



RECORDS OF THE ENDEMIC AND THREATENED CATFISH, *HEMIBAGRUS PUNCTATUS* FROM THE SOUTHERN WESTERN GHATS WITH NOTES ON ITS DISTRIBUTION, ECOLOGY AND CONSERVATION STATUS

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Abstract: The Nilgiri Mystus, *Hemibagrus punctatus*, a rare bagrid catfish endemic to the Western Ghats, has been currently listed in the IUCN Red List, as 'Critically Endangered' with a possibility that it could be extinct. The last validated record of *H. punctatus* was known to be in 1998, and several surveys since then have not been able to collect the species from its native range. In this paper, we provide information on new records of this rare catfish from the Western Ghats after a period of 14 years, and discuss its distribution, ecology and conservation. An updated conservation assessment of this species following the IUCN Red List Criteria is also provided.

Keywords: Bhavani, Cauvery, extinct, IUCN Red List, Nilgiri Mystus.

Jerdon (1849) described *Bagrus punctatus*, now included in the genus *Hemibagrus* Bleeker, 1862 from the River Cauvery in southern India, and suggested that the species occurs in the river and its various tributaries. The exact type locality, however, was not mentioned. Subsequently, Day (1867, 1878) recorded the species from the Bhavani (a tributary of the Cauvery), at the foothills of the Nilgiri Hills. Since then, very few validated records and voucher specimens of this rare catfish are

DOI: <http://dx.doi.org/10.11609/JoTT.o3427.4569-78> | ZooBank: urn:lsid:zoobank.org:pub:5F7AEDCD-8A8D-4360-BB5B-6862152D0264

Editor: Carl Ferraris, Smithsonian Institution, Portland, USA.

Date of publication: 26 July 2013 (online & print)

Manuscript details: Ms # o3427 | Received 08 December 2012 | Final received 06 June 2013 | Finally accepted 02 July 2013

Citation: Ali, A., N. Dahanukar, A. Kanagavel, S. Philip & R. Raghavan (2013). Records of the endemic and threatened catfish, *Hemibagrus punctatus* from the southern Western Ghats with notes on its distribution, ecology and conservation status. *Journal of Threatened Taxa* 5(11): 4569–4578; <http://dx.doi.org/10.11609/JoTT.o3427.4569-78>

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Funding: Rajeev Raghavan thanks the Mohammed Bin Zayed Species Conservation Fund (MBZSCF; Project 1225670) and the North of England Zoological Society (NEZS), Chester Zoo for providing financial support for the project on the 'Lost fishes of Western Ghats'. Neelesh Dahanukar thanks the Department of Science and Technology, Government of India for the INSPIRE Faculty Fellowship.

Competing Interest: The authors declare no competing interests. Funding sources had no role in study design, data collection, results interpretation and manuscript writing.

Acknowledgements: The authors thank Brawin Kumar, Keerthi Krutha, Priyanka Iyer, Payal Molur and Sanjay Molur for their encouragement and logistical support. Siby Philip thanks Allen Benziger for permission to use the photograph of *Hemibagrus cf. punctatus*. Rajeev Raghavan thanks Ralf Britz, The Natural History Museum (NHM), London for his help and support during the visits to the museum and in examining Day's material. The authors thank three anonymous reviewers and the subject editor for their critical comments on the manuscript.



The publication of this article is supported by the Critical Ecosystem Partnership Fund (CEPF), a joint initiative of l'Agence Française de Développement, Conservation International, the European Commission, the Global Environment Facility, the Government of Japan, the MacArthur Foundation and the World Bank.

available.

The Nilgiri Mystus or the Porthole Mystus, *Hemibagrus punctatus* is endemic to the Western Ghats (Dahanukar et al. 2004) where it is restricted to the tributaries and reservoirs in the Cauvery drainage (Ng & Ferraris 2000; Raghavan & Ali 2011; Ng & Kottelat 2013) (Fig. 1). Habitat degradation as a result of pollution, construction of dams and indiscriminate fishing were suggested to have led to drastic population declines (close to 100%) of this species in its native range (Raghavan & Ali 2011). Field surveys by several researchers failed to record *H. punctatus* for the last 14 years (see Raghavan & Ali 2011). Due to the heavy declines in population, and the fact that the last confirmed sighting was in 1998, the species has been listed in the IUCN Red List of Threatened Species as 'Critically Endangered' (CR) with a possibility that it could be extinct (Raghavan & Ali 2011).

During recent collection trips to various riverine fishing villages and reservoirs in the Cauvery basin, we came across several specimens of *H. punctatus* in the catches of local fishers. Here we document these records, and provide information on the distribution, ecology and conservation status of this rare catfish. In addition, using mitochondrial *cox1* gene, we show the phylogenetic relationship of *H. punctatus* (Fig. 2) (see Appendix 1 for detailed methods).

