

## CHAPTER 4: RESULT

### 4.1: GENETIC AMPLIFICATION

All the DNA were successfully extracted using AxyPrep™ Genomic DNA Extraction Kit (AxyPrep™ Genomic DNA Extraction Kit website: <http://www.axxygenbio.com/collections/vendors?page=2&q=Axygen>) and shows high purity of DNA with optical density (OD<sub>260/280nm</sub>) ratio ranged from 1.78 to 1.84 through spectrophotometric measurement of UV absorbance using a spectrophotometer (Eppendorf, Germany). All the samples included in genetic analysis shows 100% successful amplification by universal Fish F1 and Fish R1 primer set (Ward *et al.*, 2005) with optimum annealing temperature of 45°C and optimum amount of 25mM MgCl<sub>2</sub> of 2µl per reaction volume. The success of amplified COI sequence was visualized through UV gel imaging after 1% agarose gel electrophoresis and is shown in **Appendix E**. These successful amplified COI sequences were trimmed at both ends and resulted in a total length of 582 base pair amplicon for subsequent analysis.

## 4.2: DNA BARCODING

A partial fragment of mtDNA COI sequence consists of a total length of 582 base pair was generated in this study for a total of 126 barcoding specimens from 27 previously described freshwater fish species. These COI sequences were aligned with COI sequence in Genbank database and shows the absence of indels and in-frame stop codons which indicate that our entire datasets were free from existence of nuclear mitochondrial pseudogenes (numts). Hence, it is assumed that our COI barcodes are functional mitochondrial COI sequence and therefore were suitable for subsequent data analysis.

Among all 27 barcoded species, a total number of 46 unique haplotypes were generated with no overlapping haplotype distribution between different species was detected. The list of haplotypes assigned with particular species is shown in **Table 4.1**. The K2P genetic distance that present within and among each taxonomic level are summarized in **Table 4.2**. The intra-specific divergence was low for all the species investigated, ranged from 0.00% to 0.90% with mean intra-specific divergence of 0.16%. These con-specific distance were comparatively lower than con-generic distance with maximum intra-specific divergence of 0.90%, two times lower than minimum con-generic variation of 1.70%. Specifically, the divergence shown among con-specific individual of *C. striata* was 0.60%, lower than the divergence among *Channa* genus (con-generic divergence of 1.70% between *C. striata* and *C. lucius*), indicating that the divergence among these species was comparable to the divergence observed at the same taxonomic level among other species groups. This intra-specific divergence of *C. striata* was lower than other freshwater fish species such as intra-specific divergence of *Rasbora paviana* with con-specific variation of 0.90%.

Overall, the ratio of con-generic distance (mean con-generic divergence of 6.03%) to con-specific distance (mean intra-specific divergence of 0.16%) was 38 fold higher between that taxonomic level. The minimum inter-specific variation (1.70%) was almost 11 fold greater than the average intra-specific variation (0.16%). These patterns of species divergence are reflected in the phylogenetic tree shown in **Figure 4.1** where all the haplotypes within each species shows monophyletic with high statistical support of posterior probability value ranged from 0.97 to 1.00 and bootstrap value of 99% using both Bayesian and NJ inference methods.



Table continued'

Haplo-type	Position														No. of individual	Species	Individual	
	3	2	3	6	1	1	1	1	2	2	3	3	3	4				
	4	3	3	4	6	6	7	8	4	5	0	8	9	6				
	G	T	T	C	A	C	G	C	A	A	G	A	G	G	T			
24	.	.	C	.	.	.	.	T	.	G	A	.	A	.	.	2	<i>T. thynnoides</i> (Lomah)	LMH 1,2
25	T	A	A	.	.	.	.	T	.	.	A	C	A	.	.	2	<i>H. wyckii</i> (Baung Kelulang)	BAK 1,2
26	A	.	.	T	.	.	.	.	.	C	C	T	A	.	.	2	<i>C. Caudimaculatus</i> (Selimang Batu)	SLB 1,2
27	A	.	.	T	G	.	.	T	.	T	.	.	A	.	C	1	<i>C. apogon</i> (Temperas)	TEM 2
28	A	.	.	.	G	.	.	T	.	T	.	.	A	A	C	2		TEM 3,5
29	.	A	.	T	.	.	.	T	.	C	C	C	T	T	.	1	<i>A. testudineus</i> (Puyu)	PY 1
30	T	A	.	.	.	.	.	.	C	T	T	G	C	A	C	2	<i>B. dinema</i> (Gerahak)	GER 1,2
31	A	.	.	.	.	G	.	T	.	.	A	.	A	.	C	2	<i>P. bulu</i> (Tengalan)	TGL 1,5
32	A	.	.	.	.	G	.	T	G	.	A	.	A	.	C	3		TGL 2,3,4
33	.	A	A	A	.	.	.	.	C	C	.	.	.	.	.	10	<i>C. striata</i> (Haruan)	HRN(JH)8,11,15,25,30 & HRN (NS)6,11,13,14,15
34	.	A	A	A	.	.	.	.	C	G	A	.	.	.	.	6		HRN(PP)1,4&HRN(KD)1-4 &HRN(SRW)1, 3-10
35	.	A	A	A	.	.	.	T	C	G	A	.	.	.	.	3		HRN(PP) 2,3,6
36	.	A	A	A	.	.	.	T	C	C	A	.	.	.	.	4		HRN(TG) 5,6,8,16
37	.	A	A	A	.	.	.	.	C	C	A	G	.	.	.	5		HRN(PH)6,7,9,10,12
38	.	A	G	A	.	.	.	.	C	G	A	.	.	.	.	5		HRN(SL) 16,18,20,26,27
39	.	A	A	A	.	.	.	.	C	C	A	.	.	.	.	1		HRN(SRW)2
40	T	C	.	G	G	T	.	.	.	C	A	G	A	.	.	1	<i>R. paviana</i> (Seluang)	SLG(PR) 1
41	T	C	.	G	G	T	.	.	.	C	A	G	A	.	C	1		SLG(SL) 1
42	T	A	A	.	.	.	.	T	.	.	A	.	A	A	.	3	<i>H. nemurus</i> (Baung)	BAU(PR) 1,2,3
43	A	G	.	.	G	.	.	T	.	G	A	C	T	A	.	3	<i>D. regina</i> (Danio)	DNO(PR) 1,2,3
44	A	.	G	.	G	.	.	.	.	T	A	C	C	A	.	1	<i>C. lucius</i> (Bujuk)	BJK(PR) 1
45	A	.	.	T	.	.	.	T	T	.	A	.	C	A	C	3	<i>P. banksi</i> (Tengas)	PBA(SL) 1,2,3
46	A	.	.	T	.	.	.	.	G	A	.	.	T	.	.	5	<i>P. smedleyi</i> (Tengas Daun)	TGSD(SL) 2,3,4,10,11

Table 4.2: Comparison of divergence distance (K2P percent) calculated using K2P model.

