

Heteropneustes fuscus (Siluriformes: Heteropneustidae), a new catfish species from Kerala, India

MATHEWS PLAMOOTTIL[✉]

Department of Zoology, Government College, Kottayam, Kerala 686013, India. Tel/fax.: +91-944-7059690, ✉email: mathewsplamoottil@gmail.com

Manuscript received: 30 September 2021. Revision accepted: 13 December 2021.

Abstract. Plamoottil M. 2022. *Heteropneustes fuscus* (Siluriformes: Heteropneustidae), a new catfish species from Kerala, India. *Biodiversitas* 23: 87-98. *Heteropneustes fuscus*, a new catfish species, is described from Kerala, India; it is a close congener of *Heteropneustes fossilis* (Bloch, 1794) described from Tranquebar in Tamil Nadu. *Heteropneustes fuscus* has been misidentified as *H. fossilis* until now, but it differs from Bloch's species in color and many other rigid taxonomic variables. The new species can be distinguished from its relative species in the following combination of characters: deep black body and fins, 4-5 branched dorsal-fin soft rays, 72-75 anal fin rays, 58-60 total vertebrae, 26-32 total gill rakers; deeper and wider body; deeper caudal peduncle; greater pre occipital and post occipital distances and longer anal fin. The new fish is edible and found in freshwater rivers, streams, ponds and paddy fields in Kerala. It is also cultivated in some artificial impoundments. *Heteropneustes fuscus* is compared with its congeners, scientifically named and taxonomically described.

Keywords: Biodiversity, description, *Heteropneustes fossilis*, scientific naming, taxonomy

INTRODUCTION

Species of *Heteropneustes* Muller (Siluriformes: Heteropneustidae), the Asian stinging catfishes, are commercially important freshwater fishes distributed throughout the south and southeast Asian countries. *Silurus* (Bloch 1794; Hamilton 1822; Swainson 1939), *Saccobranhus* (Valenciennes 1840; Jerdon 1849; Gunther 1864) and *Clarisilurus* (Fowler 1937) are the diverse names assigned to the genus by various taxonomists. The body of *Heteropneustes* is elongated, the head is depressed and covered with osseous plates and has four pairs of barbels. The body also has a pair of accessory respiratory organs in the form of air sacs extending backward from the gill chamber on both sides of the vertebral column. The dorsal fin is small and devoid of bony spines; the pectoral spine is strong and internally serrated and the caudal fin is rounded (Nelson 2006).

The stinging catfish mostly inhabits rivers, swamps, ponds, marshlands and muddy streams (Froese and Pauly 2018). They are extensively fished and cultured because of their nutritional quality (Saha and Guha 1939; Alok et al. 1993), medicinal value (Jha and Rayamajhi 2010) and low-fat content (Rahman et al. 1982). They are also important for research studies owing to the possession of accessory respiratory organs (Burgess 1989; Jha 2009; Kasherwani et al. 2009).

Ratmuangkhwang et al. (2014) collected many specimens of *Heteropneustes* from Myanmar, Thailand and India (West Bengal, Assam, Tamil Nadu and Kerala) and conducted molecular taxonomic studies that demonstrated the existence of three distinct clades in *Heteropneustes fossilis* complex in southeast Asia and northeastern and

southwestern India and treated them as separate species. However, they could not collect *H. fossilis* from Tranquebar, its type locality. Recently this author could procure some specimens of *Heteropneustes* species from Pathanamthitta of Kerala which, on careful examination, disclosed marked differences from *H. fossilis* collected from its type locality and from its other congeners. It is described here as a new species, *Heteropneustes fuscus*.

MATERIALS AND METHODS

In connection with the SERB Major Research Project (CRG) of DST, Govt of India, this author visited many areas of south India for fish collection and taxonomic analysis. This survey resulted in the procurement of many rare freshwater fishes from various aquatic bodies of Kerala, Karnataka and Tamil Nadu. Different color variants of *Heteropneustes* were collected from various parts of south India. The black-colored *Heteropneustes* from the Pathanamthitta district showed marked differences from its original description. This led the author to conduct more taxonomical studies on this black-colored stinging catfish. Five specimens of this fish were collected, during morning hours, using gill nets, anesthetized, and fixed in 10% formaldehyde solution; they were examined and taxonomically analyzed; specimens of *Heteropneustes fossilis*, the close congener, were collected by the author from Tranquebar, its type locality, and compared with the new species. Measurements of parts of the body and head were taken using dial calipers; meristic counts were taken using a magnifying lens and a stereomicroscope; vertebral numbers were counted from radiographs. For

measurements and comparisons, Jayaram (2002) was followed; measurements and counts were made on the left side of specimens. Morphometric measurements and meristic counts were taken from the type specimens and comparative materials. Head length and other parts of the body were given as percent of standard length (SL); parts of the head were given as percent of head length (HL); distance between the occiput and dorsal-fin origin was taken as post occipital length; in the new fish and comparative specimens, the distance between anterior nostril was taken for counting inter narial width; distances from pectoral to pelvic fin and between pelvic fin and anal fin were measured from the origin of these fins; distances between fins and vent were also measured from the origin of the fins to the latter. Specimens of the new species are deposited in the Zoological Survey of India Museum at Pune, Maharashtra (ZSI/WRC); specimens of *Heteropneustes fossilis*, collected for this study, are deposited in ZSI, Shillong (ZSI/NERC).

DNA isolation, PCR, and sequencing

DNA isolation, Polymerase Chain Reaction, and sequencing of the targeted genetic marker (mtCOI) of *Heteropneustes fossilis* and *H. fuscus* sp. nov followed the standardized protocols. Genomic DNA was extracted from muscle tissue through the Kit-based method. The extracted DNA was checked in 1% agarose gel electrophoresis using a standard protocol. Approximately 648 bp was amplified from the 5' region of the mtCOI gene using the primers described in Ward et al. (2005). The thermal profile for PCR was set as per the primer specifications. Approximately 15 ng of purified PCR product was used for cycle sequencing for both reactions from the outsource services.

The generated sequences were truncated at both ends after alignment in MEGA 6.0 to get a consensus length in the dataset. The evolutionary divergences between sequences of closely related species or sister species among the congeners were estimated using Kimura 2 Parameter (K2P) in MEGA 6.0 (Tamura et al. 2013). Further, the reciprocal monophyly among the targeted congeners were tested through Neighbour Joining (NJ) tree method and Maximum Likelihood (ML) in MEGA 6.0 (Tamura et al. 2013) for species-level differentiation.

GenBank accession numbers for sequences of *Heteropneustes fuscus* and *H. fossilis* (procured from its type locality) are MZ848411 and MW684709 respectively.