The specimens referred to in this paper are deposited in the collections of the Wildlife Information Liaison Development Society (WILD), Coimbatore, India and the Conservation Research Group, St. Albert's College (CRG-SAC), Kochi, India.

***Hemibagrus punctatus* (Jerdon, 1849)
(Images 1, 2, 3 & 4)**

Synonyms

Bagrus punctatus Jerdon, 1849

Hemibagrus punctatus (Jerdon, 1849): Day (1867); Ng & Ferraris (2000); Jayaram (2006); Ferraris (2007); Ng & Kottelat (2013)

Macrones punctatus (Jerdon, 1849): Day (1877; 1889)

Aoria punctatus (Jerdon, 1849): Mukerji (1931)

Mystus (Mystus) punctatus (Jerdon, 1849): (in part) Jayaram (1954)

Mystus punctatus Jayaram (1977, 1981); Mo (1991); Talwar & Jhingran (1991); Easa & Shaji (2003)

Mystus menoda menoda (non Day) - (?) Barman (1993).

Material examined

Hemibagrus punctatus: WILD-12-PIS-026, 22.xi.2012, 1 male, 270mm SL, Athikadavu, Bhavani River, Tamil Nadu, India, 11.221°N & 76.763°E, elevation 446m, R. Raghavan et al.; CRG-SAC-2012.12.1, 12.xii.2012, 1 male, 182mm SL, stream 16km east of Malavalli, Cauvery River, Karnataka, India, 12.403°N & 77.199°E, elevation 585m, A. Ali & S. Philip; CRG-SAC-2012.12.2, 12.xii.2012, 1 male, 180mm SL, Bannur, Cauvery River, Karnataka, India, 12.320°N & 76.842°E, elevation 644m, A. Ali & S. Philip; CRG-SAC-2012.12.3, 11.xii.2012, 1 male, 232mm SL, Krishna Raja Sagar Dam, Cauvery River, Karnataka, India, 12.425°N & 76.571°E, elevation 748m, A. Ali & S. Philip; BMNH 1868.5.14:8; 1 ex., 155mm SL, Bhavani River, India, F. Day.

Morphology

Biometric data presented in Table 1, is the first such information on fresh specimens of this rare catfish. Details of body morphology, coloration, head structure and dentition of the fresh specimen are provided in Images 1, 2 and 3 respectively. The morphometric data gathered from our specimens are largely in agreement with the data taken from museum specimens provided by Ng & Ferraris (2000) as well as Ng & Kottelat (2013). The small deviations in the data that are observed could



Image 1. Specimen of *Hemibagrus punctatus* (WILD-12-PIS-026) recorded from Bhavani River. (a) lateral, (b) dorsal and (c) ventral view.

be due to the fact that the present measurements were taken from fresh specimens as opposed to museum specimens studied by Ng & Ferraris (2000) and Ng & Kottelat (2013). It is also essential to note that at least one of the specimens studied by Ng & Ferraris (2000) is not in good condition (Image 5). Further, the sample size in the current and previous studies (Ng & Kottelat 2013; Ng & Ferraris 2000) are too small (n=4 and 4/3 respectively).

Distribution

Hemibagrus punctatus has been recorded from Cauvery (Jerdon 1849; Rao & Seshachar 1927; Hora 1937) and its principal tributaries, Bhavani (Day 1877; Mukerji 1931; Rajan 1955), Moyar (Rajan 1955; Rajan 1963; Manimekalan 1998), Hemavathy (Jayaram 1977; Madhyastha & Murugan 1993) and Kabini (Easa & Basha 1995; Shaji & Easa 1995; Easa & Shaji 1997). Meanwhile, Ng & Ferraris (2000 p. 129; p. 139) gives contradicting

information on the possibility of *H. punctatus* occurring in the Krishna River system. The records of this species from the Krishna drainage is by Singit et al. (1987) and Sugunan (1995) from the Tungabhadra Reservoir. With the available information, we believe that *H. punctatus* is restricted to the Cauvery River and its tributaries, and records from Krishna River including the Tungabhadra Reservoir may actually be mis-identifications. The materials from the Krishna may actually be *Hemibagrus maydelli*.

Although there are records of *H. punctatus* from several west flowing rivers in Kerala viz. Bharatapuzha, Chaliyar (Biju 2005) and Karuvannur (Thomas et al. 2002), they are not backed by voucher specimens and have been considered to be misidentifications (see Raghavan & Ali 2011). However, one of the authors of the present study (SP) encountered a local fisherman near Kanjirapuzha (west flowing Bharatapuzha River Drainage) in Kerala with a catch of *Hemibagrus* sp.

