Indian J. Fish., 71 (4): 20-26, 2024 doi:10.21077/ijf.2024.71.4.146333-03

Available online at: epubs.icar.org.in/index.php/IJF

A new sympatric species of *Horabagrus* (Siluriformes: Horabagridae) from the Chalakkudy river basin in Kerala, India

Rahul G. Kumar, Charan Ravi, N. P. Krishnaprasoon and V. S. Basheer*

Centre for Peninsular Aquatic Genetics Resources, ICAR-National Bureau of Fish Genetic Resources, Kochi - 682 018, Kerala, India

Abstract

We describe a new species of *Horabagrus*, found in sympatry with *H. nigricollaris*, from the Chalakkudy river basin in Kerala, South India. *Horabagrus obscurus*, new species, differs from the two other known species in the genus in its colour pattern, lesser body width at the pectoral girdle, shorter barbels and total vertebral counts. Differences in partial sequences of the mitochondrial COI and CytB genes also serve to distinguish the new species from congeners. The new discovery reasserts the importance of the Chalakkudy river basin as a hotspot of piscine diversity in the Western Ghats.

Introduction

The catfish genus *Horabagrus* Jayaram currently includes two valid species endemic to west-flowing rivers originating in the Western Ghats of India. *Horabagrus brachysoma* (Gunther) is a widespread species, inhabiting the lower reaches of rivers from near Trivandrum in the south to Southern Maharashtra (Pethiyagoda and Kottelat, 1994, Bhat, 2001, Katwate *et al.,* 2012, Raghavan *et al.,* 2016) in the north. *Horabagrus nigricollaris* Pethiyagoda and Kottelat, in contrast, has a limited distribution in the middle reaches of the Chalakkudy River in Central Kerala (Pethiyagoda and Kottelat, 1994, Ali and Prasad 2007). A third nominal species, *H. melanosoma* Plamoottil and Abraham (2013), from the Manimala River in Kerala was shown to be based on dark coloured individuals of *H. brachysoma* and thus a synonym of the latter (Ali *et al.,* 2014).

Attempts to collect specimens of *H. nigricollaris* from the Chalakkudy River in 2019 yielded five specimens significantly larger than typical *H. nigricollaris* and lacking the characteristic humeral "collar" marking of that species. Fishermen who helped us catch the fish insisted they were *H. nigricollaris* and said the fish loses the collar marking as it grows in size. Additional collections yielded more specimens of this distinct form, including individuals overlapping in size with typical *H. nigricollaris*, yet lacking the distinctive marking. An analysis of mitochondrial genes and a morpho-meristic study revealed clear differences between the newly collected specimens and the previously described species, which led us to conclude it represents a distinct species, new to science. This manuscript serves to document our findings and describe the new species.

Materials and methods

Specimens were collected using gillnets or traps, euthanised with an overdose of 2-phenoxy ethanol, fixed and preserved in 10% formalin. Prior to fixation, a piece of gill tissue or a muscle plug from the right side was excised and stored in absolute ethanol for molecular analysis. Measurements were made on the left side of specimens with digital calipers, following Skelton and White (1990). Values less than 100 mm were recorded to the nearest tenth of a millimetre, while those greater were recorded to the closest millimetre. Lengths of both upper and lower caudal-fin lobes were recorded.

*Correspondence e-mail:
vsbasheer@gmail.com

Keywords:
Biodiversity, Conservation, Horabagridae, New Species, Western Ghats

Received : 13.12.2023
Accepted : 09.12.2024

Fin ray counts were taken from x-rays or with transmitted light; the last branched ray, which is split to the base, was counted as 1. Vertebral counts were recorded from x-rays and excluding the Weberian apparatus. Numbers in brackets after a count indicate the number of specimens exhibiting that value.

