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Classification of Three Crab Morphs in the Genus *Scylla* using Morphometric Analysis

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ABSTRACT

One hundred and fifty-four male mud crabs in the genus *Scylla* were collected from different coastal areas of the Gulf of Thailand and the Andaman Sea, including Trat, Ranong and Surat Thani Provinces. They were classified into three morphs, "black", "white" and "violet" based on their phenotypes and body colors. Fifty-one morphometric characters were observed and used for canonical variate analysis (CVA) and Mahalanobis' Distance analysis. CVA results showed completely separation of three morph clusters with true correlation to its phenotypes and without overlapping among different clusters. Morphological differences among crab morphs were confirmed by considerably high value of Mahalanobis' Distance in the range of 53.283 – 60.806 but those within morphs were in the range of 6.922 – 15.027. Both CVA and Mahalanobis' Distance as well as their morphological data suggested that these three crab morphs of "black" "white" and "violet" should be classified into three distinct species of *S. olivacea, S. paramamosain* and *S. tranquebarica*, respectively.

Key words: *Scylla*, mud crab, Canonical Variate Analysis, Mahalanobis' Distance analysis, phenetic tree

INTRODUCTION

Mud crabs in the genus *Scylla* inhabit brackish water and are widely distributed in Indo-West Pacific regions (Ng, 1998). In Thailand, these crabs are found both at the Gulf of Thailand and the Andaman Sea coast. Considering the fishery areas and economic value, mud crabs are very important fishery resources but recently over fishing of mud crab resulted in the drastic decline of its population (Tiensongrusmee and Pratoomchat, 2002).

Attempts have been made to prepare basic data for mud crab conservation and stock management. Pongthana *et al*. (2004) utilized CVA, Mahalanobis' Distance analysis and amplified fragment length polymorphism (AFLP) to determine morphological and genetic variation within "black" morph (*Scylla olivacea*), which collected from Trat, Ranong and Surat Thani Provinces of Thailand and also some in Myanmar. Both morphological and genetic data suggested Ranong and Myanmar "black" was closest group and Trat "black" was closely related to Ranong/ Myanmar "black" group more than Surat Thani "black". This pattern of relationship within "black" indicated that geographic distance and geographic barrier play a minor role in crab identification and grouping because Trat was far from South-West (Ranong and Myanmar) more than South-East

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(Surat Thani). Moreover, Trat and South-West area were separated by south of Thailand terrain. This result, however, based on only "black" morph but did not include the other two groups of "white" and "green" as described by Tiensongrusmee and Pratoomchat (2002).

The classification of mud crab in the genus *Scylla* in Thailand has been controversial and inconclusive for a long time. Suvatti (1950) has reported only one species, *Scylla serrata*, found in Thailand but Naiyanetr (1998) indicated the existence of two species of mud crabs, *S. serrata* and *S. tranquebarica*, in this country. Keenan *et al.* (1998), on the other hand, collected mud crab specimens from the Red Sea and throughout the Indo-Pacific region, six from all these specimens were from Thailand, and classified the crabs into four species, *S. serrata*, *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. However, Tiensongrusmee and Pratoomchat (2002) based their report on external morphology and suggested that there should be only three species of the genus *Scylla*, *S. serrata*, *S. oceanica* and *S. tranquebarica*, in Thailand.

Although most previous taxonomic reports of mud crabs in Thailand were based on external morphology, a few reports have applied statistical and molecular analysis to identify variations among crab morphs in the genus *Scylla* but most of the specimens were selectively collected from the eastern of Thailand (Chayarat and Kaew-ridh, 1984; Klinbunga *et al.,* 2000). Therefore, wide sampling areas covering their natural habitats would be an effective approach to alleviate the taxonomic confusion of mud crabs in Thailand. The aims of this paper were to assess variation of morphometric characters among crab morphs using CVA and Mahalanobis' Distance analysis, and to elucidate the distinctions and pattern of relationship among and within crab morphs. These results could lead to a more reliable classification of the mud crabs in the genus *Scylla* which based on morphometric analysis.

MATERIALS AND METHODS

Crab sampling areas

Three different phenotypes of mud crabs locally called "Poo Dum", "Poo Thong-Lang" and "Poo Thong-Long" (Tiensongrusmee and Pratoomchat, 2002) were assigned based on their body colors to "black", white" and "green" morph (which assigned as "violet" in this study), respectively (Table 1). One hundred and fifty-four mature male mud crabs (60 "black", 58 "white" and 36 "violet") of approximate 200 g body weight were directly purchased from local crab fishermen in each coastal area of Trat (eastern), Ranong (southwest) and Surat Thani (western gulf) Provinces, representing the diverse and true geographic origins of mud crab populations in Thailand (Table 1).

Morphometric characters and data analysis

Fifty-one morphometric characters (Table 2) including nineteen characters on carapace, eleven characters on each chelae, three characters on the third right periopod, five characters on right swimming leg, body depth and abdominal length, were measured to the nearest 0.02 mm using calipers (Mitutoyo Co., Japan). The data were used for canonical variate analysis (CVA) and Mahalanobis' Distance analysis. CVA was performed to classify crab sample based on quantitative variables (morphometric characters). In brief, CVA creates new variables, calling canonical variable (Z), by using special linear combination of the original variables. Therefore, canonical variables represent all of the discriminated data from a set of original variables. The linear discriminant function of CVA are derived from a linear equation,

$$Z = a_1x_1 + a_2x_2 + a_3x_3 + \ldots + a_px_p,$$

where Z is a canonical variable; $a_1, a_2, a_3 \ldots, a_p$ are the set of canonical discriminant function coefficients; $x_1, x_2, x_3, \ldots, x_p$ are the set of original variables. The numbers of canonical variables are (k-1), where k is the number of sample groups. First two canonical variables (CVA1 and CVA2) of each sample were plotted onto canonical axes to display differences among sample groups (Jobson, 1992). CVA analyses were analyzed using SPSS for windows version 12.0 statistical software (SPSS Inc., Chicago).