Comparison of K2P distances within groups	Number of groups included in comparison	Sample	K2P percentage (%)	Minimum K2P percentage (%)	Maximum K2P percentage (%)	Mean K2P percentage (%)
<b>Within species</b>	24 species	<i>Amblyrhynchichthys truncatus</i>	0.10	0.00	0.90	0.16
		<i>Bagarius yarrelli</i>	0.00			
		<i>Barbichthys laevis</i>	0.10			
		<i>Barbonymus schwanefeldii</i>	0.10			
		<i>Belodontichthys dinema</i>	0.00			
		<i>Channa striata</i>	0.60			
		<i>Cirrhinus caudimaculatus</i>	0.00			
		<i>Cyclocheilichthys apogon</i>	0.50			
		<i>Devario regina</i>	0.00			
		<i>Hampala macrolepidota</i>	0.50			
		<i>Hemibagrus nemurus</i>	0.00			
		<i>Hemibagrus wyckii</i>	0.00			
		<i>Hypsibarbus wetmorei</i>	0.00			
		<i>Luciosoma setigerum</i>	0.20			
		<i>Osteochilus vittatus</i>	0.10			
		<i>Pangasius nasutus</i>	0.30			
		<i>Poropuntius smedleyi</i>	0.00			
		<i>Pseudolais micronemus</i>	0.00			
		<i>Puntioplites bulu</i>	0.50			
		<i>Puntioplites proctozystron</i>	0.00			
<i>Puntius banksi</i>	0.00					
<i>Rabosra paviana</i>	0.90					
<i>Rasbora sumatrana</i>	0.00					
<i>Thynnichthys thynnoides</i>	0.00					
<b>Within genera (among species)</b>	4 genera	<i>Channa</i>	1.70	1.70	10.10	6.03
		<i>Hemibagrus</i>	10.10			
		<i>Puntioplites</i>	3.60			
		<i>Rasbora</i>	8.70			
<b>Within family (among genus)</b>	2 families	Cyprinidae	15.70	4.50	15.70	10.10
		Pangasidae	4.50			

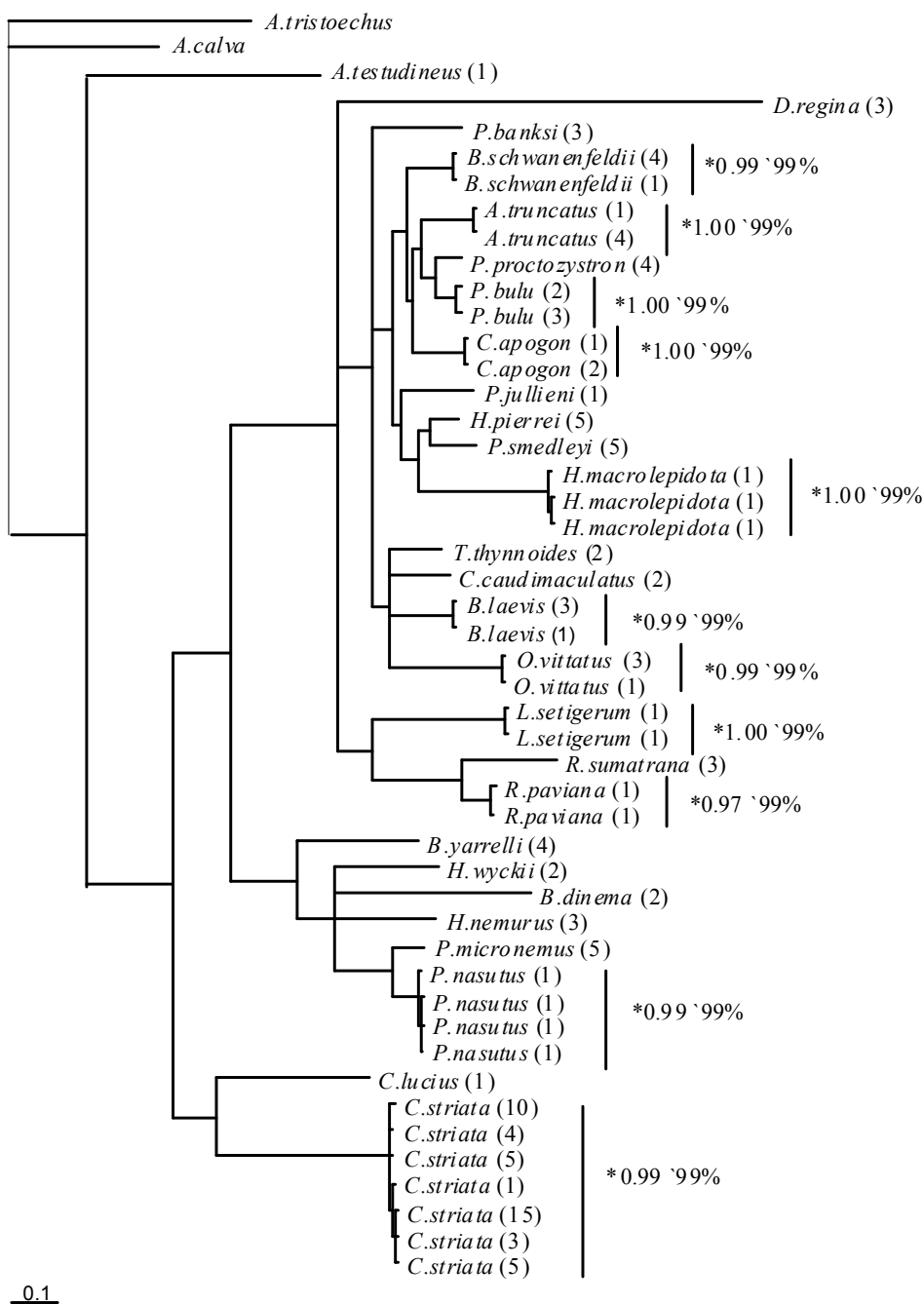


Figure 4.1: Phylogenetic tree of mtDNA COI barcodes of 27 fish species with number of individuals of each haplotype is listed in brackets after the species name. Posterior probability and bootstrap among different haplotypes of same species are shown by \* and ` respectively.

