THE BIODIVERSITY AND SYSTEMATIC RELATIONSHIPS OF THE LABEOIN GROUP OF FISHES WITHIN THE SUB-FAMILY CYPRINIDAE IN PENINSULAR MALAYSIA

EMI SHERIZAN BINTI AB RAHIM

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by

EMI SHERIZAN BINTI AB. RAHIM

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ABSTRAK

Kajian morfologi dan sistematik molekul telah dijalankan terhadap kumpulan ikan Labeoin yang diperolehi daripada Semenanjung Malaysia dan Tasik Tonle Sap di Kambodia. Ini adalah dokumentasi ulong berkenaan sistematik dan perhubungan filogenetik antara spesies Labeoin yang menggabungkan kaedah morfologi tradisional dan kuantitatif dengan teknik molekular. Spesies yang dikaji adalah *Garra taeniata, Labeo chrysophekadion, Labiobarbus festivus, L. leptocheilus, L. ocellatus, L. siamensis, Osteocheilus hasselti, O. melanopleura, O. spilurus, O. vittatus, O. waandersii* and *Tylognathus caudimaculatus.* Sebanyak 282 sampel (281 sampel untuk jaringan truss) yang terdiri daripada 11 spesies Labeoin dikira dan diukur untuk memperolehi data meristik dan morfometrik. Data kuantitatif seterusnya dianalisa berdasarkan analisis varians (ANOVA satu hala) analisis prinsipal komponen (PCA) dan analisis diskriminasi (DFA) menggunakan perisian SPSS versi 11.5. Keberkesanan diskriminasi adalah paling tinggi melalui teknik jaringan truss diikuti oleh teknik morfometrik klasikal dan meristik. Keseluruhannya, tujuh daripada lima belas ciri iaitu DR (sirip dorsal), LLS (sisik di atas garisan lateral), PRES (sisik pada kawasan predorsal), TRSL (sisik pada kedudukan melintang - bawah), CIPS (sisik pada sirkumpedunkular), LLID (bilangan sisik di atas garisan lateral, bermula dari sisik pertama sehingga ke hujung sirip dorsal) dan LLOA (bilangan sisik di atas garisan lateral, bermula dari sisik pertama sehingga permulaan sirip anal) banyak menyumbang di dalam diskriminasi spesies Labeoin berdasarkan ciri meristik. Melalui teknik morfometrik klasikal, ciri HL (lebar kepala), ED (diameter mata), SNL (lebar muncung), PRED (jarak predorsal), PAD (jarak preanal), DBL (lebar tapak sirip dorsal), CPD (kedalaman kaudal pedunkel pada kedudukan vertebra terakhir) dan CPL (lebar kaudal

pedunkel) dikenalpasti sebagai faktor diskriminasi yang penting. Dalam morfometrik jaringan truss, ciri rerangka kepala dan bahagian tengah badan adalah berkesan dalam membezakan spesies. Keputusan ini diperkukuhkan lagi menerusi kaedah penjujukan DNA sebahagian gen mitokondria 16S rRNA (730 bp) daripada 52 sampel yang mewakili spesies Labeoin untuk menyiasat perhubungan filogenetik kumpulan ikan ini. Data jujukan DNA disejajarkan dengan perisian ClustalX sebelum menjalankan analisis filogenetik dengan menggunakan perisian PAUP. Filogeni di antara takson dibina melalui kaedah 'Neighbor Joining' (NJ) dan 'Maximum Parsimony' (MP). Pokok filogenetik NJ menunjukkan persamaan topologi dan struktur populasi dengan analisis MP kecuali sedikit variasi dalam nilai 'bootstrap' dan spesies yang menjadi 'takson beradik/seinduk' kepada kumpulan takson dalaman. Dua kelompok utama telah terbentuk, yang pertama mengandungi *Garra*, *Tylognathus* dan kumpulan monofiletik *Labiobarbus* manakala yang satu lagi membentuk kumpulan monofiletik *Osteochilus*. Setiap spesies dan genus yang ditafsirkan mengikut morfologi tradisional membentuk kumpulan monofiletik tersendiri kecuali kelompok *O. waandersii* dan O. *vittatus*. Kajian yang lebih terperinci terhadap ciri morfologi dan genetik populasi dicadangkan untuk menangani masalah taksonomi kumpulan ini. *Labeo* membentuk 'takson beradik/seinduk yang jauh kepada lain-lain spesies Labeoin. Perbandingan jarak genetik di antara *Labeo chrysophekadion* dengan lain-lain spesies mencadangkan semakan semula taksonomi spesies ini mungkin diperlukan. Penggabungan kedua-dua teknik iaitu morfologi kuantitatif dan kualitatif dan analisis molekular telah ditunjukkan dapat memberikan pemahaman yang lebih baik dan seterusnya menyelesaikan beberapa isu kontroversi yang berkaitan dengan kompleks ikan Labeoin.

