

MORPHOLOGICAL AND GENETIC DESCRIPTIONS OF A NEW SPECIES OF CATFISH, *HEMIBAGRUS CHRYSOPS*, FROM SARAWAK, EAST MALAYSIA, WITH AN ASSESSMENT OF PHYLOGENETIC RELATIONSHIPS (TELEOSTEI: BAGRIDAE)

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ABSTRACT. - *Hemibagrus chrysops*, new species, is described from the Sadong and Rajang drainages in Sarawak, northern Borneo. It can be differentiated from its congeners in having a relatively long and narrow head [head width 53.4-62.5 %HL (mean 57.7 %HL)], a vivid golden-yellow colour when alive or freshly-dead, length of adipose-fin base 16.6-19.3 %SL (mean 17.0 %SL; 3.33-3.98 (mean 3.64) times adipose maximum height), dorsal to adipose distance 6.8-9.7 %SL (mean 7.9 %SL), dorsal-spine length 10.4-16.6 %SL (mean 14.4 %SL), 10-11 branchiostegal rays (mode 11), premaxillary toothband not exposed when the mouth is closed, rounded caudal-fin lobes, the absence of a broad and conspicuous dark margin around the margins of the caudal fin and a rounded anal fin. Sequence analysis of 300 bp of the cytochrome *b* gene of *H. chrysops* and that of various other *Hemibagrus* lineages revealed that *H. chrysops* is a separate lineage (mean 7.8% sequence divergence with other species of the *H. nemurus* species-complex) with an independent evolutionary history that predates the Pleistocene. *Hemibagrus chrysops* forms a sister taxon to a large clade clustering *H. nemurus* s. l. and *H. hoevenii*. *Hemibagrus chrysops* occurs sympatrically with two distinct lineages of *H. nemurus* s. l. and *H. hoevenii* in the Sadong and Rajang drainages.

KEY WORDS. - *Hemibagrus*, Borneo, new species, morphology, mitochondrial cytochrome *b* sequences, phylogenetic relationships.

INTRODUCTION

The genus *Hemibagrus* comprises large-sized bagrid catfishes that attain standard lengths of up to 800 mm. This taxon was established by Bleeker (1862) to include species with depressed heads, rugose head shields not covered by skin, slender occipital process, and

moderately long adipose fins. Subsequent authors since Günther (1864) have synonymised *Hemibagrus* with *Mystus* and only recently has the genus *Hemibagrus* been revalidated (Mo, 1991). *Hemibagrus* as diagnosed by Mo (1991) consists of species that have a depressed head with a thin, plate-like metapterygoid.

Members of *Hemibagrus* are found ubiquitously in river drainages east from the Ganges-Brahmaputra basin and south from the Yangtze basin, and reach their greatest diversity in Sundaland. There are 35 nominal species of *Hemibagrus* but the validities of many of these species is not known and it is only recently that in-depth studies have been undertaken (e.g. Kottelat & Lim, 1995; Ng & Ng, 1995; Kottelat et al., 1998). In Sarawak, the authors and their colleagues have noticed for some time now that what is called *H. nemurus* in the lowlands can easily be separated into two forms by their adult size and coloration. One 'form' grows up to 300 mm SL in size at maturity, and has a live coloration of grey to yellowish-grey. Another 'form' matures at a much smaller size (150 mm SL) and has an intense golden-yellow colour throughout the body. During a phylogeographic study using mitochondrial DNA of this '*H. nemurus*' material, genetic differences were found which suggested that the golden *Hemibagrus* found in the Sadong and Rajang rivers was unique and different from *H. nemurus* found elsewhere (Dodson et al., 1995). Further analysis revealed that these fish are morphologically distinct from *H. nemurus* s. l. and other related species and are described here, using both morphological and genetic criteria, as belonging to a new species.

MATERIALS AND METHODS

Morphological analysis - Measurements were made point to point with dial callipers and data recorded to tenths of a millimetre. Counts and measurements were made on the left side of specimens whenever possible. Subunits of the head are presented as proportions of head length (HL). Head length itself and measurements of body parts are given as proportions of standard length (SL). Measurements and counts were made following Ng & Ng (1995) with the following exceptions: head length is measured from the anterior point of SL to the posterior-most extremity of fleshy opercular flap. Length of the adipose-fin base is measured from the anterior-most point of origin to the posterior-most point of the adipose-fin base. Post-adipose distance is measured from the posterior-most point of the adipose-fin base to the posterior point of SL.

The following additional measurements were made: predorsal, preanal, prepelvic and prepectoral lengths are those measured from the anterior point of SL to the anterior basis of the dorsal, anal, pelvic and pectoral fins respectively. Lengths of the dorsal- and anal-fin bases include the respective bases of the first and last rays and the distance between them. Pelvic- and pectoral-fin lengths are measured from the origin to the tip of the longest ray. Dorsal and pectoral spine lengths are measured from the base to the tip. Dorsal to adipose distance is measured from the base of the last dorsal-fin ray to the origin of the adipose fin. Adipose maximum height is the maximum height of the adipose fin. Caudal-fin length is the length of the longest ray of the lower lobe measured from the posterior margin of the hypural complex. The length of the caudal peduncle is measured from base of the last anal-fin ray to the posterior point of SL. Nasal-, maxillary- and mandibular-barbel lengths are measured from the base to the tip. Numbers in parentheses following a particular morphometric measurement refer to the mean.

Fin ray counts were obtained under a binocular dissecting microscope using transmitted light. Vertebral counts were taken from radiographs (which were obtained using a Hitex HAC-60 x-ray radiography system) following the method of Roberts (1994). Numbers in parentheses following a particular fin-ray, branchiostegal-ray gill-raker or vertebral count indicate the number of specimens with that count. Drawings of the specimens were made with a Nikon SMZ-10 microscope and camera lucida. Institutional codes follow Eschmeyer (1998).

