Research Article

Molecular Identification and Ecology of a Newly Discovered Population of Sun Catfish *Horabagrus brachysoma* from Northern Western Ghats of India

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Received 19 August 2012; Accepted 16 September 2012

Academic Editors: R. Castiglia, V. Ketmaier, and D. Park

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*Horabagrus brachysoma*, thought to be endemic to the southern parts of the Western Ghats of India, is recorded for the northern parts of the Western Ghats, extending the species distribution range by 180 km. We have confirmed the identity of the species and the fact that the species is indigenous to this area and not an artifact of recent introductions using molecular methods. Apart from the range extension we have also provided detailed analysis regarding the nature of morphometric variations between the sexes, length-weight relationship, and a brief discussion about the potential habitat requirements and threats to this species. By documenting the possible threats to this threatened and endemic species, we have commented on the possible measures to conserve the species in the wild.

1. Introduction

Classified among the 34 global biodiversity hotspots [1], the Western Ghats of India is rich in freshwater fish diversity with more than 40% of the species being endemic to this region [2]. On one hand while new species of freshwater fish are still being described from the Western Ghats [3–6], on the other hand recent IUCN assessments have suggested that more than 58% of the endemic freshwater fish fauna of this region is threatened due to various anthropogenic stressors [7] and needs immediate conservation attention [8]. One of the major hindrances in designing and implementing potent conservation action plans for the freshwater fish in this region is the fact that they are still least understood with respect to their distribution, life history traits, population dynamics, and ecology, while many species complexes are still awaiting for proper taxonomic evaluation [7]. Limited knowledge of distribution of several fish species and continuous description of new species suggests that the freshwater fish fauna of the Western Ghats biodiversity hotspot is subject to both the Wallacean (geographical distribution of most species is poorly understood) and Linnean (most species are still not formally described) shortfalls [9].

*Horabagrus* Jayaram, 1955 is an endemic catfish found only in west flowing rivers of Western Ghats of India. In the order Siluriformes the exact familial affinities of *Horabagrus* is still debated and traditionally it has been placed under family Bagridae [10] or Schilbidae [11–13], while the recent molecular phylogeny suggests that it should belong to a new proposed family Horabagridae [14]. The genus currently comprises of two species *H. brachysoma* ( Günther, 1864) and *H. nigricollaris* Pethiyagoda & Kottelat, 1994, both of which are threatened [7].
Horabagrus brachysoma commonly called as sun catfish, yellow catfish or Günther’s catfish is characterized by anterior depressed large head; obtusely rounded snout; sub terminal transverse mouth; eyes large inferiorly visible from ventral surface of head; dorsal and pectoral fin having serrated spine with 5–7 and 8–9 branched rays, respectively; adipose dorsal fin short and well separated from caudal base; ventral fin with 6 rays; long anal fin with iii23–iii28 rays and a distinct coloration with brownish back dorsal side, pale yellow on sides, white belly, a thick black shoulder spot and semilunar thick black ring at caudal base [12, 15]. The species is heavily targeted by artisanal fishermen in inland waters of southern Western Ghats because of which it has now become vulnerable to overexploitation [16–18]. Multiple stress factors like overexploitation of wild stock for commercial fishery and international aquarium pet trade, habitat alteration, pollution, and minimum population doubling time have resulted in population decline of H. brachysoma in its native occurrence ranges and as a result of which this species has been listed as Vulnerable in IUCN Redlist [19]. Till date distribution of H. brachysoma was restricted from west flowing rivers of Kerala and Karnataka part of Western Ghats [19, 20] (Figure 1).

In the current paper we document the occurrence of H. brachysoma further north in Western Ghats of Maharashtra, with further studies regarding the molecular analysis (for taxonomic identification and molecular divergence), morphometry, length weight relationship, and behavioral aspects of this species based on in situ underwater observations. We have also documented the threats to the newly discovered population.

2. Materials and Methods

2.1. Study Area. Field surveys were conducted in Gad River basin (Figure 1) of Sindhudurga District located in south Konkan region of Maharashtra. Gad River is one of the west flowing rivers in northern Western Ghats which lies between 16° to 16° 20’N latitude and 73° 30’ to 74° longitude. Gad River originates from the hilly ranges of Sahyadri at an elevation about 600 m above sea level and drains in Arabian Sea at Malvan. The Gad River drains about 890 sq. km area in Sindhudurga District, overall passage length of Gad River from its origin to its outfall is 66 km. The present study was conducted in perennial second-order streams of Gad River near Bagayat village (16° 09’ 04.35” N and 73° 33’ 04.7” E).