THE BIODIVERSITY AND SYSTEMATIC RELATIONSHIPS OF THE LABEOIN GROUP OF FISHES WITHIN THE SUB-FAMILY CYPRINIDAE IN PENINSULAR MALAYSIA

ABSTRACT

A morphological and molecular systematics study was conducted on Labeoin group of fishes obtained from Peninsular Malaysia and Lake Tonle Sap in Cambodia. This is the first documentation of the systematics and phylogenetic relationships among Labeoin species integrating traditional and quantitative morphological and molecular techniques. The species investigated were *Garra taeniata, Labeo chrysophekadion, Labiobarbus festivus, L. leptocheilus, L. ocellatus, L. siamensis, Osteocheilus hasselti, O. melanopleura, O. spilurus, O. vittatus, O. waandersii* and *Tylognathus caudimaculatus*. A total of 282 samples (281 samples in truss network) consisting of 11 Labeoin species were counted and measured to obtain meristic and morphometric data respectively. The quantitative data were then analysed based on analysis of variance (oneway ANOVA), Principal Component Analysis (PCA) and Discrimination Analysis (DFA) using SPSS version 11.5 software. Discriminating efficiency was highest in the truss network morphometric followed by classical morphometric and meristic technique. Overall, seven of the fifteen characters namely DR (dorsal rays), LLS (lateral line scales), PRES (predorsal scales), TRSL (transverse scales - lower), CIPS (circumpeduncular scales), LLID (number of lateral line scales opposite the insertion of dorsal fin) and LLOA (number of lateral line scales opposite origin of anal fin) contributed most in the discrimination of Labeoin species based on meristic characters. Through classical morphometric technique, characters HL (head length), ED (eye diameter), SNL (snout length), PRED (predorsal distance), PAD (preanal distance), DBL (dorsal fin base length), CPD (caudal peduncle depth at end of vertebrae) and CPL (caudal peduncle length) were found to be important discriminant factors. In truss network morphometrics, the characters outlining the head and mid-body region were highly effective in discriminating the species. Results were further supported by sequencing of partial 16S rRNA mtDNA gene (730 bp) from 52 representative samples of Labeoin species to investigate the phylogenetic relationships. DNA sequence data were aligned by ClustalX software before phylogenetic analysis using PAUP was conducted. Phylogeny among taxa was constructed through Neighbor Joining (NJ) and Maximum Parsimony (MP) methods. The NJ phylogenetic trees obtained showed similar topology and population structuring to the MP analysis except for slight variation in bootstrap values and the species forming the sister taxon to the ingroup taxa. Two main clusters were formed, the first one comprising of *Garra*, *Tylognathus* and a monophyletic *Labiobarbus* lineage while the other minor clusters were formed of a monophyletic *Osteocheilus* species. Each species and genus as defined by traditional morphology form their own monophyletic assemblage apart from the *O. waandersii* and *O. vittatus* cluster. More detailed studies of the morphological characteristics and population studies is suggested to address the problematic taxonomy of this group. *Labeo chrysophekadion* formed a distant sister taxon to all other Labeoin species. Genetic distance comparisons of *Labeo chrysophekadion* with the other species suggests that a taxonomic revision of this species may be required. Merging the two techniques i.e. quantitative and qualitative morphological and molecular analysis were shown to provide better understanding as well as resolving several of the controversies associated with the Labeoin fish complex**.**

CHAPTER ONE

INTRODUCTION

South East Asia has the distinction of having the richest cyprinid fauna in the world. The dominant group is the sub-family Cyprininae. The very large sub-family Cyprininae has many genera and species in South East Asia (Rainboth, 1991). Rainboth (1991) observed that whereas South East Asia has an area 72% the size of North America it has five times as many cyprinid species and five times as many genera. Zakaria-Ismail (1994) reported that some 70 genera of cyprinids are endemic to the area.

According to Roberts (1993) the systematics and hence species identification of Labeoin cyprinids of tropical Asia is in a highly confused state. Cyprinids occur in both brackish and freshwater habitats. There are an estimated 210 genera and 2010 species in this group (Liu & Chen, 2003; http://www.fishbase.org). Generally they can withstand a temperature range of 0 - 40°C. Many species are endemic to South East Asia and to Malaysia in particular. Many of their interrelationships are questionable, whether among themselves or with other Asian genera assigned to the same sub-family. The centre of origin, evolution and radiation of Labeoin cyprinids is mostly the Malay archipelago, and the islands of Sumatra and Borneo. The relationship is mostly unclear and those of the endemic are particularly obscure (Winfield & Nelson, 1991).

The present day Asian mainland begins at the southern tip of the Malay Peninsular. The narrowest part of the Malay Peninsular is the Isthmus of Kra, which is also a point of climatic demarcation. South of the Isthmus the climate is slightly seasonal and north of it are pronounced seasons. The Isthmus marks the change from evergreen rainforest to monsoon forest to the North in Thailand and the Indo-Chinese Peninsular (Rainboth, 1991).

Studies of the Cyprinids are hampered by the poor state of systematic knowledge in Malaysia and also in South East Asia. This was evident from studies of the freshwater fishes in Malaysia (Khoo *et al*., 1987). Polyphyletic assemblages and uncertain or conflicting interpretation of genetic relationships do not lend themselves to a clear analysis. This has complicated many ecological studies of freshwater fish in Malaysia and in the Asian continent. Leading world authorities on Cyprinids referred to these taxonomic problems as "intergrades from the Malay Peninsular" (Roberts, 1993). Furthermore, there has been no agreement among fish taxonomists on the systematic status of many Cyprinidae. These taxonomic problems persist because most of the earlier investigations were solely based on classical techniques. A synthesis of classical techniques with recent morphometric analysis and modern molecular techniques examining specific portions of the fish DNA may provide a solution to the South East Asian Cyprinid taxonomic problem specifically among the Labeoin species.

The morphometric technique based on the truss network method is an important technique that is frequently applied to detect species and population differences not detected using traditional morphological technique (Strauss & Bookstein, 1982; Cavalcanti *et al*., 1999; Siti Azizah *et al*., 2001; Jayasankar *et al*., 2004; Rutaisire *et al*., 2005). It involves measuring distances between homologous landmarks over the entire length on the surface of the fish. It is a reconstruction of the original shape of the organism. It is able to detect differences in overall shape and size of individuals among species as well as populations. Both factors are known to be influenced genetically and environmentally.

