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Species Checklist and DNA Barcoding of *Baung* (Bagrid Catfish) *Hemibagrus Hoevenii* from Muar River, Johor

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Abstract: Of Asia, Africa, and the Middle East, there are 15 genera in the Bagridae family. The tropical freshwater catfish *Hemibagrus hoevenii* is found in Asian waters. Bagrids are also known as Old World pimelodids, while New World bagrids may be more accurate. In Muar, Johor, DNA barcoding has never been utilised to determine the species of bagrid catfish. Therefore, this study was done to update the species checklist of *Baung* (bagrid catfish) in Muar River, and DNA barcoding of protein-coding cytochrome c oxidase I (COI) mitochondrial gene was done for species identification and phylogenetic analyses. A number of two partial COI gene sequences ranging 674-687 nucleotide bases were successfully obtained for two specimens of *Baung Lawi* and the Nucleotide Basic Local Alignment Search Tool (BLAST) analysis suggested their species status as from the genus *Hemibagrus*. Furthermore, the results of the phylogenetic analyses showed that the neighbour joining tree, the maximum parsimony tree and the maximum likelihood tree grouped the COI mtDNA gene sequences of *Baung Lawi* from Muar River in one single cluster, thus confirming the species status and showed the presence of *H. hoevenii* in Muar River, Johor.

Keywords: *Baung* fish, Muar river, DNA barcodes, cytochrome c oxidase I gene, *Hemibagrus hoevenii*, phylogenetic analysis

1. Introduction

The Bagridae family has a wide distribution, with members found in Asia, Africa, and the Middle East. Bagrids are likewise a diverse family, ranging from *Bagrus meridionalis*, the largest fish belonging to Lake Malawi, to the tiny *Hyalobagrus flavus* of Southeast Asia, which seldom reaches one inch in length. Bagrids are commonly referred to as Old World pimelodids, and many bagrids do resemble some pimelodids [1]. However, because many scientists believe that numerous other catfish groups developed from a bagrid-like ancestor, it may be more accurate to refer to pimelodids as New World bagrids. Bagridae is made up of 15 genera in Asia. Six of the 15 genera have species that can be found in the US aquarium trade on a regular basis [1].

Hemibagrus hoevenii is a tropical freshwater catfish native to Asian waterways, found in Malaysia, Indonesia, Cambodia, Laos, Thailand, and Vietnam [2]. Ponds, swamps, streams, lakes, and rivers are where it can be found [3]. Because of its role in fisheries and aquaculture, it is economically valuable. Its high protein and omega-3 polyunsaturated fatty acid content, as well as its low cholesterol, making it an ideal aquaculture fish [4]. As we know

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that proteins have a role in building the body as well as various metabolisms that exist in the human body. The protein content in *Baung* provides benefits such as providing support to the body's metabolic process, supporting physiological functions in the body, preventing disease disorders, playing a vital role as indicator of the body's immune system and has properties to increase energy in the body [5]. In conducting this study, there are three potential limitations. There is not enough information to conduct this study because in Malaysia particularly, there are only a few studies focusing on the diversity and genetics of bagrid catfish, and no studies had been done in Johor freshwater area in *Muar* despite their unique characteristics and importance to freshwater fisheries and overall freshwater ecosystem. Most of the research that can be found are about the dietary protein and lipid level of bagrid catfish [6].

There are several methods that can be used to identify a species such as species-specific real-time polymerase chain reaction (RT-PCR), detection of single-nucleotide polymorphisms (SNPs), and DNA barcoding. COI gene-based DNA barcoding is an effective molecular technique for most species recognition; but in species identification of bagrid catfish especially in Muar, Johor this method had never been used or conducted [7]. As there is a lack of information, the species identification of bagrid catfishes cannot be determined and the species checklist of bagrid catfish in Muar, Johor cannot be monitored. Traditionally, fish were classified based on morphological and anatomical traits [8]. However, the significant physical similarities in Bagridae made species identification extremely challenging [9]. Insufficiency of data is a potential problem since this can affect the purpose of research studies for which that particular data may be of utmost importance.

