Vertebral monstrosities: Phenotypically shortened fish with deformed vertebrae in endemic fish genus Hypselobarbus (Bleeker 1860), (Teleostei: Cyprinidae) from Western Ghats, India

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Abstract

The vertebral deformity in four fish species of genus *Hypselobarbus* (Bleeker 1860), collected from three different river systems of the Western Ghats, biodiversity hotspot of India, are reported here. The radiographic images revealed reduced intra-vertebral space in comparison with the normal vertebrae. The phenotypic deformities have led to the deepening of the body with a more robust and reduced length. The deformed vertebrae were between 25 and 32. Slight genetic divergence of 1.1% between normal and deformed specimens in Mitochondrial cytochrome oxidase subunit 1 gene of *Hypselobarbus lithopidos* and *H. thomassi* and no divergence in *H. dobsoni*and *H. jerdoni* was observed. Several environmental and genetic factors might have influenced the development of these robust short-bodied phenotypes in these rivers and possess slight genetic divergence from normal specimens. The specimens were collected from areas with high anthropogenic stresses, abate water quality, and habitat, which could also be a possible reason. However, these deformities may also be the result of the stress during embryonic and early life stages.

Introduction

Monstrosities in fishes have been of great curiosity for the ichthyologists since the early days, as indicated by the description of fishes with monstrosities since the 16th century (Aldrovandi 1613). The records of monstrosities have been already published as bibliography of anomalies in fishes (Dawson 1964, 1966, 1971; Dawson and Heal 1976). In the beginning, Darwin emphasized on distinction between morphological anomalies and typical variations, but evolutionary biologists stressed more on morphological defects that are genetically based and may be influenced by natural selection as potential novelties (Darwin 1875). Many abnormalities appear to be addressed as monstrosities, specifically when the vertebral column is shortened without visible curvature, resulting in an odd, deepened short body (Golubtsov et al. 2021). Numerous physiological, environmental, xenobiotic, dietary, and genetic factors influence skeletal malformations, which have been reported in various publications (B. E. Bengtsson 1975; Gjerde et al. 2005; Madsen et al. 2001; Slooff 1982; Toften and Jobling 1996).

Further, most morphological abnormalities develop during the embryonic and post-embryonic phases due to unclear causes or poorly understood mechanisms (Houde 1972). The incidence of malformed specimens is minimal in natural aquatic environments, unlike cultured systems where deformities are quite common (Dahlberg 1970; Daoulas et al. 1991). With the reports of declining water quality already in existence, numerous research has identified deformity as the biological variable indicating contamination in natural waterbodies (B. -e. Bengtsson et al. 1997)

Fishes have dispersed all over the planet and shown an unmatched diversity in their appearance, habitat, physiology, and behaviour since their origin (Nelson et al. 2016). More than half of the known vertebrate species are represented by the 36,484 recognized species of ray-finned fish in the world (Fricke et al. 2023). In India, freshwater fishes from Western Ghats biodiversity hotspot comprise 320 species representing 11 orders, 35 families, and 115 genera (Bijukumar and Raghavan 2015; Dahanukar and Raghavan 2013). The important barbs, belonging to the genus *Hypselobarbus* (Bleeker 1860) (Cyprinidae, Teleostei; Cypriniformes) (Tan and Armbruster 2018), are native of rivers of the Western Ghats and peninsular India (Ali et al. 2013; Arunachalam et al. 2012; J. D. M. Knight et al. 2013). The genus includes 13 valid species, *Hypselobarbus bicolor* being the last species described (J. Knight et al. 2016). These fishes vary between 25 and 100cm in total length. Preliminary investigation of body shape through visual examination revealed extreme body deformity in individuals of four species of *Hypselobarbus* collected from three different rivers Tungabhadra, Netravathi and Periyar of Western Ghats. Present study aimed at a comparison of body depth and other morphometric measurements with normal and deformed specimens, radiographic investigation of the vertebral column in normal and deformed specimens, and genetic comparison of Mitochondrial Cytochrome oxidase subunit 1 for species confirmation as well as intraspecific variation between normal and deformed specimens.

Methodologies followed

Sampling site

Naturally, rivers of peninsular India are separated into west-flowing and east-flowing (Sharma et al. 2022). The east-flowing rivers are long and among the most tamed rivers that empty into the Bay of Bengal, whereas the west-flowing rivers are shorter and empty into the Arabian Sea (Lal 2001). For the present study, fishes of genus *Hypselobarbus* were collected during the pre-monsoon and post-monsoon seasons, during 2021 to 2023, from rivers Tungabhadra, originating at Gangamoola Peak in the Khudremuk range of Karnataka, Central Western Ghats (Siddaramu et al. 2009), river Netravathi, also originating at Gangamoola Peak (Gayathri et al. 2021), and river Periyar, the largest river system in southern Western Ghats, originating in Sivagiri hills in Kerala (Anjusha et al. 2020).

The fishes were collected from fishers' commercial landings used gill nets of varying mesh sizes ranging from 40 mm to 100 mm. We collected deformed and normal specimens of *H. dobsoni* from 13.843518 N, 75.697331 E in Bhadravathi and, 13.944766 N, 75.627058 E in Shivamogga, *H. jerdoni* and *H. lithopidos* from 12.873944, 75.006234 near B.C. Road, *H. thomassi* from 12.865592, 74.908957 near Mangaluru and 10.116735, 76.417786 near Aluva in Kerala. (Fig. 1) (Supp_Table. 1).

Morphology, Radiographic imaging, photographs, tissue collection, and deposition of specimens

Species were identified using the original descriptions and available literature (Day 1874, 1876, 1888; Menon and Remadevi 1995; SYKES 1839). A total of 23 morphometric characters were measured using a digital Vernier calliper with 0.1 mm accuracy and eight meristic were counted (Hubbs et al. 2004). The X-ray images of both normal and deformed specimens were captured, followed by the vertebral counts (Golubtsov et al. 2021; Naseka 1996). The tissue samples of the specimens were stored in 100% ethanol. After a day, the alcohol was discarded and filled with fresh ethanol. The fish specimens are stored in 8% buffer formalin solution and deposited at the Aquatic Biodiversity Museum and Repository ICAR-CIFE, Mumbai.

