

CHAPTER 5: DISCUSSION

5.1: DISCOVERY OF CRYPTIC SPECIES

DNA barcoding was invented in the year of 2003 by Hebert *et al.* (2003a) with the aim to assist in species identification of all animal kingdom that show complicated physical appearance through out their life cycle by assigning unique barcode to a particular species, in such a way that this molecular tag is not common between species. Hence, COI sequence was chosen as desirable barcode that evolve at a mutation rate which is sufficient to discriminate species while remaining comparatively low variation within species (Hebert *et al.*, 2003a). This recent species identification technique had successfully discovered possible cryptic species as a number of discoveries of putative cryptic species being reported in previous studies (Hebert *et al.*, 2004b; Huang *et al.*, 2007). In our study, in order to make any judgment of unidentified cryptic taxa in *C. striata*, validation of COI barcode in discriminating freshwater fish species in Malaysia is required.

Among all 27 freshwater fish species surveyed, with the evidence of non overlapping haplotype among species, low intra-specific variation (range from 0.00% to 0.90%, average intra-species variation of 0.16%) which is comparatively lower than minimum con-generic variation of 1.70%, and monophyletic divergence pattern of each species revealed in phylogenetic tree with high statistical support (99% bootstrap value and 0.97 to 1.00 posterior probability, see **Figure 4.1**), therefore, it is verified that COI barcode is able to differentiate among freshwater fish taxa in Malaysia. Specifically, one of the barcoding species, *C. striata*, which was sampled extensively across a wide geographical range of Malaysia was tend to provide a most likely extent of intra-species variation due to phylogeography influence; otherwise, the exceed of intra-specific distance over threshold

level in discriminating species might be due to phylogeography factor in stead of revealing possible cryptic taxa. In this study, the intra-species distance of this extensively sampled *C. striata* was 0.60% which is lower than the genetic distance among *Channa* genus (1.70% variation between *C. striata* and *C. lucius*), indicate that intra-specific divergence among freshwater populations in Malaysia is likely to be significantly less than divergence among congeners.

Hebert *et al.* (2004a) had proposed that the standard threshold level in discriminating species is 10X the mean intra-species variation in which this 10X method was estimated from the ratio of minimum con-generic distance over average con-specific distance. Our result on K2P distance reveal that minimum con-generic variation (1.70%) was almost 11X greater than the average con-specific variation (0.16%), hence this ratio greatly support the Hebert's *et al.* (2004a) 10X benchmark and is parallel to others finding of DNA barcoding in other animal (13X fold higher in Neotropical bats (Clare *et al.*, 2006), 18X fold higher in birds (Hebert *et al.*, 2004a), and 27X fold higher in Canadian freshwater fish (Hubert *et al.*, 2008)). Specifically, when consider particular case from entire dataset, such as in the case of *C. striata* as a representative from a wide geographical studied range, the ratio of genetic distance among *Channa* genus (1.70%) was approximately 3X more than intra-species distance of *C. striata* (0.60%). Although this 3X ratio does not follow Hebert's *et al.* (2004a) 10X inter-species cut off point, however, such case might be due to current study taxonomic sampling was relatively sparse as more *Channa* species are included in the study, the con-generic variation will increase within that study group.

Overall, our entire dataset presented here implies that this value is likely to remain under 10X threshold. In the case of cryptic taxa, our result in DNA barcoding does not reveal any possible putative cryptic taxa of *C. striata* in Malaysia due to several reasons. Firstly, all the haplotypes of *C. striata* shows monophyletic divergence pattern in phylogenetic tree with high statistical support (99% bootstrap value and 0.99 posterior probability value, see **Figure 4.1**). Secondly, the intra-specific divergence (0.60%) of this candidate species is lower than the inter-specific threshold level used in this study (1.6%, which is 10X the mean intra-species variation of 0.16%). Thirdly, this intra-specific divergence of 0.60% is comparatively lower than the con-generic variation among *Channa* genus which is 1.70%. Hence, although *C. striata* have been found to be highly diverging in other parts of its natural geographical distribution in South-East Asia (Adamson *et al.*, 2010), there is no evidence of cryptic diversity in this species discovered in Malaysia.