From the problem statements above, this study aimed to update the species checklist of *Baung* (bagrid catfish) in Muar River, Johor and to generate DNA barcodes of the fish species for species identification and phylogenetic analysis. This study managed to determine the species of *Baung Lawi* in Muar River where the result later can be updated in the species checklist of Bagridae. This study can be used as a reference to researchers and the public in the future. Database of fishes is very important because it can be used to monitor the diversity of fish in particular area and to conserve it if that species is facing any threats. This study would help to establish new information which can be used to set a priority to conserve an area to ensure the survival of the species and to avoid their status to be assessed as endangered species.

2. Materials and Methods

2.1 Study Site and Sampling

A number of two specimens of *Baung* fish (BLA & BLB, Fig. 1) were collected from Muar River, Johor, Malaysia in November 2021. The specimens were fresh prior to the transportation via land to the Molecular Biology and Genetics Laboratory, Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Campus, Muar. The morphospecies identification was done based on the external morphology and the information given by the local people of Muar. In the laboratory, the samples are stored in -20^{o}C chest freezer for long-term storage with proper cataloging.

Fig. 1 - *Baung Lawi* specimens from Muar River, Muar, Johor, Malaysia

2.2 Amplification of Cytochrome C Oxidase I Mitochondrial Gene

The total genomic DNA extraction was done using the Tissue Genomic DNA Extraction Mini Kit by Favorgen. Each PCR reaction volume is 25 µl, which includes 8 µl ultrapure water, each primer at 1 µl, 12.5 µl exTEN 2x PCR master mix, and 2.5 µl DNA template.

FishF1 (forward)	5’- TCA ACC AAC CAC AAA GAC ATT GGC AC -3’ (26 bases)
FishR1 (reverse)	5’- TAG ACT TCT GGG TGG CCA AAG AAT CA -3’ (26 bases)

Cycle parameters for the PCR run were 4 min at 95ºC for initial denaturation, 30 s at 95ºC for denaturation, 30 s at 54ºC for annealing, 45 s at 72°C for extension, repetition of step 2-4 for another 35 cycles, 10 min at 72°C for final extension and then the temperature was held at 4°C. Agarose gel electrophoresis was then used for determination of estimated yields of PCR products, the quantity and quality, on 1% agarose gel with FloroSafe DNA Stain as gel stain. The unpurified PCR products were sent for PCR fragment purification and DNA sequencing at the Apical Scientific Sdn. Bhd, Seri Kembangan, Selangor Darul Ehsan, Malaysia.

2.3 Basic Local Alignment and Phylogenetic Analysis

Online Basic Local Alignment Search Tool program for nucleotide (blastn) was used to align and match each gene sequence (i.e. the query sequence) from this study with available fish gene sequences in the GenBank, National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine. MEGA X software version 10.0.5 (BETA) [8] was used for the multiple alignment and the phylogenetic analyses [9]. Accession numbers and country of origin of the sequences downloaded from GenBank are indicated on each phylogenetic tree. Subtypes are indicated by colour and depicted in bold with the scientific name of species that related to the DNA sequences from this study. Phylogenetic trees were constructed using three methods i.e. neighbour joining, maximum parsimony and maximum likelihood. The clade credibility in the trees was tested using bootstrapping, which involved performing 1000 repeated sampling tests to determine the support values for the clade nodes [10] [11]. The phylogenetic analyses involved 18 nucleotide sequences including 17 ingroups and an outgroup of *Mystus montanus* with GenBank accession number of MF591712.

3. Result and Discussion

Three standard measurements were taken for the *Baung Lawi* samples. The total length (TL) ranged 22cm - 25cm, which was measured in a straight line from the tip of the snout or jaw to the extreme end of the tail. The next dimension is the fork length (FL) ranged 18cm - 20.5cm, which was measured from the tip of the snout or jaw to the fork in the tail. Finally, the standard length (SL) ranged 17cm - 19cm, which was measured from the tip of the snout or jaw to the end of the vertebral column.