### 4.3: MOLECULAR DATA (COI) OF *C.striata*

A total of 43 *C. striata* individuals were molecularly surveyed in this study and were characterized with a total length of 582 base pair of mtDNA COI partial fragment. These sequenced *C. striata* specimens were barcoded during barcoding analysis and all these 43 *C. striata* barcodes showed 0.60% of intra-species divergence strongly indicates that variation was present among *C. striata* populations surveyed in this study and thus these COI barcodes are suitable for subsequent genetic analysis at population level. Among the entire COI sequence obtained, a total of nine polymorphic sites had been detected with the overall nucleoside diversity of 1.55%. All the nine variable sites are parsimony informative. There are a total of seven haplotypes identified from all the *C. striata* populations investigated in molecular study and with the haplotype diversity value of  $h = 0.8018$  being recorded. Among all seven generated haplotypes (hap), hap2 was the most common haplotype and was shared among populations in Kedah, Pulau Pinang and unexpectedly, in the island of Borneo, Sarawak. Populations in the central coast of Peninsular Malaysia were characterized by a unique haplotype; hap6, hap5, and hap4 which are specific to Selangor, Pahang, and Terengganu respectively. The haplotype distribution pattern in Peninsular Malaysia was parallel to the freshwater fish division proposed by Mohsin & Ambak (1983, 1991) witnessed by the overlapping haplotype laterally between populations within the north and between populations within the south coast, with the exception of non-overlapping haplotype within the central coast. Refer **Figure 4.2** for illustration map of haplotype distribution of *C. striata* across Malaysia.



The populations structure of *C. striata* were explained by the obtained mtDNA COI Fst values with the  $p < 0.05$  as the cut off point of significant population differentiation. This population differentiation is summarized in **Table 4.3**. All the populations in Peninsular Malaysia were significantly differentiated with each other except populations which are separated in short distance, such as between Kedah and Pulau Pinang, between Kedah and Terengganu, and between Negeri Sembilan and Johor. Among the entire *C. striata* populations in Malaysia, an interesting result is the non-significant population differentiation between West Malaysia (Kedah and Pulau Pinang) and East Malaysia (Sarawak). This interesting finding coupled with the evidence of sharing of hap2 was an unexpected genetically incomplete population divergence between the Peninsular Malaysia and the island of Borneo which are geographically isolated by recent physical barrier of South China Sea.

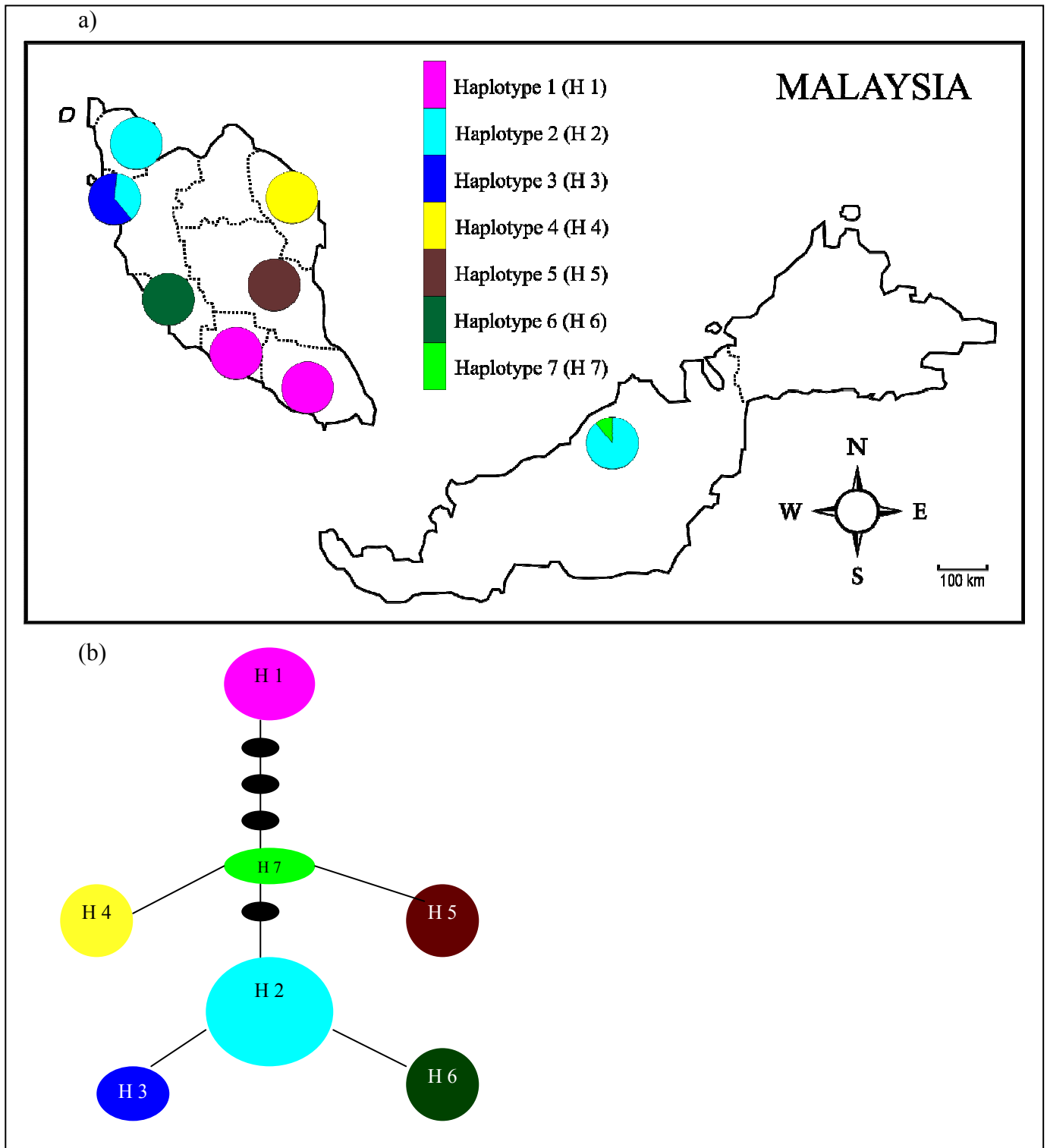


Figure 4.2(a): Haplotype distribution of seven haplotypes generated from eight populations of *Channa striata* across Malaysia. (b): Haplotype network that connecting all generated haplotypes; the size of each haplotype is proportional to the number of each individual of corresponding haplotype; each line represents one mutational step.