DNA was extracted from tissue samples using a standard salting out protocol. Around 670 bp of the mitochondrial COI was amplified using the primer pair, Fish F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al.,* 2005). The thermal profile for the PCR reaction was 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 45 s at 53°C, 60 s at 72°C and final extension for 10 min at 72°C. Around 1140 bp of the mitochondrial CytB gene was amplified using the primer pair, L14724: (5'-GTGACTTGAAAAACCACCGTTG-3') and H15700: (5'-GTTTACAAGACCGGT GCTCTGA-3') (Palumbi *et al.,* 1991). The thermal profile for the PCR reaction was 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 45 s at 49°C, 60 s at 72°C and final extension for 5 min at 72°C. Sequences were aligned using the MUSCLE (Edgar, 2004) algorithm implemented in SeaView ver.5 (Gouy *et al.,* 2010). Protein coding genes were translated to amino acids to check for premature stop codons. The generated sequences were uploaded to GenBank (Table 1). p-distances were calculated in MEGA 7 (Kumar *et al.,* 2016). The BLAST (Altschul *et al.,* 1990) tool was used to identify and download similar sequences ion GenBank. Sequences of *H. brachysoma* were also downloaded from GenBank for comparison. Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.,* 2012) was used to determine species limits separately for each gene using the online interface (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with default settings.

Results

Horabagrus obscurus, new species

Holotype: NBFGR/HORHOBS, 323 mm SL; Chalakkudy River near Vettilappara, 5 February 2020 (Figs. 1 and 2).

Paratypes: NBFGR/HORHOBS.1-8, 8 exs, 152-408 mm SL, same data as holotype. ZSI-WGRC, 3exs, 185-280 mm SL, same data as holotype (Figs. 1 and 2).

*Diagnosis***:** *Horabagrus obscurus* is distinguished from its congeners by a distinctive colour pattern in life, consisting of a greenish-brown ground colour mottled with black and a small black humeral blotch with a yellow to white region immediately anteroventral to the blotch (*vs*. a yellow ground colour with a large circular black humeral blotch, larger than eye diameter, ringed with pale yellow to white in *H. brachysoma*; and a distinct black "collar" covering the humeral region and nape, margined by a pale yellow to white band in *H. nigricollaris*). *Horabagrus obscurus* can additionally be distinguished from *H. nigricollaris* in having a larger adult size (>250 mm SL vs <200 mm SL); a narrower caudal peduncle (its least depth 8.5-10.8% SL *vs* 10.6-11.5% SL); a smaller eye (orbital diameter 13.4-19.7% HL *vs* 20.0-25.1% HL), a shorter pectoral spine (48.9–62.3% HL *vs* 65.3–72.4% SL); a more slender body (width of pectoral girdle 72.3–76.9% HL *vs* 79.5–86% HL); a shorter maxillary barbel, usually short of and sometimes just reaching the pectoral fin origin (*vs* always extending past the base of the pectoral fin) and a greater number of total post-Weberian vertebrae (modally 38 *vs* 36) (Fig. 3).

Description: Based on the holotype and 11 paratypes. The general appearance is as in Fig. 1 and 2, morpho-meristic data are given in Table 2.

The dorsal profile up to the origin of the dorsal fin is generally straight, but is sometimes convex in individuals over 350 mm SL. The body is comparatively slender (width of pectoral girdle 72.3-76.9% HL). The dorsal fin is set forward on the body (pre-dorsal length 31.9-39% SL). The caudal peduncle is longer (9.5-12.3% SL) than deep (8.5-10.8% SL).

The dorsal fin bears a spinelet, a stout spine with 10-16 serrae on the posterior margin (dorsal spine length 10.7-14.7% SL) and 5 branched rays, the last of which is split to the base. The pectoral fin has a stout spine, bearing 13-17 stout retrorse serrae on the posterior edge and 0-25 antrorse denticulations on the posterior edge, followed by 7-8 branched rays, the last of which is split to the base. The tip of the adpressed pectoral fin reaches the dorsal fin origin. The pelvic fin originates posterior to the dorsal fin base; bears one simple and 5 branched rays and reaches the anal fin origin when adpressed. The anal fin is long based and bears 3 unbranched and 25-26 branched rays, the last of which is split to the base. The caudal fin is forked, with rounded lobes, the upper usually

