. A detailed description of the data

collection and analytical procedures is presented in the methodology section of the relevant chapters, so they are not discussed further here.

Chapter Three

Descriptive taxonomy of the genus *Scylla*

3.1 Introduction

Descriptive taxonomy is defined as the discrimination and description of biological taxa (Blackwelder, 1967). It is used in defining, naming and identifying taxa, comparing similar specimens in order to group them, or conversely, distinguishing between dissimilar specimens in order to separate them (Blackwelder, 1967; Quicke, 1993). The discrimination and description of a species is based on observation, comparison, and analysis of features from a group of organisms, or from a single specimen. These features can include the presence or absence of structures; meristic characters; and functional, behavioural or developmental aspects of the organism of interest (Blackwelder, 1967). Those characters that are characteristic of the morphology of one species are known as diagnostic characters. In general, characters chosen to describe a species should be stable (*i.e.* show low intraspecific variability), be well distributed within the population, and have a clear-cut delimitation (Quicke, 1993).

In the past, descriptive taxonomy was the predominant method used to classify and record known taxa and enable others to identify them with relative ease. Even today, where advanced genetic techniques can be used to identify the species composition of a confused genus, or the relationship between closely related species, descriptive features are still important for gross identification. It is one of the most elementary procedures because until the taxonomy of a species, including a description, has been achieved, organisms can not be discussed or treated in a scientific way (Blackwelder, 1967).

The genus *Scylla* is part of the Family Portunidae Rafinesque, 1815. Members of this family are mainly characterised by the last two joints of the fifth legs being flat and oar-like; this is an adaptation for swimming and excavating (Hartnoll, 1971). Moreover, the carapace is usually wider than long and slightly convex, the frontal lobe is usually broad and cut into teeth, the orbits are complete and a rostrum is absent (Stephenson and Campbell, 1960). The family Portunidae is divided into six subfamilies. The genus *Scylla* belongs to the subfamily Portuninae Alcock, 1899. The subfamily Portuninae are characterised by their short pereopods (walking legs) compared to the chelae; the carapace has four to nine anterolateral spines; the basal joint of the second antenna is broad and its flagellum is sometimes included in the orbital socket (Stephenson and Campbell, 1960).

The genus *Scylla* has had a long history of confused taxonomy. Before the genus was officially named *Scylla*, the mud crab had four other generic names; *Cancer* Forskål, 1775, *Portunus* Fabricius, 1798, *Lupées* H. Milne-Edwards, 1834 and *Achelous* Macleay 1836. The genus name *Scylla* was allocated subsequently by de Haan in 1850. The main characters that describe the genus *Scylla* are outlined in figure 3.1.

There has been much debate as to whether or not the genus *Scylla* is comprised of more than one species. The first recorded description was by Forskål (1775) where he described *Cancer serratus* from a specimen collected from Djedda on the Red Sea. Herbst (1796) and Fabricius (1798) produced descriptions independently proposing the synonymous names *Cancer olivaceous* and *Portunus tranquebaricus* respectively. H. Milne Edwards (1834) first suggested that the genus (he called *Lupées*) contained two species.

Subsequently, there has been divided opinion for and against more than one species of *Scylla*, the chronology of which is outlined in table 3.1. Until Estampador (1949a), most of the descriptions were vague and difficult to follow. Estampador (1949a) recognised

Genus *Scylla* de Haan, 1833

de Haan, 1833: 11; Milne Edwards, A., 1861: 347; Alcock, 1899: 27; Estampador, 1949a: 98-99; Crosnier, 1962: 71; Keenan *et al.*, 1998: 228.

Diagnosis:

Carapace transverse and broad, sometimes described as two thirds as long as wide (Carapace length/Internal carapace width = 0.66 – 0.72); moderately convex carapace with smooth surface apart from “H” shaped groove marking out the mesogastric and cardiac regions. Two horizontal granular ridges from the most posterior anterolateral spines across the branchial regions and one ridge across the epigastric region. Front well separated from supra orbital angles. Frontal lobe divided into four teeth ranging from rounded to prominent. Frontal lobe width is variable between species (frontal lobe width/internal carapace width = 0.33 – 0.45). Anterolateral borders arched, bearing nine sharp spines that are roughly equal in size. Smooth posteriolateral margins. Supra-orbital margins bear one medial and one outer closed fissure; Infra orbital margins prominently toothed. Antennules folded neatly transversely, basal antennal joint is short and broad, forming lobule lying in the orbit, flagellum lying in the orbital hiatus. Eyestalks short, eyes spherical. Chelipeds massive, smooth and longer than other limbs. Chelipeds are armed with spines. Cheliped merus bears three large spines on anterior border and two smaller spines on posterior border. Cheliped carpus has one acute spine on inner angle and one to two spines on the outer ventral margin, varying from strong to obsolete. Cheliped propodus exhibits one strong tubercle at carpus articulation and a pair of dorsal spines above the base of dactylus, varying from strong to vestigial. Inner tubercle immediately behind base of gape varies from prominent to vestigial. Pereiopods stout and moderately compressed, first three pairs are similar, the fourth pair are natatorial, fringed along borders with fur-like setae. Male abdomen is narrow with third to fifth segments fused, the female abdomen is broadly arched. Colour is variable from green to brown with or without polygonal reticulation.

Figure 3.1: Taxonomic description of the mud crab (genus *Scylla*).

Table 3.1: Chronological summary of published revisions of the taxonomy of the genus *Scylla*.

“ <i>Scylla</i> is a monospecific genus”			“ <i>Scylla</i> is a polyspecific genus”		
Date	Author	Species name	Date	Author	Species name
1775	Forskål	<i>Cancer serratus</i>			
1796	Herbst	<i>Cancer olivaceous</i>			
1798	Fabricius	<i>Portunus tranquebaricus</i>			
1830	Rüppell	<i>Portunus serratus</i>	1834	H. Milne Edwards	Two species: <i>Lupea tranquebarica</i> <i>Lupea lobifrons</i>
1833 - 1850	De Haan	<i>Portunus (Scylla) serrata</i>	1852	Dana	One species plus one variety: <i>Scylla tranquebarica</i> <i>Scylla tranquebarica</i> var. <i>oceanica</i>
1887	De Man	<i>Scylla serrata</i>			
1899	Alcock	<i>Scylla serrata</i>	1907	Stimpson	One species plus one variety: <i>Scylla tranquebarica</i> / <i>serrata</i> <i>Scylla tranquebarica</i> / <i>serrata</i> var. <i>oceanica</i>
1932	Shen	<i>Scylla serrata</i>	1949a and 1952	Estampador and Serene	Three species and one variety: <i>Scylla serrata</i> (mamosain) <i>Scylla serrata</i> var. <i>paramamosain</i> <i>Scylla oceanica</i> (banhawin) <i>Scylla tranquebarica</i>
1960	Stephenson and Campbell	<i>Scylla serrata</i>			
1962	Crosnier	<i>Scylla serrata</i>			
1972	Stephenson	<i>Scylla serrata</i>			
1978	Holthius	<i>Scylla serrata</i>	1980	Joel and Raj	Two species: <i>Scylla tranquebarica</i> <i>Scylla serrata</i>
			1982	Radhakrishnan and Samuel	One species plus one subspecies: <i>Scylla serrata</i> <i>Scylla serrata serrata</i>
			1983	Joel and Sanjevaraj	Two species: <i>Scylla tranquebarica</i> <i>Scylla serrata</i>
			1984	Chayarat and Kaew-Ridh	Three species: <i>Scylla serrata</i> <i>Scylla tranquebarica</i> <i>Scylla oceanica</i>
			1984	Taylor	Two species: <i>Scylla serrata</i> <i>Scylla paramamosain</i>
			1991	Oshiro	Three species: <i>Scylla serrata</i> <i>Scylla oceanica</i> <i>Scylla tranquebarica</i>
			1998	Keenan <i>et al</i>	Four species: <i>Scylla serrata</i> <i>Scylla tranquebarica</i> <i>Scylla olivacea</i> <i>Scylla paramamosain</i>

three species and a variety of *Scylla* from the Philippines. His study was supported by Serene (1952) who also recognised three species and one variant of *Scylla*, but he reassigned the names differently. The idea of more than one species was quashed by Stephenson and Campbell (1960) who made the argument that the characters used were not evidence enough to separate the species convincingly. Crosnier (1962) and Holthius (1978) shared this view.

From 1980, interest has returned to the hypothesis that the genus *Scylla* is composed of more than one species. However, the descriptions and the species names attributed to them are still confusing. This chapter tries to elucidate the common characters in all previous taxonomic descriptions proposed, both in terms of previous descriptions and in relation to fresh material and museum specimens studied. It is also important from a practical viewpoint to clarify which characters are most reliable for field identification of the species within the genus *Scylla* (*e.g.* for research on crab fisheries where more than one species can occur in a single fishery).

3.2 Materials and methods

3.2.1 Evaluation of published descriptions of the genus *Scylla*

Published taxonomic descriptions of the genus *Scylla* were collected from the scientific literature. These descriptions were examined and a set of primary characters was selected and compared between the various authors' descriptions and their allocation of species names. The primary descriptive characters concern the external body structures of mud crabs, including the carapace, chelae and other limbs. These characters have two main attributes, namely colouration and morphology. Colouration also encompasses any patterning present on the external body structures. Morphological features are composed of meristic characters (*e.g.* number of teeth or spines) and the general morphological

appearance of these characters (*i.e.* whether a spine is vestigial or prominent). The number of valid species within the genus *Scylla* was also assessed from these descriptions.

3.2.2 Description of typed fresh specimens of the genus *Scylla*

Fresh samples of mud crab purported to be *Scylla serrata* (six individuals), *Scylla tranquebarica* (six individuals) and *Scylla olivacea* (four individuals) by Keenan *et al.* (1998) were obtained from Queensland, Australia and Sarawak, East Malaysia. These individuals were examined for external diagnostic characters that help identify these species. Both colour and morphology were taken into consideration. Notes were made of the distinguishing features and these notes were tabulated for ease of comparison between species. Each character was assessed for its ability to distinguish between species. Unfortunately there were no fresh samples of *S. paramamosain* available for this part of the study.

3.2.3 Description of typed museum specimens of the genus *Scylla*

Specimens of mud crab from the Fabricius collection (the earliest collection of mud crab) were examined at the Zoological Museum in the Department of Zoology, University of Copenhagen. Six formerly assigned syntypes of the species *Portunus tranquebaricus* (collected by Daldorff, 1798) were part of a collection described by Fabricius in 1798 which was originally divided and housed between Kiel and Copenhagen Universities (Zimsen, 1964). The complete collection currently resides at Copenhagen University. In the recent taxonomic revision of the genus *Scylla* by Keenan *et al.* (1998), one immature female specimen (ZMUC 150-2) from this collection was assigned as the lectotype of the species *Scylla tranquebarica* Fabricius. The other five specimens are believed to be composed of the other three species proposed by Keenan *et al.* (1998); namely, *Scylla serrata* (female (mature) ZMUC 150-6); *Scylla olivacea* (male ZMUC 150-1) and *Scylla*

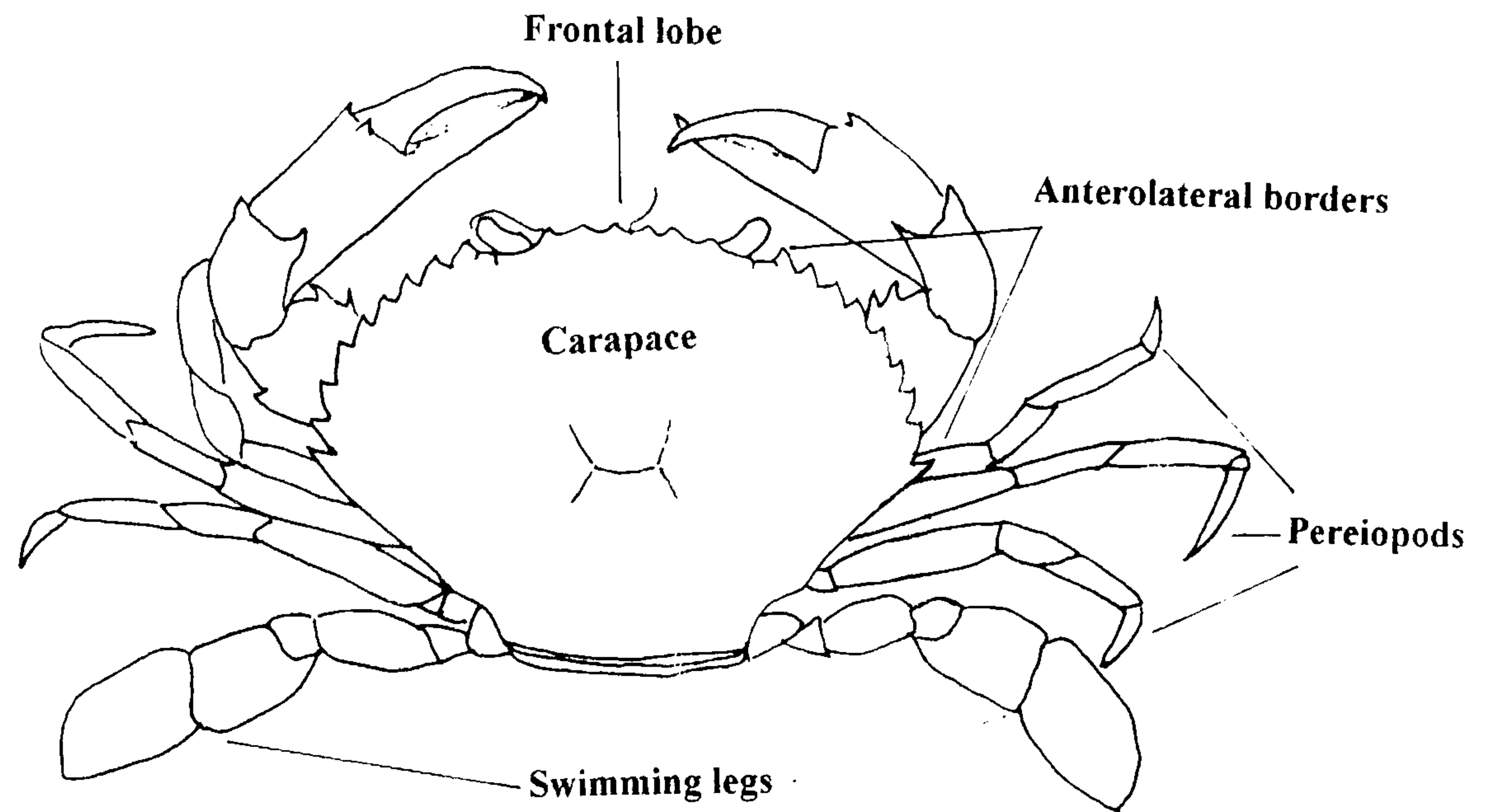
paramamosain (two females, one immature ZMUC 150-3; one with early stages of maturity ZMUC 150-5 and one male (juvenile) ZMUC 150-4). Photographs from this collection were used by Keenan *et al.* (1998) to make their evaluation.

The preserved museum specimens of *Scylla* were examined and notes were made of the main characters distinguishing the individuals within the collection. Black and white photographs were also taken as a record of these main features. All the characters described were compared and an independent assessment made of the number of species contained within the University of Copenhagen collection.

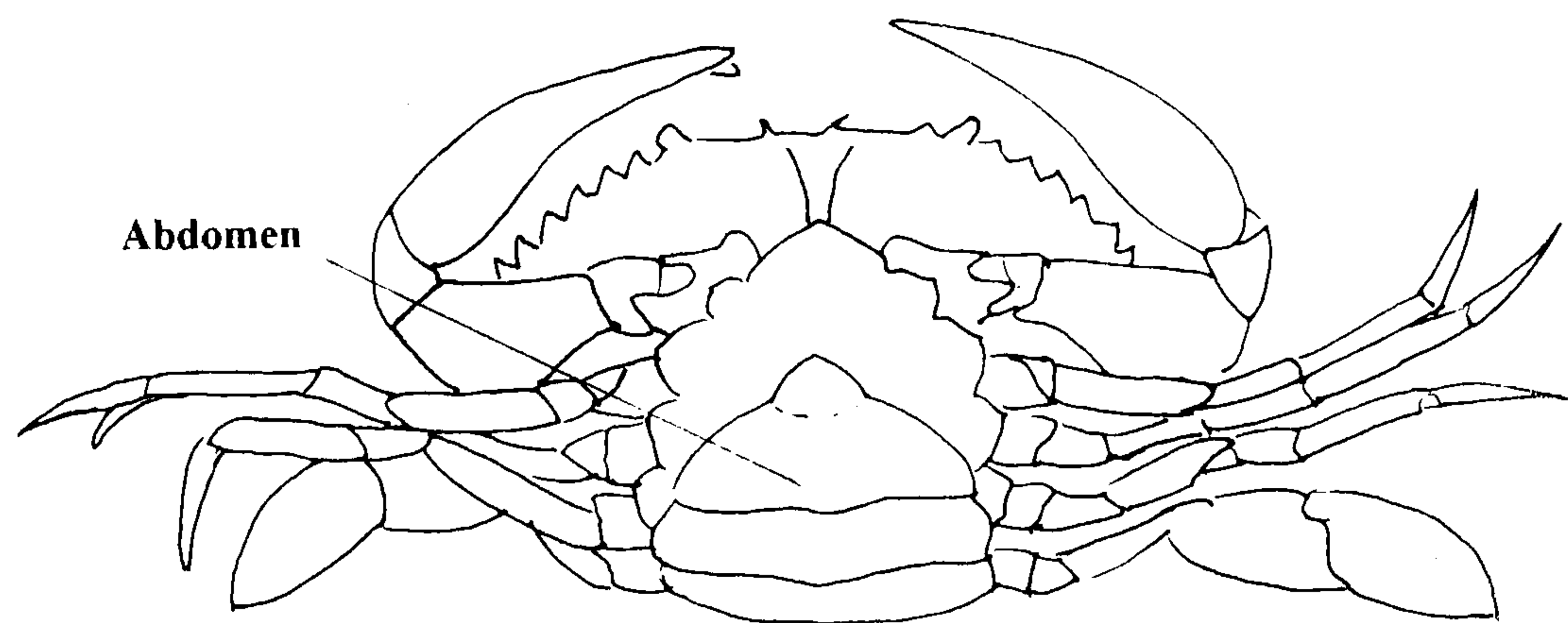
3.2.4 Evaluation of species composition of mud crabs (genus *Scylla*) from seven locations within South and Southeast Asia

Adult crabs were collected from seven locations within South and Southeast Asia, namely (A) Klong Ngao, Ranong Province, southwest Thailand; (B) Ban Don Bay, Surat Thani Province, western Gulf of Thailand; (C) Chanthaburi Province, northeast Gulf of Thailand; (D) Can Gio Province, southern Vietnam; (E) Thuy Hai Commune, Thai Binh Province, northern Vietnam; (F) Paikgasir, Sundarbans, southern Bangladesh; and (G) Sematan, Sarawak State, East Malaysia.

The general appearance of the crabs from each location was recorded. Colouration, spination and other morphological characters were tabulated for comparison. From these external characters a preliminary assessment was made as to which *Scylla* species the morphs collected seemed to be affiliated. Figure 3.2 outlines the main characters recorded in terms of the colouration and spination used to identify each *Scylla* species.



b) Ventral view



c) Chelae

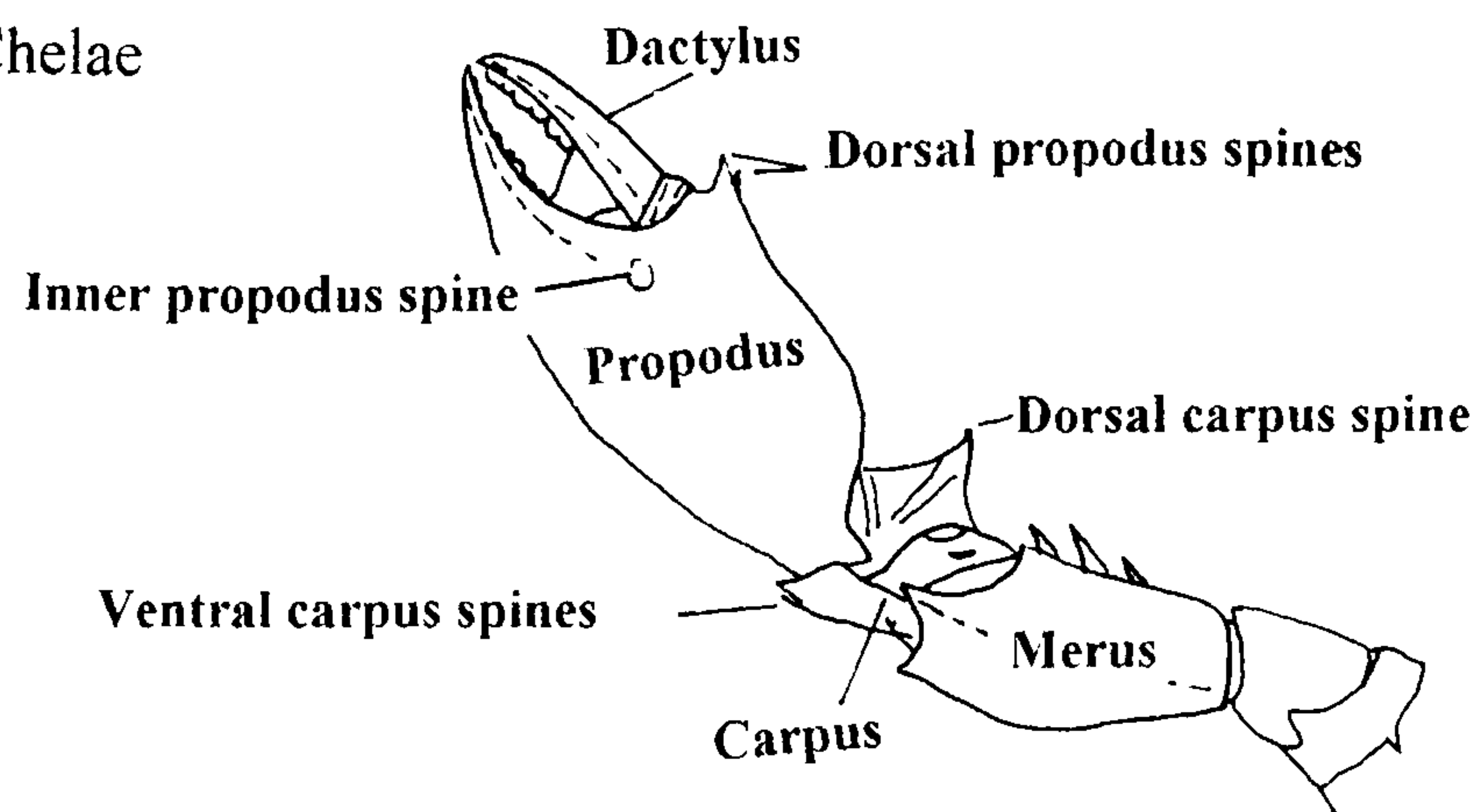


Figure 3.2: Illustration of main characters used to describe between species of the genus *Scylla* (illustrations are one-third life size).

3.3 Results

3.3.1 Evaluation of published descriptions of the genus *Scylla*

3.3.1.1 Carapace colouration and patterning

Table 3.2 outlines the use of carapace colour and patterning by authors to distinguish morphs and assign species names to these morphs within the genus *Scylla*. With reference to the dorsal carapace, the descriptions on colouration are variable and not detailed, causing some discrepancies between authors; however, there are some basic trends present that can be reviewed. Two main colour morphs are described, one green and the other predominantly russet brown. In early descriptions, including Rüppell (1830) (cited by Stimpson, 1907) and later Crosnier (1962) and Radhakrishnan and Samuel (1982), the name *S. serrata* is assigned to the morph with a green dorsal carapace. Estampador (1949a) named a green morph as *S. oceanica*. Serene (1952) and Chayarat and Kaew-Ridh (1984) also used this name. The other distinct colour morph, exhibiting a russet brown carapace, was assigned the name *Cancer olivaceous* by Herbst (1796) (cited by Keenan *et al.*, 1998).

The name of the brown morph was revised to *S. serrata* by Estampador (1949a) and maintained by Serene (1952); Joel and Raj (1980); Joel and Sanjeevaraj (1983); Chayarat and Kaew–Ridh (1984); Taylor (1984) and Oshiro (1991), until Keenan *et al.* (1998) returned to the original nomenclature, using the name *S. olivacea*.

Morphs of *Scylla* with intermediate and variable carapace colour have been described and various names proposed. A grey green dorsal carapace, sometimes with purple hues, is associated with the name *S. tranquebarica* (Milne Edwards, 1834; Estampador, 1949a; Serene, 1952; Joel and Raj, 1980; Joel and Sanjeeraj, 1983; Chayarat and Kaew-Ridh, 1984 and Oshiro, 1991). The earliest description of *S. paramamosain* describes the dorsal

Table 3.2: Chronological summary of the use of carapace colour and patterning to distinguish different species of mud crab (genus *Scylla*) (nd = no data).

Author and date	Location	Carapace		Proposed species
		Dorsal	Ventral	
Forskål, 1775	Djedda, Red Sea	Ash grey, purple red tones.	White	<i>Cancer serratus</i>
Milne Edwards, 1834	Southeast Asia.	Green grey.	nd	<i>Lupea tranquebarica</i>
Stimpson, 1907 (following Ruppell, 1830)	North Pacific	Olive green.	White	<i>Portunus serratus</i>
Estampador, 1949a	Philippines	1. Ferruginous brown to light purplish-brown. Absence of markings.	1. nd	1. <i>Scylla serrata</i> (mamosain)
		2. Greenish, grey green in big specimens.	2. Abdomen ornamented with large pigmented areas or grey bordered by purplish dark brown lines.	2. <i>Scylla oceanica</i> (banhawin)
		3. Deep purple/drab green to lighter shades.	3. Large pigmented areas on abdomen.	3. <i>Scylla tranquebarica</i> (parabanhawin)
		4. Brown/grey.	4. nd	4. <i>Scylla serrata</i> var. <i>paramamosain</i>
Serene, 1952	Nga Trang Vietnam	1. Bright green to pale green.	1. Yellow anterolateral borders.	1. <i>Scylla oceanica</i>
		2. Dark brown/reddish brown.	2. Green/olive. Less colour in subhepatic areas.	2. <i>Scylla serrata</i>
		3. Dull green with deep red/purple hues.	3. Light yellowish brown.	3. <i>Scylla tranquebarica</i>
		4. Brown/green.	4. Yellow anterolateral borders.	4. <i>Scylla serrata</i> var. <i>paramamosain</i>
Crosnier, 1962	Madagascar	Green.	nd	<i>Scylla serrata</i>
Joel and Raj, 1980	India	1. Light to dark greyish green. Some reticulation.	1. Abdomen of mature females with reticulation.	1. <i>Scylla tranquebarica</i>
		2. Rusty brown to dark green brown. No reticulation.	2. No reticulation.	2. <i>Scylla serrata</i>

Table 3.2: (continued)

Author and date	Location	Carapace		Proposed species
		Dorsal	Ventral	
Radhakrishnan and Samuel, 1982	India	1. Dark green.	1. White/cream. Abdomen of mature females with numerous yellow/white patches.	1. <i>Scylla serrata</i>
		2. Dark green.	2. Bluish with reddish tinges. Abdomen of mature females with dark bluish/black bands.	2. <i>Scylla serrata serrata</i>
Joel and Sanjevaraj, 1983	India	1. Light to dark greyish green.	1. Abdomen of mature females shows polygonal patterning.	1. <i>Scylla tranquebarica</i>
		2. Rusty brown to darkish green brown.	2. Abdomen of mature females is plain.	2. <i>Scylla serrata</i>
Chayarat and Kaew-Ridh, 1984	Chanthaburi, Thailand	1. Green/greyish brown.	1. nd	1. <i>Scylla serrata</i>
		2. Purplish/greyish green.	2. nd	2. <i>Scylla tranquebarica</i>
		3. Light green to yellow/green.	3. nd	3. <i>Scylla oceanica</i>
Taylor, 1984	Australia	1. Rusty brown.	1. nd	1. <i>Scylla serrata</i>
		2. Green with mottling.	2. nd	2. <i>Scylla paramamosain</i>
Oshiro, 1991	Japan	1. Dark brown.	1. nd	1. <i>Scylla serrata</i>
		2. Brown/blue /green.	2. nd	2. <i>Scylla oceanica</i>
		3. Slightly light green.	3. nd	3. <i>Scylla tranquebarica</i>
Keenan <i>et al.</i> , 1998	Indo-West-Pacific	1. Purple/green to brown/black.	1. nd	1. <i>Scylla serrata</i>
		2. Colour variable.	2. nd	2. <i>Scylla tranquebarica</i>
		3. Purple/green to brown/black.	3. nd	3. <i>Scylla paramamosain</i>
		4. Red to brown to brown/black.	4. nd	4. <i>Scylla olivacea</i>

carapace colour as brown grey (Estampador, 1949a). Subsequent descriptions state the dorsal carapace of *S. paramamosain* to vary from brown green (Serene, 1952) to green with mottling (Taylor, 1984). In the descriptions made by Estampador (1949a) and Serene (1952) *S. paramamosain* is classified as a variety of *S. serrata*.

Serene (1952) mentions the colouration of the ventral carapace in detail, focusing on the area around the anterolateral borders, which exhibit a pale form of the colouration seen on the dorsal carapace. Both what he calls *Scylla serrata* var. *paramamosain* and *S. oceanica* are described as having yellow anterolateral borders. *S. tranquebarica* is described as having yellowish brown ventral anterolateral borders, whereas *S. serrata* has green/olive anterolateral regions.

Other authors describe the ventral side of the crab with reference to the abdominal flap of mature female crabs. The female abdomen, when it shows reticulation, is associated with the green dorsal carapace and is described as *Scylla oceanica* (Estampador, 1949), *S. serrata* (Radhakrishnan and Samuel, 1982) and *S. tranquebarica* (Joel and Raj, 1980; Joel and Sanjeevaraj, 1983). A mature abdominal flap without reticulation, but exhibiting dark bands, is identified with the russet brown dorsal carapace of the former *S. serrata* (Joel and Raj, 1988; Joel and Sanjeevaraj, 1983). Thus the use of the mature female abdominal flap to distinguish between species is only diagnostic for the dark brown morph (most recently assigned the name *S. olivacea* by Keenan *et al.*, 1998) from the other species. However the most recent authors, namely Chayarat and Kaew-Ridh (1984); Taylor (1984); Oshiro (1991) and Keenan *et al.* (1998) have omitted this character from their descriptions.

3.3.1.2 Colouration and patterning of chelae and other limbs

Details of colouration and patterning on the limbs of the genus *Scylla* are outlined in table 3.3. Two main morphologies exist. The first is where the dorsal propodus and other limb structures exhibit a green/brown colour with the presence of polygonal reticulation. The ventral half of the propodus is pale yellow/cream with the presence of dark spots in the mid-upper half of the outer propodus. The inner propodus is orange/red colour and the dactylus also exhibits orange/red hues. This colouration is identified with the green dorsal carapace form of *Scylla* labelled as *S. serrata* by Rüppell, 1830 (cited by Stimpson, 1907), Crosnier (1962), Radhakrishnan and Samuel (1982) and Keenan *et al.* (1998); and as *S. oceanica* by Serene (1952), Chayarat and Kaew-Ridh (1984) and Oshiro (1991).

The second distinct colour morphology is with the upper propodus and other chelae structures coloured green/brown with orange/red hues on the ventral half of the propodus. Spots or reticulation are absent from the chelae; the dactylus is brown, sometimes exhibiting pinkish hues. This colouration is associated with *Scylla serrata* described by Serene (1952), Joel and Raj (1980), Chayarat and Kaew-Ridh (1984), Oshiro (1991) and *S. olivacea* described by Keenan *et al.* (1998).

Descriptions for *Scylla tranquebarica* vary, but often include purplish shades with some weak reticulation on the dorsal surface. *S. paramamosain* cannot be identified using these characters, as the descriptions of colouration by authors for this species are variable and describe intermediate forms between the two main colour morphs.

The other limbs are described with respect to the amount of reticulation present. Strong reticulation found on the chelae corresponds to the amount of reticulation on the other

Table 3.3: Chronological summary of the use of colour and different patterning on the chelae, pereiopods and swimming legs to distinguish species of mud crab (genus *Scylla*)(nd = no data).

Author and date	Location	Chelae	Pereiopods	Swimming legs	Proposed Species
Stimpson, 1907 (following Ruppell, 1830)	North Pacific	Propodus reddish, spotted with green. Dactylus red.	Pale green with darker spots.	nd	<i>Portunus serratus</i> / <i>Portunus</i> <i>tranquebaricus</i>
Estampador, 1949a	Philippines	1. Red/purple. Splashes of red occur.	1. Polygonal pigmented areas.	1. Whitish spots present.	1. <i>Scylla oceanica</i> (banhawin)
		2. Purplish. Dactylus red tipped with white.	2. Pigmented areas.	2. Large pigmented areas.	2. <i>Scylla</i> <i>tranquebarica</i> (parabanhawin)
		3. nd	3. Colour plain , same as rest of body.	3. Patterning absent.	3. <i>Scylla serrata</i> (mamosain)
		4. nd	4. Colour plain., same as the rest of the body.	4. White pigments on last pair of legs.	4. <i>Scylla serrata</i> <i>var. paramamosain</i>
Serene, 1952	Nga Trang Vietnam	1. Yellow/ green	1. nd.	1. nd	1. <i>Scylla oceanica</i>
		2. Orange/ brown/red.	2. nd	2. nd	2. <i>Scylla</i> <i>tranquebarica</i>
		3. Fine red	3. nd	3. nd	3. <i>Scylla serrata</i>
		4. Red/brown.	4. nd	4. nd	4. <i>Scylla serrata</i> <i>var. paramamosain</i>
Crosnier, 1962	Madagascar	Green. Reticulation green or brown.	Green. Reticulation green or brown.	Green or brown reticulation.	<i>Scylla serrata</i>
Joel and Raj, 1980	India	1. Outer surface grey to dark brown. Dactylus yellowish green with white tips. Propodus brownish yellow	1. nd.	1. nd.	1. <i>Scylla</i> <i>tranquebarica</i>
		2. Outer surface pinkish brown. Dactylus greenish/pinkish tinge with brown tips. Propodus pink.	2. nd.	2. nd.	2. <i>Scylla serrata</i>

Table 3.3: (continued)

Author and date	Location	Chelae	Pereiopods	Swimming legs	Proposed Species
Radhakrishnan and Samuel, 1982.	India	1. Mostly green with numerous patches and yellowish lower margin.	1. nd.	1. Numerous mosaic-like light patches.	1. <i>Scylla serrata</i>
		2. Orange with brownish green patches absent or subtle.	2. nd.	2. green or violet without patches.	2. <i>Scylla serrata serrata</i>
Chayarat and Kaew-Ridh, 1984.	Chanthaburi, Thailand.	1. Rusty red.	1. nd.	1. No clear stripes.	1. <i>Scylla serrata</i>
		2. Purplish shade.	2. nd.	2. Coarse stripes (purplish red) at base of paddle.	2. <i>Scylla tranquebarica</i>
		3. Dark green. Spots on dorsal part. Yellowish or orange on ventral half.	3. nd.	3. Fine dark green stripes on paddle.	3. <i>Scylla oceanica</i>
Oshiro, 1991	Japan	1. Propodus dark green/brown to light green on outer surface. Inner surface is pale. Reticulation present. Dactylus red.	1. Network patterning.	1. Network patterning.	1. <i>Scylla oceanica</i>
		2. Dark green /brown outer surface. Inner surface is pale. Reticulation present. Dactylus red.	2. Forth Periopod shows network patterning.	2. Network patterning.	2. <i>Scylla tranquebarica</i>
		3. Outer surface is dark brown. Inner surface is red to yellow without patterning.	3. Rarely any patterning present.	3. Rarely network patterning.	3. <i>Scylla serrata</i>
Keenan <i>et al.</i> , 1998.	Indo-west Pacific	1. Strong polygonal patterning.	1. Strong polygonal patterning.	1. Strong polygonal patterning.	1. <i>Scylla serrata</i>
		2. Weak polygonal patterning.	2. First two pairs of legs weak patterning.	2. Strong patterning.	2. <i>Scylla tranquebarica</i>
		3. Weak polygonal patterning.	3. Weak polygonal patterning.	3. Weak polygonal patterning.	3. <i>Scylla paramamosain</i>
		4. Plain.	4. Plain.	4. Plain.	4. <i>Scylla olivacea</i>

limbs. Strong reticulation is representative of the green colour morph labelled *Scylla serrata* or *S. oceanica*. Both *S. tranquebarica* and *S. paramamosain* are described as variable and intermediate and cannot be distinguished using reticulation as a character. *Scylla olivacea* (also known as *S. serrata* in some descriptions) is the species that is consistently distinguished from the others in the literature due to the absence of reticulation on any limbs.

3.3.1.3 Carapace morphology

Table 3.4 outlines the use of carapace morphology to distinguish proposed species within the genus *Scylla*. The shape of the frontal lobe teeth is a dominant character in all descriptions of the species encompassed in the genus *Scylla*. There is some discrepancy as to the number of frontal lobe teeth described for some of the early species definitions (de Haan, 1850). In reality all species within the genus *Scylla* have the same number of frontal lobe teeth. The variations in the shape of the frontal teeth described are subtle but identifiable. Three main morphologies are reported. These are: a) wide blunt rounded teeth that are equal in size; b) short, triangular sharp teeth that are equal in size; and c) protruding, long teeth that are blunt at the tips, with the medial teeth more protruding than the other frontal teeth.

The first of these three morphologies, *i.e.*, wide, blunt and rounded frontal teeth that are equal in size, is described for *Scylla olivacea* (Herbst, 1796 cited by Keenan *et al.*, 1998) also called *S. serrata* by Alcock, (1899), Estampador (1949a), Serene (1952), Joel and Raj (1980), Joel and Sanjeevaraj (1983), Chayarat and Kaew-Ridh (1984), and Oshiro (1991). This description is also associated with *S. tranquebarica* as the variety *oceanica* by Dana, 1852 (cited by Stimpson, 1907); and as *S. tranquebarica* Estampador (1949) and Keenan *et*

Table 3.4: Chronological summary of the use of morphological characters on the carapace to distinguish different species of mud crab (genus *Scylla*)(nd = no data).

Author and date	Location	Cardio thoracic region	Branchial region	Frontal lobe	Anterolateral borders	Proposed species
Forskål, 1775	Djedda, Red Sea	nd.	nd.	Six wide teeth.	Nine rounded spines.	<i>Cancer serratus</i>
Fabricius, 1798	Indian Ocean	nd	nd	Four teeth equal length.	Anterolateral spines same shape and size.	<i>Portunus tranquebaricus</i>
H. Milne Edwards, 1834	Southeast Asia	nd	nd	1. Promenant frontal lobe . six triangular teeth; broad and short.	1. Spines sharp and consistant in size.	1. <i>Lupea tranquebarica</i>
				2. Four blunt teeth.	2. Spines are small, reduced.	2. <i>Lupea lobifrons</i>
De Haan, 1850	Japan	nd	nd	Six teeth of equal length.	Nine spines of equal length.	<i>Portunus (Scylla) serratus</i>
Alcock, 1899	India	nd	Granular ridge from ninth spine.	Four blunt teeth of equal size.	Nine sharply acuminate teeth, equal in size.	<i>Scylla serrata</i>
Stimpson, 1907	1. North Pacific (Hong Kong)	nd	nd	1. Front teeth sharp.	1. All spines are of equal length.	1. <i>Portunus tranquebarical/ serratus</i> (Ruppell)
	2. Pacific Islands	nd	nd	2. Front teeth are blunt (median incision deepest).	2. Posterior spine of anterolateral margin longer than other spines.	2. <i>Portunus tranquebarical/ serratus</i> var. <i>oceanica</i> (Dana)
Shen, 1932	Hong Kong	“H” shaped impression distinct.	Faint transverse granular line from ninth spines.	Four triangular pointed teeth, the two median teeth slight more protruded.	All spines of equal size.	<i>Scylla serrata</i>

Table 3.4: (continued)

Author and date	Location	Cardio thoracic region	Branchial region	Frontal lobe	Anterolateral borders	Proposed species
Estampador, 1949a	Philippines	1. Faint “H” shaped impression.	1. Faint granular ridge from ninth spine.	1. Four lobes/bluntish teeth, equal size and prominence.	1. Nine acuminate spines of equal size.	1. <i>Scylla serrata</i> (mamosain)
		2. Deep “H” shaped impression.	nd	2. Blunt teeth, levelled.	2. Nine sharp acuminate spines of equal size.	2. <i>Scylla oceanica</i> (banhawin)
		3. Deep “H” shaped impression.	nd	3. Blunt teeth. Median incision is deepest.	3. Ninth spines are longer than other anterolateral spines.	3. <i>Scylla tranquebarica</i> (parabanhawin)
		4. Faint “H” shaped impression.	nd	4. Median pair of teeth more protuded than others.	4. Nine sharp acuminate spines of equal size.	4. <i>Scylla serrata</i> var. <i>paramamosain</i>
Serene, 1952	Nga Trang, Vietnam	nd	nd	1. Prominent pointed teeth (all ages).	1. Small spaces between spines. Spines are sharp and swollen.	1. <i>Scylla oceanica</i>
		nd	nd	2. Less pointed than <i>S. oceanica</i> .	2. Very small spaces between spines.	2. <i>Scylla tranquebarica</i>
		nd	nd	3. Teeth rounded.	3. Small spaces between spines. Spines are smooth and uniform in shape and size.	3. <i>Scylla serrata</i>
		nd	nd	4. Teeth less pointed than <i>S. tranquebarica</i> .	4. Very small spaces between spines.	4. <i>Scylla serrata</i> var. <i>paramamosain</i>
Crosnier, 1962	Madagascar	Smooth.	Granular ridges, one epibranchial two in gastric regions.	Four prominent triangular teeth.	Nine teeth of equal size and shape.	<i>Scylla serrata</i>
Joel and Raj, 1980	India	nd	nd	1. Medial frontal teeth sharp.	nd	1. <i>Scylla tranquebarica</i>
		nd	nd	2. frontal teeth blunt and level.	nd	2. <i>Scylla serrata</i>

Table 3.4: (continued)

Author and date	Location	Cardio-thoracic region	Branchial region	Frontal lobe	Anterolateral borders	Proposed species
Radhakrishnan and Samuel, 1982	India	1. Deep “H” shaped impression.	nd	1. Pointed teeth. Anteriorly projected.	1. Spines not anteriorly truncated.	1. <i>Scylla serrata</i>
		2. Faint “H” shaped impression.	nd	2. Teeth levelled.	2. Spines anteriorly truncated.	2. <i>Scylla serrata serrata</i>
Joel and Sanjeervaraj, 1983.	India	nd	nd	1. Sharp teeth, slightly protruded.	nd	1. <i>Scylla tranquebarica</i>
		nd	nd	2. Blunt teeth, equal in length.	nd	2. <i>Scylla serrata</i>
Chayarat and Kaew-Ridh, 1978	Thailand	nd	nd	1. Width short.	1. Ninth spine short.	1. <i>Scylla serrata</i>
		nd	nd	2. Width medial.	2. Ninth spine long.	2. <i>Scylla tranquebarica</i>
		nd	nd	3. Width long.	3. Ninth spine medial.	3. <i>Scylla oceanica</i>
Oshiro, 1991	Japan	nd	nd	1. Teeth wide and rounded.	nd	1. <i>Scylla serrata</i>
		nd	nd	2. Teeth pointed.	nd	2. <i>Scylla tranquebarica</i>
		nd	nd	3. Teeth intermediate.	nd	3. <i>Scylla oceanica</i>
Keenan <i>et al.</i> , 1998	Indo-West Pacific	nd	nd	1. Teeth high, bluntly pointed. with concave margins and rounded interspaces.	1. Narrow spines. Outer margins straight or slightly concave.	1. <i>Scylla serrata</i>
		nd	nd	2. Teeth are moderate in height, blunted with rounded interspaces.	2. Broad spines with outer margins convex.	2. <i>Scylla tranquebarica</i>
		nd	nd	3. Teeth high, triangular with straight margins and angular interspaces.	3. Broad spines with outer margins convex.	3. <i>Scylla paramamosain</i>
		nd	nd	4. Teeth low, rounded with shallow interspaces.	4. Broad spines with outer margins convex.	4. <i>Scylla olivacea</i>

al. (1998), although the frontal teeth are described as being more prominent than those exhibited by *S. serrata/olivacea*. Other species names associated with this morphology include *Lupea lobifrons* (Milne Edwards, 1834) and *S. serrata serrata* (Radhakrishnan and Samuel, 1982).

The second morphology, *i.e.* short sharp triangular teeth that are equal in size, is associated with *Scylla paramamosain* by Estampador (1949a) and Keenan *et al.* (1998). The third morphology is associated with *S. serrata* by Stimpson (1907), Shen (1932), Crosnier (1962), Radhakrishnan and Samuel (1982), and Keenan *et al.* (1998). This morphology also is associated with the names *S. oceanica* (Serene, 1952; Chayarat and Kaew-Ridh, 1984) and in some cases *S. tranquebarica* (Joel and Sanjeevaraj, 1983; Oshiro, 1991). Thus *S. serrata* (also known as *S. oceanica*) and *S. paramamosain* can be distinguished by examining the shape of the frontal lobe teeth.

The number of anterolateral spines is the same for all morphs considered with nine anterolateral teeth occurring on both sides of the carapace. The shape of the most posterior anterolateral spine has been noted to be more protruded in certain cases and is associated with the variety *Scylla tranquebarica* variety *oceanica* by Dana, 1852 (cited by Stimpson, 1907) and the species *S. tranquebarica* (Estampador, 1949a; Chayarat and Kaew-Ridh, 1984). Other distinguishing anterolateral spinal morphology reported includes the spaces between the spines being smaller for *S. tranquebarica* and *S. paramamosain* than for *S. oceanica* and *S. serrata* (Serene, 1952). Radhakrishnan and Samuel, (1982) describe two anterolateral spinal morphologies; *S. serrata serrata* has anteriorly truncated spines, whereas such truncation is absent for *S. serrata*.

3.3.1.4 Distinguishing morphology of chelae and other limbs

Table 3.5 outlines the use of morphological characters found on the chelae and other limbs in order to distinguish between species of the genus *Scylla*. Alcock (1899), Estampador (1949a) and Serene (1952) mention cheliped size with respect to carapace width as a distinguishing character between some species. Chelae that are not more than twice the size of the carapace width are associated with *S. serrata* by Alcock (1899) and labelled as *S. oceanica* by Estampador (1949) and Serene (1952). Chelae more than twice the size of the carapace width are associated with *S. tranquebarica* by Estampador (1949a) and with *S. serrata* by Serene (1952). This is a very subjective character, however, and its rare usage indicates its lack of consistency in providing diagnostic results.

In all *Scylla* morphologies one spine exists at the base of the propodus adjoining the wrist (carpal) joint. This spine is often described as a tubercle due to its round, bulbous appearance. The spines present on the dorsal surface of the propodus, just behind the dactylus joint, have been described as having four morphologies. These are a) two sharp spines present on the dorsal propodus; b) two spines present, one slightly reduced from the other; c) only one spine present, the other being vestigial or absent; and d) both spines vestigial or absent. The latter two of these four categories (c and d) are described for the dark brown morph labelled as *S. serrata* by Alcock (1899), Estampador (1949a), Serene (1952), Joel and Raj (1980) and Taylor (1984), and later renamed as *S. olivacea* by Keenan *et al.*, 1998. Two sharp spines are expressed by both *S. serrata* (also known as *S. oceanica*) and *S. tranquebarica*, although in some instances the second category is also described for these species. *S. paramamosain* exhibits the second category (b) where the outer spine is slightly smaller than the inner one.

Table 3.5: Chronological summary of the use of morphological characters on the chelipeds to distinguish different species of mud crab (genus *Scylla*) (nd = no data).

Author and Date	Location	Cheliped size	Propodus	Carpus	Proposed species
Milne-Edwards, 1834	Southeast Asia	Heavy chelae.	Three spines.	Three spines.	<i>Lupea tranquebarica</i>
Alcock, 1899	India	Not quite twice the width of the carapace.	Two dorsal spines, outer one often obsolescent.	One strong dorsal spine. One or two ventral spines.	<i>Scylla serrata</i>
Shen, 1932	Hong Kong	Unequal in size.	Three tubercles, one located at carpal joint, other two are side by side on dorsal surface behind dactylus. Inner spine larger than outer one.	One strong spine on upper carpus, two obtuse spines on posterior margin.	<i>Scylla serrata</i>
Estampador, 1949a	Philippines	1. Not quite twice the width of carapace.	1 Spines/tubercles. One at apex of wrist joint. Two side by side. Outer of two is obsolescent.	1. Ventral posterior surface exhibits one or two spines.	1. <i>Scylla serrata</i> (mamosain)
		2. Not more than twice the width of carapace.	2. nd	2. nd	2. <i>Scylla oceanica</i> (banhawin)
		3. nd	3. Two spines at base of dactylus. Inner spine neither obsolescent or dentiform. On big males the outer spine is vestigial.	3. nd	3. <i>Scylla serrata</i> var. <i>paramamosain</i>
		4. More than twice the width of carapace.	4. nd	4. nd	4. <i>Scylla tranquebarica</i>
Serene, 1952	Nga Trang, Vietnam	1. Twice the width of the carapace.	1. Two well developed dorsal spines.	1. Forward lower spine well developed.	1. <i>Scylla tranquebarica</i>
		2. Less than twice the width of the carapace.	2. Two well developed dorsal spines.	2. Forward lower spine less developed.	2. <i>Scylla oceanica</i>
		3. Slightly more than twice the width of the carapace.	3. Two vestigial spines.	3. Forward lower spine absent.	3. <i>Scylla serrata</i>
		4. nd	4. Two spines that are more defined than <i>S. Serrata</i> but less than <i>S. tranquebarica</i> and <i>S. oceanica</i> .	4. Forward lower spine well developed.	4. <i>Scylla serrata</i> var. <i>paramamosain</i>

Table 3.5: (continued)

Author and date	Location	Cheliped size	Propodus	Carpus	Proposed species
Crosnier, 1962	Madagascar	nd	Two spines side by side. Outer of two is superior.	One spine (no lower spines mentioned).	<i>Scylla serrata</i>
Joel and Raj, 1980	India	nd	1. Outer spine behind dactylus is smaller than inner spine.	1. Spine on outer anterolateral inferior border of carpus.	1. <i>Scylla tranquebarica</i>
		nd	2. No outer spine at base of finger or is present but dentiform.	2. No spine at outer anterolateral border of carpus.	2. <i>Scylla serrata</i>
Radhakrishnan and Samuel, 1982	India	nd	nd	1. Two stout spines on outer angle of carpus.	1. <i>Scylla serrata</i>
				2. One spine of outer angle of carpus.	2. <i>Scylla serrata serrata</i>
Joel and sanjeevaraj, 1983	India	nd	nd	1. Two spines on outer carpus.	1. <i>Scylla tranquebarica</i>
				2. One blunt spine.	2. <i>Scylla serrata</i>
Chayarat and Kaew-Ridh, 1984	Thailand	nd	nd	1. Obsolescent middle spine.	1. <i>Scylla serrata</i>
				2. Middle spine elongated.	2. <i>Scylla tranquebarica</i>
				3. Middle spine small and short.	3. <i>Scylla oceanica</i>
Taylor, 1984	Australia	nd	1. One spine on dorsal propodus behind joint to dactylus.	1. One spine on ventral margin of carpus.	1. <i>Scylla serrata</i>
		nd	2. Two spines on dorsal propodus behind joint to dactylus.	2. Two spines on the ventral margin of carpus.	2. <i>Scylla paramamosain</i>
Oshiro, 1991	Japan	nd	nd	1. Anterior ventral spine is absent.	1. <i>Scylla serrata</i>
		nd	nd	2. Two spines on ventral surface. Both are prominent.	2. <i>Scylla tranquebarica</i>
		nd	nd	3. Two conspicuous spines present.	3. <i>Scylla oceanica</i>

Table 3.5: (continued)

Author and date	Location	Cheliped size	Propodus	Carpus	Proposed species
Keenan <i>et al.</i> , 1998	Indo-West-Pacific	nd	1. Two spines on dorsal propodus behind dactylus.	1. Two obvious spines on distal half of outer margin.	1. <i>Scylla serrata</i>
			2. Two obvious spines on dorsal propodus behind dactylus.	2. Two obvious spines on distal half of outer margin.	2. <i>Scylla tranquebarica</i>
			3. Two distinct spines on dorsal surface of propodus. Two ridges run backwards from these spines.	3. One small blunt prominence. Second spine only present in juveniles.	3. <i>Scylla paramamosain</i>
			4. Two blunt spines, inner spine more prominent than outer one.	4. One small prominence. (Spines only present in juveniles).	4. <i>Scylla olivacea</i>

Chayarat and Kaew-Ridh (1984) suggest use of the shape of the dorsal carpus spine as a diagnostic character. Their diagnoses describe the dorsal spine as vestigial on *Scylla serrata*, elongated on *S. tranquebarica* and small and stout on *S. oceanica*. Other authors concentrate on the ventral spines both in number and appearance as a diagnostic character between *Scylla* species. Two main morphologies are described based on the presence or absence of the anterior spine on the outer ventral border of the carpus. This spine is present in *S. serrata* described by Crosnier (1962), Radhakrishnan and Samuel (1982), and Keenan *et al.* (1998), *S. oceanica* described by Serene, (1952) and Oshiro (1991) and *S. tranquebarica* described by Milne Edwards, (1834), Serene (1952), Joel and Raj (1980), Joel and Sanjeevaraj (1983), Oshiro (1991) and Keenan *et al.* (1998). The same spine is absent on adults and vestigial on juveniles of *S. serrata* described by Estampador (1949a), Joel and Raj (1980), Joel and Sanjeevaraj (1983), Taylor (1984) and Oshiro (1991) (also known as *S. olivacea* by Keenan *et al.*, 1998) and is present, but vestigial or in some cases

absent, on some adults of *S. paramamosain* (Serene, 1952; Taylor, 1984, Keenan *et al.*, 1998).

In summary, there are five species names for *Scylla* in circulation, namely: *S. serrata*, *S. oceanica*, *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. The use of these names is often confused between morphologies, especially in the case of the name *S. serrata* which is used for two different morphologies, one with green external features labelled *S. serrata/oceanica* and the other with brown external features labelled *S. serrata/olivacea*. In addition, there are two further morphologies that have colour and morphological traits that are intermediate between the two primary morphologies. These two intermediate morphs are labelled *S. tranquebarica* and *S. paramamosain*. There is no particular character that is singularly diagnostic for all four species/morphologies proposed. Thus a combination of characters is required to identify each proposed species.

3.3.2 Use of fresh specimens from Australia and Malaysia

Table 3.6 outlines the main characters distinguishing the specimens of *S. serrata*, *S. tranquebarica* and *S. olivacea* as designated by Keenan *et al.* (1998). Fresh samples of *S. paramamosain* were unavailable and thus this species is not considered in this section.

Colour proves to be a good method of identifying *S. olivacea* from the other two species in fresh specimens. The carapace of *S. olivacea* is clearly dark brown when compared to the olive green carapace exhibited by specimens of *S. serrata* and *S. tranquebarica*. The colour of the chelae and other limbs is also diagnostic for *S. olivacea*. The absence of reticulation on the limbs and the dark brown colour of the dorsal chelae and all other limbs,

Table 3.6: Summary of main characters distinguishing fresh specimens of *Scylla serrata*, *Scylla tranquebarica* and *Scylla olivacea*, as designated by Keenan *et al.* (1998).

Character	Species		
	<i>Scylla serrata</i> *	<i>Scylla tranquebarica</i> †	<i>Scylla olivacea</i> †
Carapace colour	Green/olive green dorsal carapace with reticulation on posterior dorsal surface and abdomen of mature females.	Green/olive green dorsal carapace.	Brown green dorsal carapace.
Chelae colour	Green dorsal propodus, carpus and merus with aqua/blue hues and reticulation. Ventral propodus is pale green.	Green chelae with purple/olive hues on whole propodus.	Brown dorsal propodus, carpus and merus. Ventral propodus is orange/red. No reticulation present.
Pereiopod colour	Green with dark green/brown reticulation.	Green with dark green/brown reticulation.	Brown/aubergine, no reticulation.
Frontal lobe teeth shape	Elongated, acute teeth with blunt tips and U-shaped interspaces. Median incision is deeper than others.	Flattened, obtuse, semicircular teeth. All teeth are equal in size.	Flattened, obtuse, semicircular teeth. All teeth are equal in size.
Spine shape on anterolateral borders	All teeth same shape and size. Acute spines with large interspaces.	Posterior spine is slightly more protruded than other spines. All spines rounded and sharp.	Flat, obtuse triangular spines. All spines equal size and shape.
General shape of chelae	Slender long propodus.	Not as slender as <i>Scylla serrata</i> but smaller than for <i>Scylla olivacea</i>	Wide, bulky propodus.
Carpus spines (number and shape)	One long angular spine on dorsal surface. Two prominent spines on ventral surface.	One long, angular spine on dorsal surface. Two spines on ventral surface.	One long angular spine on dorsal surface. One vestigial spine on the ventral surface.
Propodus spines (number and shape)	Two spines on the dorsal surface behind the dactyl joint. The inner spine is more pronounced than the outer one.	Two spines on the dorsal surface behind the dactyl joint. The inner spine is more pronounced than the outer one.	Two very blunt vestigial spines on dorsal surface behind the dactyl joint. The outer spine can be considered absent.

* *Scylla serrata* specimens were obtained from Queensland, Australia courtesy of Dr C. Keenan.

† *Scylla tranquebarica* and *Scylla oceanica* specimens were obtained from Sematan, Sarawak State, East Malaysia.

plus orange/red colouring on the ventral and lateral propodus, separates *S. olivacea* from *S. serrata* and *S. tranquebarica*. *S. serrata* and *S. tranquebarica* are very similar in colouration and only subtle differences separate the two species. Strong reticulation on all limbs and on the abdomen of mature females is diagnostic of *S. serrata*. *S. tranquebarica* also exhibits reticulation on the pereopods, but the chelae exhibit purple hues across the whole propodus.

A combination of morphological characters distinguishes the above three species of *Scylla*. The frontal lobe teeth are obtuse and rounded for *S. olivacea* and *S. tranquebarica*, unlike *S. serrata* which exhibits acute sharp frontal lobe teeth with large U-shaped spaces between teeth. The anterolateral carapace spines are very similar in all three species, although the specimens of *S. tranquebarica* examined revealed that the posterior anterolateral spines protrude more than the other anterolateral spines. Propodus shape is not conclusive as a diagnostic character, although *S. olivacea* tends to have a wide, bulky propodus compared with the other two species. Spination on the propodus and carpus is similar for *S. tranquebarica* and *S. serrata*, both exhibit two prominent dorsal spines on the propodus, the inner spine being more prominent than the outer one, and two ventral carpal spines are present. In contrast, specimens of *S. olivacea* bear two very blunt, vestigial dorsal propodus spines and only one vestigial spine on the ventral carpus, the anterior ventral spine being absent.

In summary, the frontal lobe teeth are diagnostic for *Scylla serrata* and the chela spination and colouration are diagnostic for *S. olivacea*. *Scylla tranquebarica* is identified by the protrusion of the posterior (ninth) anterolateral spine. The frontal teeth morphology is similar to that exhibited by *S. olivacea*, but the chela spination is similar to that of *S.*

serrata. With all these characters taken into consideration, a positive identification can be made for any of these three species.

3.3.3 Identification of species within the Zoological Museum, Copenhagen

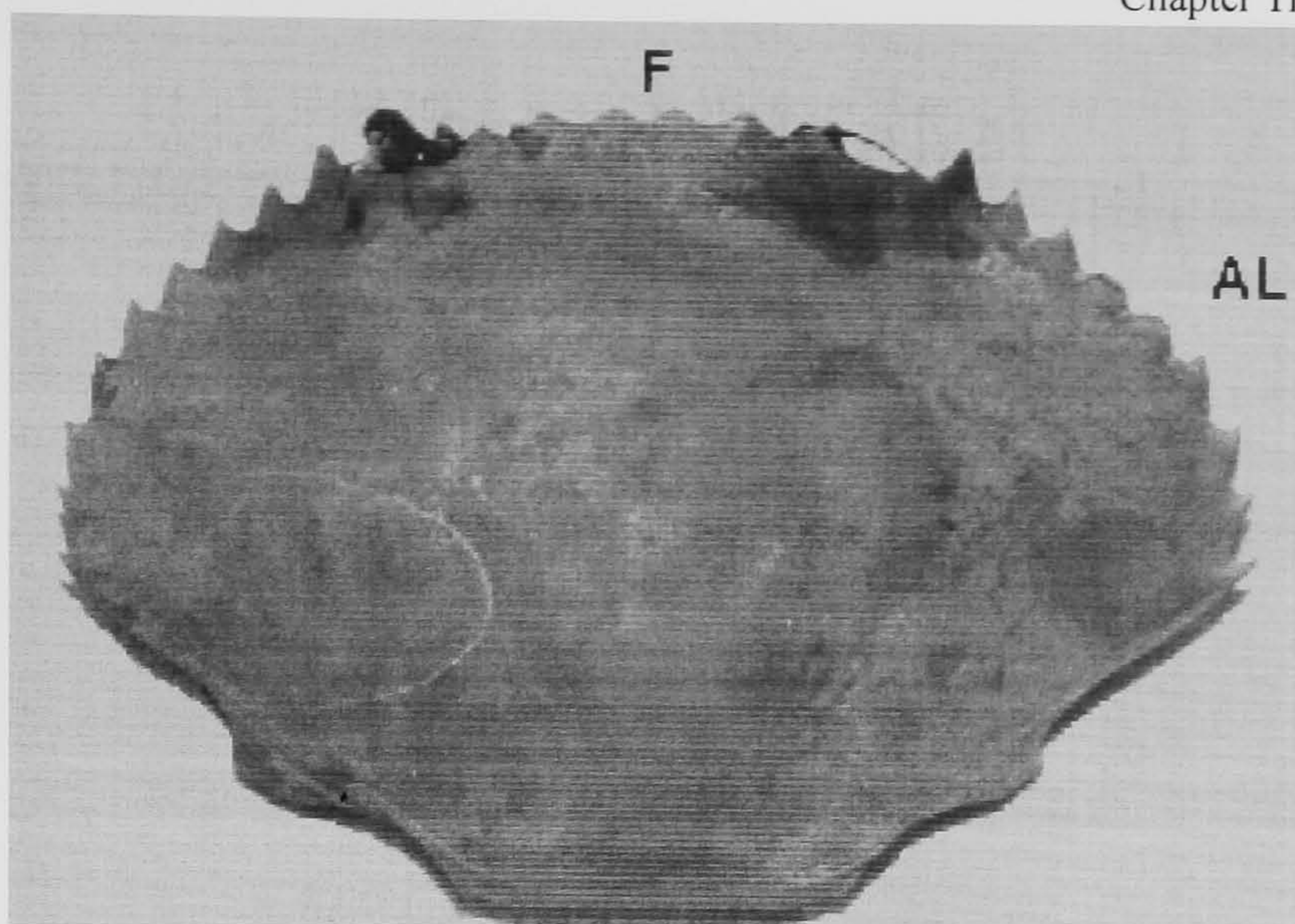
Colour characters were not taken into consideration as all the museum specimens were preserved in ethanol and their colour has been lost in the preservation process. Thus only morphological characters were used to identify these specimens. Table 3.7 summarises the main findings for the six mud crab specimens examined from the Zoological Museum, Copenhagen.

Specimen 150-1 (figure 3.3) exhibited morphological characters representative of *Scylla olivacea* as described in section 3.3.2. This specimen has obtuse, semicircular frontal teeth, short anterolateral spines, two vestigial dorsal propodus spines and one reduced, nodule-like, spine on the ventral carpus. There is a pronounced nodule on the inner lateral propodus adjacent to the apex of its joint with the dactylus. This specimen also has a convex carapace that has been described by other authors for *S. olivacea* (see section 3.3.1).

Specimen 150-2 (figure 3.4) is the lectotype of the species *Scylla tranquebarica* Fabricius. It possesses a flattened carapace. The frontal lobe teeth are obtuse and rounded with a deeper space between the medial teeth. The most posterior anterolateral spines are pronounced and needle-like. The anterolateral spines adjacent to the orbital sockets are pronounced and blunt-tipped. There are two sharp, pronounced spines on the dorsal propodus and the ventral carpus. This combination of characters is not found in the other specimens.

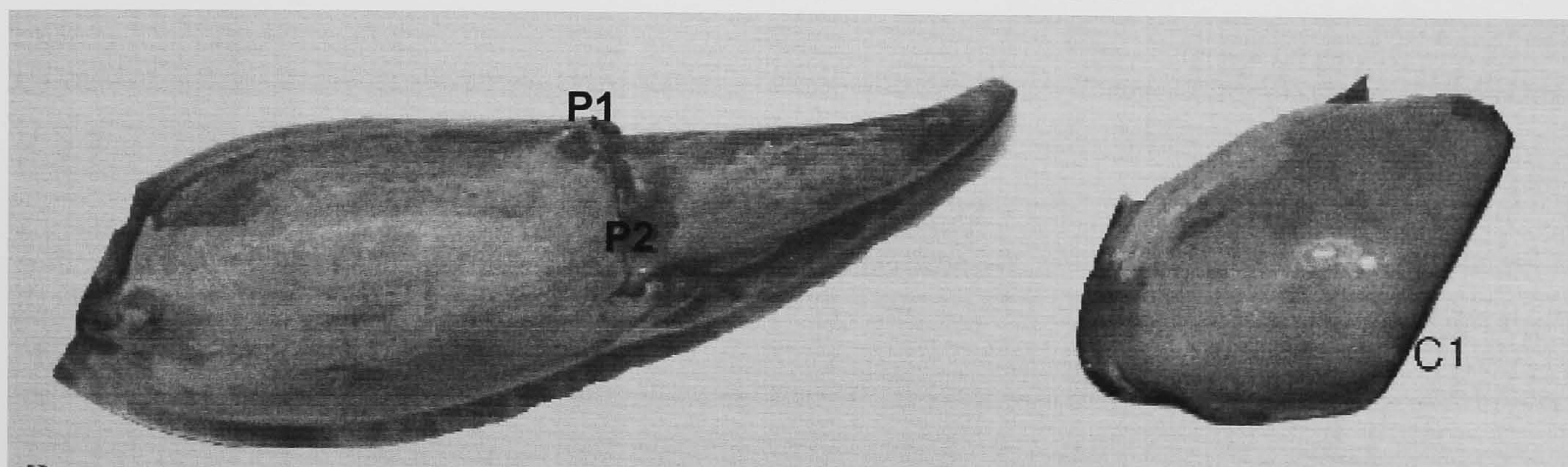
Table 3.7: Morphological description of the six museum specimens of the genus *Scylla* examined from the Fabricius collection, Zoological Museum, Department of Zoology, University of Copenhagen, Denmark.

Specimen	Character						Proposed species
	Shape of carapace	Dorsal carapace morphology	Frontal lobe teeth shape	Shape of anterolateral spines	Propodus spines (number and shape)	Carpus spines (number and shape)	
ZMUC CRU 150-1	Convex carapace with a round appearance.	Faint "H" shaped impression on the cardinal region.	Blunt obtuse semicircular teeth. All teeth are equal in size.	Short obtuse spines. All spines are equal size and shape.	Two vestigial spines on dorsal surface. A pronounced nodule on inner lateral propodus.	One pronounced spine on the dorsal surface. One reduced spine on the ventral surface of carpus.	<i>Scylla olivacea</i>
ZMUC CRU 150-2	Flattened carapace. Anterolateral region is more pronounced.	Pronounced "H" shaped impression on the cardinal region.	Blunt obtuse semicircular teeth. Central groove in deeper than other spaces.	Spines adjacent to orbital sockets are pronounced and blunt on the tips. The most posterior spines are pronounced and needle-like.	Two sharp spines on the dorsal surface. Inner spine is more pronounced than outer one. Small sharp spine on inner lateral propodus.	One pronounced spine on the dorsal surface. Two spines on the ventral surface.	<i>Scylla tranquebarica</i>
ZMUC CRU 150-3	Flattened carapace.	Pronounced "H" shaped impression on the cardinal region.	Triangular short teeth with angular small interspaces. Central teeth protruding slightly.	All spines are the same shape and size. Spines are compressed with small spaces between spines.	Two sharp spines proximal to dactylus joint. Small sharp spine on inner lateral propodus.	One pronounced spine on the dorsal surface. Two spines on the ventral surface.	<i>Scylla paramamosain</i>
ZMUC CRU 150-4	Flattened carapace.	Pronounced "H" shaped impression on the cardinal region.	Triangular but very blunt teeth with small interspaces.	All spines are the same shape and size. Spines are compressed with small spaces between spines.	Two sharp spines proximal to dactylus joint. The inner spine is more pronounced than the outer one.	One pronounced spine on the dorsal surface. One clear spine on ventral surface. The anteroventral spines are severely reduced.	<i>Scylla paramamosain</i>
ZMUC CRU 150-5	Flattened carapace.	Pronounced "H" shaped impression on the cardinal region.	Triangular but very blunt teeth with small interspaces.	All spines are the same shape and size. Spines are compressed with small spaces between spines.	Two sharp spines proximal to dactylus joint. The inner spine is more pronounced than the outer one.	One pronounced spine on the dorsal surface. One clear spine on ventral surface. The anteroventral spines are severely reduced.	<i>Scylla paramamosain</i>
ZMUC CRU 150-6	Slightly convex carapace.	Narrow pronounced "H" shaped impression on the cardinal region.	Elongated, acute teeth with blunt tips and U-shaped interspaces.	Spines adjacent to orbital sockets are pronounced and sharp. Other teeth are very sharp with large U-shaped interspaces.	Two sharp spines. The inner spine is more pronounced than the outer one. proximal to dactylus joint.	One pronounced spine on the dorsal surface. Two spines on the ventral surface.	<i>Scylla serrata</i>



A.

2 cm

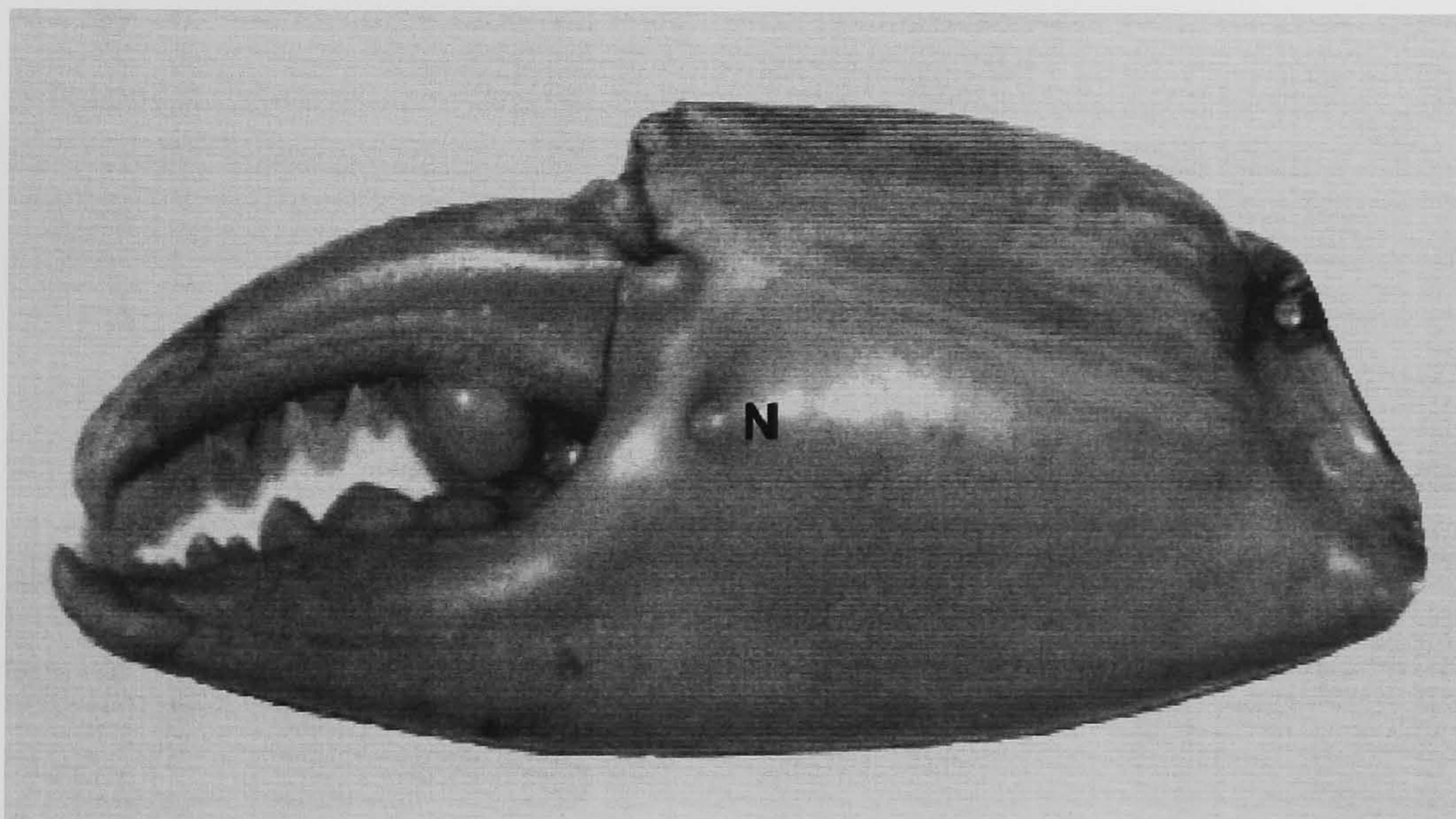


B.

1 cm

C.

1 cm



D.

1 cm

Figure 3.3: Specimen ZMUC CRU 150-1. a) dorsal carapace; frontal lobe teeth (F) and anterolateral spines (AL), b) dorsal view of right chela; dorsal propodus spines (P1 and P2), c) external lateral view of right carpus; ventral carpus spines (C1) and d) inner lateral view of right chelae; nodule on inner lateral propodus (N).

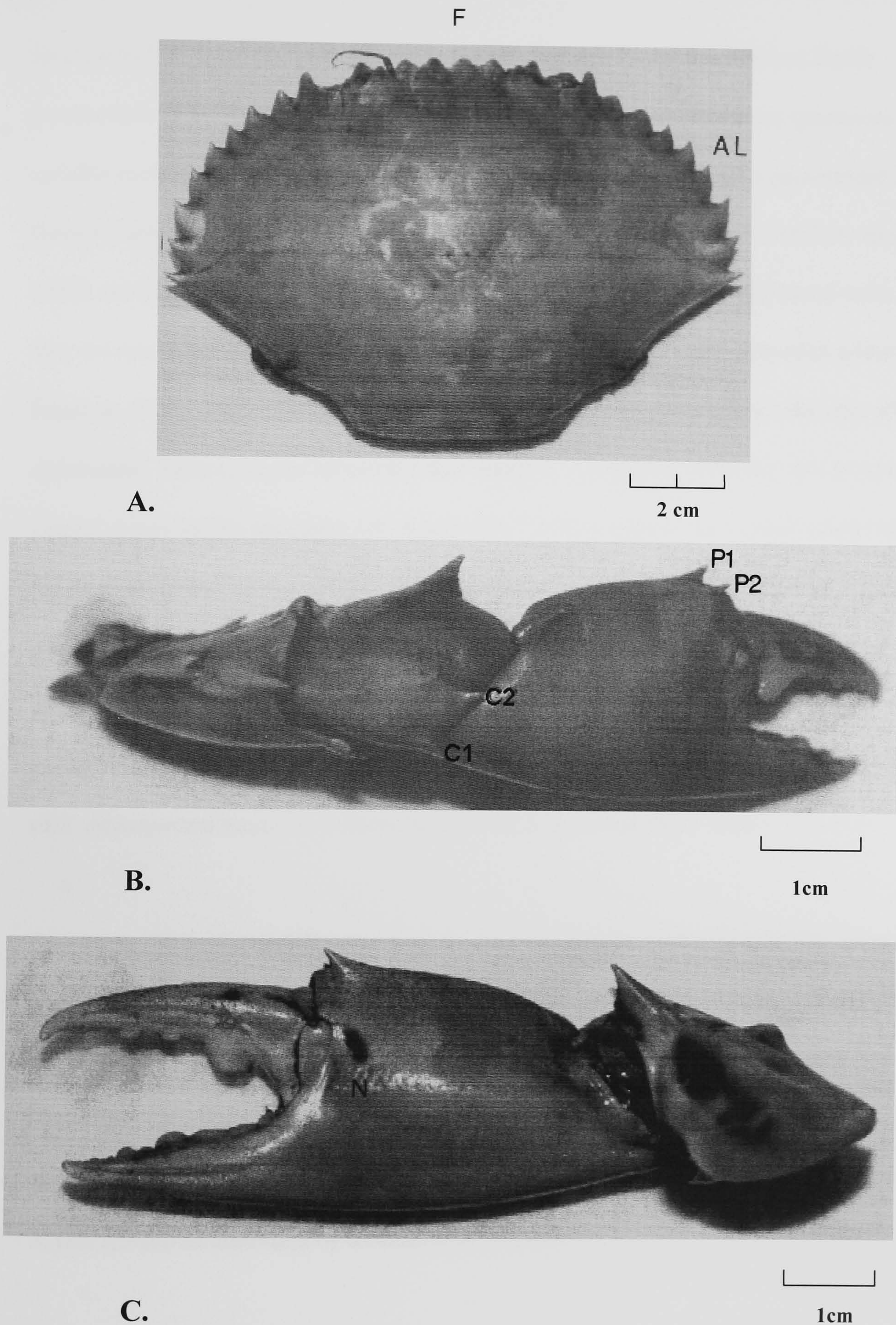


Figure 3.4: Specimen ZMUC CRU 150-2. a) dorsal carapace; frontal lobe teeth (F) and anterolateral spines (AL), b) outer lateral view of right chelae; dorsal propodus spines (P1 and P2) and ventral carpus spines (C1 and C2) and c) inner lateral view of right chelae; nodule on inner lateral propodus (N).

Specimens 150-3 to 150-5 (illustrated in figures 3.5 to 3.7) are described as *Scylla paramamosain* by Keenan *et al.* (1998). On closer inspection these three specimens are variable in their morphology. All three have a flattened carapace and a pronounced “H” shaped mark on the dorsal carapace. Specimen 150-3 has triangular frontal lobe teeth, which are short and sharp. Specimens 150-4 and 150-5 also have short frontal teeth, but they are much smoother than those on specimen 150-3. Two dorsal propodus spines are found on all three specimens, although 150-3 has more prominent spines than the other two specimens. Ventral carpus spines are also variable. Specimen 150-3 has two prominent ventral spines unlike specimen 150-4 and 150-5, which exhibit one ventral carpus spine, the anterior ventral carpal spine is severely reduced in specimen 150-4 and absent for specimen 150-5. Specimen 150-3 has all the characters of *S. paramamosain* as described by Keenan *et al.* (1998). Specimens 150-4 and 150-5 are more difficult to assign to *S. paramamosain* with any certainty. Specimen 150-4 does have many of the characteristics of *S. olivacea* and therefore is most likely to be *S. olivacea* rather than *S. paramamosain*.

Specimen 150-6 (figure 3.8) has a slightly convex carapace and a narrow “H” shaped impression in the dorsal carapace. The acute frontal lobe teeth are elongated and blunt at the tips with U-shaped interspaces. The anterolateral spines are sharp and well spaced. Two sharp spines are present on both the dorsal propodus and the ventral carpus, although they are worn quite badly. These morphological characters suggest that this specimen is *Scylla serrata* as described by Keenan *et al.* (1998).

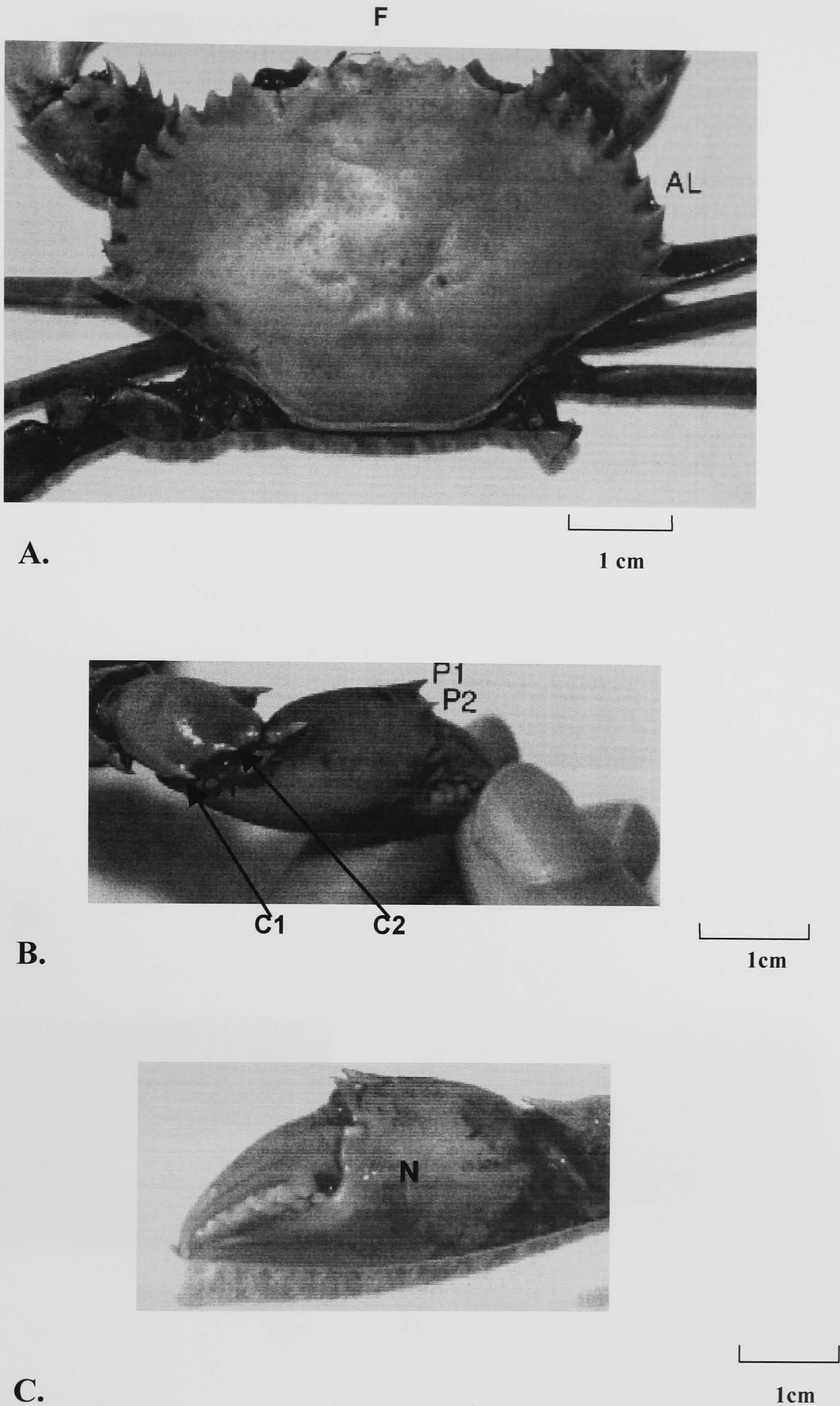
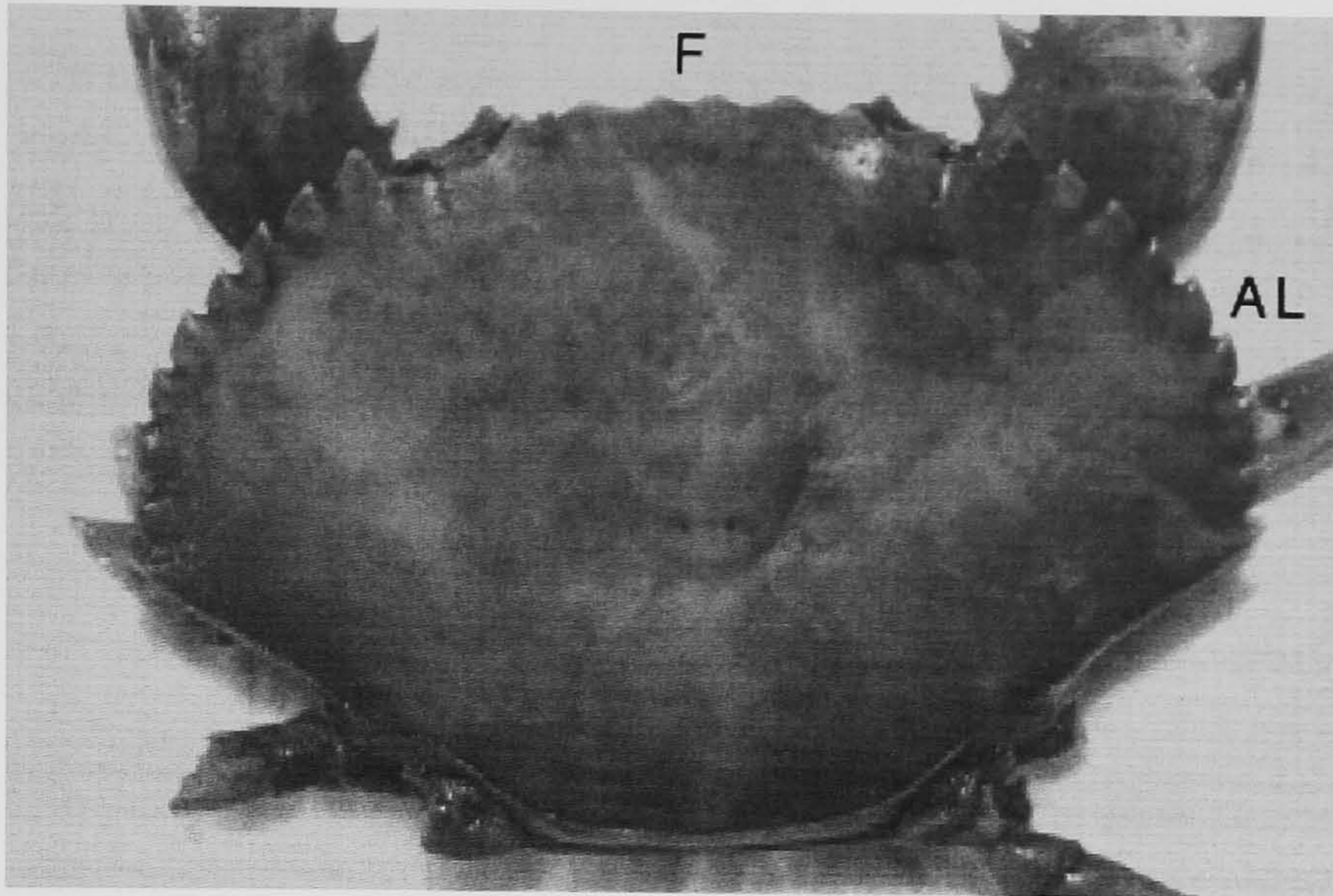
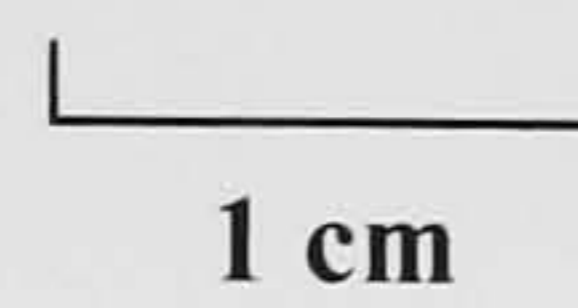


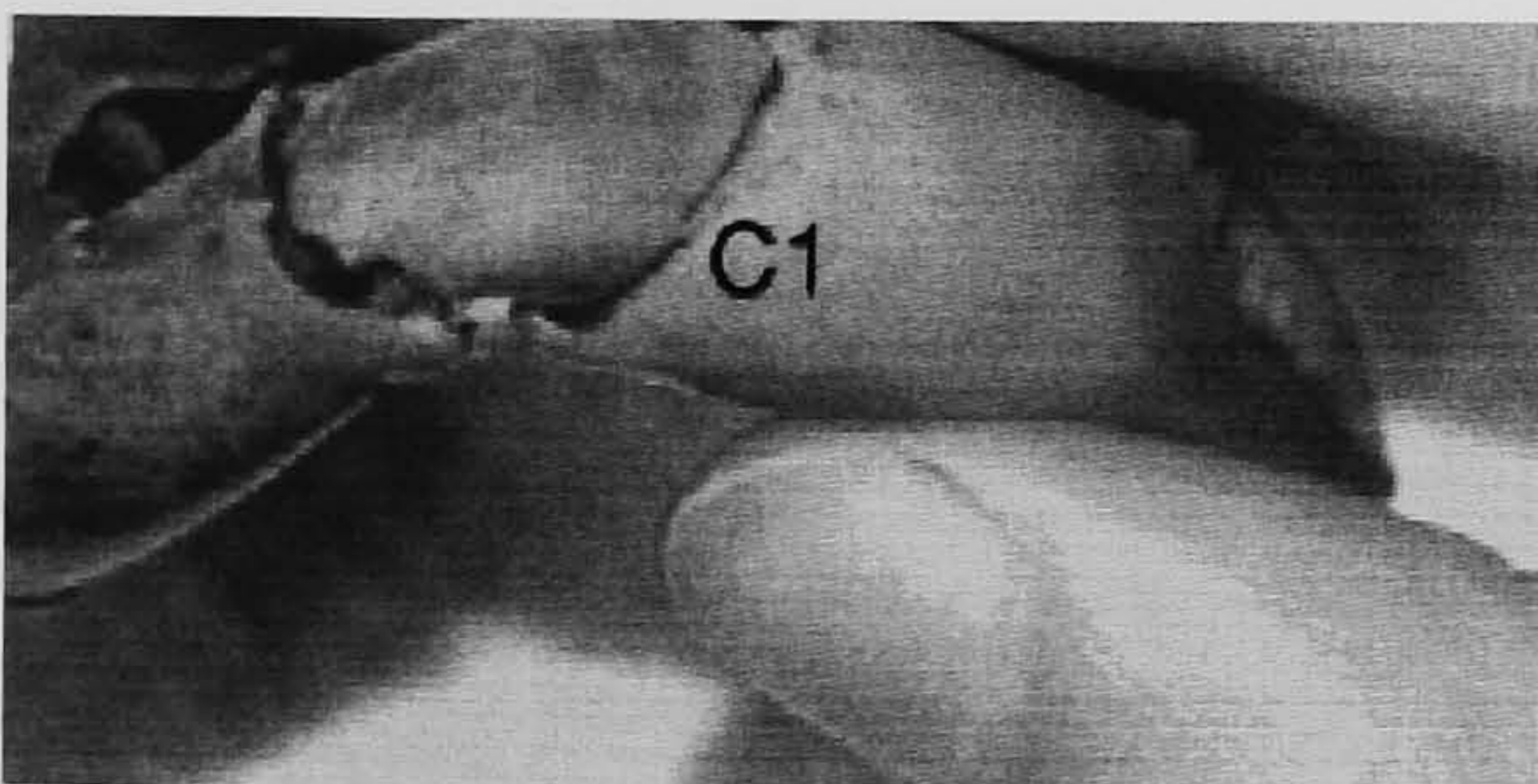
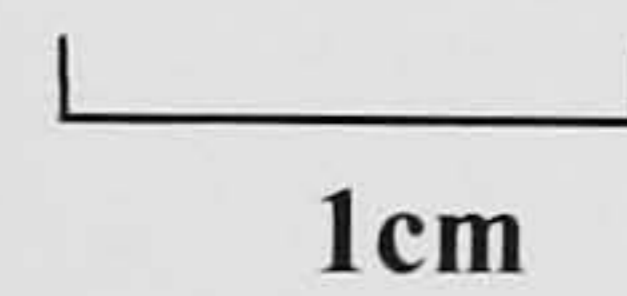
Figure 3.5: Specimen ZMUC CRU 150-3. a) dorsal carapace; frontal lobe teeth (F) and anterolateral spines (AL); b) outer lateral view of right chelae; dorsal propodus spines (P1 and P2) and ventral carpus spines (C1 and C2) and c) inner lateral view of right chelae; nodule on inner lateral propodus (N).