RESULTS AND DISCUSSION

Heteropneustes fossilis (Bloch, 1794) (Figure 1-3; Table 1)

Silurus fossilis Bloch, 1794: 46, pl. 370. Tranquebar, Tamil Nadu, India.

Saccobranthus fossilis. Day. 1878. P. 486. Pl.114. Figure 1. (Freshwater Fishes of Sind, India, Ceylon, Burma etc)

Silurus singio Hamilton, 1822: 147, 374, pl. 37. The Ganges River, India.

Silurus laticeps Swainson, 1838: 345; (1839: 393). India.

Silurus biserratus Swainson, 1839: 306. The River Ganges Basin, India.

Saccobranthus microcephalus Günther, 1864: 31. Sri Lanka.

Saccobranthus microps Günther, 1864: 31. Sri Lanka.

Description: Body elongate; laterally compressed behind pelvic-fin origin; pre pelvic region subcylindrical; head depressed, covered with osseous plates; eye lateral and with free orbital margin; nostrils wide apart; mouth small, terminal and transverse. Two cephalic fontanels, anterior one longer than posterior.

Anterior fontanel originating from level of anterior margin of eye, extending to posterior margin of orbit or slightly behind; posterior groove located on occiput. Occipital spine short and does not reach dorsal-fin base; considerable gap between occipital process and dorsal fin base. Teeth villiform in bands on jaws. Barbels 4 pairs; nasal extending one orbit diameter in front of dorsal fin base and tip of pectoral fin; maxillary barbel reaching pelvic-fin base and behind dorsal-fin base; outer and inner mandibular reaching pectoral-fin tip. Gill opening wide, gill membrane free from isthmus; 7 branchiostegal rays. Air sac tube long, originating from gill chamber and extending to one-half of anal-fin base.

Dorsal fin short and situated at anterior third of the body, in front of ventral fin origin and without spine; pectoral fin with a strong spine, inner edge serrated and outer edge granulated; pectoral fin not reaching to pelvic fin; latter fairly reaching anal fin. Anal fin is very long but not confluent with the caudal fin, with a short gap between these two. Caudal fin rounded. Skin smooth; muscle bands distinct on skin.

Color: Live colors: Body and fins pale brown to chocolate in color; barbels brown; eyes blue; fins light brown.

In 10% formalin: Body and fins lose their bright colors; eyes light blue; barbels light brown.

Distribution: Freshwater streams and ponds at Tranquebar in Tamil Nadu.

Remarks: Bloch's (1794) description of *Heteropneustes fossilis* (as *Silurus fossilis*) is brief and is of a general nature; he provided essential meristic counts and some other taxonomic details; it may be applicable to the genus as most of the diagnostic features of Bloch (1794) are common to many *Heteropneustes* species. Bloch's (1794) description was based on a single specimen, which was given to him by Mr. John, from Tranquebar. Bloch's *Heteropneustes* is still considered as a widely distributed species; in India, it is the only one valid species of the genus, excluding *H. longipectoralis*. The latter is a local species described from Tamil Nadu, not even reported from other parts of the state. Day (1878, 1889) presented a more detailed account of *H. fossilis* based on his observation of a larger number of specimens. His description was, most probably, not solely based on the type or topotypic specimens. He regarded it as a 'widely distributed species found in 'freshwaters of Sind, India, Ceylon, Burma and Cochinchina'; his many features are not of *Heteropneustes* from Tranquebar; he wrote that the body of *Heteropneustes* is 'sometimes with two longitudinal yellowish bands'; this morphological feature had also been manifested in Day's (1878, 1889) figures too. This type of yellowish longitudinal band was not at all visualized in the specimens collected by this researcher from its type locality. Day's

meristic counts are also not only of *H. fossilis* but also of many species of the genus. About body-color, Day (1878, 1889) wrote as 'leaden'; examination of specimens from Tranquebar showed a light brown color comparable to the chocolate color of the original description. Day (1878,

1889), Jerdon (1849), Misra (1976), Talwar and Jhingran (1991) and Jayaram (2010) counted 60-79 anal fin rays to *H. fossilis* in contrast to 70 in the original description (Bloch 1794).

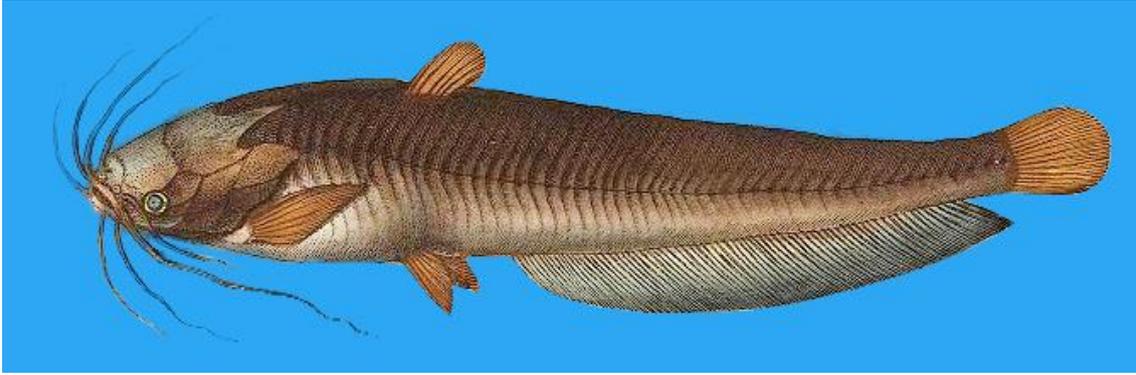


Figure 1. *Heteropneustes fossilis*, a drawing in Bloch 1794



Figure 2. *Heteropneustes fossilis* (V/F/NERC/ZSI/5135) collected from Tranquebar (showing slender body, depressed head and shorter anal fin)



Figure 3. Dorsal view of head of *Heteropneustes fossilis* (showing narrow and shallow cephalic fontanel)

***Heteropneustes fuscus* sp. nov.**

(Figure 4-8; Table 2)

urn:lsid:zoobank.org:act:753D5454-0260-488E-9444-DD5D9BA3B52A

Holotype: ZSI/WRC/P/ P/5545, 229.0 mm SL, a small water stream at Pathanamthitta, Kerala, India, coll. Mathews Plamoottil, 10.01.2017. Paratypes: ZSI/WRC/P/5546, 4 ex, 182.0-282.0 mm SL, other details same as HT.

Diagnosis: *Heteropneustes fuscus* can be distinguished from its congeners (Figure 9 and 10; Table 3 and 4) in its deep black body and fins, meristic counts and in many body proportions. The new species can be distinguished from *Heteropneustes fossilis* in having a deep black body (vs. chocolate to dark brown color in *H. fossilis*) and fins (vs. light brown). Moreover, cephalic fontanel in the new fish is broader and deeper than *H. fossilis* (Figure 8).