Development of DNA sequencing technology has provided systematists a powerful tool for elucidating evolutionary relationships among species (reviewed by Billington & Herbert, 1991; Hillis *et al.,* 1996). This technique has been widely used for nuclear (Rüber *et al*., 2004; Mayer *et al.*, 1998) and mitochondrial markers (Craig *et al*., 2001; Near *et al*., 2003; Orrell & Carpenter, 2004; Rutaisire *et al*., 2005). The mitochondrial genome is typically between 16000 and 18000 base pairs in length, haploid and is, in general, maternally inherited. Its rapid rate of evolution, clonal inheritance, and lack of recombination has made it a valuable source for studies ranging from intraspecific phylogeography and gene flow, delineation of species boundaries, and phylogeny reconstruction. As pointed out by Lansman *et al*. (1982) the level of diversity among mtDNAs appears to be uncoupled from that of morphological or nuclear gene diversity. Mutation rates appear to be often relatively constant although different genes appear to evolve at different rates. Thus, an analysis of mtDNA provides a high resolution of evolutionary processes among species. Furthermore, a number of independent studies can share the published fragment data for the estimation of genetic diversity. In addition to the sequence analysis of specific haplotypes, restriction enzyme analysis on the mitochondrial DNA (Saitoh *et al*., 1995; Ellsworth *et al.*, 1995; Chakraborty *et al*., 2005) can also be performed. However in this study only the sequence analysis of a selected gene was investigated.

Many studies have revealed that the relative efficiency of markers as population discriminators is likely to be both species and population specific, so it may be beneficial or even necessary to try various methods for a comprehensive assessment. The number of studies on the Labeoin species based on morphometrics and molecular methods have at most being limited (Zhang, 2005; Das *et al*., 2005; Rutaisire *et al*., 2005). To date, no attempt to elucidate evolutionary relationships of the Labeoin group of fishes using molecular approach has been conducted on this group. Advances in molecular biology provide potentially efficient tools in understanding the biology and the relationships among this fascinating group of fishes.

In this study two research approaches, molecular and quantitative morphological techniques were applied in order to assess the effectiveness of each method against the objectives of this study. For the quantitative morphological investigation, three methods namely classical and truss network morphometrics and meristics were applied to elucidate the interrelationships and discriminant level among this group of fishes. The fish was counted and measured for its meristic and morphometric characters respectively. The other part of this study involved molecular investigation. Partial sequencing of 16S rRNA mitochondrial (mtDNA) gene was examined for this purpose.

Due to logistic and financial constraint only a small group of Labeoin within the Cyprininae sub-family were investigated. This included three genera found in Peninsular Malaysia namely *Garra*, *Labiobarbus, Osteocheilus* and *Tylognathus*. In addition, two species namely *Labeo chrysophekadion* and *Labiobarbus siamensis* were obtained from the Tonle Sap in Cambodia. This was made possible by the collaboration fostered between The Cambodian Mekong Fish Conservation Project, Inland Fisheries Research and Development Institute (IFReDI) and School of Biological Sciences, Universiti Sains Malaysia. A study directed at solving the relationship within the Labeoin group will help solve some of the systematic and evolutionary problems posed by the Cyprinids and perhaps provide biologists and managers with better insights of the biology of the group.

The three objectives in this study were 1) To investigate the systematic status of the Labeoin group of fishes within the sub-family Cyprininae in Peninsular Malaysia and Cambodia 2) To identify and classify problematical specimen of the Labeoin group of fishes 3) To compare the discriminatory efficiency of the three different methods used (meristic, morphometric and mtDNA by direct sequencing) in differentiating the Labeoin fishes.

CHAPTER TWO

LITERATURE REVIEW

The largest family of freshwater fishes, the Cyprinidae which are commonly known as minnows or carps comprises of 210 genera and 2010 species (Liu & Chen, 2003). Approximately 1270 species are native to Eurasia, 475 species in 23 genera are native to Africa while about 270 of Cyprinid species in 50 genera are endemic to North America. Of this the greatest diversity and number of species occurs in China and Southeast Asia (Wu, 1981; Nelson, 1994).

Cyprinids appear in various sizes and shapes and can be found in almost every type of waters; small streams, rivers, lakes and pools. Their bodies are fully covered with scales but no scales are found in the head area (Sterba, 1962; Mohsin & Ambak, 1992). The family Cyprinidae as characterised by Inger & Chin, (1962) as having none, one or two pairs of barbels while Howes as noted in Winfield & Nelson, (1991) characterised the Cyprinids based on the lack of jaw teeth, possession of a protrusile upper jaw and their pharangeal teeth. Furthermore, according to Moyle & Joseph, (2000), the family Cyprinidae are distinguished by their pharaengeal teeth and thin lips with the upper jaw usually bordered only by the premaxilla.

The Labeoins, a complex within Cyprinids, which include *Garra, Labeo, Labiobarbus, Osteocheilus* and *Tylognathus* are characterised by their vomero-palatine organ, double-foraminated dilatator fossa and hypertrophied supraneural bones with the anterior usually contacting the cranium (Howes, 1991). Several members of the Labeoin are of commercial value. Some, such as the *Labiobarbus* and *Osteocheilus* are marketed as cheap protein source although fresh *Osteocheilus melanopleura* can fetch a good market price of RM 13 per kilogram (personal communication). In addition, *Labiobarbus* and *Osteocheilus* species are also commercially processed into fermented fish or ‘pekasam’. Another of its use is as fish bait for other more commercially valuable fishes. Another Labeoin i.e. the *Garra* is also popular as an aquarium fish (http://www.fishbase.org).