DNA isolation and PCR amplification

Genomic DNA was isolated from 20mg of muscle tissue stored in absolute ethanol from all four deformed samples and normal representative samples using the organic extraction method (Phenol-chloroform method) (Taggart et al. 1992). The mitochondrial partial cytochrome c oxidase subunit 1 (650 bp) gene was amplified as described in (Ward et al. 2005), using the primer FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'. PCR was performed in the 25 µl reaction volume containing 100 ng of template DNA. 10 pmol of primer, 2x master mix (Promega) scaled up and performed in 75µl. The thermocycler was set for initial denaturation at 95°C for 4 minutes, followed by 35 cycles at 94°C for 1 minute for denaturation, 54°C for 45 seconds for annealing, and 72°C for 1 minute for extension, and final extension at 72°C for 7 minutes.

The PCR products were visualized on 2% agarose gel, and the amplicons were purified using a PCR purification kit (MinElute PCR Purification Kit) following the manufacturer's protocol. The purified products were sequenced in both directions using the PCR primers from Eurofins Pvt Ltd., Bangalore, India.

Sequence Analysis

The sequence quality was assessed by estimating the Phred score of each base using Finch TV software (version 1.4.0) (Geospiza 2009). The sequences were aligned using MEGA (version 11.0): Molecular Evolutionary Genetics Analysis version 11 (Tamura et al. 2021) and were subjected to similarity analysis with the NCBI database using the BLAST tool. Sequence divergence values within the normal and deformed specimens were calculated using Kimura two Parameter (K_2P) distance model (Kimura 1980) implemented in MEGA (version 11.0), with 100 bootstrap replications. Neighbour-joining trees of K_2P distances were generated to know the divergence pattern between normal and deformed specimens (Saitou and Nei 1987).

Statistical Analysis

All analyses were performed using R- studio (R Core Team 2022). The descriptive statistics for the normal specimens of all four species were derived using the library *summarytool* (Comtois 2018). All 23 morphometric measurements (in millimeters) were size corrected using Paleontological Statistics Software Package for Education and Data Analysis PAST 4.13 (Hammer et al. 2001) for three species, except for *H. lithopidos* as the number of total specimen were not sufficient (n = 3). Univariate ANOVA was performed for all the measurements (Supp_Table. 2), and the variables with p-value < 0.05 were considered for further analysis. Principal Component Analysis (PCA) was performed as a data reduction technique to determine the variables responsible for the variation. The normal and deformed vertebrae and the total number of vertebrae (Supp_Table. 3), are represented in stacked bar graph (Supp_Fig. 2). All visualizations were done using ggplot2 (Wickham and Wickham 2016).

Results

Short definition:

The deformed specimens were found to have relatively higher body depth, head depth, and shorter body profile than other normal specimens (Fig. 2) (Supp_Table. 5). The radiographic image (Fig. 3) showed an inverse proportionate number of deformed vertebrae with shortness of the specimen. The normal specimens *H. dobsoni* have 35 vertebrae, *H. jerdoni* and *H. thomassi* have 34 vertebrae and *H. lithopidos* have 36 number of vertebrae. (Supp_Table. 3).

Morphometric differences in Normal and deformed specimens

***Hypselobarbus dobsoni* (Day, 1876):** Normal specimens (Fig 2A) had an average proportion of 36.5%, Body Depth (BD) to Standard Length (SL), while the deformed specimens (Fig 2B) had 50.5%; Head Depth (HD) to SL proportion was 17% in normal specimens and 22.5% in the deformed specimens. Caudal Peduncle Depth (CPD) to SL proportion was 13.5% in normal specimens and 17% in deformed specimens; Intra Narial Width (INW) to proportionate Head Length (HL) in normal specimens was 30%, and 36% in the deformed specimens.

***Hypselobarbus jerdoni* (Day, 1870):** *Normal* specimens (Fig 2C) had an average BD to SL proportion of 34.5%, while the deformed specimen (Fig 2D) had 53%; HD to SL proportion was 18% in normal specimens and 26% in the deformed specimen; CPD to SL proportion was 14% in normal specimens and 18% in the deformed specimen. INW to proportionate HL in normal specimens was 29%, and 34% in a deformed specimen.

***Hypselobarbus lithopidos* (Day, 1874):** Normal specimens (Fig 2G) had an average BD to SL proportion of 31%, while the deformed specimen (Fig 2H) had 42%; HD to SL proportion was 15.5% in normal specimens and 21% in the deformed specimen; CPD to SL proportion was 12% in normal specimens and 15% in the deformed specimen. INW to proportionate HL in normal specimens was 26%, and 28% in a deformed specimen.

***Hypselobarbus thomassi* (Day, 1874):** Normal specimens (Fig 2E) had an average BD to SL proportion of 31%, while the deformed specimens (Fig 2F) had 43.5%; HD to SL proportion was 16.5% in normal specimens and 22% in deformed specimens; CPD to SL proportion was 12.5% in normal specimens and 18% in deformed specimens. INW to proportionate HL in normal specimens was 25%, and 42% in deformed specimens.

The descriptive statistics of 23 morphometric variables given for normal specimens of all four species with mean, standard deviation, and range are presented (Table 1). PCA is one of the best multivariate analytical techniques which can be used to reduce morphometric data and extract the independent or explanatory variables significant for variation. The independent variables, showing significance ($p<0.05$) after univariate ANOVA (Supp_Tables 2), were subjected to Principal Component Analysis (PCA). The PCA factor loadings for each species (*H. dobsoni*, *H. jerdoni*, and *H. thomassi)* including proportion of variance and cumulative proportion of each PCA for three species shows each of the variables contributing to the PC loadings (Supp_Table 4). In PCA biplot (Fig 4), shows PC1 contribution of 77.17%, and PC2 18.84% of cumulative proportions in the case of *H. dobsoni*. The case of PCA biplot (Fig 5) of *H. jerdoni,* PC1 shows 84.23% of cumulative proportions and 9.27% in PC2. PCA biplot of *H. thomassi* (Fig 6), shows had 71.83% in PC1 of the loadings, and PC2 9.74% of the loadings.