Table 4.3: Pairwise population distance represented by Fst value calculated using mtDNA COI sequence with bold and “\*” indicate significant Fst ( $p < 0.05$ ).

Site	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1)								
(2)	0.00000							
(3)	<b>1.00000*</b>	<b>1.00000*</b>						
(4)	<b>0.95455*</b>	<b>0.95455*</b>	0.45205					
(5)	<b>1.00000*</b>	<b>1.00000*</b>	1.00000	<b>0.89992*</b>				
(6)	<b>1.00000*</b>	<b>1.00000*</b>	<b>1.00000*</b>	<b>0.93478*</b>	<b>1.00000*</b>			
(7)	<b>1.00000*</b>	<b>1.00000*</b>	<b>1.00000*</b>	<b>0.81250*</b>	<b>1.00000*</b>	<b>1.00000*</b>		
(8)	<b>0.95271*</b>	<b>0.95271*</b>	-0.12150	0.40239	<b>0.92243*</b>	<b>0.92817*</b>	<b>0.77941*</b>	

\* Site (1) = Johor, (2) = Negeri Sembilan, (3) = Kedah, (4) = Pulau Pinang, (5) = Terengganu, (6) = Pahang, (7) = Selangor, (8) = Sarawak.

#### 4.4: MORPHOMETRIC DATA OF *C. striata*

A total of 38 morphometric measurements (refer **Table 4.4**) were obtained for all *C. striata* individuals sampled in this study and the 30 successfully transformed variables were able to discriminate populations of *C. striata* in Malaysia. These patterns of discrimination were illustrated in different roots represented in two-dimensional graph in **Figure 4.3** and **Figure 4.4** with the zero cut off point in each root acted as a dividing point in discriminating populations from positive region which acquired certain unique characters in relation to other populations in negative region at the same root. A list of standardized coefficients of canonical variables underlying unique characters that could discriminate certain populations from others was generated (**Table 4.5** and **Table 4.6**) which acted as a reference for its correspondence two-dimensional graph. The eigenvalue under each root for each list was calculated representing the amount of variation that each root can explained and the value of cumulative proportion for each root was demonstrating the percentage of variation accounted by each root among all the roots. The two characters that had greatest contribution of variance to each root were represented in **Table 4.5** and **Table 4.6** whereby these characters were with the two tops value of coefficient under each root.

The first stage of multivariate discriminant analysis for the populations in Peninsular Malaysia is illustrated in the discriminant graph shown in **Figure 4.3**. For the *C. striata* populations surveyed in Peninsular Malaysia, there are four distinct groups had been discovered; there are: group A (Selangor, Pahang and Terengganu), group B (Johor, Pulau Pinang), group C (Negeri Sembilan) and group D (Kedah). These four groups are distinguishable from each other based on the obtained 30 successfully transformed morphometric measurements. The major components of variance which discriminate between the groups was underlined by root 1 axis followed by root 2 axis which contribute to percentage of variation of 58.54% and 18.49% respectively. The greatest contribution of characters that discriminated by root 1 axis were measurements of c2 and e1; whereas the most discriminant characters on root 2 axis were measurements of c3 and d2. Refer **Table 4.5** for the percentage of variance components of each axis and the most discriminated measurements reflected by each axis. Hence, within Peninsular Malaysia, group A which consists of populations of Selangor, Pahang and Terengganu were discriminated from group B, C and D by unique characters of comparative small measurement of c2 trait and large measurement of e1 trait which are the characters located on head. Similarly, group B and C which are populations of Johor, Pulau Pinang and Negeri Sembilan were characterized by unique head characters of small measurement of c3 trait and small measurement of d2 trait in relation to populations from Kedah (group D).

Table 4.4: Range of morphometric measurement of all 38 characters of *Channa striata* measured to the nearest centimeter with value in parentheses indicate mean of each measurement.

