Marine Biology (1997) 128: 55–62

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Multivariate analysis of the mud crab *Scylla serrata* (Brachyura: Portunidae) from four locations in Southeast Asia

Received: 2 October 1996 / Accepted: 5 December 1996

Abstract The large, edible mud crab *Scylla serrata* (Forskål) exhibits different phenotypes which are recognised by the fishermen of Southeast Asia and are given local names such as "white", "green", "red" or "black" crabs on the basis of their colouration. A preliminary study using multivariate techniques was undertaken in order to examine the degree of dissimilarity between the different morphs of commercially fished stocks of *S. serrata.* Twenty-two morphometric and 20 meristic characters were measured on male crabs from four locations in three countries in Southeast Asia, including Surat Thani in Thailand where "black" and "white" morphs exist sympatrically. Canonical variate analysis (CVA) on the morphometric data discriminated three groups with no chain-linking. Surat Thani "white" crabs and those from south Vietnam were phenotypically similar and formed one distinct group; a second group contained crabs from Ranong (Thailand) and Sarawak which were phenotypically "black"; the third group contained "black" crabs from Surat Thani. CVA on meristic data confirmed two groupings, but implied that the Surat Thani "black" morph may only be a variant of the Surat Thani/Vietnam "white" form, rather than a third species. These findings are discussed in relation to the ecology, fishery management and aquaculture potenial of *S. serrata.*

Communicated by J.P. Thorpe, Port Erin

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Introduction

Scylla serrata (Forskål) is the only species of the family Portunidae that is closely associated with mangrove environments. Commonly known as the mangrove crab or mud crab, *S. serrata* is distributed throughout the Indo-Pacific region mainly within tropical latitudes, but it can also be found in more temperate environments as far north as China and Japan (Macnae 1968).

The wild catch of *Scylla* is an estimated 10 000 tons annually (BOBP 1992) and contributes significantly to the coastal fisheries of many developing countries in Asia, e.g. Bangladesh, India, Sri Lanka, Indonesia, Thailand, Vietnam and the Philippines (Ferdouse 1990; Liong 1993); there is also a small but commercially valuable *S. serrata* fishery in Australia producing an annual catch of about 600 tons (Lee 1992).

There is a high consumer demand for *Scylla serrata*, particularly in countries with Chinese communities such as Malaysia, Singapore, Taiwan and Hong Kong (Liong 1993), where it is regarded as a delicacy, especially the gravid female or "egg crab" (Chen 1990); the male is also appreciated for its large chelae and high meat content (Harvey 1990). Because of this popularity, *S. serrata* has been subjected to heavy, unregulated exploitation, except in Australia which does have a fishing policy for mud crab (Heasman and Fielder 1977). Many fishing communities in Southeast Asia use unselective fishing gear and heavily exploit the available broodstock, including the gravid females. As a result of this high fishing pressure, the average size of mud crab caught in Southeast Asian countries appears to be decreasing (Macintosh 1982; Harvey 1990; Overton personal observations 1994). However, any efforts to manage the crab fisheries are hindered due to the lack of information on the population dynamics of *S. serrata* in Southeast Asia (Macintosh et al. 1993; Tan and Ng 1994).

The present impoverished status of *Scylla serrata* stocks in Southeast Asia is also compounded by other factors. These include habitat destruction due to extensive mangrove clearance; declining quality of the coastal environment (Hong and San 1993; Liong 1993) and a recent increasing interest in soft-shell crab farming in addition to the more conventional culture methods (i.e. rearing crabs in ponds for one to several months). Since there is still no commercial-scale hatchery production of *S. serrata*, all forms of mud crab culture depend on an already limited natural seed supply (Harvey 1990; Liong 1993).

Progress towards effective stock management of *Scylla serrata*, and the development of hatchery and growout techniques for crab farming, are constrained by many factors, including the uncertain taxonomic status of "*S. serrata*". Many reports have described more than one variety of *S. serrata* in local populations; for example, different colour and size forms have been recognised by fishermen and dealers in Malaysia, Thailand, India, the Philippines and Australia (Estampador 1949; Macintosh 1982; Radhakrishnan and Samuel 1982; BOBP 1992; Lee 1992). Use of common names such as black crab, golden-backed crab and green crab (Ferdouse 1990; Harvey 1990) demonstrates that there is a wide variation in the morphology of *S. serrata*. Furthermore in some markets certain morphs of *S. serrata* command a higher price than others; for example "boo khao" or white crab in Thailand is consistently more valuable than the black variety or "boo dum" (Thongkum 1988; Overton personal observation 1996).