Table 1. Morphometric characters of *Heteropneustes fossilis*, V/F/NERC/ZSI/5135 (n= 3)

Characters	Range	Mean	SD
Total length (mm)	159.0-177.0	166.0	0.75
Standard length (mm)	141.0-157.0	148.0	0.65
Head length (mm)	23.0-27.0	25.0	0.16
% Standard Length			
Head length	16.3-17.2	16.8	0.38
Head depth	4.2-7.4	5.6	1.31
Head width	13.5-14.6	14.1	0.46
Body depth at anal fin	12.9-14.6	13.7	0.70
Body width at anal fin	6.4-8.2	7.5	0.77
Predorsal length	30.5-31.8	31.2	0.53
Prepectoral	13.7-16.3	15.0	1.3
Preventral	33.7-34.4	34.0	0.35
Preanal	40.1-40.8	40.4	0.34
Preoccipital	15.6-18.5	17.0	1.45
Postoccipital	14.0-15.3	14.5	0.57
Length of dorsal fin	8.9-9.6	9.3	0.35
Length of pectoral	10.6-15.7	13.1	2.55
Length of pelvic fin	9.6-9.9	9.7	0.15
Length of anal fin	3.2-5.3	4.2	0.86
Length of base of dorsal fin	2.5-3.0	2.8	0.20
Length of base of anal fin	57.4-58.6	58.0	0.59
Length of caudal peduncle	0	0	0
Depth of caudal peduncle	3.5-4.8	4.4	0.61
Width of caudal peduncle	0.3-0.6	0.5	0.14
Length of caudal fin	12.4-12.8	12.6	0.19
Distance from pectoral to pelvic	20.2-20.7	20.4	0.25
Distance from pelvic to anal	6.4-7.4	6.9	0.5
Distance from anal to caudal	58.2-59.2	58.7	0.5
Length of air sac	44.7-49.6	47.2	0.34
Width of air sac	2.7-3.2	2.9	0.22
% Head Length			
Snout length	29.6-30.4	30.0	0.40
Post orbital length	50.0-55.5	52.7	2.75
Width of gape of mouth	22.2-34.8	28.5	6.3
Eye diameter	16.7-17.4	17.1	0.35
Interorbital width	46.3-50.0	48.1	1.85
Internarial width	24.4-28.3	26.3	1.95
Anterior fontanel length	14.8-21.7	18.3	3.45
Anterior fontanel width	11.1-13.0	12.0	0.94
Occipital fontanel length	13.0-15.2	14.1	1.1
Occipital fontanel width	8.6-9.2	8.9	0.30
Distance from ventral fin to vent	33.3-39.1	36.2	2.89
Distance from anal fin to vent	7.4-8.7	8.0	0.64
Length of nasal barbels	98.1-160.8	138.3	28.49
Length of maxillary barbel	137.0-200.0	176.1	27.87
Length of outer mandibular barbel	143.5-188.0	166.0	18.17
Length of inner mandibular barbel	100.0-168.0	144.4	31.41

The major morphometric differences and meristic counts of *H. fuscus* and *H. fossilis* are listed in Table 3 and 4 respectively. The new species differs from *H. longipectoralis* Rema and Raghunathan (1999) (Figure 9 and 10) in having a wider head (90.0-92.0% HL vs. 81.9), larger eyes (15.0-17.6% HL vs. 6.0), fewer (4-5 vs. 6) branched dorsal-fin rays and a greater number of (72-75 vs. 66-73) anal fin rays. The new species differs from *Silurus singio* Hamilton (1822), the singhi, in having a black (vs. dirty green) color, without any lateral color bands (vs. with two yellow stripes on lateral sides), only inner margin of

pectoral spine serrated (vs. both inner and outer margins of pectoral spine serrated), anal fin with 61 (vs. 72-75) rays and caudal fin with 14-16 (vs. 12) rays. *Heteropneustes fuscus* differs from *H. nani* Hossain et al. (2013) in having a greater number of anal fin (72-75 vs. 66-69) and caudal fin rays (14-16 vs. 10-12) and anal fin being not confluent with caudal fin (vs. a confluent anal and caudal fin). The new species differs from *Heteropneustes kemratensis* Fowler (1937) in having fewer anal-fin rays (72-75 vs. 75-84), fewer (8 vs. 9) pectoral fin rays, wider (90.0-92.0% HL vs. 85-86) head and a prominent orbit (eye diameter 15.0-17.6% HL vs. 6.5).

Description: Body elongate, cylindrical and laterally compressed; head small and depressed, bony plates on head; mouth terminal, transverse and non-protrusible; Teeth villiform in bands on jaws; vomerine and palatine teeth present; eye small, with free orbital margin, on lateral of head, considerably behind and above angle of jaws; nostrils 2 pairs; anterior pair located closer to snout tip than orbit; two cephalic fontanels; anterior longer and deeper and nearly of length of orbit; posterior groove located on occiput; both fontanels longer than broad. Occipital spine pointed, not reaching to dorsal-fin origin. Branchiostegal rays seven; gill openings wide. Gill rakers minute, broad and 26-32 in number; gill membrane free from isthmus. Four pairs of distinct barbels; maxillary barbel longer, reaching to one orbit diameter behind pelvic-fin tip; nasal barbel shortest, reaching to dorsal-fin base and also slightly behind pectoral-fin tip; inner mandibular extending just behind the base of ventral fin; outer mandibular reaching to tip of ventral-fin.

Dorsal fin short, located slightly behind and above ventral fin, soft and without spine; pectoral fin pointed, short and not reaching to ventral fin; its spine ossified, strong and serrated on inner margin; outer margin roughened and not serrated; ventral fin short, rounded, located slightly in front or behind or opposite to dorsal-fin origin and tip fairly reaching to origin of anal fin. Anal fin long based with tip of last anal-fin ray reaching considerably behind origin of caudal fin; anal fin not confluent with caudal fin, short gap between posterior base of anal fin and caudal fin, latter rounded. Caudal peduncle laterally compressed. Lateral line emerging as a thin line, running from head to caudal-fin base. Skin smooth; muscle bands distinct externally. Air sac, the accessory respiratory apparatus, is a long tube on each side of the body, embedded in the lateral myotomes, extends posteriorly from the ctenidial chamber and run parallel to the vertebral column and narrows to its hind end.

Color: Live colors: Body and fins deep black; eyes blue; dorsal and ventral fins have a light reddish outer margin.

In 10% formalin: Body and fins pale black.