Measurement	Measurement at each location							
	Selangor	Johor	Negeri Sembilan	Pahang	Terengganu	Pulau Pinang	Kedah	Sarawak
<b>tl</b>	18.8-32.1 (24.3)	26.7-37.8 (31.5)	26.1-42.0 (36.3)	20.1-33.0 (26.9)	17.0-38.6 (24.3)	24.0-35.1 (30.1)	14.2-19.3 (16.6)	22.0-32.8 (28.0)
<b>sl</b>	15.7-27.4 (20.4)	23.0-32.0 (27.0)	22.5-35.5 (30.6)	16.8-28.2 (22.4)	14.1-32.6 (20.4)	20.2-31.2 (25.8)	12.0-16.2 (14.1)	18.4-28.5 (24.2)
<b>Cfl</b>	2.7-6.3 (4.1)	4.0-6.7 (4.9)	3.7-7.8 (6.3)	3.4-6.3 (4.9)	2.5-6.4 (3.8)	3.4-6.0 (4.7)	1.7-3.5 (2.6)	2.4-5.0 (3.5)
<b>SnW</b>	2.0-3.8 (2.6)	2.7-4.1 (3.3)	2.8-4.5 (4.0)	2.1-4.3 (3.0)	1.7-5.1 (2.6)	2.5-4.1 (3.3)	1.3-2.1 (1.7)	2.9-4.0 (3.5)
<b>SnL</b>	5.0-9.1 (6.4)	6.7-9.8 (8.3)	7.1-12.0 (10.7)	3.8-9.2 (7.1)	4.7-10.4 (6.7)	4.3-9.5 (8.0)	4.2-5.7 (5.0)	4.0-8.5 (6.0)
<b>ed</b>	0.8-1.1 (1.0)	1.0-1.4 (1.2)	0.9-1.1 (1.1)	0.6-1.1 (0.9)	0.9-1.1 (1.0)	0.7-1.3 (1.1)	0.5-0.8 (0.6)	0.7-1.0 (0.8)
<b>a1</b>	4.6-8.9 (6.2)	6.3-8.7 (7.7)	1.4-10.7 (9.0)	5.0-8.8 (6.9)	4.4-9.0 (6.1)	5.8-8.6 (7.4)	3.5-5.0 (4.2)	5.0-7.2 (6.4)
<b>a2</b>	1.1-2.5 (1.8)	1.6-2.8 (2.2)	1.6-2.7 (2.2)	1.1-2.7 (1.9)	1.0-2.7 (1.8)	1.2-2.7 (2.1)	0.6-1.5 (1.1)	1.8-3.9 (2.6)
<b>a3</b>	10.4-17.0 (12.6)	13.6-20.3 (16.2)	13.3-21.4 (18.8)	10.3-17.0 (14.1)	8.3-19.7 (12.6)	13.0-20.0 (15.5)	6.9-9.3 (8.1)	11.2-18.4 (13.9)
<b>a4</b>	1.1-2.3 (1.5)	1.3-2.7 (2.0)	1.7-2.7 (2.3)	1.1-1.9 (1.6)	1.0-2.6 (1.6)	1.2-2.7 (1.9)	0.9-1.3 (1.1)	1.4-3.7 (2.1)
<b>a5</b>	1.7-3.0 (2.2)	2.2-3.5 (2.9)	2.6-4.5 (3.6)	1.9-3.6 (2.5)	1.5-3.6 (2.3)	1.8-3.5 (2.6)	1.0-1.7 (1.3)	1.8-3.0 (2.4)
<b>a6</b>	1.0-2.5 (1.8)	2.0-3.9 (2.6)	2.1-3.6 (2.9)	1.0-2.8 (2.1)	1.0-3.5 (1.9)	1.5-3.8 (2.4)	1.0-1.3 (1.1)	1.5-4.0 (2.5)
<b>a7</b>	6.0-10.8 (8.0)	8.0-12.5 (9.8)	8.8-17.2 (11.8)	6.7-11.5 (8.8)	6.0-12.3 (8.0)	7.4-12.4 (9.2)	3.5-5.8 (5.1)	6.5-10.8 (8.7)
<b>a8</b>	2.8-5.4 (3.8)	1.2-6.7 (5.1)	4.5-5.7 (5.2)	3.1-5.5 (4.1)	2.5-6.4 (3.9)	3.5-6.7 (5.0)	1.8-3.2 (2.6)	3.8-7.1 (5.0)
<b>a9</b>	0.6-1.5 (0.9)	1.0-1.6 (1.2)	1.0-1.8 (1.4)	0.6-1.8 (1.1)	0.5-1.4 (1.0)	1.0-1.6 (1.1)	0.4-1.0 (0.6)	0.8-1.5 (1.2)
<b>a10</b>	1.9-3.3 (2.3)	2.3-3.7 (2.9)	2.1-3.8 (3.2)	2.0-3.7 (2.5)	1.7-4.4 (2.4)	1.9-3.7 (2.9)	1.0-1.8 (1.3)	2.2-5.0 (3.7)
<b>a11</b>	2.1-4.1 (2.7)	2.3-3.7 (3.1)	3.8-4.9 (4.3)	2.1-4.2 (2.9)	1.9-4.5 (2.8)	1.7-3.7 (3.0)	1.0-1.9 (1.4)	1.8-4.9 (2.7)
<b>a12</b>	2.5-4.8 (3.1)	3.2-4.8 (4.0)	3.7-6.1 (4.9)	2.7-4.8 (3.4)	2.0-4.8 (3.1)	2.0-4.7 (3.8)	1.5-2.5 (2.0)	2.1-3.9 (3.1)

Table continued'

Measurement	Measurement at each location							
	Selangor	Johor	Negeri Sembilan	Pahang	Terengganu	Pulau Pinang	Kedah	Sarawak
<b>b1</b>	2.7-5.0 (3.4)	3.4-5.1 (4.3)	4.0-6.3 (5.4)	3.0-4.8 (3.6)	2.4-4.8 (3.3)	3.0-5.2 (4.0)	1.8-3.2 (2.6)	2.5-3.8 (3.1)
<b>b2</b>	2.0-3.6 (2.5)	2.1-3.8 (3.1)	3.1-4.7 (4.2)	2.3-3.8 (2.8)	1.3-3.6 (2.5)	2.0-3.7 (2.9)	1.6-2.4 (2.0)	2.0-3.0 (2.6)
<b>b3</b>	1.8-2.9 (2.2)	2.6-4.3 (3.3)	4.1-5.4 (4.7)	2.1-3.8 (2.8)	1.7-3.3 (2.3)	2.1-4.3 (3.2)	1.4-2.5 (2.1)	1.5-3.0 (2.6)
<b>b4</b>	3.5-5.6 (4.2)	5.5-7.1 (6.3)	4.7-8.2 (7.4)	2.8-5.9 (4.8)	2.0-5.6 (4.1)	4.8-7.1 (6.1)	1.9-3.6 (2.9)	3.4-6.6 (5.0)
<b>c1</b>	3.3-6.3 (4.3)	5.1-6.6 (5.7)	4.8-8.3 (7.4)	3.7-6.5 (5.0)	3.0-6.5 (4.4)	4.9-6.6 (5.6)	2.0-3.9 (3.0)	3.4-6.9 (4.5)
<b>c2</b>	2.5-4.5 (3.4)	5.4-7.0 (6.1)	4.3-8.3 (7.4)	2.5-5.9 (4.7)	2.4-4.5 (3.3)	5.2-7.0 (6.1)	2.1-4.6 (3.8)	3.0-5.3 (4.1)
<b>c3</b>	3.9-6.9 (4.9)	6.0-7.5 (6.6)	4.4-8.4 (7.5)	4.0-7.0 (5.8)	3.7-6.8 (4.6)	5.4-7.5 (6.5)	2.3-4.3 (3.8)	3.9-6.3 (5.2)
<b>d1</b>	3.2-5.2 (4.1)	6.1-8.5 (7.0)	4.5-8.8 (7.7)	3.2-6.7 (5.3)	3.1-5.2 (4.0)	5.5-8.5 (6.9)	2.4-4.5 (4.0)	2.9-5.5 (4.2)
<b>d2</b>	4.1-7.1 (4.9)	6.0-8.3 (6.6)	4.5-8.5 (7.5)	4.1-7.5 (6.1)	3.1-7.3 (4.9)	5.9-8.3 (6.7)	2.9-4.7 (4.0)	2.8-5.0 (4.1)
<b>e1</b>	3.7-6.1 (4.6)	6.1-9.0 (7.5)	4.7-9.3 (8.1)	4.2-7.6 (6.3)	3.8-5.9 (4.7)	5.4-9.0 (7.3)	3.0-4.8 (4.2)	3.4-5.6 (4.7)
<b>e2</b>	4.1-7.8 (5.3)	6.0-8.6 (6.9)	4.0-9.2 (7.8)	4.4-8.0 (6.6)	3.8-7.0 (5.2)	5.3-8.6 (6.8)	2.8-4.3 (3.6)	3.0-4.7 (4.0)
<b>f1</b>	5.4-9.4 (6.8)	7.8-11.3 (8.8)	7.5-10.8 (10.1)	5.7-8.9 (7.8)	5.0-8.9 (6.6)	7.0-11.3 (8.9)	2.9-5.2 (4.4)	4.6-7.8 (6.2)
<b>f2</b>	7.1-12.5 (9.3)	10.1-13.3 (11.3)	10.7-15.5 (14.6)	7.1-12.0 (10.3)	6.8-12.5 (8.9)	9.1-13.3 (11.1)	3.9-6.6 (5.6)	7.2-12.0 (9.4)
<b>g1</b>	9.4-17.3 (12.5)	14.1-18.0 (15.7)	14.4-20.6 (19.3)	10.4-15.5 (13.8)	4.0-16.4 (12.1)	13.2-18.0 (15.4)	6.8-9.3 (8.4)	9.4-15.5 (13.3)
<b>g2</b>	2.1-4.1 (2.7)	3.1-4.7 (3.7)	3.4-4.6 (4.0)	2.1-4.0 (3.3)	1.9-3.9 (2.6)	2.9-4.7 (3.5)	1.0-2.2 (1.7)	1.5-3.4 (2.7)
<b>g3</b>	2.3-4.0 (3.0)	3.0-4.9 (3.9)	3.5-5.5 (4.7)	2.3-4.4 (3.5)	2.1-4.0 (2.9)	2.7-4.9 (3.7)	1.2-2.8 (2.2)	2.1-4.5 (3.4)
<b>g4</b>	2.1-3.7 (2.6)	3.0-4.6 (3.5)	3.2-4.8 (4.2)	2.1-3.9 (3.1)	1.9-3.5 (2.7)	2.5-4.6 (3.4)	1.6-2.5 (2.1)	2.2-4.0 (3.1)
<b>h1</b>	2.6-4.7 (3.6)	4.0-5.7 (4.8)	3.8-6.4 (5.5)	3.1-4.8 (4.2)	2.8-4.7 (3.6)	3.9-5.7 (4.7)	1.4-2.8 (2.3)	2.8-5.0 (4.1)
<b>h2</b>	4.1-8.0 (5.7)	5.7-8.3 (7.2)	6.1-9.6 (8.8)	5.0-7.4 (6.4)	3.7-8.0 (5.6)	5.6-8.2 (7.0)	3.5-4.8 (4.3)	3.2-5.7 (4.7)
<b>h3</b>	1.5-3.0 (2.0)	2.1-2.9 (2.5)	2.5-3.5 (3.3)	1.3-3.1 (2.2)	1.3-3.3 (2.1)	1.5-2.9 (2.4)	1.3-1.8 (1.5)	1.5-2.5 (1.9)