Approximately 700 bp protein-coding COI mitochondrial gene fragments were successfully amplified (Fig. 2). In terms of DNA sequencing results, a range of 674- 687 nucleotide bases of the COI mitochondrial gene was successfully obtained. Moreover, the blastn results showed that the specimens of *Baung Lawi* samples were specifically identified as from the genus *Hemibagrus* with Identities scores (Ident or Percent Identity) of 97.53% and 97.60% when aligned against the corresponding sequence from the GenBank with accession number of KJ573466.1 (Table 1). The scores of Query cover for the blastn of the morphospecies *Baung Lawi* were 100% and the Expect values (E values) were 0 showing the most significant score and alignment with the corresponding sequence. The other scores were Max score and Total score both with scores of 1040 and 1074. Therefore, the findings suggested that the specimens of *Baung Lawi* from Muar River were *Hemibagrus* species. All the sequences were successfully translated to protein sequences (Appendix A).

Phylogenetic trees were constructed using the neighbor-joining method, Maximum Likehood method and also Maximum Parsimony method (Fig. 3, Fig. 4 & Fig. 5). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [12]. A total number of 17 COI mtDNA gene sequences were included in the analysis as the ingroups along with one outgroup. A number of 15 ingroups were obtained from the GenBank. The results of the phylogenetic analyses showed that the neighbor-joining method grouped the COI mtDNA gene sequences of *Baung Lawi* from Muar River in one cluster with a high bootstrap value of 98%. For maximum likelihood method and maximum parsimony method, both phylogenetic trees showed that *Baung Lawi* grouped in one cluster with strong bootstrap supports of 99%. According to Kottelat and Lim [13], *Hemibagrus hoevenii* (Bleeker, 1846) is the proper name for a largesize species of catfish that can be identified by its long, deeply forked caudal fin with a black margin all the way around the fin. The species has previously been confused with *H. hoevenii*, according to Kottelat and Lim [13]. (Valenciennes, 1839). Large rivers in Java, Sumatra, Borneo, and the Malay Peninsula are where it can be found. Therefore, the *Baung Lawi* samples were suggested as *H. hoevenii* based on their morphological characteristics as well as the results of the phylogenetic analyses. Addition of different species of bagrinid catfish could give better resolution to the genetic relationship of *Baung Lawi* from Muar River. Although the corresponding sequence of KP856825 is described as *Hemibagrus capitulum* in the GenBank, but it was grouped as *H. hoevenii* in the phylogenetic trees. This suggests that the sample of KP856825 could have been misidentified morphologically and/or genetically.

Table 1 - Basic Local Alignment Search Tool nucleotide (BLASTn) results for protein-coding cytochrome c oxidase I mitochondrial DNA sequences of *Baung Lawi* from Muar River, Johor, Malaysia

Specimen	Max Score	Total Score	Query Coverage (%)	E Value	Percent Identity (%)	GenBank Accession No. of Corresponding Sequence	Species Identity
BLA	1074	1074	100	0.0	97.60	KJ573466.1	*Hemibagrus hoevenii*
BLB	1040	1040	100	0.0	97.53	KJ573466.1	*Hemibagrus hoevenii*

Fig. 2 - Positive PCR results of protein-coding cytochrome c oxidase I (COI) mitochondrial gene sequences of BLA and BLB from Muar River, Johor. 1K - DM3100 ExcelBandTM1 KB (0.25-10 kb) DNA Ladder