Genetic analysis. - A previous restriction fragment length polymorphism (RFLP) survey of the mitochondrial DNA (mtDNA) of the *Hemibagrus* complex throughout Sundaland revealed a total of 35 haplotypes, four of which were obtained from 21 specimens of *H. chrysops*, new species, sampled in the Sadong and Rajang Rivers of Sarawak (Dodson et al. 1995). In order to establish the phylogenetic relationships of *H. chrysops* with other components of the *Hemibagrus* complex, 300 base pairs (bp) of the mtDNA cytochrome *b* (*cytb*) gene of 26 of these RFLP haplotypes were sequenced. These 26 haplotypes were chosen to represent the entire spectrum of genetic diversity of the *Hemibagrus* complex genetically analysed to date. An additional 14 specimens of *H. nemurus* were sampled in 1997 from the Sadong River, Sarawak. We amplified a 360-bp fragment of the *cytb* gene using derivatives of the universal primers of Koehler et al. (1989): 5'-CCATCCAACATCTCAGCATGATGAAA-3' (light strand) and 5'-CCCCCTCAGAATGATATTTGTCCTCA-3' (heavy strand) (Taylor & Dodson, 1994). Amplifications were carried out in 50 µl total volumes containing (final concentrations): 200 µM each of dATP, dGTP, dCTP and dTTP, 800 nM of each primer, 0.5 U of Taq polymerase, 2mM MgCl₂, 10mM Tris-HCl (pH 8.3), 50 mM KCl and 80 to 100 ng of purified mtDNA template. DNA was amplified in a programmable thermal cycler using the following profile: one single preliminary denaturation at 94°C for 120 s, 35 cycles of amplification with denaturation at 94°C for 60 s, primer annealing at 45°C for 60 s, primer extension at 72°C for 90 s, a final single extension step performed at 72°C for 5 min.

To purify amplified DNA, the 50 µl mixture was loaded on a 1% agarose gel. After electrophoresis at 75V for 2 hours, the 360-bp amplified fragment was excised from the gel and extracted and purified using the GENECLEAN II kit (Bio 101 Inc.) to a final volume of 12 µl (1 X TE buffer). A 300-bp region of the amplified fragment was sequenced in dideoxy sequencing reactions using the Sequenase kit (version 2.0, US Biochemical), according to the manufacturer's directions with the following modifications: 10 picomoles of the light strand primer was mixed with 12 µl of the purified template. This mixture was denatured by boiling (at 95°C) for 4 min and then chilled on wet ice for 1 min. Denatured DNA was mixed with 8 µl of reaction mix (2 µl of reaction buffer, 2 µl of labelling mix diluted (1:5), 1 µl of DTT, 0.5 µl of Mn buffer, 0.75 µl of [³⁵S]-dATP and 2 µl of Sequenase diluted (1:8) and then left on ice 2.5 min. Subsamples of this mixture (3.8 µl) were transferred to each of the 4 termination reaction tubes containing 2.5 µl each of ddNTP mixture, left for 5 min. at 40°C and the reaction terminated with 4 µl of stopping solution.

Sequences were separated on 6% polyacrylamide (19:1 BIS), 7.6M urea gels. Electrophoresis was performed at 1500-1600 V (ca 50 W) for either 2 to 2.5 h or 4 to 4.5 h to obtain the 5' and the 3' end of the sequence, respectively. The gels were vacuum-dried onto filter paper at 80°C for 40 to 45 min. and autoradiographed.

Both distance- and character-based data analyses were used to define the phylogenetic relationships of *H. chrysops* with other *Hemibagrus*. *Cytb* gene sequences were aligned by eye and pairwise sequence divergences (*d*; Kimura's (1980) two-parameter model) were

calculated using DNADIST of the PHYLIP 3.4 computer package (Felsenstein 1992). The distance matrix was used to construct a phenogram using the neighbour-joining clustering method (program NEIGHBOR in PHYLIP). Parsimony analysis of the sequence data, based on Wagner parsimony criteria, was carried out using DNAPARS of PHYLIP. Confidence in parsimony trees was assessed by bootstrap re-sampling of the sequence data (N=10,000) using SEQBOOT of PHYLIP. A majority-rule consensus tree was built using CONSENSE of PHYLIP. Bootstrap values equal to or greater than 70% correspond to $\geq 95\%$ probability that the corresponding clade is real (Hillis & Bull, 1993). *Hemibagrus gracilis* from the Endau River in peninsular Malaysia was used as the outgroup taxon in all analyses.

TAXONOMY

Hemibagrus chrysops, new species

(Fig. 1)

Holotype - ZRC 42653, 1 ex., 209.7 mm SL; Borneo: Sarawak, Serian wet market, from Sadong River; H. H. Tan, 31 Aug-5 Sep.1996.

Paratypes - ZRC 40489, 1 ex., 156.0 mm SL; data as for holotype. - ZRC 23008-23014, 7 ex., 172.0-210.7 mm SL; Borneo: Sarawak, Rajang River at Sibu; J. Dodson, 25-28 Jun.1992. - ZRC 39518, 18 ex., 93.2-171.0 mm SL; Borneo: Sarawak, Serian, market near Sungai Gadong; H. H. Tan et al., 5 Sep.1995.

Diagnosis. - *Hemibagrus chrysops* can be differentiated from its congeners by the unique combination of the following characters: relatively long and narrow head [head width 53.4-62.5 %HL (mean 57.7 %HL)], a vivid golden-yellow hue when alive or freshly-dead, length of adipose-fin base 16.6-19.3 %SL (mean 17.0 %SL; 3.33-3.98 (mean 3.64) times adipose maximum height), dorsal to adipose distance 6.8-9.7 %SL (mean 7.9 %SL), dorsal-spine length 10.4-16.6 %SL (mean 14.4 %SL), genital papilla in males either not reaching or just reaching the base of the first anal-fin ray, 10-11 branchiostegal rays, premaxillary toothband not exposed when the mouth is closed, rounded caudal-fin lobes, a broad and conspicuous dark margin absent around the margins of the caudal fin, a rounded anal fin, and a unique *cytb* gene sequence (see below).