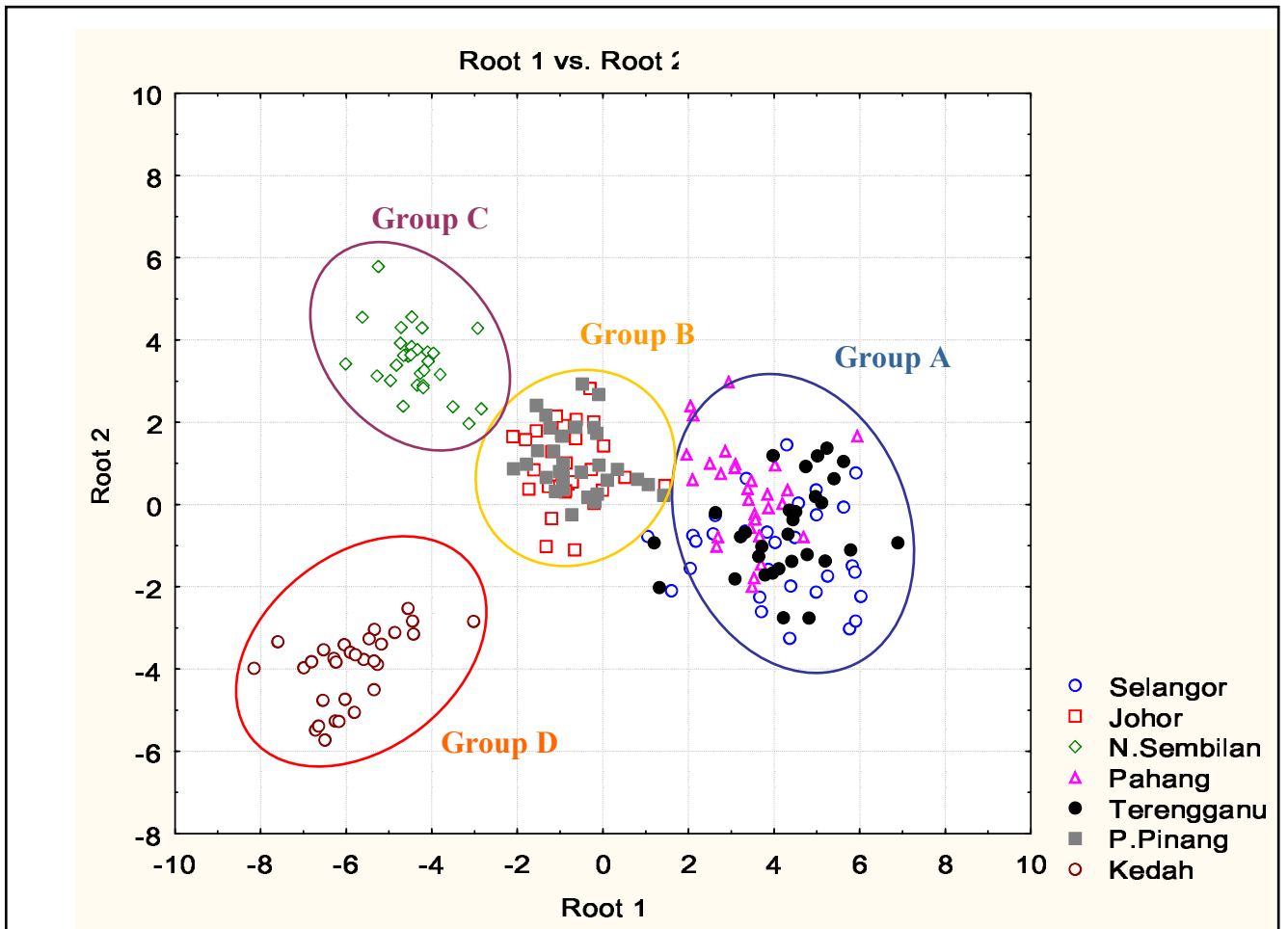
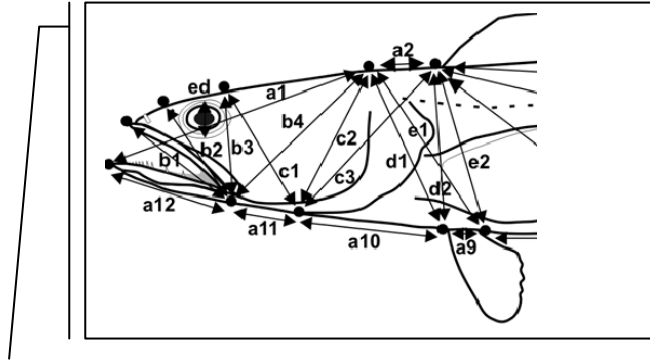