Table 1. Accession numbers of sequences used in this study

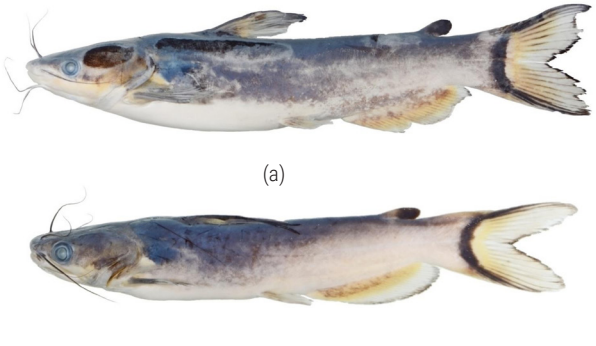
Species	Gene	Accession No.	Remarks
Horabagrus obscurus	COI	MZ351296 – MZ351301	Generated in this study
		KM874793.1, KM874794.1, KM874796.1,	From GenBank as *H. nigricollaris*
	CytB	MZ359224 – MZ359229	Generated in this study
Horabagrus nigricollaris	COI	MZ350467 – MZ350470	Generated in this gtudy
		HM579861.1, HQ009503.1, KM874797.1, MG986722.1, KM874795.1,	From GenBank
	CytB	MZ359230 – MZ359233	Generated in this study
		MG986722.1, HM579857.1,	From GenBank
Horabagrus brachysoma	COI	EU490864.1, HM579863.1, HQ009501.1, JX460967.1, KM874791.1, KU568858.1, KU568859.1, KU870467.1, MG923364.1	From GenBank
	CytB	KU870467.1, EU490913.1, HM579856.1, JX460969.1, JX460962.1,	From GenBank

(a)

(b)

(c)

Fig. 1. *H. obscurus* in life, all from the Chalakkudy River at Vettilappara. (a) NBFGR/HORHOBS.7, paratype, 248 mm SL, (b) NBFGR/HORHOBS.4, paratype, 206 mm SL and (c) NBFGR/HORHOBS.1, paratype, 152 mm SL



(a)

(b)

Fig. 2. *H. obscurus* in preservative. (a) NBFGR/HORHOBS, holotype, 323 mm SL, Chalakkudy River at Vettilappara and (b) NBFGR/HORHOBS.1, paratype, 152 mm SL, same data as holotype

longer and bears 9+9 branched rays. There are 35(1), 36(1), 37(1) or 38(3) total post-Weberian vertebrae.

The head is broad, anteriorly depressed, granulated dorsally. The mouth is sub-terminal, extends the full width of the head and is broadly crescentic. There are 4 pairs of barbels; the maxillary and outer mandibular are nearly as long as the head, the nasal and inner mandibular about 60% their length. The eye is prominent (orbit diameter 13.4-19.7% HL), set laterally and visible ventrally. The inter-orbital width (43.4-52.9% HL) is greater than the snout length (35.3-42.6% HL) The supra-occipital process is long, slender and touches the pre-dorsal plate.

Colour: Live specimens exhibit a greenish-brown body colour, mottled with black, darker dorsally. Belly white, sometimes with diffused yellow colour in the region of transition. The head is dark, almost black, above the lower margin of the orbit, white below. There is a black humeral spot, roughly about the same diameter as the eye, with a yellow to white patch immediately antero-ventral to it. The caudal fin is reddish orange, with its base, upper, lower and outer margins outlined in black. The anal fin is reddish orange, margined in black. The dorsal fin is dark brown to black, sometimes with a diffuse white distal margin.

Formalin-preserved specimens are grey with flecks of black; the belly is white. The dorsal fin is black with a hyaline to white distal margin. The caudal and anal fins are a yellowish orange, margined in black. The pectoral and pelvic fins are hyaline.

Distribution and ecology: The species is currently known from the Chalakkudy river basin between the Athirapilly waterfalls and the dam at Thumburmuzhy. The habitat in this region features rapid water flow over a hard substrate of bedrock. Sympatric species include *H. nigricollaris, Osteochilichthys longidorsalis, Sahyadria chalakkudiensis, D. filamentosa, Garra surendranathanii* and *Labeo nigrescens.*

Both *H. obscurus* and *H. nigricollaris* are primarily fished in the low-water season from deep pools, mainly using traps with the fruit of the oil palm as bait. Some live specimens of *H. obscurus* regurgitated small bivalve shells a few hours after capture.