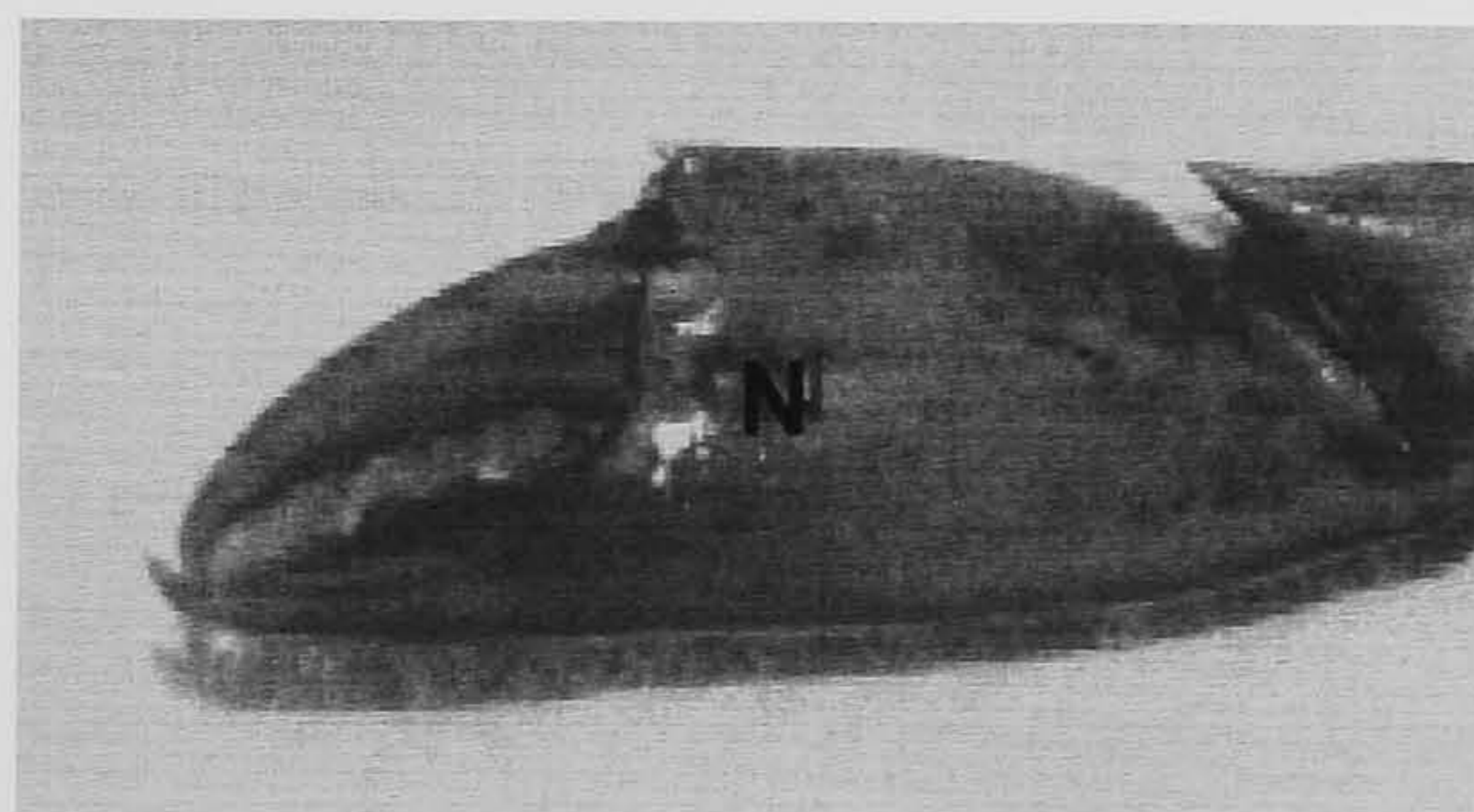
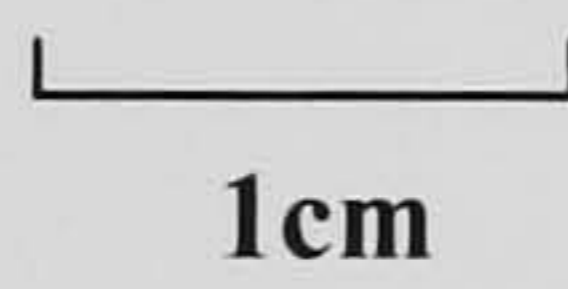
A.



B.



C.



D.

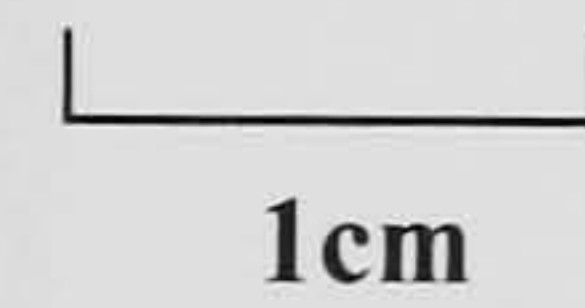
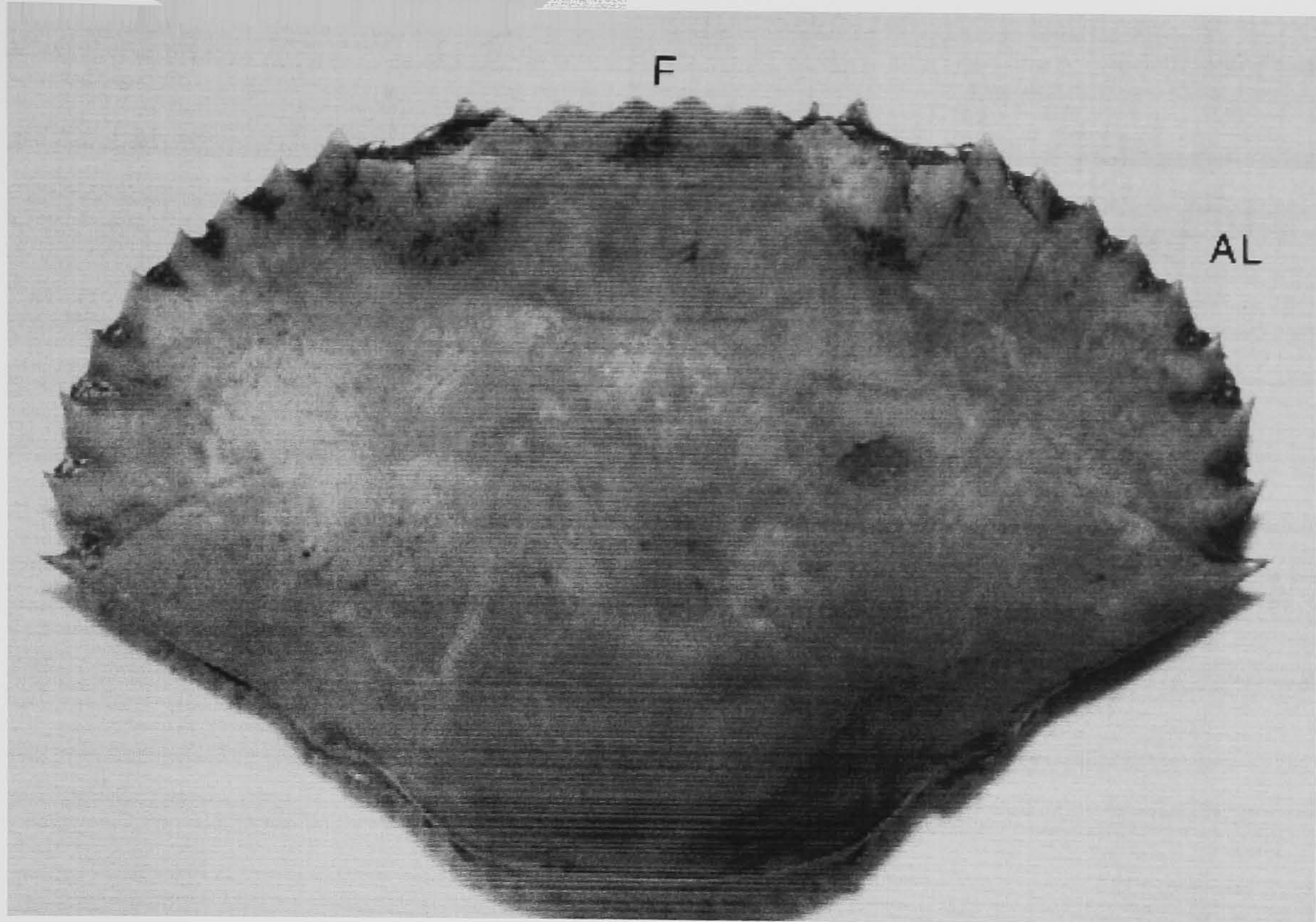
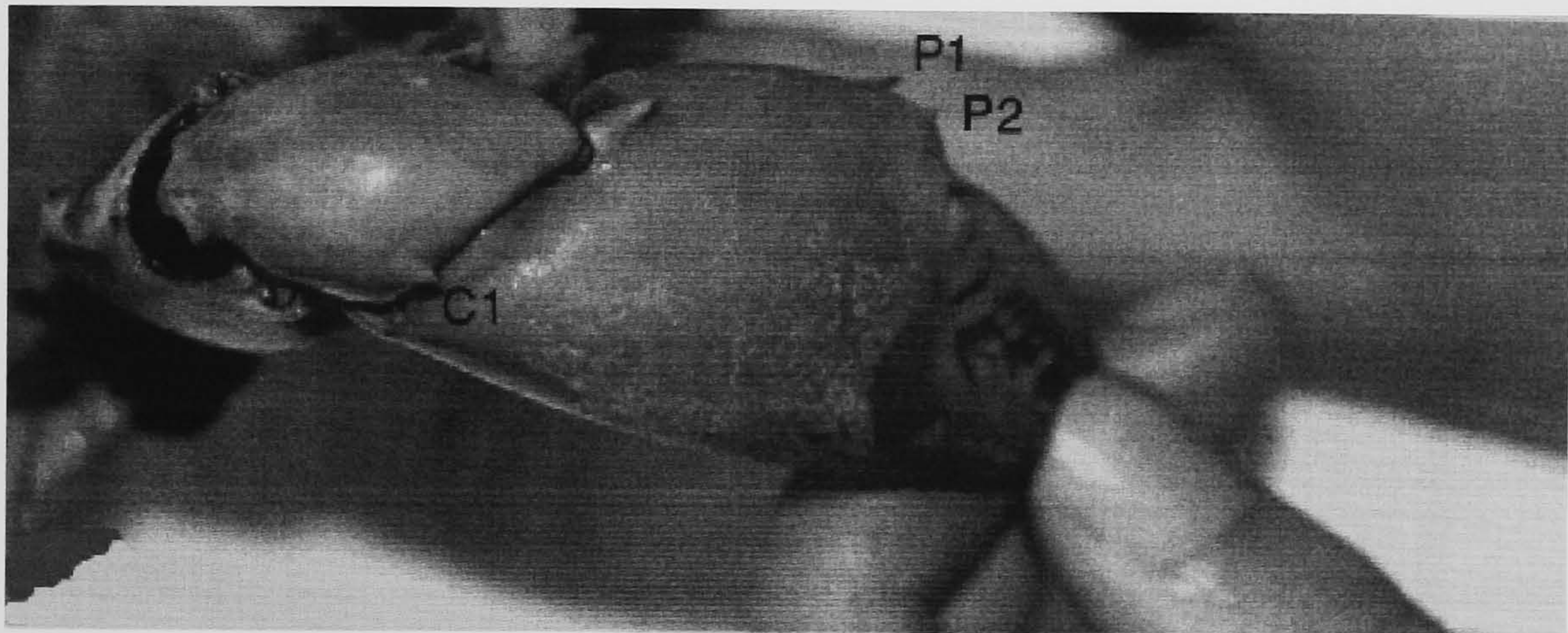


Figure 3.6: Specimen ZMUC CRU 150-4. a) dorsal carapace frontal lobe teeth (F) and anterolateral spines (AL); b) dorsal view of right chela; dorsal propodus spines (P1 and P2), c) external lateral view of right carpus; ventral carpus spines (C1) and d) inner lateral view of right chelae; nodule on inner lateral propodus (N).



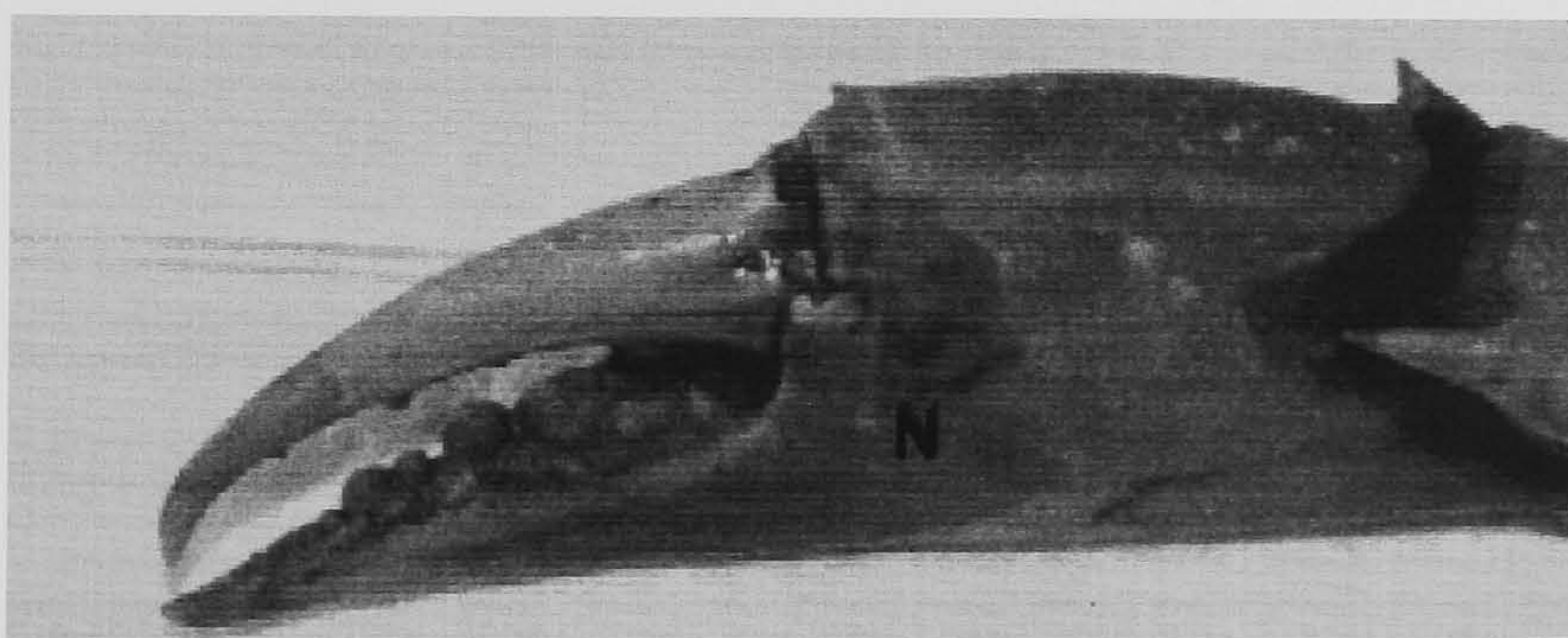
A

1 cm.



B

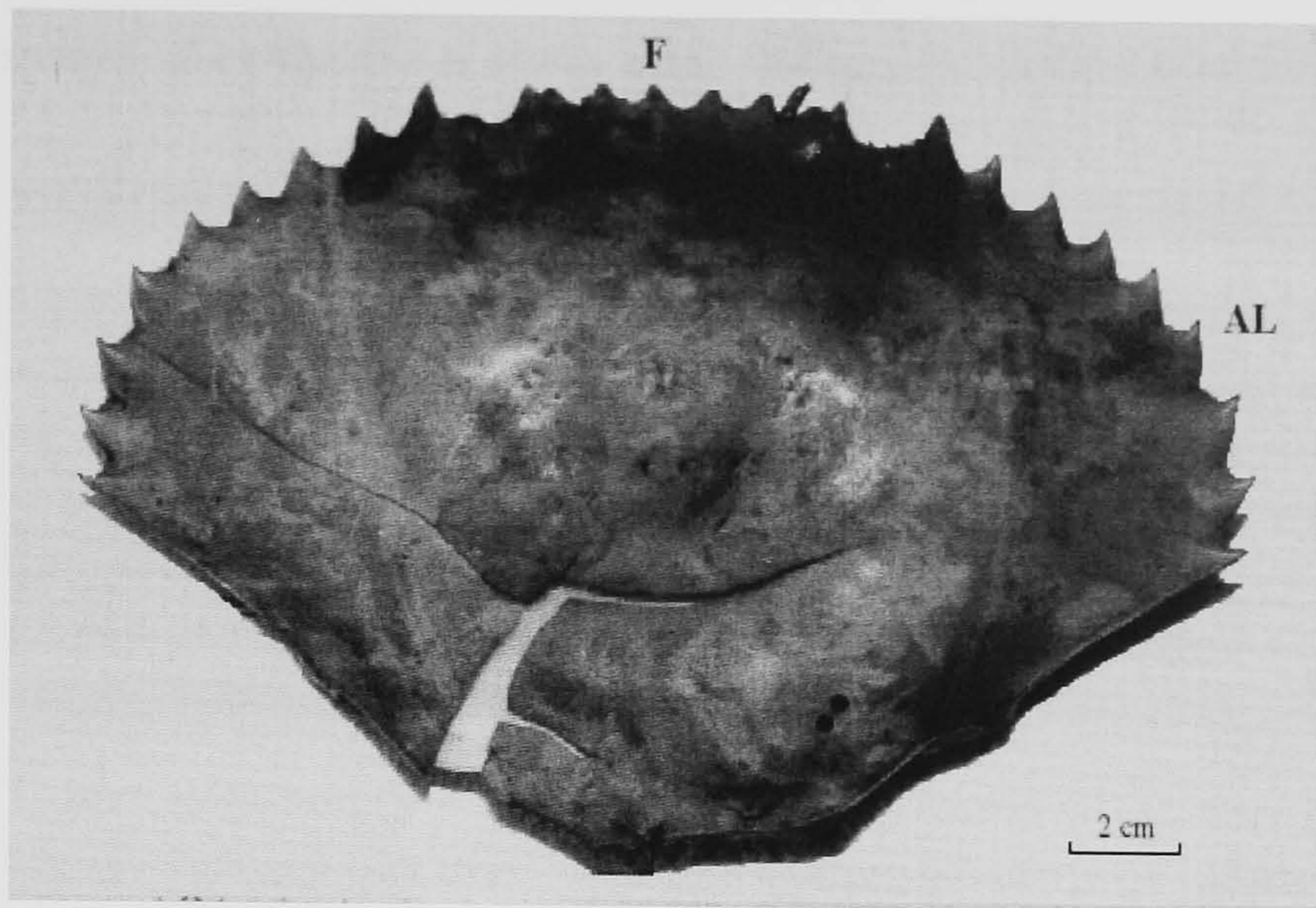
1 cm.



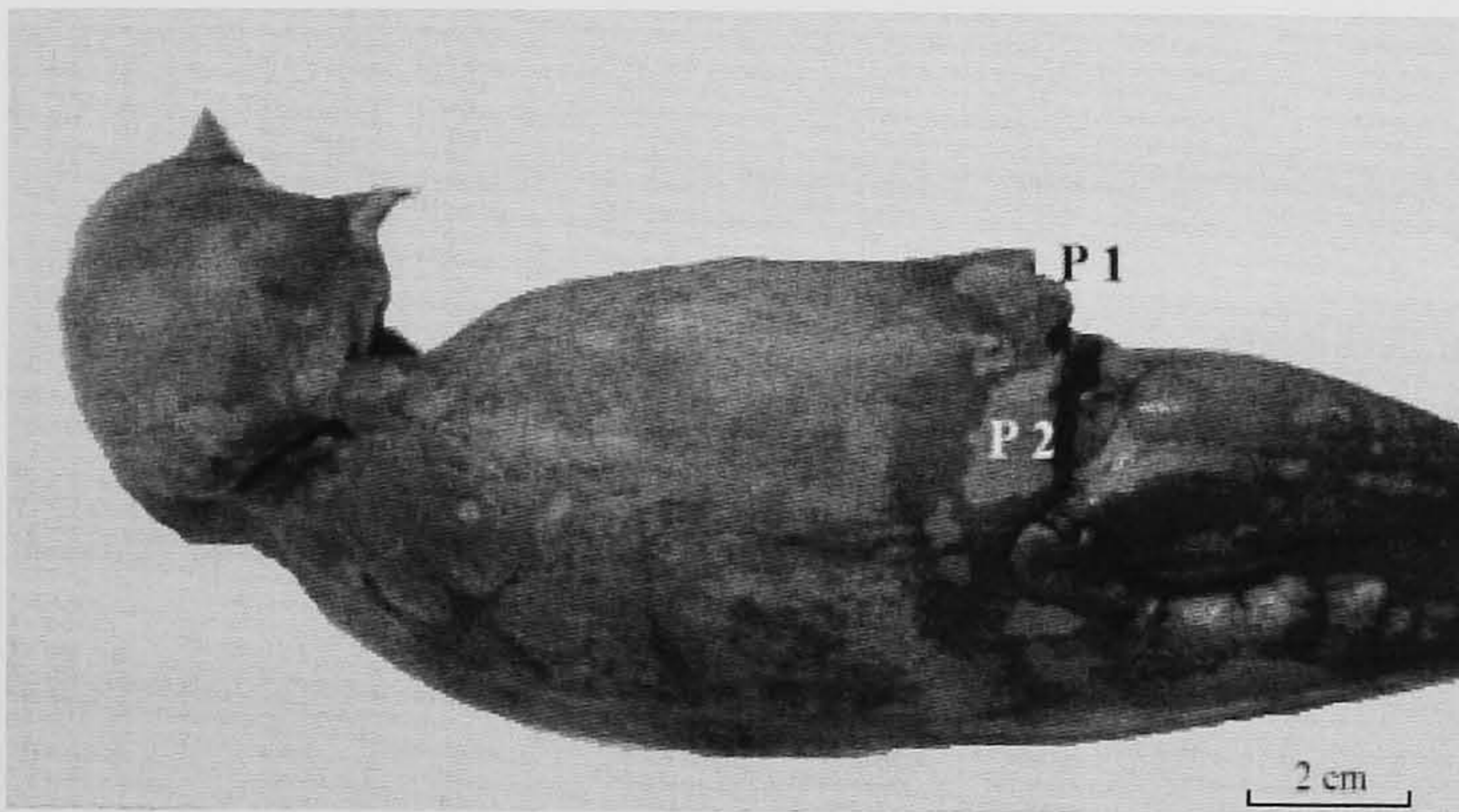
C

1 cm.

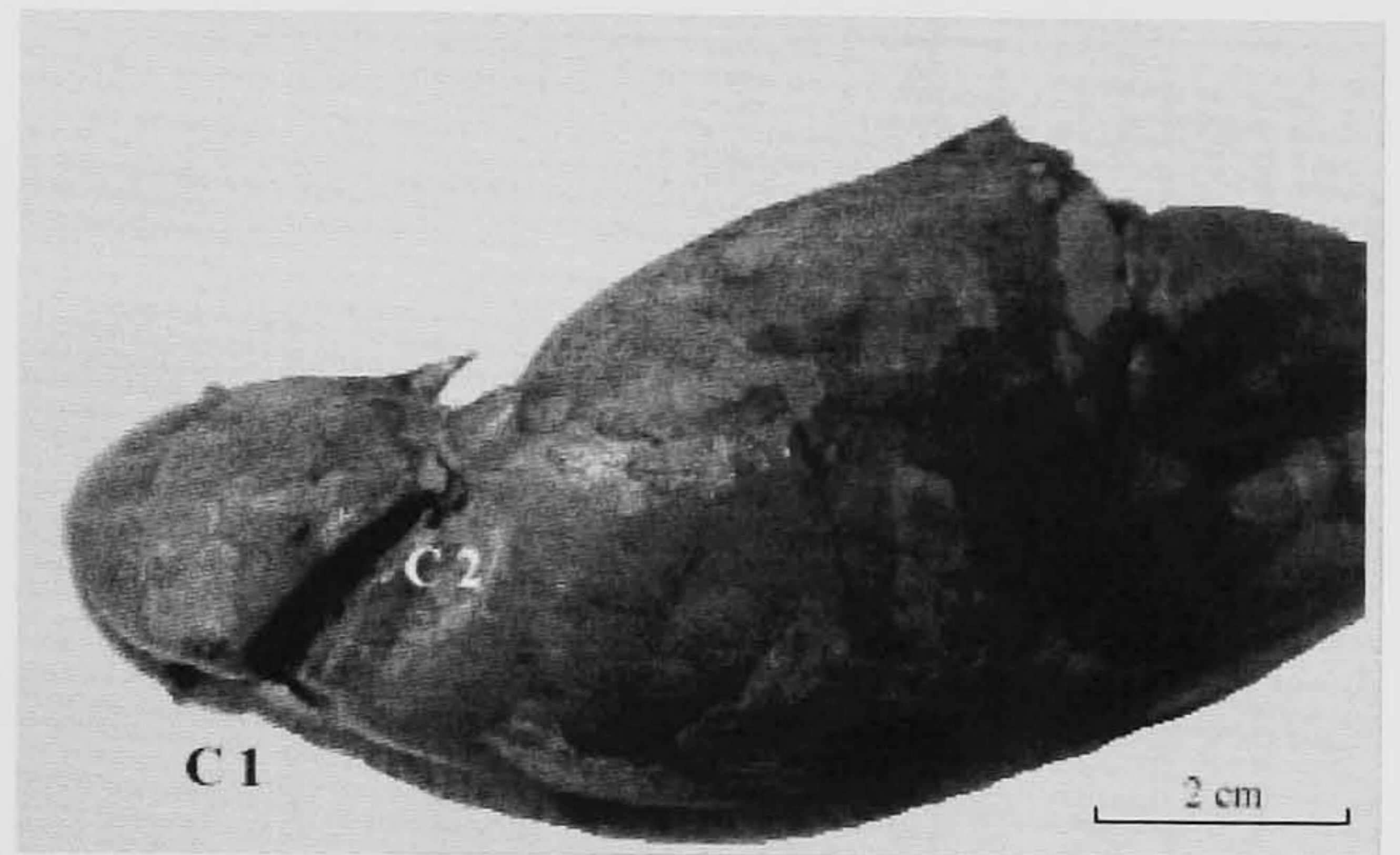
Figure 3.7 : Specimen ZMUC CRU 150-5. a) dorsal carapace; frontal lobe teeth (F) and anterolateral spines (AL), b) outer lateral view of right chelae; dorsal propodus spines (P1 and P2) and ventral carpus spines (C1) and c) inner lateral view of right chelae; nodule on inner lateral propodus (N).



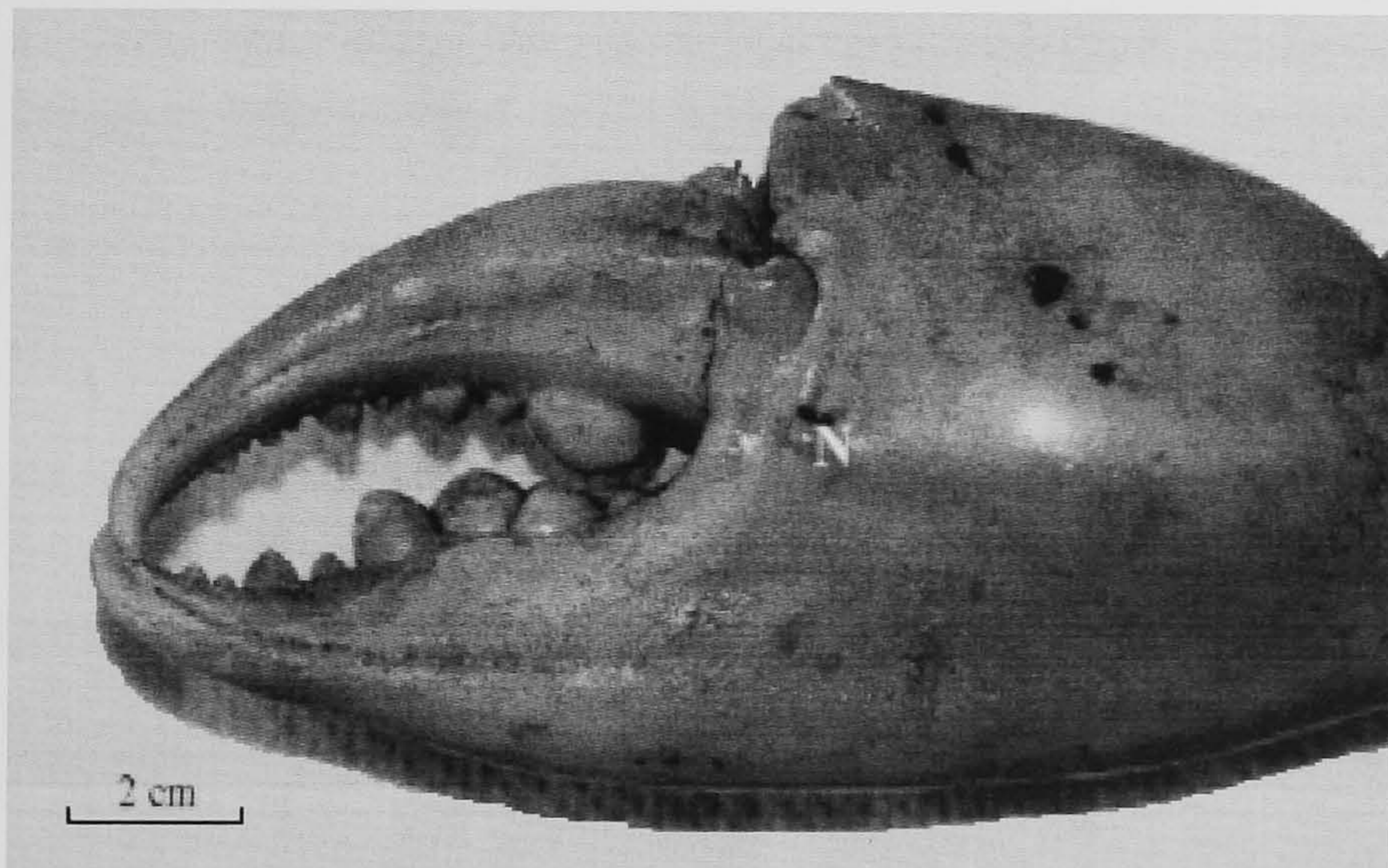
A



B



C



D

Figure 3.8: Specimen ZMUC CRU 150-6. a) dorsal carapace frontal lobe teeth (F) and anterolateral spines (AL), b) dorsal view of right chela; dorsal propodus spines (P1 and P2), c) external lateral view of right carpus; ventral carpus spines (C1 and C2) and d) inner lateral view of right chelae; nodule on inner lateral propodus (N).

3.3.4 Identification of crabs from study sites using descriptive characters

The crabs collected from seven locations within Southeast Asia represent two colour morphs of *Scylla*, denoted as “black” and “white” (represented by tables 3.8 and 3.9 respectively), common names that are associated to the two morphologies in Thailand.

The black morphs were collected from Surat Thani, Ranong, Paikgasir and Sematan. The white morphs were collected from Surat Thani, Chanthaburi, Thai Binh and Can Gio.

All black morphs exhibit dark brown to dark green dorsal colouration, incorporating the carapace and dorsal surface of all limbs. The black colour morph from Paikgasir also exhibits blue green hues on the anterolateral spines and on the points of articulation of the limbs. In the majority of individuals, reticulation is not present except for some individual mud crabs from Ranong, which exhibit faint reticulation on the dorsal merus of the chelae. Ventral body colouration is pale white cream with some variation on colour hues between locations. Crabs from Sematan have a pale orange sternum compared to the pinkish hues exhibited by crabs from the other three locations. All mature females reveal purple/brown/black abdominal flaps with a pale horizontal band on each abdominal segment.

The colouration on the chela is similar between locations. The ventral and lateral surfaces of the propodus are red/orange, although crabs from Sematan exhibit darker shades of these colours. In some cases the tips of the dactylus and propodus are pink. There is also a green trim around the dactylus joint which is also present, although more subtle, on the borders of the other chela joints.

Table 3.8: Description of characters observed in mud crabs (genus *Scylla*) with “black” colour morphology from four locations in Southeast Asia.

Character	Location			
	Surat Thani	Ranong	Paikgasir	Sematan
Dorsal carapace colour	Dark green/brown. Faint white patches on postero-branchial regions.	Dark brown/green.	Dark brown. Blue green hues around anterolateral spines.	Dark brown/green.
Ventral carapace colour	Pale pink/white. Dark blue/green anterolateral regions. Mature female abdomen is dark brown with purplish hues and pale horizontal bands on each segment.	White with pinkish hues. Mature female abdomen is black with purple hues and pale horizontal bands across each segment.	White with pinkish hues. Blue/green anterolateral borders. Mature female abdomen is black with purple hues and pale horizontal bands across each segment.	Pale, light orange sternum. Dark brown/green anterolateral borders. Mature female abdomen is brown with purple hues and pale horizontal bands across each segment.
Chelae colour	Dorsal chelae are dark green/brown. Lateral and ventral propodus and dactylus are orange/red. Green trim along dactylus joint. No reticulation present.	Dorsal chelae are dark brown/green with some faint dark reticulation on the merus. Ventral and lateral surface of propodus is tan orange/red with green trim around the dactylus joint. No reticulation present.	Dorsal chelae are dark brown green. Ventral and lateral propodus in tan orange/red. Green trim around the dactylus joint. Dactylus is brown with pink tips. Spines have pale blue/green tips.	Dorsal chelae are dark brown green. Ventral and lateral propodus is dark tan. Tips of dactylus and propodus are pink. No reticulation present.
Pereiopod colour	Dark green/brown. No reticulation present.	Dark brown/green. No reticulation present.	Brown/green. No reticulation. Blue/green edges to joints of legs.	Olive green/brown. No reticulation present.
Carapace morphology	Faint “H” shaped mark on dorsal carapace. Carapace convex.	Faint “H” shaped mark on dorsal carapace. Carapace convex.	Clear “H” shaped mark on dorsal carapace. Convex carapace.	Clear “H” shaped mark on dorsal carapace. Convex carapace.
Frontal lobe teeth shape	Blunt semicircular teeth. All teeth are equal in size and shape.	Blunt semicircular teeth. All teeth are equal in size and shape.	Blunt semicircular teeth. All teeth are equal in size and shape.	Blunt semicircular teeth. All teeth are equal in size and shape.
Anterolateral spines shape	First spines, adjacent to orbital sockets, are small and curved towards the sockets. The posterior spines are reduced compared to the other anterolateral spines.	First spines, adjacent to orbital sockets, are small and curved towards the sockets. The posterior spines are reduced compared to the other anterolateral spines.	First spines, adjacent to orbital sockets, are small and curved towards the sockets. The posterior spines are reduced compared to the other anterolateral spines.	First spines, adjacent to orbital sockets, are small and curved towards the sockets. The posterior spines are reduced compared to the other anterolateral spines.
Chelae propodus spines (number and shape)	One vestigial dorsal spine on inner dorsal surface proximal to dactylus joint. Prominent nodule on inner lateral propodus.	Two dorsal spines proximal to the dactylus. Inner spine is a nodule the outer spine is vestigial. Prominent nodule on inner lateral propodus.	Two vestigial dorsal spines proximal to the dactylus. Prominent nodule on inner lateral propodus.	One to two dorsal spines proximal to the dactylus. Prominent nodule on inner lateral propodus.
Chelae carpus spines (number and shape)	One long and sharp dorsal spine. Ranges from the absence to two vestigial ventral spines present.	One long and sharp dorsal spine. One vestigial spine on ventral carpus.	One long and sharp dorsal spine. One spine on ventral carpus.	One long and sharp dorsal spine. One vestigial spine on ventral carpus.

Table 3.9: Description of characters observed in mud crabs (genus *Scylla*) with “white” colour morphology from four locations in Southeast Asia.

Character	Location			
	Surat Thani	Chanthaburi	Thai Binh	Can Gio
Dorsal carapace colour	Olive green/pale green. Yellow spots along branchial borders.	Olive green/yellow green.	Olive green.	Olive green.
Ventral carapace colour	White/cream with yellow sternum. Green anterolateral borders. Mature female abdomen is green sometimes with weak reticulation.	White/cream with yellow hues. Mature female abdomen is green sometimes with weak reticulation.	White/cream with yellow hues. Mature female abdomen is green sometimes with weak reticulation.	White with yellow hues. Mature female abdomen is green sometimes with weak reticulation.
Chelae colour	Dorsal chelae are pale green with dark green reticulation. Brown spots on upper half of outer lateral propodus. Lower lateral propodus is pale yellow. Vertical red flashes across dactylus and propodus tip. Black patches on ventral carpus.	Dorsal chelae pale green with dark green reticulation. Brown spots on upper half of outer lateral propodus. Lower lateral propodus is pale yellow. Vertical red flashes across dactylus and propodus tip. Black patches on ventral carpus.	Dorsal chelae pale green with weak dark green reticulation. Brown spots on upper half of outer lateral propodus. Lower lateral propodus is pale yellow. Vertical red flashes across dactylus and propodus tip. Black patches on ventral carpus.	Dorsal chelae pale green with strong dark green reticulation. Brown spots on upper half of outer lateral propodus. Lower lateral propodus is pale yellow. Vertical red flashes across dactylus and propodus tip. Black patches on ventral carpus.
Pereiopod colour	Pale green with dark green polygonal reticulation.	Pale green with dark green polygonal reticulation.	Pale green with dark green polygonal reticulation.	Pale green with dark green polygonal reticulation.
Carapace morphology	Faint “H” shaped mark on dorsal carapace. Flattened carapace.	Faint “H” shaped mark on dorsal carapace. Flattened carapace.	Faint “H” shaped mark on dorsal carapace. Flattened carapace.	Faint “H” shaped mark on dorsal carapace. Flattened carapace.
Frontal lobe teeth shape	Triangular, pointed, sharp but small. All teeth are equal size and shape.	Triangular, pointed, sharp but small. All teeth are equal size and shape.	Triangular, pointed, sharp but small. All teeth are equal size and shape.	Triangular, pointed, sharp but small. All teeth are equal size and shape.
Anterolateral spines shape	Short angular sharp spines. The first spines, adjacent to orbital sockets, are straighter and blunter than others.	Short angular sharp spines. The first spines, adjacent to orbital sockets, are straighter and blunter than others.	Short angular sharp spines. The first spines, adjacent to orbital sockets, are straighter and blunter than others.	Short angular sharp spines. The first spines, adjacent to orbital sockets, are straighter and blunter than others.
Chelae propodus spines (number and shape)	Two dorsal spines proximal to dactylus joint. The inner spine is more pronounced than the outer one. Inner lateral nodule is absent or vestigial.	Two dorsal spines proximal to dactylus joint. The inner spine is more pronounced than the outer one. Inner lateral nodule is absent or vestigial.	Two dorsal spines proximal to dactylus joint. The inner spine is more pronounced than the outer one. Inner lateral nodule is absent or vestigial.	Two dorsal spines proximal to dactylus joint. The inner spine is more pronounced than the outer one. Inner lateral nodule is absent or vestigial.
Chelae carpus spines (number and shape)	One sharp dorsal spine. One to two ventral carpus spines. In some cases the antero-ventral spine is vestigial.	One sharp dorsal spine. One to two ventral carpus spines. In some cases the antero-ventral spine is vestigial.	One sharp dorsal spine. One to two ventral carpus spines. In some cases the antero-ventral spine is vestigial.	One sharp dorsal spine. One to two ventral carpus spines. In some cases the antero-ventral spine is vestigial.

Even allowing for some variation between locations, the morphological features indicate that the black colour morph is *Scylla olivacea*, as described by Herbst (1796) and from the examination of the preserved museum material. The morphological characters for this morph include frontal lobe teeth that are blunt, rounded and semicircular in shape. The anterolateral teeth are small and the same size although the most posterior tooth is reduced slightly. There is some variation in the number and shape of the dorsal spination between locations. Crabs from Surat Thani and Sematan exhibited one vestigial dorsal propodus spine. Crabs from Ranong and Paikgasir show two dorsal propodus spines, but in both cases the spines are blunt, while in the Paikgasir population both spines are vestigial on most individuals. The ventral carpus spines vary from two to none for crabs from Surat Thani. Only one vestigial spine is found on the ventral carpus of crabs from the other three sites. In summary, both the colouration and morphological characters indicate that the black colour morph is the species *S. olivacea* Herbst.

The “white” colour morph is also very similar in both colouration and morphology between the studied locations, with only very subtle differences in morphology between the populations sampled. The dorsal carapace is pale green to olive green. Some crabs of the white colour morph from Surat Thani also have faint yellow spots on the branchial regions of the dorsal carapace.

The ventral carapace is pale white/cream with yellow hues. In some cases the mature female abdomen shows some weak reticulation. In all locations the dorsal chelae and other limbs are green with dark green/brown reticulation, which is indicative of *Scylla serrata* Forskål, *S. paramamosain* Estampador and *S. tranquebarica* Fabricius. The lower half of the propodus is pale yellow with brown spots on the outer lateral surface. The intensity of

these spots varies between locations. Crabs from Thai Binh have the most intense spotting. Mud crabs from all locations sampled also exhibited black patches on the ventral carpus as well as vertical red flashes across the dactylus and propodus tips.

Morphological characters of the white morph include a flattened carapace and triangular pointed frontal lobe teeth that are sharp, but small, as described for *Scylla paramamosain* Estampador. The anterolateral spines are small and sharp although the spines adjacent to the orbital socket are somewhat blunter and straighter than the other spines. There are two sharp dorsal spines on the propodus for crabs from all locations. The inner dorsal spine tends to be more pronounced than the outer spine. In some individuals the outer spine is quite reduced, but still sharp. The ventral carpal spines vary from one to two; in some individuals the anterior ventral spine is vestigial or absent.

In summary, the external colouration for the white colour morph of *Scylla* is difficult to assign to a particular species, although it is similar to *S. oceanica* Dana described by Serene (1952). However, the morphological characters are straightforward and are diagnostic for the species *S. paramamosain* Estampador as reported by Keenan *et al.* (1998).

3.4 Discussion

3.4.1 Evaluation of published descriptions

From published taxonomic descriptions there are five species names referred to, namely *Scylla serrata* Forskål, *S. oceanica* Dana, *S. tranquebarica* Fabricius, *S. olivacea* Herbst and *S. paramamosain* Estampador. However, the same descriptions also reveal only four plausible morphologies within the genus. In many cases the names have been confused between morphologies, where more than one name has appeared for similar descriptions.

There are many reasons for this confusion between publications, some of the most pertinent are as follows. The early descriptions of the mud crab are extremely vague. The first description by Forskål 1775 of *Cancer serratus* is unreliable as he describes a specimen with no chelae. Moreover, the holotype from which he made his description was lost and thus could not be referred to in subsequent revisions of the taxonomy. Probably for the latter reason some of the subsequent authors have followed Fabricius' (1798) description of *Portunus tranquebaricus*, resulting in the two names being used simultaneously.

Even though descriptions did become more detailed when more than one species of *Scylla* was being proposed, the first proper revision by Estampador (1979a, b) claiming that there were three species and one variant in the genus *Scylla*, has used the nomenclature incorrectly and attributed the name *S. serrata* to the morphology described in previous publications as *Cancer olivaceous* (Keenan *et al.*, 1998). Moreover, as with many early descriptions, both the original publication and/or collection is difficult to obtain for comparative studies. Therefore, the more recent diagnoses by Estampador (1949a) has

been followed by many authors, making the comparisons between early and more recent descriptions difficult.

In traditional taxonomy, it is common for species diagnoses to be made from one sex, or indeed one individual of the proposed species. The first (or earliest known) description is given priority (known as the law of priority) (Quicke, 1993). Some biologists disagree with this method of attributing species names, purporting that it does not take into account the natural variation within and between conspecific populations. Thus revisions of the genus *Scylla* proposing more than one species were argued by later authors (Stephenson and Campbell, 1959; Stephenson, 1971; Holthius, 1978) to reflect only geographic variation in morphology of the same species.

The locations where many of the authors worked and collected mud crabs represent a broad geographical area throughout the Indo-West Pacific although each description is based on a restricted sampling range. Thus the descriptions between authors vary according to the location from which they sampled; those locations with sympatric morphs advocating more than one species. However, the proposal of four species made by Keenan *et al.* (1998) is based on collections from a wide geographical region and is therefore the most reliable published revision of the taxonomy to date.

The morphology of *Scylla* in published descriptions hinges on spinal architecture and in particular the frontal lobe spines. As shown in sections 3.3.1.3 and 3.3.1.4, three frontal lobe morphologies are described although there are four species proposed. This has thrown a lot of confusion into the descriptions especially when described in conjunction with chelae spine morphology.

The most recent publication revising the taxonomy of *Scylla* by Keenan *et al.* (1998) has reassigned four of the five species names (*S. serrata* Forskål, *S. tranqubarica* Fabricius, *S. olivacea* Herbst and *S. paramamosain* Estampador) based on what they believe to be the first descriptions of those species. However, there is still some debate as to which names should be assigned to the appropriate morphology (Macintosh, pers. comm.). *S. oceanica* is still a popular synonym for *S. serrata* and the name *S. serrata* will still be used in place of *S. olivacea* until the recent nomenclature is accepted, thus continuing the confusion.

3.4.2 Evaluation of diagnostic characters to distinguish four species of *Scylla*

Four morphologies were positively identified from examining both fresh material and museum specimens. Table 3.11 summarises the characters that are believed to be diagnostic for the four morphologies identified within the crab material examined in the present study. Species names have been attributed to these morphologies using the earliest known published nomenclature (Keenan *et al.*, 1998).

Of the fifteen characters described in the published taxonomic descriptions, nine ones prove to be reliable in distinguishing between four morphologies of the genus *Scylla*. The first thing to note is that no single character is diagnostic for all four proposed species, rather a combination of characters is required to identify any of the four species with certainty. This has also been recognised by other authors (Keenan *et al.*, 1998).

Colour characters are diagnostic only for separating *Scylla olivacea* Herbst from the other three *Scylla* species. Although fresh samples of *S. paramamosain* typed by Keenan *et al.* (1998) were unobtainable for comparison, the earliest taxonomic publications describe

Table 3.11: Summary of diagnostic characters for the four proposed species of *Scylla*.

Feature and Character	Proposed species			
	<i>Scylla serrata</i>	<i>Scylla tranquebarica</i>	<i>Scylla olivacea</i>	<i>Scylla paramamosain</i>
Colour;				
Carapace	Shared with <i>S.t</i> and <i>S.p</i>	Shared with <i>S.s.</i> and <i>S.p.</i>	Diagnostic	Shared with <i>S.s.</i> and <i>S.t.</i>
Chelae	Shared with <i>S.t</i> and <i>S.p</i>	Shared with <i>S.s.</i> and <i>S.p.</i>	Diagnostic	Shared with <i>S.s.</i> and <i>S.t.</i>
Pereiopods and swimming legs	Shared with <i>S.t</i> and <i>S.p</i>	Shared with <i>S.s.</i> and <i>S.p.</i>	Diagnostic	Shared with <i>S.s.</i> and <i>S.t.</i>
Ventral abdomen	Diagnostic	Shared with <i>S.p.</i>	Diagnostic	Shared with <i>S.t.</i>
Morphology;				
Frontal lobe teeth (shape)	Diagnostic	Shared with <i>S.o.</i>	Shared with <i>S.t.</i>	Diagnostic
Anterolateral spines (shape)	Shared with <i>S.o.</i> and <i>S.p.</i>	Diagnostic	Shared with <i>S.s.</i> and <i>S.p.</i>	Shared with <i>S.s.</i> and <i>S.o.</i>
Ventral carpus spines (number and shape)	Shared with <i>S.t.</i>	Shared with <i>S.s.</i>	Diagnostic	Some crabs share with <i>S.s.</i> and <i>S.t.</i>
Dorsal propodus spines (number and shape)	Shared with <i>S.t.</i> and <i>S.p.</i>	Shared with <i>S.s.</i> and <i>S.p.</i>	Diagnostic	Shared with <i>S.s.</i> and <i>S.p.</i>

the colouration to be akin to *S. serrata* and *S. tranquebarica*. Colour has proven to be important in identifying between other crustacean species (Knowlton, 1993). For example, colour patterning has been shown to be the only true distinguishing morphological feature to identify between three species of *Goniopsis* de Haan located in tropical west Africa (Sternberg, 1994). Bert *et al.* (1996) have shown how colour characters have just as important a role in distinguishing between two species of stone crab (*Menippe adina* and *M. mercenaria*) as molecular techniques.

However, colour as a diagnostic character has some limitations. In addition to being useful only for identifying fresh crustacean specimens (Holthius, 1978), colour also tends to vary due to environmental factors including diet (Maguire, 1961). Ontogenic (Bert, 1985, 1986) and behavioural changes in colour (Hantson-Parker, 1985; Landau, 1992) within populations of crustacean taxa also have to be taken into account especially during periods of sexual maturity and breeding. Colour may also differ between species and indicate social dominance (Landau, 1992). Colour polymorphism may not be indicative of separate species but instead represent ecotypes of the same species, colour being dictated by the niche within the coastline that these species inhabit.

Morphological characters and in particular carapace and chelae spination are easy characters to examine. They are the only true diagnostic characters on preserved mud crab material, which includes museum specimens. In the case of *Scylla*, spinal architecture rather than meristics defines the four species. Frontal lobe teeth are the most reliable of the morphological characters in that they cannot be autotomised and are rarely worn down and broken, unlike chelae spination. The frontal lobe teeth are also fairly definitive and conservative within a species in terms of their morphology and are not affected by ontogenetic influences. With respect to other portunids, Stephenson and Campbell (1959) found frontal lobe teeth morphology to be the only diagnostic character able to discriminate between *Portunus trituberulatus* and *Portunus pelagicus*.

Unfortunately, the frontal lobe teeth examined in both fresh samples and museum specimens defines only two of the four species with any certainty, namely, *S. serrata* and *S. paramamosain*. Keenan *et al.* (1998) describe four diagnostic morphologies for frontal lobe teeth architecture, however, the variation they describe between *S. tranquebarica* and

S. olivacea is so subtle that it could not be described as diagnostic, especially to the untrained eye. Therefore additional morphological characters have to be used in order to identify between all four species, especially if dealing with preserved specimens.

Chelae spination proves to be more complex between species. Both ventral carpal spines and dorsal propodus spines can only be seen as diagnostic for *Scylla olivacea* where they appear vestigial and in some individuals absent, unlike the other three species. Hartnoll (1971) has linked the reduction of spines with the burrowing behaviour of some brachyuran species. This view is supported by the burrowing behaviour observed for *S. olivacea* (Estampador, 1949a).

Scylla paramamosain is unusual because in older specimens the anterior ventral carpal spine may appear vestigial or be absent. This variation in chelae spination makes it an unsuitable candidate for a diagnostic character although as additional characters in conjunction with other characters it proves to be useful.

Species discrimination of *Scylla* based on chelae spination has been criticised (Stephenson and Campbell, 1959; Brown, 1993). The number and architecture of the spines can vary with the size and age of the crabs sampled (Keenan *et al.*, 1998). Moreover, wearing can reduce the spines, especially when the crab has not moulted for some time (as is the case for the *Scylla serrata* specimen from the Zoological Museum, Copenhagen). Thus it is believed that only newly moulted adult crabs show a good indication of true spinal morphology. It is also true to say that spinal morphology only is useful in adult crabs as it is difficult to distinguish between the juveniles of the genus *Scylla*. This ontogenetic change in spinal morphology is also experienced between two species of spider crab (*Maja*

crispata and *M. squinado*) where dorsal carapace spines are only useful in distinguishing between the two species when the crabs are adult size (Neumann, 1995). The same author also notes that because these spinal characters are not distinguishable for juveniles that they may not be classed as true diagnostic characters.

There are other characters that have been used as part of the identification of new crab species that have not been discussed here, including the male pleopod (gonopod) structure (e.g Neumann, 1995; Stewart and Cook, 1998) and morphology of the mouthparts (Rodriguez and Suarez, 1994; Osawa, 1998). Male gonopod morphology is dealt with in chapter seven and will not be discussed further in this chapter. Crab mouthparts are a possible additional feature to be examined in *Scylla* species. However, the mud crab is primarily a scavenger and therefore unlikely to possess differences in feeding structures between species, unlike the genus *Uca* which have specialised adaptations to filter and select food particles which are unique to particular species inhabiting different parts of the intertidal zone (Crane, 1975).

In summary, two morphologies within the genus *Scylla* are easily distinguishable. These are *S. olivacea* and *S. serrata*. The other two morphologies are much harder to justify as species. In particular *S. tranquebarica* seems to share characters from the other morphologies, as would be expected from a hybrid between two species. *S. paramamosain* has only very recently been identified properly (Keenan *et al.*, 1998; Keenan, 1999a) and its species status has still to be accepted by the scientific community. However the “white” morph collected during this study most closely resembles the morphological description for *S. paramamosain*. The black morph is very easily identified as *S. olivacea*. Figure 3.9

summarises the species diagnoses based on the present study using the earliest nomenclature.

In the present day, it is strongly recognised that descriptive taxonomy is useful but it has its limitations. Descriptive taxonomy alone is seen as being purely subjective, causing confusion rather than clarifying a species status, as seen for the genus *Scylla*. When combined with quantitative and hereditary information however, descriptive taxonomy will provide supporting evidence that there is more than one species of *Scylla*.

***Scylla serrata* (Forskål, 1775)**

Elongated acute frontal lobe teeth with blunted tips and wide, U-shaped interspaces. All anterolateral spines are very sharp with U-shaped interspaces. Dorsal propodus of chelae has two sharp spines, directly behind joint with dactylus. The outer spine is slightly more pronounced than the inner one. The ventral carpus of the chelae has two sharp spines.

Olive green carapace. Some reticulation on posterior surface of carapace. Polygonal reticulation present on abdomen of mature females. Dorsal chelae and pereopods are olive green with dark green/brown reticulation. Ventral propodus of chelae are pale green and without reticulation.

***Scylla tranquebarica* (Fabricius, 1798)**

Anterolateral regions of carapace are more pronounced than in other *Scylla* species. Blunt, obtuse, semicircular frontal lobe teeth with shallow interspaces. Central space between frontal lobe teeth is deeper than other interdental spaces. Anterolateral spines adjacent to the orbital sockets are pronounced and blunt tipped. The ninth anterolateral spines are pronounced and needle-like in some individuals. There are two sharp spines on the dorsal propodus of the chelae. The inner spine is more pronounced than the outer one. The inner lateral propodus bears a small sharp spine. Two spines on the ventral carpus of chelae.

Olive green carapace. Green chelae with purple/olive hues on whole propodus and some faint reticulation. Olive green pereopods with dark green/brown reticulation. Mature female abdomen olive green with faint or no reticulation.

***Scylla olivacea* (Herbst, 1796)**

Carapace with a round appearance. Blunt, obtuse semicircular frontal lobe teeth that are all equal in size; with shallow interspaces. Short, obtuse anterolateral spines that are uniform in size and shape. Two vestigial spines present on the dorsal propodus of chelae. In some individuals the outer spine is absent or nodule-like. A pronounced nodule is present on the inner lateral propodus. Ranges of two vestigial nodule-like spines to a total absence of spines are found on the ventral carpus of the chelae.

Brown/dark green dorsal carapace. Dorsal carapace and pereopods range from dark brown to green with no reticulation present. The ventral propodus is orange red.

***Scylla paramamosain* (Estampador, 1949)**

Short frontal lobe teeth are triangular with angular interspaces. The central pair of frontal lobe teeth protrude slightly in some individuals. All anterolateral spines are uniform in morphology; compressed with small spaces between the spines. There are two sharp spines on the dorsal propodus with ridges following posteriorly from behind the spines. Two sharp spines are found on the ventral carpus although in some individuals the anterior of the two spines is vestigial or missing.

Pale green carapace. Dorsal chelae and pereopods are pale green with prominent dark green reticulation. Reticulation breaks up and forms brown dots on the outer upper half of the propodus. Lower half of outer lateral propodus is pale yellow with orange/red flashes on the tips of the propodus and dactylus.

Figure 3.9: Diagnoses for the four species morphologies of the genus *Scylla* based on examination of fresh specimens and museum specimens (Zoological Museum, University of Copenhagen).

Chapter Four

Multivariate morphometrics of mud crabs (genus *Scylla*) from Southeast Asia

4.1 Introduction

Descriptions continue to have an important place in crustacean taxonomy because crustacean species are still identified primarily by their morphological characters (Mclaughlin *et al.*, 1982). As shown in chapter three, the majority of the taxonomic revisions for the genus *Scylla* have been based on descriptive techniques (*e.g.* Forskål, 1775; Estampador, 1949a; Kathirval and Srinivasagam, 1992). However, descriptive taxonomy has some obvious shortcomings. Firstly, there is often an element of subjectivity with the descriptive terms used; for example, there are no universal definitions for spines, teeth or tubercles, yet these are structures referred to frequently in the descriptions of *Scylla* species. Secondly, descriptive terms such as “broad”, “long” or “wide” are not quantitative in any way, but are usually applied when two or more taxa are compared at one time (Mclaughlin *et al.*, 1982). Thirdly, unless all the taxa used are available for examination, it is difficult to follow the descriptions. The problem is accentuated when translations between languages lose the subtleties in description. Finally, descriptions may vary between authors and the specimens they have managed to obtain. In many cases, only a few individuals have been examined and in some cases only one sex has been described. Thus descriptive taxonomy often has not taken into consideration the phenotypic variation at the population level, potentially resulting in taxa being falsely identified.

The limitations of descriptive taxonomy have led biologists to adopt the use of a more quantitative approach in the use of morphometric characters as a basis for classification. Morphometrics involves the use of linear proportions measured from the organism's body structures to assess differences according to their overall shape. Morphometrics were used to identify brachyuran groups in the late nineteenth and early twentieth centuries (Barnes, 1968). Tesch's (1915) complete review of the genus *Macrophthalmus* Latreille, made extensive use of relative carapace and chela proportions to distinguish and define the species (cited by Barnes, 1968). Unfortunately, one of the major drawbacks of this technique was that many of these early authors assumed that these linear proportions remain constant with the growth and maturity of the animals. It is well accepted now that this is not the case; morphology is affected by ontogenic factors, one of which is sexual maturity (Thorpe, 1976). This type of variation may obscure any racial variation that may be present and thus must be accounted for. Subsequently, bivariate techniques, such as linear regression, have been used that take into account this ontogenic variation when describing the differences between taxonomic groups. Linear regressions have already been used to answer biological questions other than taxonomy, such as the condition of fished crustacean stocks, in addition to being used as an index of sexual maturity within some crustacean species, including shrimps (Farmer, 1986), lobsters (Miller and Mohn, 1993) and crabs (Haley, 1969; Melville-Smith, 1989; Pineiro and Fransozo, 1993).

Linear regression is a form of bivariate analysis, *i.e.* the analysis of two characters simultaneously. This can be used successfully to separate groups of taxa, but it is limited in its application by the fact in that only two characters are influencing the result. As each character will have its own pattern of geographic variation and/or relative discriminatory

power between taxonomic groups, by examining only two characters at a time, the total variation is under represented.

The limitation of using bivariate methods has led to the use of multivariate methods in elucidating species status. Multivariate analysis is the use of biometric data to analyse the variation in several characters simultaneously. This rapid and relatively inexpensive method can be employed to evaluate both racial and geographic variation within and between populations and /or taxa as it allows many types of characters to be analysed at any one time.

Preceding the revolution in biochemical techniques to analyse DNA from dried and preserved materials, numerical methods had the advantage over genetic techniques such as allozyme electrophoresis that preserved museum specimens as well as fresh material could be used, which was especially important in the study of rare or extinct species (Thorpe, 1976). Currently, multivariate analysis can provide useful additional morphological data that can be used in conjunction with molecular data as well as providing a starting point for further genetic investigations and for outlining those characters that can be used to discriminate between species.

Multivariate analysis has the advantage of being able to analyse all types of data including genetic and phenotypic data, which are both quantitative and qualitative in nature. There is a range of techniques that fall under the title of multivariate analysis, one group being ordination techniques. These include principal components analysis (PCA), principal coordinates analysis (PCDA) and canonical variate analysis (CVA) or discriminant function analysis (DFA). Ordination techniques plot several characters into a

multidimensional hyperspace and then find vectors that best describe the variation in a reduced number of axes. The first axis in any of the above techniques always expresses the greatest variation.

Canonical variate analysis is by far the most popular ordination technique (Thorpe, 1983). Although both PCA and CVA are comparable in their power to analyse data, there is a subtle difference in the approach used in each of these techniques. Principal components analysis aims to explain the variation present between and within populations, or individuals, in a reduced number of dimensions by considering the information obtained from several characters at once. The first two to three vectors represent the majority of variation although the total number of vectors (or principal components) is equal to the number of characters used (Quicke, 1993). Canonical variate analysis attempts to discriminate between predefined groups of individuals (also known as operational taxonomic units or OTUs) on the basis of the available characters by maximising the distance between means of distances between taxa (Quicke, 1993). The matrix of pairwise distance values is calculated using the Mahalanobis D^2 distance statistic. The number of vectors is equal to the number of groups minus one. Canonical variate analysis has been the preferred technique in the past due to its ability to take into account any information redundancy between characters as well as not requiring the data to be standardised beforehand (Thorpe, 1983).

The use of linear measurements in the taxonomy of the genus *Scylla* first appeared in Estampador's (1949a) study on *Scylla* collected in the Philippines. He based this study on measurements of carapace width, carapace length and circumference of the propodus.

Serene (1952), however, realised that these characters are unreliable on their own, as they

would be affected by growth-dependant size. Moreover, a mixture of male and female data would further confuse the apparent racial variation due to sexual dimorphism not being taken into account. Serene concluded that there were advantages in employing biometric characters in addition to the traditional descriptive techniques. Internal carapace width has featured as a prominent character in other taxonomic descriptions of different morphs of *Scylla* (Joel and Raj, 1983; Taylor, 1984; Oshiro, 1991).

Chayarat and Kaew-Ridh (1984) successfully used regression lines to differentiate between three colour morphs of *Scylla* collected from Chanthaburi, Thailand. Regression analysis required use of larger sample sizes of mud crabs to separate these morphs of *Scylla* rather than using only several individuals, as was the case in previous descriptive taxonomic studies.

Multivariate analysis of morphometrics was used by Overton *et al.* (1996) to separate groups of male mud crabs of the genus *Scylla* into three clusters on the basis of their external morphology. Keenan *et al.* (1998) used discriminate function analysis to discover which characters best discriminated between four genetically distinct groups of *Scylla*, which they thought to be different species based on allozymes. In Keenan *et al.* (1998) morphometric ratios were used to alleviate the effects of size. Although there have been arguments for and against the use of ratios to standardise data and alleviate the problems of growth-dependant size, *e.g.* Chambers *et al.* (1980), unless the two variables are linearly related, and this regression line passes through the origin, the reliability of this method is still questionable (Thorpe, 1987; Corti *et al.*, 1988). Overton *et al.* (1996) successfully used multiple group principal components analysis (MGPCA) to remove growth-

dependant size. MGPCA has the advantage that the range of growth within groups does not need to be similar or unbiased (Thorpe, 1987).

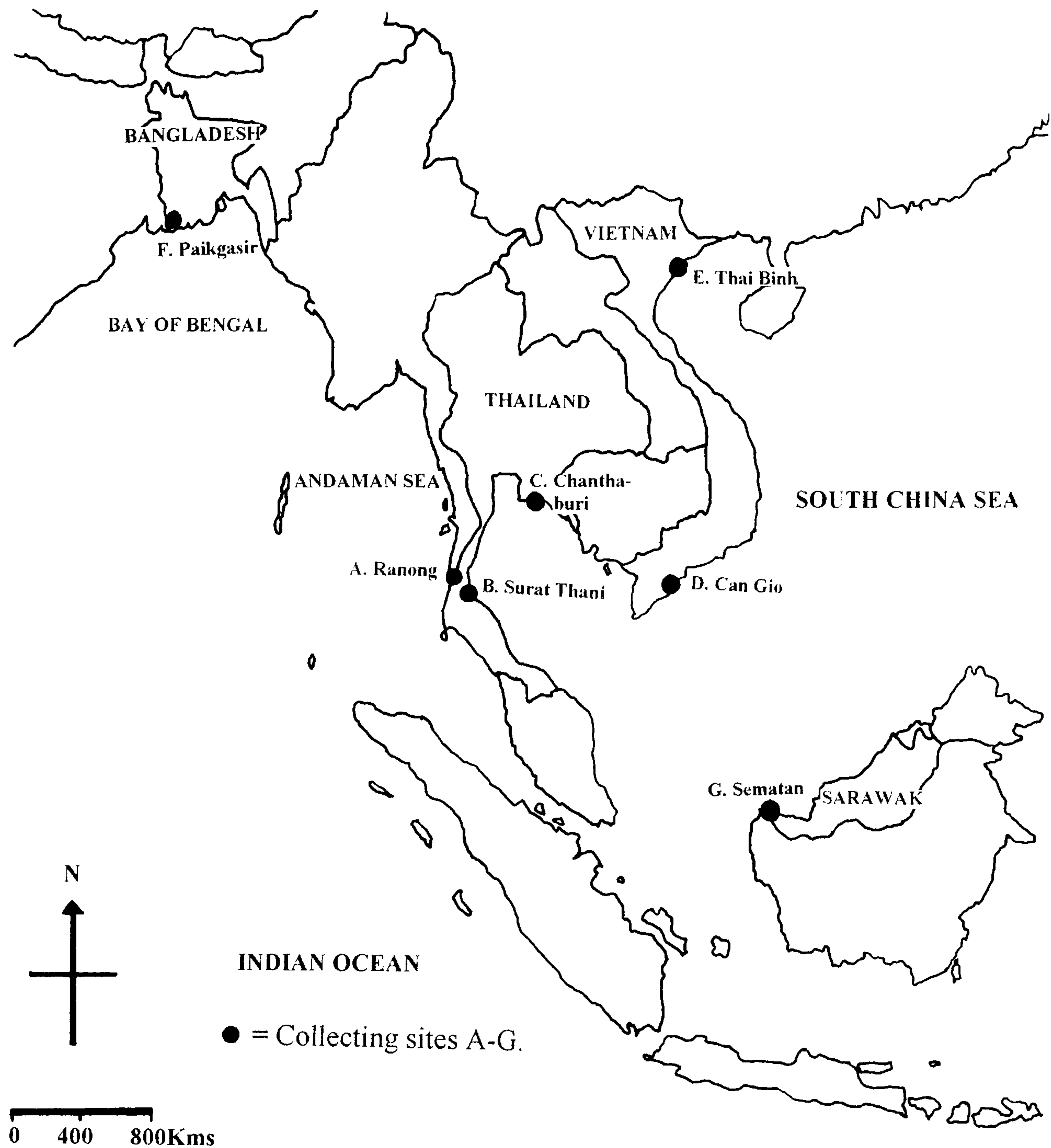
The aim of this part of the study was to discriminate between various morphs of *Scylla* collected from seven locations in Southeast Asia for both male and female mud crabs using discriminate function analysis. Multiple group principal components analysis was used to alleviate growth-dependant size variation. Discriminant function analysis was also used in an attempt to assign the two phenotypes collected in this study to mud crab specimens (fresh and preserved) believed to contain the four species of *Scylla* proposed by Keenan *et al.* (1998).

For each colour morph, the samples collected from each location represented a group in the analysis. By using multivariate methods, it was also the aim of this part of the study to see whether there is any pattern of geographic variation in the “shape” of the mud crabs collected between these study sites within Southeast Asia.

4.2 Materials and methods

4.2.1 Site selection

Adult crabs were collected from seven locations within Southeast Asia, namely (A) Klong Ngao, Ranong Province, southwest Thailand; (B) Ban Don Bay, Surat Thani Province, western Gulf of Thailand; (C) Chanthaburi Province, northeast Gulf of Thailand; (D) Can Gio Province, southern Vietnam; (E) Thuy Hai Commune, Thai Binh Province, northern Vietnam; (F) Paikgasir, Sundarbans, southern Bangladesh; and (G) Sematan, Sarawak State, East Malaysia. The geographical locations of these sites are illustrated in figure 4.1. Although technically Bangladesh is outside Southeast Asia, it was the next nearest site



Location	Distance to location (Kms)					
	Paikgasir	Ranong	Surat Thani	Chanthaburi	Can Gio	Thai Binh
Ranong	2280					
Surat Thani	4750	2470				
Chanthaburi	5500	3220	750			
Can Gio	6310	4030	1560	810		
Thai Binh	7810	5530	3060	2310	1500	
Sematan	4190 [†]	1910 [†]	1400*	1420*	960*	2170 [†]

* = Straight line distance from Sematan to location on Malay peninsula

† = Straight line distance from Sematan to nearest point on Malay peninsula plus distance along coastline to location.

Figure 4.1: Locations of collecting sites and distances between locations for multivariate analysis of mud crabs (genus *Scylla*) from Southeast Asia.

available northwest of the Andaman Sea, since Myanmar was not accessible for sample collection. Chapter two contains a more detailed description of each site.

4.2.2 Crab collection

Up to 30 male crabs, ranging from 90g to 360g fresh weight were collected from each site. Two colour morphs (black and white) were collected on the basis of their external morphology (as described in Chapter three). In Bangladesh, Thai Binh and Chanthaburi, females ranging from 67g to 491g fresh weight were also collected and measured. Within the main study site, Surat Thani, three collections were made of both males and females for the two morphs present. In other sites, where more than one morph occurred (Chanthaburi and Sematan), only the dominant morph was collected, the other morphs proving to be rare and difficult to obtain.

Care was taken to ensure that the crabs were of local origin by avoiding farms and secondary dealers who may also deal in crab from outside the immediate area. The crabs were caught principally using baited traps or gill nets. Of the crabs available, only hard-shelled crabs with all limbs intact were selected for measurement. Table 4.1 summarises the numbers of male and female crabs collected from each of the study locations.

Additional morphometric data were obtained from museum specimens of *Scylla* from the Fabricius collection, located in the Zoological Museum, University of Copenhagen and specimens from Sarawak, East Malaysia and Australia, which had been typed by Keenan *et al.* (1998) (the taxonomic features of these specimens are described in chapter three). A summary of these specimens is shown in table 4.2.

Table 4.1: Number of individual crabs (genus *Scylla*) obtained from seven locations in Southeast Asia for multivariate analysis of morphometric data.

Location	Black Number of individuals		Location	White Number of individuals	
	Male	Female		Male	Female
Paikgasir	29	21	Thai Binh	27	27
Sematan	25	nc [†]	Can Gio	30	nc
Ranong	26	nc	Chanthaburi	22	13
Surat Thani	S ₁ *	16	Surat Thani	S ₁	28
	S ₂	nc		S ₂	nc
	S ₃	28		S ₃	24
	S ₄	28		S ₄	21
Total	152	109	Total	152	121

* S₁ = male collection 1994; S₂ = female collection February 1996; S₃ = male and female collection August 1996; S₄ = male and female collection 1997.

† nc = no collection.

Table 4.2: Summary of specimens from the Zoological Museum, University of Copenhagen (ZMUC) and other specimens obtained from Sematan, East Malaysia, used for multivariate analysis for mud crabs (genus *Scylla*). These specimens have been typed is described in chapter three and by Keenan *et al.* (1998).

Typed species	Number of individuals					
	Male			Female		
	ZMUC	Sematan	Total	ZMUC	Sematan	Total
<i>Scylla serrata</i>	0	0	0	1	0	1
<i>Scylla olivacea</i>	1	3	4	0	0	0
<i>Scylla tranquebarica</i>	3	0	3	1	3	4
<i>Scylla paramamosain</i>	1	0	1	2	0	2

Unfortunately, due to bad packing, samples of the type *Scylla serrata* from Australia had all their limbs broken during transportation and were thus rendered useless for the morphometric part of this study.

4.2.3 Data collection

Twenty-seven physical characters were measured on each crab to nearest millimetre using digital callipers (Mitutoyo Absolute Digimatic, Tokyo, Japan). These measurements were taken from the carapace, both right and left chelae, and pereiopods from the right side of the crab. These characters are listed in table 4.3 and illustrated in figure 4.2. The fragility of the museum specimens reduced to number of characters that could be measured reliably from 27 to 22 characters (these are marked with an asterisk in table 4.3). As BMDP-7M does not allow for missing data, only 22 characters were used in the subsequent analysis involving the museum specimens.

4.2.4 Data analysis

The data were analysed using stepwise discriminant function analysis (also known as Canonical Variate Analysis) using the software programme BMDP-7M (BMDP Statistical Software Inc., Cork, Ireland). Although BMDP-7M is essentially a programme which carries out a stepwise discriminant analysis, a direct discrimination analysis could be carried out by forcing entry of all characters into the analysis at once and suppressing the stepwise approach except for the last step. Discriminant function analysis is one type of ordination technique that discriminates between two or more groups of data so that a minimum overlap occurs, resulting in maximum discrimination between groups. In order to perform discriminant function analysis, each individual must be pre-assigned to a group (known as operational groups or operational taxonomic units (OTUs)). In this study,

Table 4.3: Morphometric characters used in canonical variate analysis of the mud crabs (genus *Scylla*) from Southeast Asia.

Character	Abbreviation
A. Carapace	
1. Internal carapace length*	ICL
2. Internal carapace width*	ICW
3. Frontal lobe width*	FLW
4. Right antero-lateral carapace length*	RACL
5. Left antero-lateral carapace length	LACL
B. Cheliped	
6. Right dactylus length*	RDL
7. Left dactylus length*	LDL
8. Right dactylus width*	RDW
9. Left dactylus width*	LDW
10. Right propodus length*	RPL
11. Left propodus length*	LPL
12. Right propodus width*	RPW
13. Left propodus width*	LPW
14. Right carpus length*	RCL
15. Left carpus length*	LCL
16. Right carpus width*	RCW
17. Left carpus width*	LCW
18. Right merus length*	RML
19. Left merus length*	LML
20. Right merus width*	RMW
21. Left merus width*	LMW
C. Pereiopod	
22. Third pereiopod total length	3PTL
23. Third pereiopod merus length	3PML
24. Third pereiopod merus width	3PMW
25. Right paddle upper width*	5UPW
26. Right paddle lower width*	5LPW
27. Right paddle length	5PTL

* Measurements used for multivariate analysis of the museum/typed specimens.

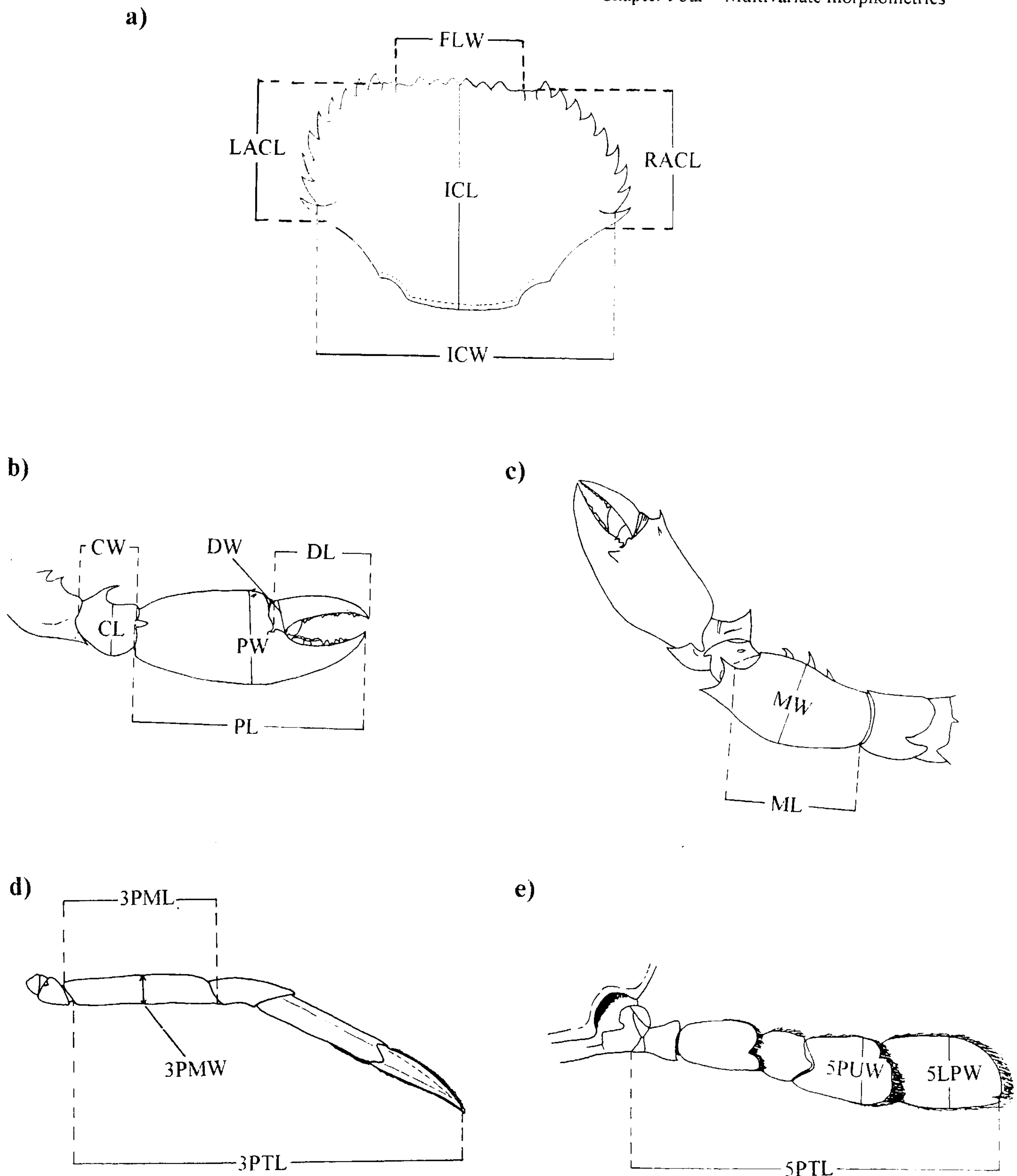


Figure 4.2: Illustration of linear characters providing data for multivariate analysis of mud crabs (genus *Scylla*). (a = carapace; b = anterior view of cheliped; c = posterior view of cheliped; d = third right pereiopod; e = fifth right pereiopod).

CL = carpus length; CW = carpus width; DL = dactyl length; DW = dactyl width; FLW = frontal lobe width; ICL = internal carapace length; ICW = internal carapace width; LACL = left antero-lateral carapace length; ML = merus length; MW = merus width; PL = propodus length; 3PML = third pereiopod merus length; 3PMW = third pereiopod merus width; 3PTL = third pereiopod total length; 5PTL = fifth pereiopod total length; PW = propodus width; 5PLW = paddle lower width; 5PUW = paddle upper width; RACL = right antero-lateral carapace length.

individuals were assigned to OTUs according to both location and the external morphological characteristics described in chapter three (frontal lobe spines, colouration and ventral carpus spines). In order to avoid the effects of variation due to sexual dimorphism, the data for male and female crabs were analysed separately (the effect of sexual dimorphism on the analysis when the data is analysed irrespective of sex is illustrated in appendix II).

Tables of means, ranges, standard deviations and coefficients of variation were used to summarise the initial differences between localities and morphs sampled according to characters measured. F-statistics computed from one-way multivariate analysis of variance indicated the contribution of each character for the overall discrimination between groups. At step 0, these F-values represent the between-group discrimination contributed by each character singly before information redundancy had been taken into consideration. The last step (equal to the number of characters) illustrates the true contribution of each character to the overall between-group discrimination after all other characters have been entered into the discriminant analysis and intra-locality correlation (information redundancy) between characters has been taken into account.

The Wilks lambda statistic was used to test whether mean population vectors were identical to the sample mean vectors for the different groups (Tabachnick and Fidell, 1983). The approximate F-ratio (a transformation of the Wilks lambda statistic) was used to test the significance of the overall between-group discrimination. The approximate F-ratio was compared with the F-distribution from statistical tables. Pairwise F statistics and associated Mahalanobis D^2 values illustrated the equality of group means and the relative "distance" between each pair of groups respectively. (Manly, 1994)

Two main colour morphs were identified in the locations sampled in this study. These were named black and white morphs due to their general appearance. Samples of mud crab with "black" morphology were dominant in four very widely dispersed locations encompassing both the Bay of Bengal and the South China Sea (see table 4.2). It was decided that it was unrealistic to draw a transect through these locations and test for patterns of geographical variation as the Thai/Malay peninsula lies in the middle of the transect and thus provides a significant geographical barrier between mud crabs from the South China Sea and those from the Bay of Bengal. The samples of the "white" colour morph, however, were collected in locations along the western seaboard of the South China Sea in a north to south transect, and were therefore suitable for a preliminary investigation on the patterns of geographic variation in *Scylla*. Pairwise values for Mahalanobis D^2 distances derived from discriminant function analysis were used to construct network diagrams between locations for male and female white morph mud crabs in order to illustrate the extent of change in morphology between these groups. In addition to the rate of change in morphology between locations, the scores from the first canonical variate were plotted against geographic distances between these locations in order to illustrate any patterns of geographic variation within the white morph.

In addition to discriminating between groups, BDMP-7M also was used to re-classify individual mud crabs. The combination of character values was used to produce a classification function for each group. Each individual was then entered into each function and subsequently reclassified into the group whose function results in the highest value from this process. This method can also be used for individuals whose group membership is unknown (Manly, 1994). Tables denoting the results of this reclassification process were produced to show the percentage of individuals that were classified correctly. A

jack-knifed classification was also applied to ensure that any bias as a result of the classification process itself was prevented (Lachenbruch and Mickey, 1968). Each individual was removed and the classification function recalculated prior to reclassification of that particular individual. From the reclassification process, large Mahalanobis D^2 distance values for individual mud crabs within their own group indicated that they were distant from their group mean. This can be due to errors in the data input, incorrect classification, or is an indication of outliers from the group. These aberrations of the output were located, assessed and where appropriate, removed from the data set and the programme re-run.

A table of eigenvalues was produced to illustrate the degree of between-group discrimination for each canonical variate (the total number of canonical variates in this instance was equal to the number of groups minus one), along with the cumulative percentage of total dispersion. In most of the analyses, the first two canonical variables accounted for the majority of between-group discrimination. Therefore a scatter plot of these two canonical variables was used to illustrate the relative position of these groups with respect to each other. As there were many data points in the analyses, only the group centroids (overall group means) were employed to summarise the relationships between the groups analysed.

In some circumstances, the differences in size range between groups of crabs may bias the results from discriminant function analysis (Corti *et al.*, 1988). Multiple groups principal component analysis (Thorpe, 1988) was used to reveal any potential affect of growth-dependent size on the linear measurements taken (indicated by similar weightings and signs for all growth-dependent variables). Re-entering the data in the form of normalised

vectors (component scores) into the discriminant function analysis resulted in the same ordination pattern as for raw data. The contribution of each normalised vector was expressed by its F-values. If the size vector showed a larger F-value than the other vectors, then growth dependent size was having an effect (Malhotra and Thorpe, 1997). By removing this vector and using the remainder for a "size-out" CVA analysis, the results were subsequently assessed free of growth effects.

The classification procedure mentioned previously to reclassify individuals within each group of crabs could also be used to allocate unknown individual crabs to one of the pre-defined crab groups. This method was used to classify the known museum specimens into the crab groups that they each most closely resembled. Thus each of the clusters described in the former part of this morphometrics study was associated with one or more of the four species of the genus *Scylla* proposed. Classification, ordination and F-values for those characters contributing to these associations show the relative similarity between crab groups and the type specimens.

4.3 Results

4.3.1 Descriptive statistics

Tables 4.4 to 4.7 summarise the statistical means, ranges, standard deviations (SD) and coefficients of variation (CV) for all the morphometric characters measured on "black" morph and "white" morph male and female mud crabs. Internal carapace width (ICW) represents the size variable for all groups of crabs sampled. Size ranges incurred within each group were comparable, with a large degree of overlap between groups for both male and female data and for both morphs. In both morphs, females were slightly larger than males (black morph: mean ICW=102mm for female crabs compared with ICW = 98mm

Table 4.4: Mean (mm), range (mm), standard deviation (SD) and coefficient of variation (CV) for 27 characters measured on groups of male mud crabs (genus *Scylla*) from Southeast Asia exhibiting "black" morphology. Abbreviations for morphometric characters are explained in Table 4.3 and Figure 4.2.

GROUP	MORPHOMETRIC CHARACTER																											
	FLW	ICL	ICW	LACL	LCL	LCW	LDL	LDW	LML	LMW	LPL	LPW	SPLW	3PML	3PMW	3PTL	5PTL	5PUW	RACL	RCL	RCW	RDL	RDW	RML	RMW	RPL	RPW	
Paikgasir N=28	mean	31.76	67.27	95.89	44.02	27.57	19.61	33.80	40.46	22.39	63.22	27.60	15.08	37.51	11.36	112.57	76.84	15.12	44.30	27.19	19.48	33.29	9.40	39.98	22.09	62.11	27.52	
	min	26.42	57.03	81.06	36.53	21.99	15.44	28.12	33.53	18.05	52.23	20.66	12.37	27.19	10.00	93.69	65.15	12.52	36.78	22.52	14.96	27.13	7.38	33.65	18.45	51.25	21.52	
	max	37.24	74.72	105.01	48.69	31.19	22.55	40.17	45.48	45.48	25.14	72.15	33.36	17.38	44.44	12.50	126.12	85.15	16.96	48.90	31.02	22.20	39.48	11.30	45.30	24.80	72.69	32.90
	SD	±2.31	±4.59	±6.27	±3.13	±2.36	±1.83	±3.25	±1.08	±3.04	±1.63	±5.36	±3.16	±1.23	±3.99	±0.72	±9.41	±5.43	±1.16	±3.19	±2.34	±1.84	±3.40	±0.95	±3.05	±1.78	±5.65	±2.71
	CV	0.071	0.068	0.065	0.071	0.086	0.094	0.096	0.115	0.075	0.073	0.085	0.115	0.081	0.106	0.063	0.084	0.071	0.077	0.072	0.086	0.094	0.102	0.101	0.076	0.081	0.091	0.098
Sematan N=25	mean	33.20	67.87	97.29	44.95	29.08	20.31	35.69	42.69	23.48	66.32	29.78	15.07	41.57	11.31	119.95	80.99	15.18	45.15	29.69	20.93	36.38	11.07	42.90	23.75	68.11	31.70	
	min	28.14	61.43	82.48	37.69	22.59	15.61	27.94	34.64	19.56	51.53	20.33	12.17	33.93	9.63	99.50	67.41	12.59	38.10	22.80	16.64	28.72	7.68	34.51	19.18	53.16	22.45	
	max	37.70	80.60	114.38	48.88	38.52	26.45	46.37	55.86	29.99	85.92	43.62	18.28	52.00	13.63	154.90	100.68	18.73	54.10	40.04	28.62	48.51	16.81	55.88	28.58	92.17	49.12	
	SD	±2.46	±5.79	±8.32	±4.03	±4.52	±3.18	±5.67	±2.19	±5.73	±2.83	±10.71	±7.07	±1.52	±4.22	±0.83	±12.86	±7.81	±1.57	±4.06	±4.33	±3.05	±5.44	±2.28	±5.58	±2.93	±10.38	±6.59
	CV	0.074	0.085	0.085	0.090	0.156	0.157	0.159	0.217	0.134	0.120	0.161	0.238	0.101	0.102	0.073	0.107	0.096	0.104	0.090	0.146	0.146	0.150	0.206	0.123	0.152	0.208	
Ranong N=26	mean	33.22	67.50	95.76	44.46	29.09	19.90	36.44	42.42	23.86	67.01	30.61	14.98	40.59	11.35	117.57	80.17	15.36	44.79	30.15	21.61	36.57	12.45	42.97	24.34	69.89	34.72	
	min	29.46	61.86	87.01	40.44	24.18	15.83	28.71	33.87	19.27	52.54	22.73	13.40	34.64	9.11	99.96	69.97	12.06	40.32	27.03	17.53	28.12	8.08	37.20	20.39	50.90	24.52	
	max	37.61	74.69	104.91	48.51	34.23	24.31	43.44	49.38	27.94	79.89	40.98	17.52	46.51	12.48	133.82	88.09	17.80	49.38	35.43	25.09	40.17	18.25	49.43	28.74	84.50	46.24	
	SD	±1.96	±3.79	±5.53	±2.57	±3.140	±2.18	±4.07	±1.48	±4.13	±2.29	±7.66	±5.07	±1.35	±0.77	±9.33	±5.84	±1.30	±2.71	±3.07	±2.12	±3.55	±2.32	±3.68	±2.32	±7.92	±6.01	
	CV	0.059	0.056	0.058	0.058	0.108	0.110	0.111	0.143	0.097	0.096	0.114	0.166	0.090	0.068	0.079	0.073	0.085	0.060	0.060	0.102	0.098	0.097	0.186	0.095	0.113	0.173	
S.T.:S ₁ N=16	mean	31.60	67.06	95.65	43.92	26.64	18.45	32.95	38.66	22.20	61.12	26.43	14.64	39.93	11.17	116.17	77.35	14.98	43.82	26.21	18.63	32.18	9.64	38.53	21.64	61.49	27.50	
	min	28.40	63.00	88.93	40.02	22.43	15.41	28.83	33.60	18.17	52.33	21.30	12.91	35.95	10.24	105.96	70.81	13.29	40.48	22.43	16.56	28.24	7.56	35.39	18.33	54.85	22.88	
	max	34.56	72.37	104.96	49.72	29.82	20.62	35.86	41.13	43.08	25.31	67.43	29.33	16.43	12.21	133.16	83.55	16.68	49.72	32.66	22.16	36.69	12.95	43.06	23.93	70.71	35.91	
	SD	±1.88	±3.28	±5.02	±2.88	±2.32	±1.52	±2.36	±1.06	±2.64	±1.83	±4.26	±2.68	±1.03	±0.51	±7.41	±4.05	±0.96	±2.48	±2.58	±1.70	±2.68	±1.62	±2.45	±1.45	±4.74	±4.19	
	CV	0.059	0.052	0.049	0.066	0.087	0.082	0.072	0.113	0.068	0.082	0.070	0.101	0.070	0.046	0.064	0.052	0.064	0.057	0.098	0.091	0.083	0.168	0.063	0.067	0.077	0.152	
S.T.:S ₂ N=28	mean	34.27	71.82	102.24	47.00	31.29	21.97	39.18	45.68	24.92	72.59	33.64	16.28	43.53	11.88	127.54	84.13	16.57	47.67	32.07	22.56	38.68	12.30	46.13	25.14	73.73	35.89	
	min	30.26	63.98	93.20	41.92	27.18	18.63	32.34	40.05	21.40	60.58	26.34	13.76	38.16	10.46	113.56	75.72	14.90	42.33	27.12	19.06	31.79	9.01	42.24	22.09	59.94	26.59	
	max	39.65	81.55	118.47	55.09	38.91	25.44	48.70	54.61	29.86	89.43	43.37	19.15	50.26	14.35	145.12	98.81	19.38	55.79	38.40	26.69	46.77	17.60	54.08	29.23	90.97	48.39	
	SD	±1.96	±4.35	±6.51	±3.14	±2.84	±2.16	±4.06	±1.68	±3.46	±1.97	±1.32	±3.28	±0.83	±4.50	±8.95	±6.03	±1.26	±3.07	±2.82	±2.05	±4.20	±2.00	±3.67	±1.95	±7.45	±5.31	
	CV	0.057	0.064	0.061	0.067	0.091	0.098	0.104	0.148	0.076	0.079	0.100	0.134	0.081	0.070	0.070	0.072	0.076	0.064	0.088	0.091	0.108	0.162	0.079	0.078	0.101	0.148	
S.T.:S ₃ N=28	mean	32.59	71.00	100.91	46.41	31.14	21.60	36.76	44.93	24.05	70.52	32.54	16.11	42.68	11.56	120.12	80.49	16.19	46.54	32.14	22.70	37.81	12.28	45.17	24.60	73.61	35.71	
	min	29.39	66.60	92.72	39.29	27.07	19.23	32.00	39.39	21.69	56.41	27.27	13.44	35.44	9.99	99.66	73.54	14.29	42.11	28.51	26.06	30.81	8.90	40.23	21.92	62.27	27.21	
	max	40.63	85.20	118.45	55.26	36.71	26.15	44.28	55.54	27.89	80.91	39.49	20.79	49.39	13.65	137.75	93.00	20.75	56.22	38.58	19.95	43.53	16.40	53.37	28.72	85.50	44.07	
	SD	±2.59	±4.33	±6.15	±3.38	±2.32	±1.71	±3.39	±1.04	±3.71	±1.61	±5.02	±3.29	±1.50	±3.71	±0.72	±8.61	±6.08	±1.51	±3.33	±2.49	±1.97	±3.00	±1.53	±3.30	±1.70	±5.84	±3.98
	CV	0.080	0.061	0.061	0.073	0.074	0.079	0.092	0.097	0.083	0.067	0.071	0.101	0.093	0.062	0.072	0.076	0.093	0.072	0.072	0.087	0.087	0.079	0.125	0.069	0.079	0.111	

S.T. = Surat Thani

Table 4.5: Mean (mm), range (mm), standard deviation (SD) and coefficient of variation (CV) for 27 characters measured on groups of male mud crabs (genus *Scylla*) from Southeast Asia exhibiting “white” morphology. Abbreviations for morphometric characters are explained in Table 4.3 and Figure 4.2.