Local name: It is locally called as *kaari* in Malayalam.

Common name: As its body and fins are deep black in color the common name 'Travancore black stinging catfish' is given.

Etymology: The specific epithet *fuscus* was taken from Latin (*fuscus* meaning-dark); it refers to black colored body and fins of the new species.

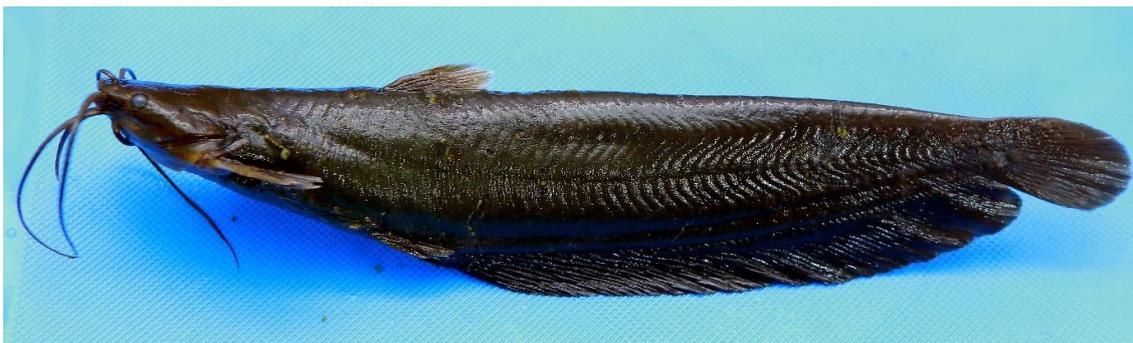


Figure 4. *Heteropneustes fuscus*, ZSI/WRC/P/ P/5545, Holotype, photographed fresh (showing deeper body and longer anal fin)



Figure 5. *Heteropneustes fuscus*, ZSI/WRC/P/5545, Holotype, dorsal view



Figure 6. *Heteropneustes fuscus*, ZSI/WRC/P/5545, Holotype, ventral view



Figure 7. *Heteropneustes fuscus*, ZSI/WRC/P/5546, Paratype, preserved

Table 2. Morphometric characters of *Heteropneustes fuscus*, ZSI/WRC/P/ P/5545 and 5546) (n= 5)

Character	Holo-type	Range	Mean	SD
Total length (mm)	263.0	209.0-323.0	280.0	40.70
Standard length (mm)	229.0	182.0-282.0	244.0	35.70
Head length (mm)	37.0	30.5-40.0	34.8	3.96
% Standard Length				
Head length	16.2	16.1-18.2	17.0	0.68
Head depth	6.6	6.6-8.2	7.7	0.75
Head width	14.8	14.2-15.5	14.6	0.46
Body depth at anal fin	15.3	15.3-16.8	15.9	0.57
Body width at anal fin	10.5	9.9-12.4	11.1	1.05
Predorsal length	34.1	32.3-34.3	33.0	0.66
Postdorsal length	67.0	66.2-69.5	67.4	1.36
Prepectoral length	13.9	13.7-14.1	13.9	0.15
Preventral Length	33.6	30.7-33.6	33.2	0.98
Preanal Length	41.0	38.9-41.8	39.0	1.05
Preoccipital length	15.2	13.2-15.2	14.8	0.70
Postoccipital Length	18.6	15.8-19.8	16.7	1.99
Length of dorsal fin	9.2	9.1-9.8	9.5	0.30
Length of Pectoral fin	12.4	12.2-13.2	12.8	0.40
Length of pelvic fin	8.7	8.5-9.0	8.8	0.15
Length of anal fin	8.3	6.5-8.5	7.5	0.95
Length of base of dorsal fin	3.6	3.5-5.4	4.2	0.72
Length of base of anal fin	61.6	57.7-61.8	59.6	1.95
Pectoral spine length	10.5	10.5-11.1	10.8	0.30
Length of caudal peduncle	1.3	1.1-2.7	1.9	0.80
Depth of caudal peduncle	5.2	5.2-6.6	5.8	0.42
Width of caudal peduncle	0.8	0.5-0.8	0.7	0.12
Length of caudal fin	14.8	12.4-15.0	13.6	1.20
Distance from pectoral to pelvic fin	20.5	19.2-20.7	20.1	0.65
Distance from Pelvic to anal fin	8.7	7.6-9.8	9.0	1.10
Distance from anal to caudal fin	58.2	58.0-59.0	58.8	1.90
Length of air sac	45.3	44.8-48.3	46.1	1.50
Width of air sac	1.8	1.8-2.9	2.2	0.50
% Head Length				
Head width	91.0	90.0-92.0	91.0	0.80
Snout length	22.0	21.6-30.0	25.8	3.50
Postorbital length	51.4	50.3-62.5	58.7	13.6
Width of mouth gape	48.5	35.6-48.6	42.7	3.60
Eye diameter	17.5	15.0-17.6	16.3	1.30
Interorbital width	55.2	47.4-55.4	53.0	1.80
Internarial width	24.3	20.3-24.3	22.1	1.5
Anterior fontanel length	21.6	21.6-27.1	25.2	1.7
Anterior fontal width	10.8	10.0-14.7	13.5	1.7
Occipital fontanel length	18.6	14.1-18.9	16.2	1.8
Occipital fontanel width	10.7	7.8-10.9	8.2	1.3
Distant from ventral fin to vent	35.3	35.1-37.5	36.4	0.9
Distance from anal fin to vent	1.9	1.9-2.1	2.0	0.1
Head depth at orbit	18.9	18.9-20.0	19.2	1.3
Head depth at snout tip	13.7	13.5-13.8	13.7	1.2
Length of nasal barbels	177.0	175.5-215.6	185.0	13.0
Length of maxillary barbels	162.2	156.2-253.0	190.0	18.0
Length of outer mandibular barbels	205.0	203.0-206.2	204.0	17.0
Length of inner mandibular barbels	176.0	175.0-182.8	178.0	16.0

**Figure 8.** Dorsal view of head of *Heteropneustes fuscus* (showing broader and deeper cephalic fontanel)**Table 3.** Morphometric differences between *Heteropneustes fuscus* (ZSI/WRC/P/ P/5545 and 5546) and *H. fossilis* (V/F/NERC/ZSI/5135)