Description. - Head depressed and broad, body moderately compressed. Dorsal profile rising evenly but not steeply from tip of snout to origin of dorsal fin, then sloping gently ventrally from there to end of caudal peduncle. Ventral profile horizontal to origin of anal, then sloping dorsally to end of caudal peduncle. In %SL: head length 30.7-34.1 (32.1), head width 17.9-20.7 (19.2), head depth 14.2-16.7 (15.1), predorsal distance 40.8-44.3 (46.5), preanal length 70.7-79.7 (73.1), prepelvic length 53.3-59.0 (55.3), prepectoral length 25.8-32.0 (27.8), body depth at anus 14.8-18.0 (16.1), length of caudal peduncle 15.0-17.5 (16.9), depth of caudal peduncle 7.6-9.4 (8.4), pectoral-spine length 15.6-18.7 (17.3), pectoral-fin length 18.8-24.0 (21.4), dorsal-spine length 10.4-16.6 (14.4), length of dorsal-fin 24.9-32.6 (29.4), length of dorsal-fin base 15.8-18.8 (16.9), pelvic-fin length 15.0-17.1 (15.9), length of anal-fin base 11.0-13.7 (12.2), caudal-fin length 22.1-26.9 (24.6), length of adipose-fin base 16.6-19.3 (17.0), adipose maximum height 4.4-5.4 (5.2), post-adipose distance 14.7-16.2 (15.8), dorsal to adipose distance 6.8-9.7 (7.9); in %HL: snout length 33.6-38.7 (36.9), interorbital distance 27.4-33.0 (29.8), eye diameter 11.9-16.3 (13.9), nasal barbel length 26.7-38.4 (32.4), maxillary barbel length 202.2-289.7 (285.6), inner mandibular barbel length 41.2-57.6 (48.8), outer mandibular barbel length 76.6-103.0 (90.7). Branchiostegal rays 10

(4) or 11 (14). Gill rakers 4+6=10 (1), 4+7=11 (2) or 4+8=12(1). Vertebrae 23+22=45 (1 24+21=45 (1), 23+23=46 (1) or 24+22=46 (5).

Fin ray counts: dorsal I,7 (18); pectoral I,7,i (5), I,8 (1), I,8,i (9) or I,9 (3); pelvic i,5 (18); anal iii,8 (11) or iii,9 (7); caudal 8/9 (18). Dorsal origin nearer tip of snout than caudal flexure. Dorsal spine stout, with 4-14 irregular serrae posteriorly. Pectoral spine stout, with 14-15 large serrae posteriorly. Anal origin slightly posterior to adipose origin. Depressed dorsal not reaching adipose fin. Caudal fin forked; upper lobe rounded with upper simple principal ray produced into a filament, lower lobe rounded posteriorly.

Colour. - Dorsal surface of head and body uniform light grey to grey (live or freshly-dead specimens have a golden-yellow hue which fades on preservation), without a humeral spot, lateral line cream to yellow; ventral surfaces of head and body dirty white; adipose fin and fin rays of all fins grey; inter-radial membranes of all fins dirty yellow.

Distribution. - Only known from the Sadong and Rajang basins in northern Borneo.

Ecology. - *Hemibagrus chrysops* is a relatively small species and is sexually mature at about 150 mm SL (compared to 300 mm SL for *H. nemurus* s. l.). It can be found living sympatrically with *H. nemurus* s. l. in the Sadong and Rajang drainages. In the Rajang River, it appears to be found mainly in the downstream sections of the river whereas *H. nemurus* s. l. appears to be found much further upstream. Thus, some form of longitudinal segregation may occur within drainages, but this remains to be confirmed.

Etymology. - From the Greek "chrysops", meaning gold-coloured. The name refers to its golden live coloration and is used as an adjective.

Remarks. - The taxonomy of material identified as *H. nemurus* is at present rather confusing, and it is highly likely that such material may consist of more than one species (Ng & Ng, 1995). Although Ng & Ng (1995) divided most of the nominal Sundaic *Hemibagrus* into four sub-groups, we feel that the use of this scheme in the discussion would complicate it unnecessarily. We have decided to follow Weber & de Beaufort (1913) and Roberts (1989) in considering *Macrones bleekeri* Volz, 1903, *Macrones bo* Popta, 1904, *Macrones fortis* Popta, 1904, *Macrones fortis* var. *capitulum* Popta, 1904, *Macrones howong* Popta, 1904, *Macrones kajan* Popta, 1904, *Mystus johorensis* Herre, 1940 and *Mystus pahangensis* Herre, 1940, junior synonyms of *Hemibagrus nemurus* (Valenciennes in Cuvier & Valenciennes,

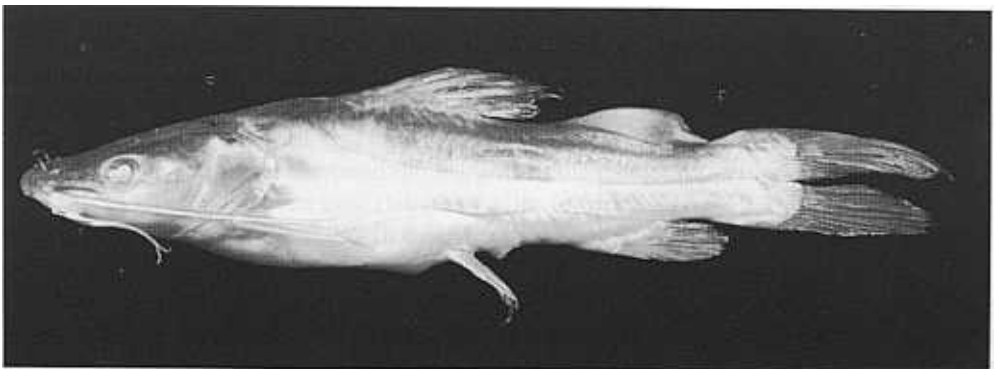


Fig. 1. *Hemibagrus chrysops*, ZRC 39518, paratype, 113.7 mm SL.

1840). As stated above, more than one species is involved in what is currently identified as *H. nemurus*. However, it is not the objective of this paper to discuss the identities and validities of the nominal species at length; to this effect, we compare *H. chrysops* only with *H. nemurus* s. l. from throughout Southeast Asia (which includes types of all the nominal species listed above; see comparative material).