GROUP	MORPHOMETRIC CHARACTER																										
	FLW	ICL	ICW	LACL	LCL	LCW	LDL	LDW	LML	LMW	LPL	LPW	SPLW	3PML	3PMW	3PTL	SPTL	SPUW	RACL	RCL	RCW	RDL	RDW	RML	RMW	RPL	RPW
Thai Binh																											
N=27	26.59	60.44	85.83	39.97	24.19	17.17	30.25	9.42	35.30	19.01	56.47	26.47	13.86	33.93	10.22	98.75	70.20	14.08	39.96	23.99	17.37	30.00	9.87	34.98	18.75	56.84	27.56
mean	23.39	51.82	73.24	30.71	15.90	13.95	24.80	7.35	29.15	16.27	41.31	20.21	11.15	26.45	8.90	80.77	58.82	11.21	33.33	16.09	14.03	24.26	7.01	28.53	15.02	44.65	19.52
min	31.00	70.35	101.14	47.48	29.66	20.89	37.17	12.13	43.24	22.76	70.28	34.01	16.88	39.76	12.04	116.08	82.94	17.32	47.47	29.66	21.89	39.19	12.93	43.99	22.67	68.27	38.29
max	±1.78	±4.49	±6.77	±3.37	±2.43	±1.87	±3.17	±1.37	±3.59	±1.60	±7.19	±3.85	±1.41	±3.26	±0.70	±8.97	±6.07	±1.47	±3.19	±2.65	±1.96	±3.48	±1.53	±4.00	±2.04	±7.20	±4.74
SD	0.067	0.074	0.079	0.084	0.101	0.109	0.105	0.145	0.102	0.084	0.127	0.145	0.102	0.096	0.068	0.091	0.086	0.104	0.080	0.111	0.113	0.116	0.155	0.114	0.109	0.127	0.172
CV	28.87	64.96	93.32	43.67	25.18	17.11	31.76	9.19	37.76	20.74	58.94	25.79	14.51	38.02	10.93	108.06	76.17	14.40	43.65	25.70	18.24	31.70	10.03	37.48	20.58	60.65	28.64
mean	25.10	57.71	82.18	38.48	14.61	14.61	26.71	7.64	33.29	18.04	50.81	21.80	12.60	33.61	9.70	92.27	67.68	12.60	38.74	21.18	15.19	27.03	7.47	32.48	18.04	50.77	21.03
min	33.29	70.89	102.47	48.09	28.44	19.48	35.77	12.07	42.70	23.20	67.08	32.44	16.68	43.32	12.41	123.39	85.15	16.71	48.41	28.65	20.82	36.92	12.05	41.63	23.27	70.67	34.05
max	±1.96	±4.28	±6.27	±3.04	±1.99	±1.43	±2.82	±1.01	±2.79	±1.33	±4.38	±2.47	±1.04	±2.82	±0.68	±8.86	±5.37	±1.07	±3.00	±2.05	±1.48	±2.85	±1.27	±2.80	±1.55	±5.53	±3.25
SD	0.068	0.066	0.067	0.070	0.079	0.084	0.089	0.110	0.074	0.064	0.084	0.096	0.072	0.074	0.062	0.082	0.070	0.074	0.069	0.080	0.081	0.090	0.127	0.075	0.075	0.091	0.113
CV	31.45	70.54	102.27	47.12	28.73	21.18	34.91	10.98	42.26	22.50	68.87	30.96	15.66	40.85	11.62	119.45	81.60	16.16	46.94	29.22	21.57	35.42	11.21	42.45	22.79	69.43	32.30
mean	29.52	63.11	93.31	42.93	24.46	18.40	28.37	8.18	36.18	19.46	56.89	25.48	13.26	34.00	10.34	104.64	70.96	13.66	42.91	25.24	18.59	31.52	8.88	36.18	20.14	60.31	26.80
min	35.42	82.96	119.23	55.44	33.25	24.68	42.00	13.71	50.09	26.69	79.62	36.56	20.29	48.25	13.66	140.73	100.02	20.18	55.51	34.77	25.48	44.13	14.70	48.45	27.91	81.96	41.97
max	±1.53	±4.48	±6.48	±3.23	±2.33	±1.69	±3.46	±1.41	±3.77	±1.85	±6.50	±3.69	±1.54	±3.79	±0.93	±9.47	±6.40	±1.47	±3.11	±2.52	±1.85	±3.57	±1.56	±3.03	±1.73	±5.83	±4.05
SD	0.049	0.063	0.063	0.069	0.081	0.080	0.099	0.128	0.089	0.082	0.094	0.119	0.098	0.093	0.080	0.079	0.078	0.091	0.066	0.086	0.086	0.101	0.140	0.071	0.076	0.084	0.125
CV	29.23	66.78	96.90	45.40	26.07	17.42	32.45	9.25	38.22	21.19	60.81	26.57	15.18	38.82	10.99	111.36	78.54	15.32	45.08	26.56	18.52	32.70	10.25	38.54	21.41	62.16	28.72
mean	25.71	62.36	90.67	42.21	22.58	15.84	24.16	7.61	35.13	19.08	54.41	23.48	12.02	35.88	9.73	101.02	72.23	13.14	41.42	23.11	16.28	29.25	8.73	34.11	16.19	55.09	24.93
min	31.34	72.64	103.05	50.31	29.58	19.80	36.26	12.39	42.64	23.74	67.40	34.03	16.80	43.53	12.02	122.17	85.41	17.40	49.00	29.60	21.66	34.89	13.44	43.64	23.09	67.88	33.12
max	±1.45	±2.88	±4.20	±2.07	±1.55	±1.05	±2.38	±0.99	±2.06	±1.23	±3.56	±2.46	±0.91	±1.83	±0.53	±5.37	±4.39	±1.01	±2.05	±1.46	±1.40	±1.61	±0.98	±2.52	±1.25	±3.34	±2.12
SD	0.050	0.043	0.043	0.046	0.060	0.060	0.073	0.108	0.054	0.058	0.059	0.093	0.060	0.047	0.048	0.048	0.056	0.066	0.045	0.055	0.075	0.049	0.095	0.065	0.058	0.054	0.074
CV	33.87	74.89	108.11	49.82	29.88	21.70	36.97	11.42	44.00	23.65	69.29	31.99	16.95	43.56	12.51	126.80	87.11	17.13	50.28	30.19	21.72	37.29	12.25	44.34	23.94	70.67	33.44
mean	29.65	66.00	93.11	42.56	25.39	18.61	30.31	8.92	37.87	21.20	56.86	24.79	13.98	36.06	10.52	104.49	72.92	14.27	43.00	25.38	19.21	30.83	9.87	38.64	21.19	57.29	25.95
min	37.52	83.08	120.25	55.41	34.99	26.68	45.59	16.21	51.02	27.94	84.22	42.16	20.10	49.78	14.38	143.49	100.13	20.62	55.93	34.53	25.71	43.02	16.19	49.96	27.13	80.69	43.53
max	±1.93	±4.18	±6.67	±3.02	±2.53	±1.94	±4.90	±1.69	±3.59	±1.94	±6.91	±3.93	±1.18	±3.54	±1.09	±9.17	±6.19	±1.26	±3.36	±2.46	±1.79	±3.11	±1.52	±3.12	±1.62	±5.83	±3.73
SD	0.057	0.056	0.062	0.061	0.085	0.089	0.133	0.148	0.082	0.082	0.100	0.123	0.070	0.081	0.087	0.072	0.071	0.074	0.067	0.082	0.082	0.083	0.124	0.070	0.068	0.082	0.112
CV	33.97	77.16	110.04	50.89	32.70	22.84	40.04	12.48	46.93	24.51	76.59	35.82	17.58	45.77	12.49	124.90	87.46	17.83	51.15	32.97	22.96	39.07	12.94	46.74	24.82	76.39	36.85
mean	28.43	64.98	80.50	42.49	26.93	19.13	32.77	9.23	40.75	20.08	63.29	27.17	13.86	38.52	10.07	156.29	71.29	14.07	41.95	27.93	18.91	32.77	9.92	38.75	20.71	62.51	29.08
min	38.90	91.89	131.04	58.20	41.34	28.78	52.40	16.81	62.29	30.25	109.44	51.13	18.78	59.51	14.57	85.58	104.64	22.49	61.77	42.27	29.63	52.40	16.79	59.71	33.85	111.04	51.79
max	±2.95	±6.71	±9.18	±4.67	±3.42	±2.45	±4.64	±1.87	±5.22	±2.51	±9.70	±5.67	±2.03	±5.41	±1.27	±16.55	±8.47	±1.91	±4.82	±3.70	±2.92	±4.50	±1.93	±5.21	±2.74	±10.26	±6.04
SD	0.087	0.087	0.083	0.092	0.105	0.107	0.116	0.150	0.111	0.103	0.127	0.158	0.115	0.118	0.102	0.133	0.097	0.107	0.094	0.112	0.127	0.115	0.149	0.111	0.110	0.134	0.164
CV																											

S.T. = Surat Thani; C'buri = Chanthaburi

Table 4.6: Mean (mm), range (mm), standard deviation (SD) and coefficient of variation (CV) for 27 characters measured on groups of female mud crabs (genus *Scylla*) from Southeast Asia exhibiting “black” morphology. Abbreviations for morphometric characters are explained in Table 4.3 and Figure 4.2.

GROUP	MORPHOMETRIC CHARACTER																										
	FLW	ICL	ICW	LACL	LCL	LCW	LDL	LDW	LML	LMW	LPL	LPW	SPLW	3PML	3PMW	3PTL	5PTL	SFUW	RACL	RCL	RCW	RDL	RDW	RML	RMW	RPL	RPW
Paikgasir																											
N=21 mean	30.88	67.11	94.96	43.46	22.46	15.69	29.41	7.42	34.33	18.58	53.62	21.77	15.68	33.23	9.76	100.69	72.85	15.12	43.50	22.61	15.70	29.50	7.80	34.24	18.43	53.79	22.37
min	27.22	57.43	80.93	36.49	19.36	13.00	23.72	5.99	29.47	15.81	44.80	17.06	12.56	27.30	9.06	82.30	60.12	12.86	36.58	19.05	12.45	24.59	6.25	28.81	15.33	45.09	17.06
max	36.61	79.81	117.38	54.28	26.33	18.74	31.50	9.11	39.75	21.51	62.66	26.56	18.04	37.75	11.42	113.83	84.11	17.79	55.49	26.52	18.63	35.63	9.20	40.34	21.51	63.75	26.56
SD	2.50	6.38	9.87	4.71	1.90	1.57	2.96	0.76	3.10	1.52	4.89	2.33	1.70	2.37	0.64	7.99	7.09	1.58	4.91	2.18	1.73	3.38	0.87	3.62	1.80	5.76	2.53
CV	0.081	0.095	0.104	0.108	0.085	0.100	0.101	0.102	0.090	0.082	0.091	0.107	0.108	0.071	0.065	0.079	0.097	0.104	0.113	0.096	0.110	0.115	0.111	0.106	0.098	0.107	0.113
S.T :S₁																											
N=28 mean	33.04	71.37	101.26	46.21	25.01	17.43	32.73	8.51	37.70	20.54	58.45	23.73	16.28	37.87	10.87	113.43	79.00	16.01	46.68	25.23	18.05	32.32	9.09	37.52	20.55	59.27	25.73
min	28.98	58.39	86.26	33.79	20.30	15.32	27.84	7.22	32.81	15.28	49.61	18.83	12.46	34.08	9.38	98.99	65.72	12.27	38.59	21.04	15.81	26.56	6.57	32.10	16.29	48.18	18.92
max	47.59	84.64	118.41	53.84	29.41	20.24	39.98	10.13	43.61	25.16	69.71	27.49	20.09	43.47	12.81	126.10	96.05	19.37	54.18	29.75	21.28	38.96	11.54	42.92	23.76	70.81	31.54
SD	2.70	6.94	9.50	4.58	1.89	1.27	3.10	0.76	2.80	1.66	4.82	2.01	2.06	2.53	0.83	8.23	7.72	1.85	4.53	2.18	1.78	3.14	1.21	2.96	1.80	5.85	3.34
CV	0.082	0.097	0.094	0.099	0.076	0.073	0.095	0.089	0.074	0.081	0.082	0.085	0.127	0.067	0.076	0.073	0.098	0.116	0.097	0.086	0.098	0.097	0.133	0.079	0.088	0.099	0.130
S.T :S₂																											
N=30 mean	33.50	77.32	109.38	51.00	26.48	18.53	34.72	9.10	40.50	22.35	62.42	25.18	18.67	39.60	11.27	117.42	84.63	17.58	51.21	27.03	19.56	34.50	10.33	40.08	22.16	63.63	28.00
min	30.86	69.27	96.67	45.17	23.10	16.54	29.61	6.88	35.85	18.07	55.70	21.41	15.94	35.31	10.05	106.89	76.62	15.18	45.71	23.57	17.04	29.82	8.30	35.07	19.43	56.36	23.13
max	38.21	90.49	130.32	59.72	32.02	21.72	42.59	10.94	49.74	25.32	75.18	30.23	21.62	46.74	13.35	139.55	102.49	22.04	60.63	32.64	23.82	43.46	12.22	48.87	25.43	79.86	34.70
SD	1.94	5.39	8.12	3.70	2.20	1.37	3.25	1.06	3.11	1.84	5.34	2.62	1.85	2.78	0.84	7.03	6.34	1.57	3.81	2.17	1.76	3.09	1.39	3.12	1.49	6.02	3.05
CV	0.06	0.070	0.074	0.073	0.083	0.074	0.094	0.116	0.077	0.083	0.086	0.104	0.099	0.070	0.075	0.060	0.075	0.089	0.074	0.080	0.090	0.090	0.135	0.078	0.067	0.095	0.109
S.T :S₃																											
N=30 mean	32.38	72.45	102.64	47.04	25.11	17.33	31.51	7.95	36.93	20.29	57.36	23.19	17.08	36.92	10.09	107.08	77.90	16.24	47.45	25.55	17.78	31.89	8.39	37.13	20.21	58.50	24.35
min	28.38	66.03	93.52	41.55	22.45	14.97	25.38	6.39	33.52	16.62	51.49	19.81	15.00	30.96	8.20	90.68	70.00	14.17	42.75	22.73	15.42	27.86	7.18	34.23	17.74	54.25	21.57
max	35.92	80.73	115.01	51.95	28.68	19.96	36.17	9.29	42.53	22.82	66.66	26.41	19.74	42.55	11.22	117.74	88.65	18.04	53.17	28.47	20.07	37.49	10.35	42.86	22.28	67.83	28.48
SD	2.03	4.11	5.24	2.62	1.52	1.27	2.33	0.86	1.88	1.31	3.58	1.87	1.04	2.53	0.72	7.00	3.98	1.11	2.60	1.44	1.30	2.20	0.82	2.27	1.17	3.21	1.89
CV	0.063	0.057	0.051	0.056	0.061	0.073	0.074	0.108	0.051	0.064	0.062	0.081	0.061	0.068	0.071	0.065	0.051	0.068	0.055	0.056	0.073	0.069	0.097	0.061	0.058	0.055	0.078

S.T. = Surat Thani

Table 4.7: Mean (mm), range (mm), standard deviation (SD) and coefficient of variation (CV) for 27 characters measured on groups of female mud crabs (genus *Scylla*) from Southeast Asia exhibiting "white" morphology. Abbreviations for morphometric characters are explained in Table 4.3 and Figure 4.2.

GROUP	MORPHOMETRIC CHARACTER																											
	FLW	ICL	ICW	LACL	LCL	LCW	LDL	LDW	LML	LMW	LPL	LPW	SPLW	3PML	3PMW	3PIL	SPTL	SPUW	RACL	RCL	RCW	RDL	RDW	RML	RMW	RPL	RPW	
C'burri N=27	mean	82.33	117.89	54.45	27.84	21.00	34.98	10.28	40.83	22.79	66.31	29.25	18.73	40.65	11.86	123.51	87.88	18.61	54.48	28.06	20.97	34.95	11.21	41.33	22.75	67.10	31.19	
	min	31.18	73.24	103.73	46.57	17.43	18.39	30.71	8.70	35.55	20.28	25.31	15.61	35.60	10.42	103.68	80.83	15.73	43.06	25.99	19.25	31.42	9.24	35.90	19.81	61.76	26.51	
	max	41.02	94.33	135.88	63.49	33.12	24.09	39.09	12.86	48.14	25.53	75.49	34.24	23.84	46.48	13.73	145.29	106.52	22.84	63.31	34.32	29.26	39.53	15.16	51.09	27.10	76.56	39.32
	SD	2.39	5.23	8.26	4.14	2.10	1.89	2.36	1.20	3.15	1.36	4.53	2.56	2.09	3.31	0.79	10.43	7.27	1.86	4.02	2.22	1.79	2.27	1.94	3.58	1.78	4.21	3.21
	CV	0.068	0.063	0.070	0.076	0.075	0.090	0.067	0.116	0.077	0.060	0.068	0.087	0.111	0.081	0.067	0.084	0.083	0.100	0.074	0.079	0.085	0.065	0.173	0.087	0.078	0.063	0.103
Thai Binh N=13	mean	28.10	65.26	91.85	42.77	21.84	28.96	8.20	32.78	18.06	52.13	23.49	15.08	31.98	9.72	96.83	72.40	14.81	42.75	22.38	16.52	28.42	8.92	33.08	18.41	53.48	25.49	
	min	24.80	57.35	80.16	36.87	19.41	14.34	25.92	6.85	29.00	15.13	45.86	19.47	12.22	27.91	8.20	85.32	64.55	12.76	36.76	20.15	13.95	20.69	7.10	29.01	16.54	46.48	21.43
	max	31.59	71.98	101.13	47.31	24.15	18.73	33.93	10.64	36.47	20.30	59.75	29.49	17.18	35.41	10.80	107.05	80.81	17.27	47.26	24.62	18.27	32.31	10.71	38.00	20.99	60.26	29.16
	SD	1.63	4.20	6.14	2.83	1.23	1.17	2.19	0.91	2.02	1.21	3.67	2.46	1.26	1.65	0.65	5.44	4.35	1.16	3.03	1.27	1.26	2.96	1.05	2.01	1.13	3.40	2.56
	CV	0.058	0.064	0.067	0.066	0.056	0.072	0.076	0.111	0.062	0.067	0.070	0.105	0.084	0.051	0.067	0.056	0.060	0.078	0.071	0.057	0.076	0.104	0.118	0.061	0.061	0.064	0.101
S.T.:S ₁ N=29	mean	33.83	78.33	111.90	51.90	26.50	33.87	10.02	39.80	22.48	62.19	27.49	17.87	38.96	11.72	117.36	85.55	17.67	52.18	27.23	19.83	33.69	10.55	40.14	22.55	63.80	29.72	
	min	22.07	49.24	72.26	32.38	18.11	13.33	23.17	6.41	27.25	14.87	42.09	17.46	11.44	8.37	80.87	55.88	10.92	33.28	17.99	13.91	22.45	7.03	25.92	14.92	53.99	22.95	
	max	41.12	96.81	135.77	64.30	32.51	24.79	42.13	14.09	49.29	28.47	76.93	38.22	22.07	47.47	14.60	141.60	105.75	14.32	64.47	32.61	24.79	41.22	13.15	49.29	28.03	86.59	42.82
	SD	4.64	12.21	16.79	8.26	3.54	2.55	4.31	1.51	5.36	3.01	8.55	4.62	3.04	5.26	1.55	16.32	13.24	3.05	8.12	3.87	2.71	4.82	1.73	5.69	3.19	8.91	4.92
	CV	0.137	0.156	0.150	0.159	0.133	0.135	0.127	0.150	0.135	0.134	0.138	0.168	0.170	0.135	0.132	0.139	0.155	0.173	0.156	0.142	0.137	0.143	0.164	0.142	0.142	0.140	0.165
S.T.:S ₂ N=26	mean	33.94	81.37	116.03	54.24	28.46	36.20	10.62	42.43	23.92	65.87	29.51	18.81	40.91	12.26	122.63	89.76	18.55	54.48	28.60	20.80	35.21	11.55	41.37	23.25	66.58	31.66	
	min	29.40	66.77	94.78	44.66	24.16	16.98	29.54	8.56	35.53	19.53	55.29	24.51	14.50	33.56	9.72	98.36	74.67	14.45	44.68	23.26	16.87	29.24	8.21	33.53	17.75	42.64	18.39
	max	41.14	100.92	145.64	69.21	35.21	23.80	47.45	13.10	52.82	29.64	38.31	38.31	23.58	48.79	14.28	150.12	110.32	23.50	70.04	35.21	26.14	14.64	14.64	51.89	31.37	79.87	38.60
	SD	3.52	10.38	14.89	7.38	2.97	2.34	4.65	1.43	4.81	2.63	7.69	4.32	3.11	4.51	1.18	14.25	11.54	2.80	7.34	3.28	2.48	1.65	4.78	3.07	8.56	5.04	
	CV	0.104	0.128	0.128	0.136	0.104	0.116	0.128	0.135	0.113	0.110	0.117	0.146	0.165	0.110	0.096	0.116	0.129	0.151	0.135	0.115	0.119	0.131	0.143	0.116	0.132	0.129	0.159
S.T.:S ₃ N=26	mean	35.99	83.29	122.17	56.81	29.54	36.20	10.53	42.66	24.05	67.98	30.27	19.72	42.24	12.24	125.53	90.40	19.42	57.11	30.17	21.34	36.19	11.65	43.27	23.77	69.61	32.66	
	min	32.86	72.05	104.30	47.56	26.25	17.81	30.21	8.73	36.46	19.99	57.98	25.08	15.58	38.23	11.18	106.17	78.89	15.88	48.23	24.45	17.63	31.60	9.56	38.20	19.41	58.18	24.56
	max	40.35	90.19	136.18	64.63	33.48	23.63	41.84	13.22	47.47	27.64	75.12	34.05	23.72	46.08	13.43	142.58	103.61	22.46	64.55	34.11	23.63	13.34	47.99	26.79	80.85	41.07	
	SD	1.75	5.44	6.85	3.56	1.76	1.45	3.15	1.08	2.98	1.99	4.63	2.48	1.84	2.19	0.59	8.53	6.75	1.55	3.20	2.36	1.77	2.54	1.24	3.04	1.95	5.68	3.85
	CV	0.049	0.065	0.056	0.063	0.060	0.070	0.087	0.102	0.070	0.083	0.068	0.082	0.093	0.052	0.048	0.068	0.075	0.080	0.056	0.078	0.083	0.107	0.070	0.082	0.082	0.118	

S.T. = Surat Thani

C'burri = Chanthaburi

for males; white morph mean ICW= 112mm for female crabs compared with ICW = 99mm for males). These data also show that for each sex, white crabs were slightly larger than the black morph specimens collected.

Standard deviation, as a measure of the amount of variation within each group sampled, showed increased values in accordance with both the increase of size range of crabs measured and the relative size of the measurement taken. Larger measurements such as the pereopod total length, ICW, and left and right propodus lengths produced the highest standard deviations in all groups of crabs. This is understandable, as the standard deviation is affected by the relative size of the animal in question; therefore, as a method of direct comparison between groups, it is a less useful statistic than the coefficient of variation (Sokal and Rohlf, 1995).

The coefficient of variation (representing the standard deviation as a percentage of the mean) can be used to compare the relative amounts of variation where groups and/or characters have different means. In the case of variation in the size variable (ICW) within each group, the male mud crab groups proved to be fairly consistent in size with CV values between 0.004 and 0.009. The female mud crab groups however showed a greater variation in size with CV values for ICW between 0.005 and 0.150. The trend in CV values that were seen for ICW was also recorded in other characters measured with respect to the carapace (RACL, LACL, ICL and FLW). Frontal lobe width (FLW) is the most homogenous character, showing the least within-group variation for all groups of crabs sampled ($CV \leq 0.080$), except for the first two white female populations sampled from Surat Thani, which show higher coefficients of variation for FLW (S_2 CV= 0.137 and S_3 CV= 0.104).

In the case of the male mud crab data, coefficients of variation are highest for measurements on the chelae, in particular the left and right dactyl widths (LDW, RDW) propodus lengths (LPL, RPL) and propodus widths (LPW, RPW) for the majority of male groups analysed ($CV \geq 0.100$). These relatively high values are indicative of variation in male claw morphometry. Interestingly, the CV values are slightly higher for these particular variables within the black male crab populations than for the white males. This is especially notable between the black and white morphs collected from Surat Thani.

The female mud crab data show high CV values for left and right dactyl widths (LDW, RDW) and right propodus widths (RPW) (tables 4.6 and 4.7) for the majority of groups. The male data set for these characters shows evidence of allometric growth in claw morphometry. Female left propodus width (LPW) does not show the same high CV value, however, revealing the presence of handedness among female mud crabs, but to a lower degree than in males.

4.3.2 Discriminant function analysis (canonical variate analysis)

4.3.2.1 Character contribution to among-group discrimination

Table 4.8 sets out the relative contribution each morphometric character makes to the between group variance (among-group discrimination) for pooled data for both male and female mud crabs. This between-group variance is recorded as an F-value. At step 0, where character values represent their own independent contribution before the other characters are taken into consideration as a whole, all characters are highly significant in discriminating between groups for both male and female data sets.

Table 4.8: F-values for 27 morphometric characters used for discriminant function analysis between groups of male and female mud crabs (genus *Scylla*) from Southeast Asia.

Character	M A L E						F E M A L E					
	Step 0 (df 11, 292)			Step 27 (df 11, 266)			Step 0 (df 8, 221)			Step 27 (df 8, 195)		
	F to enter	P	F to remove	P	F to remove	P	F to enter	P	F to remove	P		
Internal carapace width	24.80	<0.001	4.33	<0.001	24.97	<0.001	3.18	<0.001	0.005<x<0.002			
Internal carapace length	24.33	<0.001	2.94	0.005<x<0.002	19.71	<0.001	3.27	<0.001	0.005<x<0.002			
Frontal lobe width	34.35	<0.001	16.35	<0.001	17.96	<0.001	9.29	<0.001	<0.001			
Right antero-lateral length	21.22	<0.001	2.32	0.02<x<0.01	25.38	<0.001	0.95	<0.001	ns			
Left antero-lateral length	19.57	<0.001	2.88	0.005<x<0.002	24.61	<0.001	1.25	<0.001	ns			
Right dactylus length	18.37	<0.001	2.13	0.05<x<0.02	15.13	<0.001	0.95	<0.001	ns			
Left dactylus length	15.72	<0.001	3.80	<0.001	16.93	<0.001	2.70	<0.001	0.02<x<0.01			
Right dactylus width	14.54	<0.001	2.09	0.05<x<0.02	27.71	<0.001	2.46	<0.001	0.05<x<0.02			
Left dactylus width	13.14	<0.001	2.84	0.005<x<0.002	30.03	<0.001	4.62	<0.001	<0.001			
Right propodus length	20.72	<0.001	2.07	0.05<x<0.02	21.51	<0.001	0.57	<0.001	ns			
Left propodus length	19.23	<0.001	4.30	<0.001	24.34	<0.001	2.26	<0.001	ns			
Right propodus width	15.90	<0.001	4.20	<0.001	24.77	<0.001	4.96	<0.001	<0.001			
Left propodus width	15.40	<0.001	1.48	ns	27.14	<0.001	5.30	<0.001	<0.001			
Right carpus length	27.77	<0.001	2.75	0.005<x<0.002	27.80	<0.001	4.32	<0.001	<0.001			
Left carpus length	23.55	<0.001	3.04	0.002<x<0.001	32.60	<0.001	2.45	<0.001	0.05<x<0.02			
Right carpus width	23.63	<0.001	1.88	ns	26.50	<0.001	1.60	<0.001	ns			
Left carpus width	26.55	<0.001	9.46	<0.001	28.61	<0.001	3.61	<0.001	0.002<x<0.001			
Right merus length	27.16	<0.001	1.58	ns	22.10	<0.001	0.87	<0.001	ns			
Left merus length	23.23	<0.001	1.23	ns	25.61	<0.001	4.53	<0.001	<0.001			
Right merus width	26.35	<0.001	2.75	0.005<x<0.002	23.49	<0.001	1.13	<0.001	ns			
Left merus width	22.04	<0.001	5.31	<0.001	31.06	<0.001	2.08	<0.001	ns			
Third pereiopod total length	19.07	<0.001	4.02	<0.001	25.07	<0.001	1.20	<0.001	ns			
Third pereiopod merus length	21.14	<0.001	5.88	<0.001	28.52	<0.001	4.53	<0.001	<0.001			
Third pereiopod merus width	15.51	<0.001	2.96	0.002<x<0.001	30.20	<0.001	4.49	<0.001	<0.001			
Fifth pereiopod upper width	16.55	<0.001	3.33	<0.001	17.03	<0.001	1.18	<0.001	ns			
Fifth pereiopod lower width	14.98	<0.001	1.57	ns	14.41	<0.001	2.86	<0.001	0.01			
Fifth pereiopod total length	15.74	<0.001	4.67	<0.001	17.68	<0.001	1.30	<0.001	ns			

ns = not significant; df = degrees of freedom

At step 27, the last step when the information from all characters is taken into consideration, and the relative contribution of each character to the overall between group variance is calculated, five variables do not contribute significantly to the between group discrimination ($P > 0.05$) for male crabs; these are: left propodus width, right carpus width, right and left merus lengths and the fifth pereiopod lower width. Of the other variables, 11 characters are highly significant in their contribution. Only two characters, however, have strikingly high contribution values; frontal lobe width (FLW) and left carpus width (LCW), with F-values of 16.35 and 9.46 respectively.

The females data set reveals 12 of the 28 characters having no significant contribution (F-values) to the between-group variance, therefore these characters account for a much higher degree of information redundancy than in the male data set. Right and left propodus lengths, right and left antero-lateral lengths, right dactyl length, right and left merus lengths, third pereiopod total length, fifth pereiopod total length and fifth pereiopod upper width are all significant ($P < 0.05$) for the male data set, but not significant for the female data set ($P > 0.05$). Eight of the 28 characters are highly significant in their contribution (FLW, LDW, RPW, LPW, RCL, LML, 3PML and 3PMW), of which one character, namely frontal lobe width (FLW), was found to have a much more significant role in separating groups (F-value = 9.79).

4.3.2.2 Among-group discrimination

Mahalanobis distance values and associated F-values for pairwise comparisons of groups are shown for male and female crabs in tables 4.9 a and b respectively. The overall between group discrimination (tested using Wilks lambda test (Tabachnick and Fidell,

Table 4.9: Matrix of pairwise Mahalanobis D^2 (distance) values and associated pairwise F-values for groups of a) male and b) female mud crabs (genus *Scylla*) from Southeast Asia (S.T. White = Surat Thani white morph (samples 1-4); S.T. Black = Surat Thani black morph (samples 1-4)).

a. Male

Group	Pairwise Mahalanobis D^2 (distance) values between group means											
	Paikgasir	Ranong	S.T. Black S_1	S.T. Black S_3	S.T. Black S_4	Sematan	Thai Binh	Can Gio	Chanthaburi	S.T. White S_1	S.T. White S_3	S.T. White S_4
Paikgasir		10.93	3.78	6.14	8.64	5.16	32.81	22.65	14.15	21.66	16.74	25.17
Ranong	6.75		11.54	3.89	15.99	2.29	45.25	27.63	40.95	32.74	32.90	38.85
S.T. Black S_1	3.64	5.42		9.91	13.24	6.43	32.17	9.94	16.37	9.1	12.24	23.54
S.T. Black S_3	4.27	2.91	4.92		7.74	2.06	43.12	30.04	23.96	31.78	18.41	24.57
S.T. Black S_4	5.79	8.18	6.01	4.72		12.10	33.01	26.50	17.81	26.76	5.25	10.99
Sematan	4.53	2.18	4.05	2.45	5.94		48.89	26.61	30.73	29.25	22.39	33.05
Thai Binh	16.40	21.07	11.86	20.61	17.71	21.99		13.31	22.39	19.30	27.55	31.99
Can Gio	10.92	14.08	5.31	14.76	13.68	13.16	7.15		18.36	0.97	12.72	23.24
Chanthaburi	9.08	16.64	7.20	12.27	8.69	16.26	10.59	9.34		18.22	5.87	10.30
S.T. White S_1	11.40	15.65	5.44	15.66	13.44	14.11	9.43	2.20	8.90		12.17	22.15
S.T. White S_3	7.22	13.20	5.40	9.06	8.93	10.09	12.06	7.38	4.40	7.51		10.85
S.T. White S_4	10.18	15.96	8.38	10.82	5.68	13.92	13.20	10.51	5.54	9.95	4.95	

Degrees of freedom: 27 266

b. Female

Group	Pairwise Mahalanobis D^2 (distance) values between group means											
	S.T. White S_2	S.T. White S_3	S.T. White S_4	S.T. Black S_2	S.T. Black S_3	S.T. Black S_4	Chanthaburi	Thai Binh	Paikgasir			
S.T. White S_2		2.93	6.90	14.72	12.95	28.62	10.08	15.20	18.34			
S.T. White S_3	2.22		8.73	25.00	16.70	39.49	15.89	20.79	31.62			
S.T. White S_4	3.99	4.29		23.36	21.22	26.57	10.74	30.47	30.80			
S.T. Black S_2	8.14	11.35	10.89		6.67	10.08	27.20	36.52	5.60			
S.T. Black S_3	7.11	7.77	9.72	3.94		11.20	26.60	36.77	15.18			
S.T. Black S_4	14.18	17.20	12.59	5.00	6.39		36.32	47.78	11.26			
Chanthaburi	3.26	4.52	3.31	8.09	8.13	10.79		21.46	27.40			
Thai Binh	7.70	9.32	13.40	16.52	17.58	22.40	6.26		22.54			
Paikgasir	8.05	12.71	11.67	3.72	6.50	5.49	7.65	9.61				

Degrees of freedom: 27 195

1989) and the associated approximate F-statistic are significant ($P < 0.001$) for both female and male data (approximate F values = 6.602 and 7.191, respectively).

Both pairwise F values and D^2 statistics between groups exhibit the same basic trends, thus supporting the following observations. Firstly, relatively low distance values are consistently experienced between groups of the same colour morph for both male and female mud crabs. Secondly, the lowest values would be expected where comparisons were made between sampled years from the same location; *i.e.* in the main study site, Surat Thani, where three collections were made of the two mud crab morphs occurring.

However, this was not the case for the male crabs sampled. Both black and white morphs show closer associations with other sample locations than they do between years of sampling from the Surat Thani site. The female crab distance values, however, do show some geographical patterns where crabs from the same location generally have lower distance values (2.93 to 8.75 and 6.67 to 11.20 for white and black females respectively from Surat Thani) compared with groups from other locations.

Finally, the largest distance values occur where a comparison is made between two different colour morphs from the two locations geographically furthest apart. The data for male crabs from Thai Binh (white morph) and Ranong (black morph) consistently generated high distance values (32.17 to 48.89 and 13.20 to 21.07 respectively) when compared with other groups. Female crabs from Thai Binh (white morph) and Paikgasir (black morph) reveal the highest distance values when compared to the other female groups (21.46 to 47.78 and 18.34 to 30.80 respectively).

Pairwise F-values which can be tested for significance indicate that all pairwise comparisons are significant, therefore all groups provide greater between-group variance than within-group variance for both male and female mud crabs (tables 4.9a and b). The majority of these pairwise comparisons are highly significant ($P < 0.001$).

F-values for pairwise comparisons between the black and white morphs from Surat Thani are all highly significant ($P < 0.001$) for all samples collected, signifying that these two mud crab colour morphs are also different morphologically, although they live sympatrically.

4.3.2.3 Classification of individuals to groups

Tables 4.10 and 4.11 show the classification and jack-knifed classification of each individual mud crab into the group with the highest posterior probability using the Mahalanobis D^2 statistic and the percentage of individuals that are correctly classified to their original group.

Using the standard classification procedure, a high percentage of individuals can be classified correctly to their own group, the average being 84.7 % and 83.1% for male and female crabs respectively (see tables 4.10a and 4.11a). This percentage reduces when the jack-knifed procedure is used resulting in averages of 70.1% and 67.7% for male and female crabs respectively. In most cases, the misclassification of individuals is to groups with the same morphology, although there are five male crabs that are reclassified to a group with a different morphology. This is increased to 14 males using the jack-knifed classification procedure. Four female crabs are reclassified to a group with different morphological characteristics and this rises to seven crabs when the jack-knifed classification procedure is employed. Five of the seven females and nine of the 14 males

Table 4.10: Classification (a) and jack-knifed classification (b) matrices using discriminant function analysis for groups of male mud crabs (genus *Scylla*) from Southeast Asia.

a. Classification		Number of cases classified into group												Percent correctly classified
Group	Paikgasir	Ranong	S.T. Black S ₁	S.T. Black S ₃	S.T. Black S ₄	Sematan	Thai Binh	Can Gio	Chanthaburi	S.T. White S ₁	S.T. White S ₃	S.T. White S ₄		
Paikgasir	26	0	0	1	1	0	0	0	0	1	0	0	0	89.7
Ranong	1	22	0	0	0	3	0	0	0	0	0	0	0	84.6
S.T. Black S ₁	1	1	13	0	1	0	0	0	0	0	0	0	0	81.3
S.T. Black S ₃	2	2	0	20	1	3	0	0	0	0	0	0	0	71.4
S.T. Black S ₄	2	0	0	0	22	2	0	0	0	0	0	2	0	78.6
Sematan	0	2	0	2	0	22	0	0	0	0	0	0	0	88.0
Thai Binh	0	0	0	0	0	0	26	0	0	1	0	0	0	96.3
Can Gio	0	0	1	0	0	0	0	26	0	3	0	0	0	86.7
Chanthaburi	0	0	0	0	0	0	0	0	21	0	1	0	0	95.5
S.T. White S ₁	0	0	0	0	0	0	0	3	0	24	1	0	0	85.7
S.T. White S ₃	1	0	0	0	0	0	0	0	1	0	22	0	0	91.7
S.T. White S ₄	0	0	0	0	0	0	0	0	2	1	4	14	0	66.7

b. Jack-knifed classification		Number of cases classified into group												Percent correctly classified
Group	Paikgasir	Ranong	S.T. Black S ₁	S.T. Black S ₃	S.T. Black S ₄	Sematan	Thai Binh	Can Gio	Chanthaburi	S.T. White S ₁	S.T. White S ₃	S.T. White S ₄		
Paikgasir	23	0	1	2	1	1	0	0	0	1	0	0	0	79.3
Ranong	1	17	0	1	0	6	0	0	0	0	1	0	0	65.4
S.T. Black S ₁	1	1	9	0	1	0	0	1	0	1	2	0	0	56.3
S.T. Black S ₃	2	5	1	11	3	5	0	0	0	0	1	0	0	39.3
S.T. Black S ₄	2	0	0	2	19	2	0	0	0	0	0	3	0	67.9
Sematan	0	2	0	8	0	15	0	0	0	0	0	0	0	60.0
Thai Binh	0	0	0	0	0	0	25	1	0	1	0	0	0	92.6
Can Gio	0	0	1	0	0	0	0	24	0	5	0	0	0	80.0
Chanthaburi	0	0	0	0	0	0	0	0	20	0	2	0	0	90.9
S.T. White S ₁	1	0	1	0	0	0	0	5	0	19	2	0	0	67.9
S.T. White S ₃	2	0	1	0	0	0	0	0	2	0	18	1	0	75.0
S.T. White S ₄	0	0	0	0	0	0	0	0	2	1	4	14	0	66.7

S.T. White = Surat Thani white morph (samples 1 to 4); S.T. Black = Surat Thani black morph (samples 1 to 4).

Table 4.11: Classification (a) and jack-knifed classification (b) matrices using discriminant function analysis for groups of female mud crabs (genus *Scylla*) from Southeast Asia.

a. Classification

Group	Number of cases classified into group										Percent correctly classified
	S.T. White S ₂	S.T. White S ₃	S.T. White S ₄	S.T. Black S ₂	S.T. Black S ₃	S.T. Black S ₄	Chanthaburi	Thai Binh	Paikgasir		
S.T. White S ₂	18	6	0	0	1	0	1	2	1	62.1	
S.T. White S ₃	7	18	0	0	1	0	0	0	0	69.2	
S.T. White S ₄	1	2	21	0	0	0	2	0	0	80.8	
S.T. Black S ₂	0	0	0	24	1	2	0	0	1	85.7	
S.T. Black S ₃	0	1	0	2	26	0	0	0	1	86.7	
S.T. Black S ₄	0	0	0	2	1	26	0	0	1	86.7	
Chanthaburi	7	0	1	0	0	0	10	0	0	76.9	
Thai Binh	0	0	0	0	0	0	0	27	0	100.0	
Paikgasir	0	0	0	0	0	0	0	0	21	100.0	

b. Jack-knifed classification

Group	Number of cases classified into group										Percent correctly classified
	S.T. White S ₂	S.T. White S ₃	S.T. White S ₄	S.T. Black S ₂	S.T. Black S ₃	S.T. Black S ₄	Chanthaburi	Thai Binh	Paikgasir		
S.T. White S ₂	14	8	1	0	1	0	1	3	1	48.3	
S.T. White S ₃	9	15	1	0	1	0	0	0	0	57.7	
S.T. White S ₄	3	4	15	0	0	0	3	1	0	57.7	
S.T. Black S ₂	1	0	0	18	2	4	0	0	3	64.3	
S.T. Black S ₃	0	1	0	4	23	0	0	0	2	76.7	
S.T. Black S ₄	0	0	1	4	1	22	0	0	2	73.3	
Chanthaburi	2	0	3	0	0	0	7	1	0	53.8	
Thai Binh	0	0	0	0	0	0	0	26	1	96.3	
Paikgasir	0	0	0	2	0	2	0	0	17	81.0	

S.T. White = Surat Thani white morph (samples 2 to 4); S.T. Black = Surat Thani black morph (samples 2 to 4).

were reclassified from the Surat Thani white crab groups to the Surat Thani black groups and *vice versa*.

4.3.2.4 Summary of group discrimination

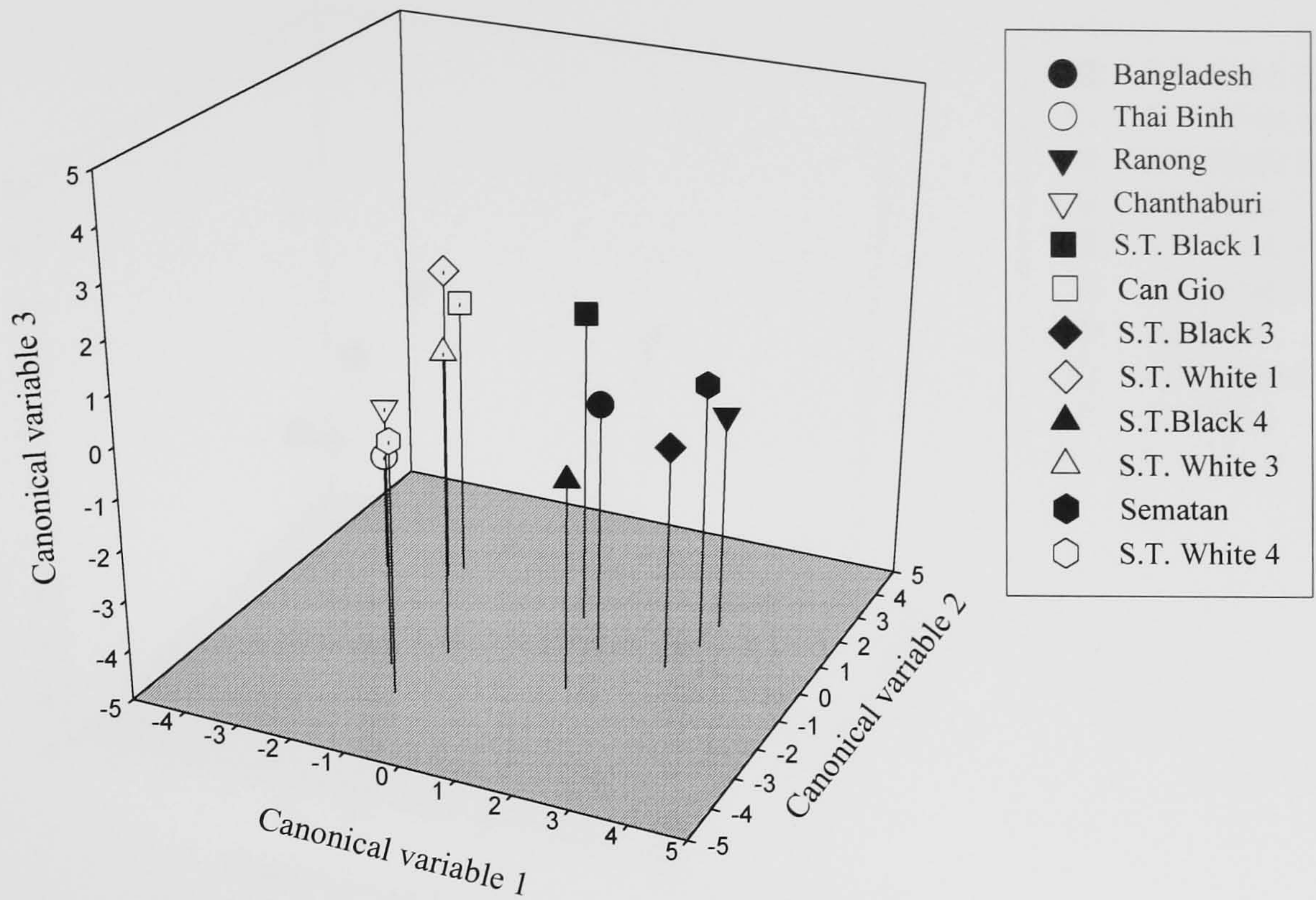
Eigenvalues, the associated canonical correlation and cumulative percentage of total dispersion are shown in table 4.12. The first three canonical variates represent 76% and 84% of the total discriminatory dispersion encountered for male and female crabs respectively, the highest proportion being accounted for by the first canonical variable (47% and 49% for male and female crabs respectively). The largest eigenvalues, which represent the between group discrimination, are expressed by the first canonical variable for both sexes.

Figures 4.3a and 4.4a show the first three canonical variables plotted as a 3D scatter plot for male and female crabs respectively. For both male and female data, the groups assigned to each location, represented by their group centroids, form two main clusters. These two clusters represent the two morphs in their entirety, *i.e.*, discriminant function analysis has successfully discriminated groups of mud crabs on the basis of their external morphological features. Based on the first canonical variable (represented by the X-axis) black males appear to have been assigned a positive canonical variable, whereas the white males have been assigned a negative canonical variable. The reverse is true for the groups of female crabs (see figure 4.4b).

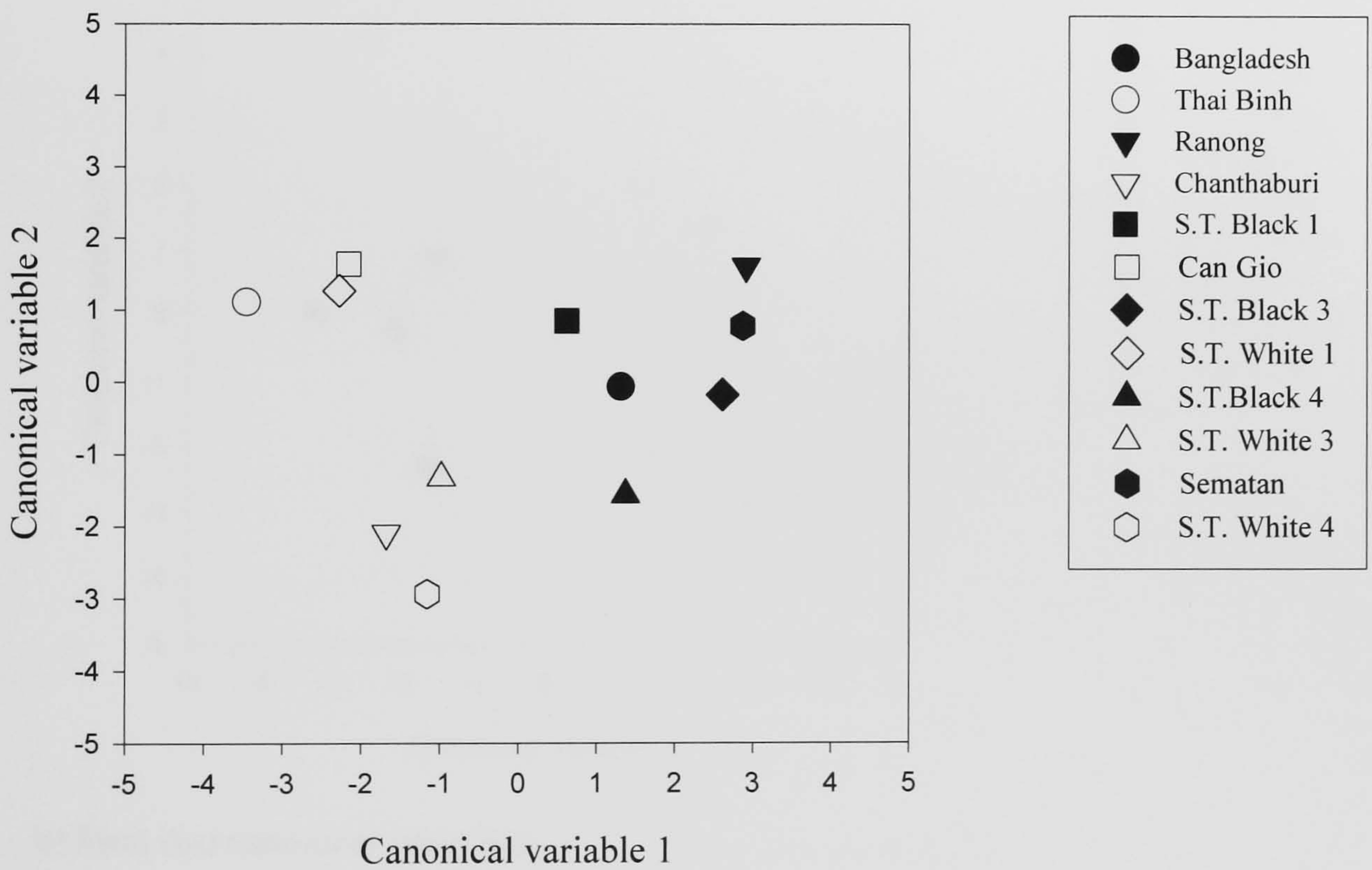
The male crab data represented in figure 4.3b also show that the groups collected from Surat Thani from different years are well interspersed within other location mean centroids, thereby supporting the evidence shown earlier in the analysis that no obvious

Table 4.12: Eigenvalues, canonical correlations and cumulative percentages of total dispersion expressed by the first five canonical variables for male and female mud crabs (genus *Scylla*) from Southeast Asia.

	Canonical variable				
	I	II	III	IV	V
Male:					
Eigenvalue	4.9567	2.1897	1.1724	0.7915	0.5428
Canonical correlation	0.9122	0.8286	0.7346	0.6647	0.5931
Cumulative percentage of total dispersion	0.4533	0.6536	0.7609	0.8333	0.8829
Female:					
Eigenvalue	4.9333	2.4254	1.1705	0.6063	0.3822
Canonical correlation	0.1998	0.8415	0.7344	0.6144	0.5259
Cumulative percentage of total dispersion	0.4889	0.7293	0.8453	0.9054	0.9433

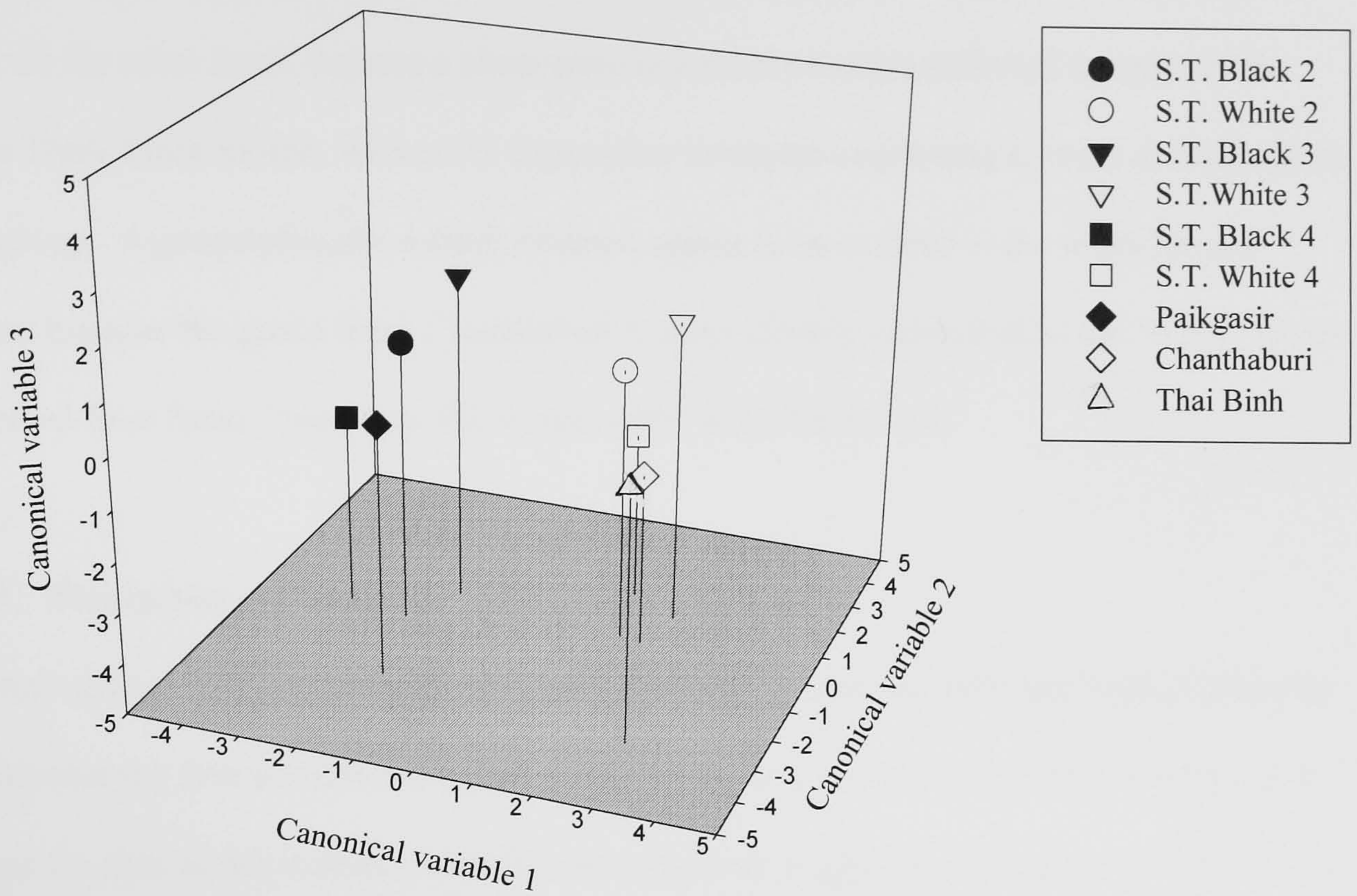


a) First three canonical variables

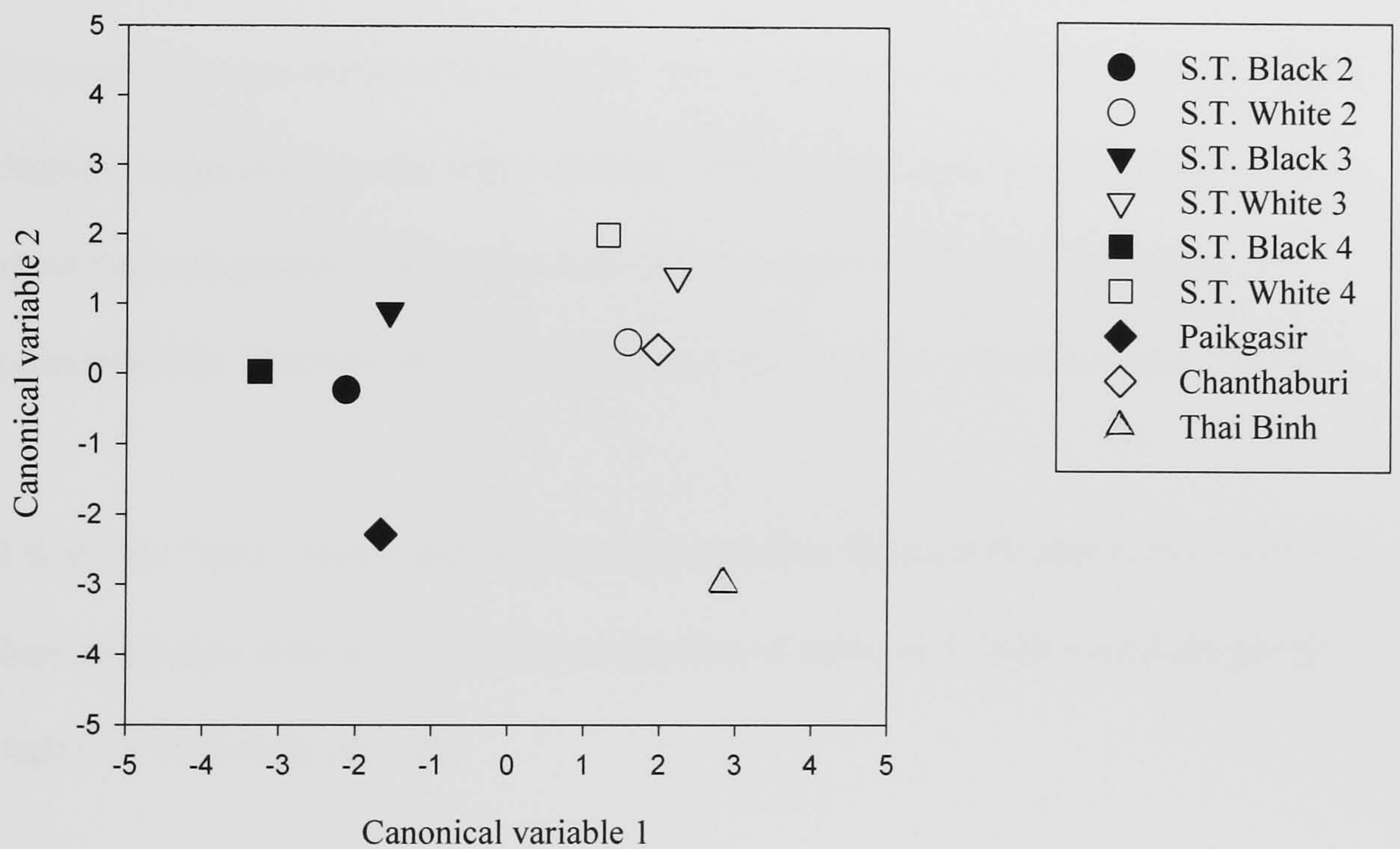


b) First two canonical variables

Figure 4.3: Ordination plots for group centroids for a) first three canonical variables and b) first two canonical variables for groups of male mud crabs (genus *Scylla*) from seven locations in Southeast Asia.



a) First three canonical variables



b) First two canonical variables

Figure 4.4: Ordination plots for group centroids for a) first three canonical variables and b) first two canonical variables for groups of female mud crabs (genus *Scylla*) from five locations in Southeast Asia.

geographical variation in *Scylla* is present between the locations sampled. The female data, on the other hand, express a close association between the different samples of the Surat Thani black morph, with crabs from other locations expressing a larger distance from this group. A geographically related distance seems to be evident in the white female cluster because the group from Chanthaburi is more closely associated to the “white” crabs collected from Surat Thani than the female crabs from Thai Binh.

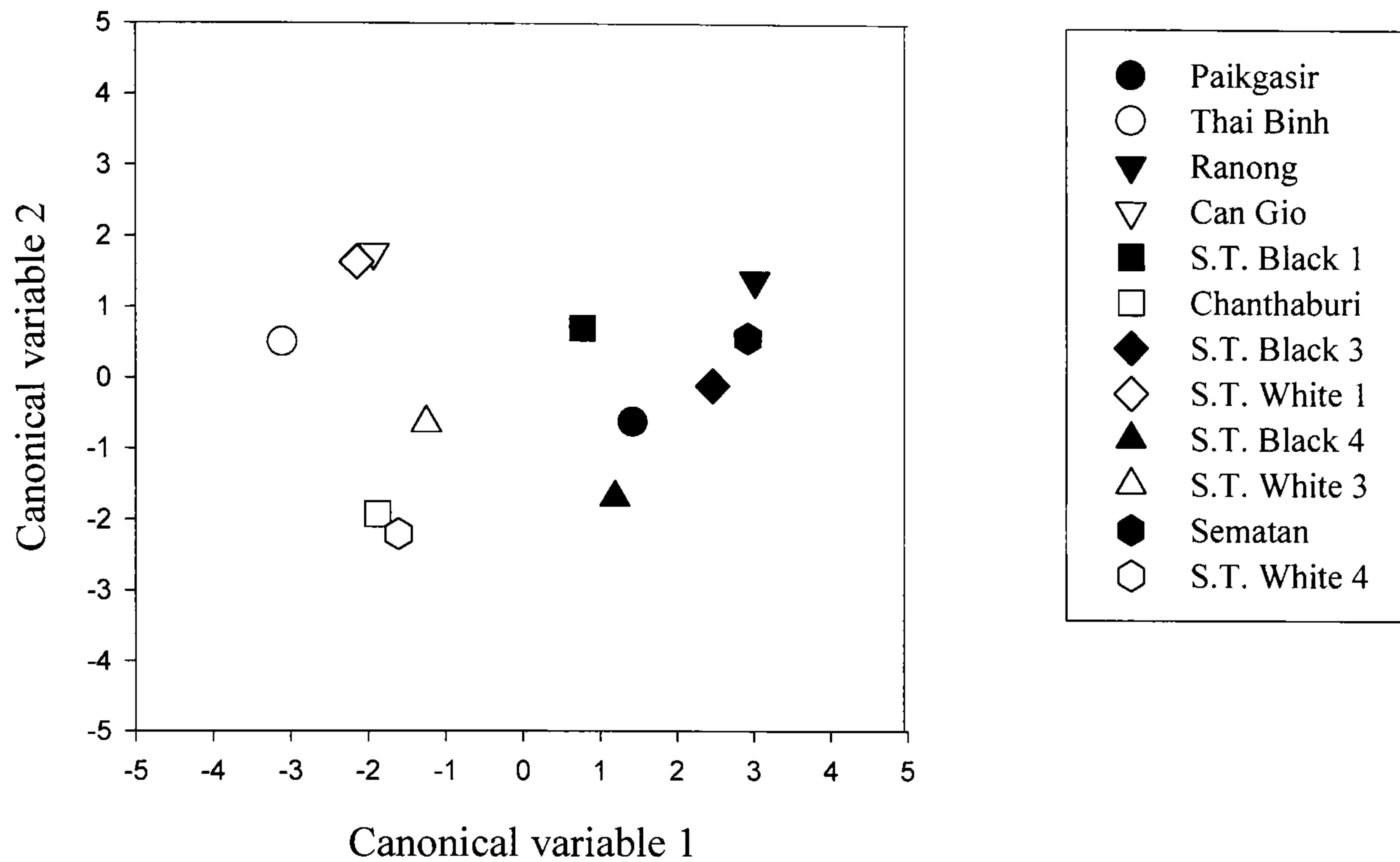
4.3.3 Size in/size out analysis

Multiple group principal components analysis (MGPCA) on the male and female data sets reveals that the first normalized vector (multiple group principal component one) is a size vector; *i.e.* this vector is responsible for any influence of growth-dependent size on the data sets. MGPC1 (multiple group principle component one) shows all growth dependant characters to be of the same sign, although the magnitude of each character varies in both male and female data sets (refer to table 4.13). Once entered into the CVA, the first vector shows a higher weighting than the other vectors. Thus size is having some effect on the ordination of the crab groups. With this size vector excluded from the input files, the resulting discriminant function analysis results in a very similar ordination pattern to that observed with this “size” vector included (as illustrated in figure 4.5a and 4.5b); therefore growth-dependant size does not effect the ordination of male or female mud crab groups even though it is obviously present.

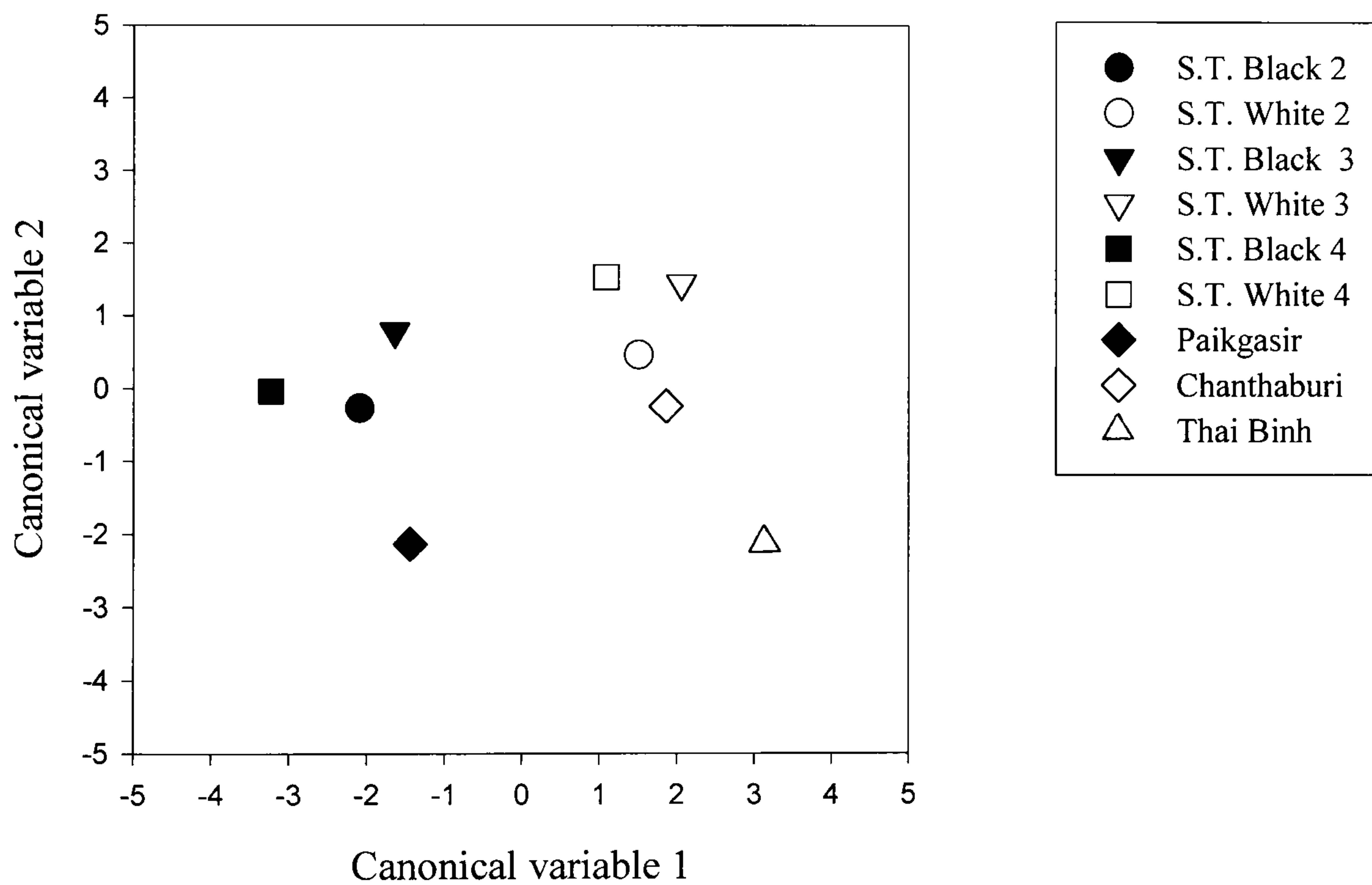
Discriminant function analysis of MGPCA scores reveal three of the 28 vectors contribute significantly to the discrimination of groups of male crabs, while four of the vectors discriminate for the groups of female crabs. On further examination of these particular

Table 4.13 : Scores for multiple group principal component one (MGPC1) for all growth-dependant variables and F-values for contribution of each MGPCA vector to the ordination pattern of groups of male and female mud crabs (genus *Scylla*), from Southeast Asia. Abbreviations for morphometric characters are explained in Table 4.3 and Figure 4.2.

Male				Female			
MGPC1 scores for characters		F-values for normalised vectors		MGPC1 scores for characters		F-values for normalised vectors	
Character	Score	Vector	F-value (step 27)	Character	Score	Vector	F-value (step 27)
ICW	-0.312	1	10.03	ICW	-0.450	1	8.87
ICL	-0.214	2	8.34	ICL	-0.316	2	2.64
FLW	-0.088	3	2.57	FLW	-0.113	3	1.71
RACL	-0.154	4	5.34	RACL	-0.216	4	1.85
LACL	-0.152	5	3.85	LACL	-0.217	5	2.97
RDL	-0.166	6	0.74	RDL	-0.135	6	3.91
LDL	-0.172	7	3.58	LDL	-0.128	7	1.89
RDW	-0.062	8	5.11	RDW	-0.042	8	3.17
LDW	-0.058	9	2.82	LDW	-0.035	9	1.75
RPL	-0.327	10	2.27	RPL	-0.251	10	2.74
LPL	-0.322	11	16.00	LPL	-0.229	11	4.34
RPW	-0.193	12	1.24	RPW	-0.129	12	7.95
LPW	-0.180	13	6.32	LPW	-0.113	13	6.34
RCL	-0.130	14	4.03	RCL	-0.100	14	1.19
LCL	-0.128	15	4.09	LCL	-0.091	15	5.06
RCW	-0.090	16	1.58	RCW	-0.071	16	1.06
LCW	-0.089	17	2.13	LCW	-0.064	17	3.22
RML	-0.173	18	4.99	RML	-0.149	18	2.87
LML	-0.178	19	5.36	LML	-0.143	19	4.21
RMW	-0.089	20	10.11	RMW	-0.076	20	3.61
LMW	-0.086	21	2.13	LMW	-0.070	21	6.40
3PTL	-0.460	22	2.39	3PTL	-0.419	22	3.77
3PML	-0.159	23	2.63	3PML	-0.130	23	1.39
3PMW	-0.033	24	2.07	3PMW	-0.034	24	0.92
5PUW	-0.062	25	2.57	5PUW	-0.082	25	6.74
5PLW	-0.060	26	3.17	5PLW	-0.085	26	1.45
5PTL	-0.293	27	2.37	5PTL	-0.347	27	0.78



a) Males



b) Females

Figure 4.5: Ordination plot for first two canonical variables on multiple group principal component analysis (MGPCA) scores with growth-dependent size removed for both a) male mud crabs (seven locations) and b) female mud crabs (four locations) in Southeast Asia.

vectors, third pereiopod total length (3PTL), right dactyl length (RDL) and left carpus width (LCW) contribute strongly to the discrimination of male data. In the case of female crabs, frontal lobe width (FLW), fifth pereiopod lower width (5PLW), left dactyl width (LDW), and right carpus length (RCL) are the most prominent characters for between-groups discrimination.

4.3.4 Geographic variation

Figure 4.6a and figure 4.7a illustrate the geographical variation observed for male and female white morph mud crabs respectively. With regards to the network diagrams, the D^2 (distance) values are much lower for sites located close to each other than for sites situated further apart for both male and female crabs. However, the sum D^2 values for locations along the coastline, *i.e.* Surat Thani/Chanthaburi and Chanthaburi/Thai Binh is much higher than the D^2 value obtained for Surat Thani/Thai Binh ($D^2 = 30.75$ and $D^2 = 38.71$ for summed values compared to $D^2 = 14.81$ and $D^2 = 20.22$ for Surat Thani/Thai Binh in white female and male crabs, respectively). This is an indication that the divergence between these groups of mud crabs is not consistent.

Scores for the first canonical variable (which accounts for 61% and 75% of total among-group variation for males and females respectively) are plotted against distance along the coastline from northern Vietnam to southern Thailand (as illustrated in figures 4.6b and 4.7b). Even with such widely dispersed locations, there is strong evidence of geographic variation within the white morph that is clinal in nature; *i.e.*, there is a gradual unidirectional change in character state. This cline is expressed in both male and female mud crab data sets; however the magnitude of this cline is negative for male crabs, but strongly positive for female crabs.

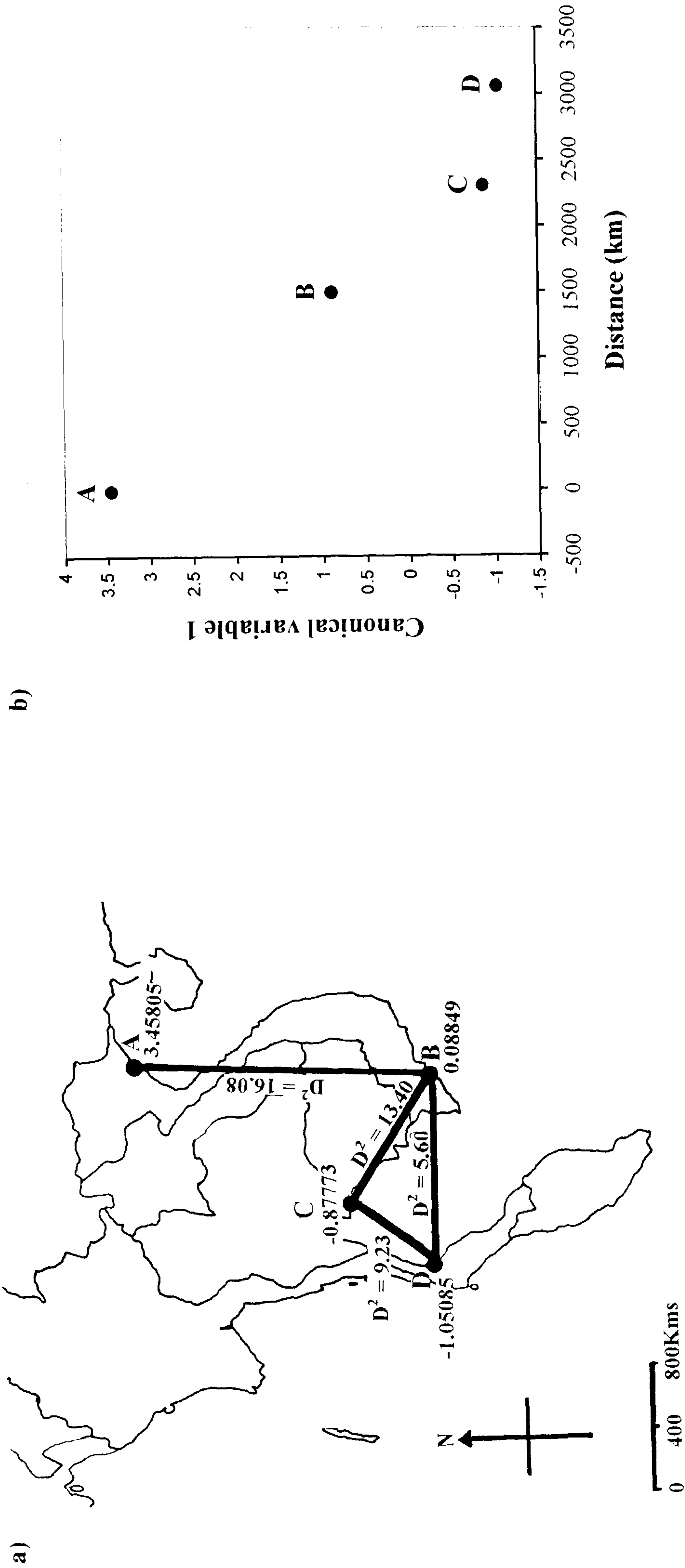


Figure 4.6: Network diagram (a) showing phenetic distances (D^2) between samples and mapped scores for the first canonical variables for groups of white morph male mud crabs (genus *Scylla*) from four locations in Southeast Asia. Canonical variables for these groups are plotted against coastal distance (b) in a north-south direction from Thai Binh to Surat Thani (A = Thai Binh; B = Can Gio; C = Chanthaburi; D = Surat Thani).

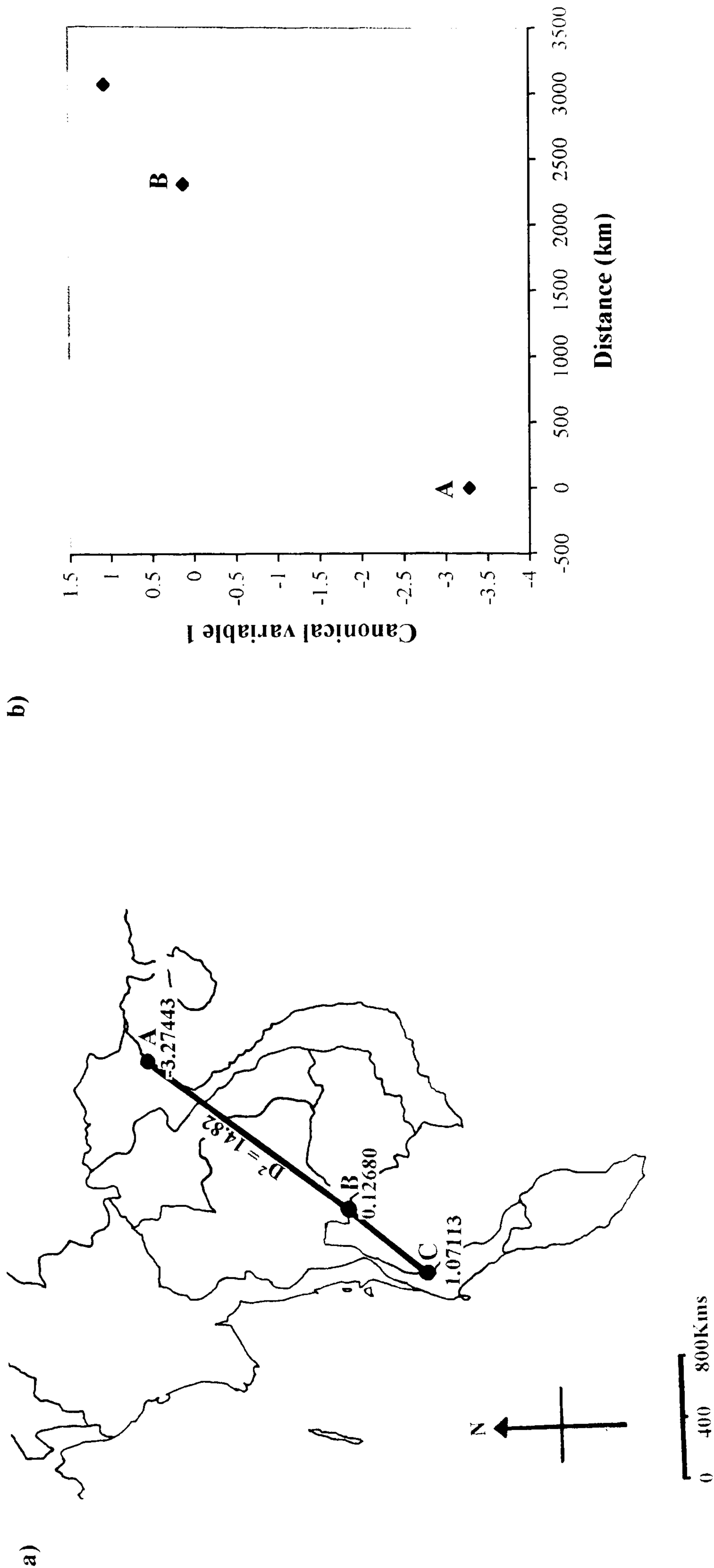


Figure 4.7: Network diagram (a) showing phenetic distances (D^2) between samples and mapped scores for the first canonical variables for groups of white morph female mud crabs (genus *Scylla*) from three locations in Southeast Asia. Canonical variables for these groups are plotted against coastal distance (b) in a north-south direction from Thai Binh to Surat Thani (A = Thai Binh; B = Chanthaburi; C = Surat Thani).

4.3.5 Classification of typed specimens

4.3.5.1 Male crabs

The results of the classification of male mud crab specimens to groups are illustrated in table 4.14. Both classification and jack-knifed classification produce the same results; thus only one set of results is shown. There were no male specimens of *Scylla serrata* available, so this species has not been included in the analysis. Before growth-dependent size is removed, one individual of *Scylla tranquebarica* is classified into the Surat Thani black morph group and the other two individuals into white morph groups, namely, Chanthaburi and Surat Thani. After size was removed, the Chanthaburi individual is reclassified to the Surat Thani white morph group.

Scylla olivacea is closely associated with the black groups sampled, especially after size is removed, all four representatives of *S. olivacea* are categorised into black colour morph crab groups and they exhibit all the characteristics of *S. olivacea*.

Only one specimen of *Scylla paramamosain* was available. This specimen is classified into the white group from Thai Binh Province; however, once size is removed, its classification changes to the Surat Thani black morph group.

F-values show that both frontal lobe width (F-value = 17.50) and right carpus width (F-value = 10.53) contributes most significantly to the classification of the male typed specimens into male crab groups.

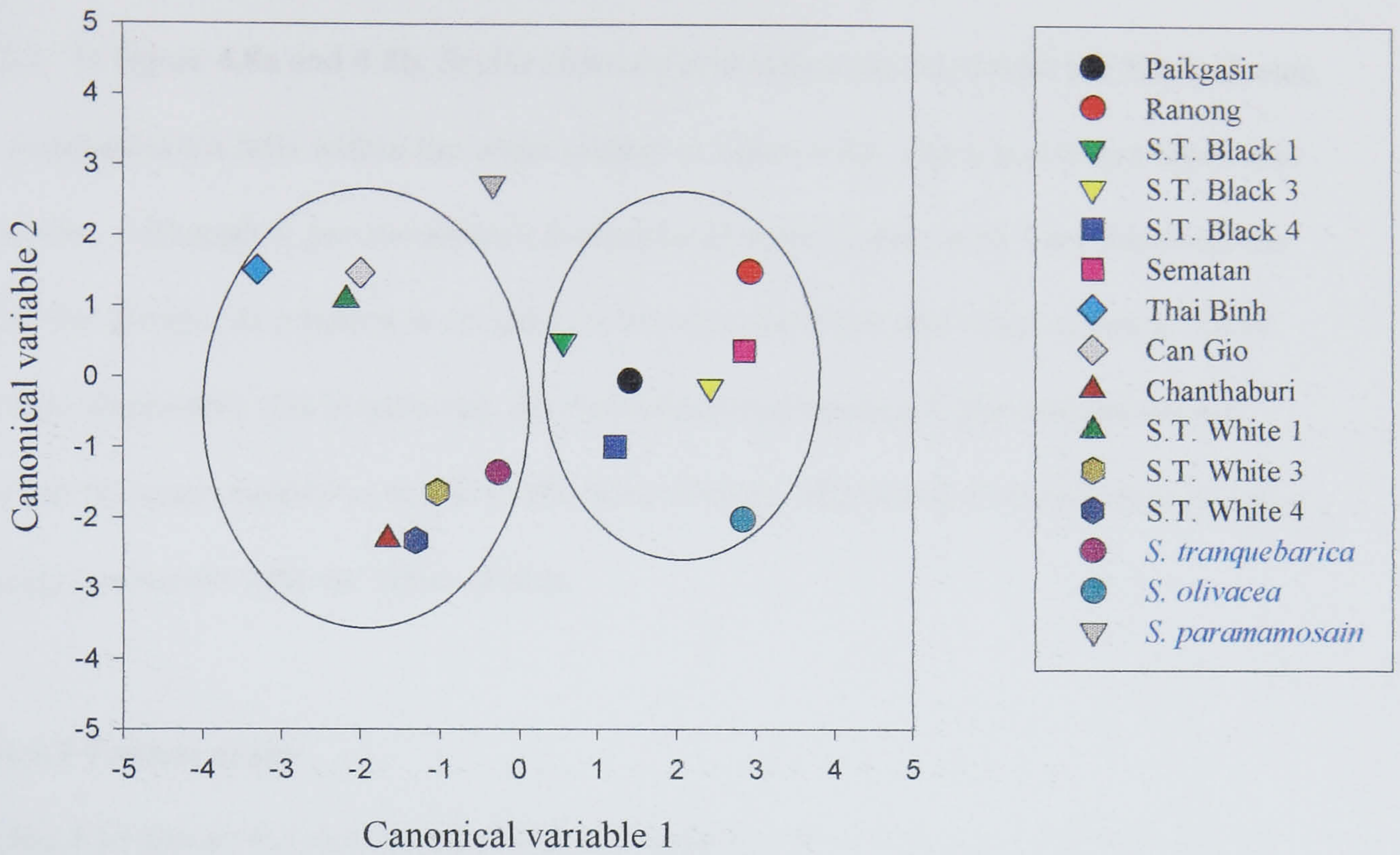
Figures 4.8a and 4.8b show the orientation of group centroids for the male mud crab groups and typed specimens. The two clusters for male crabs are as shown in section

Table 4.14: Classification (with the size vector both present and removed) of museum specimens and typed specimens of male mud crabs (genus *Scylla*) to male mud crab groups from seven locations within Southeast Asia.