One major factor that threatens the survival of wild organism including the Labeoins is habitat destruction due to human activity. Dams are constructed for flood control, a very human concern, as well as for water quality improvement and for human recreation, such as fishing, swimming and boating. However, construction of dams creates a barrier to upstream movement of fish cutting off migration routes. Eutrophication in lakes destroys the aquatic plants necessary for cyprinid spawning. Besides that, it also creates a non-riverine situation above the dam in the form of a reservoir and alters or creates new types of riverine habitats below the dam (Kott, 1997 available at http://www.adm.uwaterloo.ca/infowast/watgreen/laurelcreek/24.html). In addition, pollution (organic and inorganic), overfishing and competition for water resources such as for agricultural irrigation are also important factors threatening cyprinids and other groups (Khan *et al*., 1996).

The IUCN Red List by the International Union for Conservation of Nature and Natural Resources includes 252 species of Cyprinids of which 15 of them are categorized as extinct; one as extinct in the wild; 39 as critically endangered; 31 as endangered; 89 as vulnerable; 6 as lower risk/ conservation dependent; 23 as lower risk/ near threatened; and 48 as data deficient. Of the 15 extinct species, 12 are from the Americas, one from East Asia, one from the Middle East, and one from Europe. However, none of the species investigated in this study (Table 3.1) is listed in the Red List (http://www.iucnredlist.org).

The identification of many Cyprinidae including the Labeoin is considered taxonomically difficult and as a consequence, the classification and systematics of this group is still in a grey area by most taxonomists (Howes, 1991). Many comparative anatomical studies have been reported to clarify the taxonomic status of this group.

Most of them were generated based on traditional taxonomy which generally focused on morphology alone (Reid, 1987) without consideration of their interrelationships. However, in recent years, increasing number of studies on the phylogeny of the East Asian Cyprinids using morphological and molecular techniques have been documented by several authors (Liu & Chen, 2003; Zhang, 2005; Das *et al.*, 2005).

From their findings based on mtDNA control region data, Liu & Chen, (2003) concluded that Cyprinine and Leuciscine are basal lineages in Cyprinidae with Labeoinae at the basal position in Cyprinine. Das *et al.*, (2005) did an investigation on the relationship among 6 *Labeo* species at the nuclear DNA level of variation using the RAPD technique. However, their study did not include *Labeo chrysophekadion* which is one of the species investigated here. Zhang, (2005) investigated phylogenetic relationships of disc bearing Labeonine Cyprinids with genus *Garra* as a basal lineage.

In this study, the phylogenetics of twelve Labeoin species found in Peninsular Malaysia and the Tonle Sap in Cambodia was investigated. The hierarchical taxonomy and systematic classification of this group is presented as follows. Each species investigated is illustrated in Plates 2.1 - 2.13.

Kingdom	**:** Animalia
Phylum	**:** Chordata
Subphylum	**:** Vertebrata
Class	**:** Actinopterygii
Subclass	**:** Teleostei
Order	**:** Cypriniformes
Suborder	**:** Cyprinoidea
Family	**:** Cyprinidae
Subfamily	**:** Cyprininae
Tribe	**:** Labeonini
Subtribe	**:** Labeones
Genera	**:** *Garra, Labeo, Labiobarbus, Osteocheilus* and *Tylognathus*
Species	**:** *Garra taeniata*
	Labeo chrysophekadion
	Labiobarbus festivus
	Labiobarbus leptocheilus
	Labiobarbus ocellatus
	Labiobarbus siamensis
	Osteocheilus hasselti
	Osteocheilus melanopleura
	Osteocheilus spilurus
	Osteocheilus vittatus
	Osteocheilus waandersii
	Tylognathus caudimaculatus