This variation in the phenotype of the mud crab has led to much debate with respect to its taxonomy (Alcock 1899; Estampador 1949; Serene 1952; Stephenson and Campbell 1959; Kathirval and Srinivasagam 1992). The taxonomy of *Scylla serrata* continues to be confusing and has been further complicated by more recent descriptions based on general morphological features (Radhakrishnan and Samuel 1982; Joel and Raj 1983). Moreover, these studies have been carried out on a local basis, without taking into account the crab's morphological degree of variation on a wider geographical scale.

This paper reports on the results of multivariate analysis of morphometric and meristic data for *Scylla* collected from four commercial crab-fishing sites in Southeast Asia.

Materials and methods

Study sites

Scylla serrata (Forskål) were collected from four locations in Southeast Asia where there are significant crab landings by local fishermen: Klong Ngao in Ranong Province (9°53′ to 9°57′ N; 98°31′ to 98°37′ E : Site A), southwest Thailand; Ban Don Bay in Surat Thani Province (9°11′ to 9°21′ N; 99°14′ to 99°40′ E : Site B), western Gulf of Thailand; Can Gio near Ho Chi Minh City, south Vietnam (10°22′ N; 107°15′ E : Site C); Sematan in Sarawak, East Malaysia (1°51′ N; 109°47′ E : Site D). These locations are illustrated in Fig. 1. The sites were chosen for their degree of geographical separation as well for their importance as mud crab fishing-areas. All four study areas were located in coastal mangroves colonising fine alluvial sediments. The Ranong, Can Gio and Sematan sites are extensive mangrove delta formations with many interconnecting creeks. Surat Thani has more coastal development, but the crab fishery is centred around Ban Don Bay which still retains fringing mangroves. Care was taken to ensure that the crabs were of local origin by actually going on crab-collecting trips with the local people. The crabs were collected principally with small baited traps or gill nets.

Crab collection and measurement

In order to eliminate the effects of variation due to sexual dimorphism, only male crabs were used in this study. Thirty males were collected from each site except for Surat Thani (Site B), where two morphs are common; at this site, an extra 20 crabs were collected of the second morph type. This provided five groups of crab for measurement. To minimise ontogenic influences on body shape, the crabs collected were approximately the same size (200 g) and on dissection were noted to have mature testes. Only healthy individuals were selected, with all limbs intact.

Twenty-two morphometric characters were measured on each individual to the nearest 0.01 mm using digital callipers (Mitutoyo Absolute Digimatic, Tokyo). These characters were measured from the carapace, abdomen, both chelae and pereiopods from the right side of the crab, as illustrated in Fig. 2. Twenty meristic characters were also recorded from each individual. These included spination along the anterolateral carapace and the spination and dentition from both chelipeds (Fig. 3).

Data analysis

Morphometric data

The 22 characters measured on each crab from the five groups were analysed by canonical variate analysis (CVA) using the computer software BMDP-7M (BMDP Statistical Software Inc., Cork, Ireland).

To remove the effect of "size", MGPCA (multiple group principle-components analysis); (Thorpe 1988) was used to discover the possible size vector involved (in this instance the first vector for all 22 characters). Comparable "size in" and "size out" CVA analyses were run; in the former, the MGPCA size vector is included while in the latter it is excluded. Comparison of these analyses indicate the influence of "size" on the relationships among groups.

Meristic data

The meristic data were also analysed using CVA. In order to confirm that the CVA had not been perturbed by heteroscedasticity, principal component/co-ordinate analysis (PCA) was performed on the group means for all the characters (Thorpe 1980). Values from the first two component scores (normalised vectors from PCA) and scaled eigenvectors from principal co-ordinate analysis (PCDA) were then plotted in order to confirm the position of the group centroids from the CVA ordination plot.