Table 1. Descriptive statistics of normal specimens of all four species of *Hypselobarbus*

Species	*Hypselobarbus dobsoni*		*Hypselobarbus jerdoni*		*Hypselobarbus lithopidos*		*Hypselobarbus thomassi*	
variables	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
ABL	20.16 ± 5.01	9.78-26	11.37 ± 1.33	10.3-13.81	11.89 ± 0.31	11.67-12.11	14.8 ± 3.92	10.11-21.64
BD	76.03 ± 17.87	39.83-96.51	45.72 ± 6.66	38.66-57.19	47.27 ± 1.36	46.31-48.24	56.49 ± 13.62	36.87-77.62
BW	39.28 ± 11.37	20.6-51.67	23.7 ± 2.46	20.03-27.53	22.94 ± 1.23	22.07-23.81	28.97 ± 9.59	16.54-43.84
CPD	28.06 ± 6.27	16.32-37.01	18.79 ± 1.91	15.15-21.66	18.13 ± 0.07	18.08-18.19	23 ± 5.88	14.87-33.01
CPL	19.67 ± 3.64	15.45-25.94	11.4 ± 1.77	9.63-14.45	13.8 ± 0.24	13.63-13.98	22.77 ± 10.33	10.76-38.03
DBL	33.6 ± 8.27	16.1-42.57	22.81 ± 2.51	19.82-27.31	24.51 ± 0.89	23.88-25.14	26.36 ± 6.63	18.41-38.48
DHL	91.26 ± 26.6	40.49-119.35	52.85 ± 6.83	40.5-62.63	57.28 ± 1.68	56.09-58.48	68.4 ± 19.16	38.76-93.41
HD	34.76 ± 6.29	22.94-42.8	23.47 ± 2.01	21.85-27.47	23.53 ± 1.06	22.78-24.29	30.38 ± 8.13	17.98-43.06
HL	41.53 ± 8.34	24.18-50.22	28.16 ± 1.59	24.12-30.18	28.26 ± 1.03	27.53-28.99	39.46 ± 10.26	20.02-55.1
HW	28.73 ± 5.34	18.02-34.73	18.6 ± 1.52	15.75-20.77	17.34 ± 0.43	17.04-17.65	23.15 ± 6.06	14.87-31.37
INW	12.65 ± 2.65	7.2-15.59	8.11 ± 0.89	7.09-9.77	7.47 ± 0.19	7.34-7.61	9.94 ± 3.26	5.23-15.09
IOW	25.44 ± 5.07	14.51-29.36	16.3 ± 1.42	14.11-18.52	15.23 ± 0.51	14.87-15.6	20.8 ± 5.71	14.02-29.43
LMB	8.74 ± 3.08	4.11-13.35	10.3 ± 1.36	7.55-11.89	5.63 ± 0.35	5.38-5.88	8.19 ± 1.84	5.89-11.05
LRB	13.67 ± 5.74	6.96-23.73	12.63 ± 1.39	9.59-14.22	6.21 ± 0.02	6.19-6.23	8.5 ± 1.72	6.16-11.45
OD	11.55 ± 1.46	8.39-12.49	8.79 ± 0.75	7.56-10.05	7.34 ± 0.25	7.16-7.52	11.39 ± 3.28	7.34-16.69
PAL	160.16 ± 31.64	99.75-202.25	105.59 ± 7.74	91.16-117.03	112.98 ± 3.78	110.31-115.66	136.16 ± 36.08	82.43-199.27
PDL	95.37 ± 22.33	57.64-120.49	58.51 ± 5.08	50.39-67.63	68.01 ± 2.3	66.38-69.64	85.71 ± 28.12	42.78-126.07
PeBL	10.04 ± 3.16	4.09-13.35	6.95 ± 0.79	5.88-8.42	6.63 ± 0.05	6.59-6.67	8.59 ± 2.2	5.76-11.99
PPeL	45.21 ± 9.05	26.2-54.63	30.81 ± 1.85	26.05-33.71	31.21 ± 1.43	30.2-32.23	40.74 ± 11.14	20.12-57.43
PPvL	105.95 ±	64.58-	70.11 ±	60.52-	75.09 ±	73.81-	93.61 ±	55.6-

	20.56	129.92	4.58	77.48	1.81	76.38	24.51	134.15
PvBL	11.65 ± 3.65	5.12-15.57	7.56 ± 0.7	6.04-8.58	7.88 ± 0.16	7.76-8	8.22 ± 1.71	5.03-10.69
SL	207.58 ± 45.76	118.86-262.77	131.83 ± 12.61	113.03-152.82	151.39 ± 4.14	148.46-154.32	182.21 ± 46.88	122.62-257.66
SnL	12.3 ± 2.63	6.99-14.92	6.42 ± 0.55	5.55-7.39	6.44 ± 0.56	6.04-6.84	11.64 ± 4.91	5.49-19.43

Genetic Divergence and neighbor-joining tree

The K_2P distance estimated for deformed individuals and the normal specimens (Table 2), shows the conspecific divergence with a maximum in *H. thomassi* (1.17%), as fewer haplotypes were found in the sequences. The average congeneric divergence was 5.75%, 15 folds more than the average conspecific divergence value. The results of neighbor-joining tree (Fig 7) also showed four different clusters for all four species with bootstrap value coverage of a minimum of 99%. With the molecular sequencing data, we confirm all the deformed specimens were identified in the correct taxon (accession numbers: Supp_Table. 7).

Table 2. Genetic divergence table converted to percentage variation between normal and deformed specimen

Specimen no.	Deformed specimens	Normal specimen	Genetic divergence value (%)
1.	*Hypselobarbus dobsoni* deformed1	*Hypselobarbus dobsoni*	0.0
2.	*Hypselobarbus dobsoni* deformed2	*Hypselobarbus dobsoni*	0.0
3.	*Hypselobarbus dobsoni* deformed3	*Hypselobarbus dobsoni*	0.0
4.	*Hypselobarbus jerdoni* deformed1	*Hypselobarbus jerdoni*	0.0
5.	*Hypselobarbus lithopidos* deformed1	*Hypselobarbus lithopidos*	1.13
6.	*Hypselobarbus thomassi* deformed1	*Hypselobarbus thomassi*	1.17
7.	*Hypselobarbus thomassi* deformed2	*Hypselobarbus thomassi*	1.11

Discussion

Short and deep-bodied morphotypes are unnoticed in any of the cyprinids group from the rivers of Western Ghats. In the present study, deep-bodied phenotypic specimens with shortened inter-vertebral space are reported, the only report of such deformities in *Hypselobarbus* and the second in cyprinids. The other report on cyprinids is of a species of the genus *Labeobarbus* from Africa (Golubtsov et al. 2021). The other reports include spinal compression in *Gadus morhua* (Atlantic cod) from German wadden sea, where the seasonal prevalence and the rate of occurrence were studied (Hilger 1992; Moller 1983; Wunder 1971). In our study, we also found a slight genetic divergence of 1.13% in *H. lithopidos* and 1.17% in *H. thomassi* and no genetic divergence in *H. dobsoni* and *H. jerdoni* between normal and deformed specimens, which is insufficient to prove them as different species.