Etymology: From the Latin obscurus, meaning dark or cloudy, a reference to the colour pattern of the fish. The word obscure in english also means cryptic or ambiguous and refers to the confusion between *H. obscurus* and the sympatric *H. nigricollaris*.

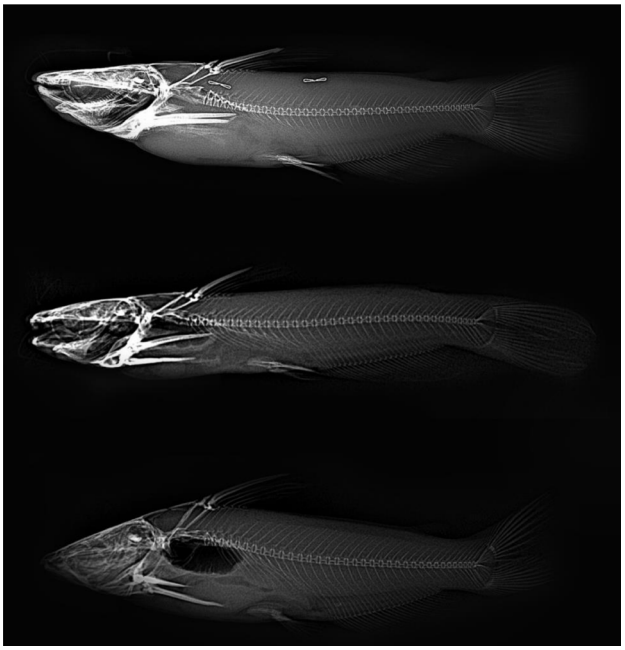


Fig. 3. X ray_ of *Horabagrus* species. Top: *H. obscurus*, NBFGR/HORHOBS.7, paratype, 248 mm SL. Middle: *H. nigricollaris*, NBFGR/HORHNIG.8, 168 mm SL. Bottom: *H. brachysoma*, 200 mm SL.

Table 2. Morphomeristic data of species of *Horabagrus*

Measurements (mm)	*H. brachysoma*			*H. nigricollaris*			*H. obscurus*			
							Holotype	Holotype + 11 Paratypes		
	Range	Mean	SD	Range	Mean	SD		Range	Mean	SD
Standard length	176-202 mm			124-168 mm			323 mm	152-408		
Pre-dorsal length	35.3-39.3	38.1	1.6	34.0-37	35.3	1.1	39.0	31.9-39.0	36.3	2.1
Pre-pelvic length	51-53.9	52.0	1.2	51.5-54.8	53.1	1.2	53.9	46.4-54.2	52.4	2.5
Pre-anal length	61.9-66.3	63.2	1.8	62.8-66.9	64.2	1.4	67.5	57.3-67.5	65.0	3.1
Length of caudal peduncle	9.3-13.3	11.2	1.6	9.0-11.7	10.8	0.8	11.4	9.1-12.3	10.5	0.9
Depth of caudal peduncle	10.1-12.3	11.3	1.0	10.6-11.5	10.9	0.3	10.4	8.5-11.1	9.9	0.7
Adipose to caudal peduncle length	13.4-16.3	15.4	1.1	11.6-16.2	14.5	1.7	14.4	11.9-17.0	14.7	1.1
Dorsal to adipose length	32.8-36.9	34.8	1.8	37.6-41.1	39.3	1.3	37.0	29.1-46.0	35.8	4.5
Adipose base length	4.8-6	5.5	0.5	3.0-3.9	3.3	0.4	3.5	2.8-6.4	3.8	0.6
Caudal total length	21.6-25.8	23.3	1.6	22.0-24.3	23.0	0.9	22.4	17.6-27.1	21.8	2.5
Caudal fork length	9.6-9.8	9.7	0.1	10.5-13	11.5	1.0	10.1	7.9-13.0	10.7	1.4
Dorsal spine length	12.1-19.1	16.4	3.4	13.7-17	15.1	1.1	13.4	10.7-15.7	13.6	1.5
Dorsal fin length	21.8-25.6	23.4	1.7	20.5-23.8	22.9	1.0	22.7	19.4-28.4	24.1	3.0
Dorsal to coracoid length	23.2-26.9	24.9	1.6	20.3-24	21.1	1.2	24.1	18.7-24.3	22.2	1.8
Head length	27.8-30.9	29.9	1.2	25.2-27.6	26.8	0.8	29.1	25.7-30.1	28.8	1.3
Snout length	37.1-40.5	38.9	1.4	33.9-40.9	38.1	2.6	41.5	34.9-42.6	39.3	2.2
Orbit diameter	16.7-18.6	17.6	0.8	20.0-25.1	22.3	1.9	14.6	13.4-19.7	16.3	2.2
Interorbital width	47.5-50.1	48.7	1.0	44.8-52.6	49.1	2.7	51.3	41.3-52.9	47.7	3.1
Width of occipital process base	9.5-13.3	11.6	1.4	8.5-16.2	10.8	2.5	11.3	7.7-11.3	9.8	1.4
Coracoid to cranium length	52-57.5	55.1	2.6	55.6-64.6	60.5	2.9	58.0	48.6-67.2	60.4	3.7
Humeral process length	31.5-42.9	37.5	4.1	38.2-46.9	41.5	3.1	33.0	31.4-39.5	34.8	2.7
Pectoral girdle width	70.4-71.8	71.3	0.6	79.5-86	81.1	2.2	72.7	72.3-76.9	74.1	1.5
Mouth width	48.1-51.8	49.6	1.4	47.5-52.8	50.4	1.8	53.2	43.6-55.1	51.3	3.1
Pectoral spine length	61.667.2	64.8	2.9	65.3-72.4	69.3	2.8	58.1	48.9-66.5	57.9	6.4