Hemibagrus chrysops differs from *H. nemurus* s. l. in having a relatively longer and narrower (vs. shorter and wider) head (head width 53.4-62.5 %HL vs. 63.1-77.1; Fig. 2), longer adipose-fin base (length of adipose-fin base 3.33-3.98 times adipose maximum height vs. 2.15-3.34), smaller dorsal to adipose distance (6.8-9.7 %SL vs. 9.1-11.7) and relatively fewer branchiostegal rays (10-11 vs. 11-12). In addition, *H. chrysops* differs from *H. nemurus* from Java (the type locality of *H. nemurus*) in having a relatively shallower adipose fin (length of adipose-fin base 3.33-3.98 times adipose maximum height vs. 2.15-2.76). *Hemibagrus chrysops* is found sympatrically with *H. nemurus* s. l. in northwestern Borneo, but the two species can be further differentiated by the size and shape of the male genital papilla. The population of *H. nemurus* s. l. in northwestern Borneo has a relatively elongate genital papilla with a rounded tip, which extends beyond the base of the first anal-fin ray in both subadult (ca. 150 mm SL) and adult males (Fig. 3A-B) while *H. chrysops* has a relatively short genital papilla with a truncate tip, which does not reach the base the first anal-fin ray in subadult males and just touches the base of the first anal-fin ray in adult males (Fig. 3C-D).

Hemibagrus chrysops also differs from all other nominal species of Sundaic *Hemibagrus* in its live coloration. The species possesses a vivid golden-yellow hue when alive or freshly dead while all other species of Sundaic *Hemibagrus* are either grey or olive-green in life. Occasionally, we have come across specimens of *H. nemurus* s. l. possessing a yellowish hue, but the colour has only been observed in specimens that have been dead for some time

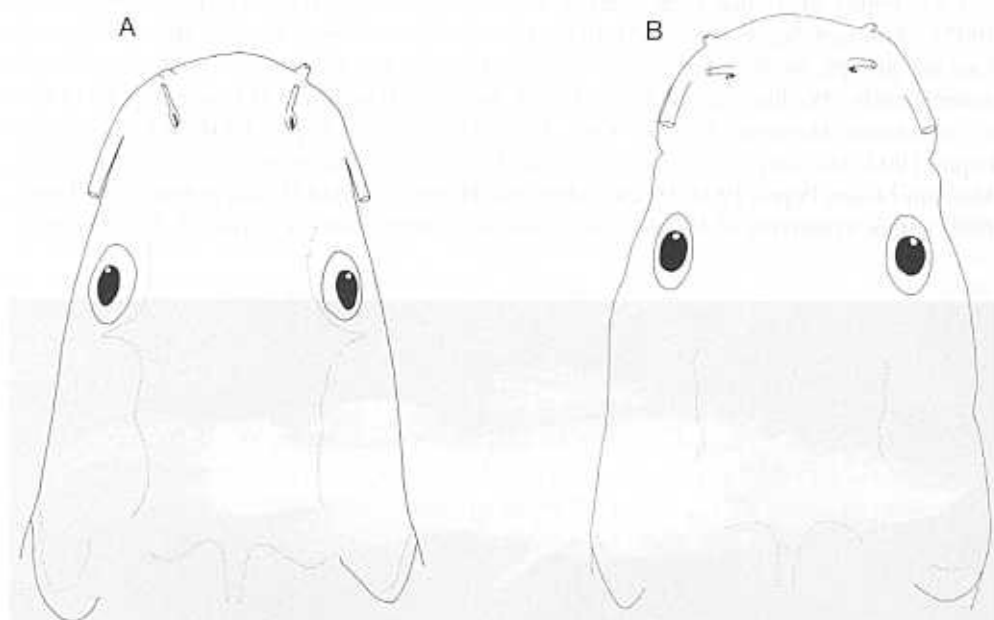


Fig. 2. Dorsal views of heads of: A. *Hemibagrus chrysops*, ZRC 39518, paratype, 150.4 mm SL; B. *H. nemurus* s. l., ZRC 38753, 131.2 mm SL; Borneo: Sarawak, Sungai Kerang at Balai Ringin.

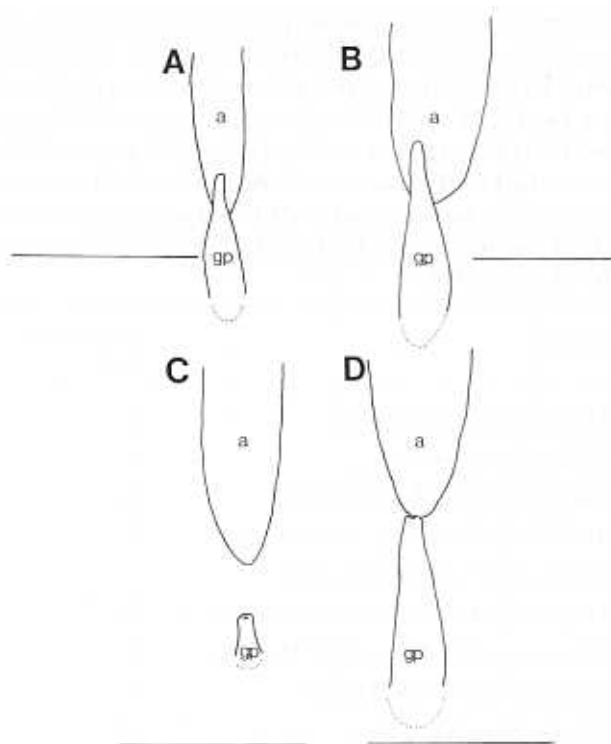


Fig. 3. Ventral view of male genital papilla (gp) and anal fin (a) of: A. *H. nemurus* s. l., ZRC 41860, 149.3 mm SL; B. *H. nemurus* s. l., ZRC 39527, 213.0 mm SL; C. *H. chrysops*, paratype, ZRC 39518, 150.4 mm SL; D. *H. chrysops*, holotype, ZRC 42653, 209.7 mm SL. Scale bars represent 10 mm.

and not in live or freshly-dead fish; in any case, it is never as intense as that of *H. chrysops*. The golden-yellow colour fades rapidly upon preservation and most preserved specimens are grey.

Hemibagrus baramensis was considered to be closely related to *H. nemurus* s. l. by Roberts (1989) and Ng & Ng (1995), but our examination of specimens indicate that this is not the case. *Hemibagrus baramensis* has a moderately long adipose fin greater than 20%SL, which is a characteristic shared by another Bornean species, *H. sabanus*, but is not seen in *H. nemurus* s. l. (which has an adipose fin less than 20%SL).