Character	<i>H. fuscus</i> (n= 5)	<i>H. fossilis</i> (n= 3)
% Standard Length		
Body depth at anal fin	15.3-16.8	12.9-14.6
Body width at anal fin	9.9-12.4	6.4-8.2
Pre dorsal length	32.3-34.3	30.5-31.8
Pre ventral length	30.7-33.6	33.7-34.4
Pre occipital length	13.2-15.2	15.6-18.5
Post occipital length	15.8-19.8	14.0-15.3
Length of anal fin	6.5-8.5	3.2-5.3
Length of base of dorsal fin	3.5-5.4	2.5-3.0
Depth of caudal peduncle	5.2-6.6	3.5-4.8
Distance from pelvic to anal fin	7.6-9.8	6.4-7.4
% Head Length		
Width of mouth gape	35.6-48.6	22.2-34.8
Inter orbital width	47.4-55.4	46.3-50.0
Inter narial width	20.3-24.3	24.4-28.3

Table 4. Meristic counts of *Heteropneustes fuscus* (ZSI/WRC/P/ P/5545 and 5546) and *H. fossilis* (V/F/NERC/ZSI/5135)

Characters	<i>H. fuscus</i> (holotype and paratypes) (n= 5)	<i>H. fossilis</i> (n= 3)
Dorsal Fin Rays	i, 4-5	i, 6
Pectoral fin rays	I, 7	I, 7
Ventral Fin rays	6	6
Anal fin Rays	72-75	69
Caudal Fin rays	14-16	16
Vertebrae	58-60	62
Gill Rakers	26-32	18-24
Pectoral spine teeth	13-16	11

Table 5. The maximum likelihood estimate of nucleotide substitution matrix of mitochondrial COI gene sequence of *Heteropneustes fossilis*

From\To	A	T	C	G
A	-	5.7165	4.8203	8.1151
T	5.1159	-	18.6598	3.0184
C	5.1159	22.1289	-	3.0184
G	13.7541	5.7165	4.8203	-

Table 6. The maximum likelihood estimate of transition/transversion bias of mitochondrial CO I gene sequence of *Heteropneustes fossilis*

From\To	A	T	C	G
A	-	4.5890	4.5890	15.8220
T	4.5890	-	15.8220	4.5890
C	4.5890	15.8220	-	4.5890
G	15.8220	4.5890	4.5890	-

Habitat: *Heteropneustes fuscus* was collected from a small water stream at Pathanamthitta of Kerala, India (Figure 14 and 15). The stream bed is dominated by mud sediments; sand occurs as discrete patches within the mud dominant deposits. Depth and width in this area are 0.3-0.7 m and 6.0-7.0 m respectively; the bank height is 2.0-3.0 m above the water level in summer season. This area is covered by dense to moderate riparian vegetation; intermittent occurrence of low riparian vegetation noticed at certain places. The major flora includes *Ochreinauclea missionis*, *Hibiscus tiliaceus*, *Bambusa bambos*, *B. vulgaris* etc. Some of the co-occurring fish species in the region are *Haludaria fasciata*, *Labeo rohita*, *L. dussumieri*, *Osteobrama bakeri*, *Amblypharyngodon microlepis*, *Rasbora dandia*, *Dawkinsia filamentosa*, *Puntius parrah*, *P. nelsoni*, *P. viridis*, *Pethia ticto*, *Hypselobarbus kurali*, *Devario malabaricus*, *Tor khudree*, *Horabagrus brachysoma*, *Mystus keralai*, *Mystus indicus*, *Wallago attu*, *Macrogathus albus* etc.

Economic importance: *Heteropneustes fuscus* is an economically important catfish in inland capture and culture fisheries. In central Travancore these fishes are caught by gill nets, cast nets, traps and hooks and sold in large quantities in live condition. Sometimes they are

transported to distant markets and are sold alive after several days due to the possession of the air breathing apparatus. They are valued as food fish due to its palatability and nutritional quality. It is also used as an ornamental fish and found in the aquarium trade.

Evolutionary affinity

(Figure 11-13; Table 5-8)

The partial coding sequence of mitochondrial cytochrome oxidase subunit 1 (COI) gene of *Heteropneustes fossilis* was PCR amplified using a forward primer with DNA sequence 5'-TCAACCAACCACAAAGACATTGGCAC-3' and reverse primer with DNA sequence 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'. The partial COI sequence of *Heteropneustes fossilis* isolated from its type locality (Tranquebar in Tamil Nadu, India) yielded a product with 642p. The DNA sequence was deposited in the GenBank with Accession No. MW684709.1. The DNA sequence obtained, its conceptual translation product and phylogenetic tree are presented in Figures 11-13.

>MW684709.1 *Heteropneustes fossilis* | cytochrome oxidase subunit I gene | voucher F05 | partial cds, mitochondrial|642bp.

**Figure 10.** Dorsal view of head of *H. longipectoralis*, Holotype, ZSI/SRC/F 4935**Figure 9.** *Heteropneustes longipectoralis* ZSI/SRC/F 4935 Holotype, Thirumurthi Dam, Tamil Nadu, India

GCCCTTAGCTTACTTATCCGGGCAGAATTAGCACAACTGGTGCCTACTGGGTGATGACCAAATTTATAACGTTATTGTTA
 CTGCTCACGCATTCGTAATAATTTCTTTATAGTAATACCAATTATGATTGGAGGCTTCGGAAACGGCTAGTACCCTAAT
 GATTGGAGCCCTGATATAGCATTCCACGTATGAATAACATAAGCTTCTGGCTACTTCCACCATCTTTCTACTACTGCTT
 GCATCTTCTGGAGTTGAAGCGGGGCAGGAACAGGATGGACAGTGTATCCACCTCTTGCTGGGAATCTTGCACATGCTGGAG
 CCTCAGTAGATTTAACCATTTTCTCCCTACACTTAGCAGGTGTCATCTATTCTAGCATCTATTAATTTTATTACTACTAT
 TATTAACATGAAACCCCGCCATCTCACAAATACAAACACCCTATTTGTTTGGTCAGTGTTAATTACAGCCGTACTACTA
 CTACTCTCCCTACCTGTACTAGCCGCTGGAATTACCATACTACTAAGTACCAGAAATCTAAACACTACATTCTTTGACCCCG
 CAGGAGGTGGAGACCCATTCTCTACCAACATCTCTCTGGTCTTTGGTCACCCTGAAGTTTTAAGC

Figure 11. The DNA sequence interpret of COI gene of *Heteropneustes fossilis*

ALSLLRRAELAQP GALLGDDQIYNVIVTAHAFV I IFFIVIPI M IGGFGNWL VPLMIAPD IAFPRMNNISFWLLPPSFLLLL
 ASSGVEAGAGTGWTVY PPLAGNLAHAGASVLTIFSLHLAGVSSILASINFITTIINMKPPAISQYOTPLFVwsVLITAVLL
 LLSLPVLAAGITILLDRNLNTFFDPAGGGDPILYQHLEWFFFGHPEVLS