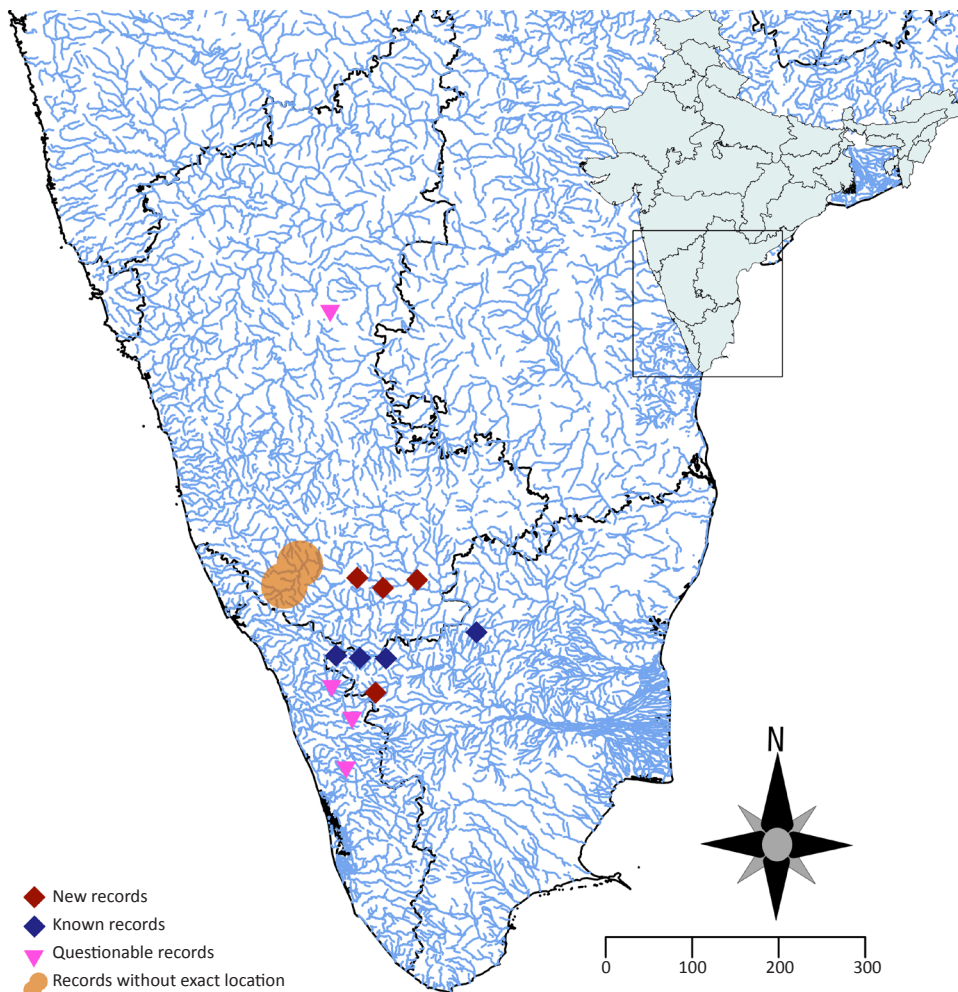


Figure 1. Distribution of *Hemibagrus punctatus* in peninsular India



Image 2. Details of (a) lateral, (b) dorsal) and (c) ventral view of head of *Hemibagrus punctatus* (WILD-12-PIS-026) recorded from Bhavani River.

(Image 6). Unfortunately, specimens could not be collected due to logistical difficulties, and therefore its validity could not be determined. Photographic evidence (Image 6), has led us to tentatively identify the specimen as *Hemibagrus* cf. *punctatus*. More surveys are therefore needed in the Bharatapuzha River to confirm the occurrence of this species. Historic and current distribution of *H. punctatus* is shown in Fig. 1.