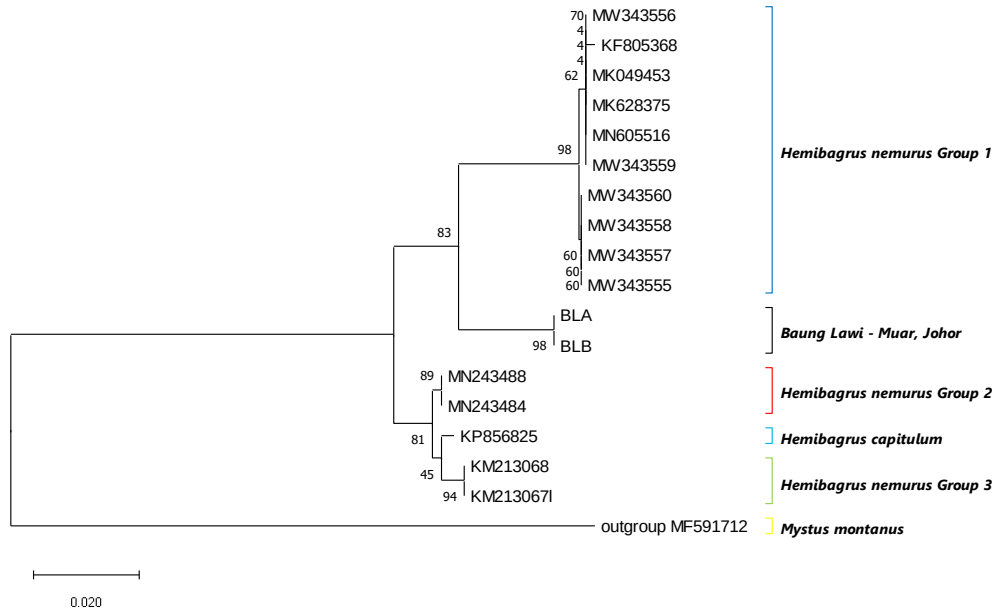

Fig. 3 - Neighbor-joining tree of *Baung Lawi* from Muar River, Johor, Malaysia using partial cytochrome c oxidase I mitochondrial DNA gene

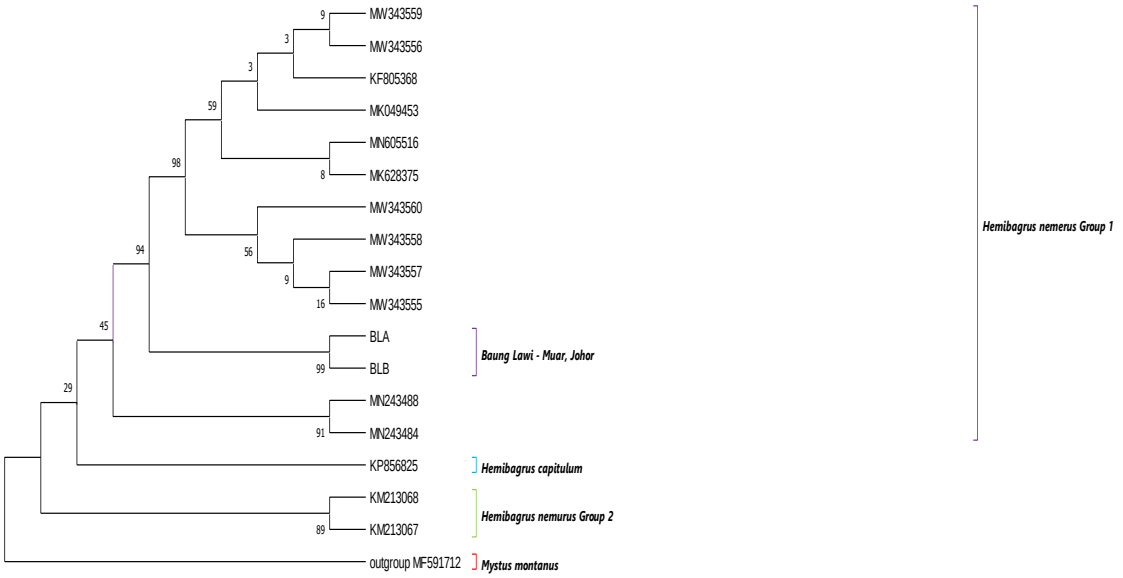

Fig. 4 - Maximum Likelihood tree of *Baung Lawi* from Muar River, Johor, Malaysia using partial cytochrome c oxidase I mitochondrial DNA gene