Figure 12. The conceptual translation product of the COI gene of *Heteropneustes fossilis*

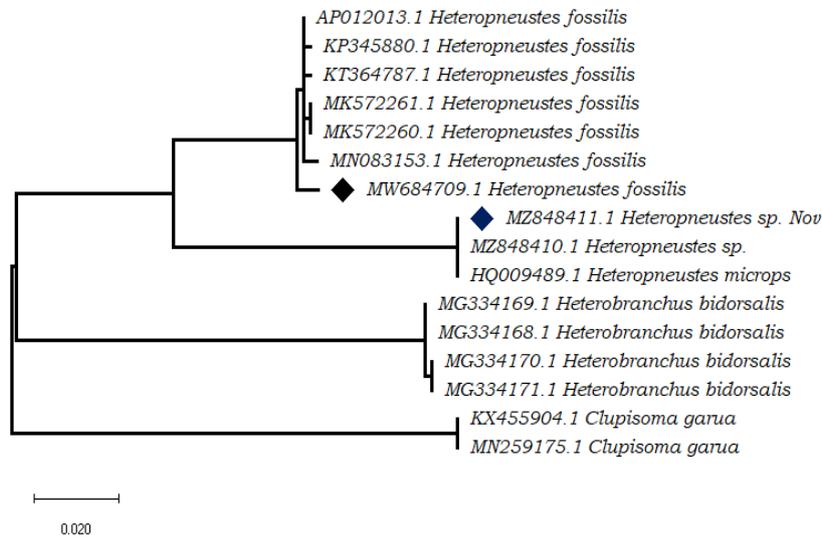


Figure 13. Molecular phylogenetic tree of *Heteropneustes fossilis* inferred by ML-Tamura-Nei-Model

The COI sequence of *Heteropneustes fossilis* showed bias to nucleotide AT, with following composition of nucleotides T: 30.8%, C: 28.8%, A: 26.4% and G: 16.8% and the average value of nucleotide composition of 16 nucleotides shows T: 30.6%, C: 25.8 %, A: 27.4% and G: 16.2% (Table 8).

The number of base substitutions per position between sequences is indicated in Table 8. using the Maximum Composite Likelihood model. Substitution patterns and rates were estimated under the Tamura-Nei (1993) model (+G). Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 100, The nucleotide frequencies are A: 27.40%, T/U: 30.62%, C: 25.82%, and G: 16.17%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was-1729.090. This analysis involved 16 nucleotide sequences. Codon positions included were

1st+2nd+3rd+Noncoding. There were a total of 613 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

The data in Table 7 reveals that the *Heteropneustes fossilis* shows 0.66% evolutionary divergence with *Heteropneustes fossilis* (AP012013.1) isolated from Japan. *Heteropneustes fossilis* species shows 0.82% of evolutionary divergence with *Heteropneustes fossilis* (KP345880.1) isolated from Bhubaneswar, Odisha, India and *Heteropneustes fossilis* (MK572261.1) isolated from Bangladesh. *Heteropneustes fossilis* species shows 11.04% of evolutionary divergence with *Heteropneustes fuscus* (*Heteropneustes* sp. nov., MZ848411.1 and *Heteropneustes* sp. MZ 848410.1, isolated from Pathanamthitta, Kerala, India). The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model. This analysis involved 16 nucleotide sequences.

The phylogenetic tree generated using ML (Tamura-Nei) method showing the phylogenetic position of

Heteropneustes fossilis (Accession No. MW684709.1) isolated from Tranquebar in Tamil Nadu, Kerala, India. Phylogenetically *Heteropneustes fossilis* (Accession No. MW684709.1) showed to be the closest relatives of *Heteropneustes fossilis* (KT364787.1) of Bangladesh. *Heteropneustes fossilis* isolated from Korea (MK572260.1) and Bangladesh (MK572261.1) seems to arise from the same clade and *Heteropneustes fuscus* (*Heteropneustes* sp. nov, MZ848411.1 and *Heteropneustes* sp. MZ 848410.1) is the nearest relative of this species present nearest branch in the phylogenetic tree. HQ009489.1 had been erroneously identified as *Heteropneustes microps*; it is nothing but *Heteropneustes fuscus*.

Here I have done molecular identification of *Heteropneustes fossilis* using DNA based taxonomy. The result confirmed that the molecular identification method strictly correlates with classical taxonomy. The cytochrome oxidase I gene of *Heteropneustes fossilis* yielded a product having 642bp amplified DNA sequence. The result of nucleotide BLAST analysis from NCBI states that *Heteropneustes fossilis* found in Bhubaneswar, Odisha, India (98.74%) and Japan (98.58%) is more closely related. Nucleotide BLAST results prove that *Heteropneustes fossilis* isolated from its type locality (Tranquebar, Tamil Nadu, India) have only 90.53% nucleotide similarity to, *Heteropneustes fuscus* (*Heteropneustes* sp. nov; MZ848411.1 and *Heteropneustes* sp. MZ 848410.1) isolated from Pathanamthitta, Kerala, India. So *Heteropneustes fuscus* is different from *H. fossilis* and can be treated as a new species.

Remarks

Species of the genus *Heteropneustes* are distributed throughout the south and southeast Asian countries including India, Bangladesh, Thailand, Myanmar, Laos, Nepal, Pakistan and Sri Lanka (Gunther 1864; Misra 1976; Talwar and Jhingran, 1991; Coad 1996). Currently, only two valid *Heteropneustes* species occur in India are *H. fossilis* and *H. longipectoralis*. The first nominal species of *Heteropneustes*, *Silurus fossilis* (now *H. fossilis*), was described by Bloch (1794) from Tranquebar in Tamil Nadu, India. It is now believed that *H. fossilis* is a widely distributed species found all over India and adjacent countries. Subsequently, Hamilton (1822) described *Silurus singio* from the Ganges River, which is now treated as a junior synonym of *H. fossilis*. *Silurus laticeps* (1838) and *S. biserratus* (1839) were described by Swainson based on the length of barbels. Description of these was so brief that one cannot confirm its identity; type materials are also lacking. So Swainson's *Heteropneustes* species is also treated as a synonym of *fossilis*. Later, Günther (1864) described *H. microps* from Sri Lanka based on the confluence of caudal

and anal fins. Pethiyagoda and Bahir (1998) and subsequently Ratmuangkhwang et al. (2014) demonstrated that it could not be regarded as a rigid diagnostic character. Therefore, *H. microps* is considered as a junior synonym of *H. fossilis*.