Hemibagrus punctatus has sometimes been

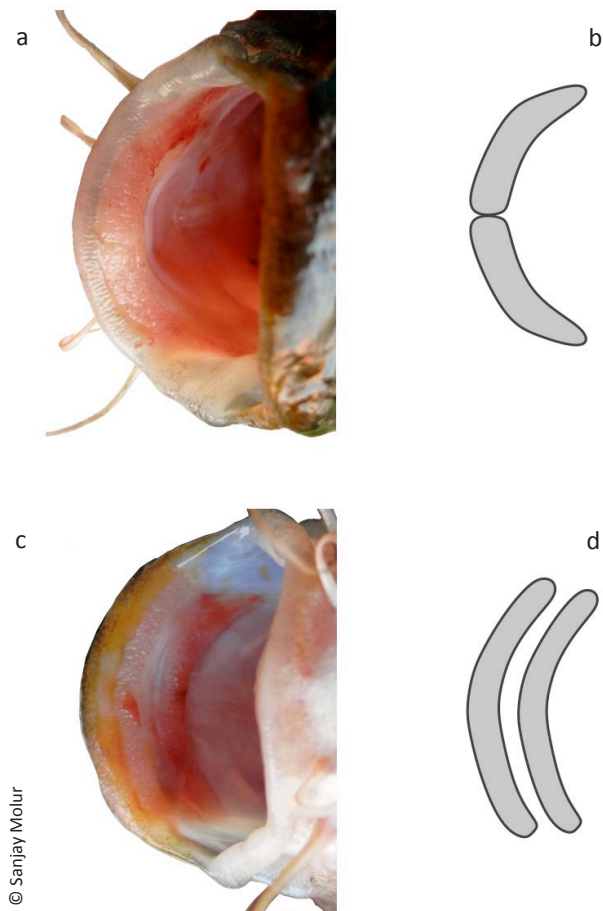


Image 3. Teeth bands on the lower (a and b) and upper (c and d) jaw of *Hemibagrus punctatus*.

confused with *H. menoda* and misidentified (see Ng & Ferraris 2000). Although the distribution of *H. menoda* is restricted to the Brahmaputra, Ganges, Mahanadi and Godavari river drainages in India, Nepal and Bangladesh (Ng 2010; Ng & Ferraris 2000), there are records of this species from the west flowing Pamba and Achankovil rivers in southern Kerala (Kurup et al. 2004; Renjithkumar et al. 2011). However, none of these records are accompanied by voucher specimens, making any validation impossible. Therefore, future studies should also focus on determining the correct identity of the specimens that have been recorded as *H. menoda* from these river systems.

Population

Members of the genus *Hemibagrus* are known to be rare, and seldom encountered in local markets (Ng & Ferraris 2000). *Hemibagrus punctatus* is the only member within the genus that is threatened and continues to face population declines (see various species accounts

Table 1. Morphometric data for *Hemibagrus punctatus* collected during the present study, and data from Ng & Ferraris (2000) and Ng & Kottelat (2013).