Kottelat & Lim (1995) have discussed the differences between *H. hoevenii* and other Sundaic *Hemibagrus* and they are applicable in this case as well. *Hemibagrus chrysops* differs from *H. hoevenii* in having the premaxillary toothband not exposed when the mouth is closed (vs. partially exposed), rounded (vs. tapering) caudal-fin lobes, a broad and conspicuous dark margin absent (vs. present) around the margins of the caudal fin, a rounded (vs. triangular) anal fin, a longer adipose fin base (length of adipose-fin base 16.6-19.3 %SL vs. 11.2-12.8), and a shorter dorsal spine (10.4-16.6 %SL vs. 16.9-19.3).

Genetic identity and phylogenetic relationships. The 26 RFLP haplotypes produced 12 *cytb* haplotypes (Table 1). The 14 new specimens of *H. nemurus* s. l. from the Sadong R., Sarawak revealed a thirteenth *cytb* haplotype. The *cytb* gene sequence distinguishing *H. chrysops* from congeners is diagnostic (Fig. 4) As the analyses of the morphological as well as the

Table 1. The RFLP haplotypes, origin, number of fish and the cytochrome b genotype assigned following sequencing 300 bp of the mtDNA cytochrome *b* gene. The RFLP haplotypes are as reported in Dodson et al (1995). Haplotype 1 is from specimens identified as *Hemibagrus gracilis*. Haplotypes 14, 15, 16 and 19 are from specimens identified as *H. chrysops*. Haplotypes 20, 24 and 32 are from specimens identified as *H. hoevenii*. All other haplotypes are from specimens identified as *H. nemurus* s. l. N.A. These fish were not subjected to a RFLP analysis. The specimens are deposited in ZRC and catalogue numbers for the RFLP haplotypes presented here are provided in Dodson et al. (1995). Catalogue numbers for *cytb* haplotype G are ZRC 38752-38753.

RFLP haplotype	Origin	Number of fish	<i>cytb</i> haplotype
1	Endau River, west Malaysia	4	A
14, 15	Sadong River, Sarawak	9	B
16, 19	Sadong & Rajang River, Sarawak	12	B
32	Musi River, Palembang, south Sumatra	2	C
20, 24	Kapuas River, west Kalimantan;		
	Musi River; Johor River, west Malaysia	12	D
4, 7	Terengganu & Kelantan, west Malaysia	4	E
8, 11	Pahang River, west Malaysia	4	F
N.A.	Sadong River, Sarawak	10	G
34	Mekong/Mun River, Thailand	3	H
3, 5, 6	Terengganu & Kelantan, west Malaysia	14	I
21, 23	Kapuas River	4	J
17, 18	Rajang River	3	K
12, 13	Perak, west Malaysia	12	L
22	Kapuas River	2	L
25, 26, 27	Cirata Reservoir, west Java	10	M

genetic data for the entire species complex are not yet completed, we offer here a preliminary picture of the number of valid species, the number of valid genetic clades and their phylogenetic relationships in the *H. nemurus* species-complex. *H. chrysops* is clearly distinct from all other *Hemibagrus* forms examined. As the clustering of haplotypes by both phenetic and cladistic procedures is identical, only the Neighbour-Joining phenogram is presented with bootstrap estimates derived from the parsimony analysis (Fig. 5). Ingroup monophyly (haplotypes B-M) was supported in 100% of the 10,000 parsimony trees generated by bootstrap resampling. In addition, a clade formed of haplotypes from specimens identified as *H. hoevenii* (haplotypes C & D) and *H. nemurus* s. l. (haplotypes B, E, F, G, H, I, J, K, L & M) occurred in 72% of the 10,000 parsimony trees generated by bootstrap sampling, corresponding to a greater than 95% probability that the clade is real. Thus, *H. chrysops* is distinct from the out-group and all other *Hemibagrus* lineages. *Hemibagrus chrysops* appears as the sister taxon to a large clade clustering *H. nemurus* s. l. and *H. hoevenii*. This clade is in turn composed of two smaller monophyletic groupings: the 'Sundaic' and 'Indochinese' clades (Dodson et al. 1995). The 'Sundaic' clade is well resolved with 89% bootstrap support in parsimony analysis. This clade occurs in various parts of Sundaland, including west Java, west Kalimantan, south Kalimantan, Sarawak and western peninsular Malaysia. The

'Indochinese' clade is relatively weakly supported, occurring in only 52% of the trees generated in parsimony analysis. Within this group, *H. hoevenii* appears as a sister species to the Indochinese *H. nemurus* s. l. genotypes, suggesting that *H. hoevenii* evolved on the Indochinese mainland (Dodson et al., 1995). The genetic distinctiveness of *H. hoevenii* was supported at the 100% bootstrap level. The Indochinese *H. nemurus* s. l. genotypes characterise fish from Thailand, eastern Peninsular Malaysia and Sarawak. Although this clade was originally associated uniquely with Indochinese mainland sites by Dodson et al.

(B)	TGAAACTTCGGCTCCCT CCTACTACTATGTTTAATAGTACAAATCCTAAC	50
(D) C T A	
(C) C T A	
(M) T T A	
(K) T T A	
(G) C T A	
(B)	CGGCCTATT CCTAGCCATACACTACACCTCCGACATCTCCACCGCCTTCT	100
(D) T G T G	
(C) T G T A	
(M) C G C A	
(K) C G C A	
(G) T G C A	
(B)	CATCCGTAGCCCACATCTG CCGAGATGTAAACTACGGCTGAATCATT CGA	150
(D) C T T T T C	
(C) C T T T T C	
(M) C T T T T C	
(K) T T T T T C	
(G) C C C T T C	
(B)	AACCTCCACGCCAACGGAGCCT CCTTCTTCTTCATCTGCATCTATCTCCA	200
(D) T T T T T C	
(C) T T T T T C	
(M) T T T T T C	
(K) T T T T T C	
(G) T T T T T C	
(B)	CATTGGTCGAGCACTTTACTATGGCTC ATACCTATATAAAGAAACCTGAA	250
(D) C T T C A C G	
(C) C T T C A C G	
(M) T C T C A T A	
(K) T C T C A T A	
(G) T C C C G T G	
(B)	ATATTGGAGTAATCCTTCTACTCCTAGTAATAATAACAGCCTTTGTTGGA	300
(D) T C G C T A C G T C C	
(C) T C G C T A C G T C C	
(M) C C G A C C T A T C C	
(K) C C G A T C T A T C C	
(G) T C G C T C T A T C C	

Fig. 4. Sequences of 300 bp from the 5' end of the cytochrome *b* gene characterising *Hemibagrus chrysops* (haplotype B), *H. hoevenii* (haplotypes C and D), *H. nemurus* from west Java (haplotype M), *H. nemurus* s. l. from Sarawak (haplotype K, 'Sundaic' clade) and *H. nemurus* s. l. from Sarawak (haplotype G, 'Indochinese' clade). The 36 variable positions in the *Hemibagrus* sequences are presented in bold.