Heteropneustes longipectoralis is restricted to the Anamalai Hills in the Western Ghats of south India. *Heteropneustes fossilis*, *H. kemratensis*, *H. longipectoralis* and *H. nani* are the valid *Heteropneustes* species described until now. *Heteropneustes fuscus* is an addition to the already existing stinging catfishes.

Heteropneustes fuscus is the black *Heteropneustes* of central Travancore. Unfortunately, it is misidentified as *Heteropneustes fossilis*. Lack of specimens of Bloch's species from its type locality might be the reason behind it. Description of Bloch (1794), which is of a general nature, was another reason behind the difficulty in the confirmation of the identity of black *Heteropneustes* species until now. The new species differs from its congeners in color, body depth, width and in many other valid taxonomic variables. In Kerala, in addition to the black stinging catfish, *Heteropneustes* appear in yellow and green colors too; all these can only be considered currently as color variants of *Heteropneustes fuscus*. Molecular-level studies are recommended for the confirmation of the identity of different color variants of this catfish genus. It is expected that more detailed taxonomic studies will be conducted on the genus in the coming days.

Table 7. Percentage of evolutionary divergence of *Heteropneustes fossilis* with its closely related species accessible from NCBI GenBank

Organism with accession No.	Percentage of divergence
MW684709.1 <i>Heteropneustes fossilis</i>	0.0%
AP012013.1 <i>Heteropneustes fossilis</i>	0.66%
KP345880.1 <i>Heteropneustes fossilis</i>	0.82%
MN083153.1 <i>Heteropneustes fossilis</i>	0.99%
KT364787.1 <i>Heteropneustes fossilis</i>	0.82%
MZ848411.1 <i>Heteropneustes</i> sp. nov	11.04%
MZ848410.1 <i>Heteropneustes</i> sp.	11.04%
HQ009489.1 <i>Heteropneustes microps</i>	11.04%
KX455904.1 <i>Clupisoma garua</i>	18.07%
MN259175.1 <i>Clupisoma garua</i>	18.07%
MG334169.1 <i>Heterobranchus bidorsalis</i>	18.47%
MG334168.1 <i>Heterobranchus bidorsalis</i>	18.47%
MG334170.1 <i>Heterobranchus bidorsalis</i>	18.72%
MG334171.1 <i>Heterobranchus bidorsalis</i>	18.72%
MK572261.1 <i>Heteropneustes fossilis</i>	0.82%
MK572260.1 <i>Heteropneustes fossilis</i>	0.82%

Table 8. Comparison of nucleotide frequencies of CO I gene sequence of *Heteropneustes fossilis* with its kin species

Domain: Data	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos #1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
MW684709.1 <i>Heteropneustes fossilis</i>	30.88	25.82	26.47	16.83	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.35	21.08	38.73	7.84	204
AP012013.1 <i>Heteropneustes fossilis</i>	30.88	25.82	27.12	16.18	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.35	21.08	40.69	5.88	204
KP345880.1 <i>Heteropneustes fossilis</i>	30.88	25.82	26.96	16.34	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.35	21.08	40.20	6.37	204
MN083153.1 <i>Heteropneustes fossilis</i>	30.88	25.65	27.12	16.34	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.35	20.59	40.69	6.37	204
KT364787.1 <i>Heteropneustes fossilis</i>	31.05	25.65	27.12	16.18	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.84	20.59	40.69	5.88	204
MZ848411.1 <i>Heteropneustes</i> sp. Nov	32.03	25.49	27.12	15.36	612.00	19.12	25.49	25.49	29.90	204.00	42.16	29.41	15.69	12.75	204	34.80	21.57	40.20	3.43	204
MZ848410.1 <i>Heteropneustes</i> sp.	32.03	25.49	27.12	15.36	612.00	19.12	25.49	25.49	29.90	204.00	42.16	29.41	15.69	12.75	204	34.80	21.57	40.20	3.43	204
HQ009489.1 <i>Heteropneustes microps</i>	32.03	25.49	27.12	15.36	612.00	19.12	25.49	25.49	29.90	204.00	42.16	29.41	15.69	12.75	204	34.80	21.57	40.20	3.43	204
KX455904.1 <i>Clupisoma garua</i>	30.23	26.47	26.14	17.16	612.00	17.16	26.96	25.98	29.90	204.00	42.16	28.92	15.69	13.24	204	31.37	23.53	36.76	8.33	204
MN259175.1 <i>Clupisoma garua</i>	30.23	26.47	26.14	17.16	612.00	17.16	26.96	25.98	29.90	204.00	42.16	28.92	15.69	13.24	204	31.37	23.53	36.76	8.33	204
MG334169.1 <i>Heterobranchus bidorsalis</i>	29.25	25.82	28.76	16.18	612.00	19.61	25.49	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	25.98	22.55	45.59	5.88	204
MG334168.1 <i>Heterobranchus bidorsalis</i>	29.25	25.82	28.76	16.18	612.00	19.61	25.49	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	25.98	22.55	45.59	5.88	204
MG334170.1 <i>Heterobranchus bidorsalis</i>	29.25	25.82	28.92	16.01	612.00	19.61	25.49	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	25.98	22.55	46.08	5.39	204
MG334171.1 <i>Heterobranchus bidorsalis</i>	29.25	25.82	28.92	16.01	612.00	19.61	25.49	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	25.98	22.55	46.08	5.39	204
MK572261.1 <i>Heteropneustes fossilis</i>	30.88	25.82	27.29	16.01	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.35	21.08	41.18	5.39	204
MK572260.1 <i>Heteropneustes fossilis</i>	30.88	25.82	27.29	16.01	612.00	18.14	26.96	25.00	29.90	204.00	42.16	29.41	15.69	12.75	204	32.35	21.08	41.18	5.39	204
Avg.	30.62	25.82	27.40	16.17	612.00	18.57	26.32	25.21	29.90	204.00	42.16	29.35	15.69	12.81	204	31.13	21.78	41.30	5.79	204



Figure 14. Type locality of *Heteropneustes fuscus*, a water stream at Pathanamthitta, Kerala, India



Figure 15. Map showing the type locality of *Heteropneustes fuscus*

Comparative materials examined

Heteropneustes longipectoralis: ZSI/SRS/F 4935 Holotype, 150 mm SL; Thirumurthi Dam, Anamalai Hills, Western Ghats, Tamil Nadu, India, coll. M.B. Raghunathan, 11 February 1996. ZSI/SRS/F 5044, Paratypes, 3, 146-154 mm SL; Thirumurthi Dam, Anamalai Hills, Western Ghats, Tamil Nadu, India, coll. M.B. Raghunathan, 11 February 1996.

Heteropneustes fossilis: V/F/NERC/ZSI/5135, 3, 141-157 mm SL, Mathews Plamoottil and Vineeth. K, Tranquebar, Tamil Nadu, 12.12.2020.