Plate 2.1 : *Garra taeniata*

Plate 2.2 : *Labeo chrysophekadion*

Plate 2.3 : *Labiobarbus festivus*

Plate 2.4 : *Labiobarbus leptocheilus*

Plate 2.5 : *Labiobarbus ocellatus*

Plate 2.6 : *Labiobarbus siamensis*

Plate 2.7 : *Osteocheilus hasselti*

Plate 2.8 : *Osteocheilus melanopleura*

Plate 2.9 : *Osteocheilus spilurus*

Plate 2.10 : *Osteocheilus vittatus*

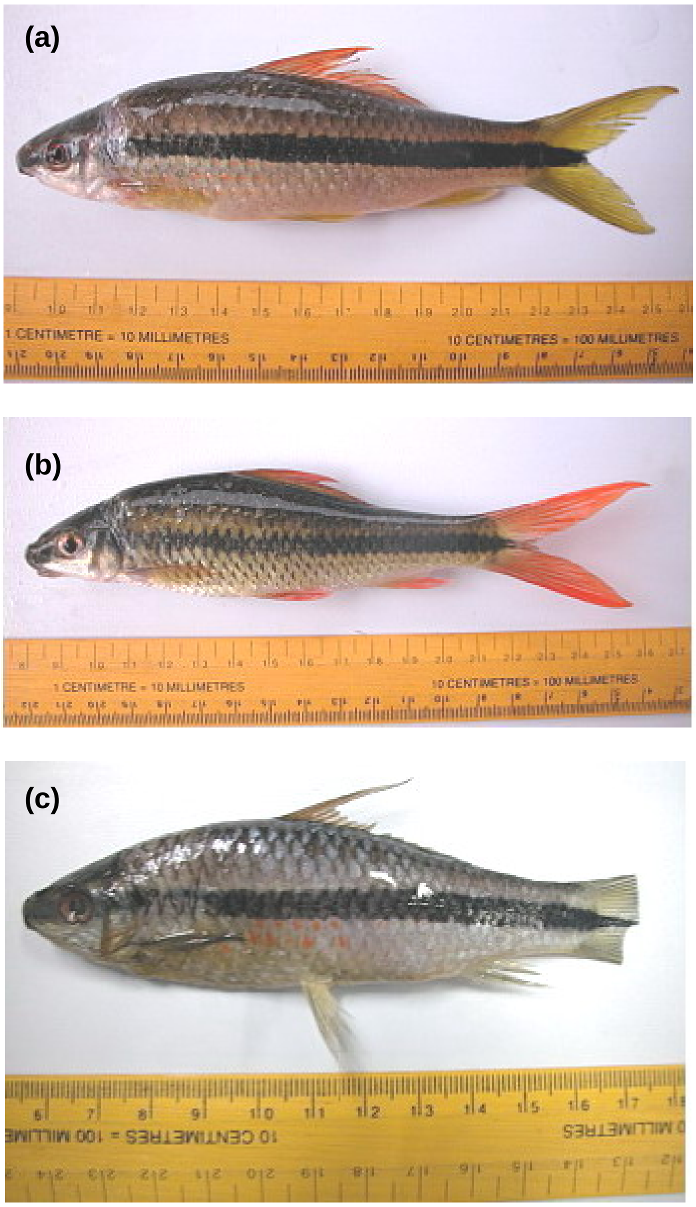

Plate 2.11 : *Osteocheilus waandersii* obtained from different sites; (a) yellow to olive green fins mostly at the pectoral, pelvic and caudal peduncle area with the exception of dorsal fin which was orange in colour and (b) orange fins - Taman Negeri (c) dusky fins - Tasik Bera.

Plate 2.12 : *Tylognathus caudimaculatus*

Plate 2.13 : *Botia modesta*

2.1 Genus *Garra*

The genus *Garra* was formally known as Discognathus (Sterba, 1962). The diagnostic character of this genus is the morphology of the upper lip. It is not separated from the snout by a groove, but continuous with the skin of the snout with the mouth conspicuously inferior (Fig. 2.1a). The lower lip is modified into a sucking disk structure (Fig. 2.1b) which enable the fish to adhere rocky bottoms in fast flowing water of streams (Sterba, 1962). There is no dorsal fin spine observed. In addition, this genus possess 8 branched dorsal fin rays and 1 or 2 pairs of barbels. The genus *Garra* is restricted to Asia; Mekong, Chao Phraya basins and the Malay Peninsular.

2.1.1 *Garra taeniata* (Smith, 1931)

Garra taeniata (Plate 2.1) or 'Stone lapping minnow' is frequently important in aquarium fish trade because of its colour pattern (Rainboth, 1996). It has a broad midlateral stripe with a width of about equal 2 scale rows. The dorsal fin has two black bands while the caudal fin is plain with no dark margins. Well-developed tubercles are found on the snout. This species feeds on periphyton, phytoplankton, insects (Roberts, 1997) and algal matter on stones (Sterba, 1962).

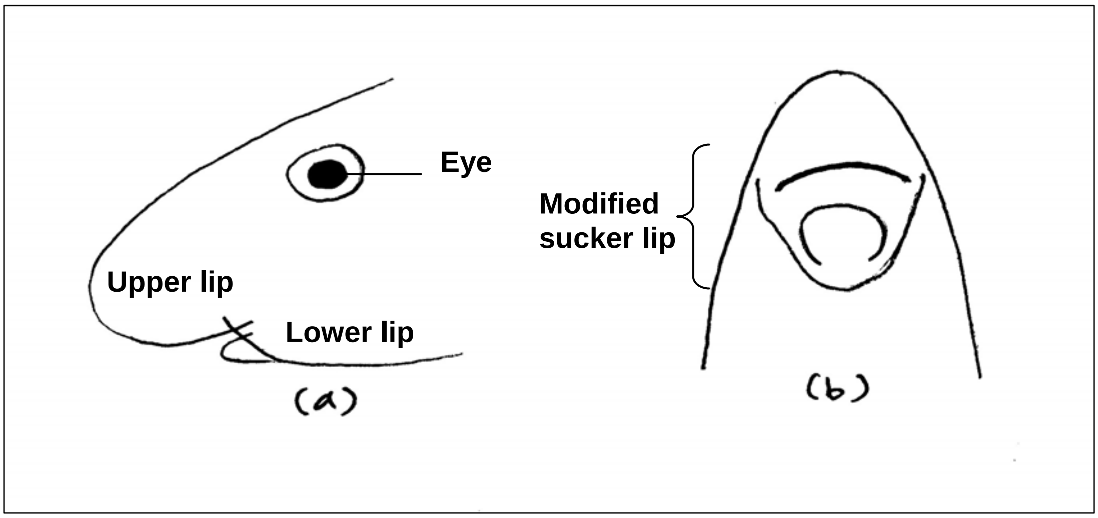

Fig. 2.1 : Side view of the upper lip structure (a) and ventral view of modified sucker lip (b) in *Garra* species.

2.2 Genus *Labeo*

About 102 species of *Labeo* has been documented. According to (Kottelat, 1998) they are characterised by having 41 to 60 scales in the lateral line. In this genus, no dorsal fin spine was observed along the very large sail-like dorsal fin. Their lower lip was separated by a deep groove. Other than that, they also possess a large rostral and 2 pairs of maxillary barbels (Rainboth, 1996). Numerous tubercles were found on their snout. Like the *Garra*, *Labeo* species also like to adhere to the bottoms of streams and rivers with fast running water.