Results

Morphometric analysis

Canonical variate analysis on the raw, untransformed data for the five *Scylla serrata* groups indicated three main clusters. This is illustrated in the scatter plot of the first two canonical variates shown in Fig. 4a. Individuals from Ranong (Site A) and Sarawak (Site D) were shown to be very similar in morphology, and phenotypically

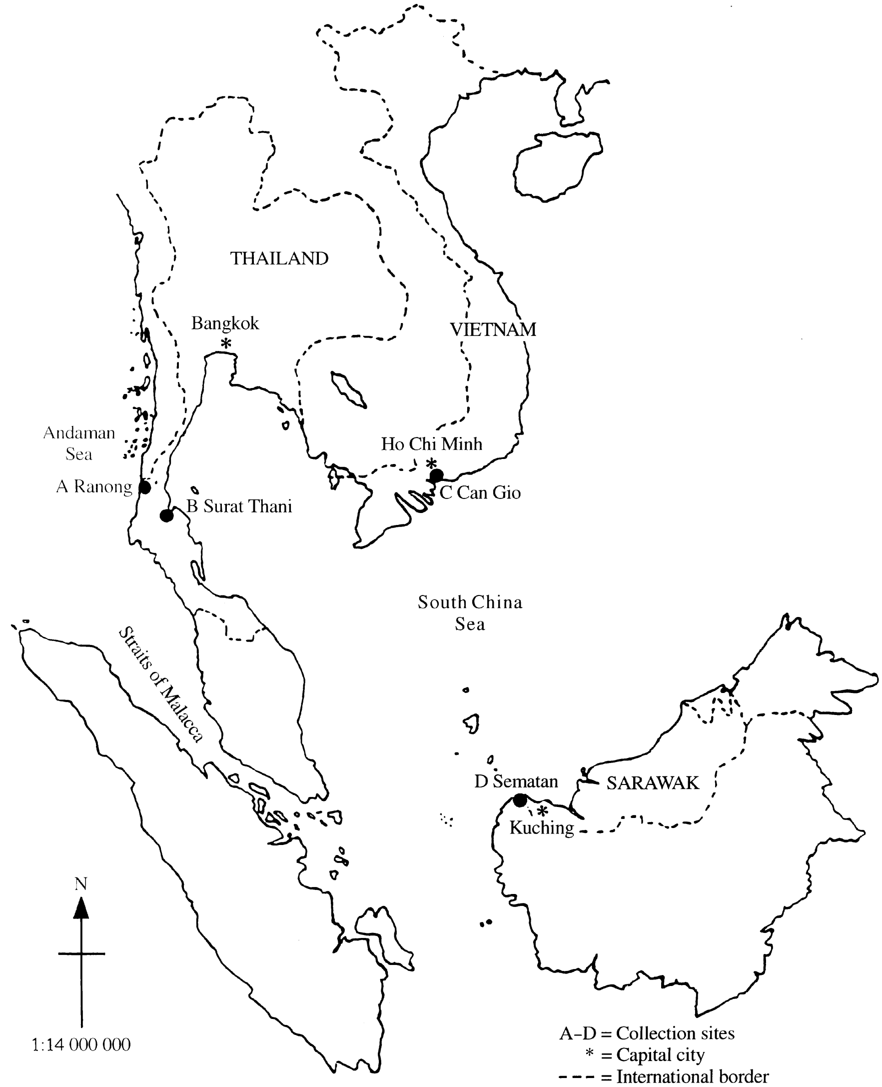


Fig. 1 *Scylla serrata*. Locations of crab collection sites in Southeast Asia

were designated as the "black" type. One of the two Surat Thani (Site B) morphs and the samples from Vietnam (Site C) also formed a discrete cluster and were designated as the "white" type. The other morph from Surat Thani physically resembled the Ranong and Sarawak individuals but formed a separate, third cluster, with its centroid equidistant between the other two clusters (Fig. 4a). This group exhibited a larger degree of scatter between the individuals indicating more within-group variance than illustrated by the other four groups. No chain-linking was discovered between the three clusters even though one of the individuals from Sarawak was reclassified as belonging to the Surat Thani "black" morph group and vice versa.