5.2: MOLECULAR POPULATION DIVERGENCE (COI)

5.2.1: GENETICS DISPERSAL OF *C. striata*

Mitochondrial gene had long been chosen as a candidate marker in understanding dispersal of a species due to its slow mutation rate compared to microsatellite markers which enable it to retain ancestral genotypes and to reveal historical dispersal pattern of certain species. In our study, hap2 being the most frequent and most connected haplotype, hence it is expected to be an ancestral haplotype (Posada & Crandall, 2001) among all *C. striata* haplotypes obtained in Malaysia. The haplotype distribution in Peninsular Malaysia which is parallel to previously reported freshwater fish division by Mohsin & Ambak (1983, 1991) with the exception of non-overlapping haplotype in central division; suggested that there might be a physical barrier located at central division which is most probably prohibiting gene flow to happen between populations at that mentioned division. In nature, populations that inhabited water ecosystem tend to diverge if there is a physical barrier such as mountain range that disconnects the drainage basin and thus the aquatic connectivity between different water sources. Briefly, with the absence of common haplotype between the west and the east coast of Peninsular Malaysia; for instance, within populations in central division of Peninsular Malaysia (between Selangor (west coast) and Terengganu (east coast)) coupled with the evidence of significant population difference between both populations, hence, these evidence are probably can be explained by significant separation of both populations by a backbone in Peninsular Malaysia – Titiwangsa Mountain, ranging from north of Perak through a north-south way to Jelebu, Negeri Sembilan with 480km mountain range length. Geographically, this natural divider had significantly divided Peninsular Malaysia into the west and the east coast with multiple

mountains being found along this Titiwangsa Mountain range with the highest elevation of 2,183m being recorded in Korbu Mountain located in Perak (Gunung Online website :<http://marina.fortunecity.com/harbourside/83/gunung.html> & OGKL website: <http://www.ogkl.com.my/infoGUNUNG/index.htm>). For slow evolving marker, in this case, mtDNA, the higher the genetic differentiation indicates that the separation event was happen at long time ago. Therefore, migration of *C. striata* which was prohibited across the mountain range which result in each population in central division of Peninsular Malaysia acquired their own unique haplotype was probably due to the geographical disconnectivity during the ancient time of separation.

On the other hand, the sharing of the same haplotype between adjacent populations (between Kedah and Pulau Pinang; between Negeri Sembilan and Johor) and the non-significant differentiation between them suggest that gene flow was most likely to happen between short distantly populations, provided that physical barrier was absence or did not significantly disconnects habitats between them. Furthermore, such lateral gene flow pattern between that mentioned populations and the absence of similar dispersal pattern within central coast of Peninsula's had further suggest the nature of laterally migration pattern of *C. striata*. Our suggestion might be explained by the possibility of migration of fish across adjacent drainage system due to flood (Wang *et al.*, 2000) which follows the one-dimensional stepping stone models (Kimura & Weiss, 1964) that allowed migration of fish to adjacent population. In addition, the migration pattern of *Acrossocheilus paradoxus* in Taiwan (Wang *et al.*, 2000) had supported this model. Our data with overlapping haplotype between adjacent locations within the north and the south division of Peninsular Malaysia could be induced by laterally migration behavior of *C. striata* in respond to the lateral dispersal towards flood plain (Hurwood & Hughes, 1998; Wang *et al.*, 2000).

The most likely dispersal patterns of *C. striata* populations, the possible migration route leading to the arrival of ancestral haplotype at the island of Borneo and the departure route of the individual from the island to Peninsular Malaysia were predicted (**Figure 5.1**). It is predicted that the ancestral population was first colonized at the north of Peninsular Malaysia rather than the island of Borneo and the departure of the island's population to the east and the south of Peninsula's was expected given that the possible initial migration event causes colonization of *C. striata* ancestral at the island (**refer section 5.2.2**). The suspected population dispersal patterns of *C. striata* were further divided into three possible consequent stages in this study.

In first stage of migration route, we expected that once the ancestral population had colonized at the north coast of the Peninsular Malaysia, there might be two ways of dispersal route with differential direction at this stage. Specifically, ancestral individuals from Kedah were suspected to migrate to the island of Borneo and then backward to Kedah followed by multi-directional way between these two populations; the second way of dispersal pattern suspected to be a random gene flow between Kedah, Pulau Pinang and Selangor laterally. The absence of ancestral haplotype at Selangor's population might be due to sample size factor, or rather due to geographical disconnectivity between Selangor and both north's populations (Kedah and Pulau Pinang) which had inhibited gene flow to happen once the ancestral individual had colonized at Selangor.