2.2.1 *Labeo chrysophekadion* (Bleeker, 1850)

Labeo chrysophekadion (Plate 2.2) is also known as the ‘black sharkminnow’, due to its black body and fins. The dorsal fin is large with 15 to 18 anterior branched dorsal rays and is normally longer than the head length (http://www.fishbase.org). Both upper and lower lips are fringed with papillae (Rainboth, 1996). Large adults are normally grey with a single spot on their scale while all juveniles are black (Kottelat, 1998). Lower lip is separated by isthmus by a deep groove.

The distribution of this species is mainly in the Asian region; Mekong and Chao Phraya basin, Malay Peninsular, Sumatra, Java and Borneo (http://www.fishbase.org). However, none was found during the sampling trips in Peninsular Malaysia. The samples collected were obtained from the Tonle Sap in Cambodia.

2.3 Genus *Labiobarbus*

The genus *Labiobarbus* is widely distributed in South East Asia and are among the most common fish obtained from the sampling sites in this study. In Malaysia, it is locally known as ikan kawan or "friendly barb". The genus is characterized by an elongated body and having 21 or more branched rays on the dorsal fin (Lim, 1975). It is important as a cheap protein source especially in the rural areas (Ismail, 1989) and is also recognised as an aquarium fish.

There are five species of *Labiobarbus* reported in Peninsular Malaysia namely *L. fasciatus*, *L. festivus*, *L. leptocheilus* and *L. ocellatus* (Ismail, 1989; Roberts, 1993) and one morphologically problematical species (intergrades of *L. leptocheilus* and *L. festivus*) (Roberts, 1993). According to Roberts (1993) and Kottelat (1994), *L. sabanus* is endemic to Sabah while *L. lamellifer* is only restricted to Kalimantan, Indonesia. *Labiobarbus* species is not in high demand in Malaysia due to its bony feature (Leong, 2002). Roberts, (1993) reported four important characters namely the scales on the lateral line series, dorsal fin colouration, number of dorsal fin rays and measurement of maxillary barbels (short or long) as guidelines in differentiating *Labiobarbus* species while Smith (1945) in Karnasuta (1993) reported that the *Labiobarbus* species are differentiated from each other based on the number of circumpeduncular scales present.

2.3.1 *Labiobarbus festivus* (Heckel, 1843)

Labiobarbus festivus (Plate 2.3) or the 'signal barb' is fairly uncommon and in Peninsular Malaysia was reported to be found in abundance only in Tasik Bera and Tasik Chini in Pahang (Khan *et al*., 1996; Leong, 2002). They have also been reported in other parts of Asia but in small numbers: Southern Malay Peninsular (Pahang, Selangor, Endau-Rompin, Johore) and Borneo, Indonesia (Kapuas, Seruyan, Kahajan and Barito). Morphologically, this species is easily identified as it possess an intense colouration compared to other species of the genus. The dorsal fin has a well defined black marginal and broad red submarginal stripes with dusky appearance at the base of the fin rays. The caudal fin has red marginal stripes. Submarginal stripe colouration of caudal and dorsal fins can be applied as species-specific morphology of *L. festivus* (Roberts, 1993). Number of branched dorsal rays observed in this species was less than 22.

2.3.2 ***Labiobarbus leptocheilus*** **(Valenciennes, 1842)**

Labiobarbus leptocheilus (Plate 2.4) is the most abundant species in this genera and can normally be found in all major streams in Peninsular Malaysia. Their dorsal ray count is 21 to 28 in total with 33 to 34 lateral line scales. Their caudal and dorsal fins are dusky. There are spots on each scale on the lateral line forming the longitudal stripes along the body (http://www.fishbase.org).

2.3.3 ***Labiobarbus ocellatus*** **(Heckel, 1843)**

Labiobarbus ocellatus (Plate 2.5) is the most common cyprinid in Tasik Chini, Pahang (Leong, 2002). It has a fairly limited distribution and are reported to be found only in Malaysia and Indonesia. Compared with other *Labiobarbus* species, they have smaller sized scales numbering 61 to 68 on the lateral series (Roberts, 1993; Kottelat, 1994). Two prominent spots are observed on their body; a black humeral spot and a black ocellated spot at the caudal area. The maximum size that has been recorded was around 22 cm in total length (http://www.fishbase.org).

2.3.4 ***Labiobarbus siamensis*** **(Sauvage, 1881)**

Labiobarbus siamensis (Plate 2.6) are well distributed in Asia: Chao Phraya, Bankpakong and Mekong basins. Caudal and dorsal fins are dusky with around 25 to 30 branched dorsal fin rays. As described by Smith (1945) in Karnasuta (1993), the circumpeduncular scales number approximately 20 with scales along the lateral line equal to 43 to 45. The caudal peduncle has a round black spot.

2.4 Genus *Osteocheilus*

The genus *Osteocheilus* as described by Sterba, (1962) is confined to the East Indies. Thirty three species have been recorded in this continent. Of the 33, five species were obtained in this study. This genus is characterised by the lips (Fig. 2.2) which is fully covered with folds and plicae (Karnasuta, 1993). Karnasuta, (1993) stressed the importance of number of lateral line scales in the discrimination of *Osteocheilus* species.

2.4.1 *Osteocheilus hasseltii* (Valenciennes, 1842)

Osteocheilus hasselti (Plate 2.7), also known as terbul locally or silver sharkminnow is widely distributed in all types of habitats and can normally be found in large streams with slow moving water and muddy to sandy substrate (Kottelat, 1998) together with *L. leptocheilus* which is also a common fish obtained during the sampling period in Peninsular Malaysia. *Osteocheilus hasselti* are recognized as having 31 to 35 lateral line scales, 12 to 18 branched dorsal fin rays, 8 anal soft rays and a round blotch on the caudal peduncle (Kottelat, 1998). They feed on roots of plants, unicellular algae, crustaceans, periphyton and phytoplankton (Rainboth, 1996).