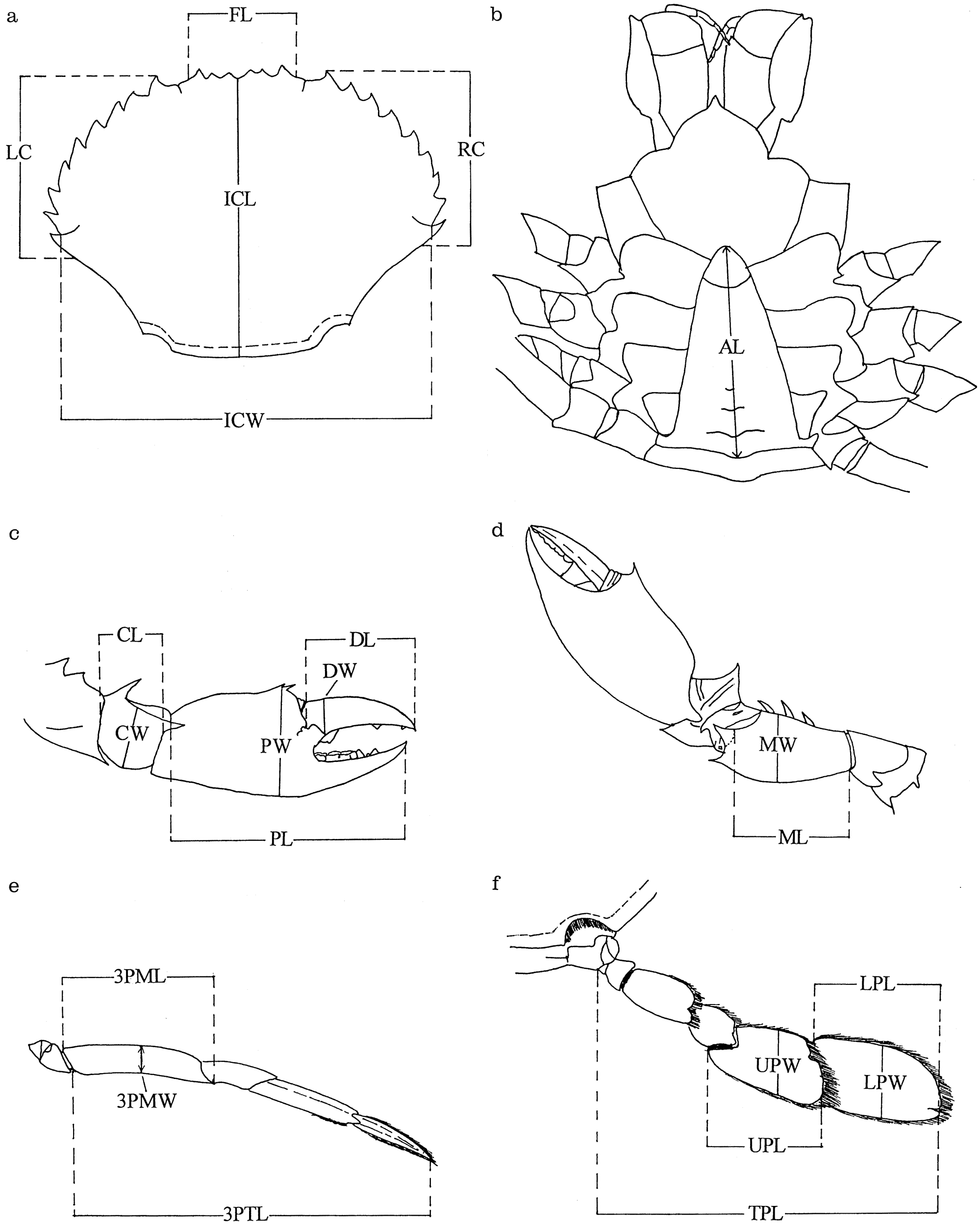
CVA on the MGPCA scores showed that 30% of the between-group variance was attributable to the sixteenth MGPCA vector. This revealed that the main characters contributing to the variance were: frontal length (between the orbital sockets); right and left antero-lateral lengths of the carapace; right dactylus width, right propodus length; right and left carpus lengths, and right merus width. The mean, range and standard deviation for these characters is presented in Table 1. The "size" character, i.e. carapace width, has been included to show the uniformity of size ranges between the five groups.