Characters	WILD- PIS-12-026	CRG-SAC. 2012.12.1	CRG-SAC. 2012.12.2	CRG-SAC. 2012.12.3	Mean (sd) (n=4)	Range (n=4)	Ng & Ferraris (2000) (n=3)	Ng & Kottelat (2013) (n=4)
Total length (mm)	355	220	216	286	269 (65.56)	216–355		
Standard Length (mm)	270	182	180	232	216 (43.3)	180–270	120.2–193.1	120.2–193.1
% SL								
Head Length	27.3	27.4	26.9	26.5	27.0 (0.41)	26.5–27.4	28.1–29.6	28.1–29.6
Head Depth	12.3	13.6	13.8	13.3	13.3 (0.67)	12.3–13.8	11.9–14.3	11.9–14.3
Maximum Head Width	18.1	19.2	19.2	19.5	19.0 (0.62)	18.1–19.5	16.4–20.5	16.4–20.5
Pre Dorsal Length	39.3	40.4	39.9	38.4	39.5 (0.86)	38.4–40.4	39.8–42.0	39.8–42.0
Anterior border of dorsal fin base to caudal fin length	62.7	63.1	64.0	64.9	63.7 (0.98)	62.7–64.9	–	–
Posterior border of dorsal fin base to caudal fin length	47.5	46.6	49.1	50.3	48.4 (1.65)	46.6–50.3	–	–
Pre Pectoral Length	20.0	23.4	22.7	23.5	22.4 (1.64)	20.0–23.5	26.2–26.6	26.2–26.6
Pre Pelvic Length	55.2	51.4	51.2	50.9	52.2 (2.03)	50.9–55.2	53.3–54.2	53.9–55.7
Pre Anal Length	70.9	69.8	68.4	67.6	69.2 (1.47)	67.6–70.9	71.0–74.2	71.0–74.2
Pre Anus Length	63.4	58.4	56.5	58.4	59.2 (2.96)	56.5–63.4	–	–
Depth at Dorsal Origin	16.6	17.9	17.7	16.8	17.3 (0.65)	16.6–17.9	–	–
Depth at Anal Origin	14.3	13.3	13.7	13.0	13.6 (0.56)	13.0–14.3	–	–
Width at Dorsal Origin	15.7	16.0	15.5	14.9	15.5 (0.46)	14.9–16.0	–	–
Width at Anal Origin	10.2	9.5	10.0	9.1	9.7 (0.5)	9.1–10.2	–	–
Least Depth of Caudal Peduncle	9.8	8.9	8.7	9.0	9.1 (0.48)	8.7–9.8	8.8–9.9	8.8–9.9
Caudal Peduncle Length	18.7	16.8	16.6	18.1	17.6 (1.01)	16.6–18.7	16.1–18.6	16.1–18.6
Dorsal Fin Length	21.0	24.8	24.0	25.3	23.8 (1.93)	21.0–25.3	24.7–27.6	24.7–27.6
Dorsal Fin Base Length	15.6	16.6	15.5	17.7	16.4 (1.03)	15.5–17.7	14.7–17.4	14.7–17.4
Pectoral Fin Length	19.5	17.3	15.7	16.2	17.2 (1.69)	15.7–19.5	18.7–21.1	18.7–21.1
Pectoral Fin Base Length	4.5	4.6	4.5	5.2	4.7 (0.34)	4.5–5.2	–	–
Pelvic Fin Length	14.2	13.1	13.2	15.2	13.9 (0.98)	13.1–15.2	14.9–17.2	14.9–17.2
Anal Fin Length	15.1	19.2	18.4	19.2	18.0 (1.95)	15.1–19.2	–	–
Anal Fin Base Length	11.3	13.7	12.3	13.3	12.7 (1.08)	11.3–13.7	11.8–14.3	11.8–14.3
Dorsal to Adipose Distance	27.7	34.5	37.8	36.7	34.2 (4.53)	27.7–37.8	16.3–19.4	16.3–19.4
Adipose Fin Length	13.6	17.3	17.0	16.3	16.1 (1.69)	13.6–17.3	–	–
Adipose Fin Base Length	10.7	13.2	12.3	12.8	12.3 (1.1)	10.7–13.2	10.1–13.2	10.1–13.2
Adipose Fin Height	3.5	3.4	3.1	4.1	3.5 (0.42)	3.1–4.1	3.9–5.4	3.9–5.4
Post Adipose Distance	16.2	16.1	16.6	15.2	16.0 (0.59)	15.2–16.6	16.2	14.8–16.2
Maxillary Barbel Length	43.0	40.6	42.6	30.0	39.1 (6.12)	30.0–43.0	–	–
Nasal Barbel Length	10.9	7.7	7.3	7.5	8.4 (1.71)	7.3–10.9	–	–
Mandibular Barbel Length	24.7	17.0	17.2	13.1	18.0 (4.85)	13.1–24.7	–	–
Inner Mandibular Barbel Length	15.3	10.4	9.2	8.4	10.8 (3.09)	8.4–15.3	–	–
% HL								
Head Depth	45.0	49.5	51.1	50.1	48.9 (2.7)	45.0–51.1	–	–
Maximum Head Width	66.3	70.2	71.2	73.8	70.4 (3.11)	66.3–73.8	–	–
Eye Diameter	13.2	15.7	14.2	14.4	14.4 (1.03)	13.2–15.7	13–15.7	13–15.7
Snout Length	35.3	39.5	40.2	41.0	39.0 (2.54)	35.3–41.0	35.7–38.9	35.7–38.9
Inter Orbital Length	35.4	35.2	33.4	33.9	34.5 (0.98)	33.4–35.4	31.3–32.5	31.3–32.5

Characters	WILD- PIS-12-026	CRG-SAC. 2012.12.1	CRG-SAC. 2012.12.2	CRG-SAC. 2012.12.3	Mean (sd) (n=4)	Range (n=4)	Ng & Ferraris (2000) (n=3)	Ng & Kottelat (2013) (n=4)
Eye to Nostril Distance	23.8	20.6	20.7	20.4	21.4 (1.62)	20.4–23.8	–	–
Inter Nostral Distance	17.9	18.6	19.3	20.6	19.1 (1.15)	17.9–20.6	–	–
Gape Width	41.7	40.5	40.8	43.9	41.7 (1.54)	40.5–43.9	–	–
Post Orbital Length	52.1	48.3	48.9	51.4	50.2 (1.86)	48.3–52.1	–	–
Maxillary Barbel Length	157.7	148.1	158.4	113.3	144.4 (21.24)	113.3–158.4	163.2–203.4	163.2–203.4
Nasal Barbel Length	39.8	28.1	27.3	28.2	30.9 (5.98)	27.3–39.8	27.5–40.3	27.5–40.3
Mandibular Barbel Length	90.6	62.0	63.7	49.7	66.5 (17.24)	49.7–90.6	68.8–80.2	68.8–80.2
Inner Mandibular Barbel Length	56.3	37.8	34.2	31.8	40.0 (11.13)	31.8–56.3	31.7–45.6	31.7–45.6



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Image 4. Specimen of *Hemibagrus punctatus* collected from Malavalli, Cauvery River, Karnataka (CRG-SAC-2012.12.1)