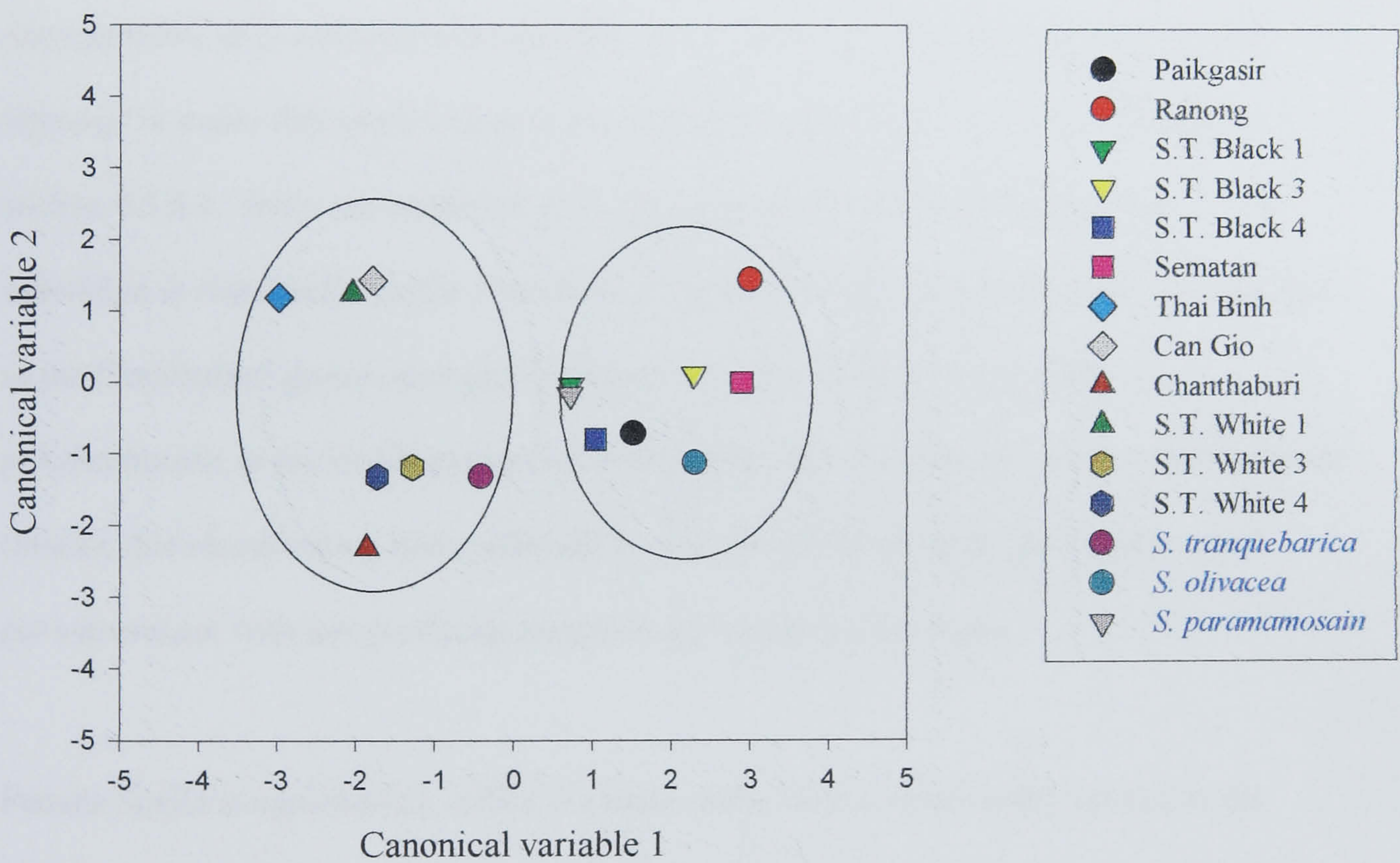
Classification groups	Museum and typed specimens					
	Classification			Classification with “size” removed		
	<i>Scylla tranquebarica</i>	<i>Scylla olivacea</i>	<i>Scylla paramamosain</i>	<i>Scylla tranquebarica</i>	<i>Scylla olivacea</i>	<i>Scylla paramamosain</i>
Paikgasir	0	0	0	0	0	0
Ranong	0	0	0	0	0	0
S.T. black S ₁	1	0	0	1	0	0
S.T. black S ₃	0	1	0	0	1	1
S.T. black S ₄	0	2	0	0	1	0
Sematan	0	0	0	0	2	0
Thai Binh	0	0	1	0	0	0
Can Gio	0	0	0	0	0	0
Chanthaburi	1	0	0	0	0	0
S.T. white S ₁	0	0	0	0	0	0
S.T. white S ₃	0	0	0	0	0	0
S.T. white S ₄	1	1	0	2	0	0

S.T. white = Surat Thani white morph (samples 1 to 4).

S.T. black = Surat Thani black morph (samples 1 to 4).



a) CVA on raw data



b) CVA on MGPCA scores with growth-dependent size removed

Figure 4.8: Ordination plots for the first two canonical variables for group centroids of male mud crabs (genus *Scylla*) from seven locations in Southeast Asia and typed specimens from the Zoological Museum, Copenhagen and from Keenan *et al*, 1998.

4.3.3. In figure 4.8a and 4.8b, *Scylla olivacea* is closely associated with the black cluster. *S. tranquebarica* falls within the white cluster in figure 4.8a, where size is not taken into account. Although *S. paramamosain* is associated more closely with Thai Binh and the Can Gio groups, its position is equidistant between the black and white clusters. Once growth-dependent size is removed, the individual representing *S. paramamosian* has moved its relative position across to the black cluster, whereas *S. tranquebarica* remains firmly associated with the white cluster.

4.3.5.2 Female crabs

Table 4.15 shows the classification of typed female crabs of the genus *Scylla* to female crabs groups sampled from four locations in Southeast Asia. Unfortunately, no female representative of *S. olivacea* was available for comparison to the crab groups sampled, although in males this species is strongly affiliated to the black cluster as explained in section 4.3.5.1. Only one specimen of *S. serrata* was available for measurement. This individual is classified with the Thai Binh population within the white cluster, but realigns to the Chanthaburi group once growth-dependent size is taken into account. *S. paramamosain*, is divided between two morphs, namely Chanthaburi (white) and Paikgasir (black); this classification is not affected by size. It is difficult therefore to associate *S. paramamosain* with any particular morphology among female crabs.

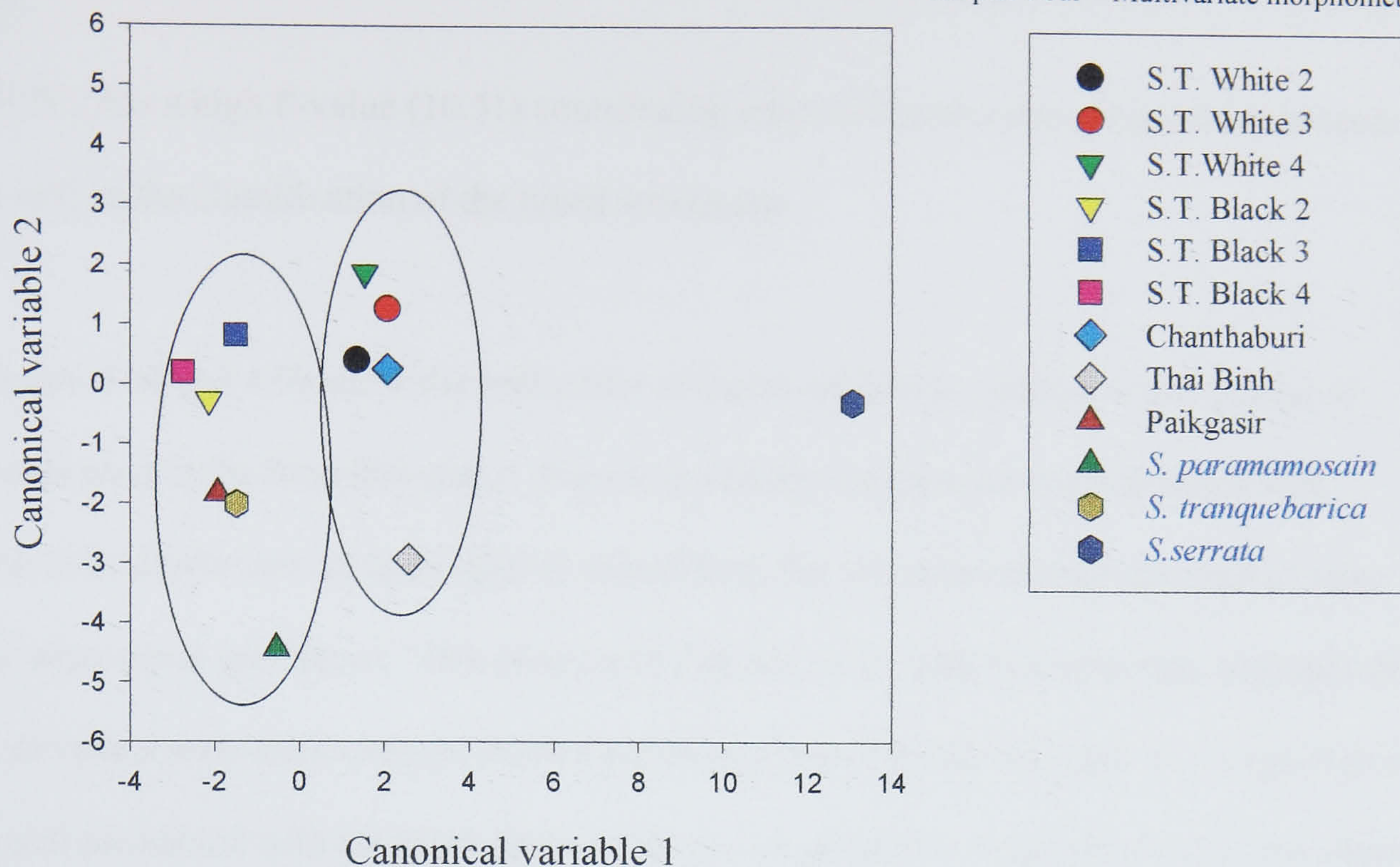
Female *Scylla tranquebarica*, unlike the male crabs, seems most closely related to the black cluster, with all four individuals classified with black groups and three individuals being classified with the Surat Thani black morph (figures 4.9a and 4.9b). The one individual which classifies to the Paikgasir sample becomes reclassified to a white group (Chanthaburi) after size is taken into account. Only one character, frontal lobe-width

Table 4.15: Classification (both with the size vector present and removed) of museum specimens and typed specimens of female mud crabs (genus *Scylla*) to female mud crab groups from seven locations within Southeast Asia.

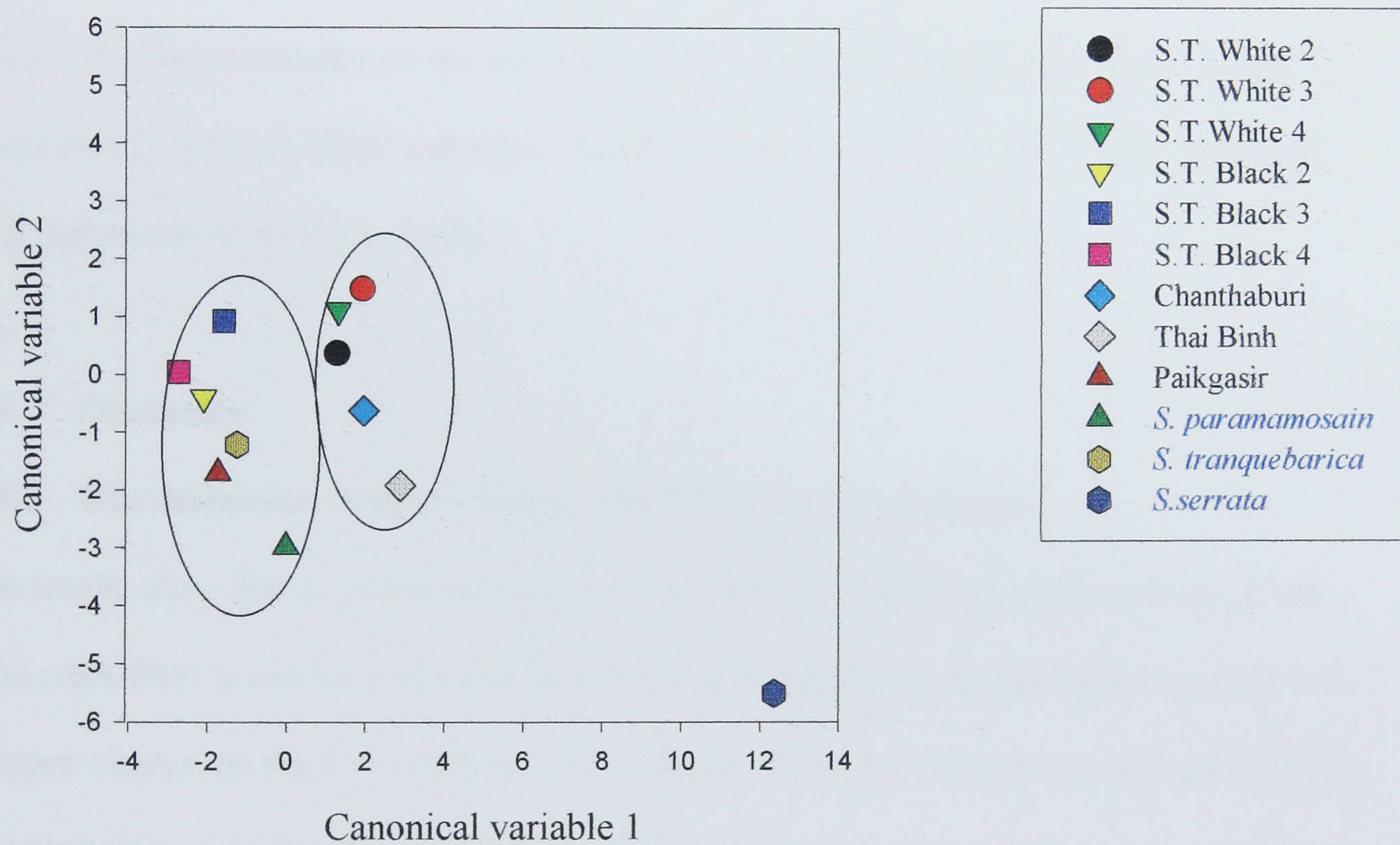
Classification groups	Museum and typed specimens					
	Classification			Classification with “size” removed		
	<i>Scylla tranquebarica</i>	<i>Scylla serrata</i>	<i>Scylla paramamosain</i>	<i>Scylla tranquebarica</i>	<i>Scylla serrata</i>	<i>Scylla paramamosain</i>
S.T. white S ₂	0	0	0	0	0	0
S.T. white S ₃	0	0	0	0	0	0
S.T. white S ₄	0	0	0	0	0	0
S.T. black S ₂	2	0	0	2	0	0
S.T. black S ₃	1	0	0	1	0	0
S.T. black S ₄	0	0	0	0	0	0
Thai Binh	0	1	0	1	0	0
Chanthaburi	0	0	1	0	1	1
Paikgasir	1	0	1	0	0	1

S.T. white = Surat Thani white morph (samples 2 to 4)

S.T. black = Surat Thani black morph (samples 2 to 4)



a) CVA on raw data



b) CVA on MGPCA scores with growth-dependent size removed

Figure 4.9: Ordination plots for the first two canonical variables for group centroids of female mud crabs (genus *Scylla*) from four locations in Southeast Asia and typed specimens from the Zoological Museum, Copenhagen and Keenan *et al*, 1998.

(FLW), has a high F-value (10.51) contributing most to the between-group variance seen as well as the classification of the typed specimens.

Figures 4.9a and 4.9b show the ordination of typed samples in relation to the groups of female mud crabs from this study. The most striking observation is the distance with which the *Scylla serrata* individual is placed from the two main clusters and indeed from the other typed specimens. This prominent distance, even with size removed, indicates that *S. serrata* is well-discriminated morphometrically from the other groups. *S. tranquebarica* is well associated with the black female cluster. Its affiliation is not affected by size, thus female black crabs show good conformity with *S. tranquebarica* in this analysis.

Female *Scylla paramamosain* are more difficult to affiliate to a specific cluster, as they lie equidistantly between black and white clusters. Finally, size did not effect the ordination of *S. paramamosain* (figure 4.9b).

4.4 Discussion

4.4.1 Discrimination between colour morphs of the genus *Scylla*

The results show that discriminant function analysis successfully separated groups of male mud crabs from seven locations and females from five locations in Southeast Asia into two primary clusters on the basis of their overall shape. These two clusters correspond with the two phenotypes identified using traditional descriptive methods of taxonomy, as presented in chapter three. Thus, morphometric analysis has reinforced the hypothesis that the two morphs of *Scylla* used in this study are discrete, with no detectable intermediate phenology, suggesting that they are two species.

Discriminant function analysis has been shown in other studies to be a very effective technique in discriminating between groups of closely related animal taxa (Thorpe, 1976). Moreover, the ease with which linear measurements can be taken from the hard body parts of crustaceans makes the collection of accurate data particularly easy for this subphylum (Davidson *et al.*, 1985; Overton *et al.*, 1996). Examples for the crustacea include the clarification of two previously undescribed species of *Procambarus* crayfish (Allegrucci *et al.*, 1992); two species of a porcellanid commensal crab, genus *Liopetrolisthes*, revealed from four colour morphs (Weber and Galleguillos, 1991); confirmation of three distinct biological species of the tropical sponge-dwelling shrimp, *Synalpheus* (Duffy, 1996); and recognition of five species of the freshwater amphipod *Paramelita* (Stewart, 1992).

In an earlier study, Overton *et al.* (1996) observed three clusters when carrying out multivariate analysis of morphometric data from populations of male *Scylla* from four locations in Southeast Asia (Ranong, Surat Thani, Can Gio and Sematan). The third cluster was composed of the black morph collected from Surat Thani. The present study includes three other locations, *i.e.* (Thai Binh, Chanthaburi and Paikgasir) plus additional samples from the original sites (including collection of data for female crabs). It was found in the present, more detailed study, that the black morph of *Scylla* from Surat Thani does indeed belong with the other locations sampled that exhibit the characteristics of the black colour morph. The concordance between the results for male and female mud crabs strengthens the view that two species are represented. This confirms well with a point made by Thorpe (1987) that general taxonomy should be robust, *i.e.* the same conclusions should be valid for both males and females within given taxa.

This study also highlights the importance of collecting material from enough locations to gain an accurate picture of the relationships between morphs of a species. The ordination of groups depends entirely on the number of groups entered into the model and how the groups are assigned. Ordination techniques only have the ability to summarise the relationships between groups and therefore cannot be used solely to make formal taxonomic conclusions; thus referral to actual similarity/distances is needed to provide more detail (Thorpe, 1980).

The significant pairwise D^2 and F-values on which the present ordination analysis was based support the pattern of two clusters. For both sexes, all mud crab groups analysed were found to be significantly different from each other, with the largest distance values observed between groups representing different morphs, including the two morphs collected from Surat Thani. This significant distance between two morphs of *Scylla* living apparently sympatrically within Ban Don Bay (the main coastal feature of Surat Thani) is strong evidence to suggest that these two morphs are in fact separate species.

Sympatric variation in morphology can be due to recent environmental selective forces, which result in dramatic morphological changes between morphs in order for the animals in question to specialise in their feeding, or to live in a particular habitat niche. A good example of this is provided by fiddler crabs, (*Uca* spp.), which show differences in their morphology, including their mouthparts and reproductive structures, which has adapted species to feed on muddy or sandy substrata and prevents hybridization between species inhabiting the same intertidal area (Miller, 1961; Crane, 1975). This is not apparent in *Scylla*, where distance values are greatest between morphs that are most geographically distant. The sympatry seen at Surat Thani among the white and black morphs of *Scylla* is

most likely to be a secondary event that has occurred after a period of allopatry and divergence, but at this stage a hypothesis is only speculative and requires further testing from molecular techniques, such as allozyme electrophoresis, to substantiate it (the subject of chapter five).

In methods that try to discriminate between groups on the grounds of morphology, other types of variation can also be expressed such as temporal, geographic and genetic variation. Variation was found between samples within the two morphs of *Scylla* from Surat Thani. Discrepancies due to such temporal variation can be explained both by natural variation and limitations in the experimental design. Often one of the problems in sampling a population is the inability to represent the phenetic range of that population (Thorpe, 1976). It is possible that 30 individuals are not enough to fully represent the population under study, that each collection is sampling only a small section of the total within-population variation; and that each sample represents a different part of the total variation. These samples were taken at different times resulting in temporal differences being observed.

The greatest temporal variation was recorded for the male crabs. Since the majority of the measurements were from chelipeds, structures which are subject to change in morphology in relation to maturity in male crabs in order to maximise their apparent size (Hartnoll, 1982; Santos *et al.*, 1995), they are most likely to be the cause of the temporal variation recorded in this analysis. Other influences, due to the sampling method, include changes in where the crabs were fished within Ban Don Bay, Surat Thani. Fishermen will change their fishing according to the tidal regime and the presence of other fishermen. Therefore, the fishing sites may not have been consistent between collections. If all these sampling

inconsistencies are taken into account, then another explanation for significant temporal variation is the effect of recruitment from crabs outside Ban Don Bay. Female mud crabs make seasonal migrations, travelling offshore to spawn (Arriola, 1940; Hill, 1975).

Recruitment of juvenile crabs into the Bay is dependent on tides and currents; thus the adult mud crabs will vary from year to year. The ideal scenario would be to sample all selected sites during the same trip. However, this was not logistically feasible and represents a limitation in this research. Similar limitations have been experienced in many studies of this kind (Thorpe, 1979).

All characters contributed to the discrimination between the mud crab groups studied, although some were more significant than others. Five male characters and 12 female characters were found to have an insignificant role in the among-group discrimination. However, canonical variate analysis has the ability to negate the effects of information redundancy by taking into account the within-group covariation between characters, thus preventing any compromising effects in the results (Thorpe, 1976; Tabachnick and Fidell, 1989).

The character that had the most fundamental effect in separating the two morphs of *Scylla* was frontal lobe width ($F=16.35$ and $F=9.29$ for male and females respectively). Overall, the frontal lobe width (the frontal section of the carapace that protrudes between the orbital sockets) was found to be wider in the black morph than the white morph. Other authors have also described the importance of frontal lobe width in discriminating between morphs of *Scylla*. Chayarat and Kaew-ridh (1984) were able to discriminate between three colour morphs of *Scylla* in Thailand using regression analysis between internal carapace width, external carapace width and frontal lobe width. Keenan *et al.* (1998) also noted frontal

lobe width to be one of the more significant characters in separating four genetically distinct groups of *Scylla* collected from a wide geographical range covering the Indo-West-Pacific region.

Right propodus width and the third pereiopod lengths were also found to be highly significant in discrimination between morphs of *Scylla* for both male and female data sets. Although all groups sampled were found to be predominantly right handed, wider claws were exhibited by the black morph in male crabs and by the white morph in females. This probably reflected the maturity of the crabs sampled, as the chelae are known to be under the influence of allometric growth with maturity (Hartnoll, 1982). This is especially the case in male crabs, which may explain the increased number of chelae-related characters significantly contributing the overall between-group discrimination in the *Scylla* male data set compared to that of the female data. This type of ontogenetic variation is difficult to avoid even with the size vector removed and has to be taken into account when assessing the results, especially when dealing with animals of indeterminate age (Thorpe, 1983).

The third pereiopod also proved to be a diagnostic character in *Scylla*. On closer inspection, the data on pereiopod length revealed that, in general, the black morph had relatively longer walking legs than the white morph. When calculating the ratio of third pereiopod merus length/internal carapace width, the ratio value is higher for the black morph (0.42=males, 0.36=females) than for the white morph (0.40=males, 0.35=females). Keenan *et al.* (1998) show comparable mean 3PML/ICW values of 0.472 for *Scylla olivacea* and 0.395 for *Scylla paramamosain* – the species most resembling the black and white morphs respectively in this study. It is also important to note that this character is also sexually dimorphic. In other studies on *Scylla*, females have been noted to have

relatively shorter legs. The males use their longer legs to carry females when the latter are in a mature premoult state before mating (Arriola, 1940). This emphasises the importance of keeping male and female data sets separate, or of identifying and removing sexually dimorphic characters when analysing crab data.

Although the character most associated with size *i.e.* internal carapace width, has been used in previous studies to discriminate between morphs of *Scylla* (Joel and Raj, 1983), the use of size as a discriminatory character has been described as being inaccurate, and in some cases even worthless, in discriminating between populations (Quicke, 1993). Body size is known to have a significant effect on a number of life history traits that are associated with the fitness of the animal and therefore has an associated genetic element (Malhotra and Thorpe, 1997). However, in marine ectotherms, size can also be affected by external environmental pressures, fishing pressure and gear selectivity, which will mask any genetically determined size (Overton, 1994). In animals with indeterminate age (such as crabs), it is especially difficult to use size as a diagnostic character and hence it should be avoided and where possible removed from analysis. The effects of size differences between groups of crabs were found to have negligible effect on the discrimination between colour morphs in this study.

4.4.2 Geographical variation

In addition to racial separation between the two colour morphs, CVA was also used to portray geographic variation between populations of the white morph of *Scylla*. The male and female mud crab network diagrams revealed levels of divergence between adjacent populations, expressed by D^2 values, was disproportionate to the geographical distance between these sites. Moreover, the sums of D^2 values were not equal to the direct D^2

values between the two most remote locations, namely, Thai Binh and Surat Thani. Thai Binh was found to be more diverged with respect to distance when compared to other locations based around the Gulf of Thailand. Clinal variation is revealed when the scores from the first canonical variate for each location is plotted against geographical distances between study sites, although the direction of the cline varies between male and female mud crab data. The difference in the direction of the cline between male and female crab data is most likely due to the discriminant function analysis extracting the first vector from a different angle through the cloud of data points, resulting in reversed signs for the first canonical variables; this does not affect the explanation of the results.

The discrepancy between distances expressed by the network diagrams and the apparent clinal variation can be explained by both genetic and environmental factors. From a genetic perspective, interbreeding between formerly isolated conspecific populations may result in clinal variation (Futuyma, 1986). *Scylla* has a pelagic larval phase of about 30 days in tropical waters (approximately 29°C), though this will vary depending on ambient water temperatures (Heasman, 1980, Brown, 1993), thus the recruitment pattern (*i.e.* dispersion and ingression) of juvenile crabs relies partly on the nature of the coastal tides and currents. The marked monsoonal current reversal in the Indo-West-Pacific (Longhurst and Pauly, 1987), especially along the western coast of the South China Sea (Dale, 1956), would provide a vector for geneflow for young recruiting crabs, both northerly and southerly, along the coastline and thus provide a vector for geneflow between populations. However, the discrepancy in the D^2 distance values suggests that there is a closer relationship between those crab locations around the Gulf of Thailand than the population from Thai Binh Province. Currents within the Gulf of Thailand are fairly complex resulting in a strong likelihood that recruitment remains within the Gulf (Sadrul, 1975).

Therefore the crabs from locations within the Gulf of Thailand could be isolated from the crab populations along the Vietnamese coastline. However, this is only an assumption and requires supporting evidence from molecular data on crabs from these locations to look at the genetic relationship between locations along the western coastline of the South China Sea, (the subject of chapter five), as well as better understanding of the recruitment process for juvenile *Scylla*.

Geographic variation in crab morphology will also be influenced by localised external environmental selection pressures (Futuyma, 1986). This includes conditions such as temperature or rainfall that change gradually according to latitude. It is common to find morphological changes in many plants and animals and correspond to such changes with latitude (Cox and Moore, 1993).

Ectotherms are more sensitive to their external environment than endotherms (Thorpe, 1980). A study on thread-fin bream from the west coast of the South China Sea showed that the presence/absence of growth rings depended on the degree of variation in seasonal water temperature (a temperature change of 4-5°C is required to produce growth rings). A clinal variation in the visibility of growth rings corresponded to the latitudinal position from where the fish were caught; the further north the more prominent the growth rings (Longhurst and Pauly, 1987).

Such clinal variation will also affect *Scylla*. Thai Binh is located in a subtropical region where the annual temperature varies by around 24°C, dropping to 14°C in the winter (Tri, 1997). It is known that *Scylla* slows down its feeding when temperatures are between 12-16°C (Hill, 1980) and thus growth is retarded. It is also possible that these temperatures

affect the variety of food items available to mud crabs. The right propodus width, left propodus length, left carpus length and right carpus width were found to contribute significantly to the cline observed for female crabs, while right propodus width and right carpus widths contributed significantly to the cline seen in the male data. The right chela was found to be smaller in both male and female crabs from Thai Binh, compared to mud crabs from those sites nearer to the equator. Seed and Hughes (1995) showed that claw size did vary with latitude in a range of brachyuran crabs; smaller claws were found on crabs from more northerly latitudes, which they believed was related to the kind of food items available. As it is difficult to age crabs, it is not known whether the observed geographic variation is an effect of maturity, genetic or external environmental influence, such as diet.

It is possible however that a few, widely scattered locations sampled resulted in a pattern of clinal variation that is purely incidental and/or is a gross simplification of a potentially more complex pattern of geographic variation. This would also explain the contradiction between a smooth cline and the discrepancies between the distance values calculated between mud crabs from different locations. Thus only limited conclusions can be drawn from these results without a much more thorough investigation.

4.4.3 Classification of individuals

In the most recent publication on the revision of the taxonomy of *Scylla*, four species are proposed within the genus; namely, *Scylla olivacea*, *S. tranquebarica*, *S. serrata* and *S. paramamosain* (Keenan *et al.*, 1998). When morphometric data from samples of these four species are entered along with the data clusters generated from this study, the

discriminant function analysis resulted in mixed success in assigning the two colour morphs to the particular species of *Scylla* proposed by Keenan *et al.* (1998).

In the analysis performed, *Scylla olivacea* was very closely associated with the black morph, but unfortunately only male crab specimens were available. *S. tranquebarica* was also found to be closely associated to the black morph for both male and female data. On closer examination, it was found that the frontal lobe width was the main contributor to the classification of typed species to the unknown clusters. On visual examination, the frontal lobe formations of the species proposed for each, namely *S. olivacea* and *S. tranquebarica*, are very similar as both exhibited rounded, blunt teeth between the orbital sockets. Therefore, it is possible that both species were collected at the same time, but identified as only one species. This highlights the problem of identifying these proposed species solely on the basis of their morphology.

The cluster representing the white morph of *Scylla* was more difficult to assign to one of the four proposed species. *S. serrata* most definitely has external characters that are similar to the white morph collected in the present study, but is not affiliated to the white cluster *per se*. Three problems prevented this species being assessed properly; a) only one individual was measured as all the other specimens were badly damaged during transportation (from Australia to Denmark) making measurement impossible; b) the one specimen measured had been preserved in fixative, and was also in poor condition; and c) it was much larger than the crabs in all other groups sampled (as there was only one individual, size could not be removed using MGPCA as one individual cannot be considered a group).

Scylla paramamosain was the most difficult to associate to any group as its affiliation jumped between the white and black morph clusters according to the analysis conducted. This outcome was not expected, as the gross external characters of *S. paramamosain* resemble the characters of the *Scylla* white colour morph (as explained in chapter three). As in the case of *S. serrata*, the specimens of *S. paramamosain* were also preserved. Shrinkage due to preserving agents, plus the small jars, in which the specimens were kept, could have distorted the original shape of the specimens making their affiliation more ambiguous. Moreover, the samples of *S. paramamosain* were very small and appeared to be juveniles. It is known that these very similar looking crab species are almost impossible to separate morphologically until they are adults (Overton personal observation; C. Keenan personal communication).

If this ordination analysis was the sole technique for interpretation of the genus *Scylla*, from Keenan *et al.*'s (1998) revision of the taxonomy to four proposed species, *S. olivacea* and *S. tranquebarica* in this study would be considered to be one species. *Scylla serrata* would be considered an outlier as there are no other individuals to support its ordination and *S. paramamosain* would be seen as a possible hybrid, lying intermedially between black and white morphs. It is only because there is genetic evidence supporting the groups assigned by Keenan *et al.* (and as shown in chapter five) that this conclusion is known to be incorrect. It is still possible, however, that *S. olivacea* and *S. tranquebarica* are more closely related to each other than to the other two *Scylla* species nominated by Keenan *et al.* (1998).

4.4.4 Limitations of multivariate morphometrics in the taxonomy of *Scylla*

Although multivariate morphometric analysis has been used successfully to discriminate between morphs of *Scylla*, this technique is limited in its capacity to identify the actual species present. The majority of examples from studies that have used multivariate techniques also combined molecular data with multivariate morphometrics to arrive at their final taxonomic conclusions. Examples where morphometrics have provided a supporting role in discriminating between crustacean species include two species of *Liopetrolisthes* (Weber and Galleguillos, 1991), five species of *Paramelita* (Stewart, 1992) and three species of *Synelpheus* (Duffy, 1996). In some cases however, multivariate morphometrics have been shown to be superior at discriminating between genetic stocks or conspecific populations than multivariate analysis of gene frequencies, as in the case of distinction between Robertsonian populations of the tobacco mouse, *Mus musculus* (Thorpe *et al.*, 1982).

A morphometrics study such as this one is also restricted in providing information about the relationship between the two colour morphs of *Scylla* other than that they are significantly different from each other. Thorpe (1987) mentions how multivariate morphometrics cannot be used for naming species, but can be used to give information on the phenetic criteria that can be employed to discriminate between populations. Although the two clusters derived from this analysis representing the two colour morphs of *Scylla* are separate, with no evidence of chain-linking, this is not proof that reproductive isolation is taking place between the two morphs, the mechanism argued to be one of the primary criteria to establishing species status (Avice, 1994). Moreover, ordination techniques (including the use of D^2 distances) do not give any systematic information, such as the rate or direction of evolution between taxa of interest (Thorpe, 1979).

Other limitations with discriminant function analysis as a technique are associated with the biological assumptions made by the use of such a technique to discriminate between groups of taxa. Discriminant function analysis requires the official taxonomic units to be predefined into groups. However, even though the data are organised into groups beforehand, it does not affect the ability of this technique to be used as a taxonomic tool, as it does not presuppose the taxonomy as long as the assumptions of the biological model used by DFA are met (Thorpe, 1983; 1987). The biological model is fairly strict and has the following assumptions. Firstly, one must deal with a single species/morph in each group assigned. Grouping samples can be difficult especially when there are subtle differences between morphs or closely related species. Secondly, CVA/DFA requires the equality of within-group covariance matrices. This may not be possible, especially when there is geographic variation in the intra-locality correlation and variance. If a character is invariant in most groups, but variable within one or two groups, there will be a distortion of the results. Heteroscedascity (*i.e.* an inequality of sign and magnitude within groups of individuals) is most likely to occur where entire taxa are used as groups, irrespective of their origin, rather than grouping individuals according to location, thereby introducing geographic variation to within-group values. This is the most likely factor affecting the results of clustering in *Scylla* groups observed in Keenan *et al.* (1998). Thirdly, ordination techniques require the data to be statistically normal (ellipsoidal) in their distribution. However, it has been shown that even when these three assumptions are not all satisfied, the results of CVA/DFA are still valid (Pimental, 1979). Tabachnick and Fidell, (1989), for example, showed that CVA is robust to skewness in the data set.

The results obtained from using ordination techniques also depend on the type of characters entered into the model. Phenotypic characters are disadvantaged in that they are

influenced both by genetic and environmental factors. A degree of morphological divergence between morphs, or locations, may not imply the same degree of genetic divergence (Lindenfelsner, 1984, cited by Weber and Galleguillos, 1991). Thus the divergence recorded in the form of D^2 distances may be only a measure of environmental differences. Moreover, if the environment is homogenous between morphs it does not mean that the difference recorded is genetic, as this assumption fails to consider the effects of the environment on earlier life history stages (Davidson *et al.*, 1985). Morphometrics, however, do have some advantages that may make them favourable as discriminatory characters. Morphometric characters are readily recorded and polygenetically controlled, thus reflecting the condition of a large number of loci that can outweigh the effects on the phenotype from external parameters (Thorpe, 1983).

Possibly it is not the use, but the misuse, of ordination techniques which have given them a poor reputation in the past (Thorpe, 1987). It is not the number of characters, but the quality of characters in discrimination between groups revealing the patterns of geographic variation that is of primary importance. Only linear measurements were incorporated in the present study. Without other types of characters being used such as meristics and colouration, it is difficult to assess what mode of speciation is taking place (as shown by Thorpe, 1985). The same geographic or racial pattern in a wide range of characters indicates that sympatric morphs are a result of secondary contact after a substantial period of time, compared to differentiation in a few characters which is often the result of adaptation to current ecological condition (Thorpe, 1987). Combinations of data on meristics and colour patterns have proved to be useful in multivariate analysis of other species (Thorpe, 1979; Bert *et al.*, 1996). It is clear therefore that the inclusion of other

types of characters, including genetic data, can result in a more holistic approach to study various aspects of geographic and racial variation in the genus *Scylla*.

Chapter Five

Allozyme electrophoresis of the genus *Scylla*

5.1 Introduction

With respect to crustacean taxonomy, traditionally crustacean species have been defined on the basis of conspicuous morphological features. However, any variation in ecology, subtle morphology and behaviour has always been suggested as representing the degree of environmentally induced phenotypic plasticity rather than evidence of new species (Knowlton and Jackson, 1994; Duffy, 1996).

Although morphological interpretation of species is still predominantly used in crustacean taxonomy, there are limitations to its application. One of these limitations is in deciphering the species status between closely related taxa, where morphological characters can be shared between species (Knowlton and Jackson, 1994).

In chapters three and four, the classical descriptive taxonomic and multivariate morphometric methods used to distinguish between *Scylla* species were found to have significant limitations, particularly with their inability to distinguish between the degree of environmental influence on the external morphology and that which is genetically inherited. Moreover, the very close resemblance between proposed species suggests that they may in fact be morphs of the same species.

The implementation of molecular techniques in order to clarify species status of crustacean taxa has become a fundamental part of modern crustacean taxonomy. The use of genetics

provides confirmation of species by providing definitive proof of non-breeding between morphs in nature and therefore whether they constitute true species (Quicke, 1993).

Protein electrophoresis is one of the most popular methods used to identify species.

Protein electrophoresis is essentially the migration of proteins under the influence of an electric field. Proteins are formed as chains of amino acids that are encoded by nucleotide sequences within the cell's DNA. Any detectable variation in the protein structure is therefore genetically related (Leary and Booke, 1990). Each amino acid chain has an electrical charge. The net charge of these proteins will vary depending on the structure of the protein molecule and therefore will affect how far these protein molecules migrate through the media with an electric gradient. For one particular group of proteins, namely enzymes, variations are visualised using a variety of staining procedures (Shaw and Prasad, 1970; Harris and Hopkinson, 1976).

Enzyme electrophoresis is capable of revealing distinct types of genetically controlled variation. At any locus (position on a chromosome) there is a code (gene) for a particular enzyme. There may be more than one form of this gene at this locus that codes for a slightly different amino acid sequence. These variants of enzyme codes are called alleles (Murphy *et al.*, 1996). Functionally similar forms of enzymes, including polymers and subunits, produced by different loci or by different alleles are called isozymes. Allozymes are a subset of isozymes expressing variant forms of polypeptides that in turn represent different allelic alternatives for the same gene locus. The procedure and background to allozyme electrophoresis is described in more detail by Richardson *et al.*, 1986; May, 1992 and Murphy *et al.*, 1996.

Allozyme patterns are interpreted in terms of mendelian genotypes at the loci of interest. Since allozymes are codominant in nature, a cross between two homozygotes will result in a clear heterozygote with a different band pattern represented on the gel. Allozyme data, therefore, consists of information on genotypes from a range of loci, which are typically unlinked and scattered through the nuclear genome (Awise, 1994).

Allozyme electrophoresis is a well-investigated genetic method of determining species status between taxa and studying population genetics (Keenan and Shaklee, 1985; Richardson *et al.*, 1986). Although more modern techniques have since been introduced to study genetic aspects of taxonomy and population structure, allozyme electrophoresis proves to be the most cost effective method of genetic investigation (Murphy *et al.*, 1996).

Allozyme electrophoresis has been employed in crustacean studies as early as the 1960s (Manwell *et al.*, 1967; Furst and Nyman, 1969). The technique is still being used in more recent crustacean studies, especially for the identification of closely related species (*e.g.* *Carcinus* spp., Bulnheim and Bahns, 1996), geographic variation (*e.g.* penaeid shrimp, Benzie *et al.*, 1992; Smith, 1999) and stock delineation (*e.g.* *Callinectes sapidus*, Bryars and Adams, 1999).

5.1.1 Identification of species boundaries

In population studies, taxonomy, phylogeny and evolutionary studies species are often referred to as the basic unit of analysis (Thorpe and Sole-Cava, 1994). The biological species concept is the most popular for defining species status (as described in chapter one). The criteria for defining a species under this concept is described as “groups of actually or potentially interbreeding natural populations which are reproductively isolated

from other such groups” (Mayr, 1963). The results of allozyme electrophoresis follow the biological species concept where a single fixed difference of relative allele mobility between two taxa of interest which are sexually reproducing indicates that there is no introgression between these two populations, advocating the recognition of two species (Thorpe and Sole-Cava, 1994). Moreover, allozymes that are fixed within populations can be used as diagnostic markers. This is particularly relevant to phylogenetic analysis, as there is a tendency for oversplitting of taxa when using other methods of species identification such as mtDNA (Moritz *et al.*, 1992). The use of allozyme electrophoresis to confirm species status in closely related crustacea is well recorded (Knowlton, 1993), *e.g.* the identification of a new species of river crab, genus *Potamonoutides* (Stewart and Cook, 1998).

Studies using genetic techniques to identify the species of the genus *Scylla* have been very recently published (Fuseya and Watanabe, 1996; Keenan *et al.*, 1998; Keenan, 1999a and Sugama and Hutapea, 1999). Both Fuseya and Watanabe (1996) and Sugama and Hutapea (1999) have identified three species within the genus *Scylla* using allozyme electrophoresis unlike Keenan *et al.* (1998) who describe a fourth species. All three publications use different nomenclature to attribute names to their proposed species. These three studies have also concentrated on identifying the number of species from a limited sample size over a large geographical range. This part of the present study however, has concentrated on collecting reasonable sample sizes and aims to confirm the species status of the genus *Scylla*.

5.1.2 Population genetics of crustacean species using allozyme electrophoresis

It is important to study natural populations of crustaceans in order to understand what forces are responsible for the maintenance of natural populations as well as evaluating what genetic resources are available to future crustacean breeders for aquaculture purposes (Hedgecock, 1987). The general purpose of conducting such studies is to determine the extent of genetic variation within and between conspecific populations. Such information provides evidence to distinct evolutionary events (Weir, 1990). This information also allows the researcher to investigate the population structure and therefore the stability of these heavily exploited crustaceans, which would not be obvious from external morphology.

Many evolutionary forces influence population structure, which also have a role in speciation events. These include the diverging effects of mutation, random genetic drift, and natural selection as opposed to the cohesive properties of geneflow (including migration). These are outlined in appendix I.

5.1.2.1 Genetic variation in crustacean populations

Genetic variation within populations is measured in terms of polymorphism and heterozygosity. Polymorphism is the frequency of polymorphic alleles with respect to the total number of alleles surveyed. Heterozygosity however, is the frequencies of heterozygous loci per individual. As these parameters are different in their perspectives they are usually both measured (Leary and Booke, 1990).

Studies of genetic variation within crustacean species have revealed large variation in the levels of both heterozygosity and polymorphism. Much of the literature describes decapod

crustaceans has having low levels of genetic variation compared to other crustacean species. In attempts to explain this low level of genetic variation, both neutral and selectionist views have been described. In neutral theory, low level genetic variation is interpreted as the result of population bottlenecks (refer to appendix I) or local extinctions (Kimura, 1983). However, the selectionist view suggests that the differences in heterozygosity between difference types of crustacean taxa are due to environmental heterogeneity where the genetic variability plays a role in the adaptation of decapod crustaceans to their environment (Nelson and Hedgecock, 1980). It is difficult to predict which interpretation is the correct one (Lavery and Fielder, 1993). In most circumstances a combination of both views have been used to interpret the geographical variation within populations of crustacea, for example, in populations of the coconut crab, *Birgus latro* (Lavery and Fielder, 1993).

5.1.2.2 Genetic differentiation between crustacean populations

In addition to the genetic variation within populations, the degree of genetic differentiation between conspecific crustacean populations may determine the degree of stock structure present between locations. The “stock”, rather than the species as a whole, has been advocated as the operational unit with respect to fisheries management of economically important crustacean species (Lavery and Keenan, 1994). Traditional approaches to studying population ecology involves marking and recapture of individuals in order to estimate population size and estimate the rate of migration between populations (Moritz and Lavery, 1996). However, the traditional approaches are limited in that they only recorded the populations of interest in a restricted area and over a limited time period (Moritz and Lavery, 1996). Molecular studies however can provide a picture of both broad-scale and long-term population processes (Avisé, 1994). Allozyme electrophoresis

is the most popular technique investigating differentiation of taxa. In particular, it has been used on crustacean species (*e.g.* penaeid shrimp, Mulley and Latter, 1981), including crabs (Kordos and Burton, 1993; McMillan-Jackson *et al.*, 1994; Bryars and Adams, 1999).

As a result of the confusion over the taxonomic status of the genus *Scylla*, population structure and geographic variation of conspecific mud crab populations using molecular techniques has not been studied. In this particular part of the study the main aims were to understand the pattern of geographical variation for the two predominant phenotypes collected from selected study sites within Southeast Asia.

5.2 Materials and methods

5.2.1 Sample collection

Adult mud crabs, ranging from 150g to 250g fresh weight, were collected from four locations in Southeast Asia, namely, Ranong (Andaman Sea coast of Thailand), Surat Thani (Western Gulf of Thailand), Chanthaburi (Eastern Gulf of Thailand) and Thai Binh Province (Western Gulf of Tongking, Northern Vietnam). These locations are illustrated in figure 5.1. Choice of location and the criteria used for crab collections have been described in more detail in Chapter two. Table 5.1 shows the number of individuals collected from each sample location. Two collections of each of the two colour morphs, denoted as “black” and “white”, were made from the main study site, Surat Thani.

Additional reference material on *Scylla* was consulted on the matter of speciation. Six female crabs that had been recently reclassified as *Scylla serrata* (Keenan *et al.*, 1998) were received from Queensland, Australia. In addition, six crabs classified as *Scylla tranquebarica* and five crabs classified as *Scylla olivacea* were received from Sematan,

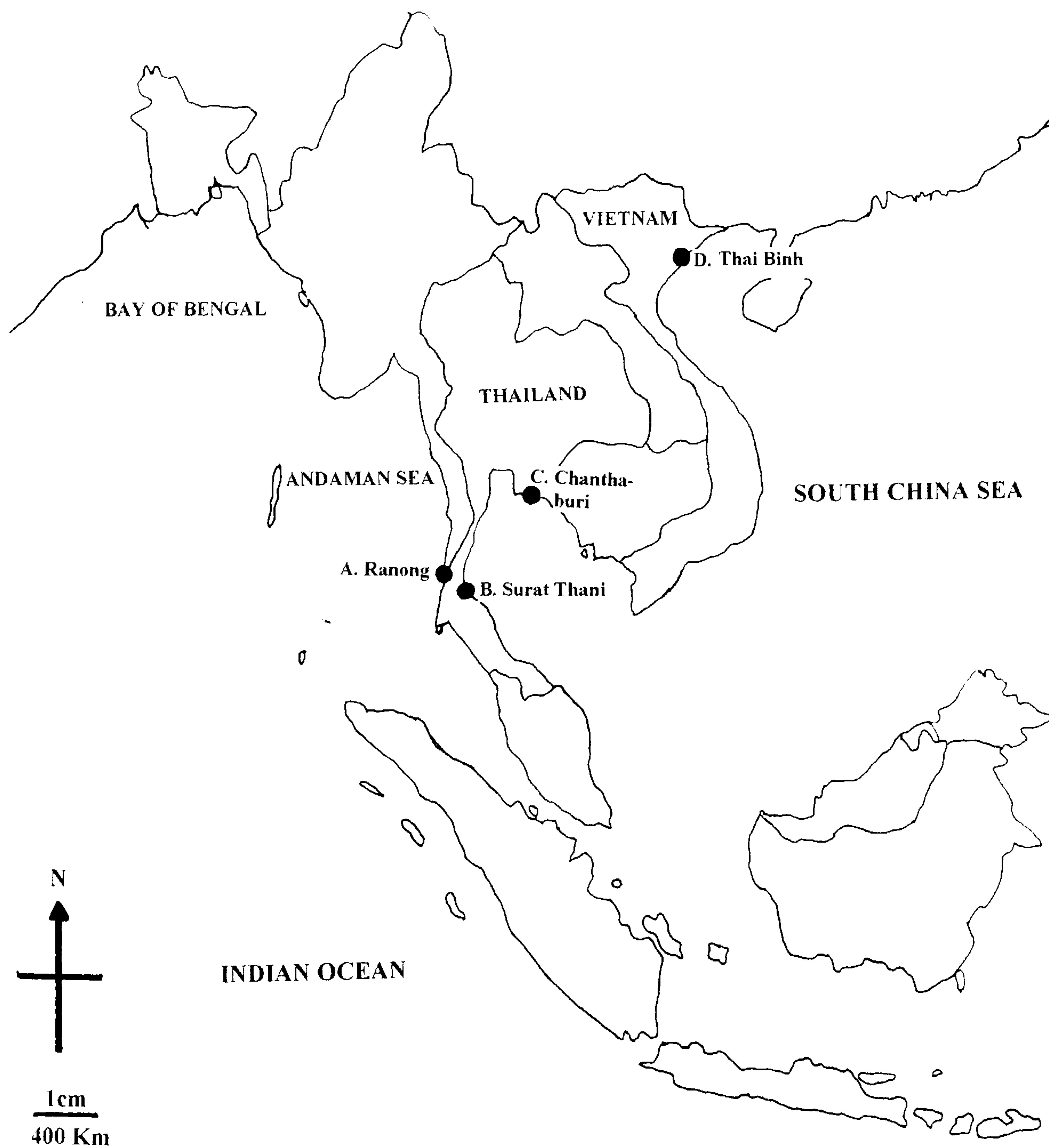


Figure 5.1: Locations of collecting sites for starch gel electrophoresis of mud crab (genus *Scylla*) from Southeast Asia.

Table 5.1: Number of individuals of the genus *Scylla* collected from four locations in Southeast Asia for allozyme electrophoresis.

Location	Number of Individuals		
	Male	Female	Total
Ranong	32	12	44
Surat Thani “black” year1*	19	25	44
Surat Thani “black” year 2	32	34	66
Thai Binh	13	29	42
Chantaburi	12	12	24
Surat Thani “white” year 1	15	25	40
Surat Thani “white” year 2	20	27	47
Total	143	164	307

* Year 1 = 1995; Year 2 = 1996

Sarawak, Malaysia. However, due to the low numbers of individuals obtainable from these sites, no population analysis was carried out on these specimens.

5.2.2 Allozyme electrophoresis

The methodology for allozyme electrophoresis has been documented extremely well. The methods used in this study were derived from a combination of sources, including Harris and Hopkinson (1976), Shaklee and Keenan (1986), Aebersold *et al.* (1987) and Pasteur *et al.* (1988). All recipes (as described in appendix III) are referenced from their original source, although they were modified in order to obtain the best results.

5.2.2.1 Tissue preparation

The crabs were dissected for two main tissue types, namely white muscle and hepatopancreas (digestive gland). The crabs were sedated before dissection using iced water. Each tissue type was dissected and placed into labelled 1.5ml polyethylene microcentrifuge tubes (Hounisens Laboreudstyr, Denmark) and immediately frozen in liquid nitrogen prior to storage at -80°C until further processing.

It is known that there may be variations in the protein and general biochemical composition of the different muscles in the crab, for example tail muscle can be significantly different in biochemical composition from walking leg muscle (Shaklee and Keenan, 1986). To ensure conformity in the results, care was taken to sample from only one part of the anatomy, namely the muscles attached to the swimming legs (pleopods) and walking legs (pereiopods).

Further processing of the crab material was necessary to a) dilute the enzymes concentrated in the tissues of interest; and b) to release the enzymes from the tissue by rupturing cells and expelling their contents into an appropriate medium. This was achieved by homogenizing an equal weight:volume of tissue with homogenising buffer (see appendix III for recipe) using a loose-fitting glass homogeniser attached to a motorised rotary system. The homogenates were then centrifuged at 7,500 rpm for three minutes in a refrigerated high-speed centrifuge to remove any excess tissue and cell debris from solution. The resulting supernatant was decanted and transferred to labelled 500 μl polyethylene tubes in 100-200 μl aliquots before being returned to frozen storage (-80°C) until required for electrophoresis.

5.2.2.2 Gel preparation

Gel buffers were prepared as described in appendix III. The starch gels were prepared using 12% weight:volume of hydrolysed starch (Connaught Laboratories Ltd, USA). Powdered starch was weighed out into a 500ml Pyrex flask and mixed with a third of the total volume of gel buffer required to make the gel. The remaining gel buffer solution was heated in a Pyrex round-bottomed flask which was seated in a heating mantle (Isopod, Labsafe). Once the heated buffer was at boiling point, the starch solution was stirred in using a rotary stirrer (RW 20 Janke and Kunkel, IKA Werk) until a homogenous gel solution was formed. The gel mixture was allowed to bubble until it turned slightly transparent and became less viscous in consistency. The gel was then degassed using a water aspirator (SUE 30, Heto) until all the air bubbles disappeared. The hot gel was poured into labelled Perspex formers (150mm x 240mm x 8mm) and allowed to set. Once firm to the touch, the gels were covered with an acetate film and cling film to prevent desiccation and were then stored at room temperature until use.

5.2.2.3 Gel loading

The prepared gels were cooled on electrophoresis chill plates set at 4°C prior to loading (LKB – MultitempII, Pharmacia). The sample extracts were removed from cold storage and placed on crushed ice, allowing them to thaw. The cold gels were then removed from the chill plates. An origin was made in the gel by cutting a parallel line through the gel 4cm from the edge of the longest side of the gel mould with a scalpel. 3mm x 6mm filter paper wicks (Whatman qualitative filter paper no.4) were used to absorb the sample extracts. These paper wicks were blotted to remove any excess fluid and placed on the origin by carefully separating the cut edges of the gel and placing the wicks on one of the cut edges using a pair of fine forceps. Care was taken not to contaminate neighbouring

individuals by providing equal spacing between each sample. Paper wicks soaked with a marker dye (0.1% bromocresol green) were used to track the migration rate of sample enzymes through the gel during electrophoresis. These were positioned at both ends and in the middle of the gel in order to check that all the samples migrated across the gel at the same rate during electrophoresis.

5.2.2.4 Electrophoresis

The loaded gels were returned to the chill plates positioned on electrophoresis baths (LKB-Multiphor II, Pharmacia) already containing the electrode buffer solution. 230mm and 110mm filter paper bridges (Whatman qualitative filter paper No.1) were soaked in the electrode buffer and placed on the longest edges of the gel forming a connection between the electrophoresis bath and the gel. Acetate sheets were placed on top of the loaded gels to help prevent desiccation during electrophoresis. The gels were run at a constant voltage or amperage depending on the buffer type being used (power supplied by electrophoresis power supply – EPS 600, Pharmacia). Electrophoresis conditions are outlined in appendix III. Once the markers had migrated across three-quarters of the gel, the electrophoresis time was noted and the gels were removed from the electrophoresis baths and prepared for slicing.

5.2.2.5 Gel Slicing

The migration of the electrophoresis markers from the origin was measured with a ruler and recorded on the data sheets. The gels were trimmed to a width of 108mm, removing the anodal and cathodal ends of the gel that had been in contact with the electrode wicks. A small diagonal notch was made in the top right hand corner of the gel as an orientation mark to the order of individuals loaded on the gel. The gel was sliced into three 2mm thick

slices and placed into Perspex staining trays (116mm x 244mm x 20mm), making sure that the cut side of the gel was facing uppermost.

5.2.2.6 Gel Staining and recording

The stains were made up in accordance to the recipes described in appendix III. In most cases the stained gel slices were incubated in an oven (Hereaus – T5042) set at 37°C, *i.e.* the optimal temperature for the enzyme reaction to take place that produces the allozyme bands which were subsequently recorded onto data sheets. Harris and Hopkinson (1976) and Richardson *et al.* (1986) give a more detailed account of enzyme systems and the staining reaction pathways.

To provide a permanent record of the results from each experiment, the stained gels were photographed on a light box using a mounted camera (Nikon, Japan) and 35mm colour film (400ASA).

5.2.3 Screening enzymes

As no standard protocols were available for *Scylla*, screening of a number of enzyme systems was necessary to locate those enzyme/buffer systems that showed an adequate level of enzyme activity and resolution to be scored accurately. Initially 41 enzymes were screened on nine buffer systems and their results noted. Those systems that showed potential were modified to improve the resolution and/or activity of the enzyme. Table 5.2 gives a summary of these particular enzyme systems, including the buffers used and the number of loci expressed.

Table 5.2: Summary of enzyme loci examined in mud crab in the present study, their subunit structure, buffer conditions and tissue type (M = muscle; HP = hepatopancreas) for each locus.

Enzyme	Abbreviation	Enzyme no.	Buffer(s)	Subunit structure	Locus	Tissue type
Aspartate aminotransferase	Aat	2.6.1.1	1	Dimeric	<i>Aat-1*</i>	HP
Arginine kinase	ArgK	2.7.3.3	2	Dimeric	<i>ArgK*</i>	M
Aconitase	Aco	4.2.1.3	1	Monomeric	<i>Aco*</i>	HP
Alkaline phosphatase	Akp	3.1.3.1	3	Monomeric/dimeric	<i>Akp*</i>	HP
Alanine aminotranferase	Alat	2.6.1.2	3	Dimeric	<i>Alat*</i>	M
Diaphorase	Dia	1.6.2.2	4, 6	Monomeric/dimeric	<i>Dia*</i>	HP, M
Formaldehyde dehydrogenase	Fdh	1.2.1.1	3, 5, 6, 8	Dimeric	<i>Fdh*</i>	M
Glucose-6-phosphate isomerase	Gpi	5.3.1.9	1	Dimeric	<i>Gpi*</i>	M
Isocitrate dehydrogenase	Idh	1.1.1.42	3, 5	Dimeric	<i>Idh*</i>	M
Leucine aminopeptidase	Lap	3.4._._	1, 7	Monomeric	<i>Lap*</i>	HP, M
Lactate dehydrogenase	Ldh	1.1.1.27	5, 6	Tetrameric	<i>Ldh*</i>	M
Malate dehydrogenase	Mdh	1.1.1.37	5	Dimeric	<i>Mdh-1*, Mdh-2*</i>	M
Mannose-6-phosphate isomerase	Mpi	5.3.1.8	3	Monomeric	<i>Mpi*</i>	M
Peptidase B	Pep-B	3.4._._	1	Monomeric	<i>PepB-2*</i>	HP, M
Phosphoglucomutase	Pgm	2.7.5.1	1, 9	Monomeric	<i>Pgm*</i>	M
Triosephosphate isomerase	Tpi	5.3.1.1	1, 7	Dimeric	<i>Tpi-1*, Tpi-2*</i>	HP, M

Buffers: **1** = Triethanolamine/citric acid pH 7.2; **2** = Tris/malate pH 8.0; **3** = Tris/malate pH 7.4; **4** = Tris/citrate pH 6.7; **5** = Tris/citrate pH 8.0; **6** = Tris/EDTA/borate pH 8.6; **7** = CAM pH 6.1; **8** = Histidine pH 6.1; **9** = CAM(EDTA) pH 6.8

5.2.4 Allozyme interpretation

Loci were labelled numerically starting with the slowest locus, *i.e.* the locus that travelled least from the cathodal end of the gel. The locus with the slowest migration was labelled one, the next slowest was labelled two, and so on. Within each locus, alleles (represented as bands on the gels) were numbered according to their relative mobilities. The most common allele was labelled 100. Other alleles were assigned numbers relative to the most common allele by measuring its distance from the origin, dividing this distance by the distance travelled by the most common allele, and multiplying the result by 100. This method follows that described by Shaklee and Keenan (1985).

5.2.5 Data analysis

Hardy Weinberg proportions (HWP) were tested using an “exact HW test”, as described by Guo and Thompson (1992), for all populations sampled. Exact tests are preferable for small samples sizes (populations fewer than 200 individuals) and/or when allele frequencies are small or zero. This test uses the complete enumeration method to calculate the exact P-value (Louis and Dempster, 1987). This method works for a maximum of four distinct alleles at each locus within each population. The exact test was used to test conformity to HWP at each locus. A global test (using Fishers’ method) across all loci was completed resulting in overall P values for each population.

Both temporal and spatial aspects of population differentiation were tested (where data was available) using exact tests for both genic and genotypic differentiation. Genic differentiation was performed on a RxC contingency table using an unbiased estimate of the P-value of the probability test (also known as Fishers exact test) (Raymond and Rousset, 1995a). Genotypic differentiation was tested using an unbiased estimate of the P-

value of a log likelihood (G) based exact test (Goudet *et al.*, 1996). This G-based test was derived from the same principles as the probability test, except that it was computed from a genic table derived from a genotypic one (Goudet *et al.*, 1996). The Markov chain method of computation devised by Raymond and Rousset (1995b) was used for both genic and genotypic tests. Global values across loci were computed using Fishers method, the main assumption being that there is statistical independence across loci. The exact tests for evaluating population differentiation were proved by Raymond and Rousset (1995a) and Goudet *et al.* (1996) to be more powerful than the traditional method of using F_{ST} -estimator tests defined by Wright (1969), especially when sampling is unbalanced. However, since the majority of authors have used F_{ST} estimator tests in their calculation of population differentiation, these have been included for comparative purposes. F statistics were calculated for single populations. Values for F_{ST} (equivalent to Nei's G_{ST}) were calculated according to Wright (1978) and θ values ($\theta = F_{ST}$) were estimated using “weighted” analysis of variance as described by Weir and Cockerham (1984). Estimates of geneflow were calculated as the number of migrants per generation using F_{ST} (or θ) values in the formula:

$$N_{em} = [(1/F_{ST})-1]/4 \text{ (Wright, 1978).}$$

Average heterozygosities (genetic diversity) were calculated using Nei's unbiased estimate (1978) in conjunction with observed heterozygosities (H_o), the average number of alleles per locus (a) and the percentage of polymorphic loci (p) for each location. The calculations for Nei's unbiased estimate (1978) of average heterozygosity are described in appendix IV.

The software packages GENEPOP version 3.1b (Raymond and Rousset, 1998) and BIOSYS-1 (Swofford and Selander, 1989) were used for the analysis of the allozyme data.

5.3 Results and discussion

5.3.1 Description and interpretation of allozymes

From a range of electrophoresis buffers and staining procedures, 18 loci were resolved and well differentiated enough to be scored accurately. Of these 18 loci, nine were found to be invariant and fixed for the same allele across all *Scylla* populations sampled. These monomorphic loci were *Dia*, *Fdh*, *Idh*, *Lap*, *Ldh*, *Mdh-1*, *Mdh-2*, *Tpi-1* and *Tpi-2*. *Argk* was also found to be monomorphic across the sample locations of this study, although this locus was fixed for an alternative allele in the reference samples from Australia. This is discussed in more detail in section 5.3.2. Four loci, namely *Alat*, *Mpi*, *PepB-1* and *Pgm*, were found to be monomorphic for all populations sampled, but were fixed for alternate alleles which related to morphology and were therefore considered species diagnostic (as described in Section 3.3.2). The remaining four loci (*Aat-2*, *Aco*, *Akp* and *Gpi*) were polymorphic for the majority, if not all the sampled locations. Each variable and diagnostic locus used in this study is described in more detail in sections 5.3.1.1 - 5.3.1.8. The relative mobilities for each allele and its frequencies in each population are summarised in table 5.3.

5.3.1.1 Aspartate Aminotransferase (*Aat*)

The crab hepatopancreatic tissue expressed two loci for aspartate aminotransferase. *Aat-1* stained too weakly to score accurately. *Aat-2* was found to be polymorphic (with two alleles; 100 and 125) in populations of the “black” morph from both the Andaman and South China Sea. The common allele was fixed for the samples collected from Chanthaburi, and the Surat Thani “white” morph collected in year one (1995). This locus was however, found to be polymorphic for Thai Binh and Surat Thani “white” in year two (1996).

Table 5.3: Summary table of allele frequencies for the mud crab, *Scylla* spp. collected from four locations in Southeast Asia.

Locus	Allele	LOCATION						
		"Black" morph			"White" morph			
		Ranong	ST year 1	ST year 2	Thai Binh	Chanthaburi	ST year 1	ST year 2
<i>Aat-2</i>	(n)	(36)	(42)	(66)	(40)	(24)	(40)	(47)
	1 125	0.403	0.060	0.015	0.050	0.000	0.000	0.011
	2 100	0.597	0.940	0.985	0.950	1.000	1.000	0.989
<i>Aco</i>	(n)	(42)	(41)	(66)	(40)	(24)	(40)	(46)
	1 115	0.131	0.012	0.030	0.600	0.646	0.575	0.446
	2 100	0.821	0.915	0.947	0.363	0.354	0.400	0.543
	3 85	0.048	0.073	0.023	0.038	0.000	0.025	0.011
<i>Akp</i>	(n)	(41)	(43)	(65)	(27)	(23)	(35)	(0)
	1 100	0.988	1.000	1.000	0.037	0.043	0.129	no data
	2 91	0.012	0.000	0.000	0.907	0.957	0.571	
	3 81	0.000	0.000	0.000	0.056	0.000	0.300	
<i>Alat</i>	(n)	(44)	(44)	(66)	(42)	(24)	(40)	(47)
	1 100	0.000	0.000	0.000	1.000	1.000	1.000	1.000
	2 75	1.000	1.000	1.000	0.000	0.000	0.000	0.000
<i>Gpi</i>	(n)	(44)	(41)	(66)	(42)	(24)	(40)	(47)
	1 130	0.239	0.110	0.121	0.036	0.021	0.063	0.053
	2 100	0.750	0.890	0.871	0.929	0.917	0.913	0.936
	3 55	0.011	0.000	0.008	0.024	0.021	0.013	0.000
	4 20	0.000	0.000	0.000	0.012	0.042	0.013	0.011
<i>Mpi</i>	(n)	(44)	(44)	(66)	(42)	(24)	(40)	(47)
	1 100	0.000	0.000	0.000	1.000	1.000	1.000	1.000
	2 95	1.000	1.000	1.000	0.000	0.000	0.000	0.000
<i>PepB-1</i>	(n)	(44)	(44)	(40)	(40)	(23)	(40)	(47)
	1 100	0.000	0.000	0.000	1.000	1.000	1.000	1.000
	2 97	1.000	1.000	1.000	0.000	0.000	0.000	0.000
<i>Pgm</i>	(n)	(44)	(44)	(66)	(42)	(24)	(40)	(47)
	1 100	0.000	0.000	0.000	1.000	1.000	1.000	1.000
	2 85	1.000	1.000	1.000	0.000	0.000	0.000	0.000

Two loci were also recorded for the Aat enzyme for *Scylla* species by Fuseya and Watanabe (1996). In Fuseya and Watanabe's study, the second (slower) of the loci, the slower of the two alleles (100) (which they named "b") was found to be fixed in the white morph (which they called *S. tranquebarica*) collected from Chanthaburi. This is in agreement with the results found for the same locus in this present study.

Both black and white morphs sampled from Surat Thani by Fuseya and Watanabe (1996) (which they call *S. serrata* and *S. tranquebarica* respectively) expressed two alleles ("a" and "b"; expressed as 125 and 100 in the present study) at frequencies of $a = 0.033$ and $b = 0.967$, and $a = 0.031$ and $b = 0.969$ respectively. These frequencies found by Fuseya and Watanabe (1996) support the frequencies found in this present study at the *Aat-2* locus for both phenotypes found within Surat Thani.

The Ranong population from the Andaman Sea was quite different from the South China Sea samples with allele frequencies of 100 allele = 0.597 and 125 allele = 0.403. Allele frequencies for populations of the black morph from Ranong and Surat Thani at the *Alat* locus conform to Hardy Weinberg proportions ($P > 0.05$). Without further sampling from both these particular populations and more extensively from either side of the Thai/Malay peninsular, it is difficult to say whether this is just a single phenomenon, or whether it is proof of east coast-west coast isolation of populations.

5.3.1.2 Aconitase (*Aco*)

Aconitase was interpreted as a single locus with three alleles in the muscle tissue of the mud crab. This locus was polymorphic for all sampled locations regardless of morphology. The common allele was found to be different among the two morphs. The

100 allele was most common for the black morph, whereas the faster 115 allele was more common within the populations of the white morph. Gene frequencies for all populations conform to Hardy Weinberg Proportions for this locus ($P > 0.05$). Aconitase was one of the least expressed allozymes across a range of phyla surveyed by May (1992), so there is virtually no comparative information available in other literature. However, it has been shown here to be a useful polymorphic locus for the study of populations of the genus *Scylla*.

5.3.1.3 Alkaline phosphatase (*Akp*)

One locus was scored for alkaline phosphatase in hepatopancreatic tissue where three alleles were expressed, namely 100, 91 and 81. The fastest (and most common) allele (100) was fixed in the Surat Thani black morph for both years and was found to have a higher frequency in the polymorphic Ranong population, which also expressed the 91 allele. The white morph populations were also polymorphic for this locus. The second allele (91) was expressed at higher frequencies than the other alleles for Thai Binh and Chanthaburi populations. Chanthaburi expressed only two alleles, namely 91 and 100. All three alleles were expressed in the Thai Binh population and in the samples collected from Surat Thani of the white morph. *Akp* would normally be expressed as a dimer with three-banded heterozygotes. During this study two banded heterozygotes were revealed in *Scylla*. However, *Akp* has also been described as having a monomeric form resulting in two-banded heterozygotes (Murphy *et al.*, 1990; 1996) and therefore the results are valid. Due to degradation of tissue samples in the second sampling year for the white populations from Surat Thani, the *Akp* locus for this sample was difficult to interpret and therefore was not scored.

5.3.1.4 Alanine aminotransferase (*Alat*)

A single locus was scored for alanine aminotransferase (*Alat*) expressing two alleles in crab muscle tissue. These alleles were monomorphic and fixed for alternate alleles in the two morphs. The slower allele (75) was fixed for black morph for both Ranong and Surat Thani black populations. The faster allele (100) was fixed for all white populations sampled. Thus *Alat* is a diagnostic loci between the two morphs sampled. Section 3.3.2 discusses and *Alat* and other diagnostic loci in more detail.

5.3.1.5 Glucose-6-phosphate isomerase (*Gpi*)

A single locus was scored for glucose-6-phosphate isomerase (*Gpi*) which was best scored in mud crab muscle tissue. This locus exhibited four alleles. The locus was polymorphic in all locations sampled. The fourth allele (the slowest allele) was only expressed in the “white” populations. Fuseya and Watanabe (1996) discovered three alleles for *Gpi* for populations of the genus *Scylla*. Keenan *et al.* (1995) found a total of six alleles for *Gpi* with a maximum of three alleles being expressed in any particular morph examined. Their three morphs, labelled green (equivalent to “white”), brown (equivalent to “black”) and spined, expressed different allele mobilities except for the common allele (100) (green = 158, 100 and 66; brown = 133, 100 and 66; spined = 100, 42). These mobilities are not concordant with the allele frequencies expressed in the present study, although the same buffer was used in both studies. The fourth allele (20), was only revealed in the present study with a combination of extended running time (over 10 hours) and an increase in the dilution of gel buffer from 1:10 to 1:24 dilution of stock solution.

This present study produced mobilities more in accordance with those discovered by Fuseya and Watanabe (1996) who found two alleles for Surat Thani black population (a and b; equivalent to 130, 100 alleles in the present study) and three alleles for Surat Thani white population (a, b and c; equivalent to 130, 100, and 55 alleles in the present study) although only two alleles were found in populations from Surat Thani whereas four alleles were discovered in the present study. The discrepancy is most likely due to Tris-citrate pH 8.0 buffer being used by Fuseya and Watanabe (1996) rather than Triethanolamine citrate pH 7.2 buffer as used in the present study. It is well known that the pH of the buffer system used can result in a different interpretation of the number of allelic variants present for that particular enzyme (Richardson *et al.*, 1986; Pasteur *et al.*, 1988; Murphy *et al.*, 1990).

Gpi is consistently found to be polymorphic for both plants and animals. May (1992) found that *Gpi* was polymorphic for all fish species and for 90% of invertebrates screened. Within the Phylum Crustacea, Hedgecock *et al.* (1982) found that *Gpi* varied in three out of every four crustaceans examined, as well as 17% of the average crustacean population being heterozygous for the *Gpi* locus.

5.3.1.6 Mannose-6-phosphate isomerase (*Mpi*)

A single locus was revealed for mannose-6-phosphate isomerase (*Mpi*) with two alleles in crab muscle tissue. These two alleles were fixed according to the morphology of the sample collected. The slower allele (95) was fixed for all black crab populations whereas the fast allele (100) was fixed for all white populations. This allele is diagnostic, distinguishing between phenotypes as seen for the *Alat* locus.

5.3.1.7 Peptidase-B (*Pep-B*)

Two loci were revealed for Peptidase-B using hepatopancreas tissue. The second locus (hence the slower in mobility of the two) was very faint, badly resolved and difficult to score but seemed to mimic the first locus and hence could have been a ghost band, although without the presence of heterozygotes it difficult to say with certainty whether this was a ghost band or a second locus. For this reason this second “locus” was not used for scoring purposes. The first locus (*PepB-1*) revealed two alleles which were fixed according to morphology of the crab populations sampled. The slower allele (97) was fixed for the black populations whereas the fast allele (100) was fixed for the white populations sampled. This is a diagnostic locus, distinguishing between the two phenotypes collected from Southeast Asia.

5.3.1.8 Phosphoglucomutase (*Pgm*)

A single locus was expressed for phosphoglucomutase using crab muscle tissue. This locus is composed of two alleles that were fixed according to morphology of the crabs sampled in the present study. The slow allele (85) was fixed for the black morph whereas the fast allele (100) was fixed for the white morph. This diagnostic locus along with *Alat*, *Mpi* and *PepB-1* are discussed in more detail in the next section.

5.3.2 Discriminating loci attributed to morphology

When an allele (gene) is found to be invariant intraspecifically in populations sampled it is said to be fixed (Maynard-Smith, 1989). Species boundaries are typically identified on the presence of diagnostic characters, in this instance the fixed alleles that are diagnostic, *i.e.* their relative mobility distinguishes between species (Richardson et al., 1986). Five of the 18 loci resolved showed fixed differences that discriminated between the two *Scylla*

morphologies, namely “black” and “white” morphs, seen in the populations of mud crab sampled. These loci have been summarised in table 5.4 together with the mobilities exhibited by the additional identified tissue samples from Queensland, Australia and Sarawak, Malaysia purported to be *S. serrata*, *S. tranquebarica* and *S. olivacea* by Keenan *et al.* (1998). Tissue samples representing the fourth species *Scylla paramamosain* were not available for the present study, therefore the mobilities for this proposed species reported by Keenan *et al.* (1998) have been added in the table for comparative purposes. The fixed differences reported by Watanabe and Fuseya (1996) and Sugama and Hutapea (1999) in similar studies have also been included.

Such a clear genetic distinction between the two *Scylla* morphs is a strong indication that two species are present in the populations sampled in this study. Moreover, the two morphs present in the main study site of Surat Thani, Thailand, are clearly two well-defined populations with little evidence of hybridization between them.

Table 5.4 also summarises the allele mobilities expressed in the sampled populations and the additional identified material. Using the same buffer, the sample of mud crabs from Australia are fixed for an alternative allele at the *Argk* locus to the morphs collected in the authors study area. This Australian group of crabs has been identified as the founder species *Scylla serrata* Forskål by Keenan *et al.* (1998).

However, it is questionable on the evidence of one discriminating locus that a group of organisms can be considered a separate species (Richardson *et al.*, 1986). Futuyma (1986) suggests that a single trait difference may only represent polymorphism in a species, although there are enough individuals sampled in this study to suggest that this is not the case. Other authors have contested that one fixed allelic difference is enough to suggest a

Table 5.4: Allozyme mobilities for discriminating loci for the two morphs (“black” and “white”) collected from four locations in Southeast Asia plus additional reference material.

Enzyme	Colour morph		Present Study				Keenan <i>et al.</i> (1998)					Fuseya and Watanabe (1996)			Sugama and Hutapea (1999)			
	Black	White	Additional reference material				Scylla olivacea	Scylla serrata	Scylla tranquebarica	Scylla serrata	Scylla tranquebarica	Scylla paramosain	Scylla serrata	Scylla tranquebarica	Scylla oceanica	Scylla olivacea	Scylla tranquebarica	Scylla paramosain
			Scylla olivacea	Scylla serrata	Scylla tranquebarica													
Number of individuals	154	153	5	6	6	Nd	Nd	Nd	Nd	Nd	Nd	150	81	111	108	72	47	
<i>Adh*</i>	Nd	Nd	Nd	Nd	Nd	75,100	100	75	100	75	75	Nd	Nd	Nd	100	100	100	
<i>Alat*</i>	75	100	75	100	100	95	100	75	100	100	100	Nd	Nd	Nd	Nd	Nd	Nd	
<i>Argk*</i>	100	100	100	133	100	75,100	100	100	100	75	75	Nd	Nd	Nd	Nd	Nd	Nd	
<i>Est*</i>	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	a	b	b	100	80	80	
<i>Lap*</i>	100	100	100	100	100	Nd	Nd	100	Nd	Nd	Nd	b	b	a	Nd	Nd	Nd	
<i>Mpi*</i>	95	100	95	100	100	95,90	100,103	100	100	100	100	Nd	Nd	Nd	100	100	90	
<i>PepB*</i>	97	100	97	100	100	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	
<i>Pgm*</i>	85	100	85	100	100	85	100	107	100	107	100	Nd	Nd	Nd	100	100	85	
<i>Sod*</i>	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	b	b	a	100	100	150	

Nd = no data

separate species provided no heterozygotes are found to suggest a polymorphism. For example, Smith *et al.* (1981) revealed two species of arrow squid based on fixed alleles at one enzyme locus. However, in the study conducted by Keenan *et al.* (1998) they found a second fixed difference at the *Adh* locus, not represented in the present study, that substantiates their claim that *S. serrata* is a separate species. This species is therefore not present in the samples from Surat Thani, Ranong, Chanthaburi and Thai Binh provinces.

The allele mobilities of the “white” populations sampled from the four locations resemble those described by Keenan (1999) for the “fourth” species *Scylla paramamosain*, although there are no data to support the fixed differences expressed at the *PepB-1* locus. The mobilities of all five diagnostic loci for crabs sampled with the “black” phenotype are concordant with those for the samples referenced as *S. olivacea* (Keenan *et al.*, 1998) which also has the typical black morphology.

Fuseya and Watanabe (1996) and Sugama and Hutapea, (1999) distinguish three species of *Scylla* in their respective studies. Fuseya and Watanabe (1996) found three discriminatory loci that were different to the ones discovered by the author and by Keenan *et al.* (1998), namely Esterase (*Est*), L-amino peptidase (*Lap*) and Superoxide dismutase (*Sod*). These loci discriminate one species out of three at each locus. The results of *Est* and *Sod* recorded by Fuseya and Watanabe (1996) are supported by Sugama and Hutapea (1999).

In all four studies, it is a combination of fixed allele mobilities that are used to identify the species of *Scylla* proposed. However, as table 5.4 clearly illustrates, there is a lot of confusion between the names attributed to the *Scylla* species identified. As morphological descriptions are absent in Fuseya and Watanabe’s (1996) and Sugama and Hutapea’s

(1999) publications it is difficult to compare their results with those of Keenan *et al.* (1998) and the present study. However, it seems from mobilities recorded for two of the loci (*Mpi* and *Pgm*) by Sugama and Hutapea (1999) that *Scylla olivacea* in Keenan *et al.* (1998) could be the same as *Scylla paramamosain* in Sugami and Hutapea's diagnoses and *vice versa*. Such findings highlight the problems of species determination solely with genetic techniques when different allozyme loci have been used to make a judgement on the speciation of a genus.

The clear-cut allozyme discrimination demonstrated between the two *Scylla* morphs collected in the present study indicates that they probably do not interbreed. Following the biological species concept, it is the importance of non-introgression that holds the status of species (Mayr, 1963), in particular where these two phenotypes are found living in an apparently sympatric manner.

The additional reference material in conjunction with the study site material resulted in four genetically distinct groups (species) being identified in relation to fixed allelic differences between them. However, no single locus used in this study could separate all four species, rather it is a combination of fixed differences at different loci that identifies the *Scylla* species. Keenan (1999) also observed this unusual pattern for closely related species. One explanation for this observation may be that the ancestral species was polymorphic for these loci. Subsequent speciation events resulted in the alleles being distributed between species (Keenan, 1999). Speciation and phylogeny of the genus *Scylla* is discussed further in chapter six.

5.3.3 Hardy Weinberg Equilibrium

All the populations sampled did not deviate from Hardy Weinberg expectations ($P > 0.05$) when tested against expected values using the exact probability test. Thus all locations sampled indicate randomly mating populations with negligible effects from selection, mutation or migration, although all sample sizes are reasonably small and may not represent the population at large. The Hardy Weinberg equilibrium has been considered as a weak test unless large sample sizes are used. For sexually reproducing Crustacea, Hedgecock *et al.* (1982) noted that there was little tendency for populations sampled to depart from Hardy Weinberg proportions although several authors have noted this phenomenon in their own studies (Beckwitt, 1985; Carvalho and Piertney, 1997). Since conformity to Hardy Weinberg proportions was the basic requirement for all further statistical analysis of the electrophoretic data, all the locations sampled could be analysed further for genetic variation, differentiation and divergence.

5.3.4 Genetic variation

The average expected heterozygosities (also known as genetic diversity measure (Nei, 1975)), observed heterozygosities, percentage of polymorphic loci (P) and mean number of alleles per locus (a) are shown in table 5.5. Percentage of polymorphic loci and mean number of alleles per locus are similar between the locations and species sampled.

For the *Scylla* populations sampled from Surat Thani in two successive years, *Scylla olivacea* was fairly consistent from year to year for average heterozygosity, number of polymorphic loci and mean number of alleles per locus denoting a stable population within the study site in Ban Don Bay, Surat Thani. *Scylla paramamosain*, on the other hand, expressed quite different values for average heterozygosity. This is primarily due to the

Table 5.5: Average expected heterozygosities (H_e) (Nei's unbiased estimate), including standard errors, observed heterozygosities (H_o), percentage of polymorphic loci (P) and mean number of alleles per locus (a) for populations of *Scylla olivacea* and *Scylla paramamosain*.

Species/location/year	H_e	H_o	P	a
<i>S. olivacea</i> (black morph)				
Ranong 1996	0.0671 (0.036)	0.0585	22.2	1.33
Surat Thani 1995	0.0262 (0.015)	0.0283	16.7	1.28
1996	0.0201 (0.014)	0.0185	16.7	1.28
<i>S. paramamosain</i> (white morph)				
Thai Binh 1996	0.0512 (0.034)	0.0384	22.2	1.44
Chanthaburi 1996	0.0396 (0.027)	0.0372	16.7	1.28
Surat Thani 1995	0.0693 (0.041)	0.0655	16.7	1.39
1996	0.0484 (0.030)	0.0331	17.6	1.35

fact that *Akp*, which is highly polymorphic for Surat Thani populations, could not be scored for the second year's sampling thus reducing the average heterozygosities available for the second year. This is also reflected in the discrepancies between years one and two for the number of polymorphic loci and the average number of alleles per locus. As far as average heterozygosities for each location are concerned, with respect to *Scylla olivacea*, the Ranong population was found to have higher expected ($H_e = 0.067$) and observed ($H_o = 0.059$) heterozygosities than those expressed in the Surat Thani populations for both years.

These values are low compared with other eukaryotes (Nevo *et al.*, 1984) but low genetic variability may be a general phenomenon in decapod crustaceans (Gooch, 1977; Busack, 1988). Table 5.6 shows a summary of allozyme variation for decapod crustaceans.

The most obvious thing to note from Table 5.6 is that the Brachyura in general have lower expected heterozygosities ($H_e = 0.039$) compared to the other decapod infraorders. The values obtained for the expected heterozygosities for *Scylla olivacea* and *Scylla*

Table 5.6: Measures of genetic variation in a range of decapod species. P = polymorphism; He = Average heterozygosity.

Species	Number of loci	P	He	Source
Crustacea	23	0.305	0.073	Hedgecock <i>et al.</i> , 1982
Decapoda	26	0.230	0.052	Nelson and Hedgecock, 1980
Penaeoidea/Caridea	25	0.267	0.051	Hedgecock <i>et al.</i> , 1982
Astacidea/Palinura	26	0.185	0.050	Hedgecock <i>et al.</i> , 1982
Anomura	23	0.271	0.068	Hedgecock <i>et al.</i> , 1982
Brachyura	24	0.200	0.039	Hedgecock <i>et al.</i> , 1982
<i>Liopetrolisthes mitra</i>	16	0.200	0.089	Weber and Galleguillos, 1991
<i>Palinurus marginatus</i>	46	0.150	0.021	Seeb <i>et al.</i> , 1990
<i>Birgus latro</i>	59	0.120	0.018	Lavery and Fielder, 1993
<i>Penaeus kerathurus</i>	34	0.265	0.055	Matthaeis <i>et al.</i> , 1983
<i>Penaeus japonicus</i>	34	0.387	0.121	Matthaeis <i>et al.</i> , 1983
<i>Metapenaeus ensis</i>	17	0.059	0.029	Tam and Chu, 1993
<i>Penaeus merguensis</i>	17	0.118	0.057	Tam and Chu, 1993
<i>Penaeus monodon</i>	17	0.177	0.054	Tam and Chu, 1993
<i>Uca minax</i>	20	0.215	0.134	Felder and Staton, 1994
<i>Sesarma reticulatum</i>	20	0.167	0.130	Felder and Staton, 1994
<i>Liocarcinus depurator</i>	16	0.282	0.058	Passmonti <i>et al.</i> , 1996/7
<i>Necora puber</i>	16	0.220	0.065	Passmonti <i>et al.</i> , 1996/7

paramamosain are generally higher than this average except for the population of *Scylla olivacea* from Surat Thani. This study shows much higher average heterozygosities compared to the results obtained for *Scylla* species by Fuseya and Watanabe (1997) from Surat Thani (0.015 and 0.014 for *S. olivacea* and *S. paramamosain* respectively). This discrepancy in heterozygosity values is most likely due to the choice of enzymes used by the two studies to investigate genetic variation in populations of the genus *Scylla*.

Various hypothesis have been put forward to account for low heterozygosity in crustaceans, including random genetic drift, trophic stability and the selective elimination of any mutational variation. This incorporates the views of both neutralist and selectionist theories.

In neutral mutation theory, heterozygosity is related to effective population size (N_e) when the effects of mutation, migration and genetic drift are in equilibrium (Kimura, 1983). A drastic reduction in the effective population size (bottlenecking) can prevent the attainment of equilibrium and thus a reduction in heterozygosity. Bottlenecking can be a result of over fishing. Surat Thani has a well established, but unmonitored mud crab fishery (described in chapter 1) thus overfishing may be taking place. Fishing pressure was one of the reasons put forward for the low values for heterozygosity experienced for species of *Penaeus* (0.039) and *Metapenaeus* (0.024) collected from the South China Sea (Tam and Chu, 1993). Seeb *et al.* (1990) found a low heterozygosity of 0.021 for the heavily exploited spiny lobster, *Palinurus marginatus*, from the Hawaiian Archipelago. However, it would take a drastic reduction in the numbers of individuals over a long period of time to create such an effect (tens of individuals remaining over a million generations) (Hedgecock *et al.*, 1982). Moreover, exploitation also does not explain the low heterozygosity found in natural populations of non-exploited brachyurans such as that found for three species of grapsid crab, *Helice tridens* ($H_e=0.019$), *Helice japonica* ($H_e=0.004$) and *Chiromantes dehaani* ($H_e=0.046$) (Irawan *et al.*, 1993).

Nelson and Hedgecock (1980) suggested a selection-based reason for the low levels of heterozygosity observed in decapod crustaceans. Their “environmental heterogeneity – trophic diversity model” predicts that decapods with “fine-grained” strategies (trophic generalists) would show higher heterozygosity in group II enzymes (*i.e.* those enzymes processing externally derived substrates) compared with group I enzymes (intracellular metabolic enzymes). This was not the case for *Scylla* even though it is a genus that fits the criteria for “fine grained” strategists. This was also found to be the case for the coconut crab, *Birgus latro* (Lavery and Fielder, 1993). Lavery and Fielder (1993) found that higher

heterozygosities are consistently observed in crustacean species with small body size and specialised trophic strategies. De Silva *et al.* (1992) pointed out that body size is inversely related to population size, therefore the neutralist theory could be involved to some extent (cited by Lavery and Fielder, 1993).

5.3.5 Genetic differentiation

5.3.5.1 Temporal differentiation

The test for both genic and genotypic heterogeneity between two years' sampling for *S. olivacea* and *S. paramamosain* in Surat Thani shows that there is no evidence of temporal differentiation for these two species ($P > 0.05$) (see table 5.7). Fishers exact test on the pooled data across years for both *S. olivacea* and *S. paramamosain* conformed to the assumptions of Hardy Weinberg equilibrium (P-values of 0.9454 and 0.4184 respectively). Results showing such strong stability between years could be influenced by the sampling regime. Only adult crabs were sampled for this study. On farm studies have shown that the mud crab could theoretically have a generation time of five years, although it is almost

Table 5.7: P-values and standard error (S.E.) for exact probability test (P_{pr}) and G-based log likelihood test (G_a) for each variable locus and global estimates (using Fishers method) to test for temporal heterogeneity for *Scylla olivaceous* and *Scylla paramamosain* collected in two consecutive years in Surat Thani, Thailand.

Locus	<i>Scylla olivaceous</i> "black"				<i>Scylla paramamosain</i> "white"			
	P_{pr}		G_a		P_{pr}		G_a	
	P-value	S.E.	P-value	S.E.	P-value	S.E.	P-value	S.E.
<i>Aat-2</i>	0.1087	0.0028	0.1020	0.0050	1.0000	0.0000	1.0000	0.0000
<i>Aco</i>	0.1747	0.0040	0.2354	0.0050	0.1302	0.0096	0.3108	0.0062
<i>Akp</i>	np	-	np	-	np	-	np	-
<i>Gpi</i>	0.8952	0.0029	0.8980	0.0027	0.8714	0.0038	0.8889	0.0026
$\chi^2(\text{all})$	8.148		7.673		4.353		2.573	
P-value	0.2381		0.2630		0.6767		0.8602	

Significant heterogeneity = $P < 0.05$

impossible to age crustaceans (Ryce, 1995). Moreover, they can take anything from 13 months (Ong, 1966) to 3 years to reach maturity, although the majority of mature adults are about two years old (Hill *et al.*, 1982). By using adult crabs rather than juveniles (which are much less apparent in coastal areas in Southeast Asia and more difficult to catch) there is a chance that the same age class (cohort) has been sampled twice. Kordos and Burton (1993) found temporal variation due to recruitment effects in the blue swimming crab, *Callinectes sapidus*, from the Gulf of Texas, although the adults were found to be much more homogenous in their allele frequencies compared with the megalopal stages suggesting that post-settlement selection takes place. As the samples of *Scylla* for both morphs from 1995 and 1996 showed no heterogeneity, they have been pooled for further analysis.

5.3.5.2 Spacial differentiation

Intraspecific spacial heterogeneity was found among populations of *S. olivacea* (section 5.3.5.2.1) and *S. paramamosain* (section 5.3.5.2.2).

5.3.5.2.1 *Scylla olivacea*

Genetic heterogeneity was found between the two populations of *S. olivacea* sampled from the east and west coasts of Thailand. From Table 5.8 it can be seen that three of the four variable loci resulted in genic (P_{pr}) and genotypic (G_a) probability values well below the $P=0.05$ threshold for homogeneity and subsequently indicate heterogeneity between locations at these loci.

Table 5.8: P-values and standard errors (S.E.) for exact probability test (P_{pr}) and G-based log likelihood test (G_a) for each variable locus, global estimates (using Fishers method) and F-statistics (F_{ST} and θ) to test for spacial heterogeneity, and derived estimates of geneflow ($N_e m$) among populations of *Scylla olivacea*.