Figure 4.3: Multivariate discriminant analysis represented by root 1 and root 2 axis based on all *Channa striata* populations surveyed in Peninsular Malaysia.



Table 4.5: List of standardized coefficients of canonical variables based on all *Channa striata* populations surveyed in Peninsular Malaysia. Measurements in “bold” and “underline” indicate greatest contribution of characters to each root. The variables are corresponding to Truss Network Measurement illustrated in Figure 3.2.



Variable	Standardized Coefficients for Canonical Variables					
	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6
c2	<b>-1.41184</b>	0.499825	0.398591	0.054463	0.119042	-0.238883
f2	0.47170	0.408845	<b>-0.615390</b>	-0.168215	-0.230770	-0.211542
b2	0.28101	-0.278591	-0.284479	0.174256	-0.42253	0.458817
a10	0.41539	0.096533	-0.367550	-0.432404	0.259111	-0.268992
c3	0.66377	<b>-0.858751</b>	0.014077	0.472265	-0.250660	-0.080690
a5	0.36065	0.314906	-0.225384	-0.008893	0.267024	0.198322
e2	0.37393	0.331761	-0.266962	<b>0.681100</b>	-0.058005	-0.360663
b4	-0.19458	0.132053	0.252415	-0.497241	-0.523356	0.359599
SnL	-0.40295	-0.046967	0.003316	-0.148526	<b>0.532470</b>	-0.133889
b3	-0.47636	0.368229	<b>-0.728886</b>	0.429959	0.008980	-0.328389
a8	0.08207	-0.149248	0.373287	-0.265477	0.015430	-0.064601
b1	-0.43399	-0.523266	-0.084456	-0.507840	-0.286558	-0.003799
SnW	0.04462	0.228839	0.184555	0.652408	-0.226785	-0.053505
e1	<b>0.67757</b>	-0.152426	0.587134	<b>0.740656</b>	0.442494	<b>0.845807</b>
d1	-0.46682	0.329635	0.231088	-0.655794	-0.133296	-0.085119
g2	0.43378	-0.040481	0.309560	0.4676630	-0.394074	<b>0.556584</b>
g4	-0.40523	-0.077577	0.026801	-0.205765	0.396488	0.091170
h2	-0.48672	0.340375	-0.166280	0.050386	0.055264	-0.023362
a3	0.27658	0.063105	0.280636	0.288647	0.163754	0.117652
a6	-0.25723	0.396127	0.321013	0.032397	-0.299612	0.211321
d2	0.07330	<b>-0.585637</b>	0.007170	-0.075163	-0.137986	-0.503075
c1	0.17793	0.309891	-0.142353	-0.043706	<b>0.811599</b>	-0.243137
a4	0.25777	0.062941	-0.042803	-0.302267	0.331285	-0.248307
g3	-0.25389	-0.259770	-0.346391	0.242604	-0.036979	0.115660
a12	0.03025	0.253880	0.294079	-0.172015	-0.278936	0.015935
g1	-0.20226	0.291106	-0.012690	0.010235	-0.403767	-0.146930
f1	0.14296	-0.046102	0.320983	-0.248611	0.212844	-0.372136
h3	-0.14463	-0.158514	-0.212149	-0.032284	-0.166438	0.353353
a9	0.08448	0.017800	-0.010266	-0.270678	0.233016	0.126766
tl	0.07500	-0.141272	-0.182243	0.040925	0.012208	0.065811
Eigenvalue	14.89929	4.705745	3.882054	1.482290	0.334059	0.148039
Cum. Prop	0.58540 (58.54%)	0.770291 (18.49%)	0.922818 (15.25%)	0.981058 (5.82%)	0.994183 (1.31%)	1.000000 (0.58%)

In second stage of multivariate discriminant analysis, in order to have balance sample size, subset of each group discovered in Peninsular Malaysia together with population from Sarawak were included in subsequent discriminant function to have a overall discrimination on *C. striata* populations across entire Malaysia. This pattern of discriminant function is shown in **Figure 4.4** in which West Malaysia populations was discriminated from East Malaysia by root 1 axis which consists of 74.42% of variance composition in discrimination function. Populations in Peninsular Malaysia were discriminated from the member of the island of Borneo whereby Peninsula's acquired a comparative large measurement of e2 trait located at head and a comparative large measurement of b1 trait located at mouth.. The degree of variance component to each axis and the contribution of discriminant characters to each axis are shown in **Table 4.6**. Briefly, populations within Peninsular Malaysia showed head polymorphism whereas populations in Malaysia showed an additive mouth polymorphism with the evidence of large b1 trait measurement.