2.2. Specimen Collection and Behavioral Study. Occurrence of H. brachysoma in streams of Gad River near Bagayat was
first observed opportunistically in December 2010 during night fish sampling. After proper identification of a single specimen by using available taxonomic literature [12, 13, 21] subsequent field surveys were conducted in the month of October to November 2011. Samplings were performed by using local fishing nets like monofilbril gill net, cast net, and local fishing traps in between down and midnight time (5.30 pm to 12.00 midnight). Representative 18 specimens were collected for morphometric and molecular phylogeny study. The specimens were preserved in 4% formaldehyde. Ten preserved specimens from study area are deposited in the museum of Bombay Natural History Society, Mumbai under the accession number BNHS FWF 1 to BNHS FWF 10. Four specimens are deposited in the museum collection of Wildlife Information Liaison Development, Zoo Outreach Organization, Coimbatore (WILD) under the accession numbers WILD-12-PIS-020 to WILD-12-PIS-023. Four specimens are deposited in the Zoological Survey of India, Western Regional Centre, Akurdi, Pune under the accession numbers P/3059.

In situ underwater observations in shallow clear stream water were performed in day as well as night time by using underwater mask and snorkel. Night time observations were performed by using UK vision head lamp. Ten minute no motion buffer time were taken by observer after entrance in the stream water to avoid human interference in natural behavior of fishes. Minimum body movements were maintained during snorkel study.

2.3. Morphometric Analysis and Length-Weight Relationship. Morphometric and meristic data were recorded following [13] for 36 morphometric and four meristic characters (Table 1). We performed the morphological analysis on the formalin-preserved specimens. Measurements were taken point to point using dial calipers (Mitutoyo No 505–626, Japan) to the nearest hundredth of an inch and then converted to millimeters. 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). Males and females were identified based on external genitalia, and morphometry of males and females was recorded separately. To understand whether the males and females differed in their morphometry we performed a Principle Component Analysis (PCA) on data expressed as %SL. We performed PCA on the correlation matrix in a freeware PAST [22].

The weight of the specimen was determined to the nearest 0.01 g using an electric balance (Anamed MX-7210A, India). We plotted length and weight of the fish to determine the power of the length-weight relationship \( W = aL^b \), where \( W \) is the weight, \( a \) is the normalization constant, \( L \) is the length, and \( b \) is the scaling power. The null hypothesis that \( b = 3 \) was tested using \( t \)-test as described by Zar [23].

2.4. DNA Isolation and Molecular Identification. Muscle tissue was harvested from two specimens, one male (WILD-12-PIS-021) and one female (WILD-12-PIS-023) and was preserved in absolute Ethanol. The tissue was digested at 60°C for two hours using the STE buffer (0.1 M NaCl, 0.05 M Tris-HCl, 0.01 M EDTA, 1% SDS) with 15μL Proteinase K (20 mg/mL) per 500μL of STE buffer. DNA was extracted using conventional phenol-chloroform method and resuspended in nuclease-free water. Polymerase chain reaction was performed to amplify two mitochondrial genes, cytochrome oxidase subunit I (cox1), and cytochrome b (cyt-b). Gene cox1 was amplified using forward primer FishF1 (5’-TCAACCAAC-CACAAAGACATTGGCCAC-3’) and reverse primer FishR1 (5’-TAGACTTCTGGTGCCAAAAGATCA-3’) [24], while cyt-b gene was amplified using the forward primer L14724 (5’-GACCTGAAACACCGTG-3’) and reverse primer H15915 (5’-CTCCGATCTCCGGATTACAAGAC-3’) [25]. PCR reaction was performed in a 25 μL reaction volume containing 5 μL of template DNA (~200 ng), 5 μL of 5X reaction buffer (100 mM Tris pH 9.0, 500 mM KCl, 15 mM MgCl₂, 0.1% Gelatin), 3 μL of 25 mM MgCl₂, 1 μL of 10 mM dNTPs, 1 μL of each primer, 0.5 μL Taq polymerase, and nuclease free water to make the volume 25 μL. The thermal profile was 10 minutes at 94°C, and 35 cycles of 1 minute at 94°C, 1 minute at 52°C (for cyt-b) or 1 minute at 54°C (for cox1) and 2 min at 72°C, followed by final 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). Sequences were edited manually using BioEdit [26]. Sequences were submitted to GenBank under the accession numbers JX460967 and JX460968 for cox1 and JX460962 and JX460969 for cyt-b. Sequences were analyzed using BLAST tool [27].