Locus	P_{pr}		G_a		F_{ST}	θ
	P-value	S.E.	P-value	S.E.		
<i>Aat-2</i>	0.0000	0.0000	0.0000	0.0000	0.201	0.4459
<i>Aco</i>	0.0013	0.0005	0.0038	0.0010	0.028	0.0569
<i>Akp</i>	0.2748	0.0029	0.2780	0.0027	0.006	0.0137
<i>Gpi</i>	0.0141	0.0025	0.0292	0.0043	0.026	0.0478
All	$\chi^2 = \infty$		$\chi^2 = \infty$		0.094	0.1936
P-value	P \ll 0.001		0.0000			
			$N_e m$		2.41	1.04

Significant heterogeneity = $P < 0.05$

With respect to the F statistics, it is only *Aat-2* which exhibits significant differentiation between the two populations for F_{ST} ($F_{ST} > 0.05$) (Wright, 1978) and *Aco* and *Aat-2* for θ ($\theta > 0.05$) (Weir and Cockerham, 1984). As all tests show strong evidence of heterogeneity, it is believed that the populations sampled from Ranong and Surat Thani represent two independent populations located on the Andaman Sea and western Gulf of Thailand coasts respectively, and that there is little evidence of mixing between these populations.

In addition to the significant genetic differentiation between the two populations, the low number of migrants ($N_e m$) shown in table 5.8 indicates that potentially little geneflow is taking place. Therefore the most likely explanation is that geographical isolation between these two sampled populations has resulted in the differentiation in gene frequencies observed, either by random genetic drift and/or localised selection events. A more in-depth study along both east and west coasts of the Thai/Malaysian peninsula would result in a better understanding of the genetic relatedness of east and west coast populations of *Scylla*. In practice, this is an important issue for fisheries management of mud crab, especially in relation to geographical movements of mud crabs to support aquaculture

development. For example, crabs were recently exported from Ranong to the East Coast of Thailand to support the mud crab farming industry (Overton, personal observation).

5.3.5.2.2 *Scylla paramamosain*

Even though the locations where samples of *S. paramamosain* were collected were from the same coastline, there is sufficient evidence to suggest that there is some population structuring between these locations. Table 5.9 illustrates the contribution of the four variable loci to the overall genetic differentiation experienced for populations of *S. paramamosain*. The overall genetic differentiation observed is attributed largely to the *Akp* locus. Genic (P_{pr}) and genotypic (G_a) probability values are below 0.00001 for *Akp* and high values were observed for F_{ST} (0.1719) and θ (0.14) indicating significant differentiation between populations at this locus.

Aat-2 is also marginal in its affect on the differentiation between populations, showing statistical significance in differentiation between populations for the G-based log likelihood test ($G_a=0.0662$) but not for the exact probability test ($P_{pr}=0.0054$). As it is believed that it is the population from Surat Thani that is genetically differentiated from the other two locations, tests of population homogeneity were applied to the Vietnam and Chanthaburi populations. Table 5.10 shows that these two populations, although separated by approximately 2300 km, are genetically homogeneous, indicating some mixing may be taking place along this coastline between these two populations.

Table 5.9: P-values and standard errors (S.E.) for exact probability test (P_{pr}) and G-based log likelihood test (G_a) for each variable locus, global estimates (using Fishers method) and F-statistics (F_{ST} and θ) to test for spacial heterogeneity, and derived estimates of geneflow (N_m) among populations of *Scylla paramamosain* from Thai Binh, Chanthaburi and Surat Thani.

Locus	P_{pr}		G_a		F_{ST}	θ
	P-value	S.E.	P-value	S.E.		
<i>Aat-2</i>	0.0054	0.0032	0.0662	0.0041	0.0274	0.027
<i>Aco</i>	0.1815	0.0110	0.1553	0.0095	0.0102	0.014
<i>Akp</i>	0.0000	0.0000	0.0000	0.0000	0.1719	0.140
<i>Gpi</i>	0.4022	0.0104	0.7189	0.0096	-0.0074	0.004
All	$\chi^2 = \infty$		$\chi^2 = \infty$		0.0503	0.052
P-value	P << 0.001		0.0000			

Table 5.10: P-values and standard errors (S.E.) for exact probability test (P_{pr}) and G-based log likelihood test (G_a) for each variable locus, global estimates (using Fishers method) for spacial heterogeneity between populations of *Scylla paramamosain* sampled from Chanthaburi (Thailand) and Thai Binh (Vietnam).

Locus	P_{pr}		G_a	
	P-value	S.E.	P-value	S.E.
<i>Aat-2</i>	0.2977	0.0028	0.2824	0.0047
<i>Aco</i>	0.5271	0.0078	0.3618	0.0058
<i>Akp</i>	0.3461	0.0044	0.3194	0.0047
<i>Gpi</i>	0.8627	0.0034	0.8638	0.0034
All	$\chi^2 = 6.121$		$\chi^2 = 7.138$	
P-value	0.6472		0.5218	

Table 5.11: Estimates of geneflow (N_m) between populations of *Scylla paramamosain* from Southeast Asia.

Locations	F_{ST}	$N_m (F_{ST})$	θ	$N_m (\theta)$
Surat Thani and Chanthaburi	0.064	3.66	0.085	2.71
Surat Thani and Thai Binh	0.043	5.56	0.049	4.82
Chanthaburi and Thai Binh	0.005	49.75	0.012	21.12

However, when the data from the Chanthaburi and Thai Binh populations were pooled together and tested for conformity to Hardy Weinberg expectations, it was found that they deviated from HWE ($P=0.0488$). This was attributed to the *Aat-2* locus where the population from Vietnam contains a rare allele that is absent from the population sampled from Chanthaburi. However, if the overall significance value of the global test is adjusted for multiple tests of the same hypothesis, using a sequential Bonferroni technique ($0.05/\text{number of tests}$) (Lessios, 1992), then the Hardy Weinberg assumptions are not violated and the Chanthaburi and Thai Binh populations show a high degree of homogeneity.

From these results, it can be seen that the Surat Thani population of *S. paramamosain* is spatially differentiated from the Chanthaburi and Thai Binh populations. This is surprising in relation to the geography of these locations since Chanthaburi is much closer to Surat Thani (approximately 750km) than it is to Thai Binh (approximately 2300km). Thus there is no evidence for isolation-by-distance population structuring for *Scylla paramamosain*. This kind of genetic differentiation has also been reported among populations of *Penaeus monodon* from the Gulf of Thailand. For example, Sodsuk *et al.* (1993) found that *P. monodon* sampled from Trat (near to Chanthaburi) were heterogenous to *P. monodon* collected from Surat Thani.

From the values of potential geneflow between the populations of *S. paramamosain* sampled in this study (table 5.11), it is apparent is that there are high levels of gene flow between the Thai Binh and Chanthaburi populations, but low geneflow between crabs in Surat Thani and in the other two locations.

Figure 5.2 illustrates the approximate monthly surface currents within the South China Sea as described by Dale (1956). This figure illustrates the complete reversal in direction of

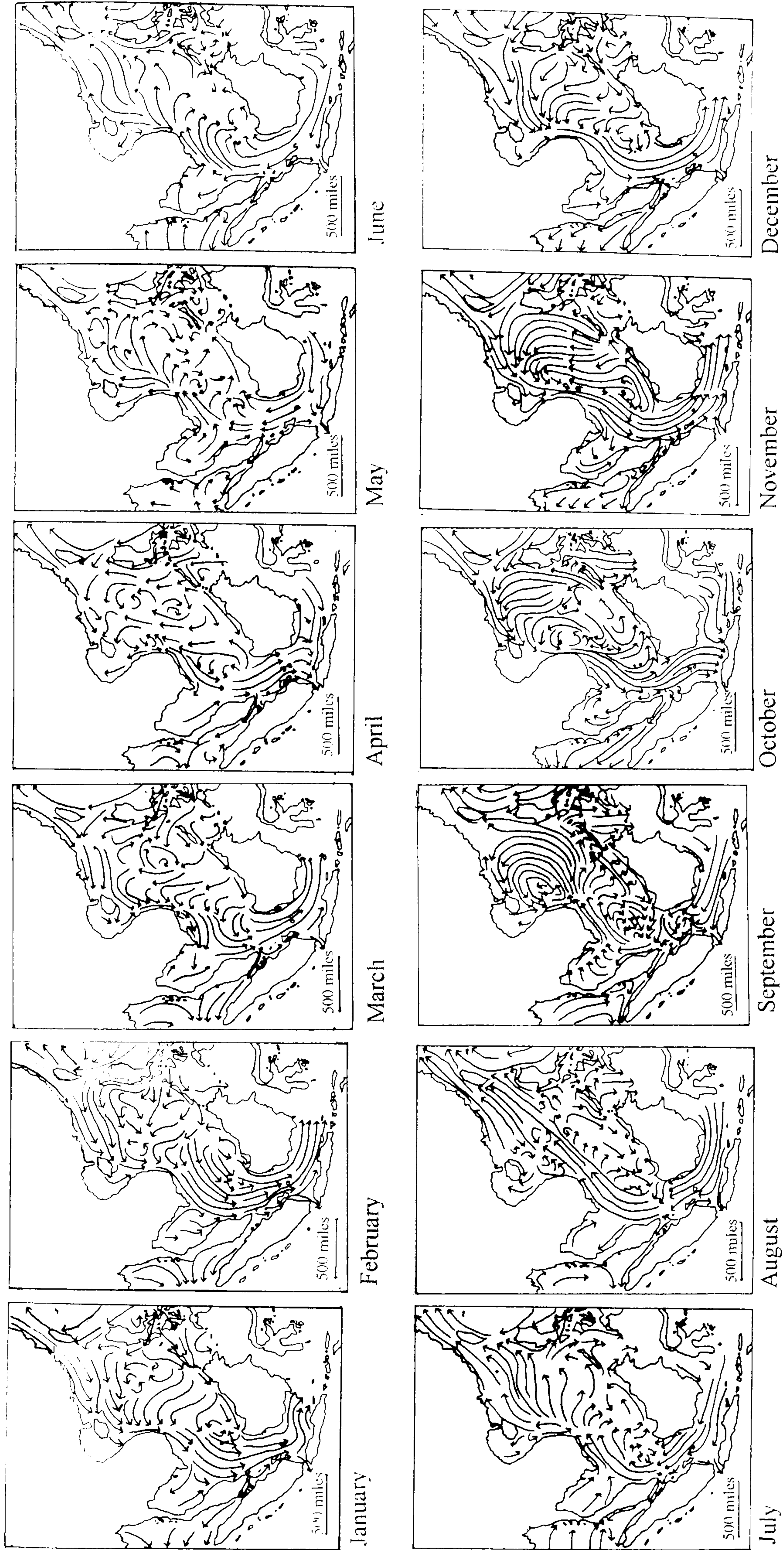


Figure 5.2: Monthly direction of the most consistent surface currents within the South China Sea as published by the Meteorological Office, the Hydrographic Department of the Admiralty and the Hydrographic Office of the United States (modified from Dale, 1956).

the coastal currents twice a year (the major changes occurring in May and October) although the main flow of current is from north to south along the western seaboard of the South China Sea. These currents are fast flowing; particularly over the Sunda shelf (from 0.27m sec^{-1} in March to 0.71m sec^{-1} in November).

It has long been believed that many marine species have the potential for great dispersal during at least part of their life cycle, resulting in intraspecific homogeneity on a broad geographic scale consistent with high gene flow (Ward *et al.*, 1994). The *Scylla* species have a pelagic larval stage that lasts approximately 30 days (Brown, 1993) providing a potential vector for geneflow. Thus the fast currents recorded along the western seaboard of the South China Sea would contribute to the high values for geneflow experienced between Chanthaburi and Thai Binh populations of *S. paramamosain*.

However, studies on marine species in recent years have shown that intraspecific differentiation can be present in what were often once thought as panmictic populations (Knowlton, 1993; Hilbish, 1996; Duffy, 1996). *Scylla* has a more abbreviated pelagic larval period than some of the temperate portunid species such as the blue crab, *Callinectes sapidus*, which has a pelagic larval period of two months depending on water temperature (Coslow and Bookhout, 1959). Thus this potential vector for geneflow may not be as affective as first thought. Moreover, the spawning period for females may vary among locations, particularly where the peak of spawning is timed with the monsoon season as recorded for *S. olivacea* in Ranong Province (Macintosh *et al.*, 1993). This subsequent recruitment will then be affected by local seasonal current patterns as to where actual recruitment may take place.

The currents within the Gulf of Thailand are quite complex, with small eddies being formed along the coastline due to the monsoon winds which sweep across the South China Sea. In particular, there is a small circular eddy that circulates around Ban Don Bay, Surat Thani Province (as seen in figure 5.3). Therefore it is quite likely that Surat Thani is receiving very little recruitment from outside the immediate area due to these current patterns. Thus the *Scylla* stock is reliant on self-recruitment instead. Modest population structuring was discovered in populations of the blue crab, *Callinectes sapidus* from the east coast of the United States (McMillen-Jackson *et al.*, 1994; Garvine *et al.*, 1997) even though a large amount of gene flow was taking place. This structuring was believed to be due to a combination of high retention of locally spawned larvae and a low incidence of long distance migration by spawning females. As well as current patterns, larval recruitment may also be affected by the ability of crab zoeae to regulate their position in the water column and therefore their control of dispersal by coastal currents (Burton, 1983).

In summary, it is most likely the level of genetic heterogeneity observed and the genetic differentiation between conspecific mud crab populations is determined largely by the source of recruited juvenile mud crabs. This is affected both by the capability for widespread larval dispersal and by both pre-settlement and post-settlement selection factors affecting recruitment success. It is therefore evident that future studies on *Scylla* stock structure should focus on recruitment processes. Moreover, with the recent clarification of species status in the genus *Scylla*, any differences in recruitment process observed between *Scylla* species may provide information about their juvenile ecology and the status of their present distribution.

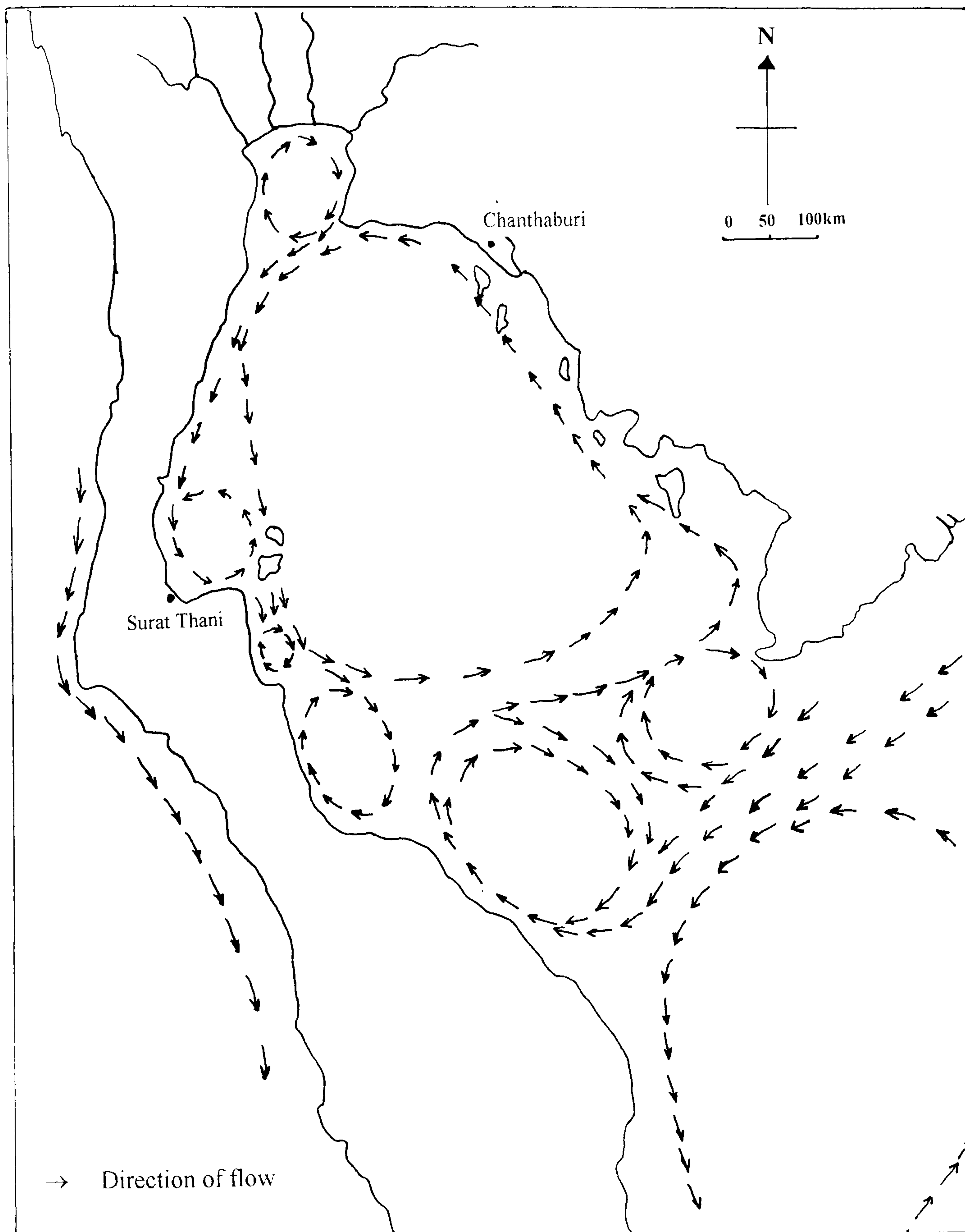


Figure 5.3: Primary currents in the Gulf of Thailand (taken from Sadrul, 1975).

Although allozyme electrophoresis proves a useful technique with adult mud crabs, from the pelagic larval stages to the first crab stages require other genetic techniques, such as mtDNA, that require smaller quantities of tissue, in order to properly represent the full extent of genetic variation. DNA techniques have been widely advocated to study stock structure in marine organisms, for example, in penaeid prawns (Lavery and Keenan, 1994) and in particular, to determine the rate of geneflow and the degree of self-retention of recruits (Ovenden, 1990).

Chapter Six

Phylogenetic study of the genus *Scylla*

6.1 Introduction

6.1.1 Definition of phylogeny and systematics

Phylogeny can be defined as the evolutionary history (or genealogy) of a group of taxa, whether they are living or extinct. Systematics is the study of the evolutionary relationships between these taxa (Wiley, 1981).

The study of phylogeny involves making the best estimate of an evolutionary history that describes the information contained within the data available and to postulate some evolutionary scenario to the resulting phylogenetic tree (Swofford *et al.*, 1996). As the name suggests, phylogenetics implies that the ancestral characteristics are inherited and that evolutionary history is responsible for changing these characteristics. Different types of characters are used to create phylogenies, including molecular, biochemical, karyological, physiological and morphological characters (Wiley, 1981; Quicke, 1993).

6.1.2 Role of electrophoresis in molecular systematics

Allozyme data from starch gel electrophoresis has been used widely to investigate phylogenetic relationships (Buth, 1984; Murphy *et al.*, 1990). However, the use of allozyme data in phylogenetic reconstruction has declined in popularity in recent years, due to the introduction of more sophisticated techniques involving the use of both mitochondrial and nuclear DNA. Allozyme data are still relevant in systematics, however,

in particular when looking at the relationship between conspecific populations, or closely related species (*i.e.* intrageneric relationships) (Hillis and Moritz, 1990; Avise, 1994; Swofford *et al.*, 1996).

Allozyme data is commonly used in systematic studies (Buth 1984). The data are normally presented as the frequency of each allele for each locus with respect to each population of the taxa of interest. Recently, there has been controversy over the methods of phylogenetic reconstruction using allozyme data. During the 1960s and 1970s it was popular to convert allozyme frequencies into matrices of pairwise distance/similarity values, thus serving as an input to ultrametric (*e.g.* cluster analysis) and additive (*e.g.* distance Wagner analysis) dendrogram construction methods. This use of pairwise distances for phylogenetic reconstruction was extrapolated from their use in population genetic studies (Richardson *et al.*, 1986). However, those opposed to the use of pairwise distances believe that their popularity has retarded the development of other approaches to phylogenetic reconstruction which made better use of the information within the data set (Swofford *et al.*, 1996).

The introduction of character-based methods brought about further controversy regarding to the use of allele frequency data directly as input data. Some authors believed that importance should be placed on the presence or absence of an allele, rather than its frequency (Mickevich and Johnson, 1976). Other authors believed that there is no reason to ignore allele frequencies when analysing allozyme data (Swofford and Berlocher, 1987).

The problem with using discrete data formats is that the assumption made by the phylogenetic reconstruction technique that the characters are independent of each other is violated (Swofford *et al.* 1996). Allele frequencies at each locus are constrained to the sum of one. Therefore as the frequency of one allele increases, one of the other alleles is

forced to reduce in frequency. This lack of independence also affects the presence/absence data required for maximum parsimony analysis where ancestors can be inferred to contain no alleles at all (Swofford *et al.*, 1996).

Buth (1984) suggested the use of the loci as the character rather than the alleles, where the different genotypes for each locus are treated as individual character states which are coded accordingly. In some situations, however, large numbers of alleles can result in more character states than the groups of taxa under study (Swofford *et al.*, 1996). Other more sophisticated methods of converting allele frequency data, to alleviate the problems of violating the assumptions of the phylogenetic construction methods used, are reviewed by Swofford *et al.* (1996).

6.1.3 Forms of allozyme data commonly used

There are two main categories of allozyme data that are most commonly used in allozyme systematics. These are a) similarity/distance data; and b) discrete characters (Swofford and Olsen, 1990).

6.1.3.1 Distance/similarity data

Similarity or distance data specify the relationship between pairs of taxa or molecules.

This was the most popular allozyme data analysis method used for phylogenetic reconstruction during the 1970s and 1980s, even though it was argued that this method did not make the most efficient use of the data (Swofford *et al.*, 1996). Although the definitions of similarity and distance are slightly different, they are often confused in the literature. Similarity values are represented most often as a value between 1 (100% similar) to 0 (0% similar); those taxa with values close to zero are said to be dissimilar.

Distance is the numerical representation of the difference between two taxa, for example, identical organisms would have a distance value of zero. There is no consensus between distance measures as to the upper limit of discrepancy between two taxa (Swofford *et al.*, 1996).

There have been various methods proposed for the conversion of allele frequency data into measures of genetic distance (Wright, 1978). The most commonly used of these are Nei's genetic distance (1972, 1978), Rogers' (1972) genetic distance, and Cavalli-Sforza and Edwards' (1967) arc distance. With respect to allozyme electrophoresis, Nei's genetic distance has a biological premise which is interpreted as the mean number of codon substitutions per locus that have accumulated since the two taxa diverged from their ancestor (Nei, 1972). Rogers' genetic distance, on the other hand, is a geometric measurement of the distance between gene frequencies summarised for all loci under investigation (Buth, 1984). Cavalli-Sforza and Edwards' arc distance attempts to incorporate some realistic assumptions with regards to the evolution of gene frequencies under the effects of random genetic drift (these distance measures are described in more detail in annex v).

6.1.3.2 Discrete character data

This is where the data are divided into characters, each character is assigned a range of character states (Swofford *et al.* 1996). There are two main assumptions tied to the use of discrete character data. Firstly, it is assumed that there is independence among characters. If this can not be assumed, then covariances must be used instead, resulting in the need for more complicated computational algorithms. Secondly, characters are assumed to be homologous *i.e.* characters must be defined in such a way that all character states observed

for that character could have been derived from a corresponding state observed in a common ancestor of the particular taxa of interest (Swofford *et al.*, 1996). Discrete character data can be divided into qualitative (discrete) and quantitative (continuous) data. Qualitative data can either be binary (typically represented as the presence or absence of alleles at a particular locus) or multistate. Quantitative data types are less popular in molecular systematics, except where polymorphic characters are coded as frequencies.

6.1.4 Methods used for phylogenetic reconstruction

The primary form of phylogenetic reconstruction involves the construction of dendrograms. These are graphical representations consisting of nodes (taxa) and branches connecting these taxa together in a way which summarises their evolutionary relationships with respect to each other (Wiley, 1981).

The main forms of phylogenetic reconstruction can be divided into phenetic and cladistic techniques. Phenetic techniques were popularised by Sokal and Sneath (1963). The basis of the phenetic approach is derived from the theory that the more similar two taxa are the more related they must be. With a minimum number of characters taxa can be grouped together in a way that explains the similarity between them (Wiley, 1981). This clustering technique has been extremely popular purely due to its simplicity. Phenetic techniques include the use of pairwise distances with clustering methods such as the Unweighted Pair Group Method using Arithmetic Averages (UPGMA).

The alternative approach, cladistics, was first proposed by Hennig (1966). Phylogenetic reconstruction using the cladistic approach is based on the theory that classification of taxa should reflect the evolutionary history of the group of organisms of interest and in

particular the way characters evolve from the primitive (plesiomorphic) state to the advanced (apomorphic) state. Parsimony and maximum likelihood are two examples of the cladistic approach (Forey *et al.*, 1995).

A recent review on the methods used to infer phylogeny using molecular data by Swofford *et al.* (1996) includes those methods, which are most commonly used with allozyme data, presented either as gene frequencies, distance values or discrete characters. Of these methods described, some are highlighted as more realistic in their evolutionary assumptions than others. The more popular methods of phylogenetic reconstruction, plus the more reliable methods that have been chosen for this study, are described below.

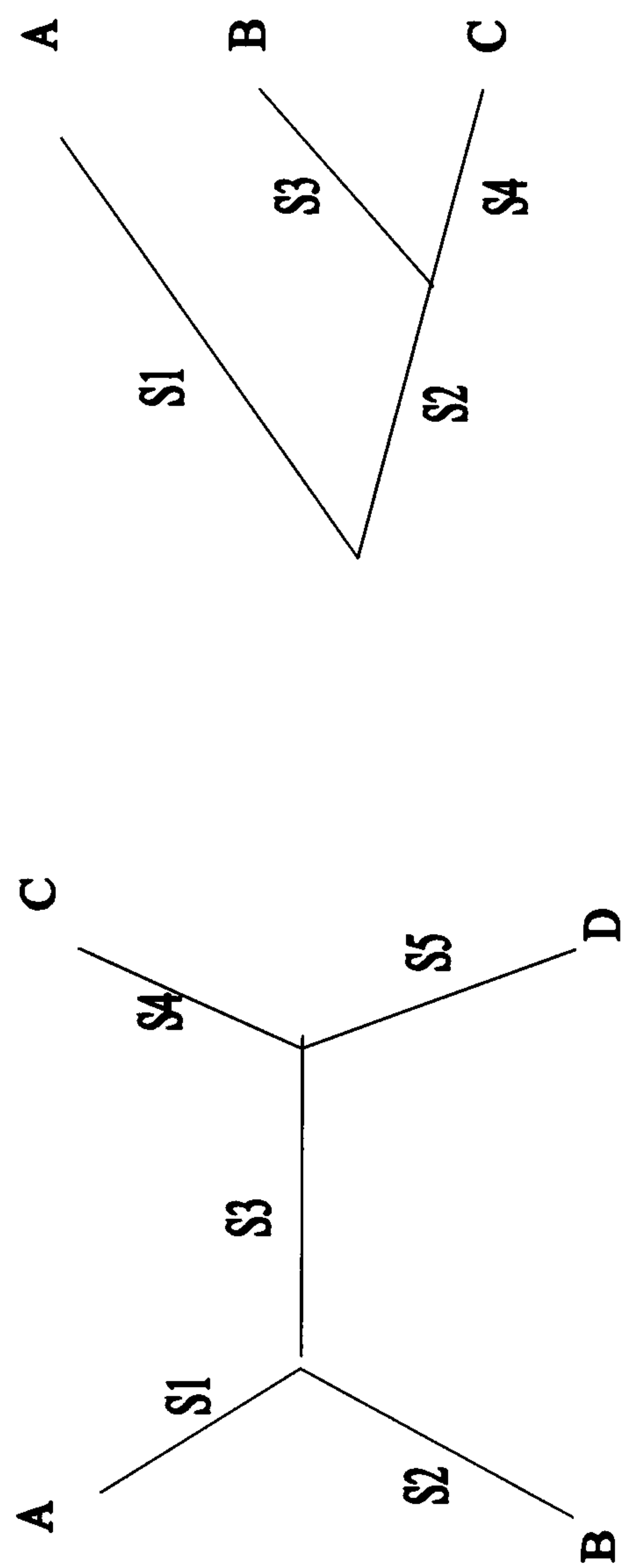
6.1.4.1 Methods of phylogenetic reconstruction based on distance measures

Distance methods attempt to fit a phylogenetic tree (dendrogram) from a matrix of pairwise distances among taxa. The distance between each pair of taxa is predicted as the sum of branch lengths in the path from one taxon to another via the tree (Swofford *et al.*, 1996). Often it is assumed that there is some time element attached to this concept of reconstruction (Nei, 1975). Felsenstein (1988) argues that this is not necessarily the case and that it is possible to assume (or not assume, as the case may be) a molecular clock when using distance methods. The most popular of methods used for the construction of trees using distance data are a) cluster analysis (Sneath and Sokal, 1973); and b) the distance Wagner procedure (Farris, 1972).

a) *Cluster analysis*

Cluster analysis is the most popular method of tree construction used in most literature published, including that on crustaceans. Its popularity centres on its computational simplicity. Cluster analysis is based on a group of similar algorithms that summarise similarity or distance data in the form of an ultrametric tree (see figure 6.1). In phylogenetic terms, ultrametric distances will fit a tree precisely where the distance between two taxa is equal to the sum of the branches connecting them. The tree can be rooted so that all taxa are equidistant from the root. The most commonly used clustering method is UPGMA (Unweighted Pair Group Method using Arithmetic averages; Sneath and Sokal, 1973). UPGMA operates by scanning the matrix of distances for the smallest distance value, then joining the two taxa involved with an internal node at an appropriate position along a distance axis. This distance is then discarded from the matrix and the process begins again. This is repeated until all the taxa are joined together, thus forming a tree. There are several points associated with UPGMA. These are a) in each cycle of clustering, clusters are grouped according to the smallest mean distance between taxa involved; b) each taxa contributes equally in the calculation of mean distance; c) all extant taxa are “right justified” along distance axis and branches are unscaled; and d) the root is automatically located where the last cluster is formed (Avice, 1994).

The major assumption associated with UPGMA is the equal rate of evolution along all branches of the dendrogram. This means that any heterogeneity in the rates of divergence between taxa would be undetected. However, even with such a strict assumption UPGMA performs well in producing trees and is still very popular (Avice, 1994; Swofford *et al.* 1996).



a) Additive tree

$$\begin{aligned}
 P_{AB} &= S_1 + S_2 \\
 P_{AC} &= S_1 + S_3 + S_4 \\
 P_{AD} &= S_1 + S_3 + S_5 \\
 P_{BC} &= S_2 + S_3 + S_4 \\
 P_{BD} &= S_2 + S_3 + S_5 \\
 P_{CD} &= S_4 + S_5
 \end{aligned}$$

b) Ultrametric tree

$$\begin{aligned}
 \text{additive properties: } P_{AB} &= S_1 + S_2 + S_3 \\
 P_{AC} &= S_1 + S_2 + S_4 \\
 P_{BC} &= S_3 + S_4
 \end{aligned}$$

$$\begin{aligned}
 \text{ultrametric properties: } S_3 &= S_4 \\
 S_1 &= S_2 + S_3 = S_2 + S_4
 \end{aligned}$$

Figure 6.1: Schematic diagram of an a) additive and b) ultrametric tree. The additive tree and ultrametric tree are relating to four taxa (A, B, C and D) and three taxa (A, B and C) respectively. Both relationships between taxa (e.g. $P_{AB} - P_{AC}$) and branch lengths ($S_1 - S_5$) are included. Note: as no assumption is made about the rooting in the additive method, the additive tree is represented as an unrooted tree (taken from Swofford and Olsen, 1990).

b) *Distance Wagner procedure*

The other commonly used method of constructing trees is the distance Wagner procedure.

This method uses distance values fitted to an additive tree where estimates can be higher or lower than the true value seen in the pairwise matrix of distance values (see figure 6.1).

The desired tree using additive data involves minimising the total of all branch lengths in the tree, while still using the distance values as the lower limits for actual evolutionary distance (Farris, 1972).

6.1.4.2 The parsimony approach

Maximum parsimony is the most widely used approach to infer phylogenies from discrete character data. Maximum parsimony works on the principle of minimising the amount of evolutionary change needed to explain a given set of data by selecting trees that minimise the total tree length; *i.e.* the number of evolutionary steps required to form the tree (Li and Graur, 1991). This method also minimises the number of extra steps (homoplasies) needed to explain the data. All parsimony methods assume that changes in character states are rare, although each method has its own set of evolutionary assumptions (Kitching, 1995).

The Wagner parsimony (based on concepts developed by Wagner, 1961, 1963 and formalised by Eck and Dayoff, 1966; Kluge and Farris, 1969 and Farris, 1978) is one of the simplest parsimony methods, with few evolutionary assumptions. Therefore it places minimal constraints on character state changes. This method allows free reversibility of character state change therefore making no assumption on the direction of evolution (Kitching, 1995). This free reversibility means that the tree can be rooted from any point without a change in tree length. Other assumptions include a) that the common ancestor is unknown; b) that different characters and lineages evolve independently; and c) other kinds

of evolutionary events such as retention of polymorphisms are less likely than a change of character state (Kitching, 1995). Those that are opposed to the use of the Wagner parsimony approach highlight the point that this method ignores information on branch lengths when evaluating the tree topology.

6.1.4.3 Maximum likelihood approach

Maximum likelihood is viewed as an alternative approach to parsimony. Maximum likelihood approach uses iteration to find a tree that has the highest probability of the dataset being derived from it (Felsenstein, 1981). The maximum likelihood method works on the assumption that each allozyme locus evolves independently according to Brownian motion. This Brownian motion is influenced by random genetic drift, although the rate of diffusion will differ in different parts of the gene frequency scale (Felsenstein, 1985). Branch lengths between taxa indicate the amount of accumulated variance between them, rather than the amount of time since divergence. However, the cost of changing the character state is also added on to the branch length (Felsenstein, 1985). Maximum likelihood assumes that every site evolves at the same rate. This means that any violation of this assumption can have severe consequences. Also the principle of finding the tree with the greatest likelihood from among a large number of possible trees for large numbers of taxa means that computationally this is slow (Siebert, 1995). Therefore it is only recommended for studies involving few taxa (Kitching, 1995). However, with respect to using gene frequencies, maximum likelihood is least affected by sampling error and is robust to most violations of assumptions used in its model (Siebert, 1995).

6.1.5 The use of phylogenetics in crustacean studies

The systematic relationships within the Subphylum Crustacea, particularly between and within families of the Order Brachyura, are still under heavy revision (*e.g.*, Bellwood, 1996; Stevcic, 1998). This has been mainly the result of an overlap in morphological features between taxa. In some cases this has been shown to be the result of congruence in adult morphology, most likely from environmental selective pressures (Spears *et al.*, 1992). Therefore it is difficult to differentiate plesiomorphic (ancestral) characters from apomorphic (derived) ones using external morphological features only. Some researchers have promoted the inclusion of other characters that are less influenced by selective pressures such as of zoeal morphology (Rice, 1980), spermatozoan ultrastructure (Jamieson, 1994) and molecular techniques (Spears *et al.*, 1992; Harrison and Crespi, 1999) in order to obtain a more accurate phylogeny of brachyuran crabs.

Within the Family Portunidae, in which the genus *Scylla* belongs, very little research has been carried out to assess the intergeneric and intrageneric phylogenetic relationships. The few studies conducted have used morphological features (*e.g.* *Charybdis* and *Thalamita*; Wee and Ng, 1995), and molecular techniques, in particular allozyme electrophoresis (*e.g.* species of *Portunus*; Bryars and Adams, 1999) and DNA techniques (*e.g.* *Charybdis*; Chu *et al.*, 1999).

Phylogenetic trees using a pairwise distances approach with gene frequency data has been conducted on few locations and a maximum of three proposed *Scylla* species, showing the potential of using this technique (Fuseya and Watanabe, 1996; Sugama and Hutapea, 1999). However, the pairwise distance approach using a phenetic technique such as clustering, cannot be used to make a judgement on the evolution of the four *Scylla* species

described in chapters three, four and five. To date, cladistic techniques, which try to evaluate the data on the basis of primitiveness of characters, have not been used to assess the phylogeny of the genus *Scylla*.

In this part of the study gene frequency data, collected using allozyme electrophoresis, were used to construct phylogenetic trees based on both phenetic and cladistic methods. Consensus between the phylogenetic reconstruction methods serves to verify the phylogeny obtained.

Consensus can also be extended to include comparisons between morphological and molecular phylogenies. Hillis (1982) reviews the advantages and limitations of both types of phylogeny. Phylogenetic reconstruction of the genus *Scylla* was also carried out on morphological, ecological and behavioural characters using cladistic techniques in order to compare the morphological relationships to those discovered using gene frequency data.

6.2 Materials and methods

6.2.1 Genetic data

Allozyme data were obtained from 18 enzyme loci examined for the four proposed species of the genus *Scylla* (refer to chapter five for detailed laboratory procedure). Samples of *S. olivacea* and *S. paramamosain* were obtained in three separate locations, whereas *S. serrata* and *S. tranquebarica* were sampled from single localities. A summary of the number of individuals sampled for each species, and the locations sampled, are outlined in table 6.1.

Table 6.1: Number of individuals of the genus *Scylla* from six locations in the Asia Pacific region.

Location	Species	Number of individuals
Thailand:		
Surat Thani	<i>Scylla paramamosain</i>	87
	<i>Scylla olivacea</i>	110
Chanthaburi	<i>Scylla paramamosain</i>	24
Ranong	<i>Scylla olivacea</i>	44
Vietnam:		
Thai Binh	<i>Scylla paramamosain</i>	42
Sarawak:		
Sematan	<i>Scylla tranquebarica</i>	6
	<i>Scylla olivacea</i>	5
Australia:		
Queensland	<i>Scylla serrata</i>	5

Swofford *et al.* (1996) highlight the importance of selecting an outgroup carefully, if one is to be used. Other authors have used *Thalamita* spp. as outgroups to root *Scylla* phylogenies (Keenan *et al.*, 1998; Keenan, 1999). Therefore it would have been desirable to use *Thalamita* spp. as an outgroup in the present study. However, *Thalamita* spp. are not a commercially fished species, and the lack of suitable resources to make personal collections of *Thalamita* spp. meant that during the present study no outgroup was used. Hence, no outgroups were used to root the dendrograms, although it is understood that it is preferable to use an outgroup.

6.2.2 Data transformation

The allozyme data resulting from starch gel electrophoresis of the mud crab populations sampled were transformed into three different forms namely: a) matrices of pairwise distances between taxonomic groups using three different distance algorithms; b) quantitative discrete characters in the form of gene frequencies; and c) qualitative discrete characters using binary coding methods. The data treatment in each case is described below.

a) *Genetic distances*

Three distance algorithms and one similarity algorithm were used to convert gene frequencies into pairwise distance/identity measures between taxonomic groups. These four algorithms were a) Nei's (1972) genetic distance (D_n), b) Nei's (1972) genetic identity (I_n), c) modified Rogers distance (D_{rog}) (Wright, 1978) and d) Cavalli-Sforza and Edwards (1967) arc distance (D_{arc}). Although there have been arguments against the use of Nei's and Rogers' distance computations, due to their unrealistic assumptions with respect to the evolution of allele frequencies, they are still both commonly used and therefore have been included for comparative purposes. The Cavalli-Sforza and Edwards (1967) arc distance measure is based on more realistic evolutionary assumptions with regard to the change in gene frequencies and therefore has been included along with the other two more popular algorithms. Gene frequencies were transformed into pairwise distances using the software BIOSYS-1 (Swofford and Selander, 1989).

b) *Quantitative discrete characters*

In this application, gene frequencies of each locus for each taxon were treated as discrete characters and were used as untransformed data to infer phylogeny.

c) *Qualitative discrete characters*

For each locus, the associated alleles were binary coded on the basis of their presence (1) or absence (0) for each taxonomic group. Therefore each allele was treated as an individual character within the data set.

6.2.3 Morphological data

Morphological and meristic characters were selected from the diagnostic characters highlighted in chapter three. In addition, information regarding the known habitat and behaviour of the four *Scylla* species was also included. The primary morphometric character that clearly discriminated between species within the genus, namely frontal lobe width, was also included in the data set. A summary of the morphological characters for the four *Scylla* species and the outgroup, *Thalamita crenata* is outlined in appendix VI.

There were no clear morphological differences between conspecific populations sampled (refer to chapter three). Therefore phylogenetic reconstruction based on morphological characters was applied solely to the observed interspecific differences.

The portunid crab *Thalamita crenata* Latreille was assigned as an outgroup for the phylogenetic reconstruction of the genus *Scylla* using morphological characters. The reasons for choosing this species were two fold. Firstly, *Thalamita crenata* shows ancestral morphological traits that are intermediate between the more primitive “crawling crab” species to the more recently evolved swimming/burrowing crab group to which *Scylla* belongs (Stephenson and Campbell, 1959). Secondly, *Thalamita crenata* is a mangrove-associated species and is therefore environmentally comparable to the *Scylla* species.

6.2.4 Morphological data transformation

The morphological data collected were transformed using a three-step process. Firstly, each morphological character was divided into various states (e.g. the character “chela reticulation” was divided into the states “absence of reticulation”, “faint reticulation” and

“highly visible reticulation”); each state was coded using the sequential numbering technique of Camin and Sokal (1965) using the outgroup rule (Wiley, 1981). The outgroup rule states that the outgroup possesses the ancestral state and is conveniently coded zero. Subsequent character states are coded sequentially from the ancestral state. Both positive and negative integers can be used, the larger numbers being associated with more derived character states.

Secondly, the coded character states are then ordered according to the hypothesis of evolutionary change from one state to another using the linear ordering recommended by Camin and Sokal (1965). Since linear ordering is too restrictive in some circumstances, branching order was also used where appropriate. The polarity was not considered as it places additional assumptions on the direction of evolutionary change between character states, which cannot be substantiated.

Finally, the ordered, coded data were recoded into binary data using the sub-program FACTOR developed by Meacham (1980), present in the software PHYLIP version 5.3c (Felsenstein, 1993).

6.2.5 Methods used in phylogenetic reconstruction

Four different methods of phylogenetic reconstruction were used in order to represent different theories on systematics, some of which are believed to be more realistic in their evolutionary assumptions than others. These four methods were a) cluster analysis, b) distance Wagner procedure; c) Wagner parsimony; and d) maximum likelihood approach.

a) *Cluster analysis*

Cluster analysis was carried out on pairwise Nei's genetic distances and Rogers' modified distance measures. The most popular clustering method is the UPGMA (Unweighted Pair Group Method using Arithmetic average). The data were treated as being ultrametric (Swofford and Olsen, 1990). The trees were rooted in a manner that ensured that all the taxa are equidistant from the root.

b) *Distance Wagner procedure*

This distance algorithm by Farris (1972) is a heuristic method of tree construction by sequentially adding taxa to the tree (Swofford and Olson, 1990). The phylogenetic reconstruction using the distance Wagner procedure was carried out on the Cavalli-Sforza and Edwards' arc distances (1967). The dendrogram was unrooted. BIOSYS-1 (Swofford and Selander, 1989) was used to construct the trees using both UPGMA and distance Wagner methods.

c) *Wager Parsimony*

Wager parsimony was used on discrete binary coded data. Therefore two phylogenetic trees were constructed; one for the presence/absence coded allele data and the other for the recoded morphological data. The trees were rooted for morphological data using *Thalamita crenata* as the outgroup, whereas the phylogenetic trees for the allozyme data were unrooted. Where large data sets are concerned, the branch-and-bound method of Wagner parsimony has been promoted to reduce computation time (Penny, 1982). As the data set in this study was not large, the ordinary heuristic (exhaustive search) method was used. The programme MIX from the software PHYLIP version 5.3c (Felsenstein, 1993) was used to construct the trees.

Bootstrap resampling was also conducted on the binary coded data, producing 100 sets of resampled data in each instance. Bootstrapping was performed using the program SEQBOOT in the software PHYLIP (Felsenstein, 1993). The bootstrapped data sets were then re-entered into the MIX programme and tested for consensus between the phylogenetic trees produced.

d) *Maximum likelihood estimate*

The restricted maximum likelihood estimate (Felsenstein, 1981) for continuous characters, used untransformed data *i.e.* allele frequencies directly as input. The resulting tree was unrooted. The program ContML was used from the software PHYLIP version 5.3c (Felsenstein, 1993).

6.3 Results

6.3.1 Genetics

The allele frequencies resulting from 18 enzyme loci scored for the eight sampled groups are summarised in table 6.2. Their compliance with Hardy Weinberg expectations, and the degree of genetic differentiation and variability within these populations, is described in chapter five.

6.3.1.1 Genetic distances

The results of the pairwise genetic distances analysis between four *Scylla* species from six locations within Southeast Asia using Nei's (1972) genetic distance, Nei's (1972) genetic identity, Rogers' distance measure (1972) and Cavalli-Sforza and Edwards' (1967) arc distance measures are outlined in tables 6.3 and 6.4.

Table 6.3: Matrix of genetic distance coefficients for populations of *Scylla* species. Above right: Nei's (1972) genetic identity; below left: Nei's (1972) genetic distance measure.

Species (location)	<i>S. olivacea</i> (Surat Thani)	<i>S. olivacea</i> (Ranong)	<i>S. olivacea</i> (Sarawak)	<i>S. paramamosain</i> (Thai Binh)	<i>S. paramamosain</i> (Chanthaburi)	<i>S. paramamosain</i> (Surat Thani)	<i>S. tranquebarica</i> (Sarawak)	<i>S. serrata</i> (Queensland)
<i>S. olivacea</i> (Surat Thani)	****	0.991	0.997	0.700	0.697	0.720	0.789	0.609
<i>S. olivacea</i> (Ranong)	0.009	****	0.989	0.691	0.687	0.709	0.780	0.602
<i>S. olivacea</i> (Sarawak)	0.003	0.011	****	0.709	0.707	0.727	0.784	0.627
<i>S. paramamosain</i> (Thai Binh)	0.357	0.369	0.344	****	1.000	0.994	0.763	0.934
<i>S. paramamosain</i> (Chanthaburi)	0.361	0.375	0.346	0.000	****	0.992	0.763	0.936
<i>S. paramamosain</i> (Surat Thani)	0.329	0.345	0.319	0.006	0.006	****	0.759	0.921
<i>S. tranquebarica</i> (Sarawak)	0.237	0.249	0.243	0.271	0.271	0.275	****	0.681
<i>S. serrata</i> (Queensland)	0.496	0.507	0.467	0.068	0.066	0.082	0.384	****

Table 6.4: Matrix of genetic distance coefficients for populations of *Scylla* species. Above right: modified Rogers' distance (Wright, 1978); below left: Cavalli-Sforza and Edwards' (1967) arc distance.

Species (location)	<i>S. olivacea</i> (Surat Thani)	<i>S. olivacea</i> (Ranong)	<i>S. olivacea</i> (Sarawak)	<i>S. paramamosain</i> (Thai Binh)	<i>S. paramamosain</i> (Chanthaburi)	<i>S. paramamosain</i> (Surat Thani)	<i>S. tranquebarica</i> (Sarawak)	<i>S. serrata</i> (Queensland)
<i>S. olivacea</i> (Surat Thani)	****	0.096	0.050	0.568	0.542	0.517	0.456	0.620
<i>S. olivacea</i> (Ranong)	0.088	****	0.102	0.536	0.544	0.521	0.461	0.619
<i>S. olivacea</i> (Sarawak)	0.069	0.115	****	0.528	0.531	0.509	0.460	0.604
<i>S. paramamosain</i> (Thai Binh)	0.527	0.522	0.521	****	0.021	0.073	0.480	0.254
<i>S. paramamosain</i> (Chanthaburi)	0.530	0.529	0.520	0.060	****	0.086	0.481	0.251
<i>S. paramamosain</i> (Surat Thani)	0.515	0.514	0.508	0.067	0.098	****	0.481	0.276
<i>S. tranquebarica</i> (Sarawak)	0.450	0.455	0.459	0.467	0.466	0.475	****	0.562
<i>S. serrata</i> (Queensland)	0.619	0.613	0.602	0.266	0.260	0.288	0.547	****

In table 6.5 the average and ranges for pairwise interspecific genetic distances are summarised for the four species of *Scylla* using Nei's genetic distance and its associated genetic identity algorithm. The largest interspecific pairwise distance (and hence where two species show the greatest divergence) is between *S. serrata* and *S. olivacea* ($D_n = 0.490$), followed by the genetic distance between *S. serrata* and *S. tranquebarica* ($D_n = 0.384$). This table also shows *S. tranquebarica* to be approximately equally diverged from *S. olivacea* and *S. paramamosain* ($D_n = 0.243$ and 0.272 respectively). *S. serrata* and *S. paramamosain* on the other hand prove to be very closely associated species relative to the other two *Scylla* species, with an average Nei's Distance $D_n = 0.075$. In general distances recorded for all pairwise comparisons are much lower for Nei's genetic distance compared with Rogers' modified distance and Cavalli-Sforza and Edwards' arc distance measures (see table 6.6) as would be expected due to the way the measures are calculated. However, the interspecific pairwise relationships proved to be concordant with those observed using Nei's distance measure.

The genetic distances between conspecific populations of both *S. olivacea* and *S. paramamosain* are summarised in table 6.7. All conspecific pairwise distances are much lower than the genetic distances observed between *Scylla* species. Intraspecific pairwise comparisons for both species show low genetic distances ($D_n < 0.012$) for all three distance algorithms used. This is reinforced by the high genetic identities also shown by the same populations ($I_n > 0.99$). The closest association was recorded between populations of *S. paramamosain* from Thai Binh and Chanthaburi ($D_n < 0.001$).

Table 6.5: Matrix of means and ranges for interspecific genetic distances between four *Scylla* species. Above right: Nei's (1972) genetic identity; below left: Nei's (1972) genetic distance measure.

	<i>S. olivacea</i>	<i>S. paramamosain</i>	<i>S. tranquebarica</i>	<i>S. serrata</i>
<i>S. olivacea</i>	**** 0.705 (0.687 – 0.727)	0.784 (0.780 – 0.789)	0.613 (0.602 – 0.627)	
<i>S. paramamosain</i>	0.349 (0.319 – 0.375)	**** 0.761 (0.759 – 0.763)	0.930 (0.921 – 0.936)	
<i>S. tranquebarica</i>	0.243 (0.237 – 0.249)	0.272 (0.271 – 0.275)	**** 0.681*	
<i>S. serrata</i>	0.490 (0.467 – 0.507)	0.072 (0.066 – 0.082)	0.384*	****

Table 6.6: Matrix of means and ranges for interspecific genetic distances between four *Scylla* species. Above right: modified Rogers' distance (Wright, 1978); below left: Cavalli-Sforza and Edwards' (1967) arc distance.

	<i>S. olivacea</i>	<i>S. paramamosain</i>	<i>S. tranquebarica</i>	<i>S. serrata</i>
<i>S. olivacea</i>	**** 0.532 (0.509 – 0.568)	0.459 (0.456 – 0.461)	0.614 (0.604 – 0.620)	
<i>S. paramamosain</i>	0.520 (0.508 – 0.530)	**** 0.481 (0.480 – 0.481)	0.260 (0.251 – 0.276)	
<i>S. tranquebarica</i>	0.455 (0.450 – 0.459)	0.469 (0.466 – 0.475)	**** 0.562*	
<i>S. serrata</i>	0.611 (0.602 – 0.619)	0.271 (0.260 – 0.288)	0.547*	****

Table 6.7: Means and ranges for four distance measures recorded between three conspecific populations of *Scylla olivacea* and *Scylla paramamosain* from Southeast Asia.

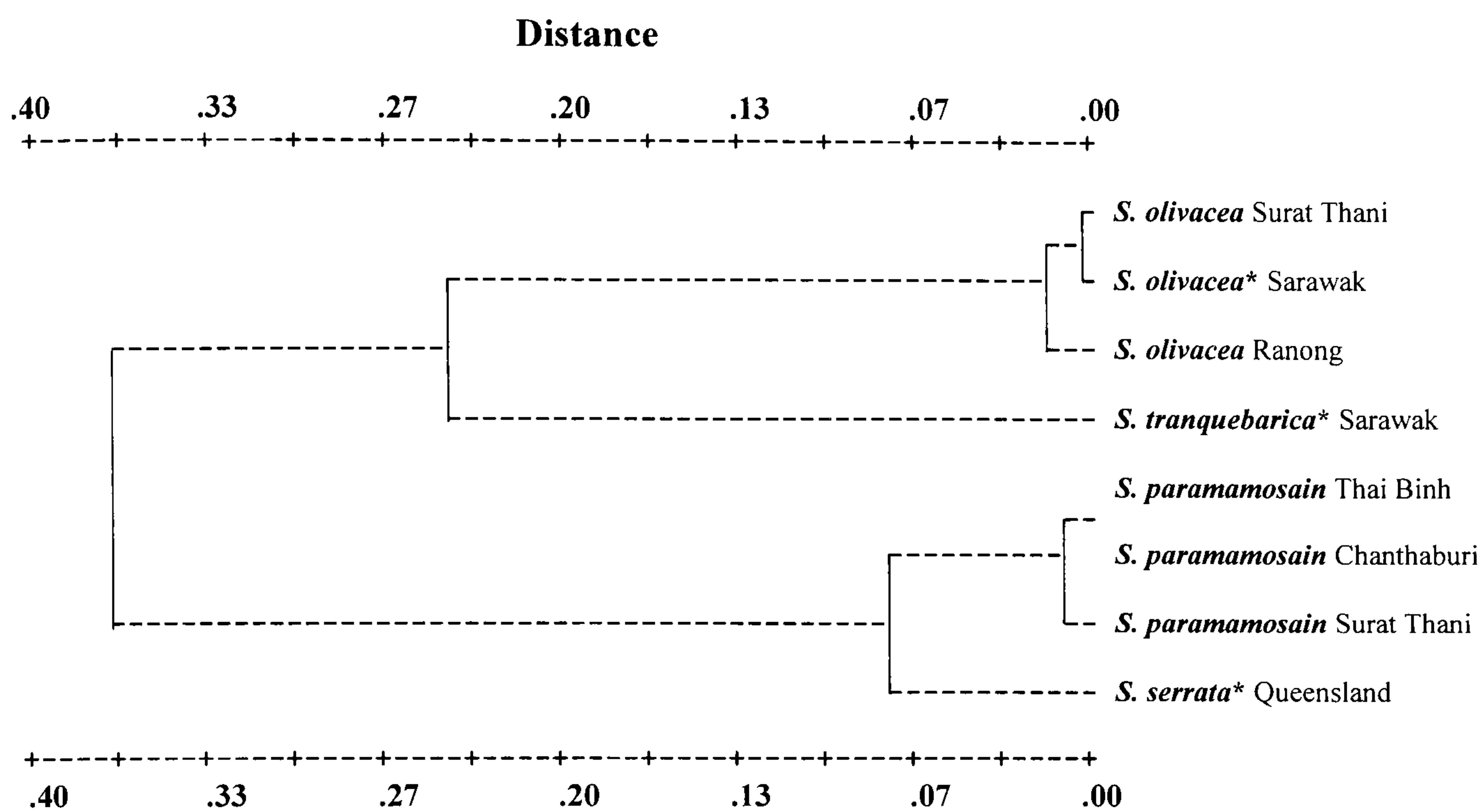
Species	Distances			
	Nei's Distance (Dn)	Nei's Identity (In)	Rogers' modified Distance (Drog)	Cavalli-Sforza and Edwards' Distance (Darc)
<i>S. olivacea</i>	0.008 (0.003-0.011)	0.992 (0.989 – 0.992)	0.083 (0.050 – 0.102)	0.091 (0.069 – 0.115)
<i>S. paramamosain</i>	0.004 (0.000-0.006)	0.995 (0.992-1.000)	0.060 (0.0021-0.086)	0.075 (0.060-0.098)

6.3.1.2 Dendrograms constructed using pairwise distances

Figure 6.2 shows two dendrograms constructed using the UPGMA clustering method on both Nei's (1972) genetic distances and Rogers (1972) modified distances for the seven groups collected representing four species of the genus *Scylla*. The data derived from both distance algorithms produced dendrograms with the same topology where all the samples cluster neatly into four species. The primary bifurcation splits the four species into two groups, namely *S. olivacea*/*S. tranquebarica* from *S. serrata*/*S. paramamosain* at $D_n = 0.372$. Secondary bifurcations result in the four species being separated with *S. tranquebarica* and *S. olivacea* diverging earlier than *S. paramamosain* from *S. serrata* ($D_n = 0.243$ and 0.075 respectively). Subsequent branches represent conspecific populations of *S. olivacea* and *S. paramamosain*. These are represented by very small branch lengths, especially when Nei's (1972) genetic distances are used compared with the more substantial branch lengths associated with using Rogers modified distances.

Figure 6.3 shows a dendrogram constructed using the distance Wagner procedure (Farris, 1972) with Cavalli-Sforza and Edwards (1967) arc distances as input data. The resulting tree topology is concordant with the dendrograms produced using UPGMA clustering methods, although the branch lengths are different. These differences in branch length show *S. serrata* to be more diverged than *S. paramamosain* from the *S. tranquebarica*/*S. olivacea* branch, while *S. olivacea* is more diverged than *S. tranquebarica* from the *S. paramamosain*/*S. serrata* branch.

a)



b)

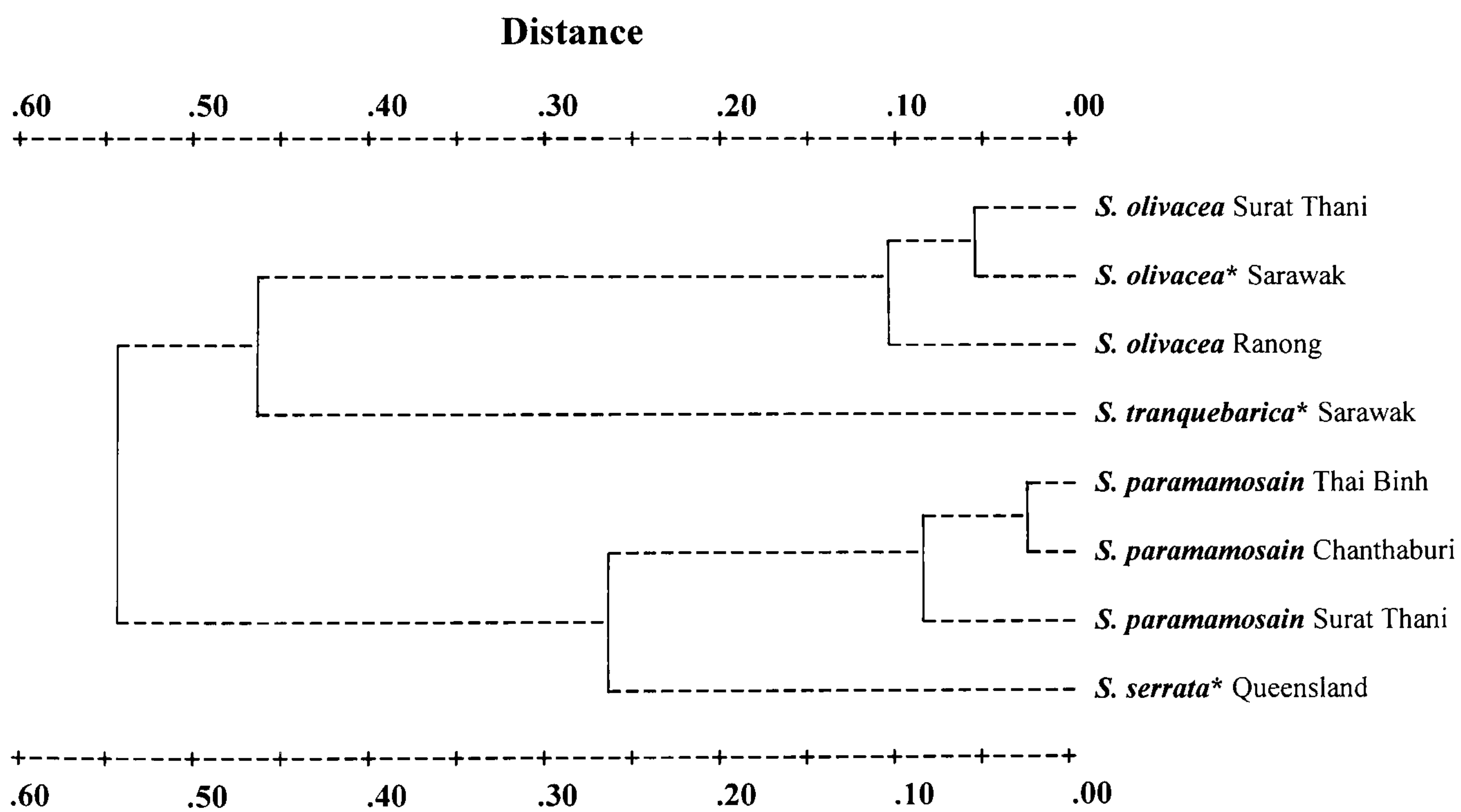


Figure 6.2: Dendrograms constructed using the UPGMA clustering method on a) Nei's distance measure (1972) and b) Rogers modified distance measure (Wright, 1978). Dendrograms are modified output from BIOSYS-1 (Swofford and Selander, 1989). Note: the asterisk represents those specimens typed by Keenan *et al.*, 1998.

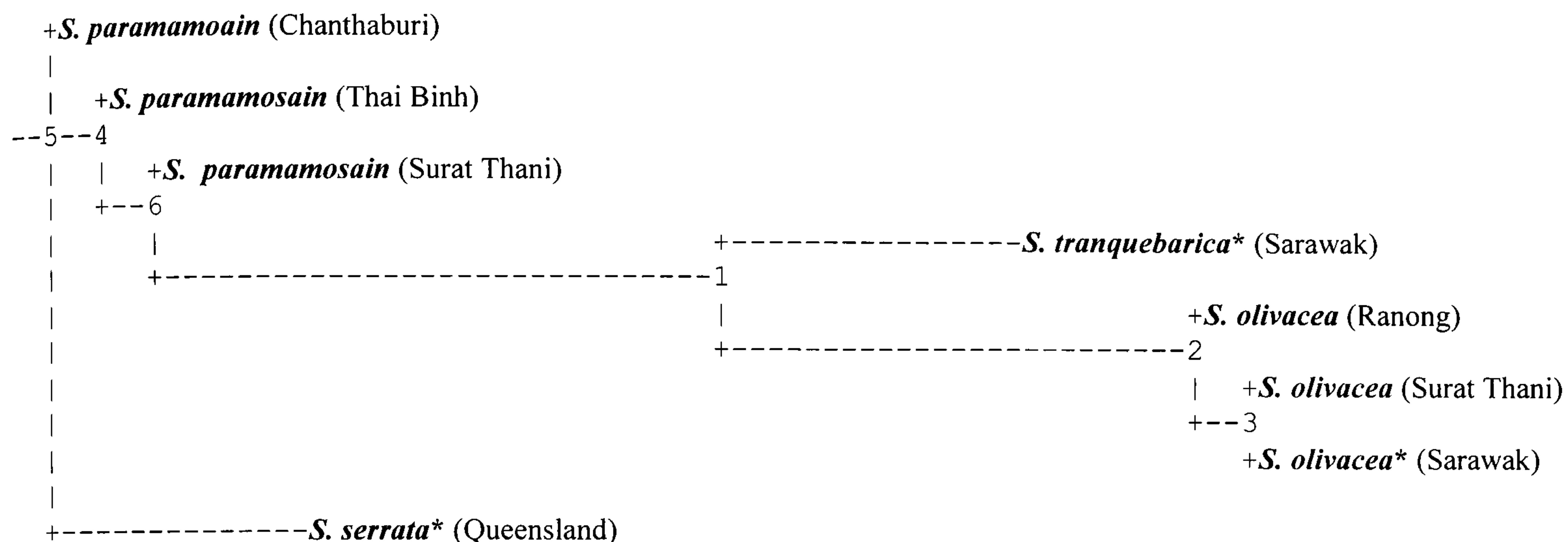
6.3.1.3 Dendrograms constructed using discrete character data

a) *Maximum likelihood estimate*

A dendrogram using the restricted maximum likelihood estimate (Felsenstein, 1981), constructed using gene frequency data, is illustrated in figure 6.4. As the dendrogram is slightly disconcerting the branch lengths associated with the dendrogram have also been included. The maximum likelihood approach reveals a primary separation between *S. serrata*/*S. paramamosain* and *S. tranquebarica*/*S. olivacea*, as seen for the methods inferring phylogeny using pairwise distance values. The relationships among the conspecific populations of *S. paramamosain* were represented differently using maximum likelihood estimate than from using pairwise distances. The maximum likelihood method revealed Chanthaburi to be slightly more differentiated from the populations from Thai Binh and Surat Thani, rather than Surat Thani being seen as the more differentiated population as shown using the pairwise distances approach.

b) *Wagner parsimony*

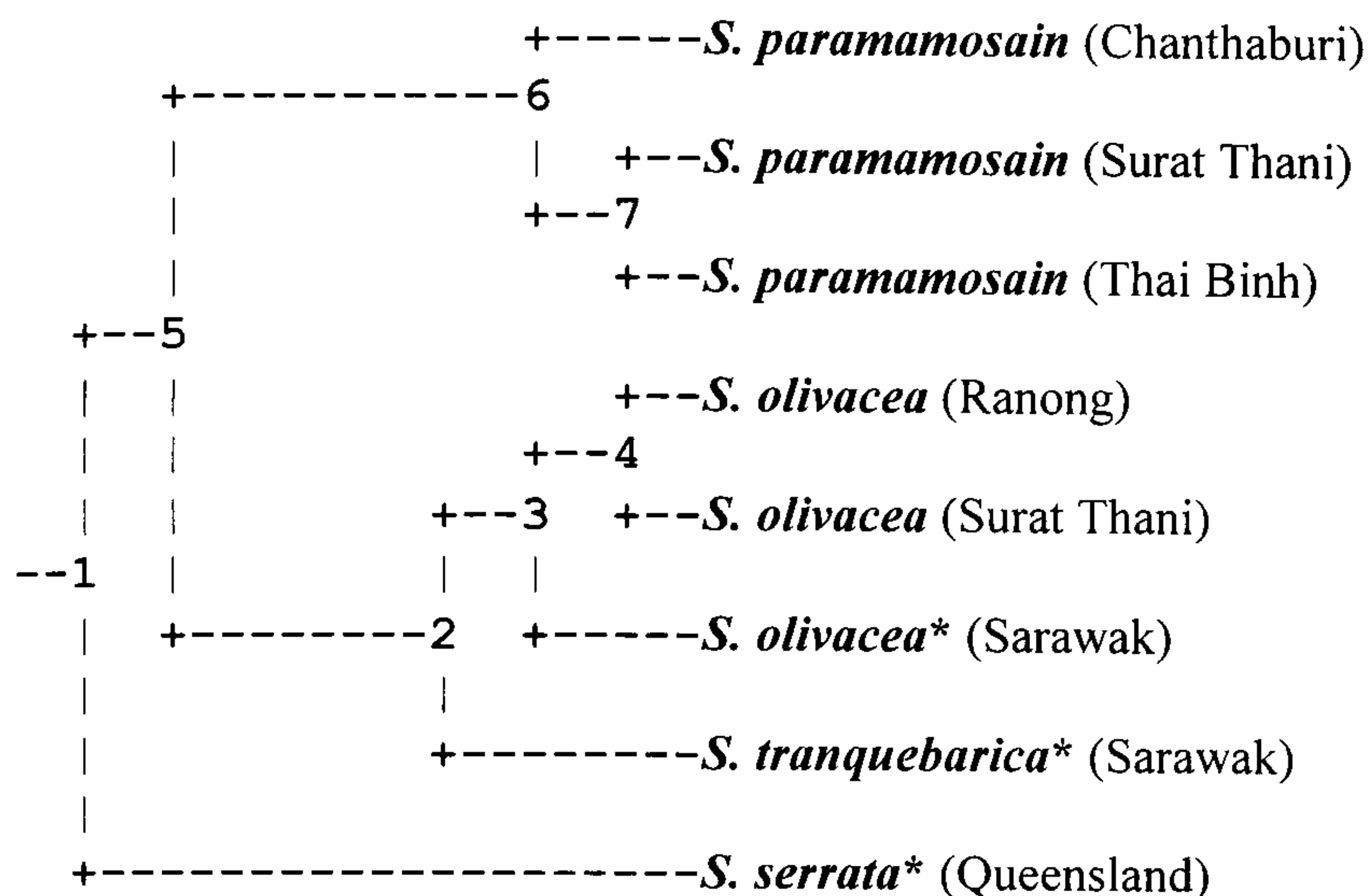
The dendrogram using the Wagner parsimony method with binary coded presence/absence of alleles is shown in figure 6.5 using the data set directly (6.5a) and with bootstrap resampled data (6.5b). The topology of both trees is the same, only the presentation of the groups is slightly different. Only one parsimonious tree was revealed using both the raw data and bootstrapped resampling, with both trees requiring 25 steps to explain the data. Both dendrograms position *S. serrata* as an outgroup to the other three species, suggesting it is the most diverged of all four species. The secondary bifurcation splits *S. paramamosain* from *S. olivacea*/*S. tranquebarica*. The tertiary bifurcation defines *S. tranquebarica* from *S. olivacea*. This overall topology is concordant with that observed using the restricted maximum likelihood approach, although no branch lengths are revealed



Between	and	Length between nodes	Confidence limits
5	Chanthaburi (<i>S. paramamosain</i>)	0.001	- 0.006, 0.010
5	4	0.011	0.004, 0.020
4	Vietnam (<i>S. paramamosain</i>)	0.000	- 0.004, 0.005
4	6	0.006	- 0.002, 0.018
6	S.T. white (<i>S. paramamosain</i>)	0.008	- 0.001, 0.020
6	1	0.374	0.072, 0.810
1	Sematan (<i>S. tranquebarica</i>)	0.206	- 0.022, 0.536
1	2	0.320	0.045, 0.717
2	Ranong (<i>S. olivacea</i>)	0.018	0.003, 0.039
2	3	0.006	- 0.008, 0.027
3	S.T. black (<i>S. olivacea</i>)	0.000	- 0.006, 0.008
3	Sematan (<i>S. olivacea</i>)	0.015	0.006, 0.028
5	Queensland (<i>S. serrata</i>)	0.179	0.072, 0.335

Figure 6.4: Dendrogram constructed using the Restricted Maximum Likelihood Estimate (Felsenstein, 1981a) on allele frequency data for species of the genus *Scylla* from six locations within Southeast Asia. The amounts of expected accumulated variance experienced between two nodes or a node and a species (location) is indicated by the branch lengths listed in the table above. The sub-program CONTML of the software package PHYLIP version 3.5c (Felsenstein, 1993) was used to produce the dendrogram. Note: the asterisk represents those specimens typed by Keenan *et al.*, 1998.

a)



b)

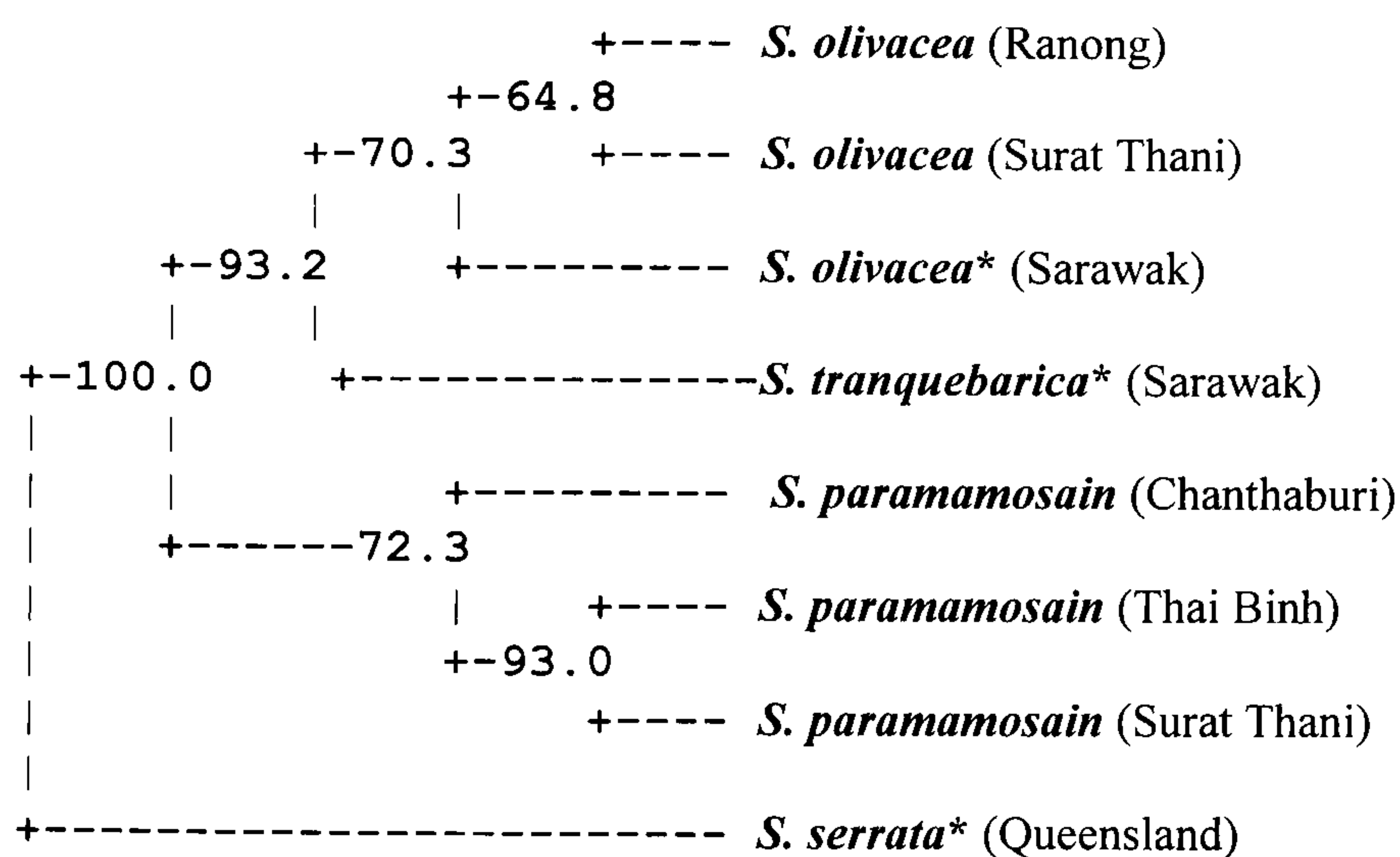


Figure 6.5: Dendrogram constructed using the Wagner parsimony method (Eck and Dayoff, 1966; Kluge and Farris, 1969; Farris, 1990) with binary coded allele data with respect to presence/absence of alleles at a locus. Both random assignment of taxon (a) and consensus of 100 bootstrapped resampled data sets (b) were used. The software package PHYLIP version 3.5 (Felsenstein, 1993) was used to produce the dendrograms. Note: the asterisk represents those specimens typed by Keenan *et al.*, 1998.

using this particular Wagner parsimony method. Thus the branch lengths observed are not indicative of genetic distance between *Scylla* species.

6.3.1 Morphology

6.3.2.1 Coding of morphological characters

Table 6.8 outlines the chosen morphological characters, their associated character states represented by the four *Scylla* species and the associated score based on the “outgroup rule” of ancestry. Subsequent multistate coding and binary recoding for the nine morphological/behavioural characters observed in the four *Scylla* species and the outgroup *Thalamita crenata* are outlined in tables 6.9 and 6.10.

6.3.2.2 Dendrogram constructed using Wagner parsimony

Only one parsimonious tree with a tree length consisting of 24 steps is observed using Wagner parsimony method with binary recoded morphological character states (see figure 6.6a). Bootstrap resampling of the binary coded data set made no impact to the resulting dendrogram topology although the presentation is slightly different (figure 6.6b). *Scylla olivacea* is revealed as being morphologically the closest to the ancestral outgroup, *Thalamita crenata*. The morphological characters they share include the lack of reticulation (characters 1 and 2); their broad frontal lobe width with respect to the internal carapace width (character 3) and the reduced smooth frontal lobe spines (character 4). *Scylla serrata* and *S. paramamosain* still remain closely associated species, as is seen in the dendrograms constructed using allozyme data. These two synapomorphic species exhibit most, if not all, the character states that are considered to be derived e.g. a high degree of reticulation and spination. *S. tranquebarica* remains intermediate between *S. olivacea* and *S. serrata/S. paramamosain*.

Table 6.8: Summary of morphological characters, their character states and associated scores (the ancestral state scored as 0), and the suggested character state order for the four species of the genus *Scylla* (with *Thalamita crenata* providing the ancestral outgroup).

Character	Character states	Score	Order
1. Chelae reticulation:	- Absence of reticulation.	0	
	- Faint reticulation.	1	0 — 1 — 2
	- Highly visible reticulation.	2	
2. Pereiopod reticulation:	- Absence of reticulation.	0	0 — 1
	- Strong reticulation.	1	
3. Frontal lobe width (FLW/ICW)	- Very broad frontal lobe.	0	
	- Broad frontal lobe (0.40).	1	0 — 1 — 2
	- Narrow frontal lobe (0.37).	2	
4. Frontal lobe teeth shape (FMSH/FLW)	- Flattened rounded teeth.	0	3
	- Flattened obtuse teeth (0.029).	1	
	- Slightly raised obtuse teeth (0.043).	2	0 — 1 — 2
	- Triangular pointed teeth (0.058).	3	
	- Elongated acute teeth with blunt tips (0.061).	4	4
5. Anterolateral spine shape	- 4 – 5 spines decreasing in size from anterior to posterior.	0	
	- 9 spines of equal size and shape.	1	0 — 1 — 2
	- 9 spines, posterior spines more protuded than other spines.	2	
6. Dorsoantero propodus spine (number)	- 1 – 2 spines	0	0 — 1
	- 2 spines	1	
7. Ventral carpal spines (number)	- absence to 1 spine	-2	
	- 1 – 2 spines	-1	-2 — -1 — 0 — 1
	- 2 small spines	0	
8. Habitat	- 2 prominent spines	1	
	- Reduced salinity inner coastal mangrove.	-1	
	- Intertidal mudflats and corals	0	-1 — 0 — 1
9. Behaviour	- Subtidal, oceanic areas including corals	1	
	- Intertidal burrowing	-1	
	- Crawl under stones	0	-1 — 0 — 1
	- Subtidal, free swimming	1	

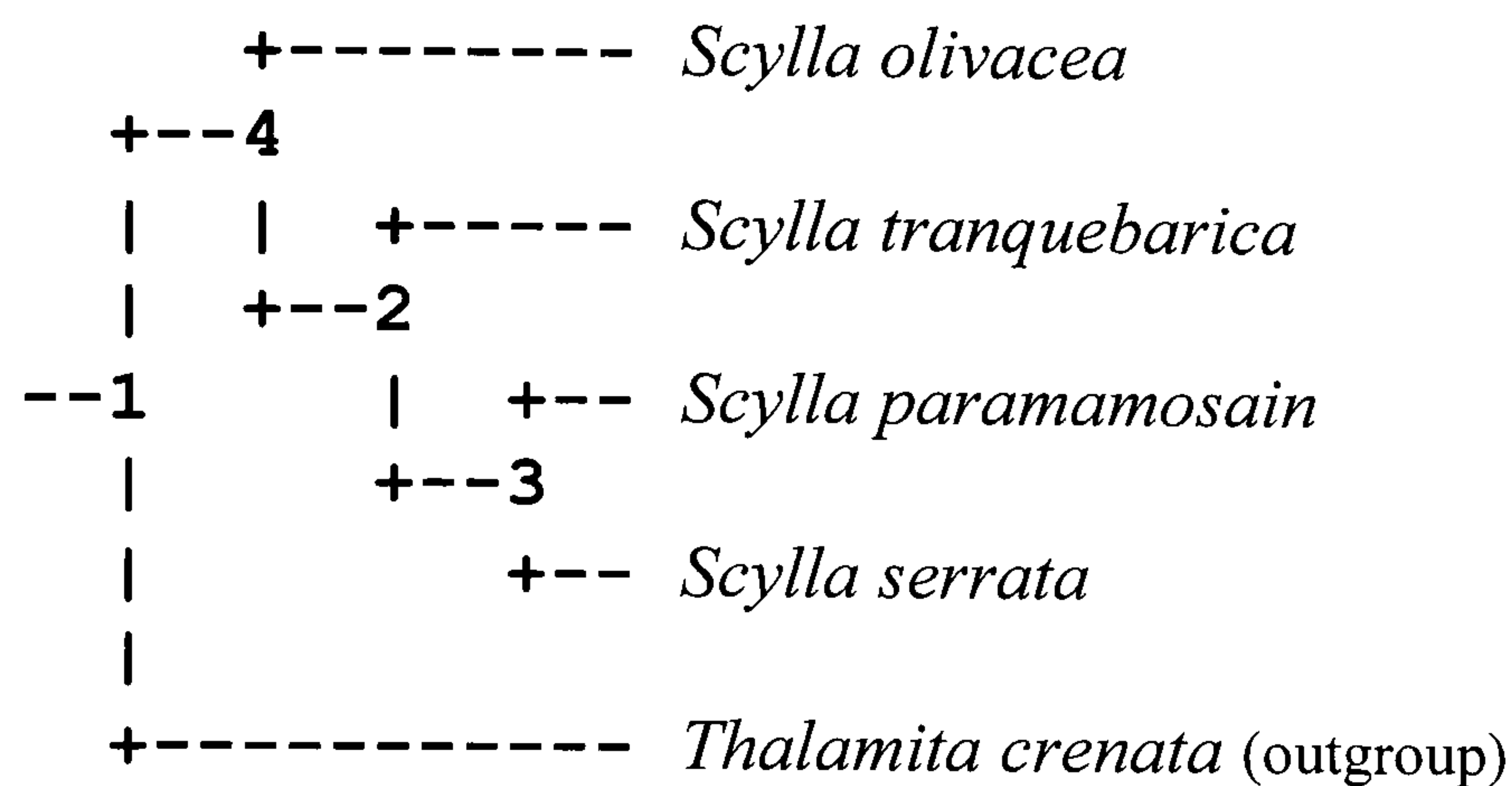
Table 6.9: Summary of continuous character states coded for the nine morphological characters collated for the four *Scylla* species and for the outgroup, *Thalamita crenata*.

Species	Character								
	1	2	3	4	5	6	7	8	9
<i>Thalamita crenata</i>	0	0	0	0	0	0	0	0	0
<i>Scylla serrata</i>	2	1	2	4	1	1	1	1	1
<i>Scylla tranquebarica</i>	1	1	1	3	2	1	1	-1	-1
<i>Scylla paramamosain</i>	2	1	2	2	1	0	-1	1	1
<i>Scylla olivacea</i>	0	0	1	1	1	0	-2	-1	-1

Table 6.10: Summary of the new recoded data into binary (0,1) format for the morphological characters collated for the four *Scylla* species and the outgroup, *Thalamita crenata*.

Species	Character								
	1	2	3	4	5	6	7	8	9
<i>Thalamita crenata</i>	00	0	00	0000	00	0	000	00	00
<i>Scylla serrata</i>	11	1	11	1101	10	1	001	01	01
<i>Scylla tranquebarica</i>	10	1	10	1110	11	1	001	10	10
<i>Scylla paramamosain</i>	11	1	11	1100	10	0	100	01	01
<i>Scylla olivacea</i>	10	0	10	1000	10	0	110	10	10

a)



b)

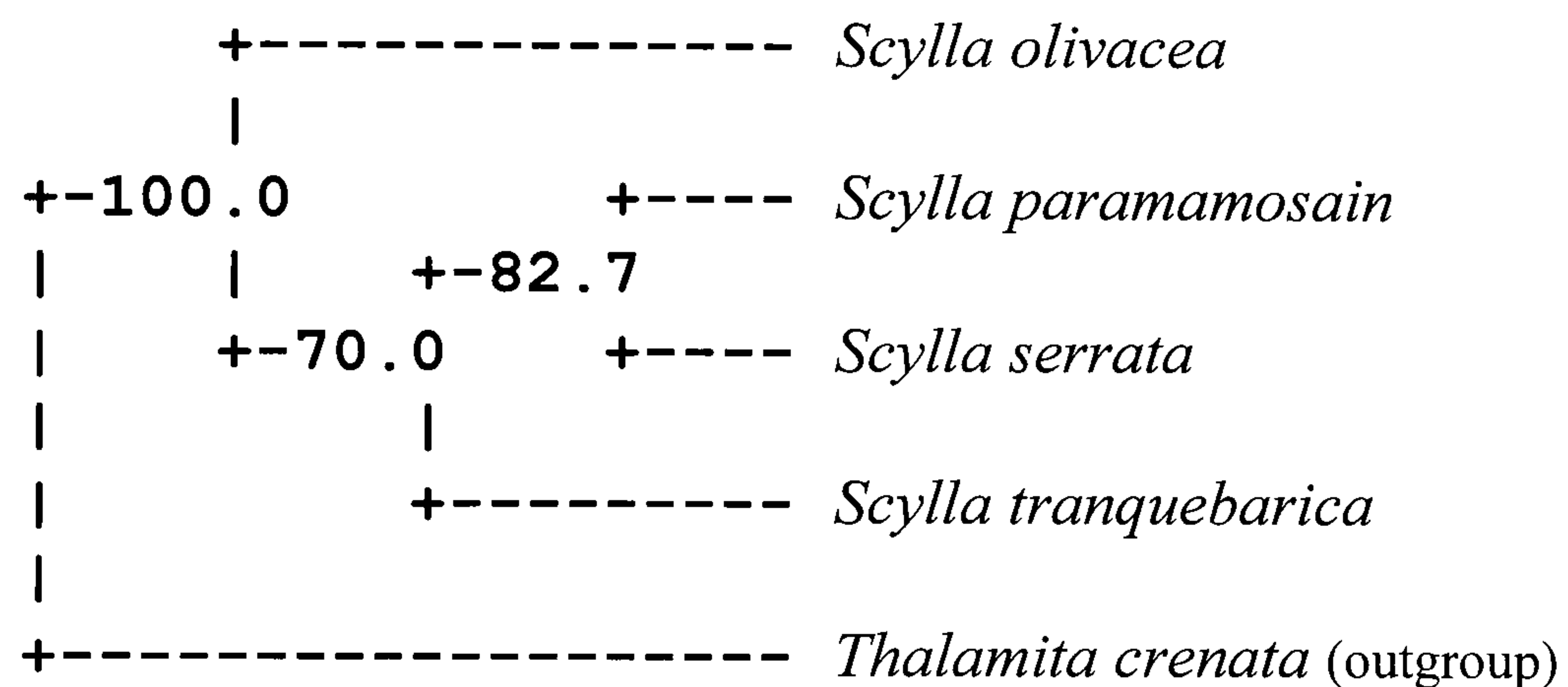


Figure 6.6: Dendrograms using a) Wagner parsimony method (Eck and Dayoff, 1966; Kluge and Farris, 1969; Farris, 1990) and b) Wagner parsimony on 100 bootstrap resampled data sets, to construct the tree with binary recoded morphological and behavioural characters for the four *Scylla* species. The dendrogram was rooted using *Thalamita crenata* as the ancestral outgroup species. The software PHYLIP version 3.5c (Felsenstein, 1993) was used to construct the dendrograms.

6.4 Discussion

6.4.1 Genetic divergence

The level of genetic divergence between the sampled locations can be divided into two categories, namely a) the divergence among the four species of *Scylla* (interspecific divergence); and b) the divergence between conspecific populations (intraspecific divergence). As one would expect, the values for divergence between the four proposed species of *Scylla* is an order of magnitude higher than between locations within a species.

6.4.1.1 Genetic divergence among *Scylla* species

Nei's genetic distance measure, revealed that among the four *Scylla* species the mean genetic distance ranged from $D_n = 0.243$ to 0.490 ($I = 0.613-0.784$) except for the genetic distance between *Scylla paramamosain* and *S. serrata* which was shown to be extremely small ($D = 0.072$; $I = 0.930$). This suggests that these two species are very recently diverged, with only one locus (*ArgK*) expressing a fixed difference in allele mobility which separates these two species. The mean Nei's genetic identity between the four *Scylla* species (excluding the *S. paramamosain/S. serrata* distance value) are in accordance with interspecific genetic identities suggested by Thorpe (1982) for a range of plant and animal species, the values ranging from $I = 0.35$ to $I = 0.85$. This was also found to be true for Nei's genetic identity between other selected crustacean species (Hedgecock *et al.*, 1982).

The associated high genetic identity value between *S. serrata* and *S. paramamosain* ($I = 0.93$) suggests that they should have subspecies, or possibly sibling species, status. Similar high genetic identity was discovered between two former species of the shore crab, genus *Carcinus* ($I = 0.89$), suggesting that they were in fact subspecies (Bulnheim and Bahns, 1996).

Table 6.11 show that in general the Nei's genetic distances recorded among *Scylla* species in the present study are higher than those quoted for three species of *Scylla* proposed by Fuseya and Watanabe (1996) and three species of *Scylla* studied by Sugama and Hutapea (1999).

Table 6.11: Comparison of Nei's (1972) genetic distances for *Scylla* species.

Pairwise comparison	Present Study	Fuseya and Watanabe (1996)	Sugama and Hutapea (1999)
<i>S. serrata</i> vs <i>S. olivacea</i>	0.490	0.200*	-
<i>S. serrata</i> vs <i>S. paramamosain</i>	0.072	0.128*	-
<i>S. serrata</i> vs <i>S. tranquebarica</i>	0.384	-	-
<i>S. olivacea</i> vs <i>S. paramamosain</i>	0.349	0.064*	0.199
<i>S. olivacea</i> vs <i>S. tranquebarica</i>	0.243	-	0.078
<i>S. paramamosain</i> vs <i>S. tranquebarica</i>	0.272	-	0.117

* The species names have been converted to the new nomenclature (Keenan *et al*, 1998) to make comparison possible.

The lower distance values found by Fuseya and Watanabe (1996) and Sugama and Hutapea (1999) are due to the low number of loci that are either fixed for different alleles, or which show differences in the dominant alleles between species (33% of alleles are close to or are fully fixed between *Scylla* species in the present study compared to 29% and 18% of loci studied by Sugama and Hutapea (1999) and Fuseya and Watanabe (1996) respectively). A lower percentage of fixed allelic differences will inherently lower the level of perceived divergence between taxa.

It is also important to note that the mean distance values between the two sympatric species located in Surat Thani, *Scylla olivacea* and *Scylla paramamosain*, show marked genetic distances from 0.329 for Nei's genetic distance to 0.515 and 0.517 for Cavalli-Sforza and Edwards' arc distance and Rogers' modified distance, respectively. This supports the view that these two species have maintained sympatry between each other; the

substantial distance value being attributed to the fixed allelic differences at three loci. The genetic distance recorded between the two sympatric species of *Scylla* are similar to that recorded for other sympatric crab species. For example, Stewart (1997) found a genetic divergence of $D_n = 0.419$ between two sympatric species of river crab, genus *Potamonautes*.