Two levels of variations were examined for mud crab classification. At the first level, CVA was calculated using morphometric data of crab specimens, which identified as “black”, “white” or “violet” morph, to indicate variation among morphs. For the second level, “black”, “white” and “violet” morph were further classified based on their geographic origins to eight morph-geographic groups, i.e., Trat “black”, Trat “white”, Trat “ violet”; Ranong “black”, Ranong “white”, Ranong “violet”; Surat Thani “black” and Surat Thani “ white” group (no “violet” was found in Surat Thani). Their morphometric data were also analyzed by CVA to display the variation within crab morphs.

Distances among crab groups were calculated using Mahalanobis’ Distance analysis and STATISTICA version 6.0 (StatSoft, Inc., USA). Phenetic trees were constructed by Unweighted Pair-Group Method Arithmetic Average (UPGMA) using NEIGHBOR program in PHYLIP package version 3.63 (Felsenstein, 2004).

RESULTS AND DISCUSSIONS

CVA of morphometric characters

Morphometric character variations among three crab morphs were determined. Statistical analyses including means, standard deviations (SD) and range of fifty-one morphometric characters are shown in Table 3. Measured data of each mud crab specimen were used as raw data for CVA. The results illustrated the significant difference among three crab morphs (Wilks’ lambda = 0.016, $F_{(32, 272)}$ = 59.644; P <

Table 1 Morph characteristics, number of specimen and sampling locations.

Morphs	Body colors	Number of specimens	Sampling areas	Number of specimens from each sampling site
Black	Carapace: black or dark brown	60	Trat	Trat “black” (*N* = 20)
	Lateral site of propodus: reddish shade		Ranong	Ranong “black” (*N* = 20)
			Surat Thani	Surat Thani “black” (*N* = 20)
White	Carapace: light green	58	Trat	Trat “white” (*N* = 20)
	Lateral site of propodus: dark green		Ranong	Ranong “white” (*N* = 18)
	spots on yellowish green ground color		Surat Thani	Surat Thani “white” (*N* = 20)
Violet[a,b]	Carapace: green	36	Trat	Trat “violet” (*N* = 20)
	Lateral side of propodus: purplish shade		Ranong	Ranong “violet” (*N* = 16)

[a] “Violet” morph was synonymous with “Green” morph used by Chayarat and Kaew-ridh (1984), Klinbunga *et al.* (2000) and Tiensongrusmee and Pratoomchat (2002). “Violet” term emphasized a distinct color purplish shade on lateral side of propodus.

[b] “Violet” morph was not found in Surat Thani.

Table 2 Fifty-one morphometric characters of crab specimens used for CVA and Mahalanobis' Distance determination.

NO.	Morphometric characters	Abbreviation	NO.	Morphometric characters	Abbreviation
1	Frontal median spine height	FMSH	27	Left carpus length	LCL
2	Distance between frontal median spines	DFMS	28	Right carpus length	RCL
3	Distance between left-pair frontal spines	DLPFS	29	Left carpus width	LCW
4	Distance between right-pair frontal spines	DRPFS	30	Right carpus width	RCW
5	Distance between left frontal spine and left orbit	DLFSorb	31	Left dactyl length	LDL
6	Distance between right frontal spine and right orbit	DRFSorb	32	Right dactyl length	RDL
7	Internal carapace width	ICW	33	Left merus length	LML
8	External carapace width	ECW	34	Right merus length	RML
9	Carapace width at spine 8	CW8	35	Left merus width	LMW
10	Total left cheliped length	TLCL	36	Right merus width	RMW
11	Total right cheliped length	TRCL	37	Left propodus width	LPW
12	Left propodus length	LPL	38	Right propodus width	RPW
13	Right propodus length	RPL	39	Third periopod merus length	3PML
14	Third periopod total length	3PTL	40	Third periopod merus width	3PMW
15	Total length of swimming leg	TPL	41	Lower paddle length	LoPL
16	Abdominal length	AL	42	Lower paddle width	LoPW
17	Frontal width	FW	42	Upper paddle width	UpPW
18	Internal carapace length	ICL	44	Upper paddle length	UpPL
19	Left carapace length	LC	45	Body depth	BD
20	Right carapace length	RC	46	Frontal margin	FM
21	Left dactyl length	LDW	47	Carapace width at spine 1	CW1
22	Right dactyl length	RDW	48	Distance between left and right frontal spine	DLRFS
23	Left pollex length	LPoL	49	Left orbit width	LOW
24	Right pollex length	RPoL	50	Right orbit width	ROW
25	Left pollex width	LPoW	51	Carapace width at spine 2	CW2
26	Right pollex width	RPoW			

Table 3 Mean, standard deviation (SD) and range of analyzed morphometric characters of crab specimens.