The MGPCA analysis also revealed the first eigenvector to be the size vector, where each coefficient had a similar sign and magnitude. When this vector was omitted to form the "size out" analysis, it was found that the ordinance plot (Fig. 4b) was tantamount to that of the "size in" plot, indicating that growth-dependent size was not having an effect on the racial expression of the five groups studied.

Meristic analysis

The resultant ordinance plot derived from the meristic data on the same five groups illustrated a different pattern from that of the morphometric data. The scatter formed two clusters rather than the three suggested by the morphometric data (Fig. 4c). The Ranong and

Fig. 2 *Scylla serrata*. Illustration of 22 characters forming data for multivariate analysis. **a** carapace; **b** abdomen; **c** anterior view of cheliped, **d** posterior view of cheliped (both right and left chelipeds measured); **e** third right pereiopod; **f** fifth right pereiopod (*AL* abdominal length; *CL* carpus length; *CW* carpus width; *DL* dactyl length; *DW* dactyl width; *FL* frontal length; *ICL* internal carapace length; *ICW* internal carapace width; *LC* left anterolateral length of carapace; *LPL* lower paddle length; *LPW* lower paddle width; *ML* merus width; *MW* merus width; *PL* propodus length; *3PML* third pereiopod merus length; *3PMW* third pereiopod merus width; *3PTL* third pereiopod total length; *PW* propodus width; *RC* right anterolateral length of carapace; *TPL* total length of swimming leg; *UPL* upper paddle length; *UPW* upper paddle width)



Sarawak groups formed the same pattern as before, but the Surat Thani "white" morph and the individuals from Vietnam had a larger within-group variance than in the morphometric analysis.

Canonical variate analysis on the meristic data also indicated a high level of congruence between the Surat Thani "black" morph and the Surat Thani white/Vietnam group, implying that the "black" morph collected from Surat Thani could be a variant of the "white" type rather than a third morph/species. Principal component/co-ordinate analysis on the character means plotted for all five groups produced the same result as the CVA analysis, thus reinforcing the two morph/species theory.

Discussion

Multivariate analysis of morphometric characters has been shown to be a rapid and effective technique in providing an insight into the discrimination of many animal species, including various types of Crustacea; moreover, the hard, well-defined body parts of crustaceans facilitate the collection of accurate data. For example, multivariate analysis of morphometric data was used to show interspecific variation between two previously undescribed species of *Procambarus* spp. crayfish discovered in a Mexican cave, confirming them as two sympatric species (Allegrucci et al. 1992). Similar studies revealed evidence of two species from four colour forms of *Liopetrolisthes mitra*, a porcellanid commensal crab

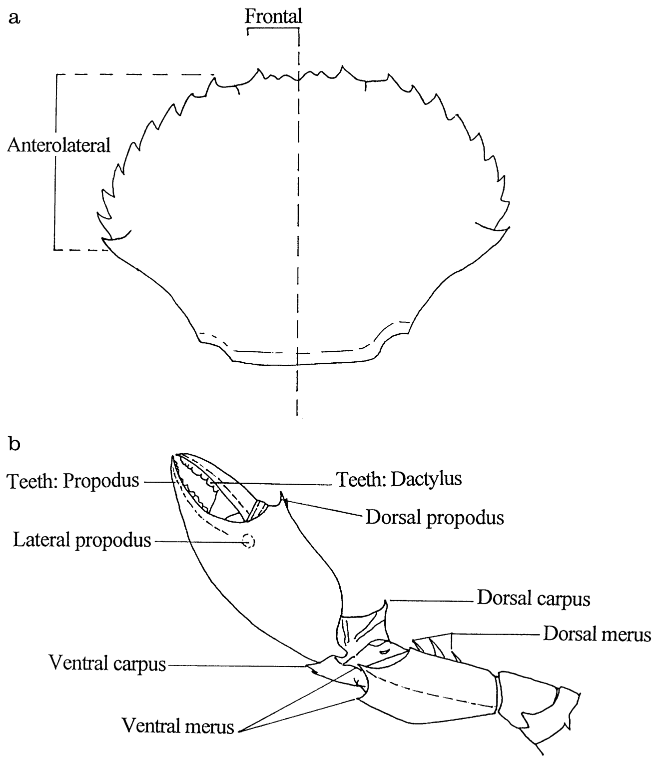


Fig. 3 *Scylla serrata.* Details of spines and dentition (teeth) on carapace (**a**) and both right and left chelipeds (**b**) forming meristic data in multivariate analysis