2.4.2 *Osteocheilus melanopleura* (Bleeker, 1852)

Locally known as kelabau, *O. melanopleura* (Plate 2.8) has a vertical black blotch above their pectoral fins. There are about 45 to 53 scales along the lateral line and 16 to 18 branched dorsal rays (Roberts, 1989). Their body is silverfish grey. The maximum size recorded was 60.0 cm (SL) based on Baird *et al*., 1999. They are fairly well distributed in the Asian region; Peninsular Malaysia, Sumatra, Borneo, Mekong and Chao Phraya basins.

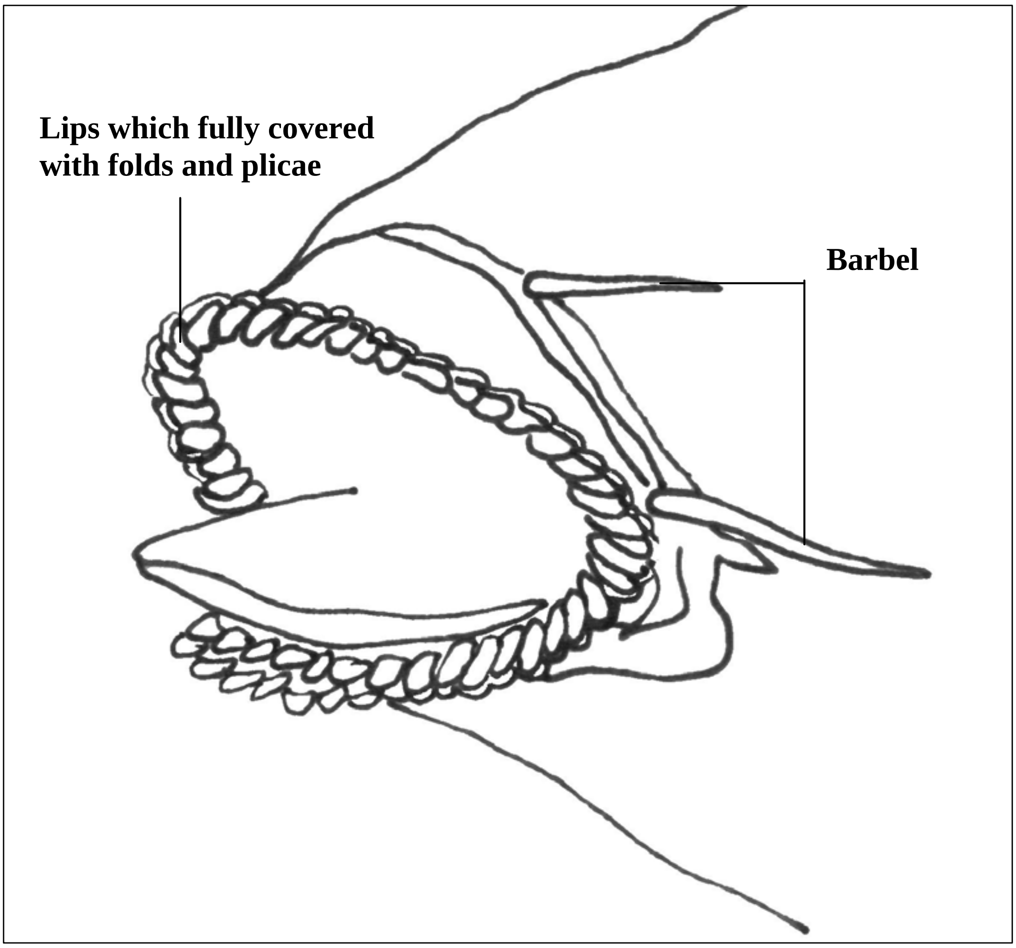

Fig 2.2 : Lips of the genus *Osteocheilus* species.

2.4.3 *Osteocheilus spilurus* (Bleeker, 1851)

Osteocheilus spilurus (Plate 2.9) is the smallest species among the genus *Osteocheilus* which can be found in the Malay Peninsular, Sumatra, Java (Indonesia) and Borneo. They have 27 - 29 scales on their lateral series. There is a round dark spot on its caudal peduncle. The reported maximum size of this fish was about 7.5 cm (SL) (Kottelat *et al.,* 1993). The *O. spilurus* obtained from Pondok Tanjung, Perak was only about 3.5 cm in SL.

2.4.4 *Osteocheilus vittatus* (Valenciennes, 1842)

Bonylip barb is the generic name given to *Osteocheilus vittatus* (Plate 2.10). It is commercially important as cheap protein source and also as an aquarium fish. In terms of morphology, it possesses a broad black longitudal stripe which originates at the tip of the snout to the end of their caudal base (Inger & Chin, 1962; Sterba, 1962), 32 - 34 lateral line scales and 11 - 13 branched dorsal fin rays numbering which is quite similar to *O. waandersii* based on morphology. Furthermore, both species share same number of lateral line scales, branched dorsal fin rays and both having tubercles on their snout. This fish can be found in Thailand, Sumatra, Borneo, Java and lower Myanmar.

2.4.5 *Osteocheilus waandersii* (Bleeker, 1852)

Osteocheilus waandersii (Plate 2.11, a-c) is also (cf *O. vittatus*) identified as having a broad black longitudal stripe which originates at the tip of the snout to the end of their caudal base. There are tubercles on their snout. Through observation, both *O. waandersii* and *O. vittatus* are morphologically very similar to each. Previous authors (Inger & Chin, 1962; Sterba, 1962; Karnasuta, 1993) have invariably described one or the other species but not both simultaneously. Therefore, distinguishing descriptions are ambiguous. In this study the two species were discriminated based on the colour of the dorsal fins (dark grey in *O. vittatus* and orange in *O. waandersii* and depth of groove under the mouth) which grouped the specimens into two groups (a group from Ahning Dam – presumed *O. vittatus and the rest as O. waandersii.* This species is well distributed in the Malay Peninsular, Sumatra, Java and western Borneo (Karnasuta, 1993). *Osteocheilus waandersii* is most likely to be found in forest streams and rivers with clear turbid water.