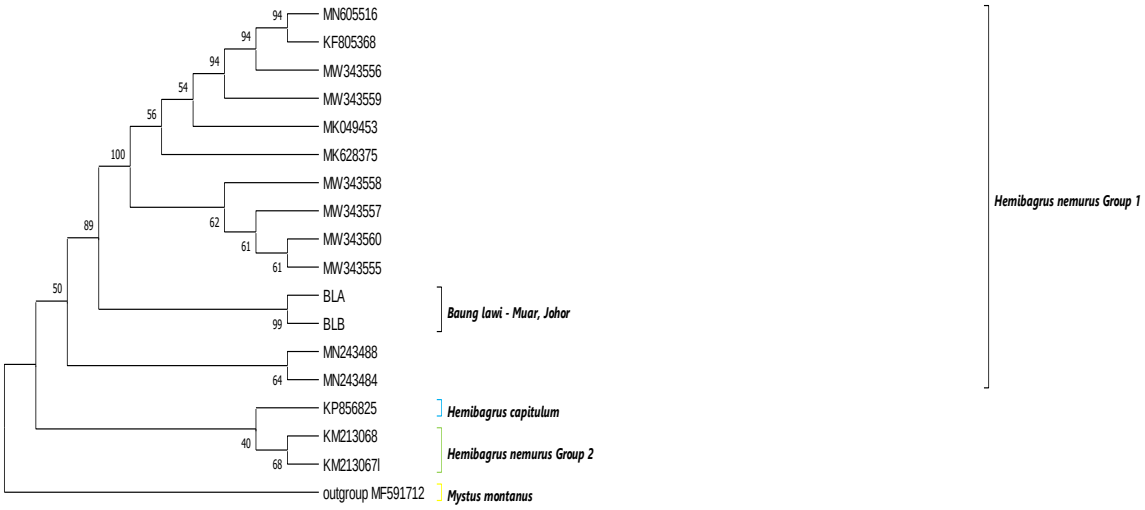

Fig. 5 - Maximum Parsimony tree of of *Baung Lawi* from Muar River, Johor, Malaysia using partial cytochrome c oxidase I mitochondrial DNA gene

4. Conclusion

In conclusion, the protein-coding COI mitochondrial gene sequence analyses using the blastn and the phylogenetic tree reconstruction resulted in the species identification of the *Baung* fish specimens as *Hemibagrus hoevenii*. The current findings gave a better insight of the importance of morphological and molecular approaches, and the present status of *Baung Lawi* from Muar River, Johor, Malaysia. For future studies, further taxonomic studies and molecular studies involving more different species of bagrinid catfish are required to better understand the genetic diversity and genetic relationship.

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Appendix A: Translation of Cytochrome C Oxidase I Mitochondrial DNA Gene Sequences of *Baung Lawi* from Muar River, Johor to Protein Sequences

Specimen	Species Identify Based On Blastn	Partial Protein Coding CO1 Mtdna Sequence	Translated Protein Sequence
BLA	*Hemibagrus* species	TGCCTGAGCCGGAATAGTTGGTACAG CCCTTAGCTTACTAATCCGGGCAGAAC TAGCCCAACCCGGTGCCCTCCTAGGCG ACGATCAAATTTACAATGTTATTGTAA CTGCTCACGCCTTTATCATAATTTTCTT TATAGTAATACCAATTATAATTGGAGG CTTCGGAAACTGACTTGTACCATTAAT GATTGGAGCACCAGATATGGCATTTCC ACGAATGAACAACATGAGCTTCTGAT TACTCCCACCCTCTTTCCTTCTACTATT GGCCTCGTCTGGTGTTGAAGCAGGCG CAGGAACAGGATGAACTGTATACCCT CCGCTCGCTGGCAATCTTGCACATGCA GGTGCCTCTGTAGATTTAACTATTTTC TCACTACATCTTGCAGGTGTATCATC TATTTTGGGGGCTATTAATTTTATTAC AACTATTATTAATATGAAACCTCCAGC TATTTCACAATACCAGACACCCTTATT TGTGTGGGCCGTCCTAATTACAGCTGT GCTCCTATTACTCTCTCTGCCAGTCCT AGCAGCTGGTATTACAATACTACTAAC TGACCGAAATCTAAACACCACATTCTT CGACCCAGCAGGGGGAGGGGACCCAA TTCTATATC	AWAGMVGTALS LLIRAELAQPGA LLGDDQIYNVIV TAHAFIMIFFMV MPIMIGGFGNW LVPLMIGAPDM AFPRMNNMSFW LLPPSFLLLLASS GVEAGAGTGW TVYPPLAGNLA HAGASVDLTIFS LHLAGVSSILGA INFITTIINMKPP AISQYQTPLFVW AVLITAVLLLLS LPVLAAGITMLL TDRNLNTTFFDP AGGGDPILY
BLB	*Hemibagrus* species	GCCGGAATAGTTGGTACAGCCCTTAG CTTACTAATCCGGGCAGAACTAGCCC AACCCGGTGCCCTCCTAGGCGACGAT CAAATTTACAATGTTATTGTAACTGCT CACGCCTTTATCATAATTTTCTTTATA GTAATACCAATTATAATTGGAGGCTTC GGAAACTGACTTGTACCATTAATGATT GGAGCACCAGATATGGCATTTCCACG AATGAACAACATGAGCTTCTGATTACT CCCACCCTCTTTCCTTCTACTATTGGCC TCGTCTGGTGTTGAAGCAGGCGCAGG AACAGGATGAACTGTATACCCTCCGCT CGCTGGCAATCTTGCACATGCAGGTGC CTCTGTAGATTTAACTATTTTCTCACT ACATCTTGCAGGTGTATCATCTATTTT GGGGGCTATTAATTTTATTACAACTAT TATTAATATGAAACCTCCAGCTATTTC ACAATACCAGACACCCTTATTTGTGTG GGCCGTCCTAATTACAGCTGTGCTCCT	AGMVGTALSLLI RAELAQPGALL GDDQIYNVIVTA HAFIMIFFMVMP IMIGGFGNWLV PLMIGAPDMAF PRMNNMSFWLL PPSFLLLLASSG VEAGAGTGWT VYPPLAGNLAH AGASVDLTIFSL HLAGVSSILGAI NFITTIINMKPPA ISQYQTPLFVWA VLITAVLLLLSL PVLAAGITMLLT DRNLNTTFFDPA GGGD