In second stage of migration route, since hap7 was formed before it mutated into hap1, hap4 and hap5; thus, this hap7 which was suspected to be formed at the island is most probably had migrated to the east (Terengganu and Pahang) and the south (Johor) of Peninsular Malaysia which are more prone to be colonization targets for those *C. striata* individuals from the island in relation to the west (Pulau Pinang, Selangor and perhaps Negeri Sembilan) of Peninsular Malaysia. This hap7 might occur at north-west location (Kedah) if more samples to be investigated in this study.

For third stage of migration route, once the hap7 had colonized at the east and the south of Peninsular Malaysia, it is suspected to be mutated spatially and overlapping haplotype pattern between Negeri Sembilan and Johor suggested that gene flow was most likely to happen between them laterally which further suggested the lateral dispersal behavior of *C. striata*. It is suspected that hap1, hap4 and hap5 were formed after separation of Peninsular Malaysia from the island of Borneo by recent formation of South China Sea due to these haplotypes are not being any haplotype leading towards formation of those Sarawak's haplotypes (hap2 and hap7).

Although some of the populations were being sampled at rice field which might be difficulties for the fish migrate towards adjacent river system, however, there was a previous report on lateral dispersal between rice field and original river system as fish migrate into rice field at the beginning of wet season to feed & spawn and return back to permanent water bodies as water level decline (Coche, 1967; Fernando, 1993; Meusch, 1996). *C. striata* which is equipped with air-breathing organ is able to migrate over wet ground (Amilhat & Lorenzen, 2005) and its migration pattern is flavored and is most likely facilitated by lateral dispersal towards floodplains and watersheds (Hurwood & Hughes, 1998; Wang *et al.*, 2000).