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Image 5. Day's material of *Hemibagrus punctatus* from Bhavani River (BMNH 1868.14:8; 155mm SL). Photograph by Rajeev Raghavan

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Image 6. Specimen of *Hemibagrus cf punctatus* recorded from Kanjirapuzha (Bharatapuzha River) in 2008. [Scale in cm].

in IUCN Red List of Threatened Species). From an extensive collection of fishes from Cauvery River, Hora (1937) recorded only a single specimen of *H. punctatus*. Similarly, Mukerji (1931) recorded a single specimen (200mm in length) from Bhavani River, Coimbatore District, Tamil Nadu, while Rajan (1955) also recorded only a single specimen (320mm in length) from Moyar River at Mangalapatti, Erode District, Tamil Nadu. The species was also reported to be rare in Ombatta swamp of Mudumalai Wildlife Sanctuary (Manimekalan 1998). The maximum number of individuals of *H. punctatus* that has been recorded is ~50 in the Cauvery Sangam near Mysore in 1996 (J. Jensen pers. comm 03 December 2012).

In the Kabini River (Wyanad, Kerala), this species has declined drastically in the last decade with not a single specimen being encountered during repeated surveys since the year 2000 (C.P. Shaji & B.M. Kurup pers. obs. In: Ali & Raghavan 2011). In Tungabhadra, the species was last recorded in the 1980's (Singit et al. 1987), while the last validated record from Moyar was during 1990–1992 (see Ali & Raghavan 2011). Surveys by Johnson & Arunachalam (2009) (actual surveys conducted in 2001–2002) in three tributaries of the Cauvery failed to collect *H. punctatus*. Overall populations in the native range were, therefore, thought to have declined close to 100% in the last 14 years (Ali & Raghavan 2011). Our record of four specimens caught by local fisherman from the Bhavani and Cauvery rivers in November and December 2012, and local knowledge of fishers in the region suggest that moderate populations of the species are still extant in both the main basin as well as the major tributaries of the Cauvery River and that the fish is not extinct as believed previously (Ali & Raghavan 2011).

Our surveys in the various fish landing centers along the Cauvery in Karnataka State, and interactions with local fishers reveal that *H. punctatus* is not 'uncommon' in the area, but represents only a very negligible part of the catch.

Habitat and Ecology

Hemibagrus punctatus is known to inhabit rapid rivers and streams (Menon 1999), as well as medium and large reservoirs. The location from where the fisherman caught his specimen of *H. punctatus* in Athikadavu, Bhavani was a fast flowing stream with deep pools, and bed rock as the substrate (Image 7). The bank on one side had a thick canopy with several wooden logs submerged in the water, while the other bank had a sandy bed with shrubs. The co-occurring species in the fisherman's catch at Athikadavu were *Hypselobarbus dubius*, *Barbodes carnaticus*, *Kantaka brevidorsalis* and *Channa marulius*. Although we encountered the fisherman at Malavalli, the catch was obtained from a stream flowing 16km east of the town, where the habitat is a fast flowing stream with deep pools and bed rock substrate, with a sandy bed and shrubs on both the shores. The co-occurring species as seen from the catches were *Channa marulius*, *Barbodes carnaticus*, *Hypselobarbus dubius*, *Oreochromis mossambicus* and *O. niloticus*. The fishermen also revealed that *H. punctatus* occur in the river at Malavalli (Image 8) but are encountered rarely. The other sites from where the specimens of *H. punctatus* were recorded in catches of local fishermen include the Krishna Raja Sagar (KRS) reservoir near Mysore and the stream near the town of Bannur.

Based on the feeding habits of related species



Image 7. Habitat of *Hemibagrus punctatus* at Athikadavu in Bhavani River.



Image 8. Probable habitat of *Hemibagrus punctatus* near Malavalli in Cauvery River.

within the genus *Hemibagrus* (Froese & Pauly 2012), *H. punctatus* is most likely to be a carni-omnivore. Rajan (1955) recorded adult specimen (103mm in length) and a severed head (25mm long) of *Garra stenorhynchus* inside the gut of *H. punctatus* (320mm in length) collected from the headwaters of the Bhavani River.

Hemibagrus punctatus is known to attain a maximum size of 450mm TL (Day 1889; Menon 1999). Records of *H. punctatus* from the Tungabhadra Reservoir with sizes of 25–35 kg (and up to 120kg) (Sugunan 1995) are misidentifications and represent *H. maydelli*, which Ng & Kottelat (2013) consider as one of the largest known species within this genus.

Phylogenetic position

The taxonomy of *Hemibagrus* is known to be confusing with the validity of many nominal species still unclear (Ng & Kottelat 2013). These authors defined eight ‘species groups’ of *Hemibagrus* based on morphological

characteristics. Here, using mitochondrial *cox1* gene, we show that *H. punctatus* and *H. menoda* are indeed sibling species belonging to the same species group as suggested by Ng & Kottelat (2013) with a 4.2% genetic distance separating them (Fig. 2).