Molecular analysis

The BLAST tool revealed sequences identical to those from the new species have previously been uploaded to GenBank as *H. nigricollaris* (Table 1). Intraspecific p-distances for the COI gene (Table 3) were less than 0.2% for *Horabagrus obscurus*, 0.0% for *H. nigricollaris* and less than 0.7% for *H. brachysoma*. Interspecific p-distances were 2.2-2.6% between *H. brachysoma* and *H. nigricollaris*, 2.6-2.8% between *H. obscurus* and *H. nigricollaris* and 2.1-2.8% between *H. obscurus* and *H. brachysoma*. Intraspecific p-distances for the CytB gene (Table 4) were less than 0.3% for *H. obscurus*, less than 0.2% for *H. nigricollaris* and less than 1.5% for *H. brachysoma*. Interspecific distances were 2.3-3.0% between *H. brachysoma* and *H. nigricollaris*, 2.5-3.3% between the *H. obscurus* and *H. brachysoma* and 2.6-3.0% between *H. obscurus* and *H. nigricollaris*.

ABGD recovered three distinct groups, corresponding to *H. brachysoma, H. nigricollaris* and *H. obscurus* for the COI and CytB genes (Fig. 4).

Discussion

All three species of *Horabagrus* are readily distinguishable from each other by their colour pattern (Fig. 5). In the field, specimens of same size class of *H. obscurus* and *H. nigricollaris* can be differentiated by the dark humeral marking, which is margined posteriorly by a pale yellow to white coloured region which extends to the dorsal region in *H. nigricollaris* but entirely absent in *H. obscurus*.

Horabagrus brachysoma is a fluviatile species known to enter brackishwaters of low salinity. It is widely distributed in the west flowing rivers originating from the southern Western Ghats. It supports a commercial fishery, especially in the Kuttanad region in the state of Kerala and is capable of growing to over 400 mm SL. *Horabagrus nigricollaris*, in contrast, has a very limited distribution, from the middle reaches of a single river system and is a comparatively smaller species, the largest being less than 200 mm SL (Pethiyagoda and Kottelat, 1994, Ali and Prasad, 2007). The discovery of a new species living in sympatry with and apparently sharing the same restricted distribution *H. nigricollaris,* raises several interesting questions, including the possibility of a hybrid. While *H. brachysoma* is primarily a lowland species, preferring deep, slow flowing water, *H. nigricollaris* is confined to rapid regime of the river with bedrock and boulders as primary substrates. Though both species do coexist in the wild but have distinct spawning periods. *H. brachysoma* spawning in May–July (Bindu *et al.,* 2012, Chandran and Prasad, 2014) while *H. nigricollaris* spawns after the north-east monsoon in November–February (Ali and Prasad, 2007). *Horabagrus obscurus* is a large species (>250 mm SL) sympatric with *H. nigricollaris* and unfamiliar to fishers in the lower stretches of the river where *H. brachysoma* occur. We hypothesise the differences in habitat preferences and reproductive cycles between *H. brachysoma* and *H. nigricollaris* are ecological barriers to prevent their hybridisation in the wild. The differences in the mitochondrial genomes of the three species also support erection of new species.