Morphometric character	Black (n_1=60)		White (n_2=58)		Violet (n_3=36)		Total (n=154)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
FMSH[a,b]	1.22±0.19	0.86-1.72	2.18±0.43	1.42-3.78	1.95±0.27	1.46-2.66	1.75±0.54	0.86-3.78
DFMS[a,b]	5.24±0.37	4.52-6.48	5.91±0.68	4.72-7.94	5.69±0.55	4.84-6.92	5.60±0.62	4.52-7.94
DLPFS	5.99±0.40	5.08-6.96	5.52±0.48	4.46-6.84	5.59±0.47	4.40-7.00	5.72±0.49	4.40-7.00
DRPFS	6.05±0.44	4.88-7.04	5.56±0.52	4.72-6.88	5.65±0.48	4.84-7.10	5.77±0.53	4.72-7.10
DLFSOrb	5.91±0.44	5.12-7.16	5.65±0.46	4.82-7.08	5.81±0.54	5.00-7.08	5.79±0.48	4.82-7.16
DRFSOrb	5.94±0.44	5.00-7.04	5.71±0.49	5.02-7.24	5.91±0.45	5.14-6.90	5.84±0.47	5.00-7.24
LPL[a]	71.62±7.17	57.94-86.60	72.17±10.10	57.00-100.40	71.03±8.67	57.38-91.44	71.69±8.67	57.00-100.40
RPL	75.22±7.94	62.84-95.98	73.31±11.40	52.04-106.64	74.12±8.91	60.04-95.42	74.25±9.57	52.04-106.64
3PTL	93.82±5.60	83.40-108.42	94.44±9.68	78.02-124.84	96.69±7.82	83.76-116.38	94.72±7.89	78.02-124.84
TPL	84.61±4.72	73.10-95.60	86.80±8.90	73.50-118.48	84.84±6.97	72.84-100.28	85.49±7.09	72.84-118.48
AL[b]	37.29±1.77	32.50-41.18	38.66±3.16	33.54-48.42	38.71±2.91	34.04-45.44	38.14±2.71	32.50-48.42
FW[b]	43.00±2.00	38.14-48.32	41.17±3.17	37.00-49.82	43.75±2.97	39.30-50.84	42.49±2.91	37.00-50.84
ICL	71.66±3.27	63.12-79.40	72.82±6.11	63.98-90.46	73.32±5.58	64.22-86.88	72.48±5.06	63.12-90.46
LC	47.73±2.57	42.80-54.20	50.17±4.88	44.58-64.54	50.19±4.07	43.46-60.64	49.22±4.08	42.80-64.54
RC	48.29±4.70	42.60-78.02	50.27±4.90	42.24-64.60	50.19±3.97	43.60-60.54	49.48±4.69	42.24-78.02
LDW[a]	10.86±1.33	8.14-13.52	11.62±1.87	8.26-16.68	11.57±1.87	8.72-16.56	11.31±1.70	8.14-16.68
RDW[a]	12.88±1.80	8.54-17.14	12.56±2.39	9.08-19.62	12.53±1.99	9.22-17.66	12.68±2.08	8.54-19.62
LPoL	33.43±3.84	27.62-49.04	32.60±4.54	25.22-46.48	32.29±4.17	25.16-41.70	32.85±4.19	25.16-49.04
RPoL	33.66±3.42	28.26-41.64	32.84±4.99	25.26-49.16	34.02±4.25	26.96-44.66	33.44±4.26	25.26-49.16
LPoW	14.42±2.16	10.88-18.86	14.37±2.57	10.30-20.14	14.23±2.64	10.46-20.64	14.36±2.42	10.30-20.64
RPoW	15.61±2.19	11.56-20.62	15.14±3.08	10.48-24.00	15.13±2.62	11.04-22.54	15.32±2.65	10.48-24.00
LCL	32.33±2.82	26.60-39.42	31.61±3.81	26.68-43.84	31.08±3.41	25.24-38.18	31.77±3.38	25.24-43.84
RCL[a,b]	33.41±2.96	28.00-40.56	32.40±4.13	25.64-45.52	31.57±3.35	26.18-39.72	32.60±3.58	25.64-45.52
LCW	22.87±2.09	19.10-28.46	22.01±2.54	17.74-30.00	21.93±2.14	17.84-26.74	22.33±2.31	17.74-30.00
RCW[a]	23.64±2.25	19.24-29.10	22.45±2.82	18.16-30.64	22.46±2.39	18.22-28.24	22.92±2.56	18.16-30.64
LDL	35.62±3.28	30.10-43.46	34.41±5.32	13.80-48.68	34.92±3.99	29.14-45.00	35.00±4.32	13.80-48.68
RDL[a,b]	35.58±3.17	30.34-44.08	34.75±5.22	16.10-50.20	35.88±4.04	28.98-45.68	35.34±4.24	16.10-50.20
LML	45.55±3.79	38.74-55.28	44.86±5.43	37.14-61.22	45.29±4.40	38.56-54.78	45.23±4.59	37.14-61.22
RML	46.21±3.80	40.70-56.06	45.90±6.61	37.06-70.76	46.37±6.48	38.88-73.78	46.13±5.61	37.06-73.78
LMW	25.33±2.13	18.90-30.92	24.17±2.69	19.82-31.32	24.47±2.19	19.82-29.54	24.69±2.41	18.90-31.32
RMW	25.95±2.13	21.68-31.82	24.42±2.76	20.20-32.42	24.73±2.14	21.82-29.92	25.09±2.47	20.20-32.42
LPW	32.67±4.50	24.00-41.36	33.37±5.83	23.66-48.88	32.79±5.56	24.40-46.28	32.96±5.26	23.66-48.88
RPW[a,b]	36.48±5.19	26.10-46.68	35.87±6.83	24.00-54.82	35.22±5.66	25.14-49.88	35.95±5.95	24.00-54.82
3PML	35.64±2.11	31.80-42.64	36.09±4.03	29.66-48.02	36.57±3.36	28.02-44.52	36.02±3.24	28.02-48.02
3PMW	11.33±0.62	10.00-13.06	11.63±1.07	10.30-14.60	11.65±0.88	10.40-13.84	11.52±0.88	10.00-14.60
LoPL[a]	33.25±1.88	29.48-39.46	33.92±3.78	27.08-48.14	32.59±2.72	27.76-38.68	33.35±2.94	27.08-48.14
LoPW	16.25±1.12	14.02-18.68	16.88±1.77	14.14-21.88	16.68±1.95	13.96-25.34	16.58±1.61	13.96-25.34
UpPW	16.41±1.05	14.16-19.00	16.93±2.04	11.56-22.06	16.55±1.46	13.86-19.76	16.64±1.59	11.56-22.06
UpPL[a,b]	25.77±1.43	22.92-29.92	27.13±2.85	22.46-35.80	26.50±2.32	22.78-33.34	26.46±2.32	22.46-35.80
BD[a,b]	42.05±2.15	36.68-47.24	42.55±4.34	27.72-54.24	40.89±3.21	36.48-48.96	41.97±3.40	27.72-54.24
FM[a,b]	28.71±1.44	25.68-32.52	27.48±2.15	24.72-34.36	28.39±1.96	25.66-33.34	28.17±1.92	24.72-34.36
CW1[a]	56.68±2.52	50.68-63.80	53.76±4.45	47.92-66.22	57.83±3.96	51.38-67.82	55.85±4.03	47.92-67.82
DLRFS	17.35±0.99	14.36-19.42	16.98±1.43	14.74-20.88	17.17±1.24	14.88-20.94	17.17±1.23	14.36-20.94
LOW	14.33±0.75	12.46-16.12	13.41±1.30	10.52-17.06	14.93±1.13	13.06-17.40	14.12±1.23	10.52-17.40
ROW	14.22±0.76	12.34-16.16	13.37±1.27	10.90-17.44	14.93±1.12	13.24-17.52	14.07±1.22	10.90-17.52
CW2	66.95±4.97	59.54-96.66	65.29±5.52	57.94-80.76	69.37±4.74	62.58-81.08	66.89±5.33	57.94-96.66
ICW[a]	102.50±4.73	96.24-113.86	105.32±9.58	94.42-133.50	105.00±7.97	91.88-124.82	104.15±7.69	91.88-133.50
ECW[a,b]	106.71±5.34	94.28-120.10	110.23±9.59	99.46-140.84	111.91±8.29	97.96-132.50	109.25±8.10	94.28-140.84
CW8	107.02±5.19	94.32-119.14	108.91±9.83	94.36-140.28	110.29±8.22	96.04-130.90	108.50±7.97	94.32-140.28
TLCL	129.85±10.70	111.24-153.32	129.22±15.90	106.36-180.18	130.05±13.38	108.58-159.28	129.66±13.39	106.36-180.18
TRCL	132.78±10.82	114.66-156.96	131.12±16.46	105.50-184.50	133.01±14.03	112.16-165.58	132.21±13.85	105.50-184.50