2.5 Genus *Tylognathus*

The genus *Tylognathus* is considered an important food fish of Thailand, Cambodia and Vietnam. Morphologically, they have 10 to 13 branched dorsal fin rays (Lim, 1975). Their rostral barbel was well developed. They migrates up small rivers and streams and out onto floodplains. This genus has a fairly wide distribution; mainly in Chao Phraya basin in Thailand (Roberts, 1997) and Tonle Sap, Cambodia. According to Rainboth, (1996) there were 5 species recorded from the Mekong.

2.5.1 *Tylognathus caudimaculatus* (Fowler, 1934)

Tylognathus caudimaculatus (Plate 2.12) have 8 to 9 branched dorsal rays (Roberts, 1997) and 32 to 35 scales on the lateral series. The fish can be recognised by its conspicuously inferior or strongly projecting snout. This morphology was not observed in other *Tylognathus* species. This group of fishes can be easily found at bottom depths in canals, ditches and small streams in large river floodplains. Basically, they feed on phytoplankton, periphyton, benthic algae, detritus and some zooplankton.

2.6 Genus *Botia*

The genus *Botia* is classified in the family Cobitidae. Morphologically, this group of fishes have at least six barbels with mouth inferior and there is also a bifid spine below the eye area. They have compressed body with subterminal mouth, forked caudal fin and mostly found in Asia.

2.6.1 *Botia modesta* (Bleeker, 1864)

In this study, *Botia modesta* (Plate 2.13) was chosen as the outgroup in the mitochondrial DNA study. *Botia modesta* or the generic name, 'redtail botia' are commercially kept as an aquarium fish and can be found in tropical climate. Their distribution is basically in Asia; Mekong and Chao Phraya basins. According to Sterba, (1962) this fish can also be found in the Malay Peninsular, Indo-China and Thailand and may grow up to 10 centimeters. They have uniformly grey body with orange fins. The *Botia* samples used in this study were obtained from the Tonle Sap in Cambodia as sampling of the species in Peninsular Malaysia proved futile.

2.7 Meristic and Morphometric

Quantitative morphological techniques have traditionally been used for the classification of fishes into numerous hierarchical taxa (family, genus, species), that, based on these observations and numerical results, taxonomists consider to be related. This taxonomic information is vital to associated research in areas such as marine biology, ecology, conservation and fisheries management (Cadrin, 2000; Cabral *et al*., 2003; Tzeng, 2004; Doherty & McCarthy, 2004).

Meristic and morphometrics are two main numerical techniques used in the process of scientific description (Taniguchi *et al*., 1996; Turan, 1999; Loy *et al*., 2000; Tzeng *et al*., 2001; Akhter *et al*., 2003; Barriga-Sosa *et al*., 2004; Pinheiro *et al*., 2005). Meristics involves counting, such as the number of fin rays, bones and cartilage in different parts of the fish. Examples of external meristic features which are commonly studied are fin-rays or spines, gill-rakers and scales while internal meristic features are vertebrae, pyloric caeca, pterygiophores, branchiostegal rays. Meristics has been often used to identify or differentiate between genera (Palma *et al*., 2002); species (Barriga-Sosa *et al*., 2004), strains and crossbred (Akhter *et al*., 2003), populations or groups within species and individuals (Cabral *et al*., 2003;

Pinheiro *et al*., 2005). Some examples of species identification based on the meristic data include the discrimination of tilapias, *Oreochromis* spp. Examples of stocks or populations differentiation of *Solea lascaris* (Pinheiro *et al*., 2005) and the Portuguese sole, *Synaptura lusitanica* (Cabral *et al*., 2003) along the Portuguese coast.

Morphometrics is a tool in the study of ichthyological systematics, or taxonomy, which looks at measurable components of fish anatomy such as the size of body parts and fins, traditionally in relation to a percentage of body length. When combined with multivariate statistical procedures, they offer the most powerful tool for testing and graphically displaying differences in shape (Loy *et al.,* 1993; Rohlf & Marcus, 1993; Rohlf *et al.,* 1996). It is a method of investigation which is conducted to express biological form in terms of physical laws (Reist, 1986). Morphometrics involves measuring the length of, or distance between physical features. Examples of morphometric methods are fourier shape analysis and Truss patterns of the general outline of the body. Observations of fish shape, size, colour and other general features are also noted during such procedures. As in meristic studies, morphometric methods are frequently used for identification of populations within species of fish (Cabral *et al*., 2003; Tzeng, 2004; Doherty & McCarthy, 2004; Pinheiro *et al*., 2005). Since the 80s, the use of truss pattern (Strauss and Bookstein, 1982; Bookstein *et al*., 1985), which is a system of measurements between many external landmarks, has become useful for this purpose. The new landmark-based technique of geometric morphometric or known as truss network morphometric poses no restriction on the directions of variation and localization of shape changes, and are much more effective in capturing information about the shape of an organism (Schweigert, 1990; Turan, 1999; Cavalcanti *et al*. 1999; Cadrin, 2000; Palma *et al*., 2002; Kassam *et al*., 2002; Jayasankar *et al*., 2004). This is considered a revolutionary tools in morphometrics since it overcomes inherent weaknesses of traditional character sets which tend to be aligned to the same horizontal axes.