The discovery of *H. nigricollaris,* 150 years after the description of *H. brachysoma* and the discovery of *H. obscurus* 25 years after

Table 3. p-distances for the COI gene in *Horabagrus*

EU490864.1 *H. brachysoma*																									
HM579863.1 *H. brachysoma*	0.6																								
HQ009501.1 *H. brachysoma*	0.0	0.6																							
JX460967.1 *H. brachysoma*	0.6	0.4	0.6																						
KM874791.1 *H. melanosoma*	0.2	0.7	0.2	0.7																					
KU568858.1 *H. brachysoma*	0.0	0.6	0.0	0.6	0.2																				
KU568859.1 *H. brachysoma*	0.0	0.6	0.0	0.6	0.2	0.0																			
KU870467.1 *H. brachysoma*	0.0	0.6	0.0	0.6	0.2	0.0	0.0																		
MG923364.1 *H. brachysoma*	0.0	0.6	0.0	0.6	0.2	0.0	0.0	0.0																	
HM579861.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4																
MZ350467 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0															
MZ350470 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0														
MZ350469 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0	0.0													
MZ350468 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0	0.0	0.0												
HQ009503.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0	0.0	0.0	0.0											
KM874795.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0	0.0	0.0	0.0	0.0										
KM874797.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0									
MG986722.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0								
MZ351296 *H. obscurus*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6							
MZ351297 *H. obscurus*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0						
MZ351298 *H. obscurus*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0	0.0					
MZ351299 *H. obscurus*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0	0.0	0.0				
MZ351300 *H. obscurus*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0	0.0	0.0	0.0			
MZ351301 *H. obscurus*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0	0.0	0.0	0.0	0.0		
KM874793.1 *H. nigricollaris*	2.6	2.4	2.6	2.1	2.8	2.6	2.6	2.6	2.6	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	0.2	0.2	0.2	0.2	0.2	0.2	
KM874794.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.2
KM874796.1 *H. nigricollaris*	2.4	2.2	2.4	2.2	2.6	2.4	2.4	2.4	2.4	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0

Table 4. p-distances for the CytB gene in *Horabagrus*

JX460962.1 *H. brachysoma*																
JX460969.1 *H. brachysoma*	0.0															
KU870467.1 *H. brachysoma*	1.0	1.0														
HM579856.1 *H. brachysoma*	1.5	1.5	0.8													
EU490913.1 *H. brachysoma*	1.0	1.0	0.2	0.8												
MZ359232 *H. nigricollaris*	2.3	2.3	2.8	2.8	2.8											
MZ359231 *H. nigricollaris*	2.3	2.3	2.8	2.8	2.8	0.0										
MZ359230 *H. nigricollaris*	2.3	2.3	2.8	2.8	2.8	0.0	0.0									
HM579857.1 *H. nigricollaris*	2.5	2.5	3.0	3.0	3.0	0.2	0.2	0.2								
MG986722.1 *H. nigricollaris*	2.3	2.3	2.8	2.8	2.8	0.0	0.0	0.0	0.2							
MZ359233 *H. nigricollaris*	2.3	2.3	2.8	2.8	2.8	0.0	0.0	0.0	0.2	0.0						
MZ359227 *H. obscurus*	2.5	2.5	2.9	3.0	2.9	2.6	2.6	2.6	2.8	2.6	2.6					
MZ359228 *H. obscurus*	2.5	2.5	2.9	3.0	2.9	2.6	2.6	2.6	2.8	2.6	2.6	0.0				
MZ359229 *H. obscurus*	2.6	2.6	2.8	2.9	2.8	2.7	2.7	2.7	2.9	2.7	2.7	0.1	0.1			
MZ359224 *H. obscurus*	2.7	2.7	3.1	3.3	3.1	2.8	2.8	2.8	3.0	2.8	2.8	0.2	0.2	0.3		
MZ359226 *H. obscurus*	2.6	2.6	3.0	3.1	3.0	2.7	2.7	2.7	2.9	2.7	2.7	0.1	0.1	0.2	0.1	
MZ359225 *H. obscurus*	2.6	2.6	2.8	2.9	2.8	2.7	2.7	2.7	2.9	2.7	2.7	0.1	0.1	0.0	0.3	0.2