[a] indicated informative characters for morph discrimination.
[b] indicated informative characters for morph-geographic group discrimination.

0.001). CVA revealed that 16 characters, including 6 characters on carapace (FMSH, DFMS, ICW, ECW, FM and CW1), 7 characters on chelae (LPL, LDW, RDW, RCL, RCW, RDL and RPW) and 2 characters on the third right periopod (LoPL and UpPL) and body depth (BD) were informative in differentiating crab morphs. Two canonical variables which contributed to 59.9 and 40.1% of variance were plotted onto canonical axes (Figure 1a). Plotting pattern showed complete separation of crab specimens into three clusters, i.e., 60 "black", 58 "white" and 36 "violet" crabs. These clusters were grouped without overlapping between them which indicated the great variation of morphometric character among crab morphs, making CVA an effective tool for crab species classification. Creech (1992) applied CVA to investigate different morphometric characters between big-scale sand smelt fishes, *Atherina boyeri* and *A. presbyter*, known to be synonymous species, and found them to be distinctively two separate species. Moreover, Parnaby (2002) could identify a new species of long-eared bat, *Nyctophilus nebulosus*, using external morphology together with CVA data to differentiate it from other two related species, *N. bifax* and *N. gouldi*. In the present study, discrete clusters of "black", "white" and "violet" morph strongly suggested that these three crab morphs should be classified into three different species.

CVA derived from morphometric data of eight morph-geographic groups, on the other hand, showed significant difference among groups (Wilks' lambda = 0.009, $F_{(77, 822.412)} = 12.778$; $P < 0.001$). Eleven characters including 5 characters on carapace (FMSH, DFMS, ECW, FW, and FM), 3 characters on chelae (RCL, RDL and RPW), UpPL on swimming leg, body depth (BD) and abdominal length (AL), were informative characters in this aspect. First two canonical variables contributing to 59.9 and 30.2% which added up to 90.1% of variance were plotted onto canonical axes (Figure 1b) and gave somewhat more scattering pattern.

The scattering pattern of CVA from eight morph-geographic groups showed complete separation among different crab morphs which were collected from the same sampling area. Although it also presented slightly geographic variations within each morph which represented by different positions of group centroids but the same morph specimens still formed three clusters of "black" (Trat/Ranong/Surat Thani "black"), "white" (Trat/Ranong/ Surat Thani "white"), and "violet" (Trat/Ranong "violet"), with no inclination to form sampling-area clusters. This strongly confirmed the three-cluster formation of CVA based on morphology alone. Moreover, it also suggested that each sampling area could support the co-inhabitance of more than one sympatric mud crab species. This data indicated species diversity of mud crab in each geographic location and, therefore, could caution the researchers from assuming having one crab species at a specific area. Lack of sampling-area clusters suggested that environmental influence of each sampling area did not produce environmental-specific characters. Our result disagreed with the observation of Stephenson and Campbell (1960) who assigned four varieties of mud crabs from Australia as one species and suggested that the morphological differences of mud crabs were produced by environmental differences (Hiroshi, 1999).