Possible factors for shortened phenotypic deformities

To the best of our knowledge, no records are available on the factors leading to such phenotypic short-bodied forms from any wild fish population. Still, several studies have been reported on farmed salmonids (Gjerde et al. 2005; Kvellestad et al. 2000; McKay and Gjerde 1986; Vagsholm and Djupvik 1998; Witten et al. 2009). Those studies also could not conclude the exact cause and explained as multiple factors viz, parasitic and bacterial infections, deficiency of micro and macronutrients, vaccination, higher temperatures during the early embryonic stage, fluctuation in photoperiod, fluctuation in water quality, and water current and environmental pollutions (Witten et al. 2009).

Possible effect of Predator-driven phenotypic plasticity

Extreme climatic events like floods during the monsoon season are very common in Western Ghats on account of heavy rainfall (Vijaykumar et al. 2021). These events caused the escape of exotic fish species under the culture system into the wild and are currently found in almost all the freshwater ecosystems of Western Ghats (Raj, Kumar, et al. 2021). Non-native species are being reported from the lakes, rivers, and reservoirs of Western Ghats (Bijukumar 2019). Reservoirs of Western Ghats have greatly been focused on stocking non-native species for capture-based culture fisheries, which became the source for exotic species to spread all over the river's catchment (Sugunan 1995). The alien fish species have outcompeted and established successfully (Raj, Prakash, et al. 2021). The accidental or deliberate introduction of exotics increase the predation stress along with the native predators particularly in the early life stages (De Leaniz et al. 2010). It is already known that smaller sized individuals have more pressure due to predation during their fast-growing phase (Sogard 1997). The predator-driven environment tends to have greater body depth (Eklov and Svanback n.d.) and also tends to diverge genetically from the normal ecosystems (Ingley et al. 2014), which is true in some species of *Brachyrhaphis* fishes. Similarly, the presence of invasive predatory fish species, such as African catfish, in huge numbers from rivers of western ghats (Pillai et al. n.d.; Raghavan et al. 2016; Raj, Prakash, et al. 2021; Ranjan 2018; Roshni et al. 2020; Sreenivasan et al. 2021), may be the reason for the occurrence of short-bodied phenotypic specimens.

Environment-driven phenotypic plasticity

Humans pose the world's greatest evolutionary force, altering global ecology and evolutionary trajectories and dramatically accelerating mutation in species associated with particular ecosystems (Palumbi 2001). There have been ample reports on phenotypic plasticity from anthropogenically altered riverine habitats and significant divergence in the body shape of fishes residing in reservoirs and streams (Brinsmead and Fox 2002; Franssen, Stewart, et al. 2013). Few fishes which occupy the reservoir have shown relatively deeper bodies and smaller heads compared to their riverine counterparts, and few others showed deeper bodies in the riverine habitat, which concludes that phenotypical evolutionary traits are purely species-specific (Franssen, Harris, et al. 2013; Haas et al. 2010). Environmental factors affect phenotypes as a single parameter or an interactive environmental variable with two or more combined parameters, such as dissolved oxygen and water flow (Langerhans et al. 2007). Alteration in riverine habitat possesses a novel selection pressure on the native fish fauna by altering body shape, evident through genetic-based morphometric plasticity in reservoir and stream fishes (Franssen 2011). The stress due to pollution or any anomalies in abiotic environmental parameters could also influence the eggs and larvae to get deformed body shapes while they grow out. During peak summer, Netravathi river dries up completely. The dammed segments show the least fish species richness due to low or no flow, as a result of small hydropower projects along the river at various places. In addition to that, waters have been characterized by elevated temperature and reduced dissolved oxygen (Jumani et al. 2018). The surface sediment and water are found to have a high load of heavy metal pollutants like lead, which may severely affect metabolic activities in riverine biota and community structures (Gayathri et al. 2021). All three species, except *H. dobsoni*, collected from Netravathi river, are in the catchment of Tumbe small hydropower project may have led to such phenotypic deformities. The location from where the specimens of *H. dobsoni* were collected is reported to have comparatively low dissolved oxygen and high biological oxygen demand as the river stretch is packed with several large-scale industries with a high inflow of effluents at Bhadravathi in Bhadra river (Shahnawaz et al. 2010). Similarly, Periyar river has about 15 impoundments that have affected the hydro flow regime, and the water quality index is reported to be poor, along with high loads of heavy metal pollutants (Abdu Rahiman et al. 2009; Mohan et al. 2019; Rajappan and Joseph 2017). All these natural and anthropogenic stresses might lead to loss of ichthyofaunal diversity of river ecosystem. Hence, it is very important to know the exact causes, as most of the species of the genus are under threatened category of IUCN. Thus, for conservation of the species, these critical riverine habitats of Western Ghats of India must be protected from anthropogenic activities.

Conclusion

The appearance of phenotypes with vertebral deformity as a result of reduced inter-vertebral space led to the excessive deepening of the body. This kind of deformity is rare in wild fish populations. There are no reports of this kind of monstrosity in any fish from Western Ghats. The presence of robust deep bodied phenotypes in four species from three different river systems of Western Ghats in a single genus is inquisitive. The slight genetic divergence in the least mutating gene in two species has made us think it is as an evolutionary trait induced anthropogenically or by natural selection. And we also observed the occurrence of these deformed phenotypes within the normal phenotypic fish population. As the number of specimens is low, we could not confirm it as an evolutionary trait. So, with no evidence on the evolutionary aspect, currently, we consider these occurrences as deformities caused due to several unknown anthropogenic or natural stresses.

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics statement

No approval of research ethics committees was required for this study as the fish samples were collected from the commercial catches of the fishers.