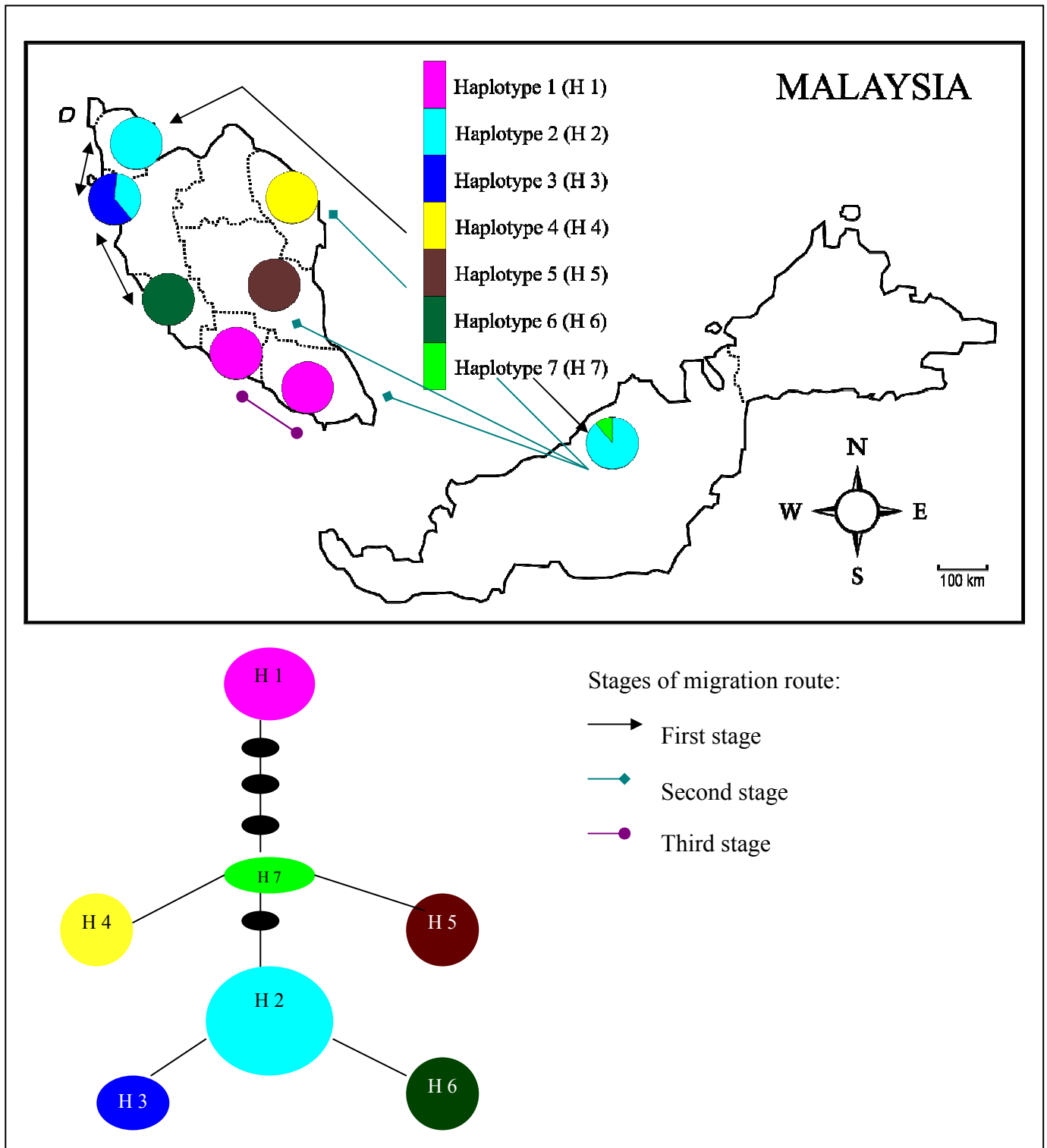


Figure 5.1: Distribution of haplotypes over entire Malaysia with predicted possible migration routes of *C. striata*. Details on the stages of migration routes can be referred to section 5.2.1.

5.2.2: PHYLOGEOGRAPHIC STRUCTURE

With the unexpected evidence of sharing of the same haplotype (hap2) between Kedah, Pulau Pinang (West Malaysia) and Sarawak (East Malaysia) together with the F_{st} value of non-significant population differentiation between both coast, these infered that Peninsular Malaysia and the island of Borneo was once interconnected during ancient period before the recent separating divider – South China Sea being formed. This incomplete population divergence pattern is strongly suggests that possible event, specifically, Pleistocene event had happen that causes historical connection between the mainland and the island. The biodiversity of fauna is greatly affected in the sense that gene flow and migration of fish is allowed across the land bridge – Sunda River during glaciation period with maximum lowering of sea-water level (Voris, 2000). In our study, *C. striata* individual might mingled in this land bridge and thus further lower the population differentiation as gene flow is apparently occur during that Pleistocene Epoch. We suspect that there is no colonization of *C. striata* at the island of Borneo and gene flow is probably happen from the ancestral population in Kedah through Sunda River and migrate to the island. This is supported by previous study of Adamson *et al.* (2010) which hypothesized that the evolution of majority of the *Channa* species including *C. striata* was happen at South-East Asia, specifically, the north part of Indochina which was due to biogeography structure between India and South-East Asia coupled with historical climatic changes that causes fishes flow through south-east way from India. Hence, it is expected that the origin of *C. striata* in Malaysia was first appear at northern region of Malay Peninsula in stead of the island of Borneo. For this candidate species, previous study had showed that the population structure of *C. striata* in Perak was an effect of Pleistocene glaciation that

causes ancient gene flow to happen at the land bridge before the recent submersion by Strait of Malacca (Jamaluddin *et al.*, 2011). Furthermore, the historical formation of Sunda River which connected both the mainland and the island had also explained most of the historical coalescence and incomplete population divergence of other freshwater fish species, such as *Hampala macrolepidota* (Ryan & Esa, 2006), *Tor tambroides* (Esa *et al.*, 2008) and *Barbonymus schwanenfeldii* (Kamarudin & Esa, 2009).

Migration of fish through the land bridge was ended during the deglaciation period with melting of the glacier that lead to flooding, submerged of Sunda River, and thus the formation of South China Sea which is now the recent physical barrier between the mainland and the island. It is suspected that this natural geographical divider is now efficiently separating the island from the mainland with the formation of hap1, hap4 and hap5 which are unique to the mainland in this study. In our current dataset, these three newest haplotypes which were found at the mainland does not lead to formation of any haplotype at the island strongly suggest that there are formed at the mainland before the formation of recent geographical divider – South China Sea which might efficiently prohibiting gene flow among the mainland and the island.