ATTACTCTCTCTGCCAGTCCTAGCAGC
TGGTATTACAATACTACTAACTGACCG
AAATCTAAACACCACATTCTTCGACCC
AGCAGGGGGAGGGGACCC

References

[1] Dignall, J. (2002). *The Catfish of Asia Series, Part 1, Family Bagridae, Geography, Shane's World*.
[2] Rainboth WJ. 1996. Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes. Rome: FAO. p 265.
[3] Inger FR, Chin PK. 1962. The freshwater fishes of North Borneo, Fieldiana: Zoology. USA: Chicago *Natural History Museum*. Volume 45. p 268.
[4] Mesomya W, Cuptapun Y, Jittanoonta P, Hengsawadi D, Boonvisut S, Huttayanon P, Sriwatana W. (2002). Nutritional evaluations of green catfish Mystus *nemurus*. Kasetsart J (Nat Sci) 36:69-74.
[5] Saputra, G. (2020, July 22). *Jenis Ikan Baung*. Akuatik.id. https://www.akuatik.id/ikan-baung/
[6] Ng, W., Soon, S., & Hashim, R. (2001). *The dietary protein requirement of a bagrid catfish, Mystus hoevenii (Cuvier & Valenciennes), determined using semi purified diets of varying protein level*. Aquaculture Nutrition, 7(1), 45-51.
[7] Zou, R., Liang, C., Dai, M., Wang, X., Zhang, X., & Song, Z. (2020). DNA barcoding and phylogenetic analysis of bagrid catfish in China based on mitochondrial COI gene. Mitochondrial DNA Part A, 31(2), 73-80.
[8] Teletchea F. 2009. Molecular identification methods of fish species: reassessment and possible applications. Rev Fish Biol Fish. 19:265-293.
[9] Li L, Liang HW, Li Z, Luo XZ, Hu GF, Zhang ZW, Zhu YY, Zou GW. 2011. Sequence and phylogeny analysis of the complete mitochondrial genome of Pelteobagrus vachelli. Hereditas. 33:627-635.
[10] Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., & Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. PloS One, 13(6), e0198109.
[11] Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547-1549.
[12] Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.
[13] Kottelat, M. & Lim, K.K.P. (1995). *Hemibagrus hoevenii* a valid species of Sundaic Catfish. (Teleostei:Bagridae). *Malayan Nature Journal*, 49: 41-47.