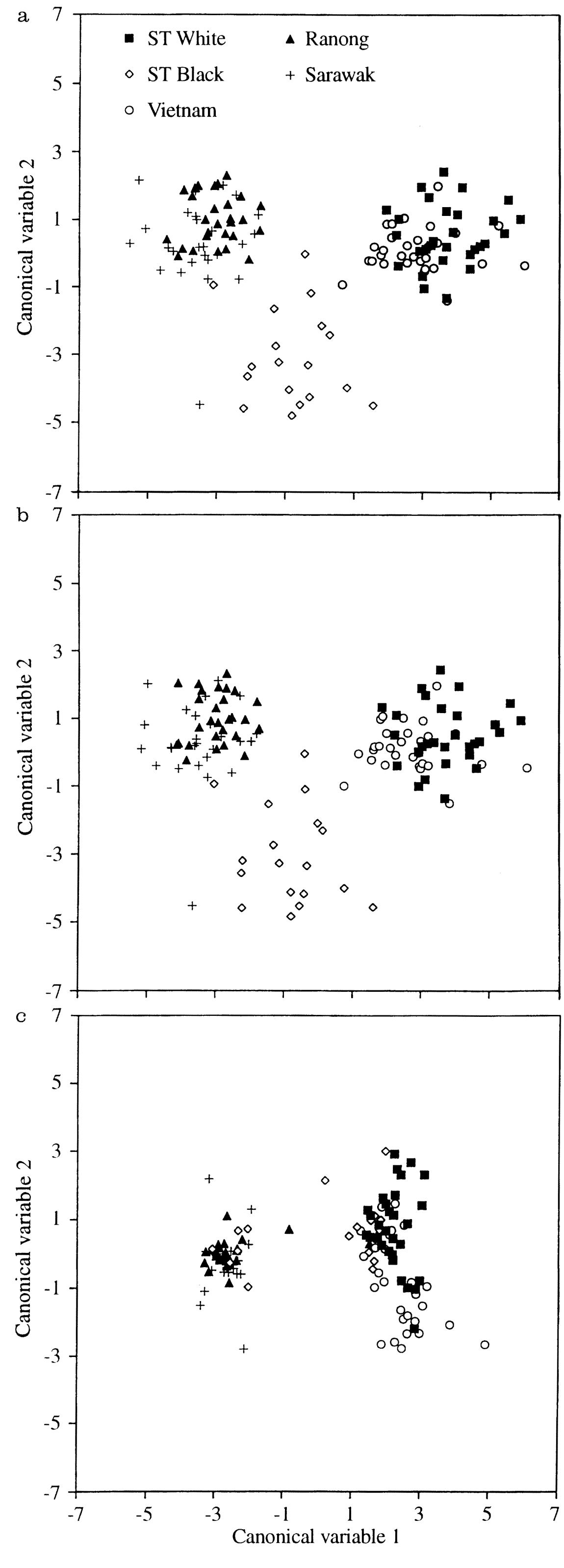


Fig. 4 *Scylla serrata.* Plots of first two canonical variables from populations collected from four sites in Southeast Asia: Ranong, Thailand (Site A), Surat Thani, Thailand (*ST* Site B), Can Gio, Vietnam (Site C), and Sematan, Sarawak (Site D). Shown are canonical variate analyses on morphometric data (**a**), on multiple-group principle-component analysis (MGPCA) scores with size vector removed (**b**), and on meristic data (**c**)

(Weber and Galleguillos 1991), but the insignificance of racial variation between different populations of the mitten crab *Eriocheir* spp., from Southern China (Li et al. 1993).

Canonical variate analysis has often been stated as being a powerful multivariate technique in the identification of unknown species (Pimentel 1979; Quicke 1993). Although there is evidence that CVA may give misleading results if the data do not conform to the mathematical assumptions (i.e. that there is homoscedasticity between the within-group covariance matrices), the ordination of the population under analysis can be checked by using principal component analysis/principal co-ordinates analysis (PCA/PCOA; Thorpe 1983). Moreover, MGPCA on the raw data collected for *Scylla serrata* indicated that "size" was not affecting the results.