Geographically, population on the east coast of Peninsular Malaysia which is closer to the island of Borneo has a priority to migrate than the west coast due to the water source draining out from the east of mainland towards Sunda River. However, Kedah, as the member of the north-west of Peninsular Malaysia, this candidate population may migrate to closer northern region of Peninsular Malaysia and then flow through the river that draining out towards Sunda River during Pleistocene glaciation. This hypothesis is supported by previous study of Esa *et al.*, 2008 on *Tor tambroides* which shows a

possibility of migration route between northern Peninsular Malaysia and Borneo due to the same haplotype between populations from Batang Ai, Sarawak and Perak, northern Peninsular Malaysia.

In short, the historical dispersal pattern of *C. striata* in this study was probably due to phylogeographic structure of Malaysia during the ancient Pleistocene Epoch with climatic fluctuation that causes changes in land mass configuration. It is expected that complete population divergence will occur after a sufficient time for population differentiation since it is expected that hap1, hap4 and hap5 were unique to the mainland and are absent at the insland in this current study.

5.3 PREVIOUS MICROSATELLITE STUDY

5.3.1: HIDDEN BIODIVERSITY OF *C. striata*

Previous study by Salah & Bhasu (accepted manuscript) on the same *C. striata* population in Peninsular Malaysia revealed possible cryptic taxa of this candidate species in Negeri Sembilan with the evidence of least genetic variability. However, this low genetic variability might be due to other possibility such as founder effect as *C. striata* sampled in Negeri Sembilan is a population represents that local rice field. It is possible that the *C. striata* surveyed in this case was a small founder population that migrated from its original ancestral population in the river and colonized a new habitat in the rice field. This newly formed population at the rice field was unstable due to its small effective population size and hence had a greater chance for genetic drift to occur in addition to high inbreeding depression to further reduce its genetic variability. The rice field landscape may be undergone man-induced alteration that disconnects aquatic habitat connectivity with surrounding river system which might prohibit gene flow between population in rice field and population from surrounding river, and thus increase population differentiation between them.

This Negeri Sembilan population in rice field might be potential cryptic taxa given that the continuation of disconnectivity between the rice field population to the surrounding population over a long period. If, for example, differential selection is acting on genes involved in mate choice, then it is possible that the population in that rice field is reproductively isolated from surrounding populations or even from its original population during founder event. Therefore, such population which is reproductively isolated from its

former breeding population might be a potential hidden cryptic species of *C. striata* in relation to other populations in the future after a sufficient time of population isolation.

5.3.2: RECENT DISPERSAL PATTERN OF *C. striata*

The F_{st} value which underlines population differentiation using microsatellite markers in previous study (Salah & Bhassu, accepted manuscript) does not give a similar population differentiation pairs compared to mtDNA markers used in this study. This is probably due to differential mutation rate of both markers which will reveal different dispersal pattern at different time point. Specifically, microsatellite F_{st} value revealed a significant differentiation among all population in Peninsular Malaysia (except between Pulau Pinang and Pahang) and some significant population pairs was not significantly differentiated using mtDNA markers. Such case is most probably due to recent population separation might causes a significant divergence as microsatellite acquired faster mutation rate which will lead to quicker divergence in allelic composition. Furthermore, all population pairs with significant difference using both markers shows a different extent of differentiation as microsatellite apparently gave a lower significant F_{st} value compared to mtDNA. This is probably due to smaller effective population size of mtDNA as if there are more samples from each site, then the chance of finding haplotypes in common among sites will increases if there truly are some haplotypes in common. Besides, a population pair (Pulau Pinang & Pahang) which shows significant difference using mtDNA in our study does not give a significant differentiation record in recent population dispersal using microsatellite markers. As suspected in previous study (Salah & Bhassu, accepted manuscript), such case can be explained by the possible of illegal man-induced transferring of fish as both locations are distantly apart.

5.4: MORPHOLOGICAL POPULATION DIVERGENCE

5.4.1: ECOLOGICAL HETEROGENEITY

Negeri Sembilan and Johor are genetically similar (sharing of common haplotype and non-significant population difference); however, these locations together with Pulau Pinang were discriminated from others, specifically, Kedah population by root 2 in discriminant function shown in **Figure 4.3** which suggests that environmental factor had significant impact on morphological divergence. In general, morphologically, terrestrial animal and member of aquatic habitat tend to diverge in respond to environmental heterogeneity as morphology is an additive effect of both genetic component and environmental factor. In nature, differential evolution in different environment habitat is common for an animal to increase survival rate and the subsequent natural selection is acting on to select beneficial adaptive character of certain trait.