Overton *et al.* (1997), on the other hand, applied CVA on two mud crab morphs, "black" and "white", from four areas in Southeast Asia and found that Surat Thani/Can Gio (Vietnam) "white" group and Ranong/Sarawak "black" group were two distinct groups. Surprisingly, CVA on morphometric data separated Surat Thani "black" group from other two similar phenotypic groups (Ranong/Sarawak "black"), but it was implied as a variant of the "white" group based on meristic data (number of spines and teeth on carapace and both chelaes). The authors, however, suspected that this unexpected result could be due also to the

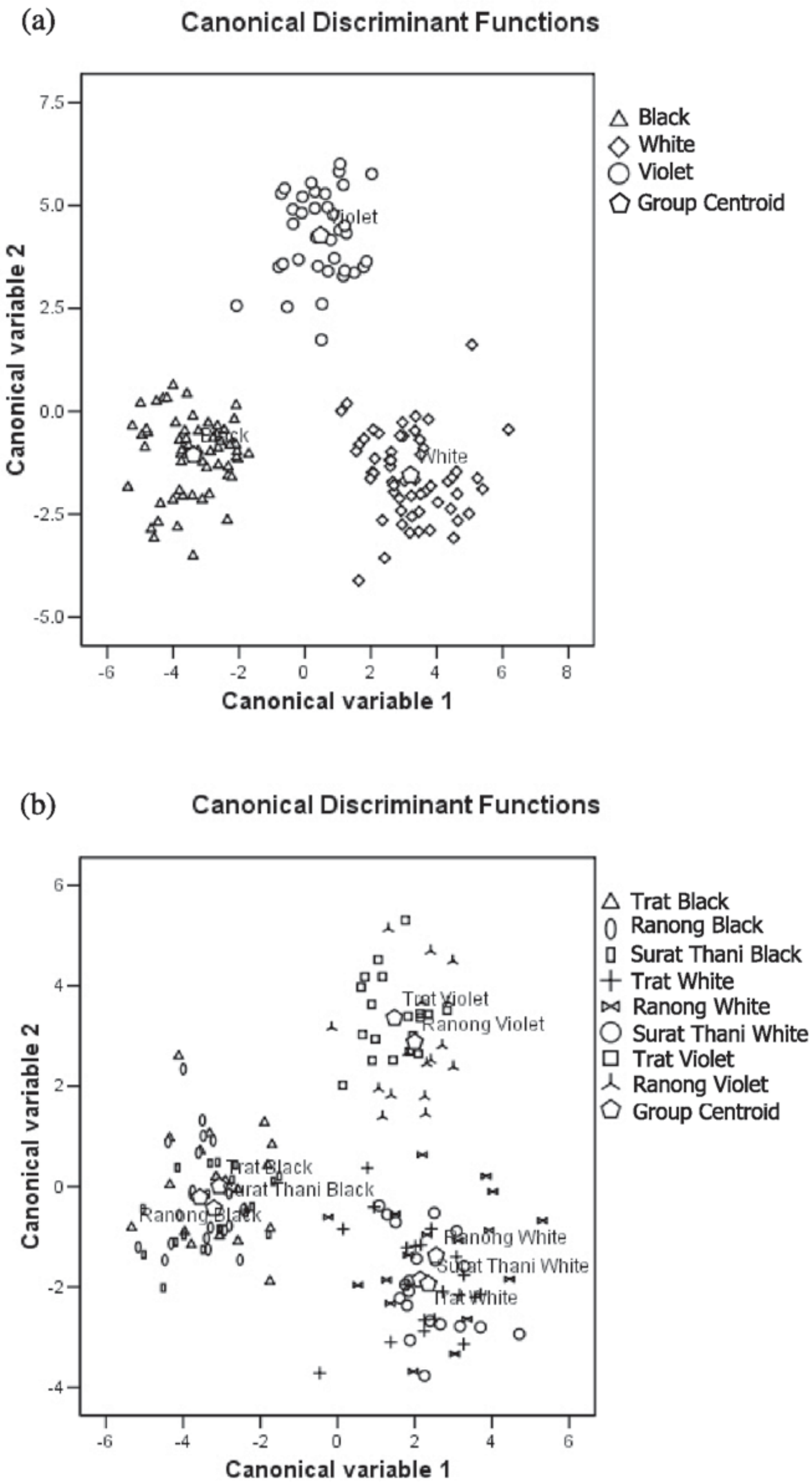

Figure 1 CVA of mud crab morphometric characters. Plot of three crab morphs (a) and eight morphogeographic groups (b) on the first two canonical axes.

limited sampling location of mud crabs. In this study, scatter plot of CVA on morphometric data of eight crab groups collected from diverse areas in Thailand clearly revealed three discrete clusters corresponding to their phenotypes. This indicated that Surat Thani "black" is actually a member of "black" morph, not a variant of "white" morph as previously proposed by Overton *et al.* (1997).

It should be noted here that nine characters including frontal median spine height (FMSH), distance between frontal median spine (DFMS), external carapace width (ECW), frontal margin (FM), right carpus length (RCL), right dactyl length (RDL), right propodus width (RPW), upper paddle length (UpPL) and body depth (BD) were common characters showing distinctively different for both morph and morph-geographic discrimination. This indicated that these nine characters were crucially affected both species difference and population difference. Seven other characters including internal carapace width (ICW), carapace width at spine 1 (CW1), left propodus length (LPL), left dactyl length (LDW), right dactyl length (RDW), right carpus width (RCW) and lower paddle length (LoPL) were specified to morph differentiation. Considerably, FMSH and ICW were also suggested by Keenan *et al.* (1998) to be informative characters in term of morphological ratios as related to frontal width (FW), i.e., (FMSH/FW and FW/ICW), while FW itself along with abdominal length (AL) were restricted to morph-geographic discrimination in this study.