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Figures

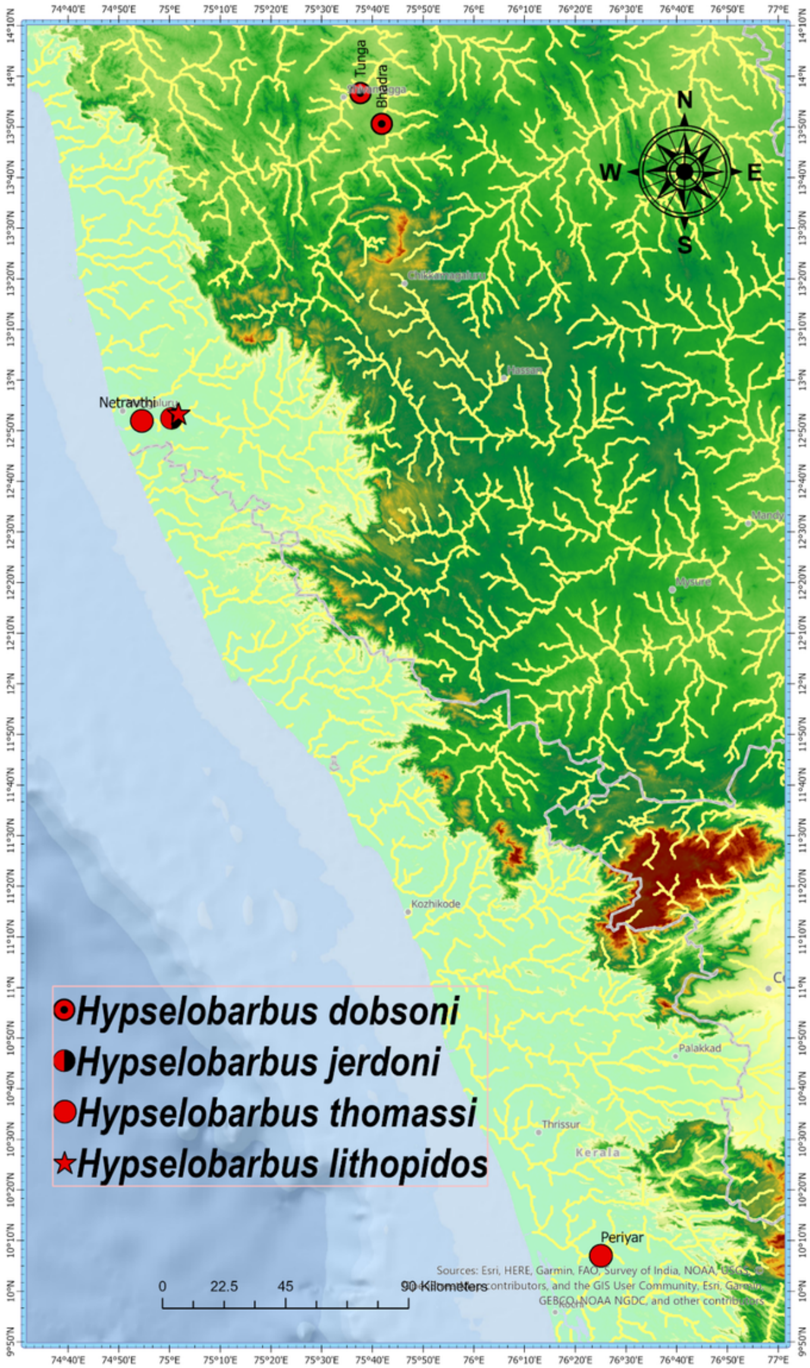

Figure 1

Map of sampling sites from Tungabhadra River, Netravathi River, and Periyar River of Western Ghats, the occurrence of deformed specimens of four species are given with different shapes.

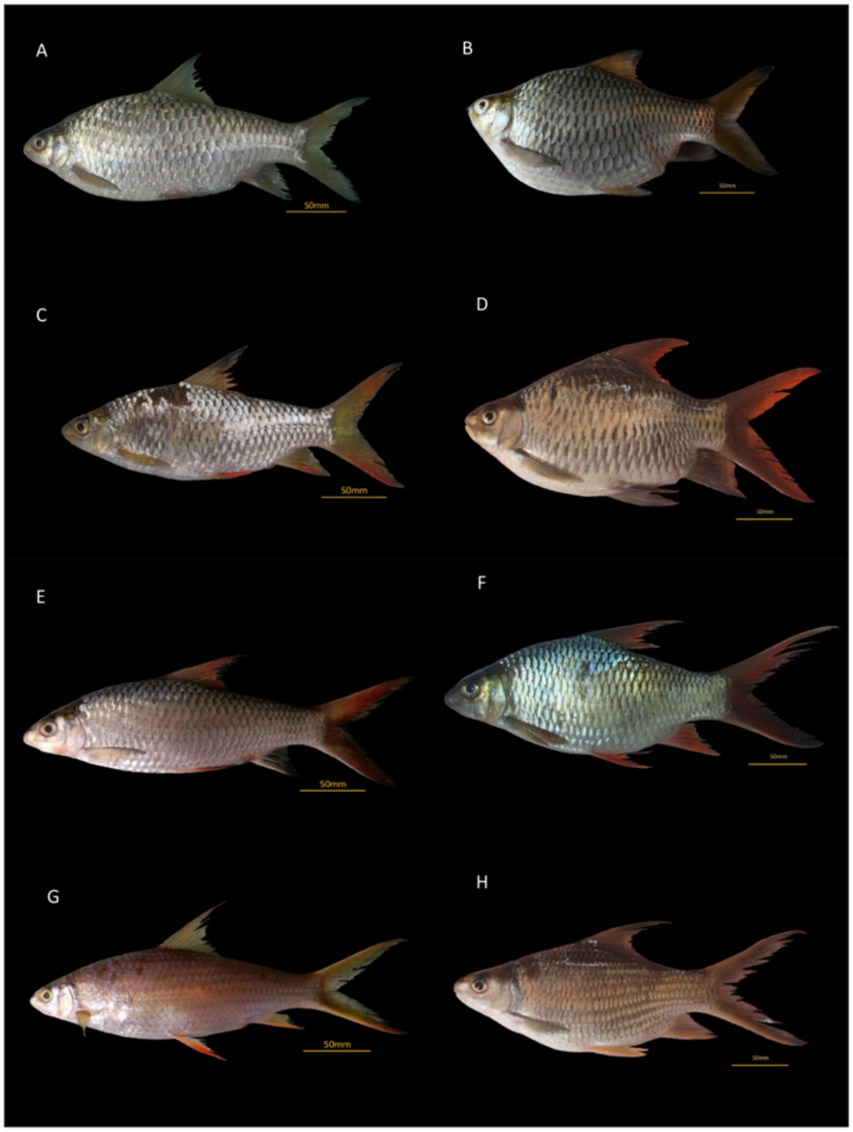

Figure 2

External appearance of Normal (Left) and deformed (Right) specimens. A: Normal specimen of *H. dobsoni*, B: short, deformed specimen of *H. dobsoni*, C: Normal specimen of *H. jerdoni*, D: short, deformed specimen of *H. jerdoni*, E: Normal specimen of *H. thomassi*, F: short, deformed specimen of *H. thomassi,* G: Normal specimen of *H. lithopidos*, H: short, deformed specimen of *H. lithopidos*