(1995), the discovery of this clade in Sarawak (genotype G) suggests that this clade has succeeded in spreading from the mainland. As such, western Borneo appears to be home to at least four genetic groups of sympatric *Hemibagrus*: *H. chrysops*, two distinct lineages of *H. nemurus* s. l., and *H. hoevenii*, the last probably a relatively recent arrival. Given the widespread dispersal of two of the RFLP haplotypes characterizing *H. hoevenii* (20 & 24 in Table 1), apparently insufficient time has elapsed to allow the evolution of site-specific haplotypes. This suggests a relatively recent expansion of *H. hoevenii*, probably during low sea levels caused by some recent Pleistocene glaciation event (Dodson et al. 1995).

The genetic distances separating *H. chrysops* from other *Hemibagrus* are substantial and suggest origins that predate the Pleistocene (mean of 7.8 % sequence divergence with congeners in *H. nemurus* species-complex (containing both the 'Sundaic' and 'Indochinese'

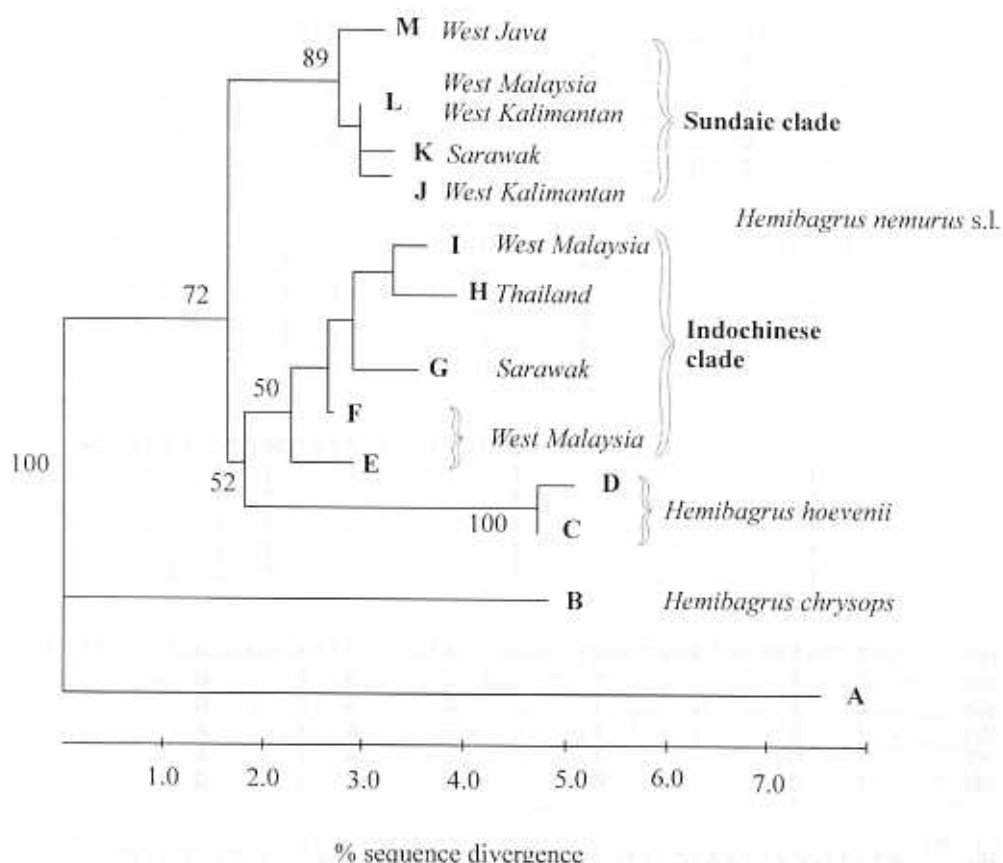


Fig. 5. Neighbour-joining phenogram clustering the distance matrix, based on 300 base pairs of cytochrome *b* sequence, of percent sequence divergence among 13 genotypes of the *Hemibagrus* complex. Letters at branch tips refer to the haplotypes identified in Table 1. The geographic origin of haplotypes characterising the two major clades (Sundaic and Indochinese) of *Hemibagrus nemurus* s. l. are indicated. The two haplotypes characterising *H. hoevenii* were found in south Sumatra (haplotype C), west Kalimantan and west Malaysia (haplotype D). Numbers at the forks indicate the percentage of times the group consisting of the genotypes located to the right of the fork occurred among 10,000 parsimony trees generated by bootstrap resampling of the cytochrome *b* sequence data. *Hemibagrus gracilis* from the Endau R., west Malaysia, (genotype A) was used as the outgroup taxon for both the Neighbour-joining and Parsimony analyses.

clades of *H. nemurus* s. l.) vs. 0.25-1.3 % sequence divergence within the 'Sundaic' and 'Indochinese' clades). Estimates of divergence times based on *cytb* sequence divergence (2.8% per million years; Orti et al. 1994), indicate that *H. chrysops* shared a common ancestor with the *H. nemurus*-*H. hoevenii* clade approximately 2.8 million years ago. Given the heterogeneity of evolutionary rate among taxa, any such calculation must be considered as a first-order estimate. Furthermore, the *H. nemurus* species-complex presented here almost certainly involves at least two species. To illustrate, the Bornean 'Sundaic' *H. nemurus* s. l. genotype sampled in the Rajang River (*cytb* haplotype K) and the 'Indochinese' *H. nemurus* s. l. haplotype sampled in the Sadong River (*cytb* haplotype G) are separated by 3.75% sequence divergence. Thus these two lineages appear to have last shared a common ancestor during the early Pleistocene approximately 1.3 million years ago. Although further analyses are required to clarify the identity and phylogenetic relationships of the various lineages composing *H. nemurus* s. l., *H. chrysops* is clearly a separate lineage with an independent evolutionary history that probably predates the Pleistocene.