In this study, for the *C. striata* survey in Peninsular Malaysia, populations in the mainland tends to show head polymorphism and this trait divergence is not concordant to geographical distance and is not parallel to genetic divergence pattern. Hence, we predicted that morphology of *C. striata* was diverged in respond to ecological diversification in terms of water quality and dissolved chemical component. Previous study by Rahim *et al.* (2009) on *C. striata* showed that there is a significant difference of physical–chemical parameters in different environmental localities inhabited by *C. striata* and we predicted that this differences may not correlated to geographical distance, but, appropriately might be due to the extent of habitat destruction in different localities as a result of human development such as agriculture, urbanization and factory development. Such human activities are most likely to causes river pollution as the waste product such as organic

waste, acidic waste, waste particle and other toxic waste will flow into the river basin and will lead to changes in water parameter.

In our case study, populations from Pulau Pinang, Negeri Sembilan and Johor that characterized by one of the head size polymorph (comparative smaller c3 trait and comparative smaller d2 trait) in relation to others locality are not caught in their native river habitat, but, specifically, they are being sampled from local rice field and dam. Briefly, samples from Pulau Pinang and Negeri Sembilan were collected from rice field which might had greater chance to be contaminated by organic chemical, and samples from Johor were caught at nearby dam in which construction of dam might causes changes in water temperature, nutrient levels and plankton production (Ward & Stanford, 1983). This suggests that changes in water parameter in respond to the man-induced habitat contamination will have significant impact on deformity on certain trait, in this case, character on head size. Previous study had reported that deformity of fish morphology had occurred in contaminated river (Sun *et al.*, 2009) and skull deformation is one of the reported deformity fish traits in previous study (Ferguson, 1989; Lindesjoo & Thulin, 1992) in addition to other fish trait such as fin erosion (Sindermann, 1979; Reash & Berra, 1989). Although genetically, genotypic of *C. striata* tends to diverge in relation to physical barrier separation, however, head size polymorphism in this case could explain the morphological divergence of *C. striata* in this study is regardless to its inhabited physical geographical structure; on the other hand, is related to ecological parameter in different locality and hence the differential extent of human development in different sites.

5.4.2: FEEDING BEHAVIOR AND PREDATION

When speculating overall morphological divergence of *C. striata* in Malaysia as a whole, these populations tend to show mouth polymorphism due to the west Malaysia representative were characterized by large mouth (comparative large measurement of b1 trait) which is one of their unique characters in relation to Sarawak population. In this case, polymorphism of mouth trait is probably indicates the feeding behavior of *C. striata* and hence, predation and available food source in different localities. Geographically different sampling sites might reflect the differential amount of food source for certain carnivorous fish, in this case, *C. striata*. The polymorphism in mouth size might be due to the competition on small amount of food source or in certain case whereby the food source is quite abundance but inadequate for larger number of population in certain place. Briefly, the demand on food source and the available food source sometimes is not in equivalent in certain place. In other words, competition will happen if the former is greater than the latter in which the Peninsula's might be at this kind of situation. Hence, in the case of limited available food source, population in Peninsular Malaysia might had acquired larger mouth to be benefited in food competition and this unique character is probably a naturally evolving character which might be coupled with natural selection that had long been selecting this beneficial adaptive character to increase survival rate. In the existing environment habitat, natural selection is expected to select and to maintain adaptive character which is observable as selection is acting on the phenotype. Therefore, the phenotypic changes are probably induced by existing inhabited environment factor.

Our expectation is parallel to previous study on *Neolissochilus soroides* by Roberts & Khaironizam, 2008. Their study reveals that polymorphism in mouth trait will also happen in population occupying same geographical habitat. The three morphs of *N. soroides* in a single river system are highly associated to feeding behavior of the fish and hence, lead to evolution of the mouth structure. Indeed, in addition to freshwater species, terrestrial animal and insect will also acquired morphology polymorphism due to the adaptive variability in different ecology (Grant *et al.*, 1976; Hebert *et al.*, 2004b; Mullen *et al.*, 2009).

In summary, the morphological divergence pattern of *C. striata* can be explained by environmental heterogeneity that induced polymorphism in certain trait as a result of trait deformity in respond to environmental factors. These divergence pattern of morphology is not parallel to the genetically divergence pattern due to morphology is often deal with the additional environment condition in stead of genetic component alone.