The relationships between *Scylla* species in this study are different to those found by Fuseya and Watanabe (1996) on three of the four proposed species. Fuseya and Watanabe (1996) found lower genetic divergence between *Scylla paramamosain* and *Scylla olivacea* than between *S. paramamosain* and *S. serrata*, contrary to the results of this study. This is probably due to the different loci examined between the two studies. The loci that exhibited variable allelic mobilities that were fixed between species in this study (*ArgK*, *Alat*, *Mpi*, and *Pgm*) were not the same as those discovered by Fuseya and Watanabe (*Est*, *Lap-2* and *Sod*). This highlights the importance of using as many loci and locations as possible in order to give a realistic representation of the interspecific relationships between taxa. It is also possible that the four species recognised in this study have been incorporated into three species in Fuseya and Watanabe's study, making comparison between the two studies almost redundant.

Sugama and Hutapea's (1999) study does reflect the same trend in genetic distances as those seen in the present study, where *S. tranquebarica* is more closely associated with *S. olivacea* than *S. paramamosain*. However, closer examination of their table of allele frequencies shows that the names *S. paramamosain* and *S. olivacea* have been used to name different species to those allocated by the author and by Keenan *et al.* (1998). Allele mobilities for *Mpi* and *Pgm* associated to the species names *S. paramamosain* and *S.*

olivacea are reversed to those associated both by Keenan *et al.* (1998) and the present study. Further study of their samples and location would be required to clarify the species and therefore the divergence between them.

6.4.1.2 Genetic divergence among conspecific *Scylla* populations

Intraspecific pairwise comparisons of populations of both *Scylla olivacea* and *S. paramamosain* prove to have low genetic distances that are in accordance with intraspecific studies on other portunid crabs. Table 6.12 summarises the level of intraspecific genetic divergence for a range of crustacean and non-crustacean species, recorded using Nei's genetic distance. The range of genetic distances for conspecific populations of both species supports the results of genetic differentiation discussed in chapter five; therefore, to minimise repetition, the possible reasons for the divergence observed between conspecific populations will only be discussed briefly here.

Nei's genetic distance between populations of *S. paramamosain* from Thai Binh and Chanthaburi ($D=0.0000$) indicates that there is total panmixis (*i.e.* wide spread mixing of genes) between these populations. This has been strongly supported in other studies for marine species, which have an extended pelagic phase within its life history (most commonly the larval phase) allowing extensive gene flow between conspecific populations (*e.g.* Ward *et al.*, 1994). The strong surface currents that flow along the eastern seaboard of the Vietnam/Thai-Malay peninsula (Dale, 1956) in conjunction with the duration of the pelagic larval phase of the mud crab (approximately 30 days; Ong, 1966), supports this hypothesis.

Table 6.12: Measures of Intraspecific population divergence using Nei's (1972) genetic distance measure for a range of decapod crustaceans and other marine species.

Species	D-value	Author
<i>Menippe mercenaria</i> (stone crab)	0.010 - 0.120	Bert, 1986
<i>Pinnotheres novaezelandiae</i> (pea crab)	0.004 - 0.249	Stevens, 1990
<i>Callinectes sapidus</i> (blue crab)	0.000 - 0.007	McMillan-Jackson <i>et al</i> , 1994
<i>Uca minax</i> (fiddler crab)	0.035	Felder and Staton, 1994
<i>Sesarma reticulatum</i> (sesarmid crab)	0.000.-0.276	Felder and Staton, 1994
<i>Nephrops norvegicus</i> (Norwegian lobster)	0.002 - 0.008	Passamonti <i>et al</i> , 1997
<i>Tridachna gigas</i> (giant clam)	0.0007	Benzie and Williams, 1992
<i>Acanthaster planci</i> (crown-of-thorns starfish)	0.010 - 0.050	Benzie and Stoddart, 1992

The population from Surat Thani however is differentiated from these two populations ($D = 0.006$) suggesting some form of isolation from the Chanthaburi and Thai Binh populations. This differentiation, although small, may be the result of the complex tidal eddies taking place within the Gulf of Thailand (as described in more detail in chapter five).

The populations of *Scylla olivacea* were also differentiated ($D_n = 0.003$ between Surat Thani and Sarawak, $D_n = 0.009$ Between Surat Thani and Ranong and $D_n = 0.011$ between Sarawak and Ranong populations). These seem to show an isolation-by-distance model of divergence. Both geological barriers (the Thai-Malay peninsula providing a geographical land barrier between Ranong and the other two populations) and local selection pressures on the recruited juvenile mud crab will result in differentiation taking place.

6.4.2 Phylogeny of *Scylla* species using allozyme data

Phylogenies based on UPGMA clustering, distance Wagner and maximum likelihood approaches resulted in the four well defined *Scylla* species dividing into two primary groups, namely *S. serrata*/*S. paramamosain* and *S. tranquebarica*/*S. olivacea*. This

primary grouping is supported by observations from the behaviour and ecology of these mud crab species. Both *S. olivacea* and *S. tranquebarica* inhabit estuarine mangrove areas, whereas *S. serrata* and *S. paramamosain* have a predominantly subtidal existence and do not make proper burrows into the sediment (Keenan *et al.*, 1998).

Dendrograms using UPGMA clustering constructed from pairwise sequence divergence of *Scylla* species using the COI (Cytochrome oxidase I) and 16S-rRNA subunits of mtDNA (Keenan, 1999) show some disparity to the phylogeny resolved in the present study (see fig 6.7). In Keenan's study *S. paramamosain* is more closely related to *S. tranquebarica* rather than to *S. serrata* as seen in the present study using allozyme data. As both COI and 16S-rRNA subunits are universally conserved genes within the mitochondrial genome (Simon *et al.* 1994), their consensus in the position of *S. paramamosain* in the phylogeny of *Scylla* is most likely to be the more correct interpretation, even though morphological characters and gene frequencies suggest *S. paramamosain* to be more closely related to *S. serrata* in the present study. The most likely explanation for the lack of consensus is due to the small number of individuals sampled that represented both *S. tranquebarica* and *S. serrata* (five and six individuals respectively). The effects of few individuals on the interpretation of phylogenetic reconstruction has been highlighted by Keenan (1991) who suggests at least six individuals should be used to pick up any polymorphisms which may not be detected in small samples sizes. It is also well documented that the number of loci used in a phylogenetic study should also be substantial so that the genome is properly represented (Richardson *et al.*, 1986; Leary and Booke, 1990).

Apart from technical reasons to explain the discrepancy, another interpretation could also be that the rates of evolution for the subunits of mtDNA used in Keenan *et al.*'s (1998)

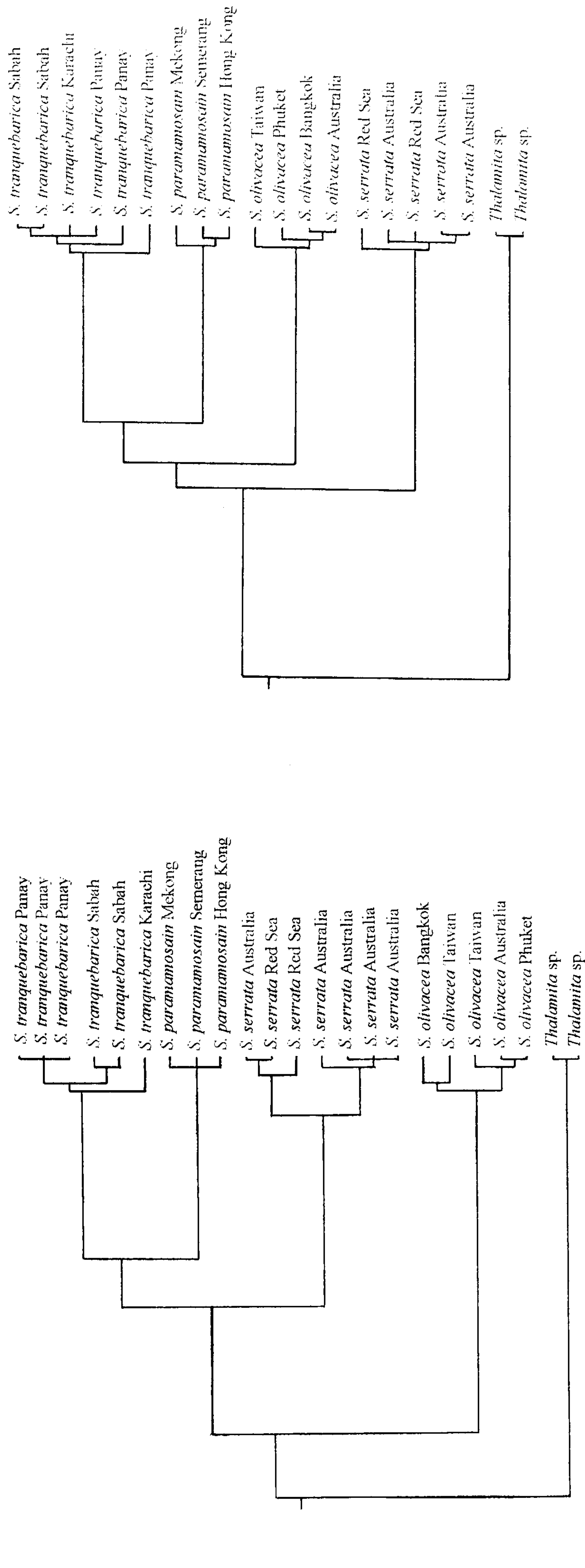


Figure 6.7: UPGMA dendrogram for Tamura's (1992) genetic distances among *Scylla* species using a) cytochrome oxidase I and b) 16S-rRNA mtDNA subunits (taken from Keenan, 1999a).

study and allozymes tested in the present study are different. This difference in the rate of evolution between mitochondrial DNA and Nuclear DNA has been stressed in other studies on marine stocks (Ovenden, 1990).

From the dendrograms produced using molecular and morphological data two species show the greatest divergence, *S. serrata* and *S. olivacea*. However the direction of evolution is open to interpretation. This discrepancy between which of these two species is the most diverged was also found between dendrograms of the mtDNA subunits 16S-rRNA and COI as shown in figure 6.7 (Keenan *et al.*, 1998).

Evidence pointing to *Scylla olivacea* as the primitive species can be based on the following deductions. Morphologically, *S. olivacea* is the most similar to the outgroup species chosen, *Thalamita crenata*. *Thalamita crenata* is also a portunid species which has morphological characters between the ancestral crawling species, such as *Carcinus* and the more recently formed swimming species such as *Portunus pelagicus* (Stephenson and Campbell, 1959). Therefore the main structural developments that are associated with the descendent swimming crab species, *i.e.* increase in size, broadening of carapace, sharp spination and reticulated exoskeleton (Hartnoll, 1971), are present on *Scylla serrata*, but are absent from *Scylla olivacea*. Therefore, one hypothesis could be that *Scylla olivacea* is the most ancestral of the four *Scylla* species, the other three species being formed during subsequent evolutionary events, such as glaciation, *S. serrata* and *S. paramamosain* adapting to a more subtidal environment.

Alternatively, the evidence pointing to *S. serrata* as the primitive species, as is observed in the maximum likelihood and Wagner parsimony using gene frequency data, is equally

valid. In particular, it is possible that the morphological features of the outgroup chosen and *S. olivacea* are similar because both these species have been selected to live in the intertidal environment and therefore exhibit analogous features as a result of the environment they inhabit. Avise (1983) also noted that the choice of outgroup could seriously affect the result of a cladistic analysis, particularly with the choice of polarities. It is possible that if another portunid genus, such as the swimming crab *Portunus pelagicus*, had been chosen that the resulting phylogeny would be quite different.

The particular adaptations to the mangrove environment adopted by *S. olivacea* include small body size and dark brown exoskeleton, aiding in camouflage especially at the juvenile and sub-adult stage. The fattened, oar-like swimming legs are equally useful for burrowing, a particular behaviour observed for both *Scylla olivacea* and *Scylla tranquebarica*. The ability to burrow is also seen as an advanced characteristic (Hartnoll, 1971). However, the vestigial spination on the carpal and propodal segments of the chelae observed on *S. olivacea* is a good indication that this has been derived from sharp spines originally. If the direction of evolution was expected in the reverse direction and *S. olivacea* was the ancestral species, then the spines would not be vestigial, but would instead be absent. The scenario in this instance is that the ancestral taxon would have been a subtidal species that later diverged to produce a sub-group that utilised the safety of the estuarine mangrove habitat and evolved to continue to live in this habitat in the adult stage. These advanced species were selected from those that could burrow during low tide and could withstand huge fluctuations in salinity, characteristic of an estuarine environment. Burrowing is regarded as highly advantageous for crustaceans living in the tropical intertidal zone (Atkinson and Taylor, 1988; Macintosh, 1988). At the present stage it is difficult to say which, if any, of these scenarios is correct. A more detailed investigation

into both of these theories may reveal further information on the sequence of events that lead to these four very closely related species.

Glaciation events have been well documented as one of the most plausible reasons for divergence between closely related species which otherwise have the potential for high gene flow (Palumbi, 1994) including that of decapod crustaceans (Bert, 1986; Lavery *et al.*, 1995).

The geographic distribution of the *Scylla* species, as shown in figure 6.8, shows that *S. serrata* is wide ranging in its distribution from the east coast of Africa to the southwest Pacific whereas the other three *Scylla* species are located around south and east Asia, indicating that the South China Sea is the most likely location where divergence of the *Scylla* species has taken place.

The South China Sea is extremely shallow and forms a continental shelf between Borneo, the Philippine islands and peninsular Malaysia/Thailand, known as the Sunda platform. During the Pleistocene epoch, periods of glaciation caused water from oceans to be locked into the ice cap which formed over northern Europe, subsequently lowering the sea level around the equator by as much as 150 metres (Dobby, 1966); this resulted in the Sunda platform being periodically exposed. Figure 6.9 illustrates the topography of the Indo-West Pacific during such a glaciation event. Early Pleistocene fossils of *Scylla* found both in Japan (Muroka, 1976) and Africa (Cooper and Kensley, 1991) indicate that this crab had a wide dispersal over most of the Indo West Pacific during this period. It is possible therefore that populations of an ancestral *Scylla* species were separated by the land

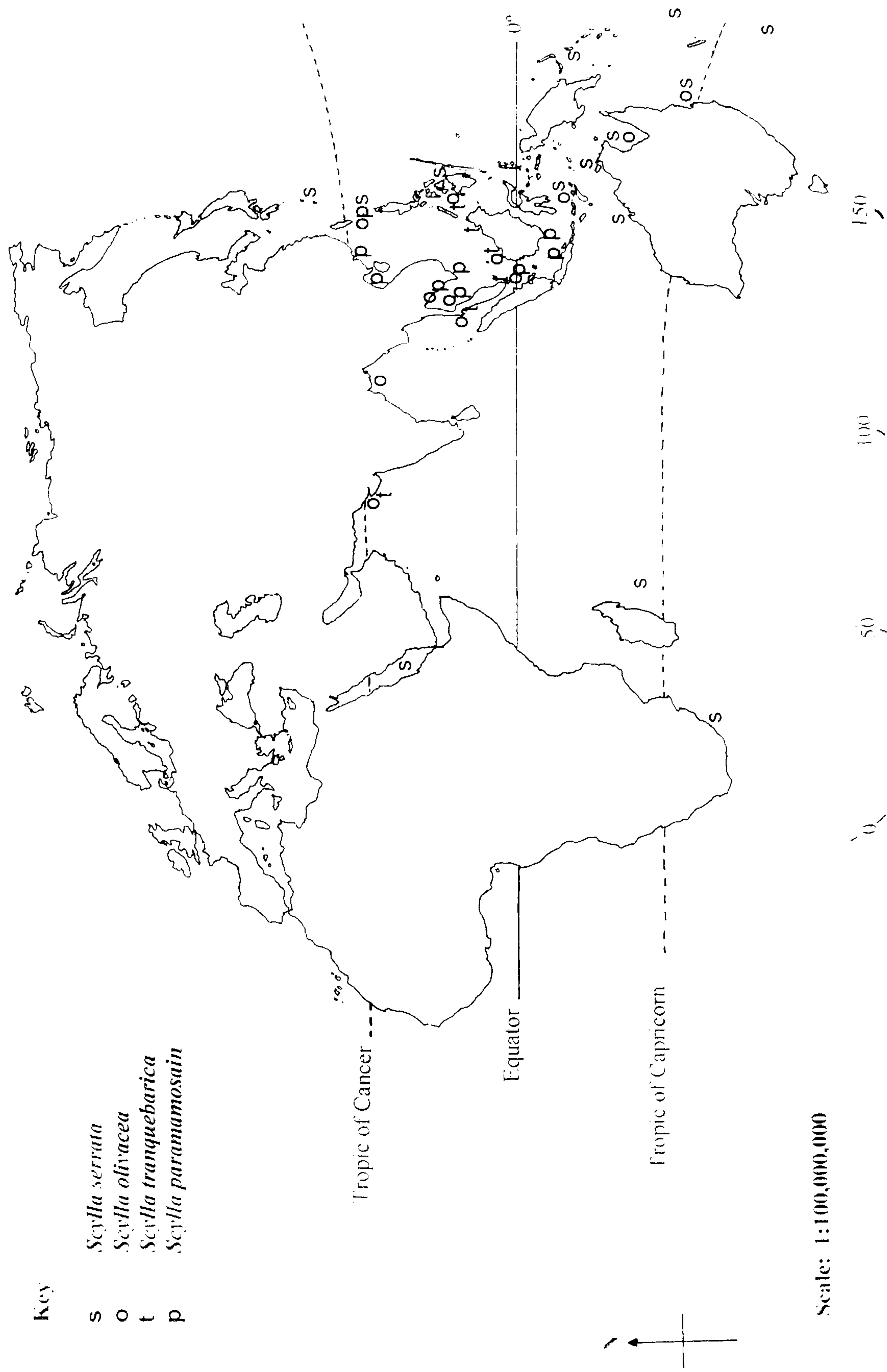


Figure 6.8: Geographical distribution of the four species of the genus *Scyllia* within the Indo-West Pacific (produced with a compilation of data from this study, Fuseya and Watanabe, 1996 and Keenan *et al.*, 1998).

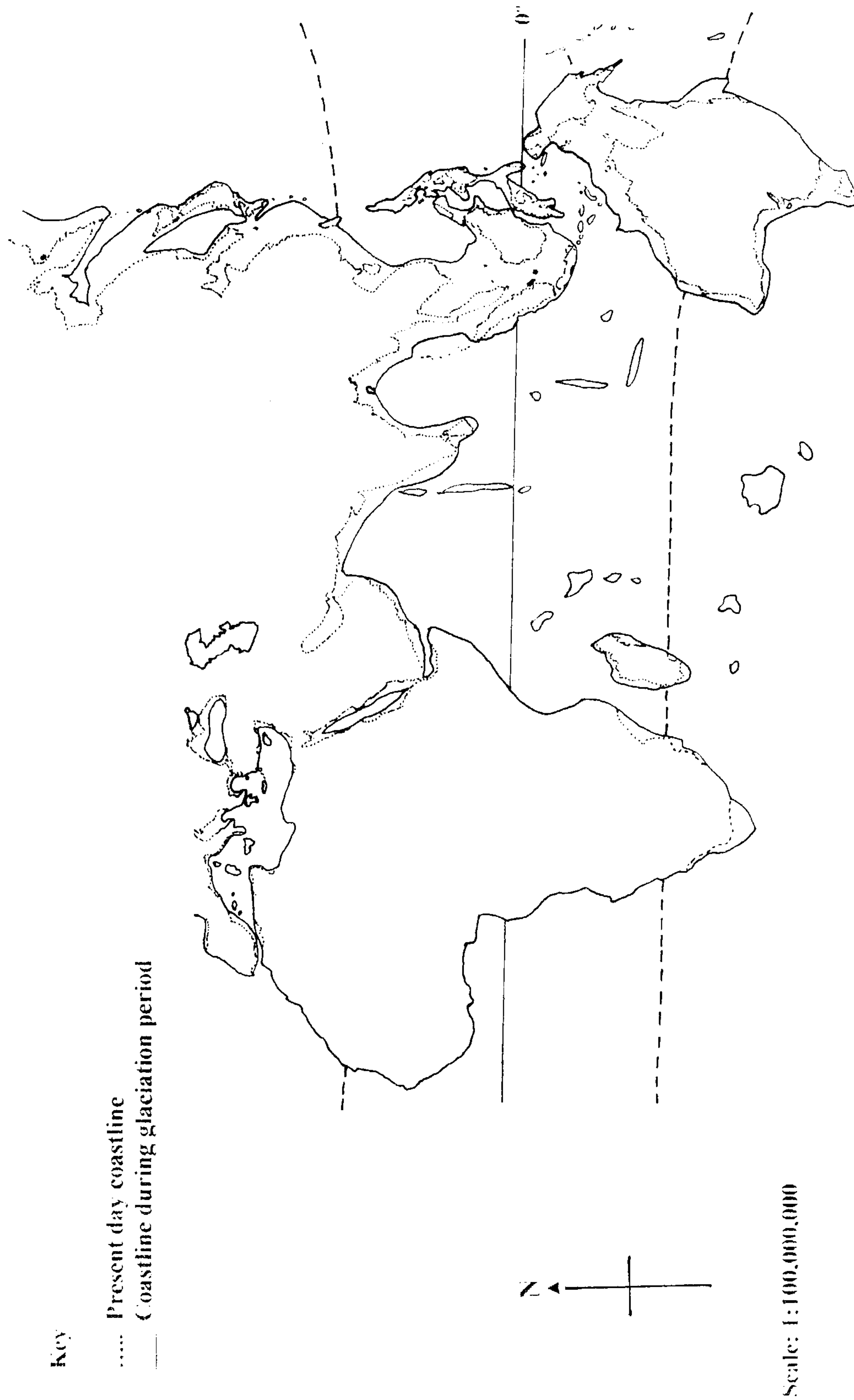


Figure 6.9: Land formation in the Indo-West Pacific during the most recent ice age where the sea level was lowered by 150m.

formation during these glaciation events and subsequently reunited when the glacier retreated and the Sunda Platform was flooded again.

The usefulness of a “molecular clock” approach to estimate the time of divergence between taxa has been well debated in the literature (Thorpe, 1982; Avise, 1994; Thorpe and Sole-Cava, 1994). However, for many invertebrates species there is a lack of fossil evidence to ascertain a time of divergence of taxa, therefore the “molecular clock” approach can prove useful in obtaining an approximate time frame for evolutionary events (Thorpe and Sole-Cava, 1994). Using Nei’s (1972) genetic distance measures between *Scylla* species (shown in table 6.5), table 6.13 shows the range of values for the time since divergence.

Table 6.13: Values for time since divergence between four species of the genus *Scylla* using two calibrations for the conversion of Nei’s genetic distance measure.

Species comparison	Nei’s genetic distance measure (D).	$t \sim 5 \times 10^6 D$ Nei, 1972	$t \sim 18 \times 10^6 D$ Yang <i>et al.</i> 1974
<i>S. serrata</i> vs <i>S. olivacea</i>	0.490	2.45mya	8.82mya
<i>S. olivacea</i> vs <i>S. tranquebarica</i>	0.243	1.22mya	4.42mya
<i>S. serrata</i> vs <i>S. paramamosain</i>	0.072	0.36mya	1.30mya

Mya = million years ago.

Nei’s calibration places the divergence of the genus *Scylla* within the Pleistocene epoch (3mya to 10,000ya). However, using the other calibration measure, the primary split between *S. olivacea*/*S. tranquebarica* and *S. serrata*/*S. paramamosain* and the subsequent divergence between *S. olivacea* and *S. tranquebarica* is estimated as taking place during the Pliocene epoch (12mya to 3mya). Both calibrations may be supported by fossil evidence of the presence of *Scylla* during the early Pleistocene epoch.

Recent mtDNA sequencing studies on the biogeography of the species *S. serrata* purported by Keenan *et al.* (1998) suggest that there was a rapid radiation of this particular species from the west Pacific across the Indo-West Pacific about one million years ago (Gupurenko *et al.*, 1999) as a result of recent glaciation events. As the other species do not show the same extent of radiation, it is possible that the other three species of *Scylla* have formed more recently, or that their geographical distribution at that time *i.e.* possibly restricted within landlocked areas of the South China Sea, sub-region made it impossible for them to radiate to the same degree as *S. serrata*. However, the conclusions made by Gupurenko *et al.* are based on small sample sizes collected from Africa compared with the samples from Southeast Asia and Australia. It is highly likely that a more detailed study would reveal other species of *Scylla* are present on the African continent. Moreover, it is unlikely that one species would have such a wide geographic panmictic population, particularly with the recent evidence of high larval retention and other post-settlement selection processes in crab populations (Ovenden, 1990).

All of the above is somewhat speculative, however, until further data on the possible sequence of events resulting in the present day distribution of *Scylla* species. Research on embryology and early developmental stages of the *Scylla* species may help clarify the true evolutionary status of *Scylla*. The use of embryology has been criticised in the past due to the high degree of variation between species (Anderson, 1982), although it may have some use in closely related species. Larval morphology has been described as being under-utilised in elucidating the phylogentic relationships within the crustacea (Marques and Pohle, 1995). Comparative larval studies have been carried out in brachyurans crabs (Rice 1980; 1981) and therefore may provide an avenue of further research on the phylogeny of *Scylla* species.

Chapter Seven

An assessment of reproductive characteristics in two sympatric species of *Scylla*.

7.1 Introduction

The research results presented and discussed in chapters three to five support the view that there are four species in the genus *Scylla*. Two of the four proposed species, *Scylla olivacea* Herbst and *S. paramamosain* Estampador, are present in Ban Don Bay, Surat Thani Province; Thailand, where they seem to coexist in sympatry. There are many examples of the coexistence of two or more sympatric species of *Scylla* in other countries such as the Philippines, India, Vietnam, Australia and Papua New Guinea (Estampador, 1949a; Serene, 1952; Radhakrishnan and Samuel, 1980; Joel and Raj, 1983; Taylor, 1984; Quinn and Kojis, 1987).

Based on the work by Keenan *et al.* (1998) and the present study, evidence of diagnostic morphological characters (Chapter Three) and fixed genetic differences (Chapter Five) suggest that hybridisation is not taking place between these very closely related species, although there is an overlap in geographical distribution (Chapter Six). Joel and Raj (1983) noted that in Pulicat Lake, India, where there are two phenotypes (species?) of mud crab present living sympatrically, breeding pairs of mud crabs are only composed of two individuals of the same phenotype. This suggests that there must be reproductive isolating mechanisms (RIMs) operating to maintain species differentiation in the genus *Scylla*.

Until recently, the genus *Scylla* was believed to monospecific; consequently there has been no research into the possible reproductive barriers to hybridization between morphs (species). Now that it is accepted that there is more than one species of *Scylla*, this part of the study examined some of the reproductive factors that may explain the maintenance of sympatry between the two mud crab species found in Ban Don Bay (*S. olivacea* and *S. paramamosain*).

As part of the biological species concept (Dobzhansky, 1939; Mayr, 1963), reproductive isolating mechanisms (RIMs) are viewed as the method used in nature to demarcate species by their inability to interbreed. RIMs fall into two main categories, namely prezygotic and postzygotic barriers (Futuyma, 1986).

From an ecological perspective, pre-mating or prezygotic barriers are represented through seasonal variation in breeding cycles; mechanical isolation; ecological or habitat isolation; and finally sexual or ethological isolation (Avice, 1994). All or some of these factors may be influencing the sympatric relationship between *S. olivacea* and *S. paramamosain* in Ban Don Bay.

Mechanical isolation involves the physical inability to mate between two individuals.

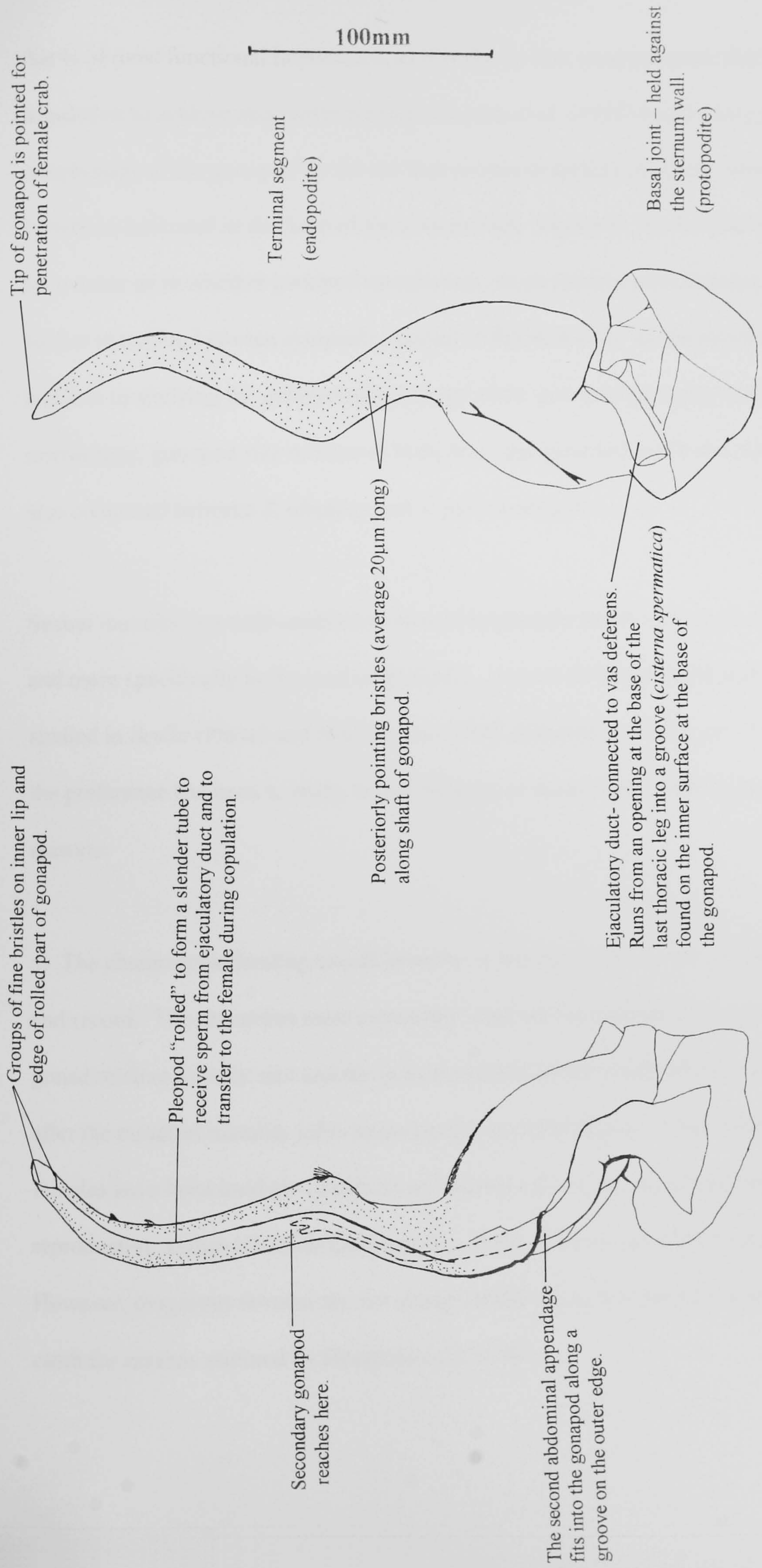
With respect to crab morphology, it is the formation of the male sexual organs that is one of the main physical factors affecting mating success. This is especially relevant to true crabs (brachyurans), such as *Scylla*, where fertilisation is internal; unlike anomurans and macrurans, which perform external fertilisation (Ezhilarasi and Subramonian, 1980).

In brachyurans, the male abdomen is much narrower than that of the female and houses only two pairs of pleopods; these have become modified as copulatory organs. The anterior pair of pleopods are known as gonopods. The gross morphology of the male gonopod in *Scylla* has been described briefly by Estampador (1949b) and Stephenson and Campbell (1960). The main structural features are outlined in figure 7.1.

Male gonopod morphology has been used previously for taxonomic purposes (Beninger *et al.*, 1991) as it is not affected by environmental conditions and is intraspecifically stable in its morphology (Chambers *et al.*, 1980). The hard external structure of the male gonopod makes it easy to examine and suitable for scanning electron microscopy (Abele, 1971). Stephenson and Campbell (1960) used the male gonopod to describe subfamilies, genera and species within the family Portunidae inhabiting the Australian coastline, including *Scylla*.

In addition to using features of the male gonopod for taxonomic purposes, the variation in gonopod morphology may also give some indication regarding the possibility of mating between two sympatric crab species. For example, gonopod morphology as a physical barrier to mating has been described as one of the factors sustaining sympatric species of fiddler crabs (*Uca* spp.); like *Scylla*, fiddler crabs are also found in mangrove habitats throughout Southeast Asia (Crane, 1975).

Some variation in gross gonopod morphology between two species of *Scylla* has been described previously in Pulicat Lake, India (Joel and Raj, 1983). The features described include variation in spinulation and setation near the basal joint of the gonopod and variation in the presence of chromatophores. However, it is the distal tip of the gonopod



A. Frontal view (against abdomen of crab)

B. Distal view (against sternum of crab)

Figure 7.1: General features of the male gonopod (first abdominal appendage) of the mud crab (genus *Scylla*) observed using camera lucida.

that is of most functional importance, as it is the tip that must penetrate the female during copulation to achieve successful mating. Keenan *et al.* (1998) briefly describe the morphology of the gonopod tip for the four proposed species of *Scylla*, with some subtle variations indicated in the form of the gonopod tip. However, no detailed investigation has been made as to whether gonopod morphology, or its relative size, may act as a physical barrier to mating between sympatric species of the mud crab. In the present study, in addition to studying the detailed morphology of the gonopod tip using scanning electron microscopy, gonopod size relative to body size, and gonopod width and curvature, were also compared between *S. olivacea* and *S. paramamosain*.

Sexual maturity is a well-established area of research in brachyuran crabs (Hartnoll, 1982), and more specifically in the mud crab, *Scylla*. Aspects of male sexual maturity have been studied in *Scylla* (Prasad and Neelakantan, 1990; Robertson and Kruger, 1994); however, the preference has been to study sexual maturity in female mud crab for the following reasons:

a) The characters indicating sexual maturity in the female mud crab are easy to identify and record. The characters most commonly used are the increase in size of the female gonad relative to body size and the radical increase in the width of the female abdomen after the moult of maturity (also known as the pubertal moult). Ovigerous (egg-bearing) females have been used previously as an indicator of female sexual maturity in reproductive studies of *Scylla* (Joel and Raj, 1980; Pollock and Melville-Smith, 1993). However, ovigerous females are not always available, as they have proved difficult to catch for reasons outlined by Heasman *et al.* (1985).

b) The interest in the maturity status of female mud crabs is also due to their economic importance. Females with mature gonads (also known as “egg crab”) are more valuable than immature females, irrespective of species; egg crabs fetch up to twice the market value of female mud crabs with immature or spent ovaries. In Surat Thani, *Scylla paramamosain* “egg crab” fetch THB350 kg⁻¹ (USD10 kg⁻¹) compared to THB160 - THB230 kg⁻¹ (USD4.57 - USD6.57 kg⁻¹) for female crabs of the same size but without mature ovaries (Author, unpublished data, 1996).

The size at which crabs attain sexual maturity is viewed as an important aspect of their reproductive biology (Campbell and Fielder, 1986). Knowledge of the size at which female mud crab reach sexual maturity is especially valuable for fisheries management strategies and for conservation purposes; for example, by understanding the size at which female maturity occurs a minimum catch size can be imposed (Somerton, 1980; Heasman *et al.*, 1985; Campbell and Fielder, 1986).

There has been substantial research on the size at which female *Scylla* reach sexual maturity (*e.g.* Quinn and Kojis, 1987; Brown, 1993; Macintosh *et al.*, 1993; Robertson and Kruger, 1994 and references therein). However interpretation of maturity data is constrained by the fact that most studies until now have treated *Scylla* as a monospecific genus. Moreover, the use of different parameters and methods of estimating sexual maturity by different authors has caused some confusion as to the true size of female maturity in *Scylla* (Robertson and Kruger, 1994). Three of the most popular methods used to estimate the size at maturity for female crabs are described below.

7.1.1 Minimum size at maturity

Somerton (1980) and Wenner *et al.* (1985) have reviewed the different methods used for estimating size at maturity in brachyuran crabs. The simplest method used is the minimum size of female maturity. This method, although well used in many reproductive studies on *Scylla* (reviewed by Brown 1993), is strongly influenced by sample size; larger sample sizes result in a smaller size at first maturity being observed (Wenner *et al.*, 1985).

7.1.2 Breakpoint analysis

Hartnoll (1978) describes the manner in which the relative growth of various body dimensions in crabs changes with their ontogeny. Within each phase, the size of one body dimension relative to another can be described by the allometric growth equation:

$$Y = bX^a$$

Where Y represents the abdomen width of the female crab, X represents the carapace width and a and b are undetermined coefficients. When plotted on a logarithmic scale the allometric growth equation forms a straight line.

$$\text{Log } Y = \text{Log } b + a \text{ Log } X$$

A break in the plotted straight lines represents a critical change in relative growth of the female abdomen, due to the onset of sexual maturity as the abdomen becomes wider in order that it can transport and incubate crab embryos (Hartnoll, 1982). This break in the straight line can be used to estimate the size at which female crabs reach the point of functional maturity. Breakpoint analysis has been used to ascertain the size of maturity

using male and female sexual characters in other crab species (Brown and Powell, 1972), including *Scylla* (Prasad and Neelakantan, 1989).

7.1.3 Probit analysis (SM_{50})

Estimation of the size of maturity for 50% of female crabs (also known as SM_{50} or M_{50}) has been the basis for computer modelling studies to predict the size of maturity in male and female crabs (Somerton, 1980; Gonzalez-Gurriaran and Freire, 1994). SM_{50} can be interpreted as the size at which 50% have reached maturity. Moreover, the size of crabs at 50% maturity is also the median size of maturity (Somerton, 1980). Quinn and Kojis (1987) used SM_{50} to estimate the size of maturity for two species of *Scylla* found in Papua New Guinea. Unfortunately small sample size made it difficult to make any realistic judgement on the size of maturity. Robertson and Kruger (1994) used probit analysis as a method to successfully ascertain the SM_{50} for female and male *S. serrata* from Natal, South Africa. However, this method has not been applied to compare the size at maturity between two sympatric species of *Scylla* in Thailand.

All three methods described above were used to establish the size at maturity for female *Scylla olivacea* and *S. paramamosain* from Ban Don Bay, Surat Thani Province. This was done in order to assess whether any differences in size at maturity, if present, may represent a barrier to hybridisation.

Seasonal trends in female gonad maturity have been studied previously in *Scylla* over a large geographic area, including India (Prasad and Neelakantan, 1989), Australia (Heasman *et al.*, 1985), South Africa (Robertson and Kruger, 1994), Ranong Thailand (Macintosh *et al.*; 1993) and indeed Surat Thani, Thailand (Khaonuna and Rachanachote,

1994). However, in all these studies, *Scylla* was still believed to be a monospecific genus (*S. serrata*), thus variations in seasonal maturity between different *Scylla* morphs (species) was not considered.

Other studies have shown distinct and displaced reproductive periods between sympatric species of crustaceans, including three species of hermit crabs (Reese, 1968) and populations of gammarid amphipods (Kolding and Fenchel, 1979). This kind of reproductive isolating mechanism has been associated with sympatric speciation events, or to the maintenance of sympatry where selective mating is linked to habitat preferences between species (Stanhope *et al.*, 1992). Joel and Raj (1983) suggested that there is variation in peaks of female gonad maturity between two sympatric morphs of *Scylla* found in Pulicat lake, but no data were presented to support their observations.

In the present study, female *S. olivacea* and *S. paramamosain* were collected in Surat Thani over an eighteen month period to try and elucidate whether there is any variation in the seasonality of sexual maturity between these two sympatric species and to look for any additional evidence as to how the integrity of the sympatric relationship between these two closely related species is maintained.

7.2 Materials and methods

7.2.1 Study site selection

Ban Don Bay, in Surat Thani Province, Southern Thailand was selected for a comparative study of the reproductive characteristics of *Scylla olivacea* and *S. paramamosain* which occur sympatrically at this site. These two species occur in the bay in approximately equal numbers all year round, making it an ideal location for a comparative study of their

reproductive biology. Moreover, Surat Thani has a long history of crab farming and fishing where these species make up a substantial part of the total fisheries within Surat Thani; thus the use of local knowledge and the collection of crabs were readily achievable.

The Surat Thani Coastal Aquaculture Development Centre, based in Kanchanadit, Surat Thani Province, provided facilities and manpower to aid in the collection and processing of crab samples for female maturity as described below in section 7.2.2.

7.2.2 Seasonal maturity of female mud crab

7.2.2.1 Crab sampling programme

The two common species in Ban Don Bay, *Scylla olivacea* Herbst and *S. paramamosain* Estampador, were identified using external morphological features. These included exoskeletal colour and patterning, frontal lobe teeth morphology and carpal spine morphology (details of the distinguishing features for these two species are discussed in chapter three).

Female crabs, ranging in size from 50g to 700g, were collected fortnightly from Ban Don Bay (one collection was made during the spring tide period, the other during neap tides) over the eighteen months from August 1995 until January 1997. A maximum of 30 female crabs of each species (up to 60 crabs in total) were selected from unsorted catches that best represented the size frequency of the crab population fished on that particular day. The unsorted catches were obtained from local fishermen who catch and sell crabs to the local market in Surat Thani town. Care was taken to ensure the crabs were caught locally. The fishermen mainly use box traps, although some gill nets and pole traps are also used to catch mud crab in Ban Don Bay.

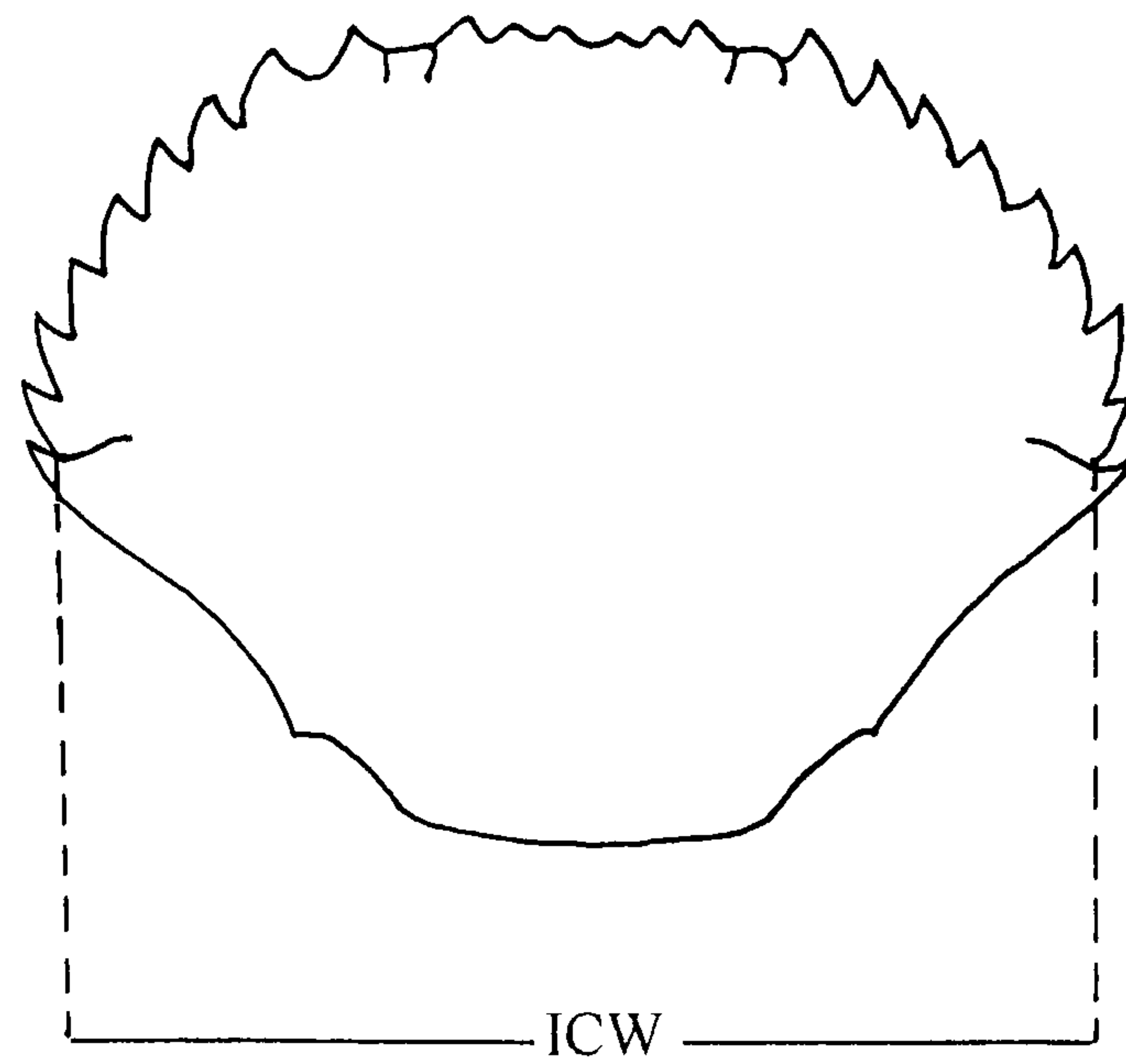
7.2.2.2 Parameters measured

The sampled female mud crabs were taken to the Surat Thani Coastal Aquaculture Development Centre at Kanchanadit. The crabs were cleaned using damp paper towels to remove any mud from the exoskeleton. Internal carapace width (the distance across the carapace measured between the eighth and ninth anterolateral spines) and maximum abdomen width of the fifth abdominal segment were recorded to the nearest 0.5mm using nylon vernier dial callipers. These morphometric measurements are illustrated in figure 7.2.

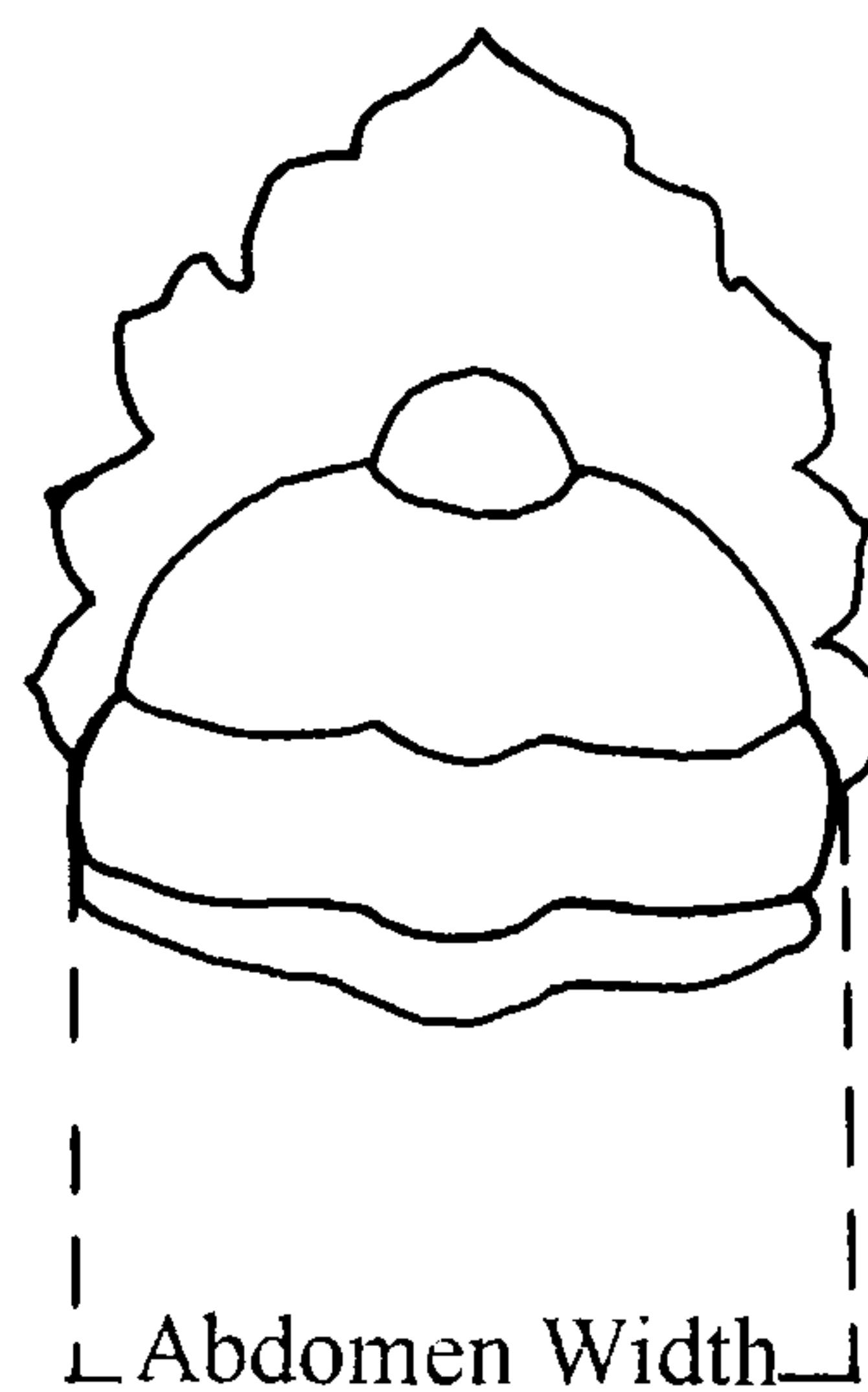
The crabs were untied and weighed to the nearest 0.01g using a top pan balance. Any missing limbs were noted and accounted for in the total fresh weight by removing the limb from the opposite side and weighing it separately, then adding it to the total fresh weight. The crabs were then cooled down in a freezer for approximately 20 minutes until they were in complete torpor. The crabs were then dissected in order to remove the ovary. The colour of the ovary and the percentage of the body cavity filled with ovary were used to allocate the female crabs to one of four stages of maturity as suggested by Prasad and Neelakantan (1989). This scale is outlined in figure 7.3. The gonad removed from each female was weighed in preweighed plastic weighing boats using a top pan balance. Ovary weights were recorded to the nearest 0.01g. Gonadosomatic index (GSI) was calculated as a percentage of the total body weight, using the equation:

$$\text{GSI (\%)} = \frac{\text{Fresh weight of female ovary (g)}}{\text{Total fresh weight of female crab (g)}} \times 100$$

(Giese and Pearse, 1974)

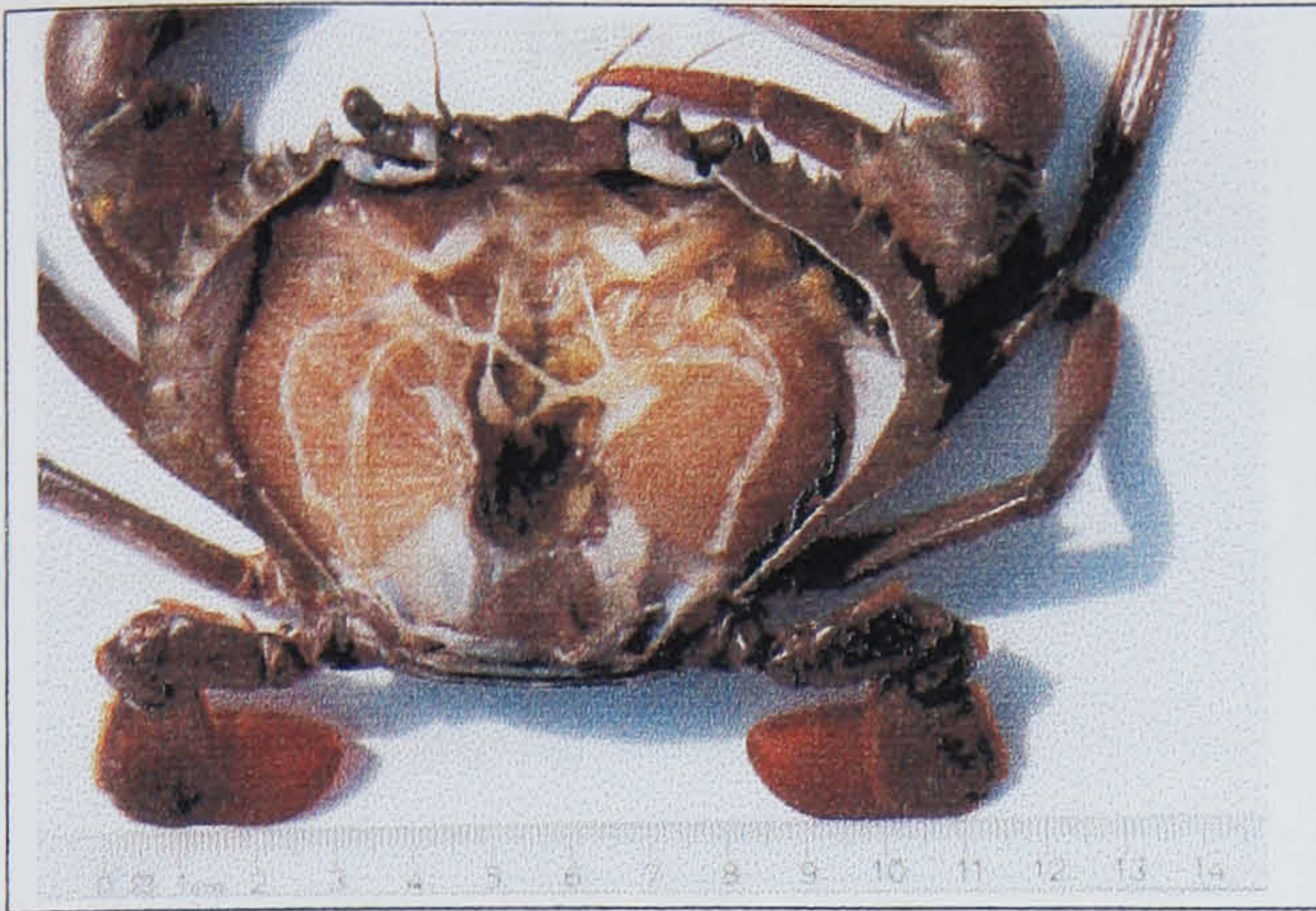


a) Internal Carapace Width (ICW)



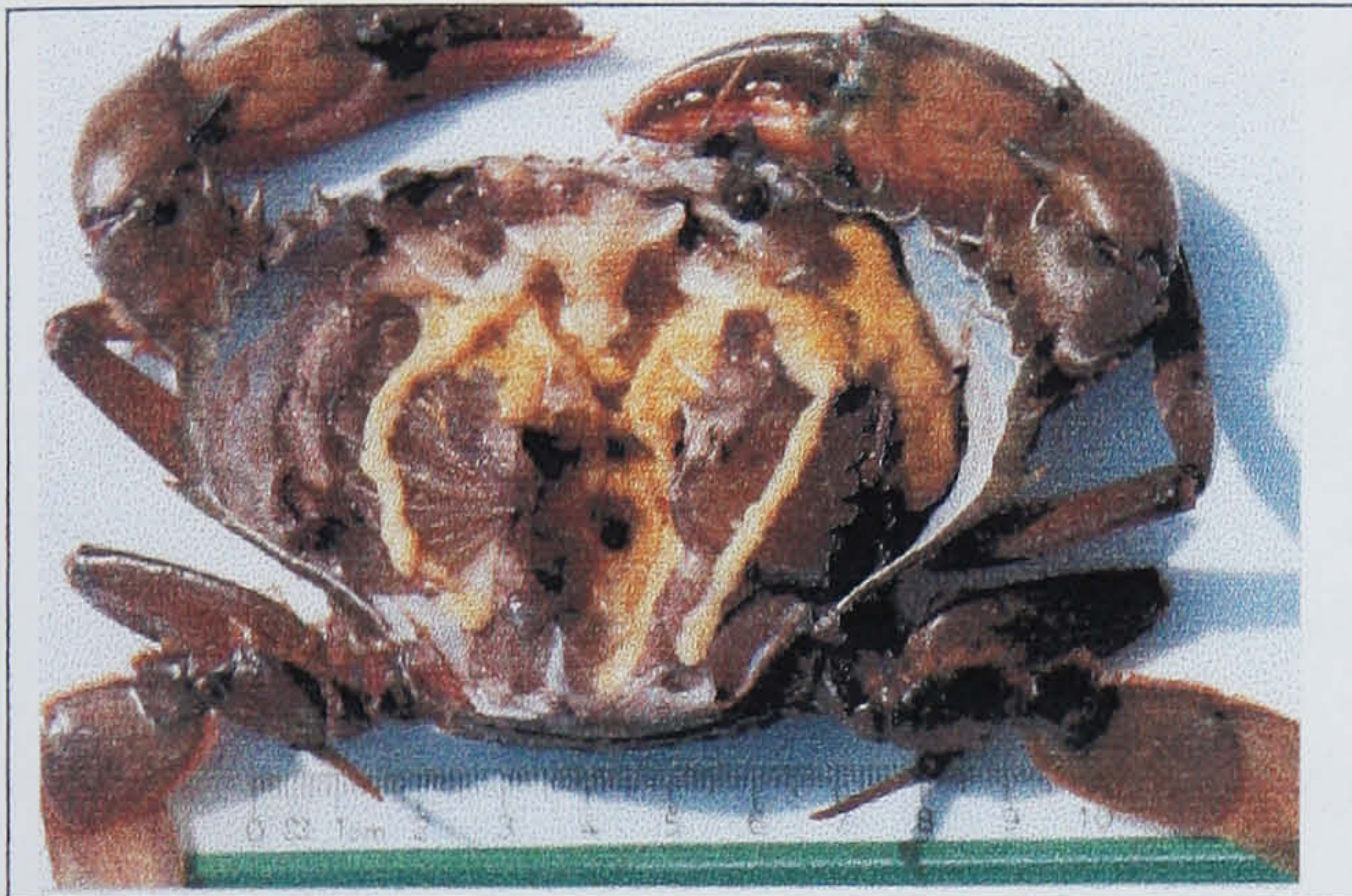
b) Abdomen Width (fifth abdominal segment)

Figure 7.2: Illustration of measurements for the estimation of size at first maturity for female mud crabs from Ban Don Bay, Surat Thani Province, Thailand (scale is actual size of subadult female; internal carapace width = 70mm).



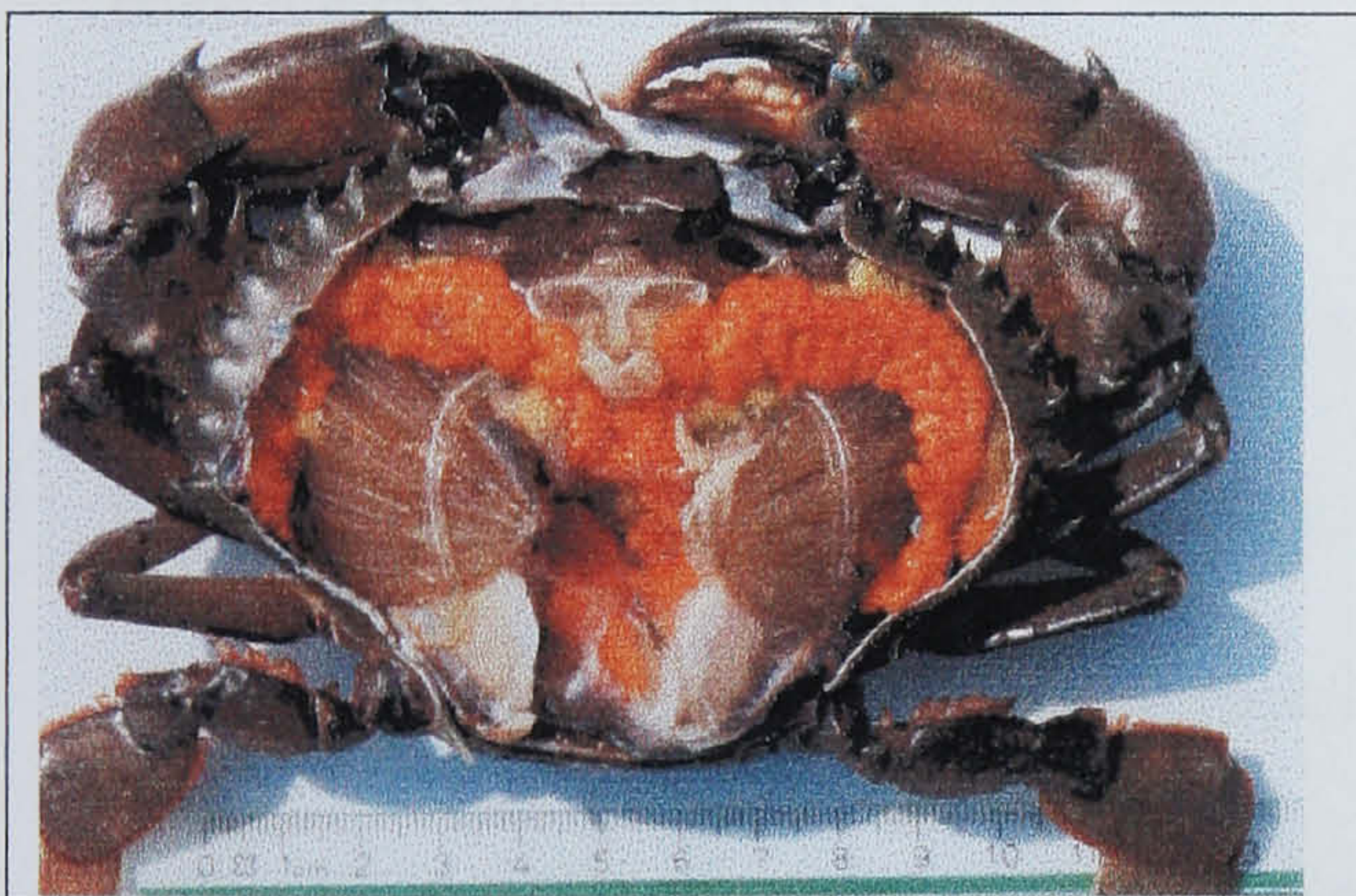
Stage I

The ovary is transparent to white, thin and thread-like, occupying less than one tenth of the body cavity. There is no prominent seminal receptacle. Ova diameter ranges from 0.01mm to 0.06mm, with a modal value of 0.05mm.



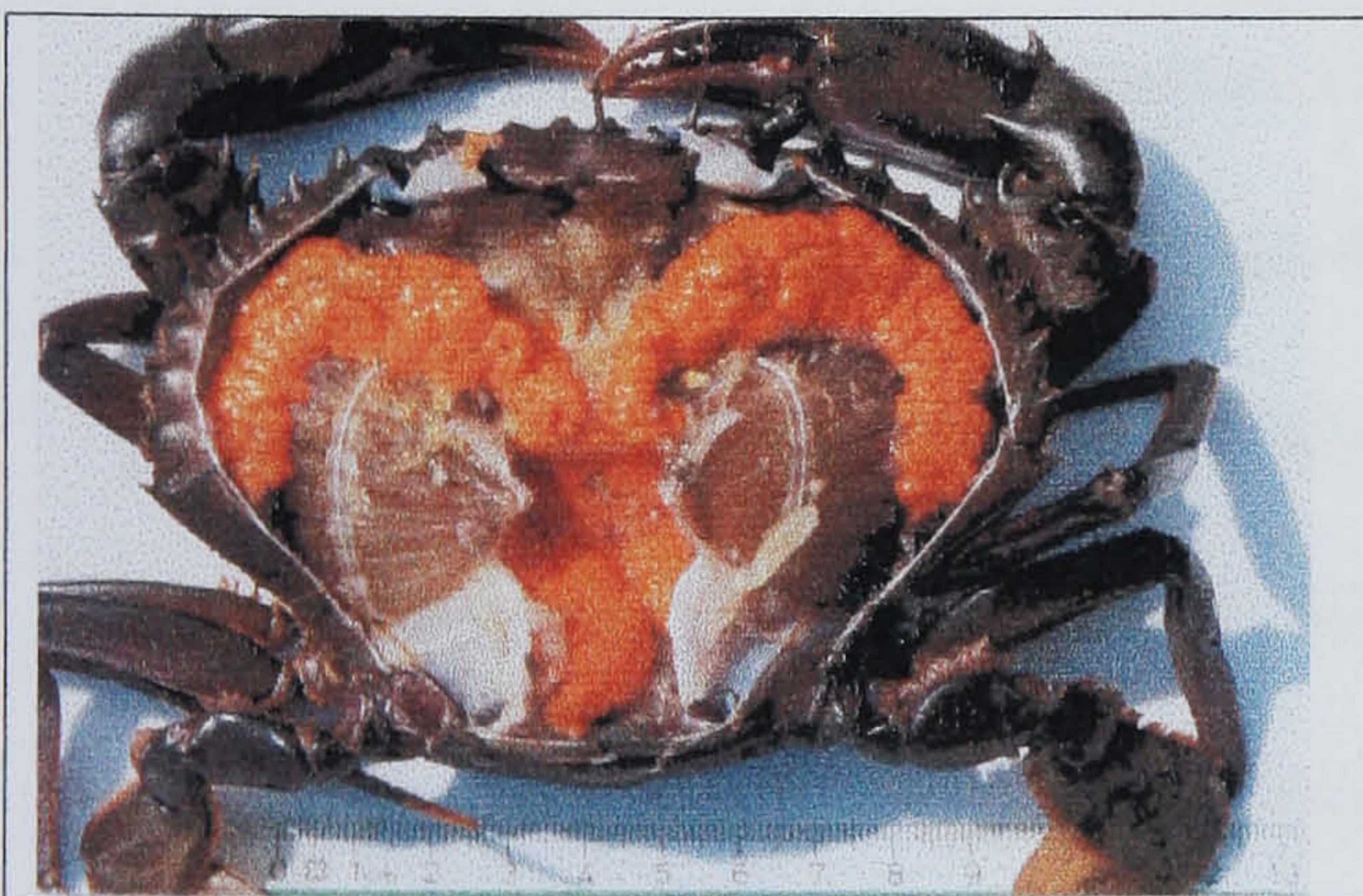
Stage II

Partial development of ovaries. The ovary is yellowish to pinkish, occupying one fifth of the body cavity. Ova diameter ranges from 0.1 – 0.3mm, with a modal value of 0.2mm.



Stage III

This stage denotes the maturing condition of the ovaries which extend into the anterolateral regions of the carapace, occupying in total approximately half of the body cavity. The ovaries are orange in colour. Ova diameter ranges between 0.4 and 0.9mm, with a modal value of 0.8mm.



Stage IV

There is a well-defined seminal receptacle. The ovary occupies approximately more than three-quarters of the body cavity. Ovaries are bright red/deep orange in colour. Ova diameter ranges from 0.7 – 1.3mm, with a modal value of 1.15mm. The crab illustrated is in the early part of stage IV; ovulation occurs rapidly after stage IV is reached.

The spent stage is indicated by a flaccid, speckled appearance to the ovaries resulting from the aggregation of unspawned ova, which vary in colour from red to brown.

Figure 7.3: Guidelines for staging gonad maturity for female crabs, genus *Scylla* (based on Prasad and Neelakantan, 1989); photographs: D.J. Macintosh.

7.2.2.3 Data analysis

7.2.2.3.1 Size frequency analysis of sampled crabs

Size frequency distributions were constructed for the sampled female *S. olivacea* and *S. paramamosain* allocated into 10mm size classes according to their internal carapace width (ICW). Maturity frequency distributions were also plotted using gonadosomatic index (GSI) classes (2% GSI class intervals). These frequency distributions were tested for normality and heterogeneity using a Minitab statistical package (Release 8.1, Minitab Inc. USA) in order to assess whether parametric or non-parametric tests were to be applied. A Shapiro-Wilk test (Shapiro and Wilk, 1965) was used to test for normality of the size frequency and GSI distributions. The normality of the data was determined by calculating normal scores (N-scores) for each sample and comparing the resulting correlation coefficient with tabulated values. Significant correlation at $P = 0.05$ is consistent with normality. The GSI data were transformed using arcsine transformation from percentages to radians (Sokal and Rohlf, 1995) to allow the comparison of mean GSIs between *S. olivacea* and *S. paramamosain*. As size frequency and GSI data for both species were found to be normally distributed and possessing equal variances, a parametric test using one way analysis of variance was used to test whether sample means could have been from populations with the same parametric mean (Sokal and Rohlf, 1995). One way ANOVA was used to compare means of the size frequency distributions between *S. olivacea* and *S. paramamosain* and their mean GSI values. A frequency distribution was also constructed for the number of individuals allocated to the four stages of maturity for the 10mm size classes used for *S. olivacea* and *S. paramamosain*.

7.2.2.3.2 Estimation of size at first maturity

Three methods were used to estimate the size at maturity for *S. olivacea* and *S. paramamosain* to reveal any differences in the size at maturity between the two species. The most simple and direct method was to record the smallest mature female and largest immature female encountered in the samples of each species.

For the other two methods used to determine the size at first maturity, female mud crabs were separated into two subgroups depending on their state of maturity. Female crabs at stage three and four were classified as fully mature. Those female crabs at stages one and two were considered to be immature. This degree of maturity was based on both gonad maturity (GSI) and the allometric change in abdomen width corresponding to sexual maturity of female *Scylla*.

Break point analysis was the second method employed. The maximum abdomen widths for both mature and immature female crabs were plotted against internal carapace width, resulting in two regression lines for each species. These regression lines were tested for equality of slope and intercept (the intercept is also known statistically as adjusted means) using one-way analysis of covariance (using the statistical programme BMDP-1V, BMDP statistical software Inc., Cork, Ireland). All data were \log_{10} transformed prior to the analysis to ensure linearity of the data (Sokal and Rohlf, 1995). To visualise the slopes of regression, log transformed data were plotted in addition to raw data for mature and immature females from both species. Significant differences between the slopes and intercepts justify the use of two regression lines to represent the data, rather than a single regression line. Comparisons of the slopes and intercepts of the lines were also made between immature female *S. olivacea* and *S. paramamosain* and between the equivalent

data sets for mature females. The carapace width at which the regression lines for mature and immature crabs intersected was taken as the size at which female mud crabs attain sexual maturity.

The third method used was to ascertain the size at which 50% of female *Scylla* attain sexual maturity (M_{50}) with respect to abdomen width and gonadosomatic index using probit analysis (Roberston and Kruger, 1994). Female crabs were allocated to 5mm size classes and the percentage of mature mud crabs for each size class was recorded. This percentage maturity was converted to a probit scale and plotted against size classes for internal carapace width. A best-fit regression line was fitted to the data points. A line was extrapolated from probit 5 (corresponding to 50% of the females attaining sexual maturity) to the regression line, and then to the size axis, to ascertain the size at which this 50% maturity level was attained.

7.2.2.4 Seasonal variability

Mean GSIs for the sampled female mud crabs were plotted for *S. olivacea* and *S. paramamosain* for each collection date to reveal seasonal trends in reproductive activity and any differences between the two species. As mean GSI can be unrepresentative of the actual state of maturity in the sample collected (*e.g.* two mature crabs and many immature ones could give a mean GSI equivalent to that for an intermediate state of maturity) two other indices of maturity were also included: a) percentage maturity and b) the frequency of the four stages of maturity in each sample collected were also plotted for each sampling date to compare with the mean GSI data.

Tide tables for Surat Thani (Hydrographic Department, Royal Thai Navy) were used to calculate the tidal amplitude (in metres) on each collection date. Mean GSIs for *S. olivacea* and *S. paramamosain* were plotted against the tidal data to see if there was any significant correlation between tidal amplitude and the level of sexual maturity observed in *S. olivacea* and *S. paramamosain*.

7.2.2.5 Physical characteristics

Average monthly temperature, total rainfall and monthly river discharge were plotted against mean GSIs for *S. olivacea* and *S. paramamosain* to compare the seasonal variation in female maturity to environmental parameters that may have an effect on sexual maturity and spawning in female mud crabs. Average monthly temperature and total rainfall were collected from a weather station at the Surat Thani Coastal Aquaculture Development Centre in Kanchandit for one year from August 1995 to July 1996. The data obtained were compared to the ten year data set collected by Rattakul from the Surat Thani Meteorological station (Rattakul, 1995) to ensure that the climatic conditions within the study period followed the normal seasonal trends for Surat Thani Province.

The majority of the freshwater discharge into Ban Don Bay is via the Tapi-Phum Duang River catchment system. The monthly discharge data for the Tapi River were extracted from a study on the coastal environmental profile of Ban Don Bay (Kositratana, 1988).

7.2.3 Male mud crab gonopod morphology

7.2.3.1 Animals selected

Primary gonopods, collected from five male crabs of each of two species found in Surat Thani, were used in a preliminary study involving scanning electron microscopy (SEM).

Once a suitable protocol had been established, six male *Scylla olivacea* and six male *S. paramamosain* representing a range of sizes from 93g to 125g were collected. Fresh weight (g), internal carapace width (mm) and gonopod length (mm) were recorded for each crab prior to the removal of the gonopods for SEM.

7.2.3.2 Preparation of samples

Dissected gonopods were placed in glass staining pots containing 10ml of 1% glutaraldehyde (made up to solution with 0.1M Na cacodylate buffer) for one hour before being transferred to 10ml of 3% glutaraldehyde solution, pH 7.5. The gonopods were left in 3% glutaraldehyde solution for three days. After this primary fixation, the gonopods were trimmed, leaving a 1cm tip for scanning, before being washed in cacodylate buffer for a minimum of four hours. Secondary fixation was carried out by submerging the tissues in 1% osmium tetroxide (diluted in cacodylate buffer as before) for two to four hours. These fixed samples were then transferred to 70% ethanol overnight. The concentration of ethanol was raised to 90% for two hours and then to 100% ethanol (absolute alcohol) for two hours before being placed in fresh ethanol overnight. The ethanol was changed one more time before samples were dried using a critical point dryer.

The dried samples were then mounted on to 1-cm aluminium stubs using double-sided adhesive tape before being sputtered with gold. The prepared stubs were viewed on a scanning electron microscope (Joul JSM-840). Photographs were taken using black and white film (Ilford FP4 plus, 125 ASA).

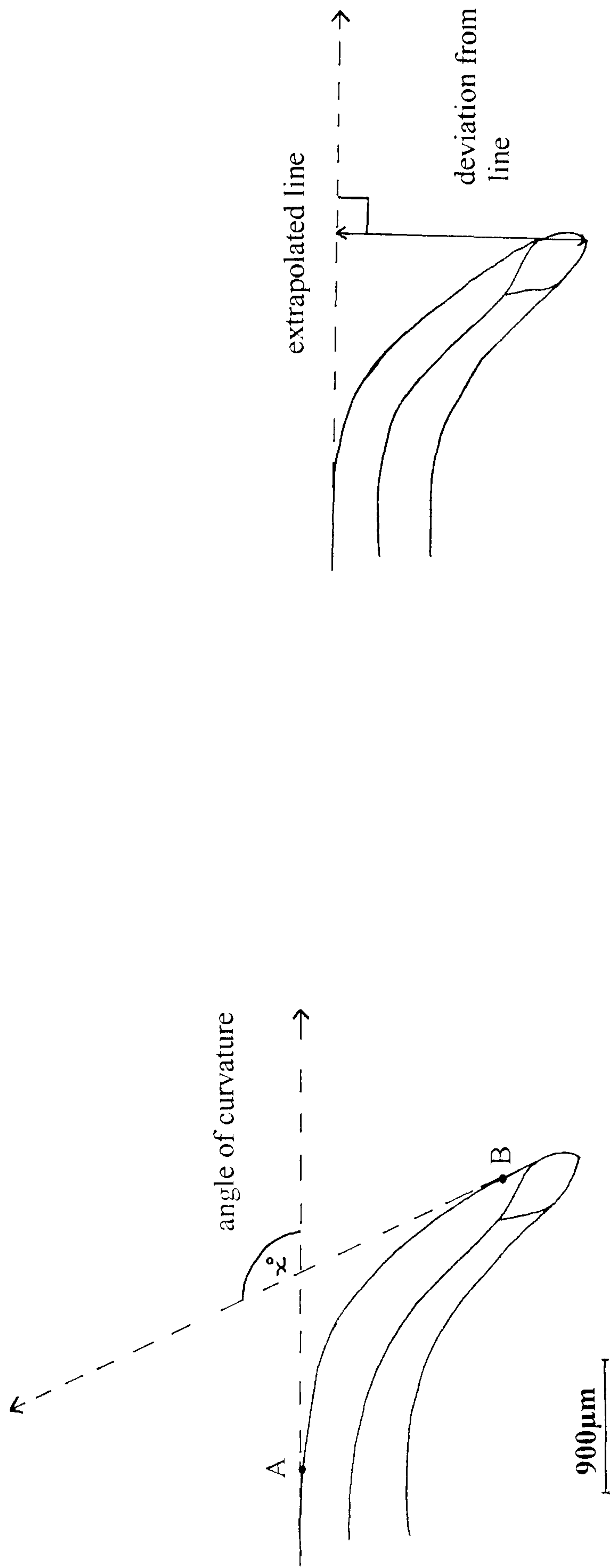
7.2.3.3 Data collection and analysis

Two magnifications were used to compare between gonopods of individual male crabs.

The lower magnification (x15) was used to view and measure features of the whole section dissected. The second magnification (x60) was used to compare the morphology of the tip of the gonopod, in particular the unfolded “ear” shaped distal part of the gonopod used to transfer spermatozoa to the female spermatheca (a receptacle used to store sperm within the female until fertilisation takes place). Photographs taken at both magnifications were used to take general measurements in order to compare a) the curvature in the tip of the gonopod b) the width of the gonopod tip and c) the area of the “ear” of the gonopod, between male specimens of *S. olivacea* and *S. paramamosain*.

The curvature of the gonopod tip was estimated in two ways. The first method was to measure the deviation between the straight part of the shaft of the gonopod tip and the end of tip by extrapolating a line from the shaft and using the perpendicular line to this extrapolation until it reaches the tip. This perpendicular is measured as the deviation (see figure 7.4a). The second method was to use the geometric method of calculating the degrees of change in direction of the gonopod by taking two points on the gonopod at the point where the curvature begins and ends. Lines extrapolated from these points intersect. At the intersection of these two lines the opposite angle is measured using a protractor, resulting in a rough estimate of the angle of curvature in the gonopod tip (see figure 7.4b).

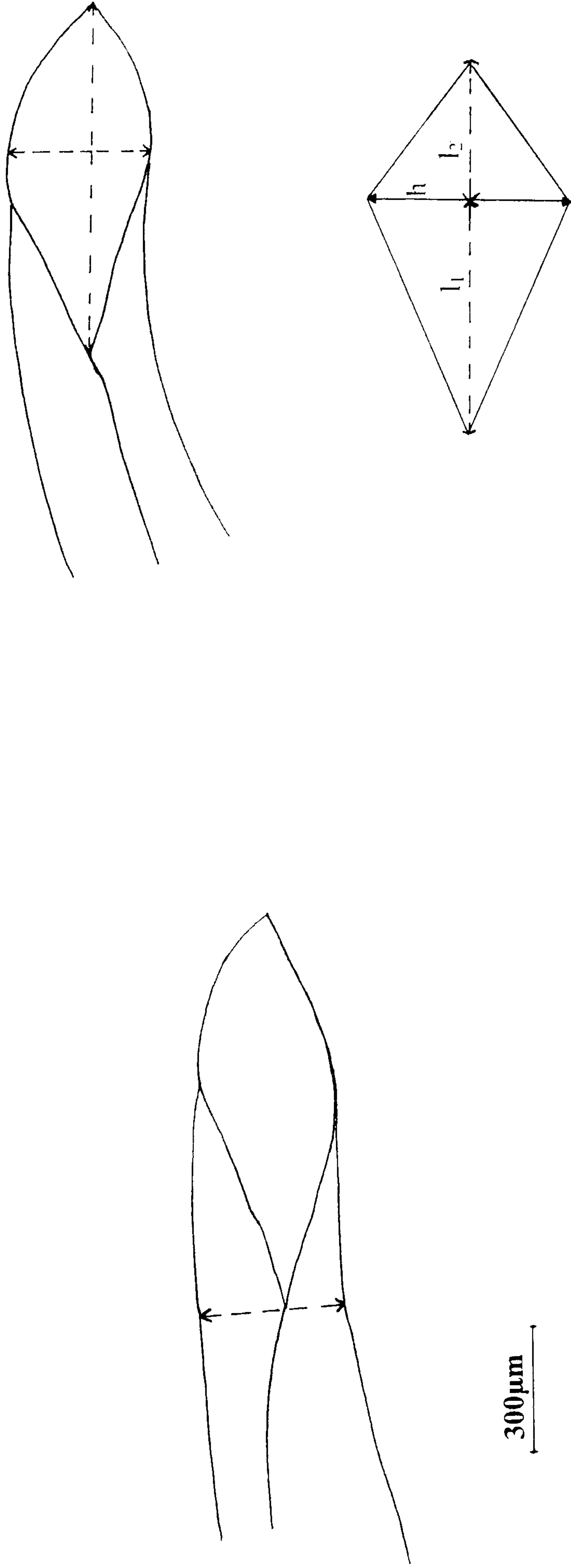
The width of the gonopod tip was measured at the point at which the gonopod ear begins. The area of the open part, or “ear”, of the tip was calculated using the geometric equation for a kite. Both these measurements are illustrated in figure 7.5.



a. Degrees of curvature

b. Deviation from a straight line

Figure 7.4: Illustration of two methods for the estimation of curvature of the gonopod tip in male *Scylla olivacea* and *Scylla paramamosain* collected from Ban Don Bay, Surat Thani Province, Thailand.



$$\text{Area of kite} = (0.5h \times l_1) + (0.5h \times l_2)$$

a. Gonopod tip width

b. Calculation of area of gonopod opening

Figure 7.5: Illustration of a) the width of the gonopod tip and b) the area of the open tip (or “ear”) used to compare gonopod morphology between male *Scylla olivacea* and *Scylla paramamosain* collected from Ban Don Bay, Surat Thani Province, Thailand.

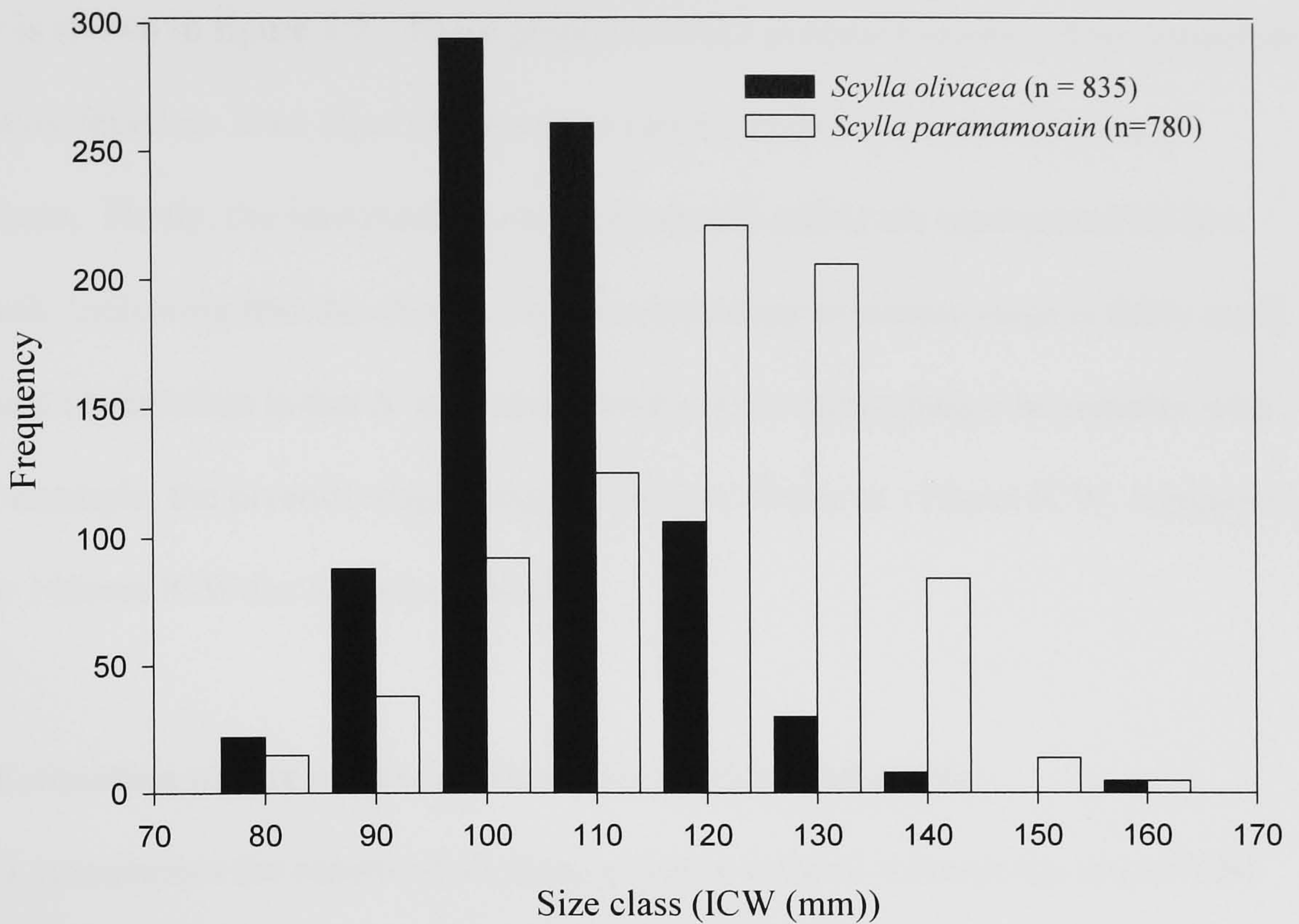
Total curvature (degrees), deviation from a straight line (mm), gonopod width (mm) and gonopod “ear” area (mm²) were plotted against internal carapace width in order to illustrate any differences between gonopod tip morphology of *S. olivacea* and *S. paramamosain* from Ban Don Bay. For the latter three measurements, the values were divided by internal carapace width prior to being plotted to remove the variation in these characters attributed to the size of the crabs measured.

7.3 Results

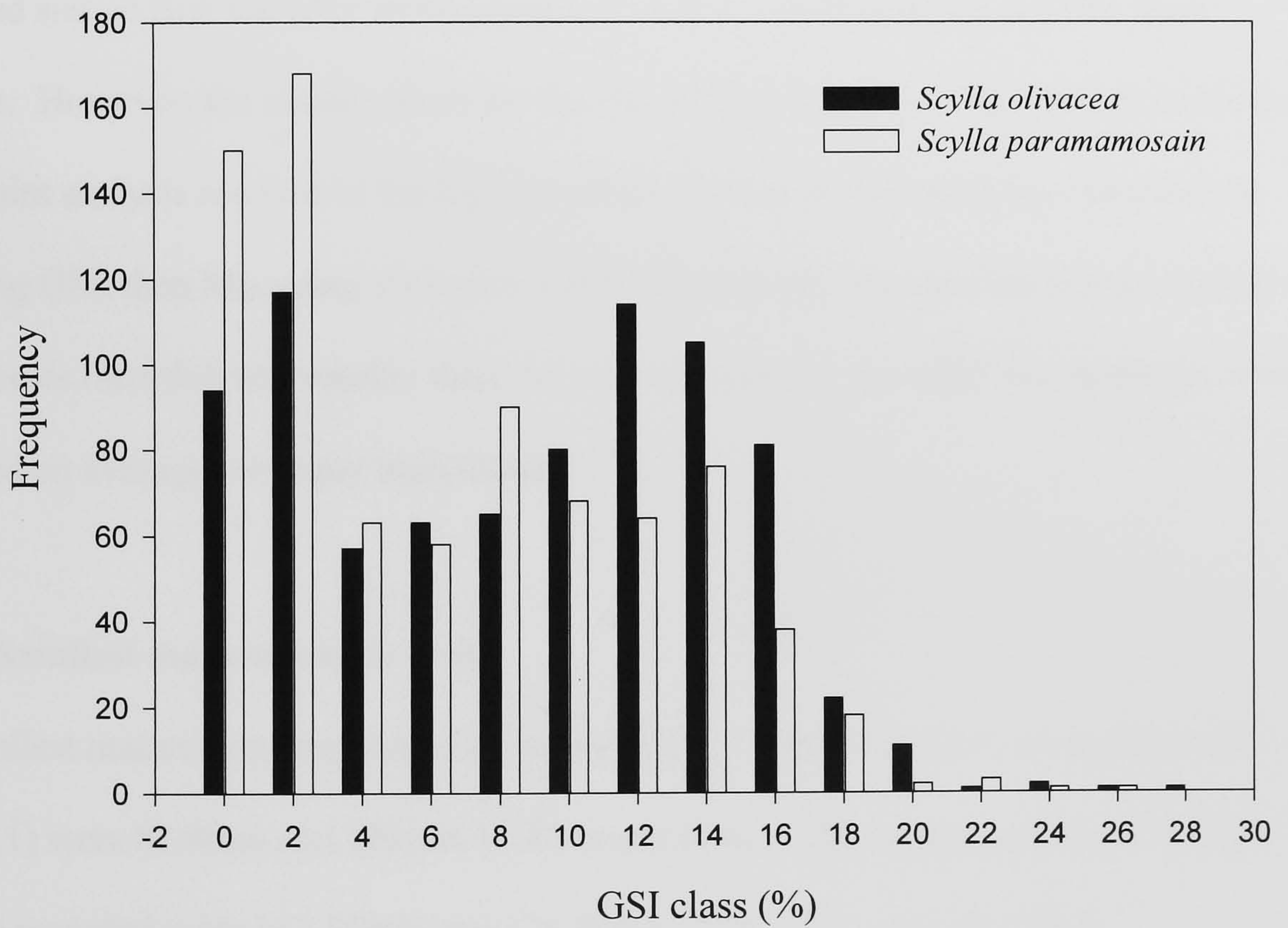
7.3.1 Size frequency analysis of sampled crabs in relation to sexual maturity

Figure 7.6 shows histograms of internal carapace width (ICW) and gonadosomatic index (GSI) for female *S. olivacea* and *S. paramamosain* from Surat Thani. ICW frequency distributions for both species were found to be normally distributed. The modal classes were 100mm and 120mm ICW for *S. olivacea* and *S. paramamosain* respectively, showing that in general female *S. paramamosain* are larger than female *S. olivacea*. The statistical difference between the means for these two distributions is found to be significant ($F = 101.11$, $P < 0.001$).

The GSI frequency distributions for both *S. paramamosain* and *S. olivacea* show a bimodal form. The first peak encompasses females with zero to 2% GSI, *i.e.* immature females. This peak is much higher in *S. paramamosain* than for *S. olivacea*. The second, less pronounced peak, represents the mature female crabs. The modal class for this second peak is 12% and 8% GSI for *S. olivacea* and *S. paramamosain* respectively. There is a significant difference between mean GSIs for *S. olivacea* and *S. paramamosain* ($F = 57.31$, $P < 0.001$) where *S. olivacea* has a higher mean GSI than *S. paramamosain*, even though they are significantly smaller in size. A breakdown of the stages of maturity for each size



a) Female crab size



b) Gonadosomatic index

Figure 7.6: Histograms of female crab sizes and gonadosomatic indexes for *Scylla olivacea* and *Scylla paramamosain* sampled from Ban Don Bay, Surat Thani Province, Thailand.