Interestingly, it was also observed that the characters on the right chelae were more related to crab discrimination than the characters on the left chelae (Table 3). Moreover, the average length of right chelae characters were higher than those of left chelae except only one character, dactyl length, in black morph showing RDL (35.58 mm) to be slightly shorter than LDL (35.62 mm). This indicated that all crab morphs are bilaterally asymmetrical. Significant unequal in length of characters on the left and the right chelae may due to "handedness" which related to behavioural preference for one chelae as suggested by Palmer (2004). However, Overton *et al.* (1997) claimed that "black" morph was bilaterally asymmetrical but "white" morph was bilaterally symmetrical.

Mahalanobis' distance analysis

Mahalanobis' Distances between "black" and "white", "white" and "violet" and "violet" and "black" were 53.283, 55.694 and 60.806, respectively. These values were considerably high indicating their true separation (Table 4). Phenetic tree showed a closely related group of "black" and "white" morph separating from "violet" morph (Figure 2a). However, Fuseya and Watanabe (1996) presented the genetic variation and relationship of mud crab in the genus *Scylla* using electrophoretic analysis of allozymes. They concluded that the genus *Scylla* comprised at least three species including *S. serrata*, *S. oceanica* and *S. tranquebarica* which corresponded to "black", "white" and "violet" morph, respectively, but indicated that *S. serrata* ("black") and *S. tranquebarica* ("violet") were closely related groups separating from *S. oceanica* ("white"). Klinbunga *et al.* (2000), on the other hand, presented a phylogenetic tree of the genus *Scylla* based on randomly amplified polymorphic DNA (RAPD) showing the closely related groups of "white" and "green" ("violet" morph in this study)

Table 4 Mahalanobis' Distance between crab morphs.

	Black	White	Violet
Black	-		
White	53.283	-	
Violet	60.806	55.694	-

separating from “black” morph. The three different and independent methods, i.e., morphometric analysis (this study), allozyme divergence (Fuseya and Watanabe, 1996) and RAPD (Klinbunga *et al.*, 2000) ironically gave inconclusive evidences of relationships within the genus *Scylla*.

Further analysis on Mahalanobis’ Distances of eight morph-geographic groups

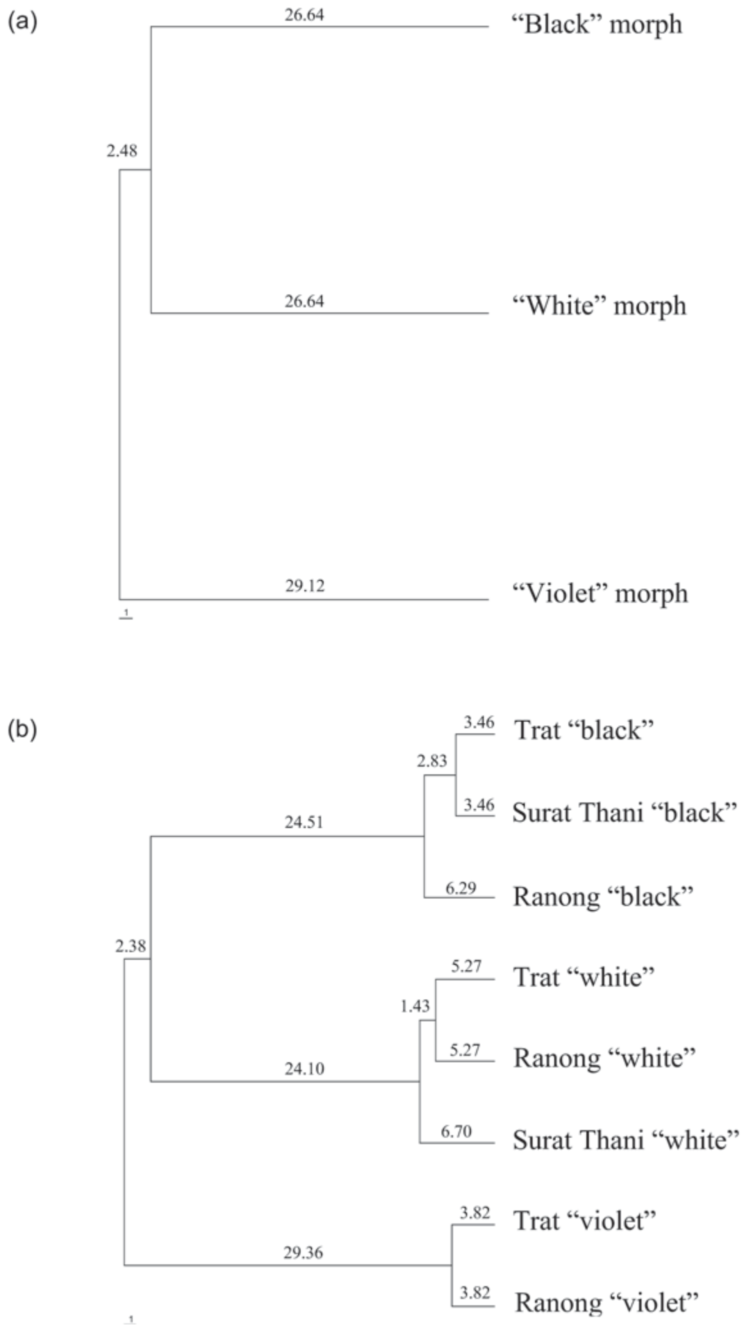

Figure 2 Phenetic trees based on Mahalanobis’ Distance between crab morphs (a) and eight morph-geographic groups (b). Numbers above branches indicated Mahalanobis’ Distance.

indicated that the distances within and among morphs were in the range of 6.922 – 15.027 and 51.878 – 76.933, respectively, of which the average values within “black”, “white” and “violet” were 10.693 and 12.443 and 7.631, respectively (Table 5). The differences of morphometric characters within morphs were obviously smaller than those among morphs implying the true identity within each crab morph. However, it is interesting to find that geographic origins played less role in grouping them as seen from the topology of phenetic tree (Figure 2b), i.e., Trat and Surat Thani “black” morphs were closely related while Trat and Ranong “white” morphs were closely related.