The results show that CVA can discriminate between different groups of *Scylla serrata* regardless of their geographical origin. All 22 morphometric characters were shown to have some contribution to this variance, however, larger contributions were attributed to the frontal length (the measurement between the two orbital sockets), the right and left antero-lateral lengths, and a significant proportion of the characters from the right chelae. In a previous study, Chayarat and Kaew-ridh (1984) demonstrated, using regression analysis, that frontal length is wider in the "white" morph than in other morphs from Ranong which they described as "red" and "green".

Table 1 *Scylla serrata*. Means, standard deviations (*SD*) and ranges for morphometric characters contributing significantly to between-group variance using canonical variate analysis on multiple-group principle-component analysis scores for five groups of mud crab collected from four locations in Southeast Asia (*ICW* internal carapace width; *FL* frontal length; *RC* right anterolateral length of carapace; *LC* left anterolateral length of carapace; *RDW* right dactylus width; *RPL* right propodus length; *RCL* right carpus length; *LCL* left carpus length; *RMW* right merus width; *ST* Surat Thani)

Group	Character								
	ICW	FL	RC	LC	RDW	RPL	RCL	LCL	RMW
Thailand									
ST white									
mean	96.92	29.29	45.02	45.35	10.13	61.59	26.34	26.12	21.23
(range)	(90.67–104.26)	(25.71–31.34)	(41.42–49.00)	(42.21–50.31)	(7.06–13.44)	(45.79–67.88)	(20.23–28.59)	(22.58–29.58)	(16.19–23.09)
SD	±4.12	±1.46	±2.04	±2.04	±1.12	±4.47	±1.86	±1.55	±1.56
ST black									
mean	96.00	31.82	44.03	44.17	9.64	61.50	25.91	26.86	21.56
(range)	(88.93–104.96)	(28.40–43.56)	(40.48–48.55)	(40.02–49.72)	(7.56–12.95)	(54.85–70.71)	(20.12–32.66)	(22.01–29.82)	(18.33–23.93)
SD	±5.03	±1.90	±2.44	±2.83	±1.57	±4.89	±3.37	±2.27	±1.59
Ranong									
mean	93.32	33.13	44.75	43.67	12.45	69.98	31.18	28.90	24.36
(range)	(87.01–104.91)	(29.46–37.61)	(40.32–49.38)	(40.44–48.51)	(8.08–18.25)	(57.39–84.50)	(27.03–35.43)	(24.18–35.23)	(20.39–28.74)
SD	±8.01	±1.89	±2.51	±2.38	±2.23	±7.52	±2.91	±3.23	±2.19
Vietnam									
mean	94.68	28.86	43.64	44.50	10.03	60.68	25.70	25.12	20.60
(range)	(82.18–102.47)	(25.10–33.29)	(38.74–48.01)	(38.46–47.97)	(7.47–12.05)	(50.77–70.67)	(21.18–28.65)	(21.83–28.44)	(17.32–23.27)
SD	±6.27	±1.96	±3.00	±3.04	±1.27	±5.52	±2.05	±1.99	±1.52
Sarawak									
mean	97.05	33.19	45.09	44.89	11.00	67.84	29.62	28.83	23.69
(range)	(82.48–114.38)	(28.14–37.70)	(38.10–54.10)	(37.69–48.88)	(7.68–16.81)	(53.16–92.17)	(22.80–40.04)	(22.59–38.52)	(19.18–28.58)
SD	±8.21	±2.41	±3.99	±3.93	±2.27	±10.26	±4.27	±4.60	±2.88

The discrimination due to the measurements of the right chelae indicates a variance due to "handedness". The group means of these characters suggest that the right chelae is enlarged in the "black" morph type whereas the chelae of the "white" morph were found to be bilaterally symmetrical. In a study of "handedness" in the brachyuran crab *Cancer productus*, phenotypic plasticity due to environmental induction was demonstrated whereby claw size was found to increase in order to eat hard-shelled diets (e.g. molluscs). There is a suggestion that bilaterally symmetrical species develop a behavioural preference for one chelae, allowing it to increase in size in such a manner that the induction of this slight asymmetry becomes inheritable (Smith and Palmer 1994). Thus, behavioural stimulation of handedness, especially with respect to dietary preferences, merits closer examination in *Scylla serrata.*

Based on the measured characteristics, crabs from the four locations show three discrete groupings with no evidence of chain-linking. The "white" morph from Surat Thani and the crabs originating from Vietnam formed their own group, which was consistent with their phenotypic likeness. Similarly, the Ranong and Sarawak groups also formed their own cluster, while the third grouping comprised of "black" morph crabs from Surat Thani.