COMPARATIVE MATERIAL

H. baramensis - BMNH 1895.7.2:50, 1 ex., holotype, 133.9 mm SL; Borneo: Baram district; C. Hose, 1894. - FMNH 51825, 9 ex., 57.0-140.9 mm SL; Borneo: Sabah, Sandakan district, Sapagaya forest reserve, tributary of Sapagaya River; R. F. Inger, 26 Jul.1950. - FMNH 68059, 12 ex., 17.7-168.5 mm SL; Borneo: Sabah, Tawau district, Kalabakan, Sungai Marikut; R. F. Inger, 16 Jun.1956. - FMNH 68060, 19 ex., 29.8-129.5 mm SL; Borneo: Sabah, Tawau district, Kalabakan, Sungai Tibas camp, Sungai Tawan (4°25'N 117°28'E); R. F. Inger, 8 Jun.1956. - FMNH 68091, 2 ex., 109.1-128.7 mm SL; Borneo: Sabah, Tawau district, Kalabakan, Sungai Tibas camp, Sungai Tawan (4°25'N 117°28'E); R. F. Inger, 6 Jun.1956.

H. hoevenii - ZRC 37472, 1 ex., neotype, 116.2 mm SL; Malaysia: Johor, Muar River at Kampong Bukit Lepong; M. Kottelat & K. K. P. Lim, 25 Jul.1992. - ZRC 29573-29582, 10 ex., 89.1-112.3 mm SL; Malaysia: Johor, Kota Tinggi; P. K. L. Ng, 1992. - ZRC 38002, 2 ex., 193.8-223.9 mm SL; Borneo: Sarawak, market at Marudi; M. Kottelat & T. Tan, 18-20 Jun.1994. - ZRC 40021, 2 ex., 216.7-252.0 mm SL; Borneo: Kalimantan Selatan, Banjarmasin, Pasar Lima Beton; H. H. Ng & O. Chia, 30 May-1 Jun.1996. - ZRC 40499, 3 ex., 153.9-210.1 mm SL; Borneo: Sarawak, Miri, Kampung Bakung from Sungai Bakung, turnoff after 62 km to Batu Niah; H. H. Tan & Y. Y. Goh, 25 Sep.1996. - ZRC 40511, 8 ex., 176.7-255.5 mm SL; Borneo: Sarawak, Marudi market, from Batang Baram; H. H. Tan & Y. Y. Goh, 28 Sep.1996. - ZRC 40590, 10 ex., 169.1-292.0 mm SL; Malaysia: Johor, Muar, Seram III, Tanjung Selabu port; Y. Y. Goh, 20 Nov.1996. - ZRC 41526, 2 ex., 234.2-240.0 mm SL; Sumatra: Jambi, Pasar Angso Duo; H. H. Tan & H. H. Ng, 23-29 Jul.1995.

H. nemurus s. l. - ZRC 29583-29596, 14 ex., 177.6-214.2 mm SL; Thailand: Bangkok; J. Dodson, Dec.1992. - ZRC 29659-29665, 7 ex., 132.3-158.0 mm SL; Thailand: Bangkok; J. Dodson, Dec.1992. - ZRC 39345, 4 ex., 95.2-135.3 mm SL; Thailand: Yasothon province, Amphoe Muang Yasothon; S. Kuntarphrung, 16 Oct.1993. - ZRC 40896, 3 ex., 59.2-101.2 mm SL; Thailand: Nakhon Ratchasima province, drained irrigation ponds or padi fields along Highway 24, fed by Klong Kabau (14°44'34.8"N 102°12'31.7"E); H. H. Tan et al., 16 Jan.1997. - CAS 133026, 1 ex., 213.1 mm SL; Malaysia: Johor, Sungai Kayu, 16 miles north of Kota Tinggi; A. W. Herre, Feb.1937 (holotype of *H. johorensis*). - CAS 133025, 1 ex., 192.2 mm SL; Malaysia: Pahang, Sungai Garam near Karak; A. W. Herre, Mar.1937 (holotype of *H. pahangensis*). - ZRC 9555, 1 ex., 128.7 mm SL; Malaysia: Johor, Mawai district; P. K. L. Ng, 6 Dec.1992. - ZRC 11642, 1 ex., 190.0 mm SL; Malaysia: Johor, near Layang Layang; P. K. L. Ng, 14 Mar.1990. - ZRC 14899, 1 ex., 153.7 mm SL; Malaysia: Selangor, North Selangor peat swamp forest, irrigation canal on W boundary; NUS 1991-92 Zoology Honours class, 20 Jun.1991. - RMNH 7551, 4 ex., 54.4-272 mm SL; Borneo: Bo River; A. W. Nieuwenhuis, May-Aug.1900 (syntypes of *H. fortis*). - RMNH 7552, 1 ex., 197 mm SL; Borneo: Bo River; A. W. Nieuwenhuis, May-Aug.1900 (holotype of *H. fortis* var. *capitulum*). - RMNH 7549, 1 ex., 134.5 mm SL; Borneo: Bo River; A. W. Nieuwenhuis, May-Aug.1900 (holotype of *H. bo*). - RMNH 7547, 1 ex., 106.4 mm SL; Borneo: Howong River; A. W. Nieuwenhuis, May-Aug.1898 (holotype of *H. howong*). - RMNH