This study gave strong evidences which revealed variation of morphometric characters among three mud crab morphs collected from three main coasts of Thailand. Based on CVA and Mahalanobis’ Distance analysis, they indicated that “black”, “white” and “violet” should be classified into three different species. This result supported Chayarat and Kaew-ridh (1984), Klinbunga *et al.* (2000) and Tiensongrusmee and Pratoomchat (2002), who also recognized “black”, “white” and “violet” morphs as three different species.

Species classification

There have been only two main and widely referred groups working on the mud crab species classification. Estampador (1949) classified mud crabs from the Philippines, based on gametogenesis and external morphology, into three species *S. serrata, S. oceanica, S. tranquebarica,* and a variety of *S. serrata*; *S. serrata var. paramamosain.* He suggested that the color of carapace, chelae and leg, spine on finger bases and polygonal pigment areas could be the crucial characters differentiating these four taxonomic groups of the genus *Scylla*. Keenan *et al.* (1998), on the other hand, classified mud crab based on several factors, i.e., external morphology, genetic variations and multivariate analysis of morphometric characters and suggested that crabs in the genus *Scylla* should be classified up to four species, *S. olivacea, S. paramamosain S. tranquebarica,* and *S. serrata*. However, Keenan’s classification was mainly relied on two types of diagnostic tools, i.e., external morphologies and morphological ratios. The external morphologies included spines on carpus of chelae, shape of frontal spine and position of polygonal pigment areas on crab body, while the morphological ratios included ICS/OCS (inner carpus spine/outer carpus spine), FMSH/FW (frontal median spine height/frontal width) and FW/ICW (frontal width/internal carapace width).

To designate reasonable scientific name for each crab morph, we considered both standards used by Estampador (1949) and Keenan *et al.* (1998). Based on Estampador (1949), we compared the color of carapace, chelae, and leg, spine on finger bases and polygonal pigment areas of our crab specimens to those suggested by Estampador and found that “black”, “white” and “violet” morphs in this study are more correlated

Table 5 Mahalanobis’ Distance of eight crab morphogeographic groups.

	Trat “black”	Ranong “black”	Surat Thani “black”	Trat “white”	Ranong “white”	Surat Thani “white”	Trat “violet”	Ranong “violet”
Trat “black”	-							
Ranong “black”	11.241	-						
Surat Thani “black”	6.922	13.916	-					
Trat “white”	55.569	70.969	62.803	-				
Ranong “white”	60.364	74.622	65.494	10.544	-			
Surat Thani “white”	51.878	59.600	53.038	11.762	15.027	-		
Trat “violet”	53.838	73.975	65.838	69.378	63.270	64.590	-	
Ranong “violet”	60.589	76.933	74.413	69.071	61.253	63.056	7.631	-

to *S. serrata* and *S. serrata var. paramamosain* and *S. tranquebarica*, respectively. This observation of "black" and "violet" morph also agreed with Chayarat and Kaew-ridh (1984), Klinbunga *et al.* (2000) and Tiensongrusmee and Pratoomchat (2002). Although "white" morph (Poo Thong-Lang) has been assigned as *S. oceanica* by all above authors but we found that it lacks the *S. oceanica* characters of large polygonal pigment areas on all legs, including chelipeds as specified by Estampador (1949). Our observation distinctively clarified that "white" morph contained polygonal pigment area only on the swimming leg and the third periopod. Therefore, it should be included as *S. serrata var. paramamosain* since brownish gray color with patches of bluish green at joints of legs and cheliped, median pair of frontal teeth (slightly produced anteriorly) were clearly seen (Estampador, 1949). In addition, "white" morph should be assigned as species instead of a variety of *S. serrata* ("black" morph) since it was found to be completely separated from "black" cluster and also from "violet" cluster. Moreover, Mahalanobis' Distance between "black" and "white" (53.283) were roughly equal to those between "black" and "violet" (60.806) and "white" and "violet" (55.806). Therefore, both CVA and Mahalanobis' Distance indicated that "white" should be a species in the genus *Scylla*, not a variety of *S. serrata* as suggested by Estampador (1949).

Keenan *et al.* (1998), on the other hand, classified mud crabs based on morphological ratios and external morphologies. Only two morphological ratios, FMSH/FW and FW/ICW, were used in this study (Table 6). ICS/OCS ratio was not included in our analysis because ICS and OCS were very short spine and difficult to locate on the spine base. Moreover, ICS of "black" morph was obsolete spine. Therefore, ICS and OCS were hard to measure and prone to induce error for analysis. Based on the standard measurements proposed by Keenan *et al.* (1998) "black", "white" and "violet" morphs in this study should be assigned to *S. olivacea*, *S. paramamosain* and *S. tranquebarica*, respectively. Our suggested nomenclature of the three species of mud crabs are shown in Table 7 which could be used as a cross-reference to those of Estampador (1949) and Keenan *et al.* (1998). As a matter of fact, this recent revision of the genus *Scylla* by Keenan is feasible

Table 6 Comparisons of FMSH/FW and FW/ICW ratio determining in this study and those of Keenan *et al.* (1998).