H. nigricollaris are testament to the aquatic diversity still waiting to be discovered in the rivers of the Western Ghats. This discovery also adds to the diversity described from the stretch of the Chalakkudy River between the Athirampilly waterfalls and Thumburmuzhy, which are highly adapted to life in these fast flowing, oxygen rich waters.

Acknowledgements

The authors would like to express their sincere thanks to the Director, ICAR-NBFGR, Lucknow for providing facilities to carry out this study. The present work was done under the project on Exploration of Western Ghats fishes. The authors would also like to

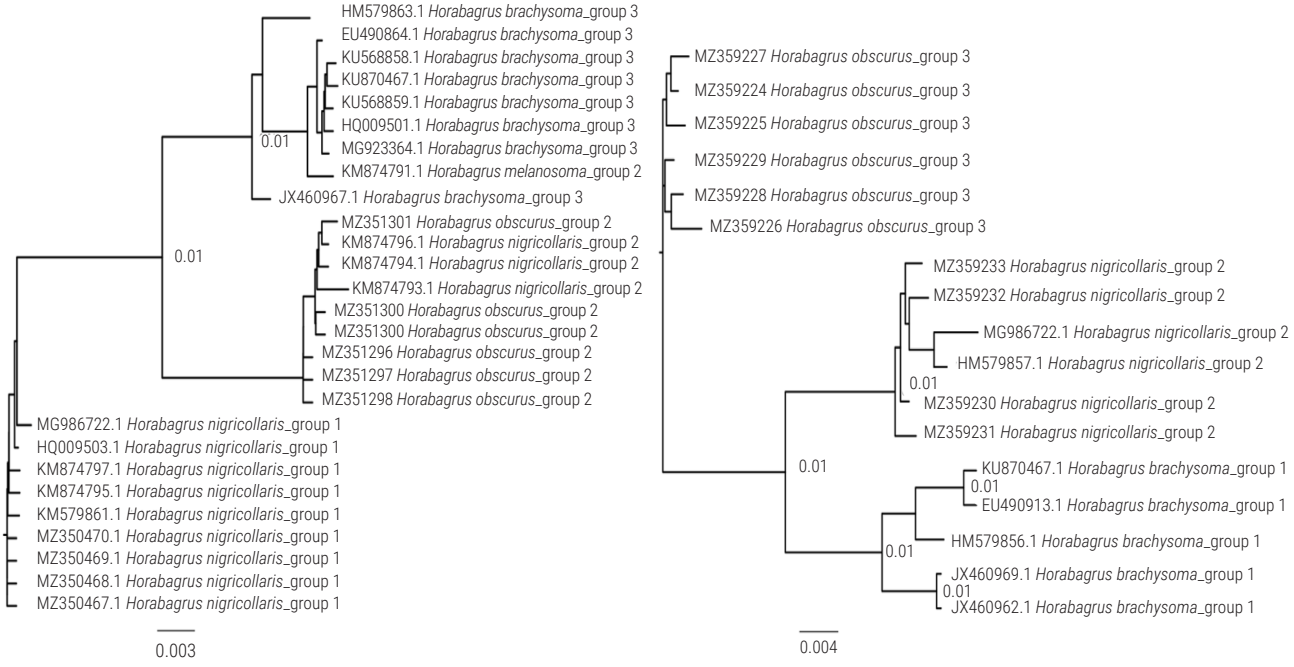


Fig. 4. *Horabagrus* species groups as determined by ABGD. (a) COI and (b) CytB

Fig. 5. Species of *Horabagrus* in life. (a) *H. obscurus*, NBFGR/HORHOBS.7, paratype, 248 mm SL, (b) *H. nigricollaris*, NBFGR/HORHNIG.8, 168 mm SL and (c) *H. brachysoma*, 200 mm SL, Periyar river at Varapuzha, not preserved

express their gratitude to Dr. J. K. Jena, DDG (Fy. Sc.), ICAR, New Delhi for the support, guidance and encouragement.