We retrieved additional sequences on other related species from NCBI (http://www.ncbi.nlm.nih.gov/) GenBank database (Horabagrus brachysoma cox1: HQ009501, HQ009502, EU490864, EF014947, HM579863; H. brachysoma cyt-b: EU490913, GQ398123, HM579856; H. nigricollaris cox1: HQ009503, HM579861; H. nigricollaris cyt-b: HM579857, GQ398127; Mystus bocourti cox1: JX420129; M. bocourti cyt-b: EU490912; Glyptothorax poonaensis cox1: JN092397; G. poonaensis cyt-b: JN092396). Sequences were aligned using MUSCLE [28]. Molecular phylogeny was performed using the freeware MEGA 5 [29]. Best fit model for nucleotide substitution was selected from 24 models available in MEGA 5 based on minimum Akaike Information Criterion (AIC) value [30]. Phylogenetic trees were built using four methods, namely, maximum likelihood (ML), maximum parsimony (MP; close-neighbor-interchange algorithm), minimum evolution (ME), and neighbor joining (NJ, maximum composite likelihood method). Reliability of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations. Evolutionary divergence between the sequences was computed using maximum composite likelihood method with bootstrap values run for 1000 iterations.

3. Result and Discussion

3.1. Taxonomic Identification and Molecular Phylogeny. Detailed morphological characters for Horabagrus brachysoma provided by Günther [15] and Jayaram [10, 12] matched
perfectly with the specimens collected in the current study (Figure 2) indicating that the specimens in the current collection were conspecific with *H. brachysoma*. However, while all the morphometric and meristic counts of the current collection were in the prescribed range for *H. brachysoma* as provided by Jayaram [10, 12] our specimens were small sized (maximum 50.8 mm SL) as compared to the adult sizes recorded by studies in Kerala (150 mm SL) [16]. Therefore, we further confirmed the identity of the current collection using molecular methods.

### Table 1: Morphometric and meristic data of *Horabagrus brachysoma*.

| Character | Male (*n* = 13) | Female (*n* = 5) |
|-----------|----------------|-----------------|
| **Morphometric** | | |
| Total length (mm) | 53.76 (4.47) | 53.33 (1.4) |
| Standard length, SL (mm) | 42.42 (3.59) | 42.84 (0.94) |
| % SL | | |
| Head length, HL | 31.11 (1.71) | 30.04 (1.31) |
| Depth of body at dorsal fin origin | 23.73 (1.22) | 21.25 (0.66) |
| Depth of body at anus | 18.97 (0.72) | 15.16 (1.37) |
| Width of body at dorsal fin origin | 16.25 (0.76) | 9.47 (0.51) |
| Width of body at anus | 10.13 (0.62) | 9.47 (0.51) |
| Predorsal length | 37.59 (1.4) | 37.8 (1.09) |
| Dorsal origin to caudal distance | 63.16 (1.79) | 62.58 (1.33) |
| Prepectoral fin length | 23.99 (0.66) | 27.53 (1.33) |
| Preventral fin length | 51.53 (1.29) | 52.96 (1.07) |
| Preanal fin length | 61.92 (1.24) | 64.28 (1.35) |
| Preanus length | 59.01 (1.27) | 59.99 (2.4) |
| Ventral fin to anus distance | 7.55 (0.58) | 7.57 (0.77) |
| Anus to anal fin distance | 3.73 (0.4) | 4.03 (0.59) |
| Dorsal fin length | 25.25 (1.64) | 23.97 (1.94) |
| Length of dorsal fin base | 10.79 (0.63) | 9.82 (1.3) |
| Pectoral fin length | 22.92 (1.08) | 22.67 (0.79) |
| Ventral fin length | 12.69 (0.87) | 12.36 (1.43) |
| Length of anal fin base | 25.47 (0.9) | 24.69 (1.36) |
| Caudal peduncle length | 14.46 (1.16) | 13.87 (1.06) |
| Caudal peduncle depth | 10.24 (0.54) | 9.53 (0.52) |
| Dorsal fin to adipose fin distance | 31.39 (1.82) | 30.38 (2.11) |
| Adipose fin length | 10.9 (1.39) | 10.87 (0.71) |
| Adipose fin base length | 6.13 (0.93) | 5.91 (0.66) |
| Postadipose distance | 17.87 (0.96) | 17.23 (0.81) |
| Outer maxillary barbel length | 22.6 (1.86) | 15.4 (4.1) |
| Outer mandibular barbel length | 20.45 (1.89) | 13.25 (2.19) |
| Inner mandibular barbel length | 16.03 (2.45) | 8.93 (1.94) |
| Nasal barbel length | 17.6 (1.57) | 8.57 (1.05) |
| % HL | | |
| Head depth | 57.15 (3.01) | 65.56 (3.58) |
| Head width | 72.67 (4.44) | 82.16 (2.71) |
| Eye diameter | 21.57 (0.95) | 24.86 (1.68) |
| Snout length | 25.22 (2.66) | 21.16 (2.26) |
| Interorbital length | 49.82 (2.05) | 43.33 (4.21) |
| Gape of mouth | 46.57 (2.81) | 43.43 (2.9) |