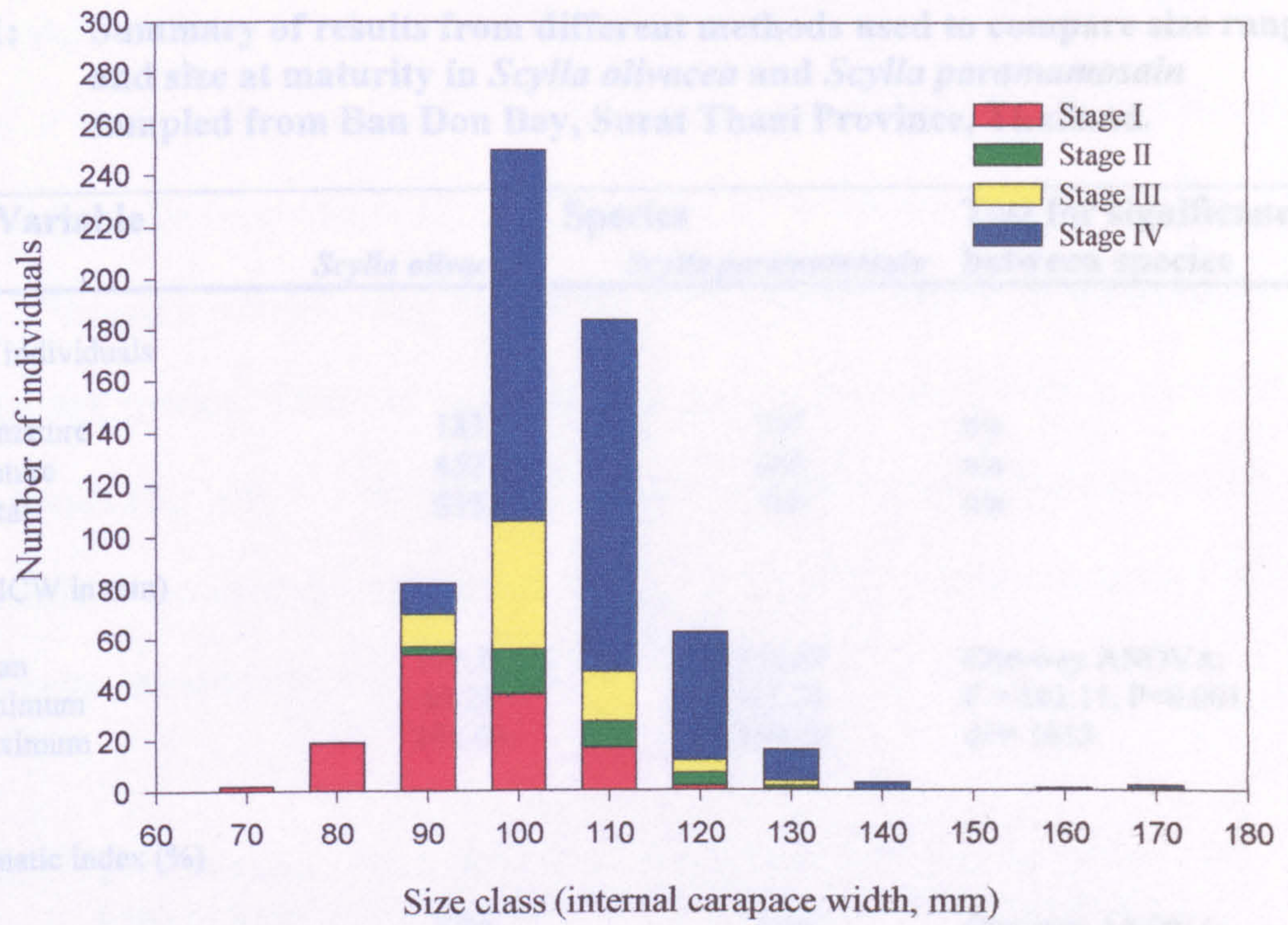
category is shown in figure 7.7. These graphs reveal a gradual transition from immature to fully mature females. Two clear observations can be made from these frequency distributions. Firstly, the intermediate stages (stages II and I) are represented by few individuals, indicating that the change from the immature to mature stage is fairly rapid. The second observation is that *S. olivacea* shows a more rapid change in maturity with size; for example, the juvenile stage (stage 0) is from 70mm to 110mm ICW, compared to 70mm to 140mm ICW for *S. paramamosain*.

7.3.2 Estimation of size of first maturity for female mud crabs

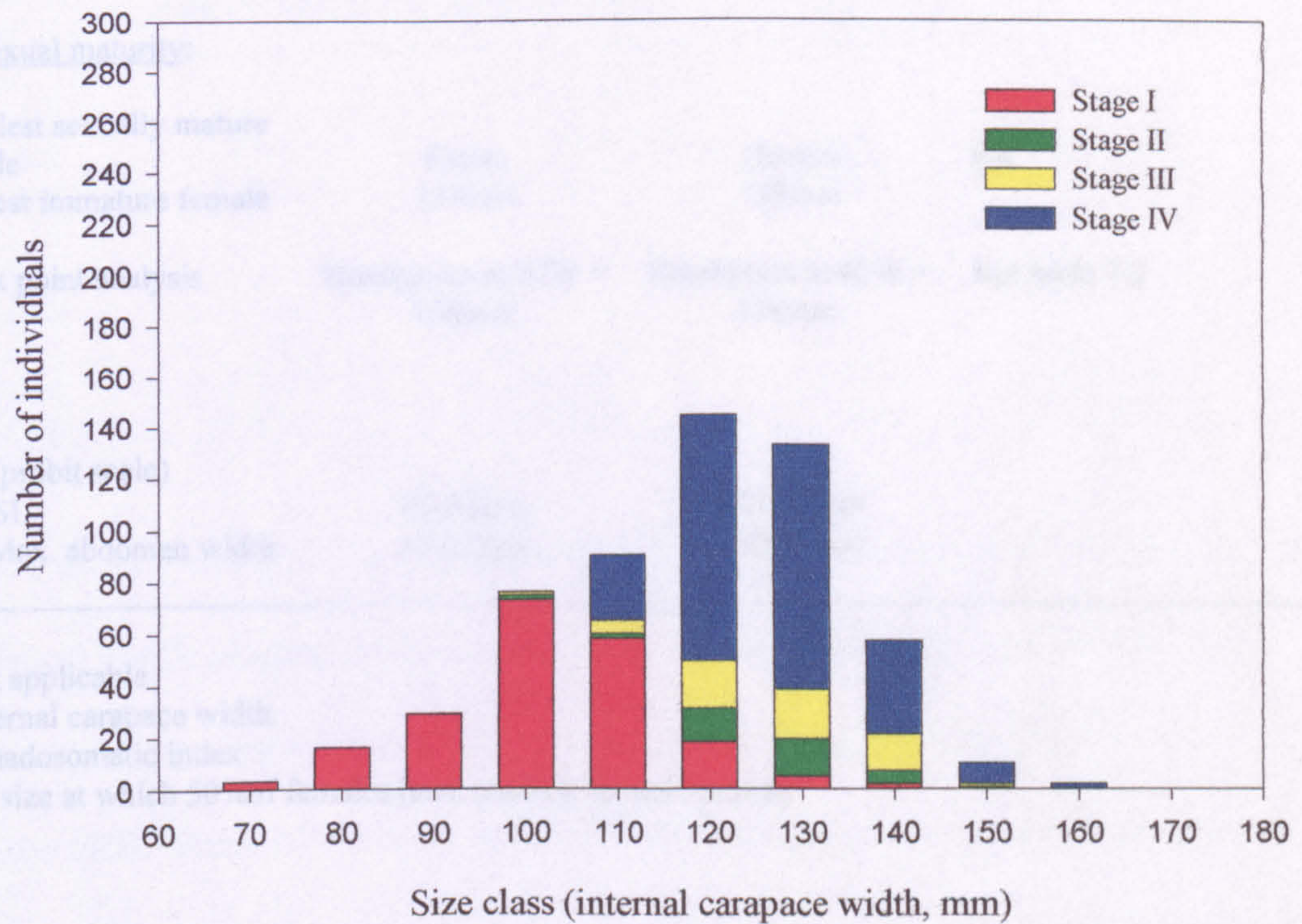
Table 7.1 summarises the results of all three methods used to estimate the size of first maturity. What is striking from the analysis is that female *S. paramamosain* have a larger estimated size of first maturity when compared with *S. olivacea* by each of the three methods. However, the actual values for the size of first maturity vary between methods. Breakpoint analysis resulted in the highest values for size of first maturity, followed by M_{50} using GSI, then M_{50} using abdomen width. Predictably, the smallest mature female of each species recorded was smaller than the size estimates by the other two methods, which are based on averages or many individuals.

7.3.2.1 Smallest mature female crab

The smallest mature females (stage IV) recorded for *S. olivacea* and *S. paramamosain* (table 7.1) were 82.9mm and 101mm ICW, respectively. The largest immature females (stage I) recorded were 117.56mm and 136.80mm ICW for *S. olivacea* and *S. paramamosain*, respectively. This indicates that there is an overlap in the size of immature and mature crabs of approximately 35.8mm and 34.16mm ICW for *S. paramamosain* and *S. olivacea*, respectively.



a) *Scylla olivacea*



b) *Scylla paramamosain*

Figure 7.7: Size frequency for four stages of female gonad maturity for a) *Scylla olivacea* and b) *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand.

Table 7.1: Summary of results from different methods used to compare size range and size at maturity in *Scylla olivacea* and *Scylla paramamosain* sampled from Ban Don Bay, Surat Thani Province, Thailand.

Variable	Species		Test for significance between species
	<i>Scylla olivacea</i>	<i>Scylla paramamosain</i>	
Number of individuals			
Immature	183	285	n/a
Mature	652	495	n/a
Total	835	780	n/a
Crab size (ICW in mm)			
Mean	101.33	115.67	One-way ANOVA: F = 101.11, P<0.001, df = 1613.
Minimum	69.20	65.20	
Maximum	195.00	180.40	
Gonadosomatic index (%)			
Mean	7.70	5.66	One-way ANOVA: F = 57.31, P<0.001, df = 1613.
Minimum	0.00	0.00	
Maximum	26.21	24.46	
<u>Index of sexual maturity:</u>			
A. Smallest sexually mature female			
	83mm	101mm	n/a
Largest immature female			
	118mm	139mm	
B. Break point analysis			
	Breakpoint at ICW = 108mm.	Breakpoint at ICW = 138mm.	See table 7.2
C. M ₅₀ (probit scale)			
I. GSI	93.94mm	111.78mm	
II. Max. abdomen width	91.21mm	110.45mm	

n/a = not applicable

ICW = internal carapace width

GSI = gonadosomatic index

M₅₀ = the size at which 50% of females have reached sexual maturity

7.3.2.2 Breakpoint analysis: abdomen width

The results of analysis of covariance on data for mature and immature female *S. olivacea* and *S. paramamosain* (as shown in table 7.2) reveal that the comparison of slopes and intercepts of the regression lines between mature and immature females are significantly different in each species. Thus two regression lines are required to represent mature and immature females for both *S. olivacea* and *S. paramamosain*. In mature females of both species growth of the abdomen slows down relative to that of the internal carapace width compared to the immature females.

Table 7.2: F-values and probabilities for comparison between regression lines used in breakpoint analysis for female *Scylla olivacea* and *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand.

Source of variation	Regression lines			
	<i>Scylla olivacea</i> mature vs immature	<i>Scylla paramamosain</i> mature vs immature	Immature <i>S. olivacea</i> vs <i>S. paramamosain</i>	Mature: <i>S. olivacea</i> vs <i>S. paramamosain</i>
Equality of Slopes:				
F-value	374.07	199.48	10.85	73.6247
Probability	P<0.001	P<0.001	P<0.001	P<0.001
Equality of adjusted means:				
F-value	316.84	184.93	21.29	208.0447
Probability	P<0.001	P<0.001	P<0.001	P<0.001

Figures 7.8 and 7.9 show the fitted regression lines for mature and immature female crabs of *S. olivacea* and *S. paramamosain* on raw data. The fulcrum represents the point at which the two regression lines meet (*i.e.* the breakpoint). The breakpoint occurs at a smaller size (108.19mm ICW) for *S. olivacea* when compared to *S. paramamosain* (138.37mm). The regression lines between *S. olivacea* and *S. paramamosain* (as shown in table 7.2) are significantly different in slope and intercept for both mature and immature females, although the F-value between immature females of each species is smaller (10.85 and 21.29 for equality of slopes and intercept, respectively) than for the other comparisons

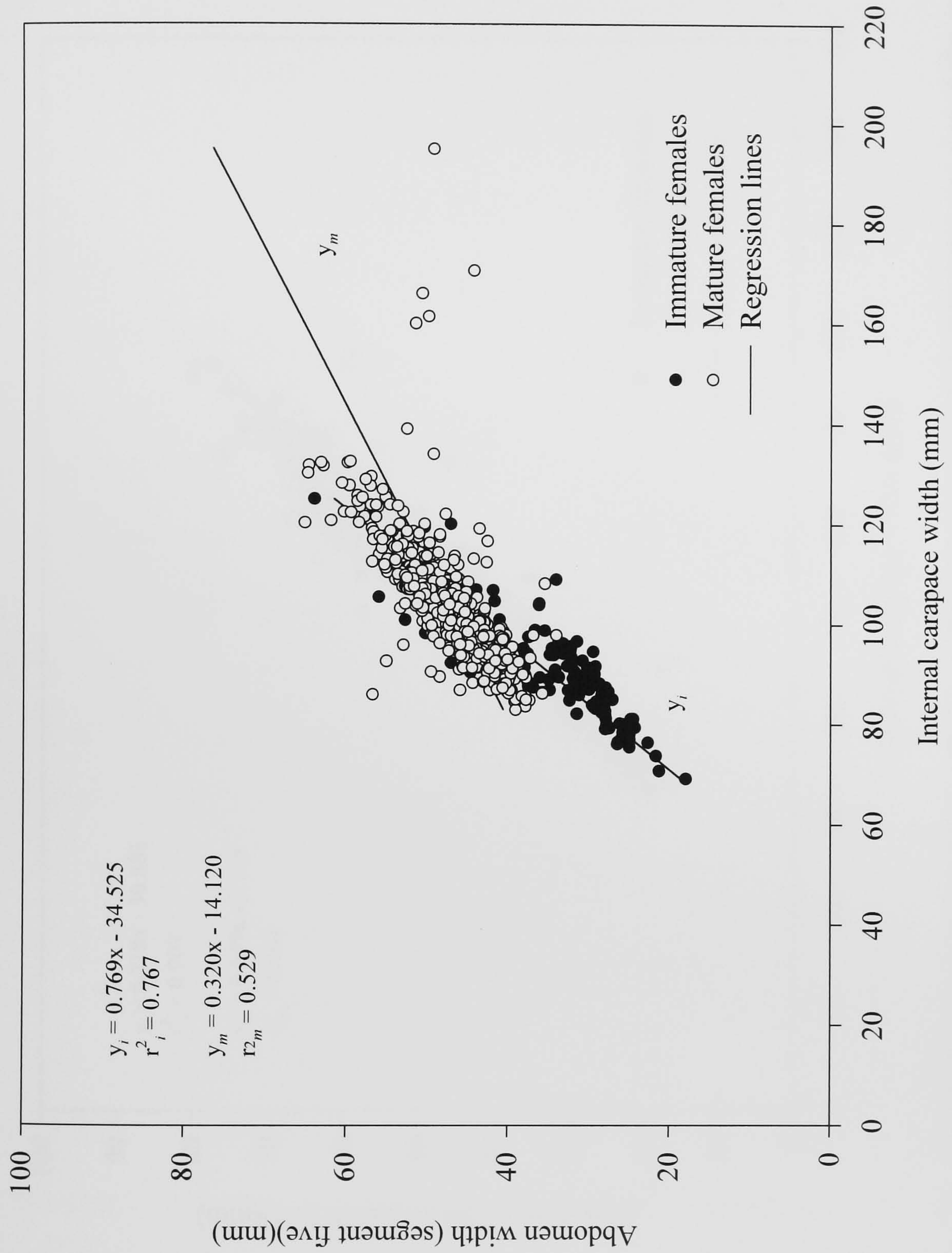


Figure 7.8: Regression of abdomen width against carapace width plotted to calculate the size of the moult at maturity using breakpoint analysis for female *Scylla olivacea* sampled from Ban Don Bay, Surat Thani Province, Thailand ($n_i = 183$ and $n_m = 652$).

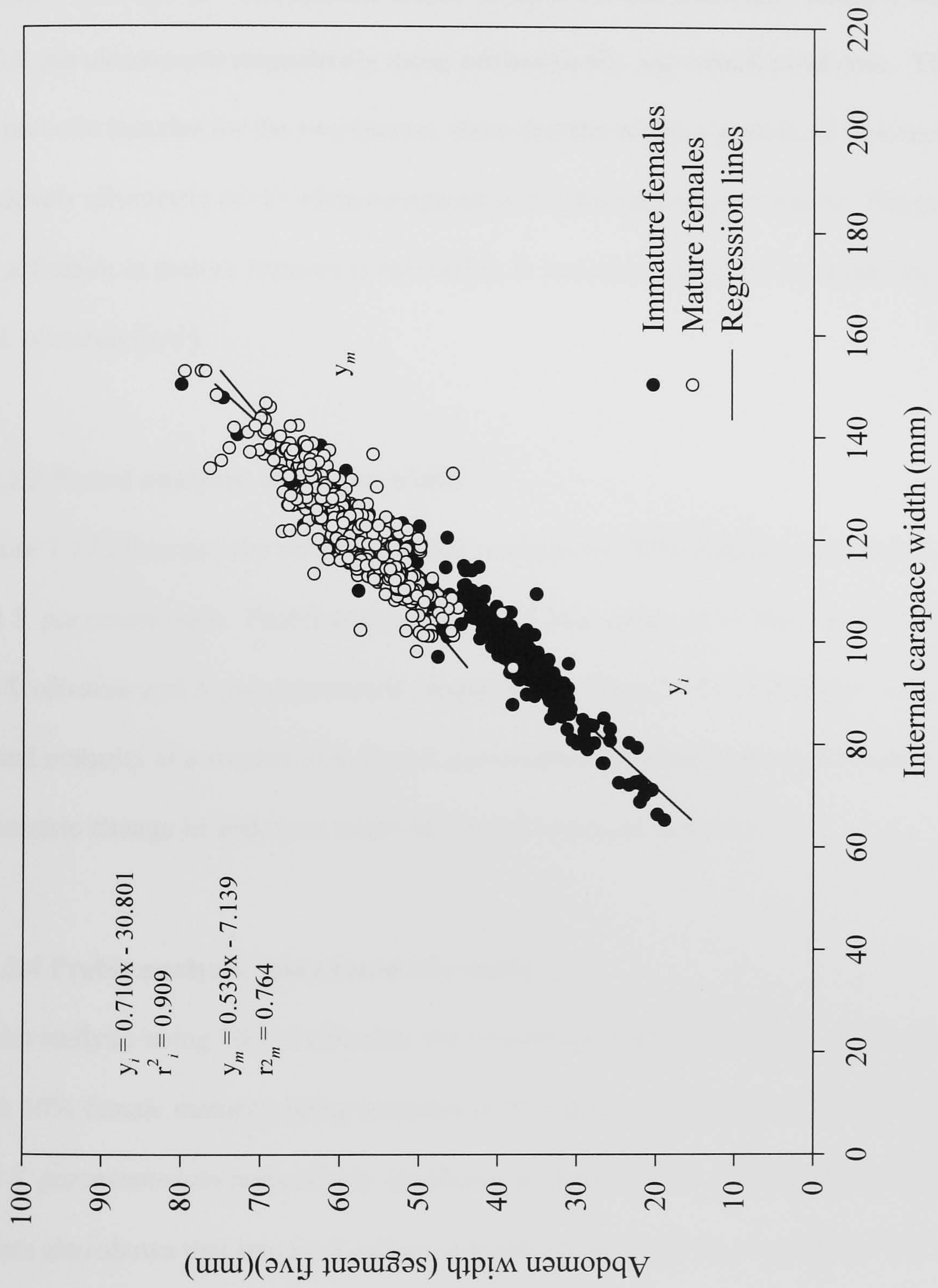


Figure 7.9: Regression of abdomen width against carapace width plotted to calculate the size of the moult at maturity using breakpoint analysis for female *Scylla paramamosain* sampled from Ban Don Bay, Surat Thani Province, Thailand ($n_i = 285$ and $n_m = 495$).

made. This similarity is also reflected in the slopes and elevation of the regression lines shown in figure 7.8 and figure 7.9.

Figures 7.10 and 7.11 illustrate the slopes for juvenile and immature female *S. olivacea* and *S. paramamosain* respectively using arithmetically log-transformed data. The slopes for juvenile females for the two species show that the relative growth of abdomen width is positively allometric ($b > 1$) when compared with internal carapace width. The growth of the abdomen in mature females is reduced in *S. paramamosain* and is negatively allometric in *S. olivacea* ($b < 1$).

7.3.2.3 Probit analysis: abdomen width

Figure 7.12 illustrates the results of probit analysis for 50% maturity in female *S. olivacea* and *S. paramamosain*. Probit analysis revealed M_{50} values of 93.9mm and 111.8mm ICW for *S. olivacea* and *S. paramamosain*, respectively. Thus 50 % of female *S. olivacea* reach sexual maturity at a smaller size than *S. paramamosain* when maturity is based on the allometric change in abdomen width attributed to sexual maturity.

7.3.2.4 Probit analysis: gonadosomatic index

Probit analysis using GSI to calculate the percentage maturity revealed smaller M_{50} values with 50% female maturity being recorded at 91.2mm and 110.5mm ICW for *S. olivacea* and *S. paramamosain* respectively (as shown in figure 7.13). Probit analysis of the GSI values also shows that female *S. olivacea* reach sexual maturity at a smaller size than female *S. paramamosain* as seen for probit analysis on abdomen width.

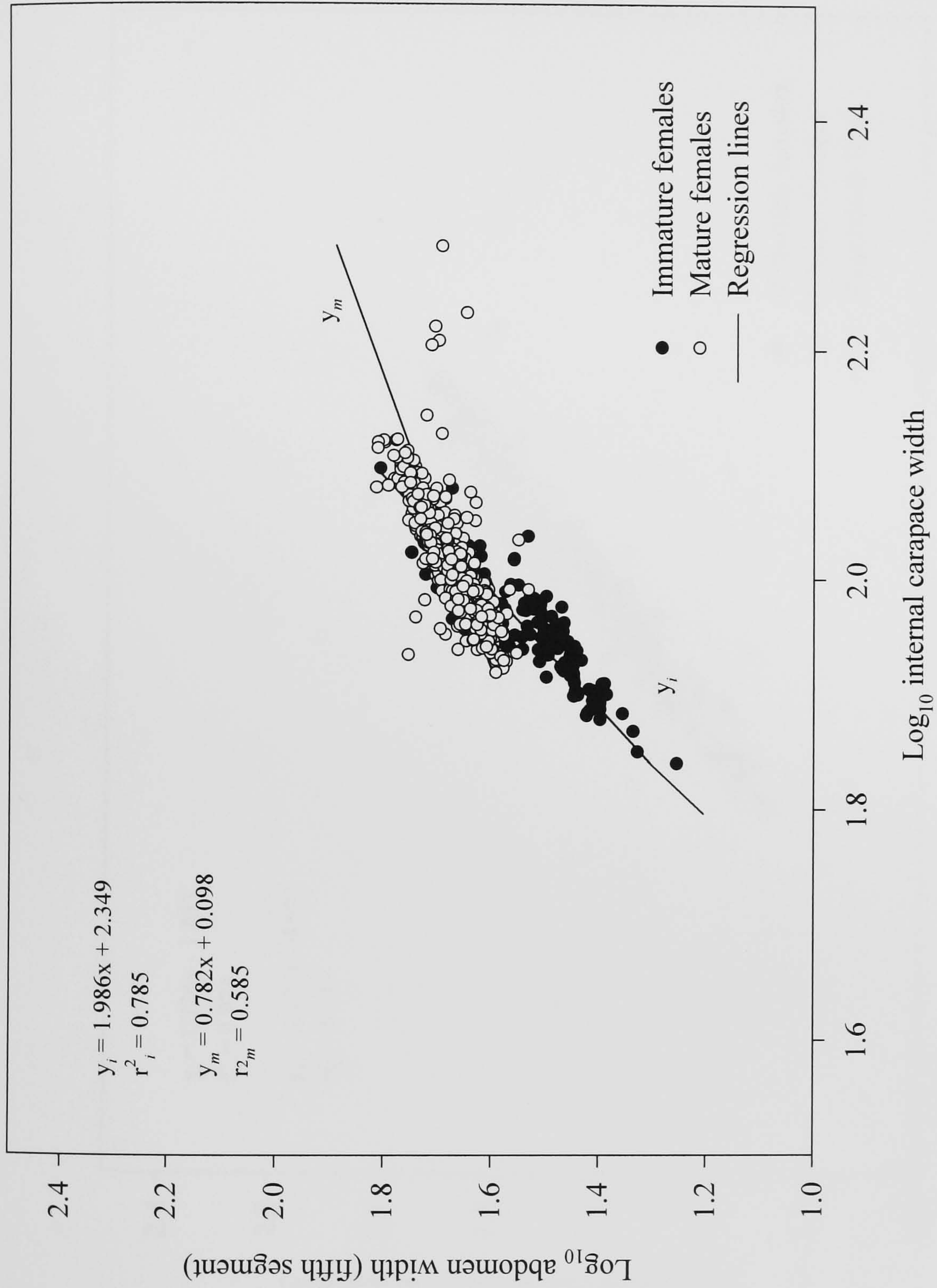


Figure 7.10: Illustration of regression lines of log₁₀ abdomen width plotted against log₁₀ internal carapace width for *Scylla olivacea* from Ban Don Bay, Surat Thani Province, Thailand.

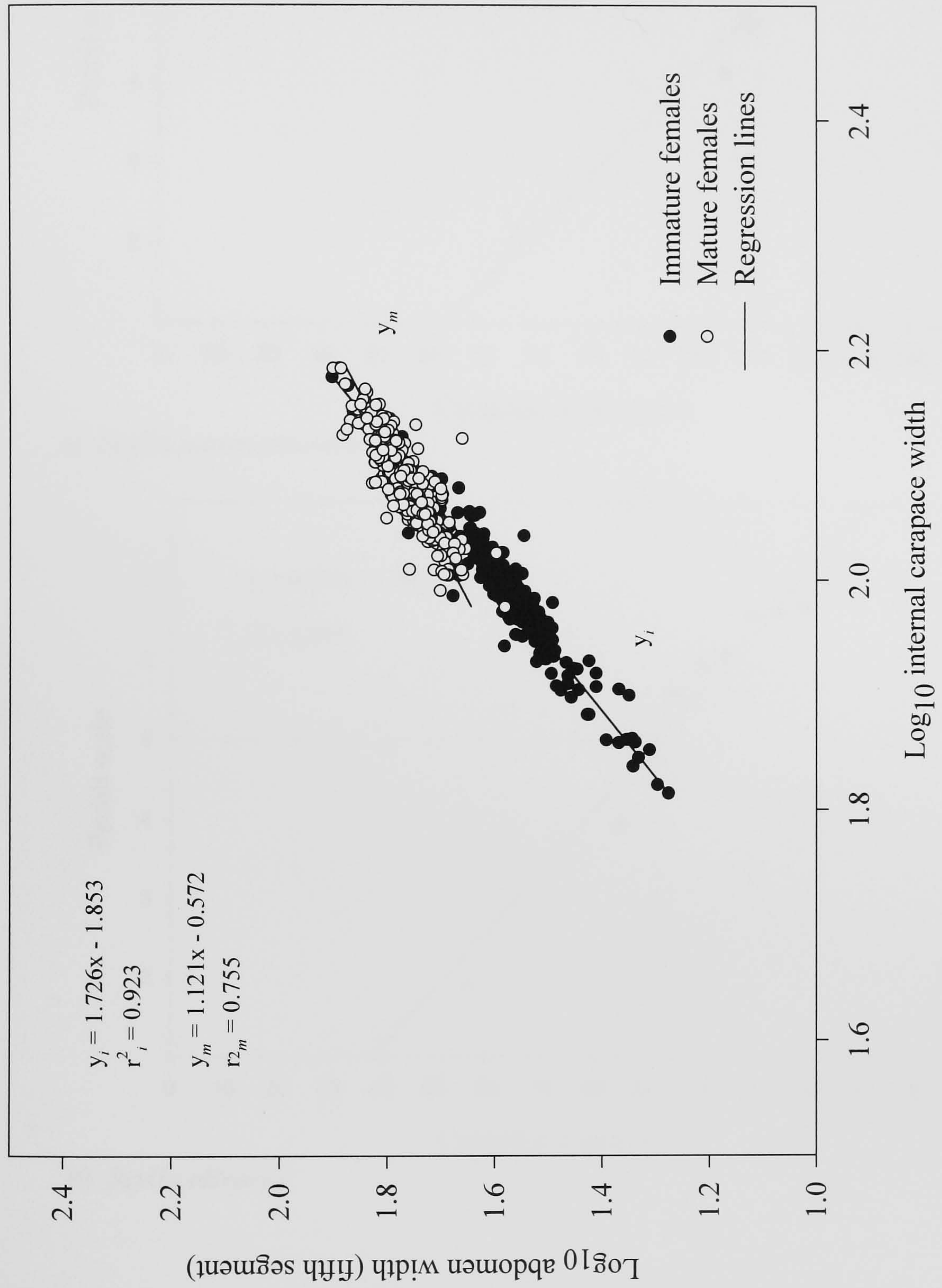


Figure 7.11: Illustration of regression lines of log₁₀ abdomen width plotted against log₁₀ internal carapace width for *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand.

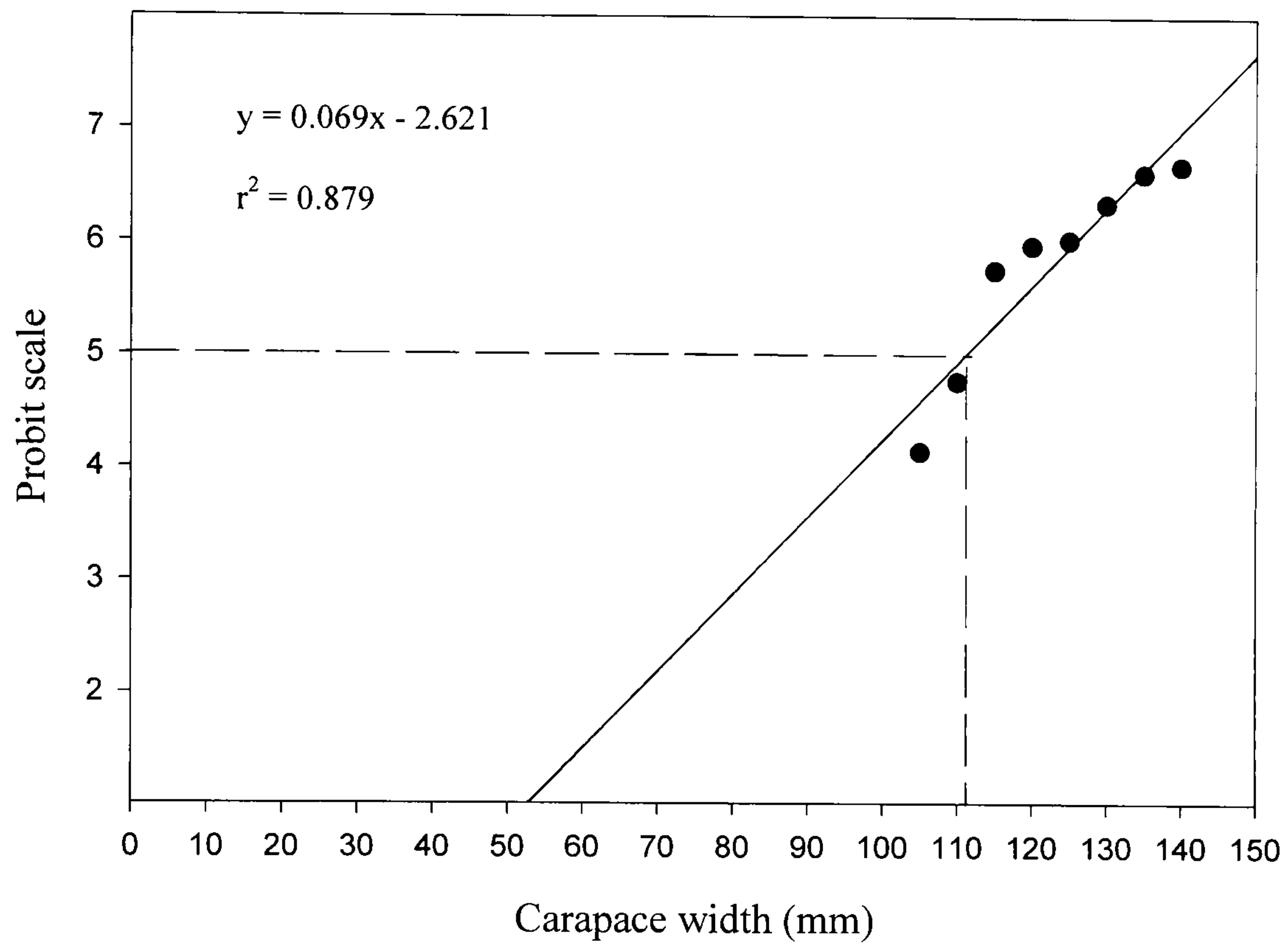
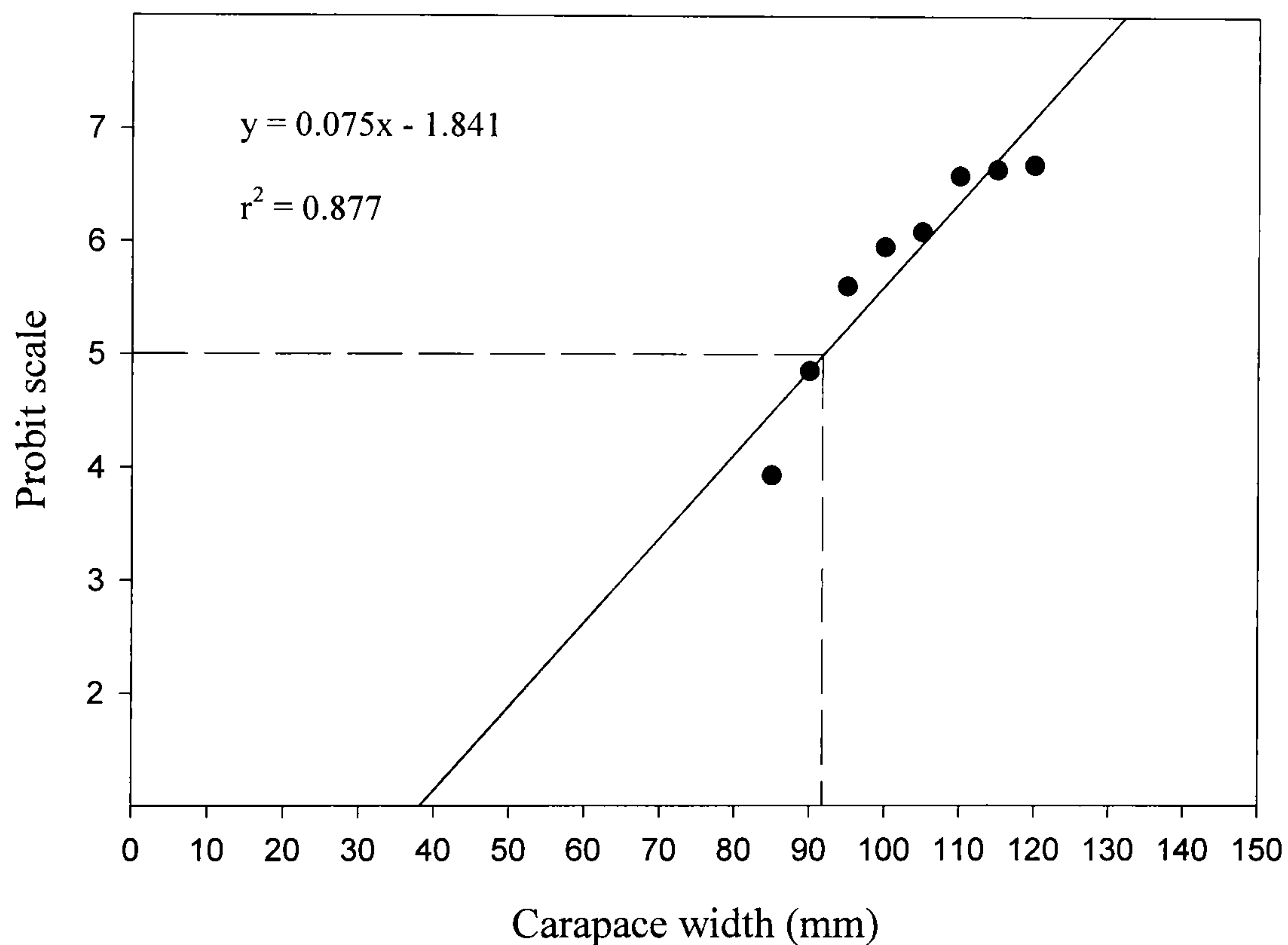
a) *Scylla paramamosain*b) *Scylla olivacea*

Figure 7.12: Percentage maturity, using abdomen shape to identify mature females, as a probit scale plotted against carapace width to obtain the carapace width for M50 (the point at which 50% of female crabs are mature) for a) *Scylla paramamosain* and b) *Scylla olivacea* sampled from Ban Don Bay, Surat Thani Province, Thailand.

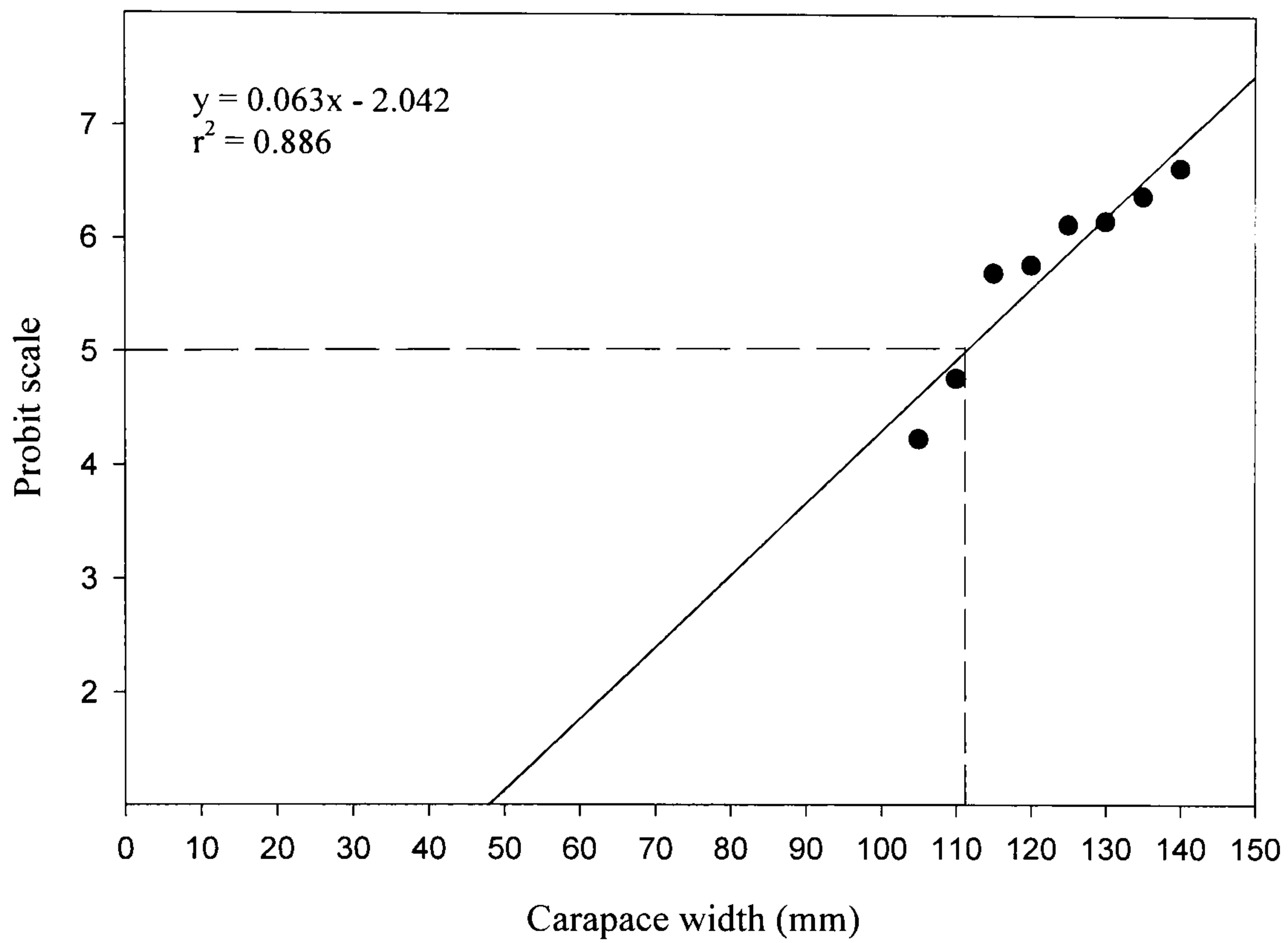
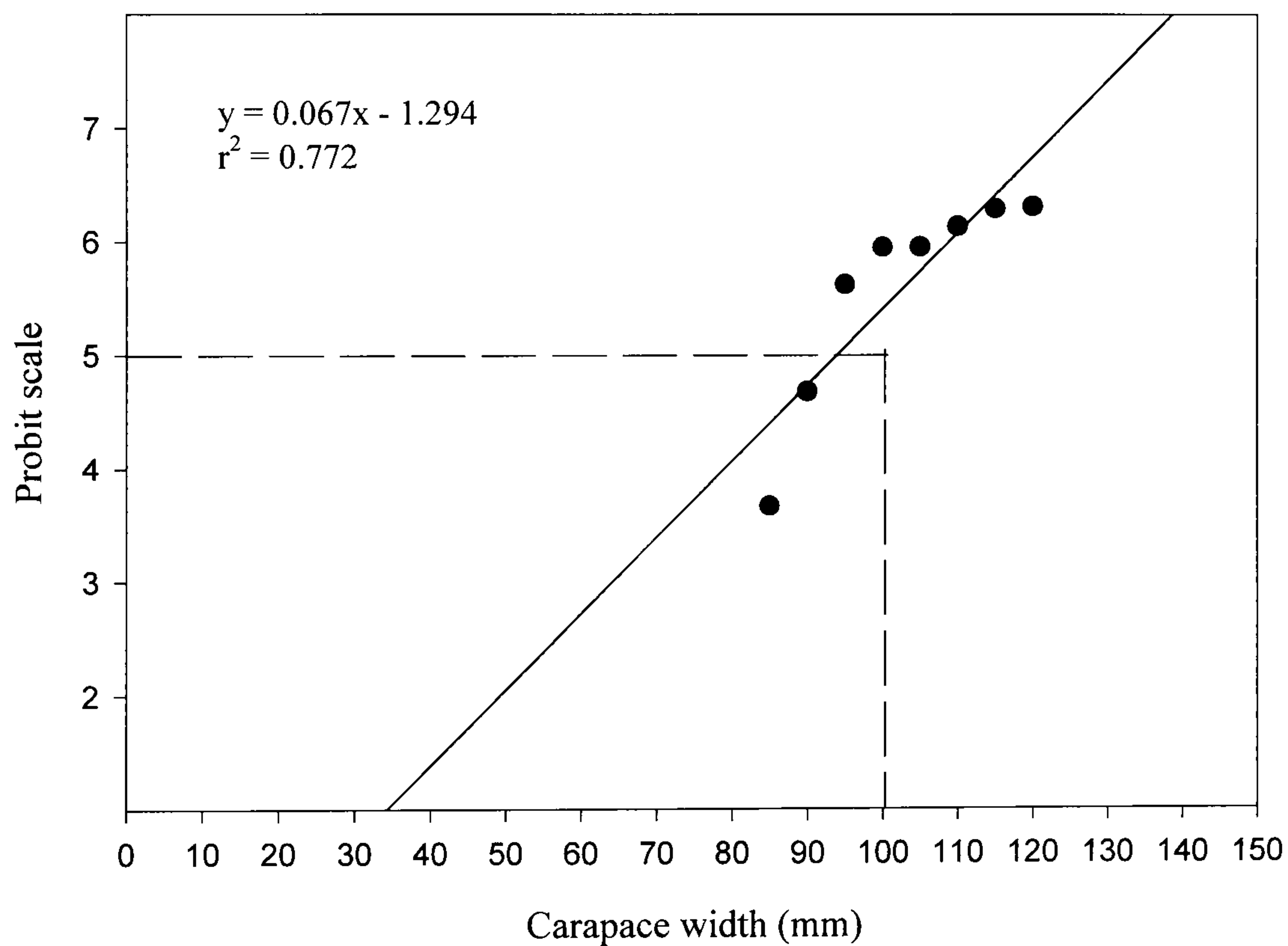
a) *Scylla paramamosain*b) *Scylla olivacea*

Figure 7.13: Percentage maturity, using gonadosomatic index to identify mature females, as a probit scale plotted against carapace width to obtain carapace width for M₅₀ (the point at which 50% of female crabs are mature) for a) *Scylla paramamosain* and b) *Scylla olivacea* sampled from Ban Don Bay, Surat Thani Province, Thailand.

7.3.3 Seasonal variation in reproductive indices for female mud crabs

7.3.3.1 Seasonal variation in mean GSIs

Figure 7.14 shows the seasonal change in mean GSI recorded for female *S. olivacea* and *S. paramamosain* from Surat Thani over eighteen months of study. A “sawtoothed” profile is revealed in the plotted data for both species. Three main peaks, reaching an average GSI of 13%, occurred in a) September-October; b) March; and c) July for both species. There was a large drop in GSI from May to June with mean GSIs ranging from 0 to 2% for both species. A decrease in GSI also occurred again between November and January, occurring in November for *S. paramamosain*, but later (January-February) in *S. olivacea*. Mud crabs of both species were scarce in the same period (November 1995 to February 1996).

7.3.3.2 Seasonal variation in percentage of mature females

Figure 7.15 denotes the seasonal change in the percentage of mature female *S. olivacea* and *S. paramamosain*. The highest percentage of mature crabs occurred from February to April and from July to October. There was a drop in the percentage of mature crabs in May for both species. The seasonal trends in maturity are similar for both species, although *S. olivacea* did exhibit a higher percentage of mature crabs in the majority of samples collected. The frequency of female crabs in each sample collected that represent the four stages of maturity (as seen in figure 7.16) reinforces the observations regarding percentage maturity. Stage I female crabs were present in relatively high numbers from May to June and were in abundance in July. Mature females dominated the catches from August to October. Juvenile crabs seemed to be more abundant in November for *S. paramamosain* than for *S. olivacea*. In the second year (1996/97), the intermediate stages (stages II and III) were dominant in December and January.

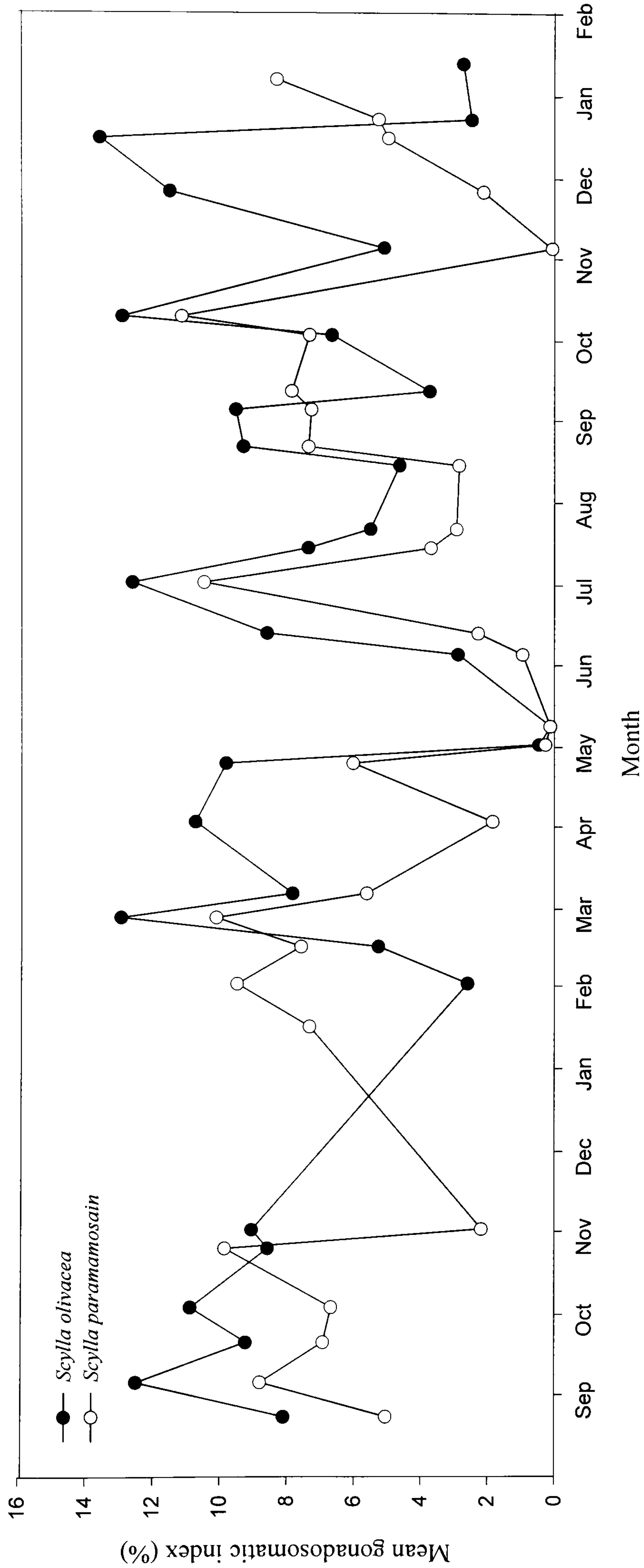


Figure 7.14: Seasonal change in mean gonadosomatic index of female *Scylla olivacea* and *Scylla paramamosain* sampled from Ban Don Bay, Surat Thani Province, Thailand (24/08/95 - 13/01/97).

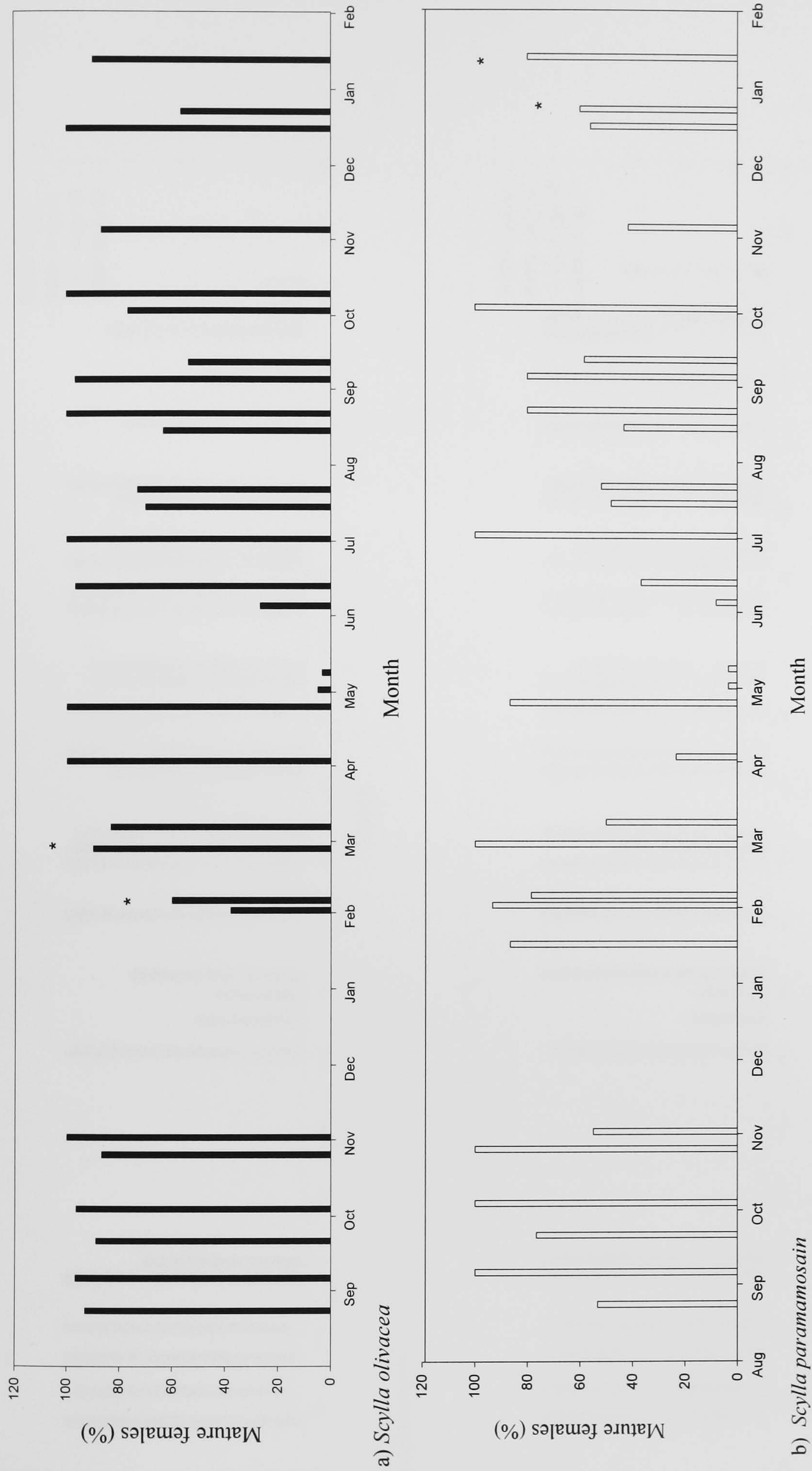
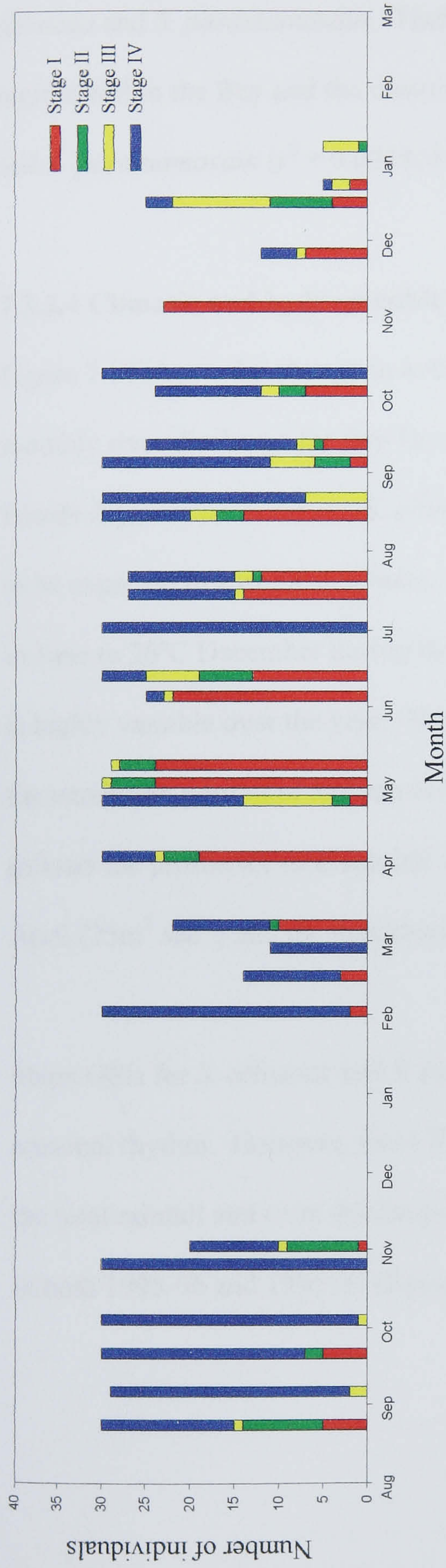
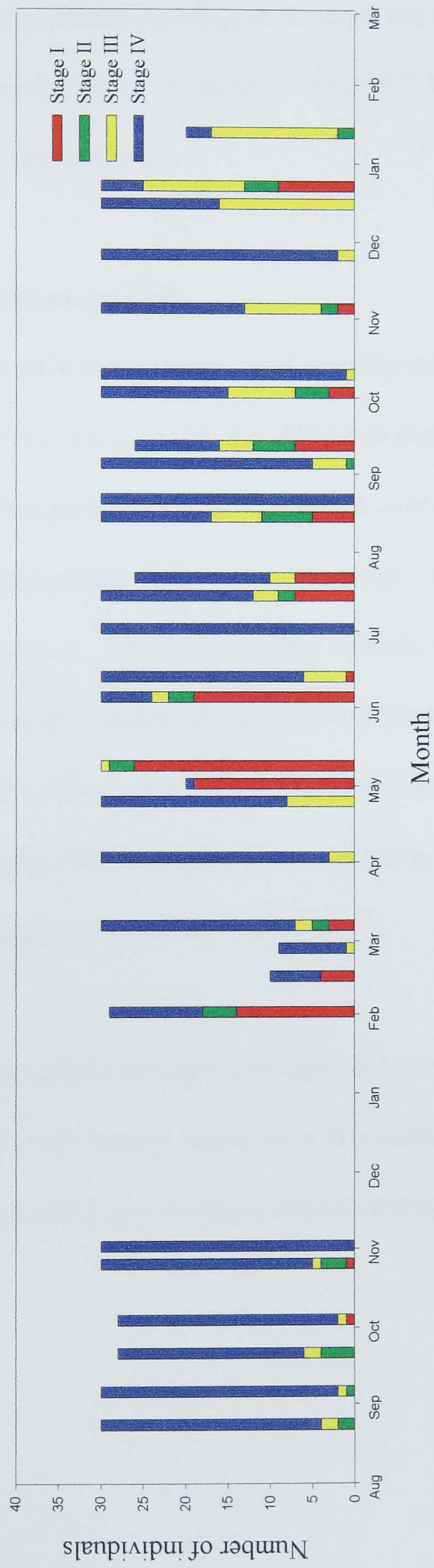


Figure 7.15 : Percentage of mature female mud crabs in fortnightly samples of a) *Scylla olivacea* and b) *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand (24/08/95 - 13/01/97). Note asterisks mark those percentages calculated from samples where n crabs < 10.



a) *Scylla paramamosain*



b) *Scylla olivacea*

Figure 7.16: Frequency of female mud crab a) *Scylla paramamosain* and b) *Scylla olivacea* in relation to stage of gonadal maturity sampled fortnightly from Ban Don Bay, Surat Thani Province, Thailand (24/08/95 - 13/01/97).

7.3.3.3 Effect of tidal amplitude on GSI

Figure 7.17 shows tidal amplitude in Ban Don Bay plotted against mean GSI for *S. olivacea* and *S. paramamosain*. There is no significant correlation between the tidal regime within the Bay and the mean GSI recorded for *S. olivacea* ($r^2 = 0.0097$; $P > 0.05$) and *S. paramamosain* ($r^2 = 0.0048$; $P > 0.05$), respectively.

7.3.3.4 Climatic and hydrographic effects on mean GSI

Figure 7.18 shows the change in average monthly temperature, total monthly rainfall and monthly river discharge for Ban Don Bay in conjunction with the change in mean GSIs for female *S. paramamosain* and *S. olivacea*. Temperature is almost constant over the year, as to be expected in a tropical climate. Average monthly air temperatures range from 29.4°C in June to 26°C December during the rainy season. The total monthly rainfall, in contrast, is highly variable over the year. Minimum rainfall (6mm) is recorded in February, whereas the maximum rainfall of 329mm is recorded in November. The amount of river discharge follows the profile for total rainfall very closely, the minimum discharge being recorded in April ($25\text{m}^3 \text{sec}^{-1}$) and the maximum discharge in November ($310\text{m}^3 \text{sec}^{-1}$).

Mean GSIs for *S. olivacea* and *S. paramamosain* do not seem to follow any particular seasonal rhythm. However, from November to February, when there is a sudden drop in the total rainfall and river discharge, the mean GSI also declined sharply for each species in both 1995-96 and 1996-97 (figure 7.18).

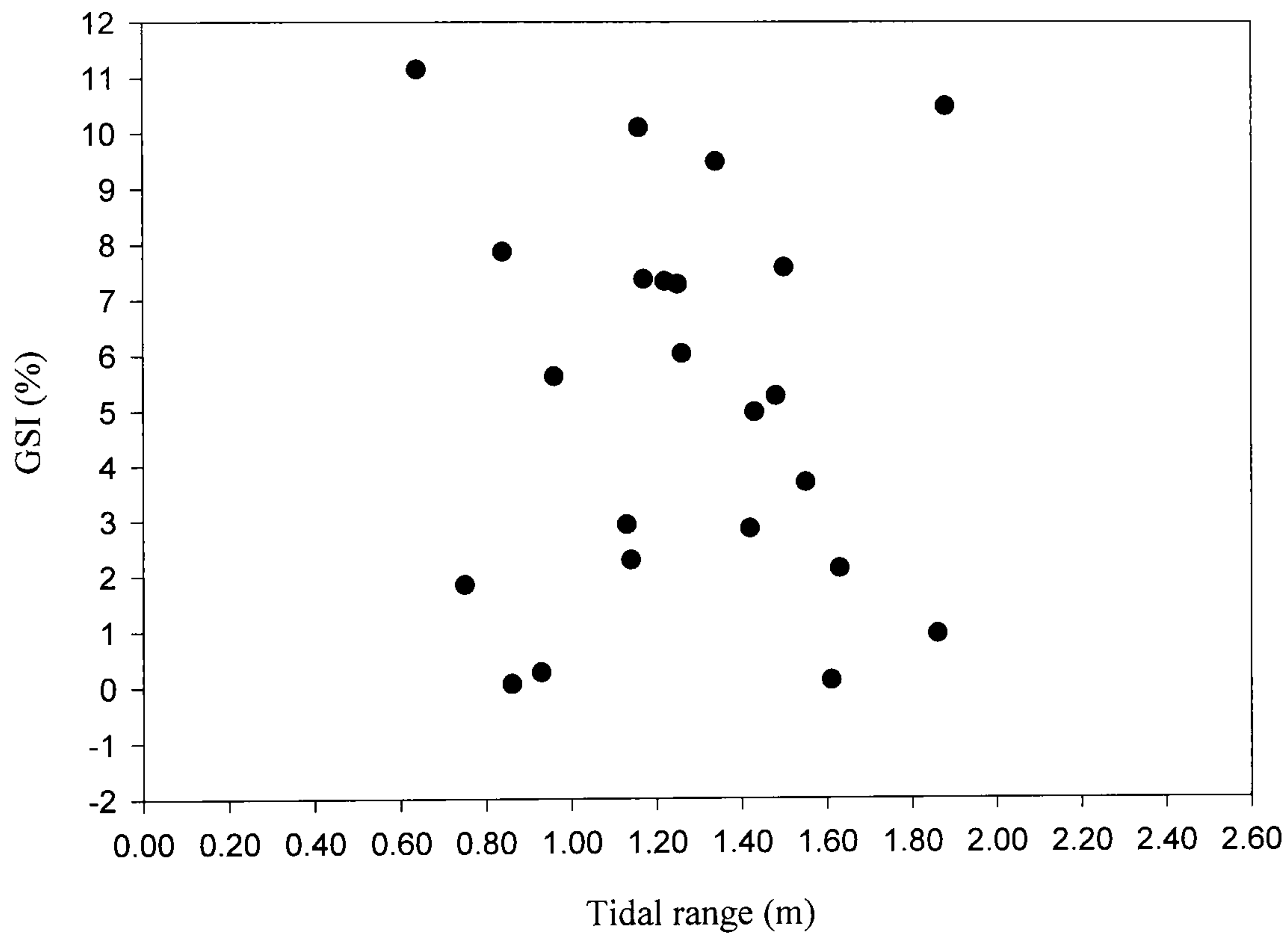
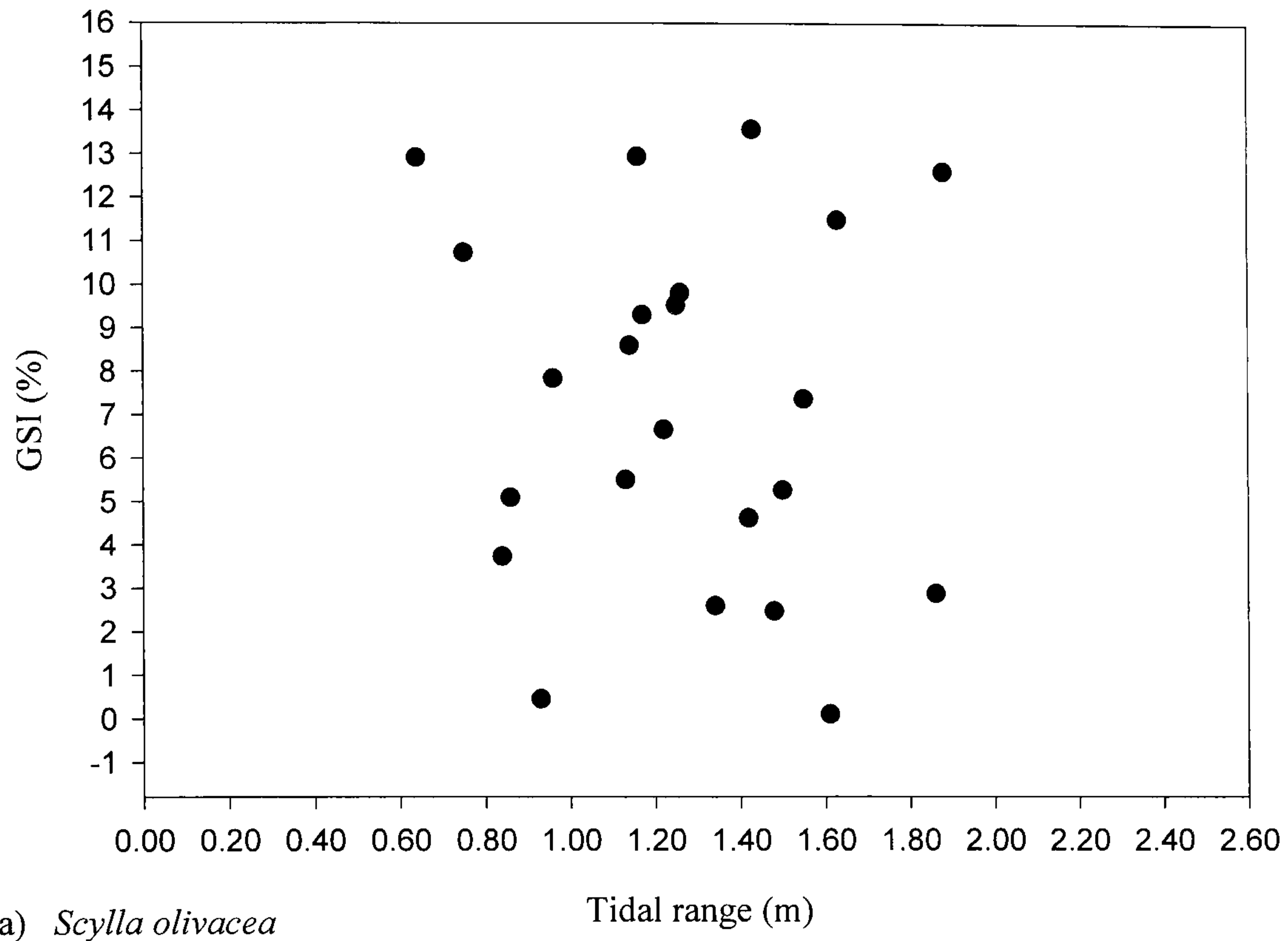


Figure 7.17: Mean GSI of female mud crabs plotted against tidal amplitude on each sampling date from 01/01/96 to 31/12/96 for a) *Scylla olivacea* and b) *Scylla paramamosain* sampled from Ban Don Bay, Surat Thani Province, Thailand. Correlation coefficients are $r^2 = 0.0097$ and 0.0048 for *S. olivacea* and *S. paramamosain* respectively. Both correlations are not significant ($P > 0.05$).

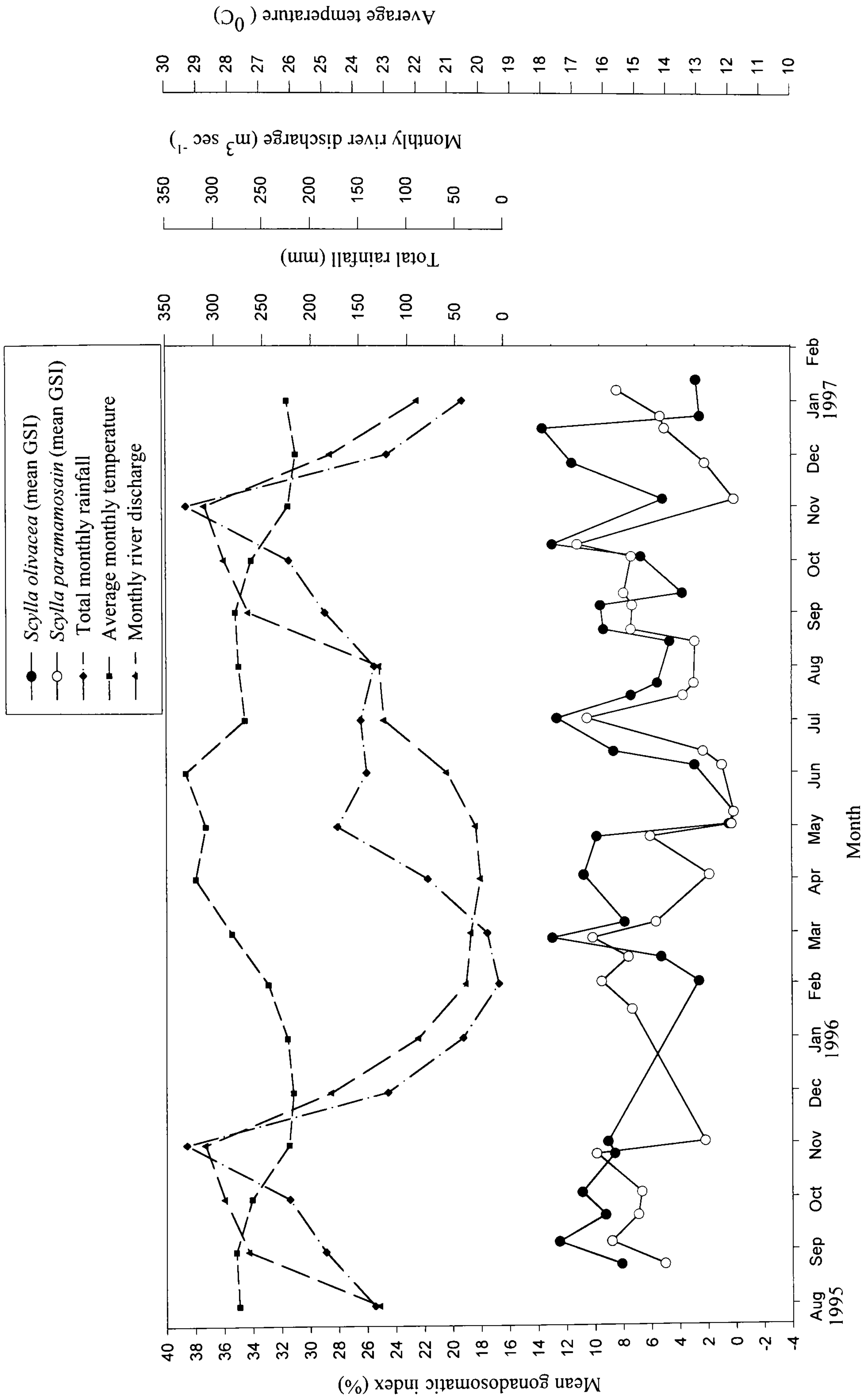


Figure 7.18: Seasonal change in mean gonadosomatic index of female *Scylla olivacea* and *Scylla paramamosain* sampled from Ban Don Bay, Surat Thani Province, Thailand (24/08/95 - 13/01/97). Climatic data collected from STCAC, Kanchanadit (average monthly temperature and total monthly rainfall) and hydrological data from Kositratana, 1988 (monthly discharge of the Tapi River) are included for reference.

7.3.4 Male gonopod morphology

Table 7.3 summarises the size data recorded for gonopods from male *S. olivacea* and *S. paramamosain* in relation to the body sizes of the specimens obtained. Although the size range was larger for *S. paramamosain* (31.9mm in ICW between the largest and smallest specimens) than for *S. olivacea* (only 18.9mm in ICW between the largest and smallest specimens), the mean gonopod sizes are comparable between the two species when adjusted by dividing the gonopod length by internal carapace width.

Table 7.3: Summary of sizes (internal carapace width and fresh weight), gonopod length and adjusted gonopod length, for male *Scylla olivacea* and *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand.

Species and individual crab	Size (ICW) mm	Fresh weight (g)	Gonopod length		Gonopod length/ICW	
			Right	Left	Right	Left
<i>Scylla olivacea</i>						
Crab 1	94.1	209.7	22.7	22.6	0.241	0.240
Crab 2	95.2	200.7	20.0	20.5	0.210	0.215
Crab 3	98.8	244.5	22.2	21.0	0.225	0.214
Crab 4	107.4	296.0	24.1	23.0	0.224	0.214
Crab 5	111.6	393.9	24.5	25.6	0.220	0.229
Crab 6	112.7	382.5	27.9	25.2	0.248	0.224
Mean	103.3	287.9	23.6	23.0	0.228	0.223
S.D.	8.30	84.68	2.66	2.10	0.014	0.011
<i>Scylla paramamosain</i>						
Crab 1	92.9	183.8	21.7	21.1	0.234	0.227
Crab 2	96.1	234.2	23.1	23.1	0.240	0.240
Crab 3	110.4	292.3	25.9	25.9	0.235	0.235
Crab 4	119.1	412.5	25.9	25.9	0.217	0.217
Crab 5	124.8	558.0	27.7	28.4	0.222	0.228
Mean	108.7	336.16	24.9	24.9	0.230	0.230
S.D.	13.19	150.48	2.41	2.83	0.010	0.009

Figures 7.19 and 7.20 illustrate the morphology of the right gonopod tip from each male examined using scanning electron microscopy for *S. olivacea* and *S. paramamosain*, respectively. It can be seen from these two figures that there is more variation intraspecifically than between male mud crabs from the two species. Careful examination of the photographs revealed no gross morphological differences that could be used to separate the two species.

The curvature in the tip of the male gonopod, measured using a) total curvature and b) the deviation of the gonopod tip from a straight line when plotted against internal carapace width (as shown in figure 7.21) showed no discernible species differences. Finally, gonopod tip width and the areas of the gonopod “ear” also proved to be similar between male *S. olivacea* and *S. paramamosain* (as illustrated in figure 7.22).

7.4 Discussion

Scanning electron microscopy revealed that the apex of the male gonopods of *Scylla olivacea* and *S. paramamosain* has similar morphology in both species. This finding agrees with some previous descriptive taxonomic studies in which gonopod morphology was reported to be homogenous between the species of *Scylla* proposed (Estampador, 1949b; Radhakrishnan and Samuel, 1982).

Subtle variations in the gonopod apex are shown by Keenan *et al.* (1998) for the four species of *Scylla* they have recently described. However, the range of gonopod tip morphology they describe was represented among individuals of both *S. olivacea* and *S. paramamosain* examined in this study. In fact, the apex form varied more intraspecifically than interspecifically for *S. olivacea* and *S. paramamosain*. As the tip is the fundamental

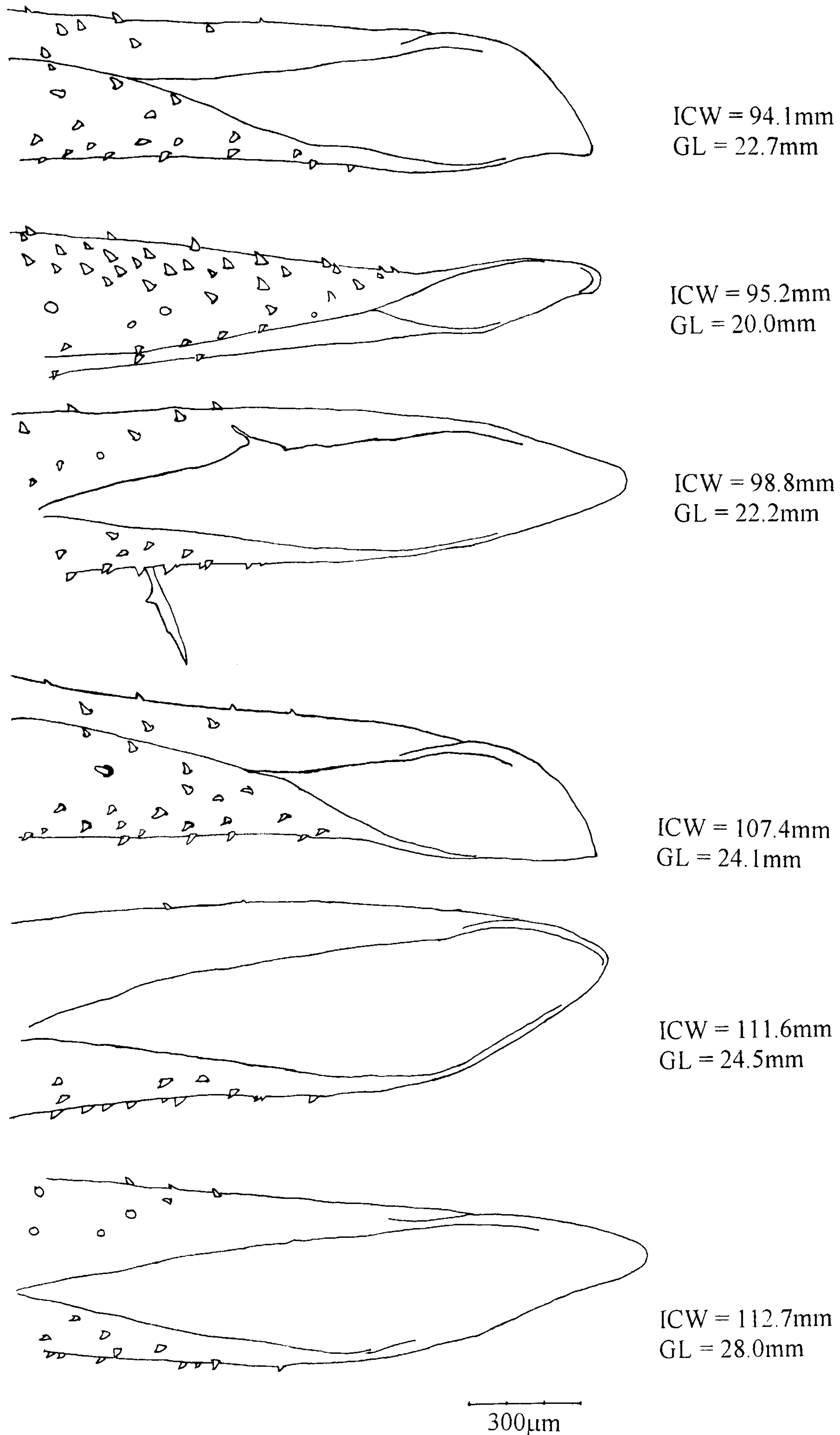


Figure 7.19: Illustration of right gonopod tips from male *Scylla olivacea* from Ban Don Bay, Surat Thani Province, Thailand (ICW = internal carapace width; GL = gonopod length). Drawn from SEM photographs of the gonopods at 60x magnification).

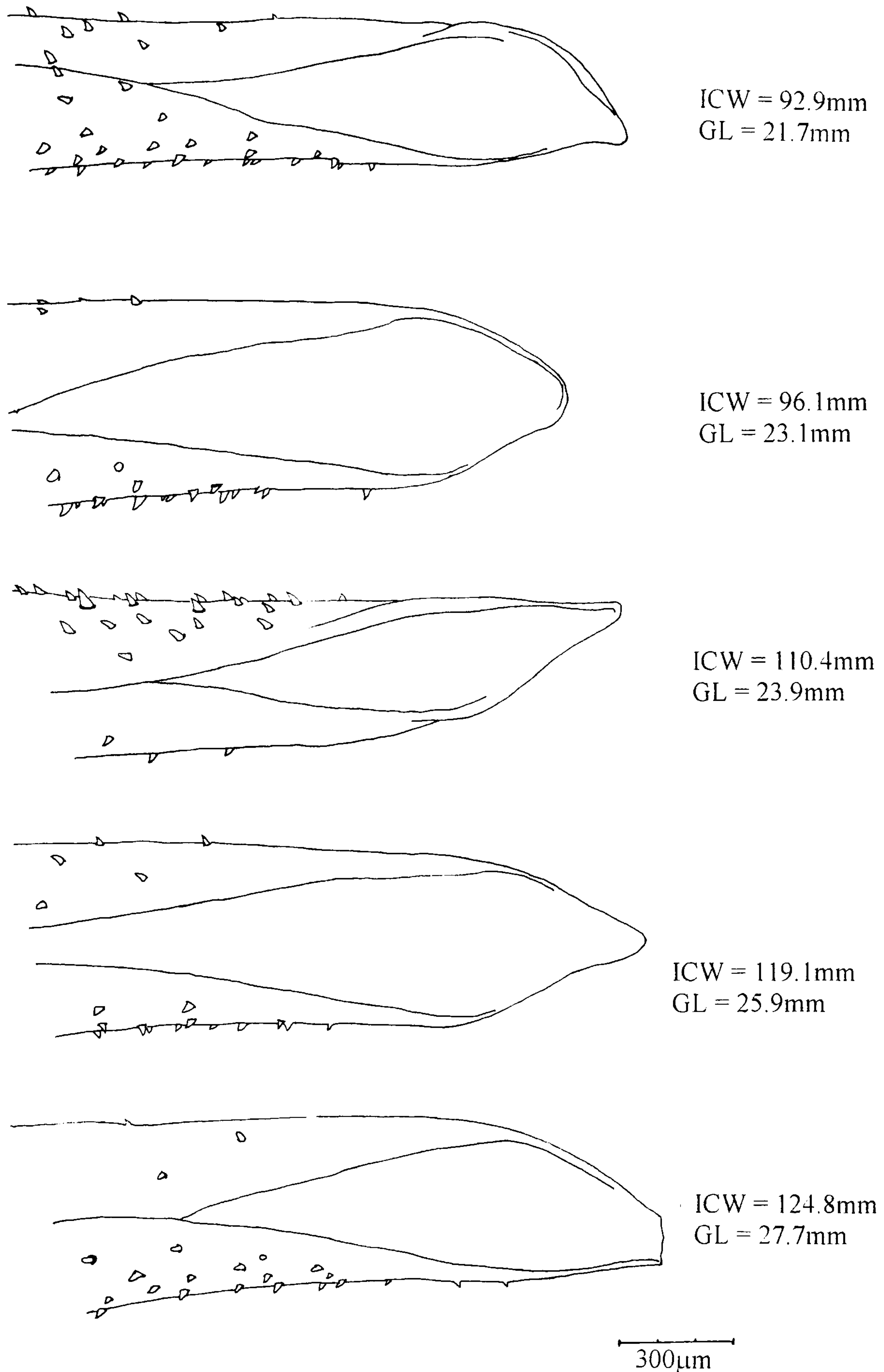
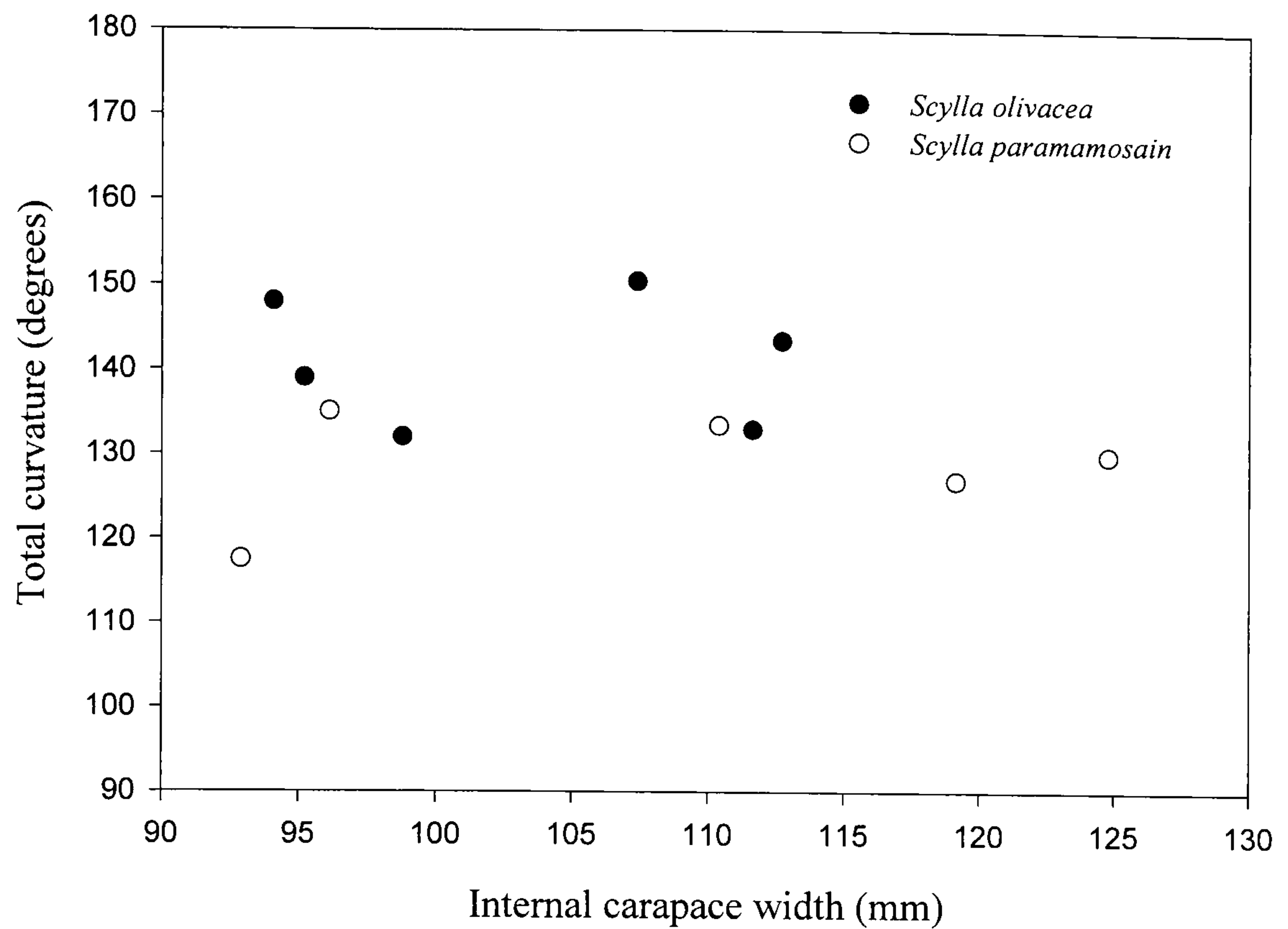
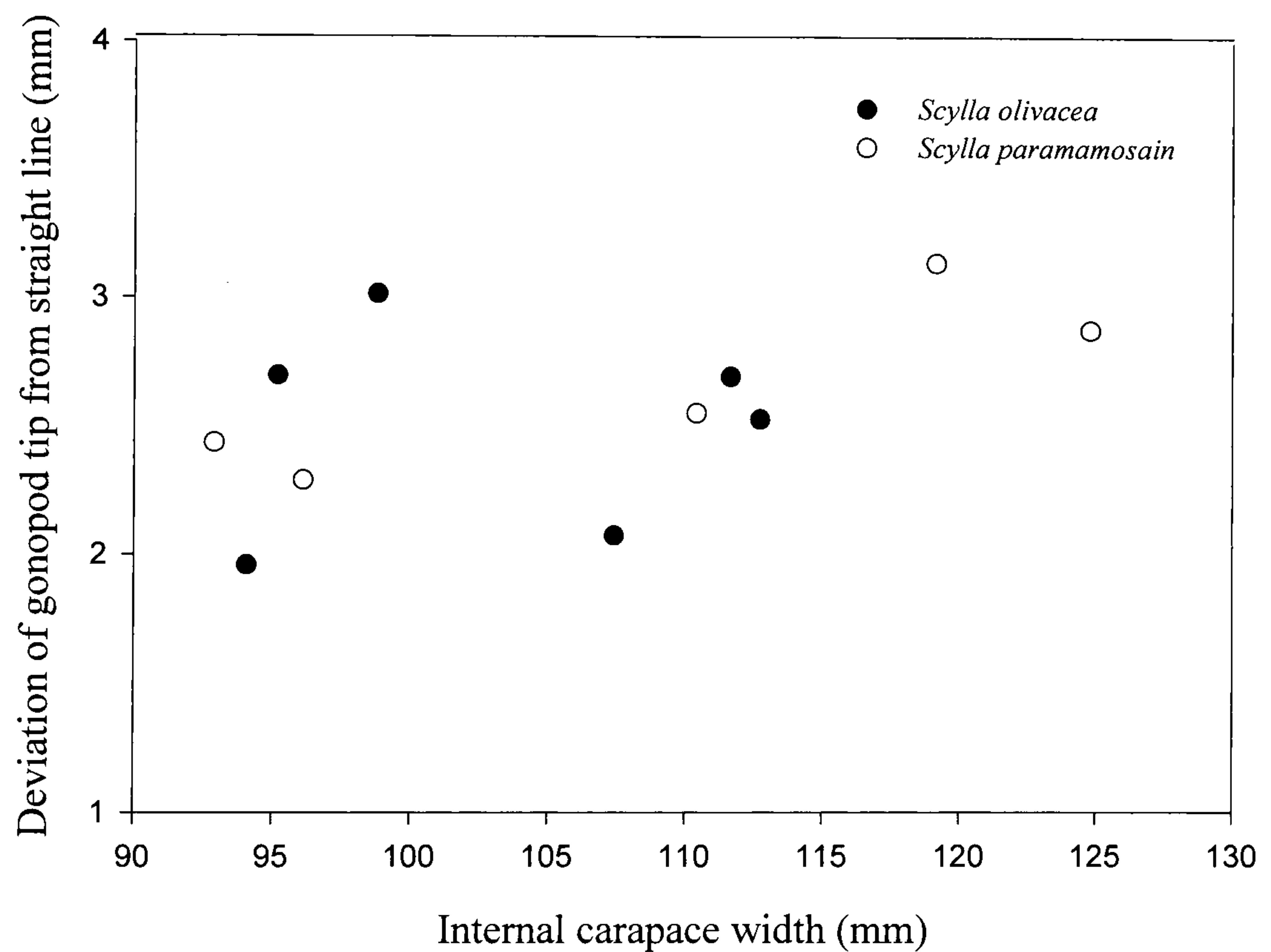


Figure 7.20: Illustration of right gonopod tips from male *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand (ICW = internal carapace width; GL = gonopod length). Drawn from SEM photographs of the gonopods at 60x magnification).