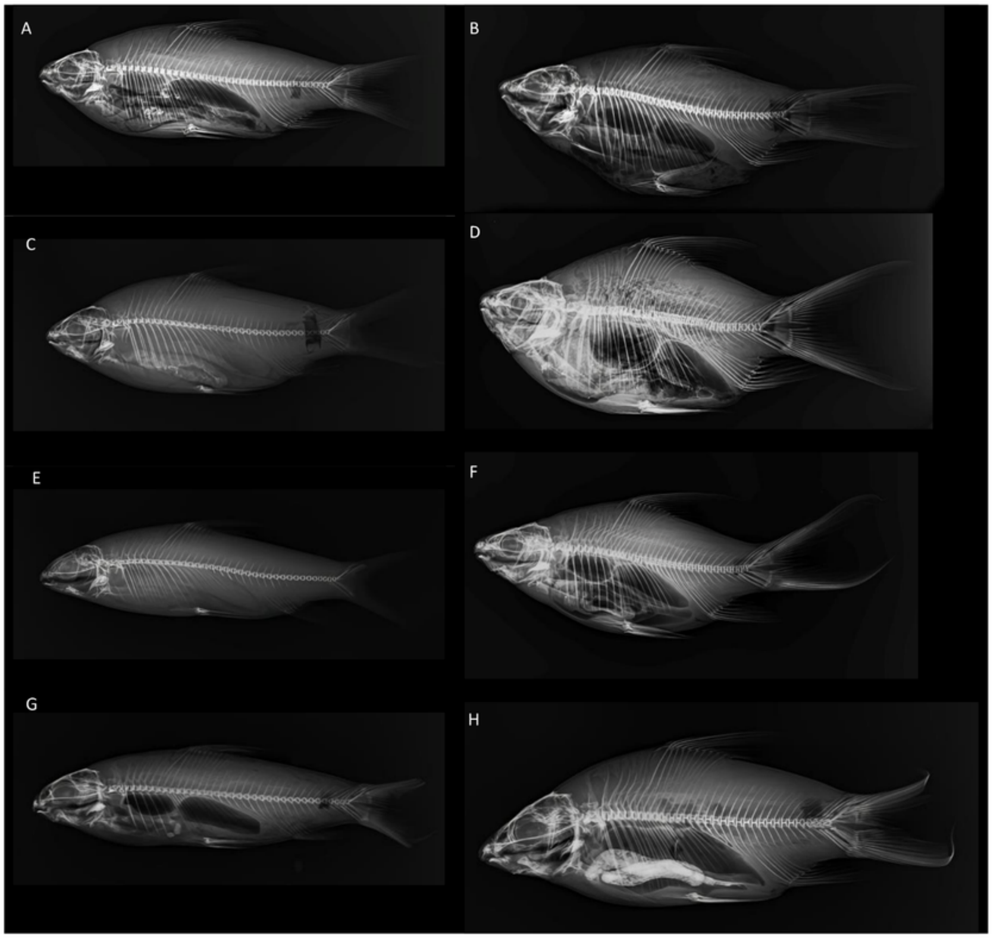

Figure 3

Radiographic images of Normal (Left) and deformed (Right) specimens. A: Normal specimen of *H. dobsoni*, B: short, deformed specimen of *H. dobsoni*, C: Normal specimen of *H. jerdoni*, D: short, deformed specimen of *H. jerdoni*, E: Normal specimen of *H. thomassi*, F: short, deformed specimen of *H. thomassi,* G: Normal specimen of *H. lithopidos*, H: short, deformed specimen of *H. lithopidos*