Heteropneustes nani: Original description from Hossain et al. (2013).

Heteropneustes kemratensis: Original description from Fowler (1937).

Sulurus singio: Original description from Hamilton (1822).

ACKNOWLEDGEMENTS

The author is grateful to DST-SERB, Government of India for sanctioning Core Research Grant for funding this research work. I thank Dr. Boni Amin Laskar, Scientist, Zoological Survey of India, Hyderabad for his scientific assistance. I am indebted to Dinesh (Maramon) and Akhilesh (Calicut) for the invaluable help extended to this work. The author thanks the officer-in-charge, Zoological Survey of India, Southern Regional Centre, Chennai for providing permission to examine the specimens of *Heteropneustes longipectoralis*. Also, I thank Dr. Jayashree Tilak, who is in charge of the freshwater fishes in ZSI/SRC, Chennai, Tamil Nadu. I am thankful to two anonymous reviewers for their insightful commentary on the manuscript.

REFERENCES

- Alok D, Krishnan T, Talwar GP, Garg LC. 1993. Induced spawning of catfish, *Heteropneustes fossilis* (Bloch), using D-Lys6 salmon gonadotropin-releasing hormone analog. *Aquaculture* 115: 159-167. DOI: 10.1016/0044-8486(93)90366-7.
- Bloch ME. 1794. *Naturgeschichte de Ausländischen Fische*. Siebenter Theil, Morino, Berlin.
- Burgess WE. 1989. *An Atlas of Freshwater and Marine Catfishes: A Preliminary Survey of the Siluriformes*. T.F.H. Publications, Neptune City, New Jersey.
- Coad BW. 1996. Exotic fish species in the Tigris-Euphrates basin. *Zool Middle East* 13 (1): 71-83. DOI: 10.1080/09397140.1996.10637707.
- Day F. 1878. *The Fishes of India: Being a Natural History of the Fishes Known to Inhabit the Seas and Fresh Waters of India, Burma, and Ceylon*. Bernard Quaritch, Piccadilly, London.
- Day F. 1889. *Fauna of British India including Ceylon and Burma*. Volume I, Taylor and Francis, London.
- Fowler HW. 1937. Zoological result of the third De Schauensee Siamese Expedition. Part VIII, Fishes obtained in 1936. *Proc Acad Nat Sci Phila* 89: 125-264.
- Froese R, Pauly D. 2018. *Fish Base 2018*, World Wide Web electronic publication. Available at: <http://www.fishbase.org> (accessed on 25 April 2018).
- Günther A. 1864. *Catalogue of the Fishes in the British Museum. Catalogue of the Physostomi, Containing the Families Siluridae, Characinidae, Haplochromidae, Sternoptychidae, Scopelidae, Stomiidae in the Collection of the British Museum*. Trustees, London.
- Hamilton BF. 1822. *An Account of the Fishes Found in the River Ganges and its Branches*. Archibald Constable and Company, Edinburgh, London.
- Hossain MS, Sarkar S, Sharifuzzman SM, Chowdhury SR. 2013. New species of stinging catfish *Heteropneustes nani* (Siluriformes: Heteropneustidae) from Noakhali, Bangladesh. *Vertebr Zool* 63 (3): 259-267.
- Jha BR. 2009. *Fish Ecological Studies in Assessing Ecological Integrity of Rivers: Application in Rivers of Nepal*. VDM Verlag Dr. Muller Aktiengesellschaft & Co. KG, Germany.
- Jha BR, A Rayamajhi. 2010. *Heteropneustes fossilis*. The IUCN Red List of Threatened Species. Version 2014.2. www.iucnredlist.org. (Accessed on 10 October, 2014).
- Jayaram KC. 2010. *Fresh Water Fishes of the Indian Region*. Narendra publishing House, Delhi.
- Jerdon TC. 1849. The Fishes of Southern India. *Madras J Liter Sci* 15: 147.
- Kasherwani D, Lodhi HS, Tiwari KJ, Shukla S, Sharma UD. 2009. Cadmium toxicity to freshwater catfish, *Heteropneustes fossilis* (Bloch). *Asian J Exp Sci* 23: 149-156. DOI: 10.31018/jans.v10i2.1758.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111-120. DOI: 10.1007/BF01731581.
- Misra KS. 1976. *The Fauna of India and the Adjacent Countries, Pisces, Vol. III. The Controllor of Publications, Delhi*.
- Nelson JS. 2006. *Fishes of the World*. 4th Edition. John Wiley and Sons, Hoboken, New Jersey.
- Pethiyagoda R, Bahir MB. 1998. *Heteropneustes microps*, a junior synonym of *H. fossilis* (Osteichthys: Heteropneustidae). *J South Asian Nat Hist* 3 (1): 113-114.
- Rahman MA, Gheyasuddin S, Rashid MH, Chowdhury MFZ. 1982. Proximate composition and nutritive quality of freshwater Zeol fishes of Bangladesh. *Bangladesh J Fish* 2 (5): 37-43. DOI: 10.4236/jms.2011.210145.
- Ratnuangkhwang S, Muiskasinthorn P, Kumazawa Y. 2014. Molecular phylogeny and biogeography of air sac catfishes of the *Heteropneustes fossilis* species complex (Siluriformes: Heteropneustidae). *Mol Phylogenet Evol* 79 (1): 82-91. DOI: 10.1016/j.ympev.2014.05.009, DOI: 10.1016/j.ympev.2014.05.009.
- Rema DK, Raghunathan MB. 1999. *Heteropneustes longipectoralis* (Siluriformes: Heteropneustidae) a new species from the Anamalai Hills, in the Western Ghats. *Rec Zool Surv India* 97 (3): 109-115.
- Saha KC, Guha BC. 1939. Nutritional investigation of Bengal fish. *Indian J Med Res* 26: 921-927.
- Swainson W. 1838. *The Natural History and Classification of Fishes, Amphibian, & Reptiles or Monocardian Animals, Vol. 1*. Green and Longmans, London.
- Swainson W. 1839. *The Natural History and Classification of Fishes, Amphibian, & Reptiles, or Monocardian Animals, Vol. 2*. Green and Longmans, London.
- Tamura K, Stecher G, Peterson D, Filipinski A, Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30: 2725-2729. DOI: 10.1093/molbev/mst197.
- Talwar PK, Jhingran AG. 1991. *Inland Fishes of India and Adjacent Countries*. Oxford-IBH Publishing Co. Pvt. Ltd., New Delhi.
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding of Australia's fish species. *Philos Trans R Soc Lond B Biol Sci* 360: 1847-1857. DOI: 10.1098/rstb.2005.1716.