References

Ali, A., Katwate, U., Philip, S., Dhaneesh, K., Bijukumar, A., Raghavan, R. and Dahanukar, N. 2014. *Horabagrus melanosoma*: A junior synonym of *Horabagrus brachysoma* (Teleostei: Horabagridae). *Zootaxa*, 3881(4): 373-384. https://doi.org/10.11646/zootaxa.3881.4.5.

Ali, P. H. A. and Prasad, G. 2007. Bionomics of a critically endangered and endemic catfish, *Horabagrus nigricollaris* from its type locality in Kerala. *J. Bombay Nat. Hist. Soc.*, 104(2): 165–169. https://doi.org/10.1007/s10641-006-0022-4.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.*, 215(3): 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Bhat, A. 2001. New report of the species, *Horabagrus brachysoma* in the Uttara Kannada District of Karnataka. *J. Bombay Nat. Hist. Soc.*, 98(2): 294-296.

Bindu, L., Padmakumar, K., Sreerekha, P. and Joseph, N. 2012. Reproductive biology of the golden catfish, *Horabagrus brachysoma* (Günther, 1864), an endemic species of the Western Ghats, India. *J. Appl. Ichthyol.*, 28(5): 772-777. https://doi.org/10.1111/j.1439-0426.2012.02026.x.

Chandran, L. R. and Prasad, G. 2014. Reproductive characters of yellow catfish *Horabagrus brachysoma* (Horabagridae) from Periyar River, Western Ghats. *J. Aquat. Biol. Fish.*, 2(1): 154-161.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32(5): 1792-1797. https://doi.org/10.1093/nar/gkh340.

Gouy, M., Guindon, S. and Gascuel, O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.,* 27(2): 221-224. https://doi.org/10.1093/molbev/msp259. Epub 2009 Oct 23. PMID: 19854763.

Katwate, U., Raut, R., Khot, M., Paingankar, M. and Dahanukar, N. 2012. Molecular identification and ecology of a newly discovered population of sun catfish *Horabagrus brachysoma* from northern Western Ghats of India. *Int. Sch. Res. Notices*, 2012(1): 419320. https://doi.org/10.5402/2012/419320.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol.*, 33(7): 1870-1874. https://doi.org/10.1093/molbev/msw054.

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L. and Grabowski, G. 1991. *The simple fools guide to PCR Honolulu. Special publication*, Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, USA.

Pethiyagoda, R. and Kottelat, M. 1994. Three new species of fishes of the genera *Osteochilichthys* (Cyprinidae), *Travancoria* (Balitoridae) and *Horabagrus* (Bagridae) from the Chalakudy River, Kerala, India. *J. South Asian Nat. Hist.*, 1(1): 97-116.

Plamoottil, M. and Abraham, N. P. 2013. *Horabagrus melanosoma*, a new fish species (Actinopterygii: Siluriformes) from Kerala, India. *Int. J. Pur. Appl. Zool.,* 1(4): 280-288.

Puillandre, N., Lambert, A., Brouillet, S. and Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.*, 21(8): 1864-1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x.

Raghavan, R., Philip, S., Ali, A., Katwate, U. and Dahanukar, N. 2016. Fishery, biology, aquaculture and conservation of the threatened Asian Sun catfish. *Rev. Fish Biol. Fish.*, 26(2): 169-180. https://doi.org/10.1007/s11160-016-9418-1.

Skelton, P. H. and White, P. N. 1990. Two new species of *Synodontis* (Pisces: Siluroidei: Mochokidae) from southern Africa. *Ichthyol. Explor. Freshw.* (3): 277-287.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. and Hebert, P. D. 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B, Biol. Sci.*, 360(1462): 1847-1857. https://doi.org/10.1098/rstb.2005.1716.