Threats

The Cauvery and its tributaries from where *H. punctatus* has been recorded are subjected to several on-going threats including pollution, construction of barrages and indiscriminate fishing (Dahanukar et al. 2011). Athikadavu, from where we recorded one of our specimens is relatively free of threats barring gill net fishery by the local communities for sustenance.

Conservation actions

No species level conservation plans are currently in place. There is a need for organized surveys throughout the known range of *H. punctatus* to determine the

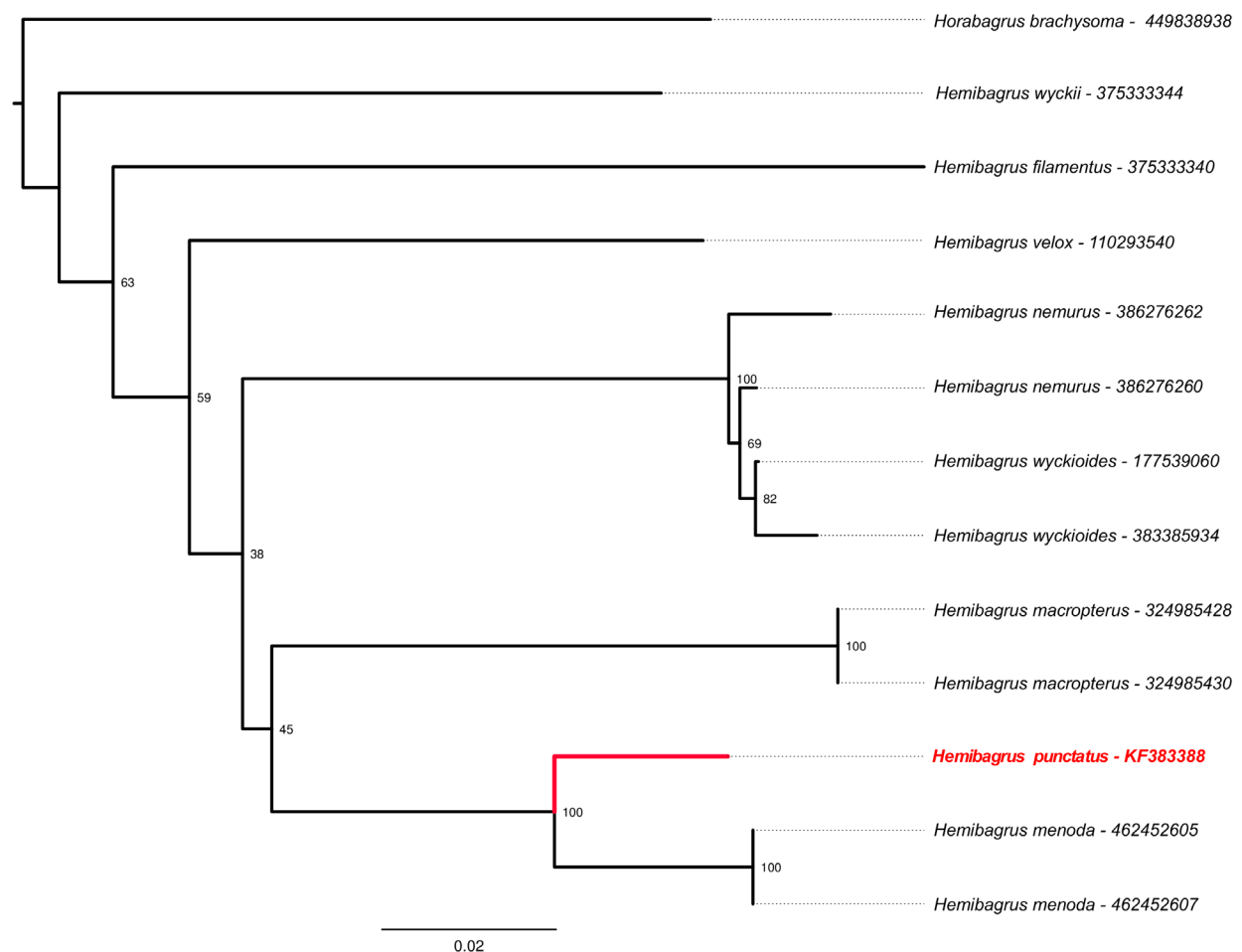


Figure 2. Neighbor Joining phylogenetic tree based on observed distances of cytochrome oxidase subunit 1 (*cox1*) gene sequences of different *Hemibagrus* species showing the phylogenetic position of *Hemibagrus punctatus*.

current distribution and population status of this species. We are currently carrying out such surveys in the Cauvery and its tributaries including Bhavani and Kabini as part of a project to determine the status of missing fishes of Western Ghats. In addition, there is a need to undertake detailed taxonomic studies to clear the ambiguities related to the identity of specimens that have been recorded as *H. punctatus* (and *H. menoda*) from many west flowing rivers of Kerala. Local Ecological Knowledge surveys (see Kanagavel & Raghavan 2012) could also help gather important information on the natural history of the species in its native range.