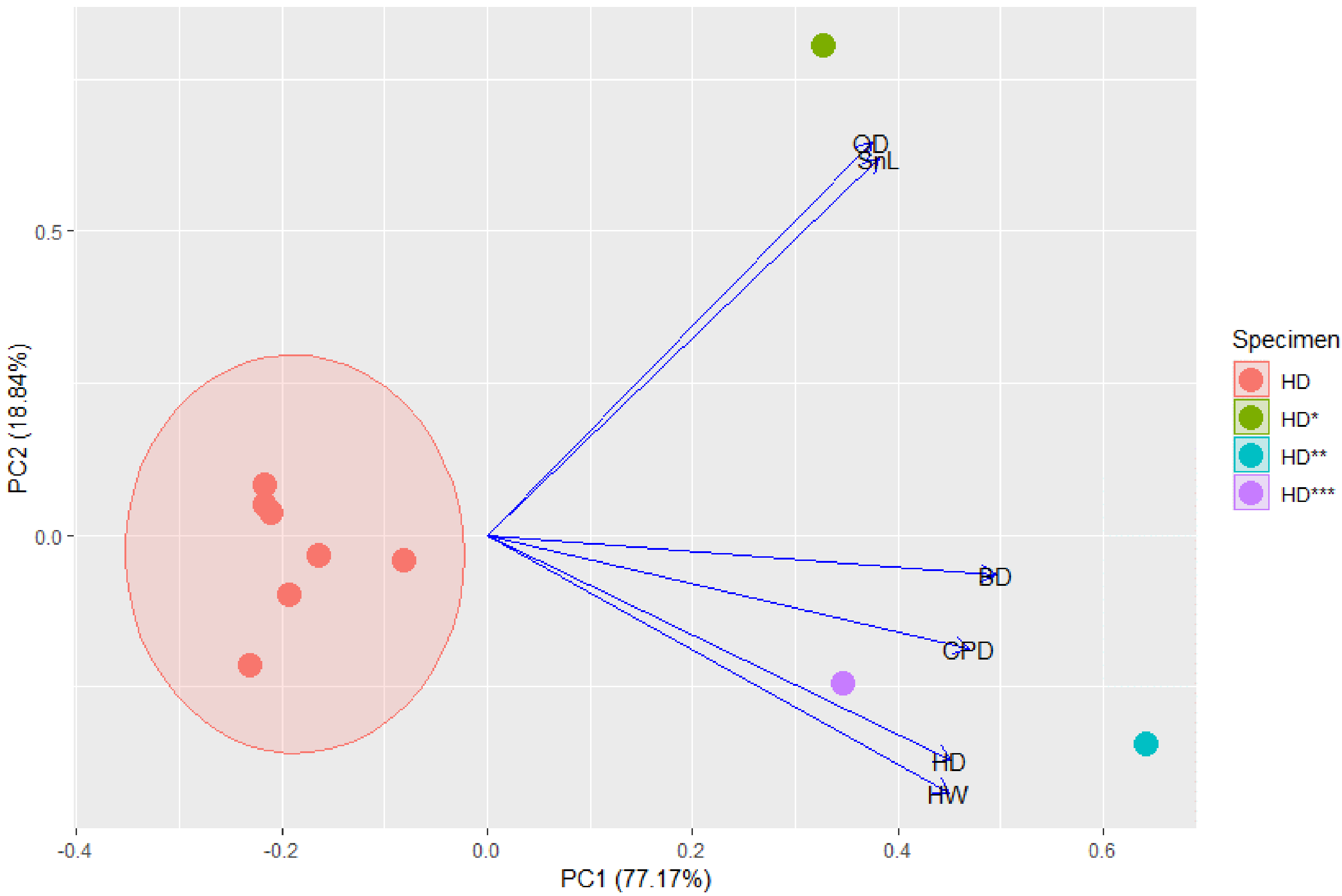

Figure 4

PCA plot distinguishing normal and deformed specimens of *H. dobsoni*

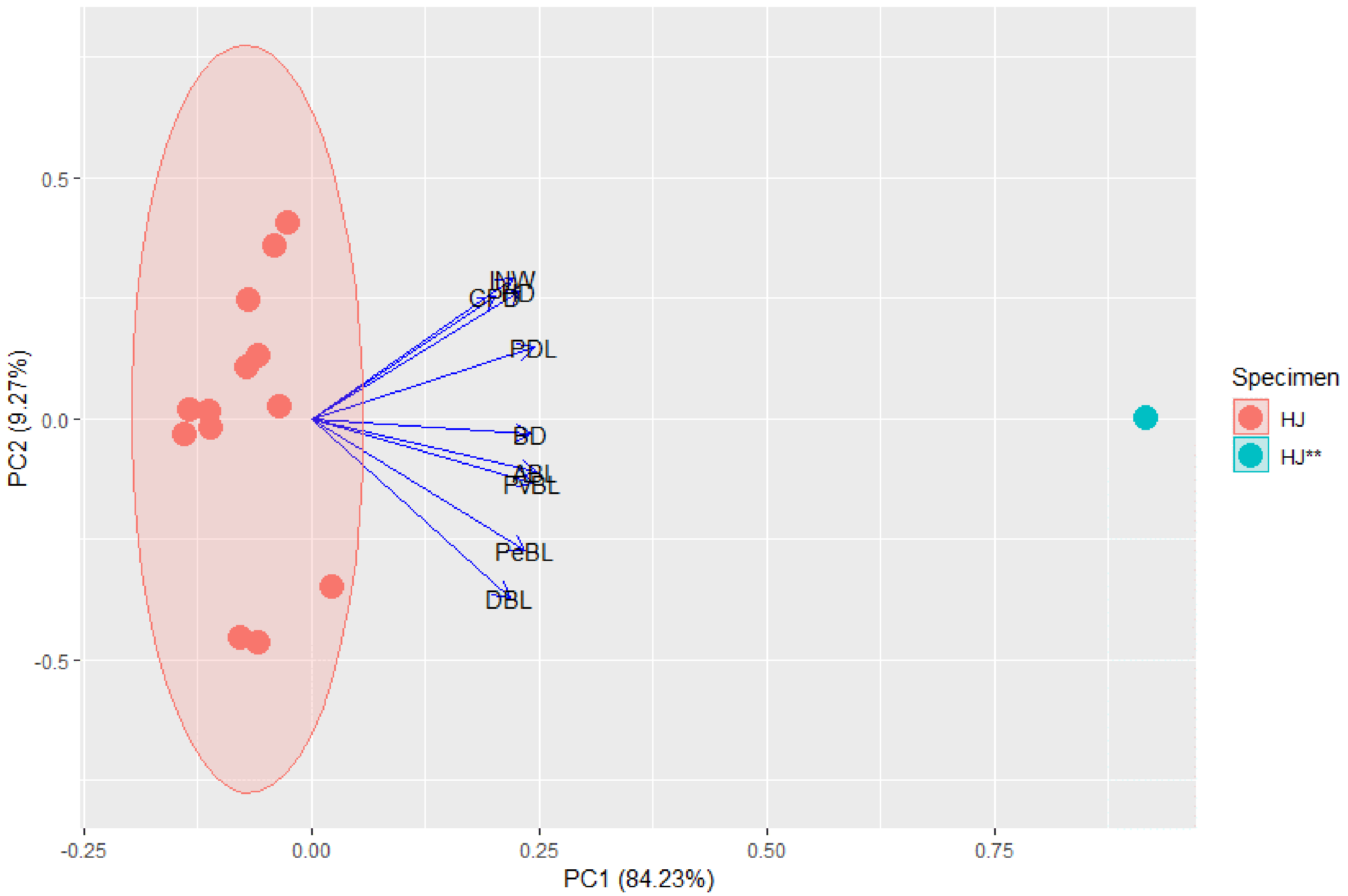

Figure 5

PCA plot distinguishing normal and deformed specimens of *H. jerdoni*

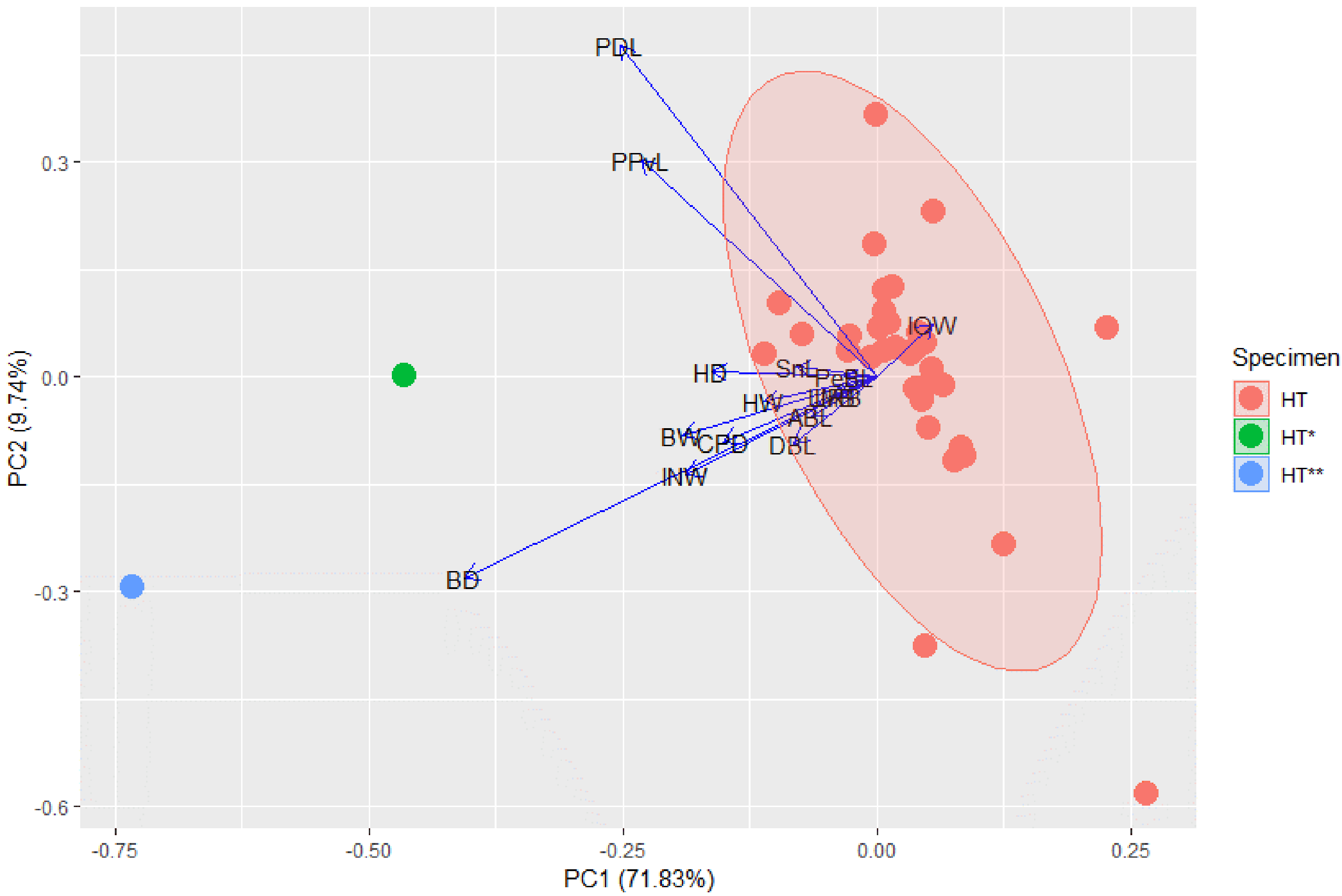

Figure 6