| Meristic | | |
|-----------|-------|-------|
| Dorsal fin rays | I 5-6 | I 5-6 |
| Pectoral fin rays | I 7-8 | I 7-8 |
| Ventral fin rays | i5 | i5 |
| Anal fin rays | iii22-23 | iii22-23 |
Model Test in MEGA 5 [29] suggested that models HKY+G (AIC = 2685, lnL = -1318, Gamma = 0.1273) and GTR + G (AIC = 5220.85, lnL = -2586.36, Gamma = 0.38292) explained the nucleotide patterns in the cox-1 and cyt-b gene sequences, respectively. Phylogenetic trees based on all four methods (ML, MP, ME, and NJ) showed similar tree topologies. A consensus phylogenetic tree (Figure 3) that compared known sequences of Horabagrus with the current collection, suggested that the specimens in our collection were closely related to H. brachysoma. This was further supported by the low genetic distances between the current specimens from the known H. brachysoma sequences (genetic distance, cox1: 0.006 ± 0.005, cyt-b: 0.013 ± 0.004) as compared to H. nigricollaris sequences (genetic distance, cox1: 0.041 ± 0.019, cyt-b: 0.024 ± 0.006). While the cox1 gene sequences showed little deviations from the known sequences (Figure 3(a)) the cyt-b gene sequences showed that the specimens in the current study formed a different cluster, which was supported with high bootstrap value (Figure 3(b)). However, based on the low values of branch lengths in ML (branch length 0.00495) as compared to the variation in the southern Indian populations of H. brachysoma (mean branch length 0.002942 with standard deviation 0.000949) indicates that the specimens in our collection are not phylogenetically drastically distinct from the known H. brachysoma. The fact that the current specimens show some phylogenetic deviation also vouch for the fact that the current population is not just a recent introduction from southern Western Ghats.

3.3. Morphometric Analysis and Length-Weight Relationship. Morphometry of female and male Horabagrus brachysoma is provided in Table 1. PCA revealed the sexual dimorphism in the female and male H. brachysoma (Figure 4(a)). Males and females were separated on the first PCA axis which explained 32% of the total variation in the data. As a percent of SL the first PCA axis had high positive correlations for eigenvectors related to length of different types of barbel, caudal peduncle depth, interorbital distance, and snout length and high negative correlations for head depth, head width, eye diameter, prepectoral fin length, and preanal fin length. Thus, as a percent of SL, males had longer barbels, deeper caudal peduncle, wider interorbital distance, and longer snout as compared to females. While, as a percent of SL, females had higher body depth, head width, head depth, eye diameter, prepectoral fin length, and preanal fin length. Prasad et al. [16] have also mentioned that the male and female have sexual dimorphism; however, to our knowledge there are no previous attempts to actually document the nature of this sexual dimorphism.

Power of the length-weight relationship (Figure 4(b)) of the collected specimens was 2.7716 (SE = 0.3968), and it was not significantly different from cubic value ($t = 0.5755$, df = 16, $P = 0.5729$). Anvar et al. [31] suggested that the power of length-weight ranged between 2.7623 and 3.17968 in the case of specimens collected from Chalakudy River in Kerala. Our report is within the same range. Anvar et al. [31] further suggested that the slope of males and females differed significantly. However, due to very small sample size for females we could not check the gender difference in the current study.

3.4. Behavioral Observation. Several stream habitats like runs, riffles, pools, and adjacent aquatic vegetation were scanned for presence of H. brachysoma. In first attempt of snorkeling during day time we failed to observe any active individual of H. brachysoma. During further extensive exploration in assistance with local fishermen we could see shoals of H. brachysoma refuge inside submerged roots of Pandanus vegetation along stream banks (Figures 5(a) and 5(b)). Shoals of individuals of H. brachysoma could be seen during snorkeling. Mature full grown individuals of H. brachysoma were not recorded in entire study. Shoals of H. brachysoma have been found to be inactive during day time. In the early down time (1730 h–1830 h) remarkable activity of hidden individuals of H. brachysoma was recorded. Emergence of these cat fishes began from dusk. After sunset all individuals started emerging in shoals and were seen searching for food. Approximately 30–50 individuals were recorded in single shoal. During night shoals of H. brachysoma were found to be occupying all niches present in streams for feeding and foraging. In night time individuals of H. brachysoma were mostly found to be feeding on crustaceans present in leaf litter. During early morning dives very few individuals were seen moving and hiding in Pandanus roots. Underwater observations well demarcate nocturnal foraging and feeding behavior of H. brachysoma. Our study also indicates that dense Pandanus vegetation across the stream banks is found to be excellent microhabitats for H. brachysoma to take