IUCN Red List Assessment

Current status: Critically Endangered (CR) A2ac (Raghavan & Ali 2011).

Proposed status: Vulnerable (VU) A2ace, B2ab(iiii)

Justification for the proposed status: Population decline of ~100% due to habitat loss and several other threats, which are not yet clearly understood, were suggested by researchers after carrying out comprehensive surveys in its native range (see Raghavan & Ali 2011). While the species may have been extirpated from certain locations, surveys carried out after this assessment have now led to the collection of four specimens from multiple sites in the River Cauvery. However, except for these locations (Athikadavu, Krishnaraja Sagar Dam, Bannur and Malavalli), there are no validated records and voucher specimens of *H. punctatus* since the late 1990s. Although, the local knowledge of fishers indicate that moderate populations of *H. punctatus* exist in the Cauvery, out of the current seven confirmed locations where the species is currently known from, in at least three locations it has been suggested that the population of the species has declined close to 100 percent (Raghavan & Ali 2011). Even if we consider that the population in the remaining four sites is stable, the average population decline is still more than or equal to 30%. Further, the habitats that are likely to harbour *H. punctatus* in these regions are currently threatened as a result of pollution and dams. The estimated area of occupancy (AOO) is not more than 2000km because of very fragmented and restricted distribution of this rare species. Further, the fragmented populations are likely to fall under not more than 10 locations based on the on-going threats to the habitat and populations as mentioned earlier. Therefore, *Hemibagrus punctatus* needs to be categorized as a 'Vulnerable' species. However, it is advised that further studies on the distribution and population status of this species should be carried out along with detailed

taxonomic studies to verify the records of the species from the west flowing rivers of Kerala, and the Krishna River system.

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Appendix 1. Morphometry

Morphometric and meristic data were recorded following Ng & Ferraris (2000). Measurements were taken point to point using dial calipers to the nearest 0.1mm. Measurements of body parts are reported as percentage of standard length (SL) and measurements of subunits of head are reported as percentage of head length (HL).

DNA isolation and molecular phylogeny

Muscle tissue was harvested from one fresh specimen (WILD-12-PIS-026) and was preserved in absolute ethanol. The tissue was digested at 60°C for two hours using the STE buffer (0.1M NaCl, 0.05 M Tris-HCl, 0.01M EDTA, 1%SDS) with 15µl Proteinase K (20mg/ml) per 500ml of STE buffer. DNA was extracted using conventional phenol–chloroform method and re-suspended in nuclease free water. Polymerase chain reaction was performed to amplify mitochondrial cytochrome oxidase subunit I (cox1) gene, using the forward primer Fish R1 (5'- TCAACCAACCACAAAGACATTGGCAC-3') and reverse primer Fish R1 (5'- TAGACTTCTGGGTGGCCAAAGAATCA -3') (Ward et al. 2005). PCR reaction was performed in a 25µl reaction volume containing 5µl of template DNA (~200ng), 2.5µl of 10X reaction buffer (100 mM Tris pH 9.0, 500 mM KCl, 15mM MgCl₂, 0.1% Gelatin), 2µl of 25mM MgCl₂, 1µl of 10mM dNTPs, 1µl of each primer, 1µl Taq polymerase and 16.5µl nuclease free water. The thermal profile was 10 minutes at 95°C, and 35 cycles of 1 minute at 94°C, 1 minute at 54°C and 2 min at 72°C, followed by extension of 10 min at 72°C. Amplified DNA fragments were purified using the 'Promega Wizard Gel and PCR clean up' system and sequenced. The purified PCR products were sequenced using ABI prism 3730 sequencer (Applied Biosystems, USA) and Big dye terminator sequencing kit (ABI Prism, USA).

BLAST tool (Altschul et al. 1990) was used to analyze the integrity of the sequence. The sequence was submitted to NCBI GenBank and accession number for this sequence is KF383388. We retrieved additional sequences for other related species from NCBI GeneBank database (<http://www.ncbi.nlm.nih.gov/>). GenBank GI record numbers for the sequences used for the analysis are provided in Figure 2. Sequences were aligned using MUSCLE (Edgar 2004). A neighbor joining phylogeny with observed distances was built using SeaView (Gouy et al. 2010).