Present study			Keenan *et al.* (1998)		
	FMSH/FW	FW/ICW		FMSH/FW	FW/ICW
Black morph (*N*=60)	0.028±0.0043	0.420±0.014	*S.olivacea*	0.029±0.005	0.415±0.017
White morph (*N*=58)	0.053±0.0083	0.391±0.0115	*S.paramamosain*	0.058±0.012	0.377±0.007
Violet morph (*N*=36)	0.045±0.0048	0.417±0.0082	*S.tranquebarica*	0.043±0.006	0.412±0.016
			S.serrata	0.061±0.010	0.371±0.016

Table 7 Cross – reference of mud crab morphs, local names, and suggested scientific names to those of Estampador (1949) and Keenan *et al.* (1998).

Mud crab morph	Local name	Estampador (1949)	Keenan *et al.* (1998)
Black	Poo Dum	*S. serrata*	*S. olivacea*
White	Poo Thong-Lang	*S. serrata var. paramamosain*	*S. paramamosain*
Violet	Poo Thong-Long	*S. tranquebarica*	*S. tranquebarica*

and generally accepted by crab taxonomists as well as by FAO (Ng, 1998). It is, therefore, reasonable to assign “black”, “white” and “violet” crabs of Thailand to the species of *S. olivacea*, *S. paramamosain* and *S. tranquebarica*, respectively.

CONCLUSIONS

Mud crabs, “black”, “white” and “violet” morph, in the genus *Scylla* were collected from three different coastal areas, Trat, Ranong and Surat Thani Provinces, in Thailand. Although “black” and “white” were found from all sampling areas, “violet” was found only from Trat and Ranong Provinces. CVA and Mahalanobis’ Distance analysis on fifty-one morphometric characters showed great phenotypic variations among these crab morphs and suggested that they should be assigned as three different species. Species classification was done based on the standards set by both Estampador (1949) and Keenan *et al.* (1998). Although “black”, “white” and “violet” seemed to be comparable to Estampador’s classification of *S. serrata, S. serrata var. paramamosain* and *S. tranquebarica*, respectively, considering external morphology alone, recent revision of the genus *Scylla* by Keenan group gave more concrete evidences on morphological ratios as well as external morphologies to clarify and support our morphometric analysis that these three morphs should be assigned to *S. olivacea*, *S. paramamosain* and *S. tranquebarica*, respectively.

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LITERATURE CITED

Chayarat, C. and B. Kaew-ridh. 1984. **Some Possibilities of the Mud Crabs. *Scylla* spp. (Crustacea: Portunidae), Identification in the Vicinity of Chantaburi Fisheries Station.** Department of Fisheries, Bangkok, Thailand.

Creech, S. 1992. A multivariate morphometric investigation of *Atherina boyeri* Risso, 1810 and *A. presbyter* Cuvier, 1829 (Teleostei: Atherinidae): morphometric evidence in support of the two species. **J. Fish Biol.** 41: 341-353.

Estampador, E.P. 1949. Studies on *Scylla* (Crustacea: Portunidae) I. Revision of the genus. **Philipp. J. Sci.** 78: 95-108.

Felsenstein, J. 2004. **PHYLIP version 3.63.** Department of Genetics, University of Washington, Seattle, USA.

Fuseya, R. and S. Watanabe. 1996. Genetic variability in the mud crab genus *Scylla* (Brachyura: Portunidae). **Fisheries Sci.** 65: 705-709.

Hiroshi, F. 1999. Problems in species identification of the mud crab genus *Scylla* (Brachyura: Portunidae). **UJNR Technical Report** 28: 9-13.

Jobson, J.D. 1992. **Applied Multivariate Data Analysis.** Springer-Verlag Inc., New York. 731 p.

Keenan, C.P., P.J.F. Davie and D.L. Mann. 1998. A revision of the genus *Scylla* DE HAAN, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). **Raffles Bull. Zool.** 46: 217-245.

Klinbunga, S., A. Boonyapakdee and B. Pratoomchat. 2000. Genetic diversity and species-diagnostic markers of mud crabs (Genus *Scylla*) in eastern Thailand determined by RAPD analysis. **Mar. Biotechnol.** 2: 180-187.

Naiyanetr, P. 1998. **Checklist of Crustacean**

Fauna in Thailand (Decapoda and Stomatopoda). Integrated Promotion Technology Co., Ltd., Bangkok. 161 p.

Ng, P.K.L. 1998. Crabs. pp.1045-1155. *In* K.E. Carpenter and V.H. Niem (eds.). **The Living Marine Resources of the Western Central Pacific Volume 2 Cephalepods, Crustaceans, Holothurians and Sharks**. Rome: FAO.

Overton, J.L., D.J. Macintosh and R.S. Thorpe. 1997. Multivariate analysis of the mud crab *Scylla serrata* (Brachyura: Portunidae) from four locations in Southeast Asia. **Marine Biol.** 128: 55-62.

Palmer, A.R. 2004. Symmetry breaking and the evolution of development. **Science** 306: 828-833.

Parnaby, H.E. 2002. A new species of long-eared bat (Nyctophilus: vespertilionidae) from new Caledonia. **Aus. Mammal.** 23: 115-124.

Pongthana, N., S. Pripanapong, K. Youngvanichset, B. Sumritthivej, K. Sompong and A. Aunrerk. 2004. **Research and development project for commercial production of mud crab.** Report on the seminar at Maruay Garden Hotel, Bangkok, Thailand. July 29, 2004.

Stephenson, W. and B. Campbell. 1960. The Australian Portunids (Crustacea: Portunidae) IV. Remaining genera. **Aus. J. Freshwater Res.** 11: 73-122.

Suvatti, C. 1950. **Fauna of Thailand.** Department of Fisheries, Bangkok. 1,100 p.

Tiensongrusmee, B. and B. Pratoomchat. 2002. **The Mud Crabs: Biology, Conservation of Resources and Sustainable Culture**. The Thailand Research Fund. Bangkok. 264p.