Figure 2: Horabagrus brachysoma collected from Bagayat stream in Gad River basin.
refuge in day time. As per local fishermen knowledge small streams like Bagayat in Gad River basin have abundant Pundanus vegetation and mostly dominated by subadult individuals of *H. brachysoma*, adults are mostly confined to large third order and main riverine flow of Gad River. Preference of Pundanus roots as a refuge microhabitat by subadult individuals of this threatened cat fish indicates that small rivulets like Bagayat are possibly breeding and nursery grounds of *H. brachysoma*. It also suggests that adults possibly track this secondary streams during breeding period in monsoon.

3.5. Threats and Conservation Measures. Various anthropogenic threats like overexploitation, habitat alteration, and pollution are already known to be major stressors for population decline of *H. brachysoma* which makes this species vulnerable [19]. It is also known that *H. brachysoma* is popular in international aquarium pet trade [32], which could be a potential threat to the species. Our personal observations and discussions with the local fisherman revealed that, locally called as “Ghag,” most of the extensive fishing of *H. brachysoma* is carried out during monsoon. Monsoonal trawling ban in marine water and delicacy of *H. brachysoma* are possible reasons for this extensive fishing of *Horabagrus* during this season. Given that monsoon during June and July is the breeding season of the species [33], extensive fishing in early monsoon may alter the population structure of *H. brachysoma* in Gad River basin. A seasonal ban on riverine fishery during early monsoon may safeguard the viable breeding population of *H. brachysoma*. Extensive exploitation of wild immature population of *H. brachysoma* for aquarium trade is one of the prominent

**Figure 3:** Phylogenetic position of *Horabagrus brachysoma* from Malvan compared to known cox1 (a) and cyt-b (b) gene sequences. Maximum likelihood trees, based on nucleotide substitution models given in the text, are shown in the following figures. Bootstrap values are provided for maximum likelihood/maximum parsimony/minimum evolution/neighbor joining methods run for 1000 iterations. GenBank accession numbers are provided after the species name. Mystus (family Bagridae) and Glyptothorax (family Sisoridae) are used as outgroups following Sullivan et al. [14]. Specimens in the present study are highlighted in red.
threats we have observed in the study area. Captive breeding programs for *H. brachysoma* may overcome the problem of high demand of this species in international aquarium trade.

Industrial pollution, urbanization, mining, and laterite quarrying are increasing anthropogenic stresses in Konkan region of Maharashtra. Extensive riparian deforestation by slash and burn (Figure 5(c)) for mango and cashew cultivation, use of excessive pesticides in farms and nearby mango plantation, heavy siltation, and laterite boulder quarrying (Figure 5(d)) are major threats we have observed in study area. Recently constructed large Mahamadwadi Dam on main river channel [34] may block seasonal upstream migration of fishes. Further studies on these aspects, however, are needed to support our claims. Nevertheless, site area protection is needed to overcome these threats and conserve the population of this threatened endemic species of the Western Ghats.
4. Conclusions

We have reported a new population of threatened and endemic catfish *Horabagrus brachysoma* extending the distribution of the species by about 180 km in the northern parts of the Western Ghats. We have confirmed the identity of the species using molecular methods. Along with new locality record and range extension study we have also discussed here some natural history and conservation aspects of *H. brachysoma*, which would be useful to set some conservation measure for this threatened and endemic catfish in Western Ghats. To our knowledge, in the current study, we have provided the detailed account of nature of sexual dimorphism and behavioral observations on *H. brachysoma* for the first time.

Acknowledgments

The authors thank Rajeev Raghavan, Anvar Ali, and Shrikant Jadhav for helpful discussions. They also thank members of Wild Explorers (WE), India, especially Abhijit Gharat and Harshal Rikame, for their unstinted help in the field. They are grateful to museum curators of Bombay Natural History Society, Mumbai; Zoological Survey of India, Western Regional Center, Akurdi, Pune; Wildlife Information Liaison Development, Zoo Outreach Organization, Coimbatore, India, for help in vouchering studied specimens. U. Katwate is thankful to the Deputy Director, Conservation, Bombay Natural History Society for constant encouragement. R. Raut is thankful to the Principal, Elphinston college, Mumbai for providing infrastructure facilities to carry out this work.

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