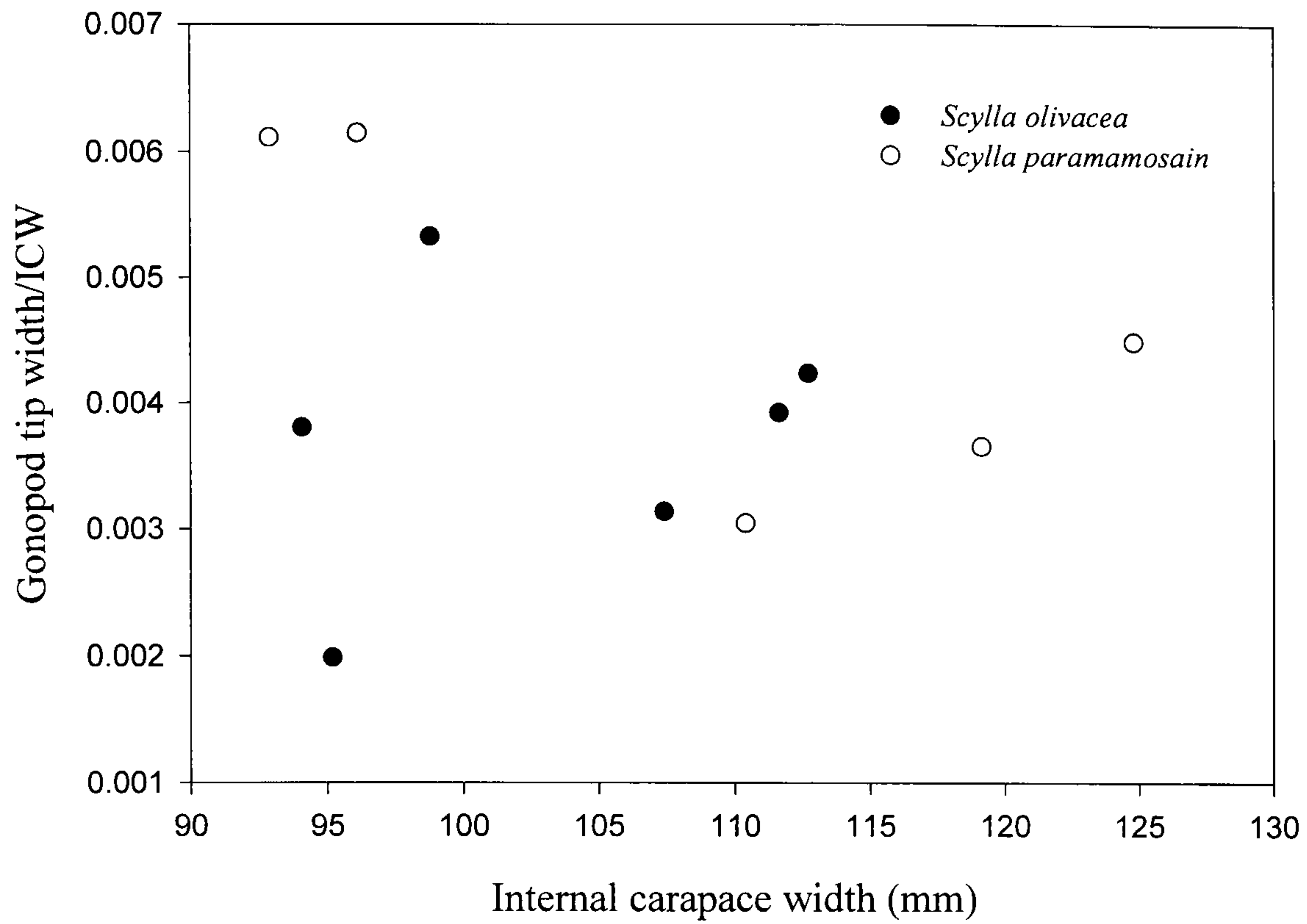


a) Total curvature

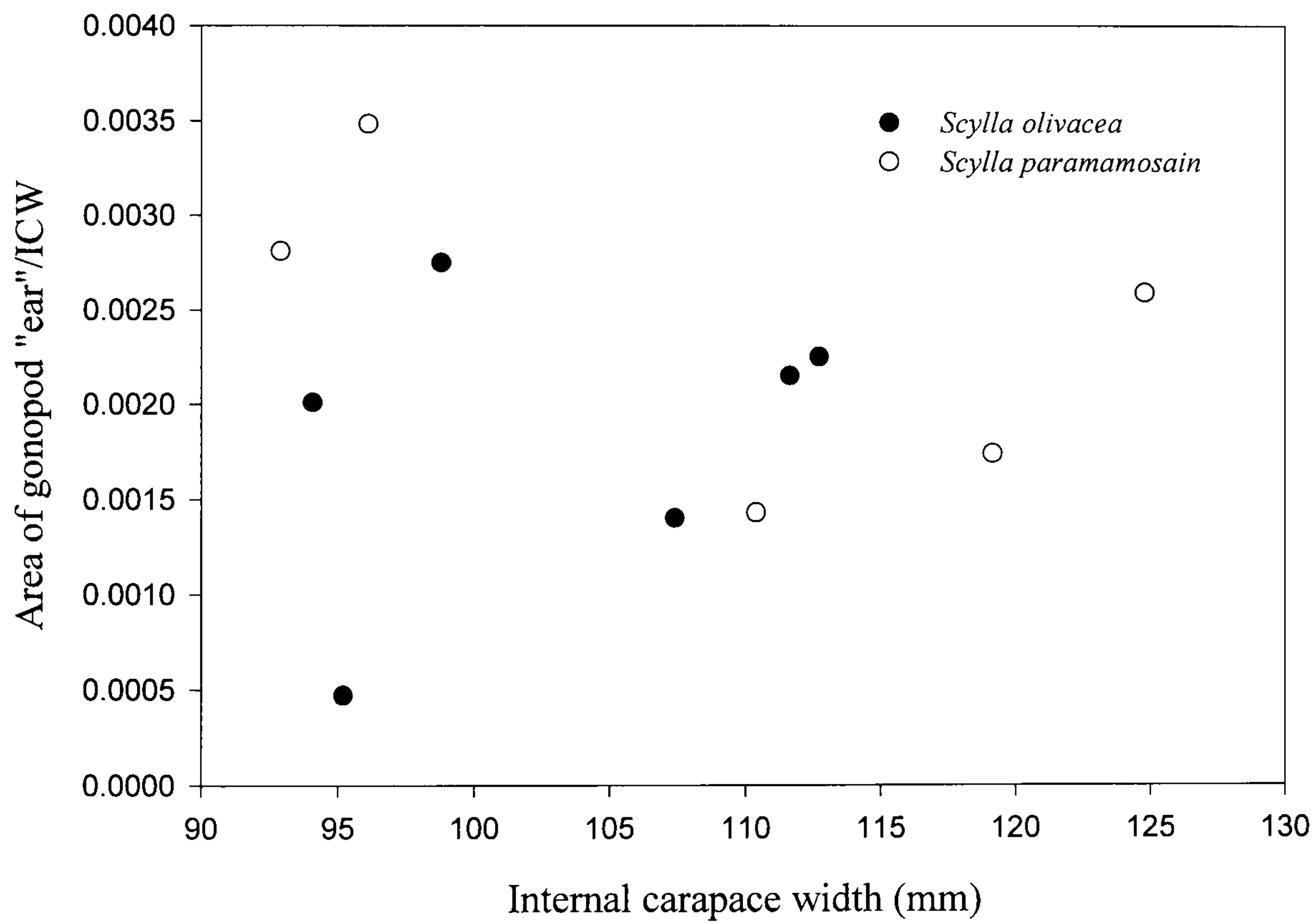


b) Deviation from a straight line

Figure 7.21: Estimation of curvature of male gonopods using a) total curvature and b) deviation from a straight line plotted against internal carapace width for *Scylla olivacea* and *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand.



a) Width of gonopod tip



b) Area of gonopod "ear"

Figure 7.22: Width of gonopod tip and area of the gonopod "ear" plotted against internal carapace width for *Scylla olivacea* and *Scylla paramamosain* from Ban Don Bay, Surat Thani Province, Thailand.

structure which penetrates the female (the distal third of the male gonopods are sometimes broken off, most probably from attempts to mate with female mud crabs; personal observation), it appears that there is no obvious mechanical barrier to mating between these two closely related species. The additional measurements made on the crabs examined: gonopod tip width, the extent of curvature and area of the gonopod tip, all confirm the lack of any consistent difference in gonopod morphology between *S. olivacea* and *S. paramamosain*.

Homogeneous gonopod morphology is often seen in many closely related crustacean species, as illustrated in Stephenson and Campbell's (1960) review of Portunid crabs. Nevertheless, the fiddler crabs (*Uca* spp.) are a good example where several species show sympatric associations, including mangrove species such as *Uca dussumieri*, *U. coarctata* and *U. forcipata*, but have differences in gonopod form which help to reinforce the reproductive barriers between them (Crane, 1975).

Even in the absence of a physical pre-zygotic isolating mechanism, prior to the event of mating other pre-zygotic mechanisms come into play which will usually prevent two species ever reaching the stage of attempted copulation (Ridley, 1993). One such isolating mechanism is the displaced reproductive seasons between sympatric crustaceans as shown by populations of gammarid amphipod species (Kolding and Fenchel, 1979).

Mean bimonthly GSIs recorded over eighteen months revealed a protracted period of reproduction for both *S. olivacea* and *S. paramamosain* in Ban Don Bay. This finding is consistent with previous studies on *Scylla* species (Arriola, 1940; Pillai and Nair, 1968; Brick, 1974; Hill 1975; Macintosh *et al.*, 1993; Roberson and Kruger, 1994) and other

tropical brachyuran crabs (Pillai and Nair, 1968). An extended period of reproduction is consistent with the constant warm water temperatures characteristic of equatorial latitudes, compared to more temperate regions where light and water temperature are the prominent environmental factors which influence the peak time of maturity and spawning of crustaceans (Pillay and Ono, 1978). Heasman (1980) and Quinn and Kojis (1987) have discussed the effect of increasing latitude in reducing the seasonal periodicity of female gonad maturity in *Scylla*. An increase in the length of period of high gonad maturity and spawning with decreasing latitude has also been recorded in the blue crab, *Callinectes sapidus*, along the western seaboard of the United States (Steele and Bert, 1994).

Even with protracted periods of female mud crab sexual maturity there are often peaks in mean GSI values (Roberson and Kruger, 1994). Peaks of mean GSI in March, July and November to January were recorded for *S. olivacea* and *S. paramamosain* within Ban Don Bay in the present study. This coincides with the results obtained by Khaonuna and Ratanachote (1994) showing that maximum numbers of gravid female mud crab were recorded in February and in July to August in Ban Don Bay in their study from January to September 1993. This suggests that they also did not find any variation between the peaks of reproductive activity for these two mud crab species.

On the west coast of Thailand, peaks in reproductive activity were recorded in May and September, both in Ranong (Macintosh *et al.*, 1993) and other coastal areas along the Andaman Sea (Poovichiranon, 1992) where *S. olivacea* is the only prominent mud crab species.

The differences in peak reproductive activity for *S. olivacea* between Ranong and Surat Thani are not a result of latitude as these locations are latitudinally similar (9°53'N and 9°11'N for Ranong and Surat Thani respectively). In this case it is most likely that the peak of reproductive activity in *S. olivacea* is influenced by two separate monsoons affecting the two coastlines of Thailand. Ranong, located on the western seaboard of Thailand, is affected by the Southwest monsoon, whereas Surat Thani (located in the western Gulf of Thailand) is affected by the Northeast monsoon. Peak rainfall occurs from June to September in Ranong compared to Surat Thani where peak rainfall takes place around November and to a lesser extent in May (Bunapong and Paw, 1988). High rainfall also coincides with an increase in freshwater runoff (as illustrated in figure 7.16). This also coincides with a rapid decrease in the mean GSI in May and November and a reduction in the number of female crabs present within Ban Don Bay from mid November to December in both 1995 and 1996 for each species. High levels of freshwater runoff may result in a lowering of the salinity in the bay. Thus the decrease in GSI and of the numbers of females present in November to December is most likely to be the result of the female mud crabs migrating offshore to spawn where the salinity is closer to full strength seawater.

Such migrations are well documented for *Scylla* (Arriola, 1940; Ong, 1966; Brick, 1974, Hill, 1994); in fact it is known to occur in all estuarine portunid crabs (Norse, 1977). The periods of migration and offshore spawning by female mud crabs are believed to maximise the use of a) high nutrient inputs due to monsoonal upwellings (Pillai and Nair, 1968; Heasman, 1980); b) the more stable, higher salinity environment found offshore (Hill, 1975; Hill 1994); and c) the improved larval dispersal capability provided by offshore sites (Hill, 1994) by strong wind driven currents.

There was no obvious separation in the seasonality of female sexual maturity between *S. olivacea* and *S. paramamosain*, although a subtle variation was recorded for the two species in peak and then reduction in mean GSIs, possibly related to migration of the females. Female *S. olivacea* shows peak gonad maturity one month in advance of *S. paramamosain* recorded in November to February.

The constant warm water temperatures associated with tropical latitudes provides optimal conditions for maturity and spawning all year round, therefore it is not unexpected that there is a prolonged period of sexual maturity for both species. In higher latitudes it is possible for variations in spawning periods between *Scylla* species to be more noticeable. Joel and Raj (1983) recorded the breeding season for *S. olivacea* (formerly known as *S. serrata*) to be two months in advance of *S. tranquebarica* from Pulicat lake, five degrees further north than Surat Thani.

Keenan *et al.* (1998) suggest that the breeding seasons for all four species of *Scylla* are similar regardless of locality. However, it is possible that until recently the genus *Scylla* was believed to be monospecific, therefore variations in female maturity and spawning seasonality data have not been separated by species.

There was an almost complete overlap in breeding season between the two species studied from 1995 to 1997, although some interspecific variation from December to January was recorded. Thus variation in seasonal reproductive activity is not a factor influencing the sustainability of sympatry between *S. olivacea* and *S. paramamosain* from Ban Don Bay.

As expected, there was no correlation found between tidal amplitude and the mean GSI's for female *S. olivacea* or *S. paramamosain*; a result also observed by Quinn and Kojis (1987). Even though they are semi-terrestrial, female mud crabs migrate to sea to spawn, thus there is no need for selection for spring or neap tides in order to release their larvae. This is also the case in the shore crab, *Carcinus maenas* (Paula, 1989) although diurnal rhythms were found to be important for spawning in the shore crab, its spawning efforts being synchronised to nocturnal high tides (Zeng and Naylor, 1997).

The more terrestrial crab species, such as the grapsid, *Sesarma haematochier* and the fiddler crab *Uca pugilator* (also a mangrove species), have both been shown to have spawning migrations that follow the lunar cycle (Saigusa, 1986; Christy, 1978). Female *Uca pugilator*, in particular, have been shown to adjust the initial stages of their reproduction to ensure larval release on the spring tides (Christy, 1978).

It was found in the majority of samples collected from Ban Don Bay (Gulf of Thailand) that the mean GSI was higher for *S. olivacea* than for *S. paramamosain*. The range of 1% to 14% in mean GSIs recorded for *S. olivacea* in the present study corresponds to the range found by Macintosh *et al.* (1993) for the same species from Ranong on the Andaman coast of Thailand. The higher mean GSI values recorded for *S. olivacea* may reflect differential use of the coastal habitat where the two species are caught. From interviewing the local fishermen from Ban Don Bay, Surat Thani, it was discovered that *S. paramamosain* are more subtidal than *S. olivacea*, the mature females being found further offshore for *S. paramamosain* than for *S. olivacea*. Therefore mature female *S. paramamosain* are less frequently caught than mature female *S. olivacea* in coastal sites, where the majority of artisanal mud crab fishing takes place. Apart from sampling bias, biological reasons may

also explain the apparent difference in GSI's between the two species of *Scylla*, for example, *S. olivacea* may have a higher fecundity than *S. paramamosain*.

All three methods used to determine the estimated size of maturity, namely, minimum size of maturity, Probit analysis (M_{50}) and breakpoint analysis for mud crabs from Ban Don Bay, revealed that female *S. paramamosain* are larger (on average) at the point of maturity than female *S. olivacea*. Although these methods of analysis have their own limitations, their agreement makes the result credible. A discrepancy with regards to estimated size of maturity was also noted by Radhakrishnan and Samuel (1982) for one species and one proposed subspecies of *Scylla* found in the Cochin Backwaters. Previous authors have linked the variation in size of female maturity to the effects of latitude when *Scylla* was considered to be a monospecific genus (reviewed by Quinn and Kojis, 1987 and Brown, 1993). However, this phenomenon was also presented as evidence to support the existence of different species, or at least of genetically distinct stocks (Brown, 1993). As these two species are living sympatrically, the results of this study show that difference for the size at first maturity is not simply the result of geographical variation.

Breakpoint analysis revealed that the slopes of the regression lines representing the rate of relative growth in abdomen width are significantly higher in female *S. olivacea* than in female *S. paramamosain* for juvenile crabs up to the size of maturity. This does not mean, however, that *S. olivacea* reaches sexual maturity before *S. paramamosain* as the age of the crabs used could not be determined. Determination of age in crustaceans is hindered by the fact that they have an external skeleton, which is moulted during growth (Hartnoll, 1982). Thus any external marks or tags are lost during moulting. Nevertheless, two scenarios can be put forward to explain the observed difference in the size of maturity of *S.*

olivacea and *S. paramamosain* due to different growth rates. If both species reach the moult of maturity at the same age, then it is possible that female *S. paramamosain* grow faster and therefore reach a larger size than *S. olivacea* at sexual maturity. Conversely, female *S. olivacea* may be the more precocious of the two species, thus reaching sexual maturity at a smaller size than *S. paramamosain*. These two theories apply if the moult increment and/or moult frequency are different between the two species (Hartnoll, 1982).

In addition to the rate of growth, the number of moults to the pubertal moult may be different between *S. olivacea* and *S. paramamosain*. The observed difference in size at first maturity between these two species could be easily compared to the size increment for one moult stage. Mounsey (1990) found that female mud crabs, genus *Scylla* increase by as much as 30mm carapace width (CW) after moulting. Female *S. olivacea* of 90mm to 99mm CW increase by approximately 11mm in CW between moults (Ryce, 1995).

A study to compare the growth to first maturity of *S. olivacea* and *S. paramamosain* from Ban Don Bay is required to elucidate which, if any, of these factors is responsible for the difference in the size at maturity; however this would have to be carried out using captive animals, as in the study conducted by Ryce (1995).

One of the important issues surrounding the variation in size at maturity is whether it has any functional significance in preventing cross breeding between *S. olivacea* and *S. paramamosain* in Ban Don Bay. In the shore crab, *Carcinus maenas*, both the physical ability to mate and competition from other, larger males, determines the size of females chosen that result in successful mating (Reid *et al.*, 1994). However, this does not explain

why a male mud crab that comes into contact with a size compatible female of the other sympatric species does not mate successfully.

One other explanation could be that the male size at maturity might also be different between the two mud crab species under investigation. This was not examined in the present study but may be important in determining if size has a role as a pre-zygotic reproductive isolating mechanism separating the two species. The size of maturity for male *S. serrata* has been reported to be close to the size of maturity of females (Robertson and Kruger, 1994; Robertson, 1996). Even though the males could produce sperm at a relatively small size (92mm ICW), they were not able to mate successfully until they were much larger. The “eligible” male size for successful mating was also found to be boosted by competition from larger males (135mm ICW was the minimum size recorded for reproductively successful males).

Other studies have reported examples of *Scylla* species living sympatrically (*e.g.* Estampador, 1949a; Radhakrishnan and Samuel, 1982; Joel and Raj, 1983), confirming that the observed sympatry between *S. olivacea* and *S. paramamosain* in Ban Don Bay is not an isolated phenomenon. For these sympatric species to maintain their separate populations and avoid hybridisation there must be some form of reproductive isolating mechanism operating. The present study showed that there are no obvious physical constraints, or seasonal variations in female reproductive condition, to prevent interspecific mating between these two species, therefore other factors must be preventing interspecific hybridization.

The factors operating could include courtship and behavioural differences and/or habitat partitioning between *S. olivacea* and *S. paramamosain*. Prolonged courtship is common in portunids (Hartnoll, 1969). Recognition of a mate tends to be by chemical or tactile sensing in submerged species, which includes the majority of portunids (Shone, 1968). Mud crabs, however, are semi-terrestrial and therefore may also use auditory and visual stimuli indicative for crabs of the intertidal environment (Hartnoll, 1969). Sympatric species of fiddler crabs (genus *Uca*) are a good example of intertidal brachyurans in which claw waving display, and to a lesser extent claw colour and acoustic behaviour, are all used in courtship (Crane, 1975).

Behaviour and preference for different habitats within the mangrove zone have been mentioned previously by Estampador (1949a) to explain how the species of *Scylla* he proposed remain separate, but to date there has not been a proper investigation into habitat preference or the comparative courtship behaviour of sympatric species of *Scylla*. A full study of comparative courtship behaviour and habitat partitioning by the species of *Scylla* is required in order to elucidate the role of these potential pre-zygotic barriers to the maintenance of sympatric species of mud crabs.

Chapter Eight

General Discussion

8.1 Identification and speciation processes in *Scylla* species

8.1.1 Identification of *Scylla* species

Since the first description of *Cancer serratus* by Forskål in 1775 from a single specimen, there has been much confusion as to whether the genus *Scylla* is composed of more than one species. In the present study, a thorough assessment was made of mud crab samples from Southeast Asia using general descriptive taxonomy, multivariate morphometrics and allozyme electrophoresis. The results obtained agree with the most recent taxonomic revision proposed by Keenan *et al.* (1998), confirming that there are four species of *Scylla*; namely *S. serrata* Forskål, *S. tranquebarica* Fabricius, *S. olivacea* Herbst and *S. paramamosain* Estampador.

Allozyme electrophoresis was found to be the only one of the three methods chosen that could truly determine species status. Under the biological species concept, the identification of a species can be based on the presence of fixed genetic differences between two groups, indicating that there is no genetic exchange (Richardson *et al.*, 1986). Thus, fixed differences in alternate alleles provide conclusive evidence that there are four distinct species in the genus *Scylla*, with no evidence of interspecific hybridisation. However, both phenotypic methods employed (*i.e.* descriptive taxonomy and multivariate morphometrics) provided results that support the molecular findings that these are closely related species.

One phenomenon common to all three of the methods applied for species identification is that no single character was found to be diagnostic for the four species of *Scylla*; rather a combination of characters (whether genetic or morphological) are required to make any formal identification. With respect to general descriptive taxonomy, the subtle differences in spinal architecture and colour patterning between the species makes it relatively difficult to separate them, especially to the untrained eye. This is particularly a problem with preserved specimens (as was seen with the museum material in Copenhagen University) where the colour has been lost due to the preservation process. Thus initial identification using fresh specimens is recommended.

Gross identification can be used to differentiate *S. olivacea* and *S. tranquebarica* from *S. paramamosain* and *S. serrata*. However, it is difficult to further separate *S. serrata* from *S. paramamosain* as they share many of the same morphological features, as do *S. olivacea* and *S. tranquebarica*. The difficulty in discriminating between *S. tranquebarica* and *S. olivacea* is further exacerbated by their tendency to share the same niche within the mangrove zone (Keenan *et al.*, 1998), thus adding to the likelihood of incorrect identification. In these circumstances, confirmation of species status requires the use of genetic markers such as those available using allozyme electrophoresis (Richardson *et al.*, 1986).

The results of discriminant function analysis (DFA) revealed an overlap in external morphology between the four *Scylla* species. Keenan *et al.* (1998) used DFA to find those characters that discriminated between the four species of *Scylla*, which they had already identified genetically. Their plots of the first three discriminant functions also show

overlap between species, in particular between *S. serrata*, *S. tranquebarica* and *S. paramamosain*.

The external morphological characters used to distinguish between adult forms of *Scylla* species are not applicable to juveniles. This is because the distinguishing morphological characters only became well formed in adult mud crabs. Neumann (1996) also noted the late formation of distinguishable characters, such as spines, when identifying two closely related species of spiny lobster, *Maja crispata* and *M. squinado*. The use of genetic techniques has the advantage of being able to identify the genotype, which remains constant throughout the individual's life and therefore can be tested at any stage of the lifecycle (Hedgecock *et al.*, 1982). Allozyme electrophoresis is still the most cost effective method to test large sample sizes and it is especially useful in identifying closely related species (Avisé, 1994). Bryars and Adams (1999) used allozyme electrophoresis to provide evidence of a new cryptic species of the blue swimming crab, *Portunus*, in the coastal waters of South Australia.

However, there are some limitations to allozyme electrophoresis. Firstly, zoeae and megalopae stages of the crab's life cycle are too small to collect enough animal tissue from each individual to identify species status, although the use of cellulose acetate rather than starch gel requires significantly less sample tissue. Modern DNA techniques, particularly with DNA amplification using PCR techniques, can include the use of juveniles and crab larvae, and allows studies such as determining the parental origin of pre-settlement larvae, or to follow the effects of selection on post-settlement recruits to be conducted (Ovenden, 1990). Secondly, allozymes are limited in their usefulness in elucidating the phylogeny of these species, particularly when proposing a date for initial species divergence. In these

types of studies a more stringent technique, such as mtDNA sequencing, may be more appropriate.

8.1.2 Speciation of the genus *Scylla*

Such strong genetic differentiation between these morphologically similar species suggests that they have evolved from a common ancestor during an allopatric speciation event, which has resulted in the fixation of alternate alleles during a substantial period of physical isolation (Keenan *et al.*, 1998). In many locations more than one species of *Scylla* may be found living sympatrically (Radhakrishnan and Samuel, 1982, Joel and Raj, 1983, this study); however, there is no evidence to suggest that the speciation of *Scylla* has resulted from a sympatric speciation event, such as morphological specialisation to feeding resulting from habitat partitioning and/or physical reproductive barriers.

On examining the geographical distribution described for the four species of mud crab, it is observed that *S. serrata* inhabits the deeper oceanic waters of the Pacific and Indian oceans, whereas the other three species are located within the much shallower regions of the South China Sea, Java Sea and Bay of Bengal (as discussed in chapter six). This present day distribution suggests that the initial species divergence took place within Southeast Asia. Periods of rise and fall in sea level within the tropical belt during a succession of glaciation events within the Pleistocene period, has been held responsible for both the processes of extinction and generation of new marine species within the Southeast Pacific region (Palumbi, 1994).

Deep basins within the South China Sea and Java Sea would have been isolated for some considerable period of time when the sea level was reduced during the last glaciation

period to 150m below its present day level (Dobby, 1966). Thus smaller populations of *Scylla* would have remained isolated from each other for extended periods of time where the effects of genetic drift and/or selection would result in divergence between these sub-populations. The restriction of larval dispersal between these groups would have increased the degree of divergence between them. Keenan *et al.* (1998) have also suggested that during this period of reduction in sea level, significant freshwater inflows to these isolated seas would have changed the salinity considerably, selecting for those individuals tolerant of low salinity conditions. This has been concluded from the present day distribution of *S. olivacea* and *S. tranquebarica* in estuarine deltas, compared to the offshore oceanic preference of *S. serrata*. As yet there has been no evidence to support a direct link between the habitat preference of the four species and their differences in salinity tolerance. One valuable future area of investigation would be to test the environmental requirements for these species at all stages of their life cycle.

8.1.3 Reproductive isolating mechanisms among *Scylla* species

The overlap in geographical distribution of three of the four *Scylla* species suggests the South China Sea is a potential zone for introgression, yet the numbers of fixed differences between species indicates that hybridisation is not taking place in nature. This is particularly relevant to Ban Don Bay, Surat Thani Province where *Scylla paramamosain* and *Scylla olivacea* are found living sympatrically with no evidence of interspecific hybridisation between the two species. This phenomenon must result from conditions operating presently that maintain species integrity. Following the biological species concept (Mayr, 1967), potential reproductive isolating mechanisms, composed of pre- and postzygotic barriers, include a) habitat specialization; b) mate preference; c) spawning synchrony; d) physical mechanisms preventing intercourse; and e) reduced fitness and/or

viability of progeny after successful fertilization (Palumbi, 1994). It was once thought that reproductive isolating mechanisms were probably necessary for sympatric speciation (Schluter, 1996). However, it is more likely that they are the products of speciation, thus maintaining sympatry between two or more species that have accumulated adaptations to alternative niches (Nagel and Schluter, 1998).

From the results of chapter seven it was evident that there are no differences between the male reproductive structures to prevent mating between *S. olivacea* and *S. paramamosain*, and little evidence of shifts in breeding season between the female crabs of these two species. Therefore both physical barriers to mating and spawning seasonality can be ruled out as these isolating mechanisms for these two species of *Scylla* in Ban Don Bay, Surat Thani Province.

Sympatric sibling species in marine environments are often found to have distinct habitat preferences defined by depth, salinity, substratum type and/or degree of exposure (Knowlton, 1993). With *Scylla* species, it was evident from interviews with fishermen at collection sites during the present study, and from personal observation, that *S. olivacea* is found well within the mangrove zone, where it inhabits a brackishwater environment and burrows at low tide in order to prevent desiccation and to gain protection, especially when moulting. *Scylla paramamosain*, in contrast, is found to inhabit subtidal coastal areas, preferring sandy substrata and is less likely to burrow. However, the dietary items identified from the stomach contents of both species are similar, being composed mainly of estuarine fauna found throughout the mangrove fringe (Overton, personal observation). This means that both species have a large area of overlap in their niches where interspecific contact is possible.

Although there is an overlap in habitat of the four *Scylla* species, differences in the burrowing behaviour between the four species may influence mate choice. It has been suggested that mud crab burrows may provide shelter during mating (Brown, 1993). Basic descriptive studies have described *Scylla olivacea* and *Scylla tranquebarica* as predominantly burrowing species (Estampador, 1949a). Moreover, *S. olivacea* is believed to have one burrow entrance, whereas *S. tranquebarica* has two burrow entrances (Joel and Raj, 1983). *S. serrata* and *S. paramamosain*, on the other hand, are less likely to burrow (Estampador, 1949a). To date, these observations have not been researched adequately, but may lead to some evidence on the role of burrows for attracting mates of the same species.

Another prezygotic reproductive isolating mechanism is that of sexual selection. Mate preference may take different forms including size selection and sexual display. Mating in mud crabs is fairly complex, involving the male carrying the female for three to four days prior to the female moulting and copulation taking place (Arriola, 1940). This is expensive in terms of energy cost to the male crab. Secondly, there will only be a certain percentage of females receptive to mating at any one time, thus competition between males is likely to occur, as described for the shore crab, *Carcinus maenas* (Reid *et al.*, 1994).

Botton and Loveland (1992) cite several examples in the Crustacea where size is important in sexual selection, especially where there is competition between males. The results from chapter seven reveal that female *S. paramamosain* mature at a larger size than *S. olivacea*. It is therefore possible, provided male sexual maturity is also affected in the same manner, that size is important size differences inhibit interspecific mating between the two species of mud crab. Size preference has also been shown to be an important factor in preventing interspecific mating between sympatric species of sticklebacks (Nagel and Schluter, 1998).

The variation in body size of stickleback species is a highly divergent trait resulting from their adaptation to alternative niches within a freshwater reservoir. In order to maintain separation between species, size preference has subsequently become a prerequisite to successful mating. However, interspecific matings were found to occur between the smallest of the largest species and the larger of the smaller species. This could occur between *S. paramamosain* and *S. olivacea* if size preference is the only criterion for mate preference. However, it is likely that there are other additional prezygotic mechanisms that exist which collectively prevent interspecific mating taking place.

Mature *Scylla* males often have enlarged chelae (weighing up to 45 % of their total body weight; Hill, 1976). The most obvious advantage for enlarged chelae is for defence, although escape may be difficult with such a cumbersome weight to carry. Another use of the male chelae is to attract females. The colouring and patterning on the male chelae are different for the two sympatric species of *Scylla* found in Surat Thani. *S. paramamosain* has yellow claws with brown/black spots on the upper half of the chelae, whereas *S. olivacea* had red/orange claws without spotting (as described in chapter three). *Scylla* has been shown to have the ability to discriminate colour (Leggett, 1979) and therefore sexual selection through colour discrimination is a feasible hypothesis. Behavioural studies on mud crabs, such as colour preference, chemosensory and vocal communication have not been investigated, due mainly to the confusion with their taxonomy. Now that the species status has been clarified (Keenan *et al.*, 1998; this study), research on mud crab behaviour can be undertaken reliably. For example, polypheromonal systems have been already been shown to be important in insects (Cardé, 1986) and have been implicated in the maintenance of the observed sympatric subdivided population structure in different races of gammarid amphipod (Stanhope *et al.*, 1992).

Postzygotic reproductive barriers have rarely been investigated in marine species due to the difficulties of raising offspring through complex life cycles (Palumbi, 1994). As yet, there are no conclusive studies of cross-fertilization in mud crabs to show whether hybridisation is possible between *Scylla* species. Karyological studies on “*Scylla serrata*” have resulted in a range of values from $2n = 106$ (Niiyama, 1959) to $2n = 94$ (Vishnoi, 1972) (both cited by Mittal and Pahawa, 1982). One possible reason for this discrepancy is that these two researchers were working on different species of mud crab. Such differences in karyology have also been recorded in closely related species of the freshwater prawn, *Macrobrachium*, (diploid ($2n$) chromosomes numbers are 104 and 114 for *M. rosenbergii* and *M. nipponense* respectively, Wong, 1993). Now that more than one species of *Scylla* has been established, the karyotyping of the genus *Scylla* is one of the most obvious steps forward in discovering whether there are any postzygotic barriers to successful, fertile progeny.

Even if the karyology does not show evidence of postzygotic isolation barriers, then cross fertilisation experiments, such as those carried out between different penaeid shrimp (Benzie *et al.*, 1995), may reveal something about the potential for hybridisation between mud crab species. If no potential postzygotic barriers are found between *Scylla* species, then prezygotic reproductive isolating mechanisms are the sole methods of preventing hybridisation between *S. paramamosain* and *S. olivacea*. Estampador (1949b) revealed comparative differences in spermatogenesis and oogenesis for the four species of *Scylla* proposed in his studies. However, he also mentions that his studies are limited in deciphering whether interspecific hybridisation is feasible between *Scylla* species.

8.2 Intraspecific geographic variation

The results of multivariate analysis on morphometric data and genetic differentiation of allele frequencies from the present study revealed both morphological and genetic differentiation between conspecific populations of *Scylla olivacea* and *S. paramamosain* within Southeast Asia. As with many marine species, genetic stock differentiation has been presumed to be quite low, with the view that even though the life cycle for most of these marine organisms may be sedentary, some part of their life history and in most instances this is the pelagic larval phase, has the potential for large scale dispersal (Hilbish, 1996). In some cases widespread genetic homogeneity has been shown to be present in marine species, in particular fish species (Ward *et al.*, 1994). However, in marine invertebrate species, fine-scaled genetic divergence between populations are observed due to their limited dispersal capacity both as adults and as pelagic larvae, for example, the sponge-dwelling Alpheid shrimp (Duffy, 1996) or to selection after larval settlement as seen for the Barnacle, *Balanus glandula* (Hedgecock, 1982)..

The factors that influence both geographic differentiation and speciation in marine species has been extensively reviewed (*e.g.* Knowlton, 1993; Avise, 1994; Palumbi, 1994; Hilbish, 1996). Firstly, where there is a geological barrier between locations it is highly unlikely that geneflow will occur between two populations. This is most likely to be the case for *S. olivacea*, where the populations sampled were taken from both sides of the Thai/Malay peninsula which provides a substantial geological barrier to potential geneflow between these populations. As there were only two locations studied using allozyme electrophoresis, this conclusion is speculative; however further sampling along both sides of the Malay Peninsula would confirm the effectiveness of the peninsula as a geographical barrier.

Where populations are not separated by geography, differentiation between populations must be due to other, more subtle factors. In Chapter Five, the complex system of surface currents within the South China Sea was revealed, which change their direction depending on the time of year (from Dale, 1956), was mentioned. These currents will affect the dispersal and subsequent location of settlement of mud crab larvae. As the mud crabs from Surat Thani were shown to have a prolonged period of sexual maturity (chapter seven), it is possible that the location of recruitment of individuals from both species may vary throughout the year. Therefore, the genetic population structure may vary from one time of sampling to another. This may account for the unusual genetic distances revealed between conspecific populations of *S. paramamosain* sampled along the western seaboard of the South China Sea between Thai Binh (North Vietnam), Chanthaburi (Thailand) and Surat Thani (Thailand).

In addition to the stock structure identified in *Scylla olivacea* and *S. paramamosain*, low levels of heterogeneity were recorded for both species. Although not uncommon for brachyuran species (Hedgecock *et al.*, 1982), this may indicate that a high percentage of self-recruitment is taking place. The degree of self-recruitment which may take place in estuarine crustacean populations could be due to a number of factors occurring at all stages of the lifecycle, where some or all could be operating at any one time. Firstly, it has been reported that mud crabs do not travel far from their own estuarine system (Hyland *et al.*, 1984) and even though it is well recognised that the gravid females migrate seawards to spawn (Arriola, 1957; Hill, 1996). Secondly, it has been shown that crab larvae can control their position in the water column with respect to light, salinity and pressure such as that reported in the blue crab, *Callinectes sapidus* by Tankersley *et al.* (1995). This control of depth may allow retention of mud crab larvae in the same estuaries, or return them to the

same coastal habitat, as their parents after their initial dispersal offshore (Palumbi, 1994). Finally, factors within the local habitat may cause post-settlement selection to take place among juvenile crabs during recruitment. Macintosh *et al.* (1991) reported a survival rate of 0.05% of brachyuran crab larvae to megalopae stage within Klong Ngao estuary, Ranong, Thailand, indicating that selection factors, such as predation, are high. This was also suggested as an important factor in explaining the low level of genetic variation observed in adult blue crab populations compared to the larvae collected from the same locations in the Texas Gulf coast (Kordos and Burton, 1993).

Environmental factors at each location not only place selection pressures on individuals entering the population but also may influence the external morphology of mud crabs. The clinal variation observed between populations of *S. paramamosain* using multivariate analysis of the morphometric data was not mirrored in the allele frequencies of the same populations, suggesting that *S. paramamosain* exhibits phenotypic plasticity. This is a common occurrence in ectothermic animals and plants (Thorpe, 1980). In particular the latitudinal variation in climate and the regional differences in the range of food items available may affect the morphology of the mud crabs between locations. Claw morphology, in particular the range of hard-shelled dietary items, (Seed and Hughes, 1995) have been shown to be the most likely factors affecting the claw morphology of brachyuran crabs (Smith and Palmer, 1994).

In relation to mud crab biology and ecology, all the suggestions above are presently conjectural, however now that the species status of the genus *Scylla* has been established, research into the biology and ecology of each species, in particular their recruitment

processes and stock structure, would provide vital information for fisheries management and the development of mud crab culture.

8.3 Implications for the future management of mud crab fisheries and aquaculture

8.3.1 Fisheries

The mud crab is widely regarded as a high value delicacy. This attribute has resulted in a keen interest in mud crab fisheries and aquaculture in all the countries sampled within the present study. However, the desire to harvest and culture *Scylla* has resulted in already heavy exploitation of the mud crab stocks (Macintosh *et al.*, 1993). Other contributing factors confounds this situation. Firstly, most of these countries have no fisheries management policy for mud crab. Secondly, the mud crab is treated as a single species fishery. Thirdly, the gravid females are the most valuable products from the mud crab fishery, so no conservation measures exist to protect the breeding population. This is also the most sensitive part of the fishery in terms of the effects on future recruitment of mud crabs and therefore sustainability of the mud crabs stocks.

The results of the present study show that the mud crab fisheries within the locations studied in Southeast Asia are made up of predominantly two *Scylla* species, *S. olivacea* and *S. paramamosain*. In addition, it is confirmed that the genus *Scylla* is made up of four species within Southeast Asia and Australia. Up until recently, the genus *Scylla* was treated as a monospecific genus. It is well known from other studies, that closely related marine species can be misinterpreted as a single species, which in turn can confound any fisheries study or biological investigation into that species (Knowlton, 1993). Therefore, it is important to confirm which species of *Scylla* is being studied in order to gain a better

insight into the fisheries biology of correctly identified species, and to allow valid comparisons between studies, as up until now the stock composition of the Genus *Scylla* has been misrepresented.

The results in chapter five reveal low levels of genetic variation within the populations of the two species of *Scylla* sampled and low gene flow recorded between these conspecific populations, in particular *Scylla paramamosain*. Marine species have often been shown to be composed of large panmictic species with a high degree of gene flow between stocks due to life history traits that result in large population sizes, and species having large geographic ranges (Palumbi, 1994). However, a large number of marine species have been shown to reveal differentiation between conspecific populations and less geneflow between them than was first suspected (Palumbi, 1994; Hilbish, 1996).

Scylla olivacea, *S. tranquebarica*, and to a lesser degree *S. paramamosain* and *S. serrata*, are estuarine species, and therefore may prove to show more stock structure than true marine crab species. In tagging experiments, movement between populations inhabiting neighbouring mangrove creeks has been shown to be reduced where an area of unsuitable habitat for *Scylla* exists between populations (Hyland *et al.*, 1984). As more area of mangrove is converted for other uses, so the areas suitable as habitat for mud crab become more isolated, thus accentuating the barriers to geneflow between populations. It is also possible that there is a high degree of self-recruitment, even though the females are reported to migrate seawards to spawn (Hill, 1994). However, as yet little is known about the recruitment process in *Scylla* populations. The present study has recorded some differentiation between mud crab stocks, however a more thorough study covering more locations, possibly using a more sensitive techniques, such as PCR (polymerase chain

reaction) amplification microsatellite DNA loci, would give a better picture of the degree of differentiation in *Scylla* stocks between river deltas. Once the genetic differentiation of *Scylla* stocks is known, then a continuation of monitoring the degree of genetic variation could continue as a way of deciding whether some form of fishing policy is required and also assessing the effectiveness of any fisheries management policy put into practice. However, it is clear, even at this early stage of investigation, that some form of fisheries policy would be beneficial to the sustainability of mud crab fisheries within the Southeast Asian region.

One method of providing a sustainable fishery is to have a closed season for catching females when the gravid females are most abundant, thus protecting the spawning population. In Ranong, where the peak of spawning is clearly seasonal (Macintosh *et al.*, 1993), this is a feasible management measure. However the results in Chapter Seven suggest that it would be more complicated for the mud crab fishery in Surat Thani, as there is a protracted breeding season with peaks of abundance of gravid females throughout the year for both species, lending a closed season impractical as a fishery management option.

The second method of protecting the spawning females is to have a minimum legal catch size as has already been implemented as part of the Australian mud crab fishing policy (Heasman and Fielder, 1977). In Surat Thani, where two species live sympatrically, female *S. paramamosain* have a larger size of first maturity than female *S. olivacea* (as discussed in chapter seven). This makes setting a minimum catch size for mud crabs more complicated. In Queensland, Australia, where two colour morphs (now known to be two species) were identified as having different size ranges, two separate minimum catch sizes were implemented successfully (Taylor, 1984). A mangrove research project conducted in

Ranong, Thailand (UNDP/UNESCO, 1991) recommended a minimum size of 10cm carapace width of mud crabs caught in the local fishery where the levels of exploitation were deemed unsustainable. This is still a recommended policy, but to date there is still no official fisheries management policy in Thailand for mud crab (Macintosh *et al.*, 1993). This is a concern particularly with soft-shell crab farming which prefer to farm pre-adult crabs as they moult more frequently and are cheaper to purchase than mature adults. In a study of soft shell crab farming in Ranong Province, one soft-shell crab farm was calculated to purchase over nine tonnes of juvenile crabs a month (Ryce, 1995).

In a review, Brown (1993) highlighted the inconsistency in mud crab fisheries policy throughout the Indo West Pacific. In reality, both a closed season and minimum size of catch are difficult to regulate in Southeast Asia. Firstly, unlike the commercial status of crab fisheries in Australia, in Southeast Asia mud crab fishing is a subsistence activity based on using artisanal methods and the market structure is complex; thus the collection of reliable catch data is impossible. Although an individual fisherman may catch few crabs at any one time, it is the large numbers of families that are carrying out this type of fishing which leads to a widespread exploitation of mud crab. As suggested by Macintosh *et al.* (1993), any management policy requires some form of accompanying monitoring system and extension programme to the fishermen. Moreover, any management policy for protection of the mud crabs stocks must also include the conservation of the remaining mangrove areas. This was one of the principal policies within the Queensland fisheries act (Heasman and Fielder, 1977) and should also be considered in Southeast Asia.

8.3.2 Aquaculture

Table 8.1 summarises the links between the main results of this study and the management needs for the development of mud crab farming. Confirmation that the genus *Scylla* is

composed of four species represents a major step forward for future research leading to the development of mud crab culture. It is the prerogative of researchers to decide which of the four species to concentrate on as a potential aquaculture candidate, since research results can now be compared for known species.

It is now understood that in Australia most previous studies have concentrated on the local species, *S. serrata* (Cann and Shelley, 1999), while in the Philippines (Fortes, 1999), most trials have used *S. tranquebarica*. As the present study shows that *S. paramamosain* and *S. olivacea* are the most prominent species in the South China Sea, future aquaculture research in Thailand, Malaysia and Vietnam should concentrate on these two species as both have desirable qualities for aquaculture. *Scylla paramamosain* has a larger adult size and, due to its more limited tendency to burrow, is both cleaner looking and easier to harvest than *S. olivacea*. It is also the more desirable of the two species in China, reaching a much higher price than *S. olivacea*, in all size categories (Author, personal observation). Female *S. olivacea*, however, reach maturity at a smaller size and generally have higher GSI values (as seen in Chapter Seven); this species is therefore generally more desirable for marketing as "egg crab".

One of the major setbacks in crab culture has been the lack of success in producing a consistent supply of larvae and juveniles in an economically viable hatchery system (Mann *et al.*, 1999). As yet little is known about the ecology of the larval and juvenile stages. This in part has been due to the uncertainty regarding the taxonomy of the genus *Scylla*.

Table 8.1: Table linking results from this study to research and management needs for the development of mud crab farming.

Aquaculture development issue	Knowledge generated from this Study	Future research priorities
<p>Reproduction and genetics:</p>	<p>Confirmation that there are four <i>Scylla</i> species. Therefore careful selection of wild broodstock needed pertaining to the species selected for aquaculture.</p> <p>Size at maturity is smaller and the GSI higher for female <i>S. olivacea</i> than for <i>S. paramamosain</i>. Thus relative fecundities may be different.</p>	<p>Study the differences in reproductive biology between species e.g. relative fecundities, fertilization rates, egg quality in culture conditions. Identify optional conditions for spawning for selected species.</p>
<p>Seed supply:</p>	<p>In Surat Thani there is a prolonged period of gravid females. In such locations, hatcheries could supply seed all year round.</p> <p><i>S. paramamosain</i> is known to be more subtidal than <i>S. olivacea</i>, which prefers a more estuarine extistance.</p>	<p>Any seasonal differences in the quality of seed produced from wild broodstock.</p> <p>Compare differences in larval development between species. in particular their tolerance to different environmental parameters.</p>
<p>General husbandry:</p>	<p>The knowledge of four <i>Scylla</i> species means that all aspects of husbandry including, stocking densities, nutrition, disease, water quality, have to be examined for each species, where requirements may vary between species. Some differences between species are as follows,</p> <p><i>S. olivacea</i> has a smaller size at maturity than <i>S. paramamosain</i>. This could mean that <i>S. paramamosain</i> has faster growth rates or that <i>S. olivacea</i> is more precocious and has a shorter cycle to maturity. Both characteristics are desirable for aquaculture.</p> <p><i>S. paramamosain</i> is less likely to burrow than <i>S. olivacea</i>. However, some of the other species e.g. <i>S. serrata</i> is known to migrate far during the night and requires strong holding facilities to prevent escapeses.</p> <p>Burrowing makes harvesting mre difficult and damages the pond walls.</p> <p><i>S. olivacea</i> is more estuarine and may be able to withstand variable water quality better than the more subtidal species (e.g. sudden reduction in salinity due to monsoon rains).</p>	<p>Carry out comparative growth trials between species in culture conditions to determine which results in the best performance at harvest.</p> <p>Find optimum conditions for growout that prevent behaviours such as burrowing and movement out of the ponds. Moreover, selection of species may depend on which of the four species have a lesser tendency to burrow or migrate. This could be tested experimentally.</p> <p>Ecologically studies into their habitat and in particular their food preferences may aid in understanding the nutritional requirements of each species.</p>
<p>Marketing and economics:</p>	<p>Each species has its own marketing features whether its size (<i>S. serrata/tranquebarica</i>), attractiveness (<i>S. paramamosain</i>) or high GSI content (<i>S. olivacea</i>) all of which are desirable depending on the crab product being marketed.</p>	<p>Market research into the regional demands of particular crab products.</p> <p>Studies in the present market chains for mud crab. Identify which sectors of the community are employed from crab aquaculture and from that make some economic analysis as to its importance to the local community.</p>
<p>Extension of technology</p>	<p>In areas where there is a multispecies mud crab fishery, many farmers/fishermen are aware of different colour morphs of <i>Scylla</i> and the differences in the behaviour between these morphs. However, the fact that they are different species needs to be explained to them; also how the biological differences between these species may be important to the culture methods employed.</p>	<p>Encourage local opendays and workshops where researchers and farmers/fishermen may exchange experiences and ideas.</p> <p>Research different methods of improving growout facilities that can compliment the traditional methods already used for crabfarming rather than changing the methods currently used.</p> <p>Once a feasible crab hatchery is available, encourage the farmers to use hatchery produced seed rather than wild seed supplies.</p>
<p>Sustainability</p>	<p>Both <i>S. paramamosain</i> and <i>S. olivacea</i> showed population structure between locations. Thus transfers between locations of wild caught seed for stocking ponds has the potential to change the stock structure within locations, especially when a significant proportion of losses in crab culture from escapeses. Moreover, local broodstock should be used in hatchery production of crab seed.</p> <p>In locations like Surat Thani, where the fishery is composed of more than one species of <i>Scylla</i>, care must be taken in formulating a reasonable fishing policy that allows collection of crabs for fattening, growout of crabs but does not affect the sustainability of the fisheries.</p>	<p>Research into the degree of stock structure between local river systems and the degree of self recruitment taking place.</p>

Now that four species have been identified, the ecological requirements of each one can be studied, which in turn will help develop optimum culture conditions for the species selected for aquaculture.

Without proper hatchery production of seed crab, crab farmers will still rely on wild seed supplies, putting further pressure on an already overexploited fishery. Use of more advanced aquaculture techniques, such as hormone induction for controlled moulting, also have the potential once the biology of the selected mud crab species is better known. The control of the moulting process is desirable for both the production of soft-shell crab and egg crab, as well as inducing broodstock to spawn. Similarly, nutritional requirements and other factors important to successful crab husbandry can be researched for the species in question.

The protracted season of reproduction for female *S. olivacea* and *S. paramamosain* from Surat Thani suggests that there is a supply of potential broodstock all year round, unlike other locations where the peak of gravid females is more seasonal. Consequently, Surat Thani may be a good location for the establishment of a mud crab hatchery.

The degree of genetic differentiation recorded between the crabs in this study suggests that some consideration should be made to use local broodstock if restocking is to take place from hatchery produced juveniles. Presently, crabs are transported from Ranong on the west coast of Thailand to supply the crab farms in Surat Thani on the east coast. Such transfers threaten the present mud crab stock structure in Surat Thani. The possible detrimental effects of broodstock transfer for restocking programmes as has been highlighted for other aquatic species, *e.g.* Salmon stocks (Altukhov and Salmenkova,

1987). However, until a viable hatchery for mud crab is established in Thailand, it is most likely that transfers of wild caught juvenile mud crabs from Ranong to Surat Thani will continue to take place.

In summary, with careful species selection and development of sustainable aquaculture techniques, mud crab has the potential to be as attractive as Penaeid shrimp in terms of coastal aquaculture. However, some fisheries management policy is required to be in operation in order to protect the mud crab fishery from serious over exploitation, particularly since it is presently supporting the mud crab farming industry.

Chapter Nine

Conclusions and Recommendations

9.1 Conclusions

From the results obtained, the following conclusions can be made with respect to the species status, geographical variation and factors influencing reproductive isolation in populations of the genus *Scylla* collected from Southeast Asia.

1. A thorough investigation into the taxonomic status of *Scylla* using descriptive taxonomy, multivariate morphometrics and allozyme electrophoresis confirmed that there are four species within the genus, namely *S. serrata*, *S. tranquebarica*, *S. olivacea* and *S. paramamosain*.
2. Two of the four species dominated the study sites sampled, *S. paramamosain* and *S. olivacea*. Both these species were found living sympatrically in Surat Thani, Thailand.
3. For all three methods used to compare the species of *Scylla*, no single character was found that distinguished between all four species, rather a combination of characters was required to make a positive identification.

4. Both descriptive taxonomic characters and multivariate analysis of morphometric characters revealed an overlap in the morphology of the four *Scylla* species, although genetically they proved to be quite different, expressing fixed differences between species, indicating that they are distinct, but closely related species.
5. It is valuable to use fresh specimens when identifying the species of *Scylla* so that their colouration can also be used to distinguish between species. However, the meristics and architecture of spines are the only characters that can be used to identify preserved specimens, in particular the frontal lobe teeth and the spines on the chelae.
6. Discriminant function analysis (DFA) successfully discriminated between groups of male crabs from seven locations and female crabs from five locations in Southeast Asia. The data formed two discrete clusters with no detectable intermediate phenology indicated. However, there was mixed success in assigning the two phenotypes collected in the seven locations to typed species of *Scylla* proposed by Keenan *et al.* (1998). Frontal lobe width and pereopod length contributed most to the discrimination between the morphs of *Scylla* examined.
7. The morphological similarity between these species suggests that they were formed recently from a single ancestor by a succession of allopatric speciation events, most likely during the last glaciation. A subsequent overlap in distribution of these recently formed species has resulted in the sympatry observed between two or more species of *Scylla*.

8. Phylogenetic reconstruction using gene frequency data suggest that *S. serrata* is the most primitive species among the four species identified. Phylogenetic reconstruction using the outgroup rule of ancestry to code morphometric characters revealed *S. olivacea* as the primitive species. However, phylogeny based on morphological characters could equally be interpreted as showing *S. olivacea* is the most derived of all four species, exhibiting specialisation in body morphology (*e.g.* a reduction of body spines), in order to inhabit estuarine conditions (in particular the ability to burrow).
9. Both multivariate morphometrics and allozyme electrophoresis revealed differentiation between conspecific populations of *S. olivacea* and *S. paramamosain* indicating that there is some stock structure, possibly due to a combination of restricted pelagic larval distribution and post-settlement selection events.
10. The lack of evidence of genetic or morphological hybrids between the two sympatric species of *Scylla* sampled in Surat Thani (*S. olivacea* and *S. paramamosain*), Thailand indicates that reproductive isolating mechanisms are operating between them. However, examination of male gonapod morphology revealed that there are no obvious mechanical reasons to prevent interspecific matings.

11. Female *S. olivacea* and *S. paramamosain* exhibited a similar protracted seasonal pattern of sexual maturity; however three methods used to determine the size at first maturity revealed that female *S. olivacea* are significantly smaller in size at sexual maturity than *S. paramamosain* (mean internal carapace width of 83 - 118mm versus 101-139mm respectively).

9.2 Recommendations

Recommendations for further research into the biology of *Scylla* are prioritised as follows;

1. The juvenile stage of the mud crab is the most crucial stage of the life cycle both in terms of recruitment into the local fishery and as seed supply for use in aquaculture. Therefore research into characters that can be used to identify between juvenile mud crab species would be extremely valuable to ascertain what effect the fishing is having on the recruitment into the mangrove forest, as well as for local mud crab farmers to know which species are being stocked.
2. The present study indicated that there is some stock structuring between conspecific populations of both *S. olivacea* and *S. paramamosain*. Further collections of mud crab from intermediate locations, in combination with the use of molecular techniques, could provide more detailed information on the genetic relationships between different stocks and their degree of mixing. Moreover, the testing the relationship of recruiting juveniles to the present adult populations would be important in order to understand the level of self-recruitment taking place.

3. A study into the differences in salinity tolerances for the four *Scylla* species may indicate how some niche separation is occurring. This may also indicate the environmental conditions required for successful hatchery production and growout of cultured mud crab.
4. With respect to breeding biology, research into whether there are any post-zygotic reproductive isolating mechanisms would a) confirm the importance of prezygotic isolating mechanisms in preventing interspecific hybridisation; and b) reveal the potential for producing hybrids selected for desirable characters for aquaculture *e.g.* fast growth rates, large body size, high fecundity in females.
5. The present study suggests that female *S. olivacea* and *S. paramamosain* may have different rates of growth. A project to examine growth rates between the *Scylla* species in various aquaculture conditions would aid selection for the best performing species for a given culture system (ponds, pens, cages and compartments are all potential methods for rearing mud crab).

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Appendix I

Evolutionary factors affecting population genetics

Various evolutionary forces influence the structure of a population. Effects of mutation, natural selection and random genetic drift increase variability and differentiation between populations whereas geneflow (including migration) is the main opposing force; having a cohesive effect on the genetic differentiation between populations with a tendency to homogenise genetic composition among conspecific populations (Futuyma, 1986).

Apart from these forces, historical events (i.e. ice ages) forming permanent or extended but temporary geographic isolation between populations, and drastic changes in population size also influence the rate of change in genetic composition as well as the phenotypic variation of populations (Palumbi, 1994).

Mutation

Mutation is the ultimate source of genetic variation. There is the possibility of bidirectional mutation (both forwards and backward mutation). In mutation theory the “infinite alleles model” assumes that every time a mutation occurs, a new allele is created which does not already exist in a finite population (Hartl and Clark, 1989). This kind of mutation increases the potential number of alleles within the population balancing the loss of alleles that may occur due to evolutionary forces such as random genetic drift or natural selection. The hypothesis surrounding mutation-random genetic drift balance is known as the “neutrality hypothesis”. This hypothesis also suggests that many mutations have a negligible effect on the ability of organisms to survive and reproduce. The frequency of neutral alleles is not determined by the forces of natural selection, but by geneflow, and random genetic drift. This is one of the primary hypothesis in population genetics.

Selection

Evolution by means of natural selection has three assumptions, namely; a) that every species can produce more offspring than can possibly survive and/or successfully reproduce, thus causing the population to rise exponentially, b) that they vary in their ability to survive and reproduce and c) that part of the variation in the ability to survive and reproduce is hereditary. This greater ability to survive and reproduce is called fitness. Thus under natural selection those genotypes with greater fitness have greater reproductive success as a result and will be over represented with successive generations. The average fitness is very difficult to measure, as it is the complex interaction of factors that gives the individual the selective advantage. Survival, growth, condition and mating success have all been used as indicators of fitness in scientific trials. Components of fitness include;

- a) Zygotic selection: including the survival of prenatal, juvenile or adult individuals
- b) Gametic selection: where there is a differential success of gametes
- c) Sexual selection: where some genotypes mate more often than others
- d) Fecundity selection: where some genotypes produce more young than others.

Assuming that there are no other evolutionary forces acting on a population, there are three main models of selection (listed below) that may act upon the genotype or phenotype of a normally distributed population.

a) **Stabilising/balancing selection:** This is where the intermediate phenotypes are considered most fit. There is a stabilising effect around the mean where extreme phenotypes (which are also rare) are selected against. Under this kind of selection the population tends to remain in balanced polymorphism.

b) **Disruptive selection:** This is where the rarer genotypes/phenotypes are favoured over the mean genotypes/phenotypes that are intermediate in nature. This would result in the splitting of genotypes/phenotypes which between the two extremes, often thought of as the basis for allopatric speciation.

c) **Directional selection:** This is when one of the extreme phenotypes is most fit (being fixed in the population) and the other extreme phenotypes/genotypes and some of the intermediates are lost. This then shifts the mean of gene frequencies towards the advantageous phenotype/genotype.

Of these three methods of selection it is only the stabilising selection that could potentially maintain genetic variation and thus the range of genotypically controlled phenotypic variation within a population.

Where there is selection against a recessive homozygote, mutation may reintroduce the allele back into the population. This will maintain an unfavourable allele within the population at a low frequency. This is the basis of the selection mutation balancing theory.

Selection pressures may be divided into biotic (e.g. competition, food availability, parasites/diseases and predation) and abiotic (e.g. Temperature, pH, climate, substrate and salinity) factors. The formers are believed to be density dependent and the latter density independent.

Genetic drift

Changes in gene frequency can be the result of chance events. As allele frequencies do not alter in any predetermined way, the chance process of sampling is called random genetic drift (Hartl and Clark, 1989). In neutral theory, much of the molecular variation observed is neutral. Therefore much of the divergence among species and populations has been caused by genetic drift (Kimura, 1983; Nei, 1983). Genetic drift in alleles which are selectively neutral, new alleles are substituted at a constant rate by mutation which results in large numbers of formally polymorphic alleles being lost or fixed in a population over time. Thus the heterogeneity will also reduce over time. Effective population size, N_e , determines the rate at which this takes place;

$$H_T = H_t (1 - 1/2 N_e)^t$$

Where H_T and H_t are the present and founding heterozygosity levels within the population and t is the number of generations under study. Thus, in a small population the rate at which fixation or loss may take place is much more rapid than a population with a larger population size.

Geneflow (migration)

Geneflow is the transfer of genetic information between populations which is successfully introduced into the recipient populations, that is to say, they are accepted as part of the reproducing population; producing viable offspring (Hartl and Clark, 1989). Geneflow has a homogenising effect on the genetic composition between populations and introgression between them. The vector for geneflow in sexually reproducing species is via the migration of individuals between populations. The degree of differentiation between conspecific populations depends on the geneflow between them. The degree in which geneflow is successful depends on both the rate of migrant exchange and the fitness of those migrants. Under the assumptions of negligible genetic drift and selectively neutral alleles, geneflow would cause all populations involved to converge to the point that all these populations had the same allele frequencies; they effectively have become one single panmictic population whose population size is equal to the sum of all populations involved.

There are several models to describe the populations structures observed with regards to geneflow (Richardson *et al.*, 1986; Futuyma, 1989). Four of the main models are described below;

1. **Continent-island model:** This is the one way migration of individuals from a large population to a small isolated population.
2. **Island model:** This was first suggested by Wright (1931) where migration occurs at random between a group of small populations. In Wright's model there is an infinite number of populations or "islands". This model was modified to a finite number of islands by Latter (1973). This model later became to be known as the n-island model (Slatkin, 1984).
3. **Stepping stone model:** Purported by Malecot (1975), each population only received migrants from neighbouring populations.
4. **Isolation-by-distance model:** Geneflow only occurs in neighbourhoods, the likelihood of which diminishes with increasing distance between populations.

Geneflow has often been reported as being a very strong cohesive force between populations. In extreme cases geneflow is the main force in events such as recolonisation of local populations that have become extinct.

Effective population size

The genetic variation between populations can be affected directly when there is a change in effective population size. The effective population size governs the way in which the various forces described in the previous sections (mutation, selection, random genetic drift and geneflow) affect both genetic composition and the variation between populations (Avice, 1994). Not all individuals of a population contribute genetically to the next generation. However, effective population size refers to the idealised size of population that that would have the same genetic properties as observed in the real population. Effective population size (or N_e) is normally smaller than the actual population size due to the following reasons (Avice, 1994).

a) Unequal sex ratios

In organisms that have separate sexes, sometimes one sex is more prevalent than the other resulting in an unbalanced sex ratio. If a breeding population reveals a census count of N_m males and N_f females then the effective population size is represented by the equation;

$$N_e = 4N_mN_f/(N_m + N_f)$$

Unless $N_m = N_f$, the effective population size is less than the census population size, N (Avisé, 1994). In *macrobrachium* species where there is a dominant hierarchy in males, the polygamous mating behaviour or the dominant males may reduce effective population size (Chen, 1993).

b) Overlapping generations

In overlapping generations the offspring have the potential to mate with their parents, thus lowering the effective population size (Futuyma, 1986).

c) Variation in number of progeny

In a stable population with equal numbers of both sexes, it is possible for some individuals to leave more progeny than others, resulting in a large variance between families in the number of offspring surviving to reproduce. If the variance proves to be larger than the mean number of surviving offspring then the effective population size is smaller than the census size. Large variations in numbers of offspring between individuals is especially important in marine invertebrate species where fecundities are generally high (Avisé, 1994).

d) Fluctuations in population size between generations (bottlenecking)

Fluctuation in population size may be with regards to the variation in numbers of offspring produced by the adult population, resulting in an unequal genetic contribution to the next generation. The equation that supports this is;

$$N_e = \frac{4N - 4}{V_k + 2}$$

Where, N is the number of adults in the population and V_k is the variance in progeny per parent to the next generation.

There may also be a fluctuation in total population size, particularly when there is a fluctuation in population size between generations. In this instance, effective population size is represented as the harmonic mean of the population sizes recorded between generations. This is represented by the equation;

$$\frac{1}{N_e} = \frac{1}{t} \sum_{i=1}^t \frac{1}{N_i}$$

Where t is the number of generations and N_i is the population size of the i th generation. The harmonic mean tends to be closer to the lower population sizes that are being averaged; therefore the population size is lower than the actual census size. The severe reduction in population size is called a “population bottleneck” (Avisé, 1994). Both natural and artificial forces may cause bottlenecks. This includes ice ages and other

geographical changes. With respect to commercially important crustaceans, overfishing of isolated crustacean populations may have devastating effects on the effective breeding population.

Appendix II

Example of the effect of sexual dimorphism on discrimination between OTU's when both sexes are analysed together using discriminant function analysis.

Both figure 4.10 and table 4.16 illustrate the importance of separating male from female individuals in order to eliminate the effects of sexual dimorphism when conducting multivariate morphometrics. When both sexes for black and white morphs of the mud crab are analysed together, it can be observed that the first canonical variate (accounting for 95% of the total between-group dispersion) divides the twelve groups sampled into two clusters according to sex, irrespective of their phenotype. The second canonical variate (accounting for only a further 2% of total dispersion) separates the groups according to phenology.

The canonical variates outlined in table 4.16 show that the first canonical variate shows positive values for all female groups and negative values for male groups. The second canonical variate shows negative values for those groups representing the black morphology and positive values for those groups exhibiting the white phenology. This result perfectly illustrates the overriding effect of sexual dimorphism compounding the results of discriminating between taxa. However, the mud crabs are still easily discriminated on the basis of their phenotype using the second canonical variate.

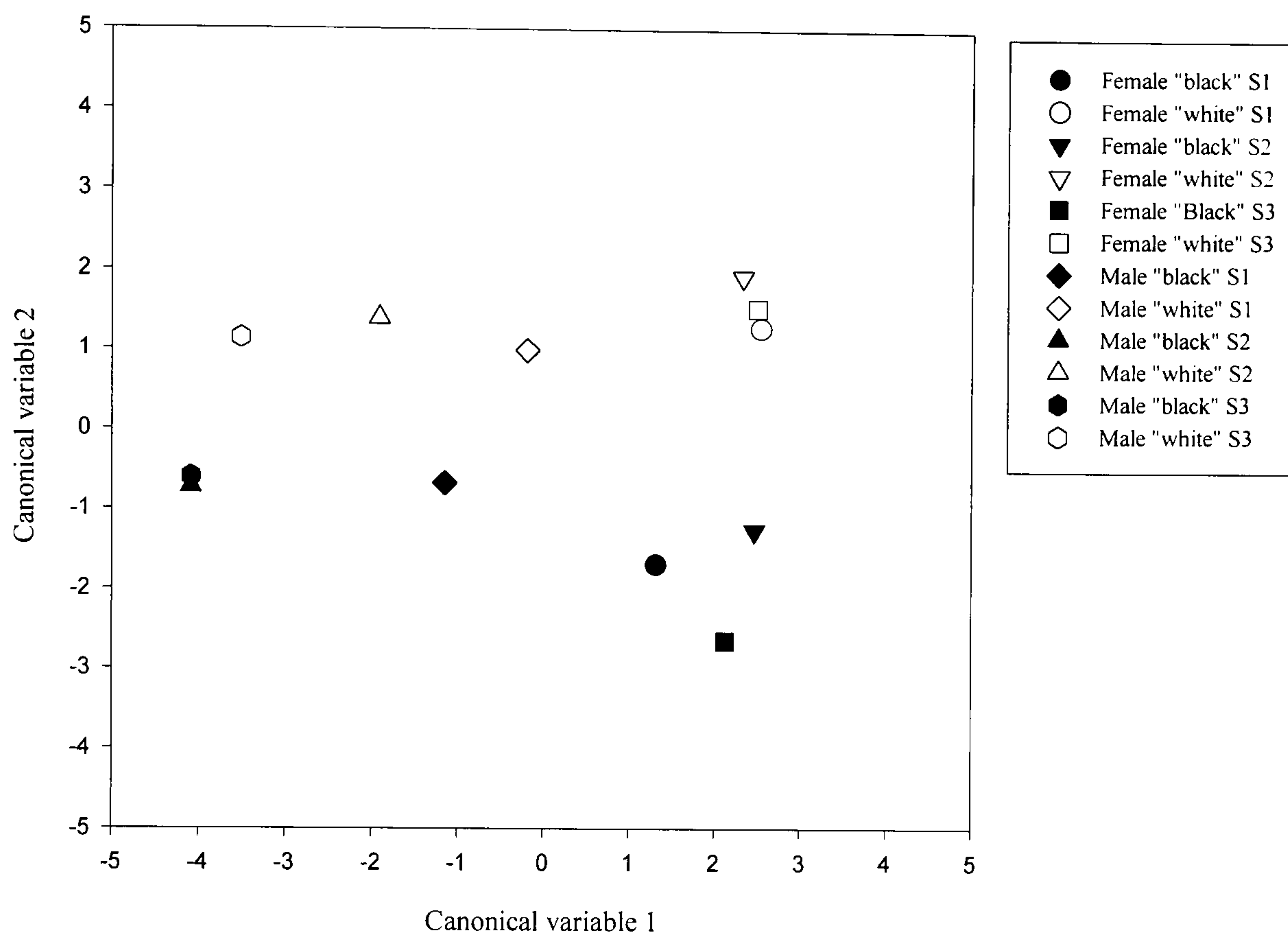


Figure 4.10: Ordination plot for the first two canonical variables for male and female crabs of both "black" and "white" morphs from Ban Don Bay, Surat Thani, Thailand.

Table 4.16: First two canonical variables evaluated from group means for male and female mud crabs of both "black" and "white" morphs from Ban Don Bay, Surat Thani, Thailand.

Group	Canonical variate	
	I	II
Female "white" S1	2.53	1.28
Female "white" S2	2.32	1.93
Female "white" S3	2.49	1.53
Female "black" S1	1.31	-1.68
Female "black" S2	2.46	-1.25
Female "black" S3	2.12	-2.64
Male "black" S1	-1.15	-0.67
Male "black" S2	-4.09	-0.73
Male "black" S3	-4.09	-0.60
Male "white" S1	-0.19	0.99
Male "white" S2	-1.91	1.39
Male "white" S3	-3.51	1.14
Cumulative percentage of total dispersion	95	97

Appendix III

Buffers and staining recipes for allozyme electrophoresis

A. Buffer recipes

1. Triethanolamine/Citric acid (TRIC), pH 7.2 (Clayton and Trefiak, 1972)

Stock solution:

0.04M Citric acid

8.4 g l⁻¹

Triethanolamine

≈20 ml l⁻¹

Adjust pH with Triethanolamine

Electrode: undiluted stock solution

Gel: 1:19 dilution of stock solution

2. Tris/Maleic acid/EDTA/MgCl/NaOH, pH8.0 (Murphy *et al.*, 1990)

Stock solution:

0.1M Tris

12.10g l⁻¹

0.1M Maleic acid

11.60g l⁻¹

0.01M EDTA(Na₂)

3.36g l⁻¹

0.01M MgCl

2.03g l⁻¹

0.12M NaOH

6.34g l⁻¹

Electrode: undiluted stock solution

Gel: 1:19 dilution of stock solution

3. Tris/Maleic acid/EDTA/MgCl/NaOH, pH7.4 (Murphy *et al.*, 1990)

Stock solution:

0.1M Tris

12.10g l⁻¹

0.1M Maleic acid

11.60g l⁻¹

0.01M EDTA(Na₂)

3.72g l⁻¹

0.01M MgCl

2.03g l⁻¹

Adjust pH with NaOH to pH 7.4

Electrode: undiluted stock solution

Gel: 1:10 dilution of stock solution

4. Tris/Citrate, pH 6.7 (TC-6.7 or Selander 4) (Pasteur *et al.*, 1988)

Electrode buffer (pH 6.3):

0.223M Tris	13.50g l ⁻¹
0.086M Citric acid	9.04g l ⁻¹
adjust to pH 6.3	

Gel buffer (pH 6.7):

0.008M Tris	0.97g l ⁻¹
0.003M Citric acid	0.63g l ⁻¹
adjust to pH 6.7	

5. Tris/Citrate, pH 8.0 (TC-8 or Selander 5) (Pasteur *et al.*, 1988)

Stock solution:

0.687M Tris	83.20g l ⁻¹
0.143M Citric acid	30.00g l ⁻¹
adjust to pH8.0	

Electrode buffer: Undiluted stock solution

Gel buffer: 1:30 dilution of stock solution

6. Tris/EDTA/Borate pH 8.6 (Pasteur *et al.*, 1988)

Stock solution:

0.9M Tris	109.00g l ⁻¹
0.5M Boric acid	30.90g l ⁻¹
0.02M EDTA (Na ₂)	7.40g l ⁻¹

Electrode buffer: 1:6 dilution of stock solution

Gel buffer: 1:19 dilution of stock solution

Adjust pH with 1M Tris or 1M Boric acid

7. Citric acid/Amino-propylmorpholine pH6.1 (CAM-6.1 or Fred 4)

Stock solution:

0.04M citric acid	8.41g l ⁻¹
0.068M Aminopropylmorpholine	≈45ml l ⁻¹
add aminopropylmorpholine to citric acid solution to adjust pH to 6.1	

Electrode buffer: undiluted stock solution

Gel buffer: 1:20 dilution of stock solution

Argenine kinase (ARGK)	EC:2.7.3.3
α -D-Glucose	40 mg
Phosphoargenine	10 mg
ADP	5 mg
Hexokinase (1000units in 0.2ml)	15 μ l
MgCl (25g/100ml)	1 ml
Glucose-6-phosphate dehydrogenase (0.1mg/ml)	25 μ l
MTT (5mg/ml)	1 ml
PMS (2mg/ml)	1 ml
NADP (10mg/ml)	1 ml
0.5M Tris/HCl, pH 8.0	25 ml
2% agar	25 ml
Aconitase (ACON)	EC:4.2.1.3
Cis-Aconitic acid	7 mg
Dissolve in 20ml 0.4M Tris/HCl, pH8.0 and adjust pH to 8.0 with 1M Tris. Then add;	
0.6M MgCl ₂	5 ml
NADP (Na ₂)	5 mg
Isocitrate dehydrogenase (20 units/ml)	100 μ l
MTT (5mg/ml)	1 ml
PMS (2mg/ml)	1 ml
2% agar	25 ml
Alkaline phosphatase (AKP)	EC:3.1.3.1
α - Naphthyl acid phosphate	50 mg
Fast blue BB salt	50 mg
0.5M MgCl ₂	1 ml
0.25M MnCl ₂	0.6 ml
0.05M Tris/NaCl/HCl, pH8.7	50 ml
Alanine aminotransferase (ALAT) - UV	EC:2.6.1.2
L-Alanine	500 mg
α -Ketoglutaric acid	40 mg
NADH	10 mg
L-Lactic dehydrogenase	200units
0.2M Tris/HCl, pH 7.5	25 ml
2% agar	25 ml

Diaphorase (DIA)	EC:1.6.2.2
NADH (Na ₂)	10 mg
2,6 Dichlorophenol-indophenol (dissolved in 2.5ml H ₂ O)	5 mg
MTT (10mg/ml)	1 ml
0.025M Tris/HCl, pH8.5	50 ml
Formaldehyde dehydrogenase (FDH)	EC:1.2.1.1
Glutathione, reduced	40 mg
NAD (10mg/ml)	1 ml
MTT (5mg/ml)	1 ml
PMS (2mg/ml)	1 ml
37% Formaldehyde	3 drops
0.2M Tris/HCl, pH 8.0	25 ml
2% agar	25 ml
Glucose-6-phosphate isomerase (GPI)	EC:5.3.1.9
Fructose-6-phosphate	25 mg
Glucose-6-phosphate dehydrogenase (0.1mg/ml)	25 μ l
MgCl ₂ (25mg/100ml)	1 ml
NADP (10mg/ml)	1 ml
MTT (10mg/ml)	1 ml
PMS (2mg/ml)	1 ml
0.06M Tris/HCl, pH 8.1	25 ml
2% agar	25 ml
Isocitrate dehydrogenase (IDH)	EC: 1.1.1.42
DL-Isocitric acid (Na salt)	50 mg
NADP (10mg/ml)	1 ml
MgCl ₂ (25g/100ml)	1 ml
NBT (10mg/ml)	1 ml
PMS (10mg/ml)	1 ml
0.5M Tris/HCl, pH 8.0	5 ml
Distilled H ₂ O	45 ml
Leucine aminopeptidase (LAP)	EC: 3.4. _._
L-Leucyl- β -naphthylamide	10 mg
Fast garnet GBC salt	25 mg
0.05M Tris/HCl, pH 7.0	50 ml

Lactate dehydrogenase (LDH) EC:1.1.1.27

1M Lactate solution, pH 7.0	10 ml
0.1M Sodium pyrophosphate	10 ml
0.005M MgCl ₂	10 ml
NAD (20mg/ml)	1 ml
MTT (10mg/ml)	1 ml
PMS (10mg/ml)	1 ml
0.5M Phosphate buffer, pH 7.4	20 ml

Malate dehydrogenase (MDH) EC:1.1.1.37

1M Malic acid	10 ml
0.1M Sodium pyrophosphate	10 ml
0.005M MgCl ₂	10 ml
NAD (20mg/ml)	1 ml
MTT (10mg/ml)	1 ml
PMS (10mg/ml)	1 ml
0.5M Phosphate buffer, pH 7.4	20 ml

Mannose-6-phosphate isomerase (MPI) EC:5.3.1.8

D-Mannose-6-phosphate	40 mg
NADP (5mg/ml)	1 ml
MgCl ₂ (25mg/ml)	1 ml
Glucose-6-phosphate dehydrogenase	10 units
Glucose phosphate isomerase	10 units
MTT (5mg/ml)	1 ml
PMS (2mg/ml)	2 ml
0.2M Tris/HCl, pH 8.0	25 ml
2% agar	25 ml

Peptidase-B (PEP-B) EC:3.4. _._

L-Amino acid oxidase	5 mg
Horseradish peroxidase	10 mg
Leucyl glycyl glycerine	20 mg
O-Dianisidine	5 mg
0.2m Tris/HCl, pH8.0	25 mg
2% agar	25 mg

Phosphoglucomutase (PGM) EC:2.7.5.1

Glucose-1-phosphate (with 1% glucose-1,6-diphosphate)	100 mg
Glucose-6-phosphate dehydrogenase (0.1mg/ml)	10 μ l
MgCl ₂ (25g/100ml)	1 ml
NADP (5mg/ml)	1 ml
MTT (5mg/ml)	1 ml
PMS (5mg/ml)	1 ml
0.06M Tris/HCl, pH 8.1	25 ml
2% agar	25 ml

Triosephosphate isomerase (TPI) UV EC:5.3.1.1

Fructose-1,6-diphosphate 50 mg
 (dissolve in 2ml of stain buffer and add 5 μ l aldolase)
 incubate at 37°C for one hour.

Then add,

NADH	20 mg
α GPDH (80 units/ml)	20 μ l
0.1M Tris/HCl, pH8.0 + 0.005M EDTA)	20 ml
2% agar	25 ml

Appendix IV

Formula for calculating expected heterozygosity (Nei's unbiased estimate, 1978).

For a single locus, the equation for an unbiased estimate of heterozygosity (h) is the following;

$$h = 2n(1 - \sum p_i^2) / (2n - 1),$$

where p_i is the frequency of the i th allele of the locus in question in a sample population. The sample size n may vary between groups.

The corresponding unbiased estimate of heterozygosity averaged over all loci (H) is as follows;

$$H = \sum h_k / r,$$

Where h_k is the value of h for the k th locus, and r the total number of loci investigated in the sample group.

In order to calculate the associated standard error (S.E.) the variance must be calculated first. The variance is calculated using the equation,

$$\text{Var} = \sum (h_k - H)^2 / r(r - 1)$$

$$\text{S.E.} = \sqrt{\text{Var}}$$

Example

Mud crab sample from Ranong ($n=42$)

Locus : Aconitase	allele frequencies are	p_{115}	= 0.1310
		p_{100}	= 0.8214
		p_{85}	= 0.0476

Therefore;

$$h_{aco} = (2 \times 42) [1 - (0.1310^2 + 0.8214^2 + 0.0476^2)] / (2 \times 42 - 1) \\ = 0.310$$

h values for the other loci are calculated in the same way, therefore the average heterozygosity for a total of 18 loci is;

$$H = (0.310 + 0.385 + 0.488 + 0.024 + 0 + 0 + \dots + 0)/18 \\ = 0.0671$$

$$\text{Variance} = [(0.310 - 0.067)^2 + (0.385 - 0.067)^2 + (0.488 - 0.067)^2 + (0.024 - 0.067)^2 + (0 - 0.067)^2 + \dots + (0 - 0.067)^2] / 18(18 - 1) \\ = 0.001314$$

Thus;

$$\text{S.E.} = \sqrt{0.001314} \\ = 0.036$$

Appendix V

Description of different methods used to determine pairwise genetic distances.

There have been various methods proposed for the conversion of allele frequency data into measures of genetic distance (Wright, 1978). The most commonly used of these are *i*) Nei's genetic distance (1972, 1978), *ii*) Rogers (1972) genetic distance, and *iii*) Cavalli-Sforza and Edwards (1967) arc distance.

i) Nei's Genetic distance (D_n) (1972, 1978)

This is still the most frequently used method of calculating genetic distance. Nei's (1972) standard equation for genetic distance is;

$$D_n = \ln [J_{XY}/\sqrt{(J_X J_Y)}] \quad (\text{Nei, 1972})$$

Where, J_X , J_Y and J_{XY} are arithmetic means across all loci for $\sum x_i^2$, $\sum y_i^2$ and $\sum x_i y_i^2$, respectively. However, it was discovered that this equation resulted in biased estimates of genetic distance when the sample sizes were small. Modification to this equation to obtain an unbiased estimate was made by replacing $\sum x_i^2$ and $\sum y_i^2$ with $(2n\sum x_i^2 - 1)/(2n_x - 1)$ and $(2n_y\sum y_i^2 - 1)/(2n_y - 1)$ respectively (Nei, 1978). The new equation assumes that the rate of gene substitution per locus is uniform across loci and lineage. This has been shown to be an unrealistic assumption, therefore Hillis (1984) recommended the following change to D_n to alleviate the problems of non-uniform rates of change.

$$D_n^k = \ln[\sum(x_i y_i / \sqrt{\sum x_i^2 \sum y_i^2}) / L], \quad L = \text{total number of loci.}$$

Nei's distances (including the modified forms shown above) are non-metric and therefore violate the triangle inequality. Farris (1981) argued that if the distance measures are non-metric then it is meaningless to fit branch lengths using an additive tree model where branch lengths are seen to be relative to rates of evolutionary change. However, Felsenstein (1984) believed that if branch lengths were interpreted as expected and not actual rates of change, then the method is viable.

ii) *Rogers distance measure (1972)*

The popularity of this method of calculating distance measure is based around its simplicity as well as being easy to interpret on a geometric basis. The equation used to calculate Rogers' distance measure is as follows;

$$D_r = (1/L)\Sigma[\sqrt{\Sigma(x_i - y_i)^2}]/2$$

It is essentially the euclidean distance between allele frequency vectors for each locus of the two taxa being compared. Roger's distance is heavily influenced by within-taxon heterozygosity - the same limiting factor experienced using Nei's genetic distance measure.

iii) *Cavalli-Sforza and Edwards' arc (chord) distances*

This is an alternative euclidean distance measure that does not have the same limitations as experienced by both Nei's and Rogers distance measures. The equation to calculate Cavalli-Sforza and Edwards arc distance measure is as follows;

$$D_{arc} = \sqrt{(1/L)\Sigma(2\theta/\pi)^2} \quad \text{where, } \theta = \cos^{-1}\Sigma\sqrt{x_i y_i}$$

This distance measure incorporates an angular transformation of genic frequencies in an attempt to make the variances of the transformed frequencies independent of the ranges in which they fall (Swofford and Olsen, 1990). This transformation standardises the distance with respect to random drift, thus the rate of increase in genetic distance under the influence of drift is nearly independent of the initial gene frequencies. The Chord distance is a relative of the Arc distance. Both incorporate some realistic assumptions about the nature of evolutionary changes in gene frequencies.

Appendix VI

Details of morphological, ecological and behavioural characters used to reconstruct the phylogeny for the genus *Scylla*. *Thalamita crenata* represents the outgroup.

Table 6.14: Colour characters for four proposed species of the genus *Scylla* and *Thalamita crenata* (the outgroup).

Character	Species				
	<i>Scylla serrata</i>	<i>Scylla olivacea</i>	<i>Scylla tranquebarica</i>	<i>Scylla paramamosain</i>	<i>Thalamita crenata</i>
1.1 Carapace colour	Green/olive green carapace	Brown/green carapace.	Green/olive green carapace.	Green/olive green carapace.	Brown/green carapace.
1.2 Chelae colour	Green dorsal propodus, carpus and merus with aqua/blue hues and reticulation. Ventral propodus is pale green.	Brown dorsal propodus, carpus and merus. Ventral propodus is orange/red. No reticulation present.	Green dorsal propodus, carpus and merus with purple olive hues on whole propodus. Some reticulation on dorsal chelae.	Green dorsal propodus, carpus and merus with prominent dark green reticulation. Lower lateral propodus is pale yellow. Vertical red flashes across dactylus and propodus.	Brown/green propodus, carpus and merus. No reticulation present.
1.3 Pereiopod colour	Green with dark green/brown reticulation.	Brown/aubergine. No reticulation.	Green with dark green/brown reticulation.	Green with dark green/brown reticulation.	Brown green. No reticulation.

Table 6.15: Morphological characters for four proposed species of the genus *Scylla* and for *Thalamita crenata* (the outgroup).

Character	Species				
	<i>Scylla serrata</i>	<i>Scylla olivacea</i>	<i>Scylla tranquebarica</i>	<i>Scylla paramamosain</i>	<i>Thalamita crenata</i>
2.1 Frontal lobe width (FLW/ICW).	0.29 (0.371)	0.40 (0.415)	0.31 (0.412)	0.33 (0.377)	Very broad frontal lobe compared to carapace width (no value).
2.2 Frontal lobe teeth shape (FMSH/FLW)	Elongated, acute teeth with blunt tips (0.061).	Flattened obtuse teeth. All teeth are equal in size (0.029).	Flattened obtuse teeth. All teeth are equal in size (0.043).	Triangular, pointed, sharp but small teeth (0.058).	Flattened, rounded teeth (no value).
3. Anterolateral spine shape.	9 spines. All spines are equal in size and shape. Acute spines with large interspaces.	9 spines. Posterior (ninth spine) more protruded than other spines. All spines sharp.	9 spines. All spines are equal in size and shape. Flat, obtuse triangular spines.	9 spines. Short angular sharp spines.	4-5 spines decreasing in size from anterior to posterior. Short angular sharp spines.
4. Number and architecture of chelae dorso-antero propodus spines	Two spines. The inner spine more pronounced than the outer one.	One to two spines. Both vestigial. Often the outer spine is absent.	Two spines. The inner spine more pronounced than the outer one.	Two spines. The inner spine is more pronounced than the outer one.	One large inner spine proximal to dactyl articulation. The outer spine is vestigial or in some cases absent.
5. Number and architecture of chelae ventral carpal spines.	Two prominent spines. (0.940)	One vestigial spine. The anterior spine almost always absent. Sometimes both are absent. (0.006)	Two prominent spines. (0.980)	One to two spines. The anterior spine is vestigial or absent on some individuals. (0.352)	Two small spines.

Table 6.16: Habitat and biogeography of the four proposed species of the genus *Scylla* and for *Thalamita crenata* (the outgroup).

Character	Species				
	<i>Scylla serrata</i>	<i>Scylla olivacea</i>	<i>Scylla tranquebarica</i>	<i>Scylla paramamosain</i>	<i>Thalamita crenata</i>
5. Habitat	Associated with mangrove forests inundated with full salinity oceanic water for greater part of the year. Can tolerate reduced salinity.	Associated with mangrove forests and coastline inundated with reduced salinity seawater during the wet season.	Associated with mangrove forests and coastlines inundated with reduced salinity seawater for part of the year. Berried females found within estuaries.	Associated with shallow denuded coral in Singapore; shallow subtidal ponds and flats in central Java, and in mangrove forests in the lower Mekong delta, Vietnam.	
6. Biogeography	Red sea, South Africa, Mauritius, Western Australia, Indonesia, Fiji, Solomon Islands, New Caledonia, Western Samoa, Panay Islands, Philippines, Okinawa, Southern Taiwan.	Found in Indian ocean, Pacific Ocean South China Sea and Arafura Sea.	Found in South China Sea and specific locations around the Indo-Pacific	Found along continental coast of South China Sea south to the Java Sea.	

Appendix VII

Abbreviations and acronyms

<u>Abbreviation/acronym</u>	<u>Full title</u>
BOBP	Bay of Bengal Project
BSC	Biological Species Concept
CP	Concordance Principles
CV	Covariant
DANCED	Danish Cooperation for Environment and Development
DFA	Discriminant Function Analysis
DRC	Danish Red Cross
CPUE	Catch per Unit Effort
CVA	Canonical Variate Analysis
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
GSI	Gonadosomatic Index
ICW	Internal Carapace Width
MGPCA	Multiple Group Principle Components Analysis
MYR	Malaysian Ringgit (currency)
PCA	Principle Components Analysis
PCDA	Principle Co-ordinates Analysis
PSC	Phylogenetic Species Concept
RIM	Reproductive Isolating Mechanism
THB	Thai Baht (currency)
TCEP	Tropical Coastal Ecosystems Project
UNESCO-MAB	United Nations Education, Science and Culture Organisation – Man and Biosphere Programme
UPGMA	Unweighted Pair Group Method with Arithmetic Averages
USD	American dollars
VND	Vietnamese Dong (currency)