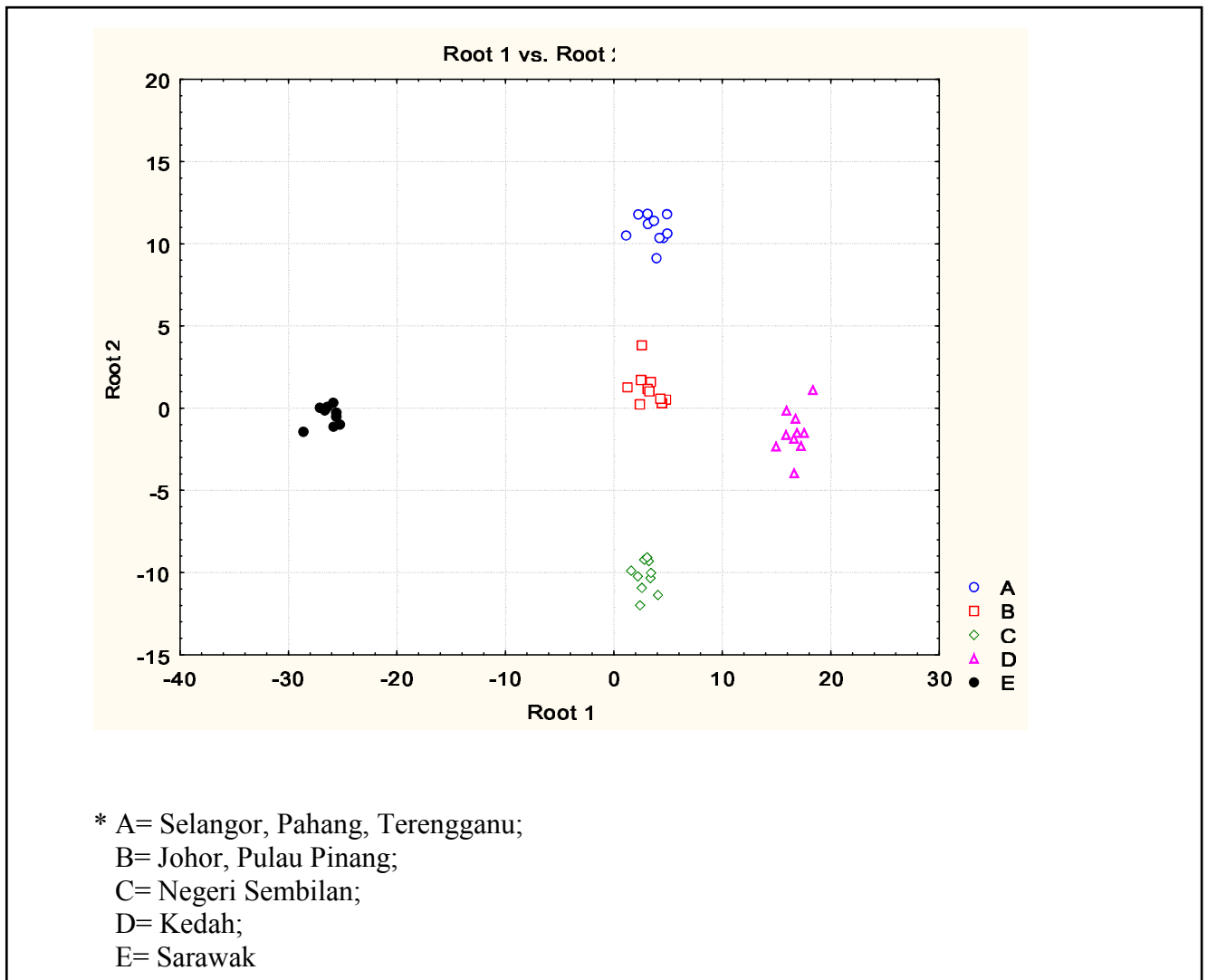
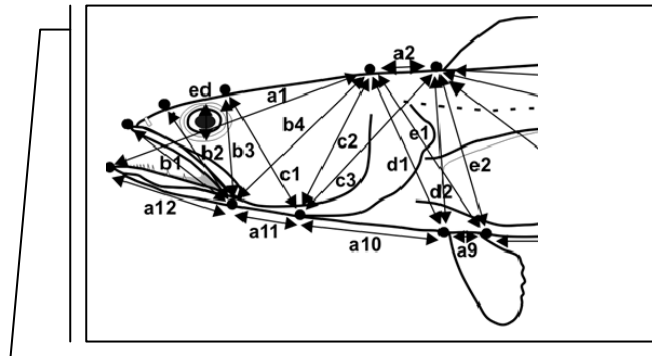


Figure 4.4: Multivariate discriminant analysis represented by root 1 and root 2 axis based on all *Channa striata* populations surveyed across entire Malaysia.

Table 4.6: List of standardized coefficients of canonical variables based on all *Channa striata* populations surveyed across entire Malaysia. Measurements in “bold” and “underline” indicate greatest contribution of characters to each root. The variables are corresponding to Truss Network Measurement illustrated in **Figure 3.2**.



Variable	Standardized Coefficients for Canonical Variables			
	Root 1	Root 2	Root 3	Root 4
e2	<b><u>2.1872</u></b>	<b><u>3.91106</u></b>	-0.20063	0.21126
c2	0.4818	-0.84864	<b><u>-1.70885</u></b>	0.08811
a5	-0.8042	-2.37991	1.35470	-0.82680
b1	<b><u>2.2417</u></b>	-0.37858	0.06038	-0.61243
a10	0.0702	0.13883	-0.14192	0.04250
f2	-1.0685	-0.79491	0.75069	-0.53315
a4	-0.0686	-2.99113	0.23824	<b><u>-1.09478</u></b>
g4	-2.0462	-0.56517	-0.82163	-1.07446
c3	1.0960	3.83485	-1.40422	0.20379
d1	-2.1132	<b><u>-5.06293</u></b>	1.15619	0.83281
SnL	1.7179	0.77327	-1.08747	0.41713
a3	-2.0655	0.90394	-0.30638	1.06254
b4	-1.6337	0.72442	0.09383	<b><u>1.44342</u></b>
g2	0.1400	1.89031	-0.14720	0.51732
a6	-2.0150	0.96549	0.42037	-0.75643
h2	1.4260	-2.70404	0.60591	0.13971
e1	1.2073	0.65500	0.18580	0.41229
g3	1.3471	1.36426	-0.57506	-0.92315
a9	-0.1035	-0.83578	0.18981	0.35164
a12	0.2179	-0.71198	0.24452	0.91279
a8	-0.5912	1.89025	<b><u>-1.70222</u></b>	0.92930
c1	0.9959	0.06944	1.27592	-0.77418
d2	1.3882	-0.48643	0.69451	-0.86227
SnW	-0.3964	-0.58803	0.77795	-0.50801
a1	-1.4941	-0.67406	0.28236	-0.67413
tl	0.9918	-0.12765	-0.75142	0.35329
a7	-0.7121	0.48801	0.72751	0.40213
<b>Eigenvalue</b>	222.9919	50.48606	19.21761	6.94981
<b>Cum. Prop</b>	0.7442 (74.42%)	0.91267 (16.85%)	0.97681 (6.41%)	1.00000 (2.32%)