Even though this analysis indicates that groups of *Scylla serrata* can be discriminated racially (irrespective of geographical distribution), it is hard to know how much of this observed variation is due to direct environmental induction (phenomic plasticity) and what is genetically controlled. Heterogeneous coastal environments would be expected to have a significant influence on the phenotypic expression of *S. serrata*; however two well discriminated morphs were found in the same location (Surat Thani), suggesting that there is more than environmental induction influencing the expression of these two apparently sympatric morphs. Environmental temperature is one of the main variables known to affect morphometric features. The four locations have only a 9° latitudinal range, and all lie within a warm equatorial region where the dominant climatic feature in the coastal belt is the monsoons rather than temperature fluctuation. The average monthly temperatures at Ranong and Can Gio, the most northerly sites, each vary by <4 C°. Moreover, the two sites furthest away from each other, i.e. Ranong and Sematan, formed their own separate group on the basis of morphology even though they were the most geographically distant sites.

Different morphs of *Scylla serrata* have been described as inhabiting different niches within the mangrove coastal zone. The "white" type has been described as being more subtidal and less likely to burrow than the "black" type (Estampador 1949). However, in the case of Surat Thani the two morphs occupy the same geographical area (Ban Don Bay), suggesting other barriers to prevent interspecific matings. Segregation of morphs has been noted in Pulicat Lake by Joel and Raj (1983) where tandems were observed between male and female *S. serrata* of the same morph type.

The separation of the Surat Thani "black" morph into a third group was surprising, as phenotypically it resembled the populations collected from Ranong and Sarawak that were also of the "black" type. It could be that the CVA discriminated between the predetermined groups that are actually part of a cline. Thorpe (1983) indicated that because CVA can discriminate between predetermined categories, it does not mean that the variation is absolute. A more extensive sampling programme around the eastern seaboard of Thailand may help answer this question.

The meristic analysis conducted in this experiment provides evidence for only two distinct morphs and not three as illustrated by CVA on the morphometric data. Meristically, the "black" morph from Surat Thani is closely associated with the "white" Surat Thani group and the group collected from Vietnam. Most of the between-group variance underlying the discrimination is attributable to the nodule found on the inner lateral propodus. However meristics seem to be a weak form of identification between species of Crustacea where spination and dentition on the chelae are the paramount characters used. Ranges of spines have been indicated for many species of Brachyura (e.g. Crane 1975). Spines are often broken or are subject to ontogenic variation, and increase in number as the animal gets larger or older. Dentition is often worn down with usage, which makes it difficult to count the teeth with any certainty.

Obviously morphometric data cannot be expected to give all the answers alone. The fact that phenotypic expression is controlled by many external factors, e.g. diet and habitat, indicates the need for an understanding of the level of endogenous intervention in the observed phenotypic variation of *Scylla serrata*. The taxonomy and biology of *S. serrata* merits further study, since the presence of more than one species or morph has major implications for both the fisheries management and aquaculture development of this crab. In addition, there is a growing risk of genetic contamination of *S. serrata* stocks [already a problem for certain other farmed aquatic species, e.g. salmon (Altukhov and Salmenkova 1987)], due to the large-scale transportation of live mud crabs not only from one area to another to support aquaculture, but also between consumer and producer countries (Ferdouse 1990; BOBP 1992).

Acknowledgements This study was funded by Contract No. TS3-CT92-1052 awarded to the University of Stirling by the European Community Science and Technology for Development Programme (STD-3). We are also grateful to the Natural Environment Research Council (NERC) for scholarship funding to J.L Overton. We particularly wish to thank staff of the Royal Thai Forestry Department and Department of Fisheries, Thailand; Forest Department of the Ho Chi Minh City Agricultural Service, Vietnam; and the Department of Agriculture, Sarawak; for their kind assistance during the fieldwork in Southeast Asia.

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