Although sexual dimorphism is common in animal kingdom particularly in the case of sexual selection and fishes had been reported to show sexually distinct trait (Kodric-Brown, 1990); however, our current data set did not provide sexual information on *C. striata* specimens due to previous informative karyotyping data on this candidate species did not reveal any sexually distinguishable sex chromosomes (Supiwong *et al.*, 2009). This suggests that sexually distinct character may not present in *C. striata*.

5.5: CONSERVATION ASSESSMENT

While *C. striata* tend to withstand in harsh environment and so had a greater survival rate compared to the other freshwater taxa, conservation planning for this candidate species in Malaysia still required as for example, over-fishing will causes the number of population to decline due to this freshwater species had a great commercialized value to the local. Although our study did not reveal any high diversity population in one of the surveyed locations, however, conservation of this species at its natural variation level is required as *C. striata* forms a diverse group of taxa across Malaysia with seven haplotypes distributed across Malaysia and majority of populations show significant differences among each other.

Although our study did not reveal any commercialized character such as larger body size which will produce more flesh, but our result on morphological divergence pattern of *C. striata* indicated that environment factor had a significant impact on the evolution of certain trait. Thus, this study tends to provide information to further evaluate the water quality in Malaysia on deformity of fish trait due to the developmental of fish trait potentially respond to environmental stresses. To go further, as the country is developing, the extent of human activities such as agriculture and industrial are actively processed leaving heavy-metal contamination in freshwater system which had drawn a significant attention to the conservationists as this may causes aquatic biota and human health being threatened (Paolette *et al.*, 1986; Winston & Di Giulio 1991; Stegemen *et al.*, 1992). The toxicity of heavy-metal which is undestroyable by biological degradation and uncontrollable in aquatic environment (Cleary *et al.*, 2002; Farombi *et al.*, 2007) may causes induction of disease or death in fish which further decrease the wild stock of *C. striata*. Brood-stock management of *C. striata* through cultivation of this species in pond

may increase fish stock for market demand and the excess amount of copper used by farmer to eradicate filamentous algae in pond (Chen & Lin 2001) may be controllable if biomarker of specific fish trait had been identified in the future. Hence, our data in morphology which is suspected diverged in respond to environmental condition was an initial step to further propose the future study of ecological parameter on the developmental of fish trait in selecting specific biomarker for specific contaminated chemical.

CHAPTER 6: CONCLUSION

To date, all the populations of *C. striata* surveyed in this study remain as single taxa across Malaysia and variation did exist by using both molecular and morphological approaches to examine the presence of variability by both aspects in natural occurring population of *C. striata*. However, previous study using microsatellite markers did not revealed a parallel divergence pattern when compared to our mtDNA divergence pattern of *C. striata*. This incongruent divergence pattern is probably due to differential mutation rate of both markers and hence mtDNA tends to reveal historical dispersal pattern of *C. striata* whereas fast evolving rate of microsatellite markers is able to reveal recent population dispersal pattern. However, both genetically divergence pattern is not congruent to morphological divergence pattern of *C. striata*. In such case, environment factors, for example, available food source and changes in dissolved chemical compound or temperature may change the phenotype of certain organism by influencing its development. Such development in respond to inhabited environment conditions is not correlated with underlying genetic differences. Briefly, two genetically divergent individuals may have the same morphology if they are raised in the same environmental conditions, while two genetically identical individuals raised in different environments may turn out phenotypic observed different.

Our study was an initial step in proposing spatial conservation assessment to conserve this candidate species in different aspect. For instant, genetically divergence pattern in this study tends to provide information to further conserve this species genetically, in other words, to maintain the population speculated through the aspect of

haplotype diversity in order to retain its biodiversity richness and population stability from certain genetic drift. In terms of conserving this species in brood-stock management inferred using potential biomarker, different conservation assessment should be proposed regardless to haplotype diversity as the phenotypic variability is not correlated with underlying genetic differences. Since water quality have high impact on the morphological divergence pattern of *C. striata*, in order to ensure high quality production of this species in aquaculture, management planner should speculate on the issue of water quality. Our study provides information to the ecologist to further evaluate the water quality on the trait deformity of this possible bio-indicator fish, so that optimum brood-stock management strategy can be proposed. The potential strategy, for instance, using biomarker inferred environmental stresses in pond if specific biomarker for particular contaminated chemical compound is identified in the future. Henceforth, to achieve this strategy, future study on the correlation between the contaminated water parameter and the deformity of certain fish trait is probably the suggested study to be conducted.