7550, 1 ex., 122.7 mm SL; Borneo: Kajan River; A. W. Nieuwenhuis, Sep-Oct.1900 (holotype of *H. kajan*). - ZRC 37709, 1 ex., 189.2 mm SL; Borneo: Sarawak, Lanjak Entimau Wildlife Sanctuary; C. H. Diong, 17-23 May.1994. - ZRC 38752, 5 ex., 165.5-222.7 mm SL; ZRC 38753, 4 ex., 131.2-196.5 mm SL; ZRC 39527, 3 ex., 210.3-220.8 mm SL; Borneo: Sarawak, Balai Ringin, stalls by roadside next to bridge over Sungai Kerang; H. H. Tan et al., 19 Feb.1997. - ZRC 39526, 5 ex., 198.2-219.8 mm SL; Borneo: Sarawak, Serian market; H. H. Tan et al., 19-20 Feb.1997. - ZRC 39747, 2 ex., 135.4-146.0 mm SL; Borneo: Sarawak, Serian market, from Batang Kerang; P. K. L. Ng et al., 14-17 Jan.1996. - ZRC 40488, 7 ex., 131.4-212.7 mm SL; Borneo: Sarawak, Serian market; H. H. Tan et al., 31 Aug-5 Sep.1996. - ZRC 40498, 7 ex., 153.0-232.5 mm SL; Borneo: Sarawak, Miri, Kampung Bakung from Sungai Bakung turnoff after km 62 to Batu Niah; H. H. Tan & Y. Y. Goh, 25 Sep.1996. - ZRC 41860, 3 ex., 97.4-162.0 mm SL; Borneo: Sarawak, Balai Ringin, stalls by roadside next to bridge over Sungai Kerang; H. H. Tan et al., 24 Oct.1997. - ZRC 40521, 1 ex., 190.0 mm SL; Borneo: Sabah, Danum, Sepat Kalisun stream; H. H. Tan & Y. Y. Goh, 1 Oct.1996. - ZRC 40522, 1 ex., 126.9 mm SL; Borneo: Sabah, Danum, Sungai Palum Tambun, tributary of Sungai Segama, upstream of Danum Valley field centre; H. H. Tan & Y. Y. Goh, 1 Oct.1996. - ZRC 40529, 1 ex., 74.5; Sabah: Danum Valley; H. H. Tan & Y. Y. Goh, 1 Oct.1996. - ZRC 40022, 1 ex., 192.2 mm SL; Borneo: Kalimantan Selatan, Banjarmasin, Pasar Lima Beton; H. H. Ng & O. Chia, 30 May-1 Jun.1996. - ZRC 40070, 1 ex., 228.3 mm SL; Borneo: Kalimantan Selatan, Banjarmasin, Pasar Kuin; H. H. Ng & O. Chia, 30 May.1996. - ZRC 40084, 4 ex., 158.8-187.8 mm SL; Borneo: Kalimantan Selatan, market at Negara; H. H. Ng & O. Chia, 31 May.1996. - ZRC 40042, 1 ex., 224.6 mm SL; Borneo: Kalimantan Tengah, Muara Teweh, Pasar Pendopo; H. H. Ng & O. Chia, 3-4 Jun.1996. - NMBE 1020756, 1 ex., 129.1 mm SL; Sumatra: Palembang; W. Volz, Oct.1900 (holotype of *H. bleekeri* Volz (non Day)). - ZRC 39032, 7 ex., 122.6-227 mm SL; Sumatra: Riau, Sungai Bengkwan, tributary of Indragiri (Batang Kuantan), 4 hrs downstream from Rengat; P. K. L. Ng et al., 14 Jun.1995. - ZRC 29513-29531, 19 ex., 204.0-380.0 mm SL; Sumatra: Jambi; J. Dodson, 1992. - ZRC 38663, 1 ex., 113.9 mm SL; Sumatra: Jambi, Batang Hari, near Tanjung Johor; M. Kottelat & H. H. Tan, 5 Jun.1994. - ZRC 39151, 1 ex., 115.2 mm SL; Sumatra: Jambi, Berbak Nature Reserve, Sungai Air Hitam Dalam; H. H. Ng & S. H. Tan, 16 Jun.1995. - ZRC 39181, 1 ex., 132.4 mm SL; Sumatra: Jambi, Sungai Alai at 19.5 km Muara Bungo-Muara Tebo road; coll. S. H. Tan & H. H. Ng, 22 Jun.1995. - ZRC 40534, 3 ex., 230.8-247.9 mm SL; Sumatra: Jambi, Pasar Angso Duo; H. H. Tan et al., 5-8 Jun.1996. - ZRC 42261, 10 ex., 71.7-123.2 mm SL; Sumatra: Jambi, ca. 15 mins. After Kampung Rantau Panjang along Batang Hari confluence; H. H. Tan et al., 6 Jun.1996. - ZRC 42554, 17 ex., 33.5-70.0 mm SL; Sumatra: Jambi, Pijoan, Danau Souak Padang; H. H. Tan et al., 8 Jun.1996. - ZRC 41504, 1 ex., 255 mm SL; Java: Sungai Sokan at Cibalagung, a probable inlet of the Cirata Reservoir at Citarum; Y. Y. Goh & T. B. Lim, 21 Jun.1997. - ZRC 40554, 6 ex., 339-390 mm SL; West Java; J. Dodson, 1992.

H. sabanus - FMNH 68088, 1 ex., holotype, 118.5 mm SL; Borneo: Sabah, Kinabatangan District, Kinabatangan river at Deramakot camp; R. F. Inger, 7 May.1956. - FMNH 68061, 1 ex., paratype, 97.5 mm SL; Borneo: Sabah, Kinabatangan District, Kinabatangan river at Deramakot camp; R. F. Inger, 24 Apr.1956. - FMNH 68062, 5 ex., paratypes, 47.7-93.3 mm SL; Borneo: Sabah, Kinabatangan District, Kinabatangan river at Deramakot camp; R. F. Inger, 8 May.1956. - FMNH 68085, 1 ex., paratype, 151.6 mm SL; Borneo: Sabah, Kinabatangan District, Kinabatangan river at Deramakot camp; R. F. Inger, 12 May.1956. - FMNH 68086, 1 ex., paratype, 130.7 mm SL; Borneo: Sabah, Kinabatangan District, Kinabatangan river at Deramakot camp; R. F. Inger, 29 Apr.1956. - FMNH 68087, 1 ex., paratype, 94.8 mm SL; Borneo: Sabah, Kinabatangan District, Kinabatangan river at Deramakot camp; R. F. Inger, 6 May.1956. - ZRC 40493, 1 ex., 104.9 mm SL; Borneo: Sabah, lower Segama river at Bukit Belacong; K. M. Martin-Smith, 15 Jun.1996. - ZRC 41190, 1 ex., 93.2 mm SL; Borneo: Sabah, Sungai Kinabatangan at Sukau; B. M. Manjaji, Mar.1997.

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