PCA plot distinguishing normal and deformed specimens of *H. thomassi*

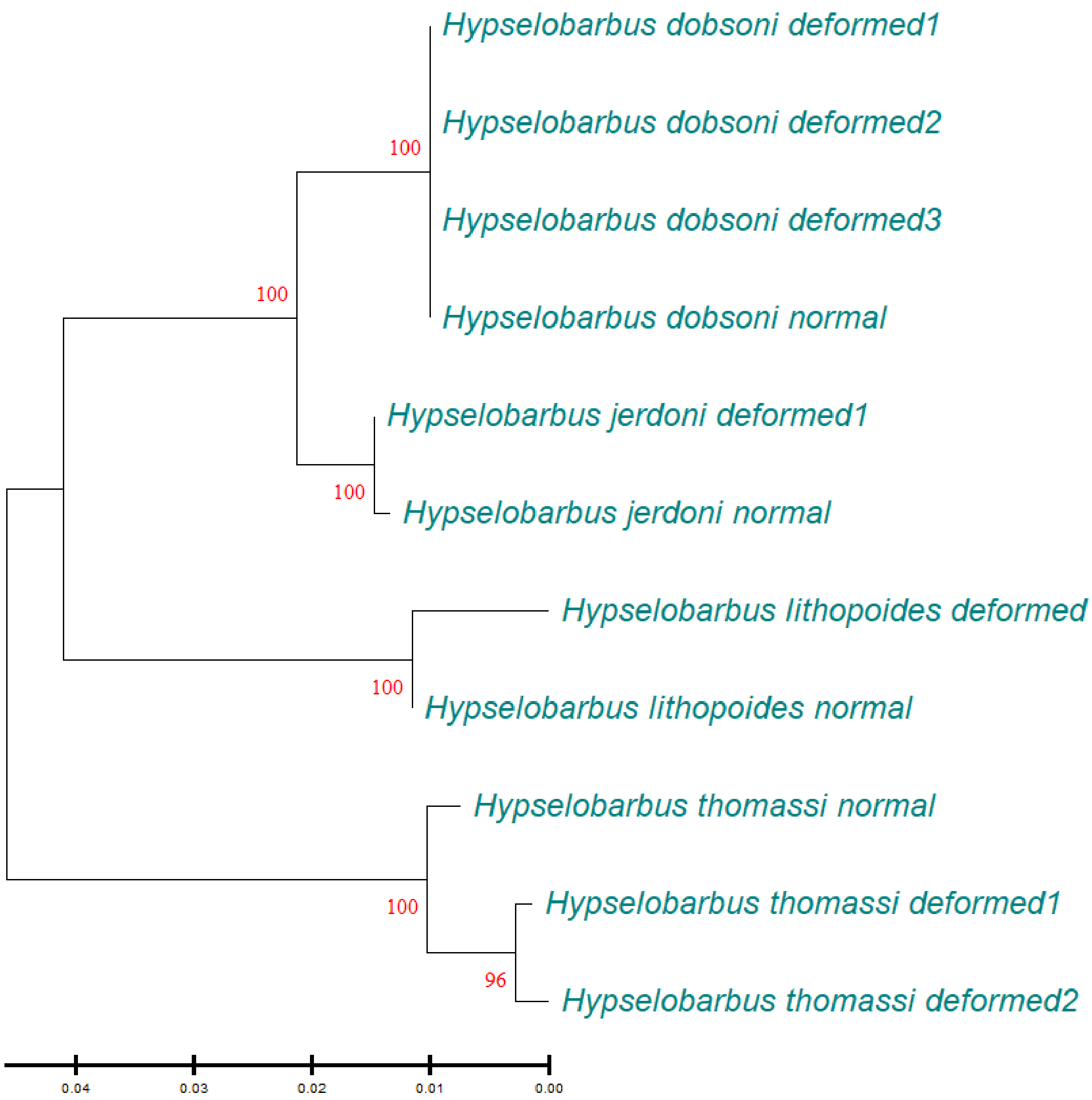

Figure 7

Neighbor-joining tree based on COI sequences using K2P distances for normal and deformed specimens of genus *Hypselobarbus*

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