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Research Article

RADIOGRAPHIC AND GENETIC CHARACTERIZATION OF PHENOTYPICALLY DEFORMED TELEOST FROM GOMTI RIVER, UTTAR PRADESH, INDIA

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ABSTRACT: In the present study, we recorded two morphologically abnormal wild fishes from the Gomti river, Uttar Pradesh. Deformed *Puntius chola* (Hamilton, 1822) and *Heteropneustes fossilis* (Bloch, 1794) were observed in an experimental catch of the Gomti river at Lucknow and collected along with normal individuals of the same species for further investigation. Externally, the caudal region of fishes was completely deformed and fused. Abnormal and normal specimens of both species were identified using integrative taxonomy (traditional taxonomy and DNA barcoding). We created molecular signatures of each species using a partial sequence of the mitochondrial gene Cytochrome C oxidase subunit I (COI). Radiography is a non-lethal and non-invasive approach that can provide useful information about skeleton abnormalities in fishes. The radiographic images revealed different vertebral deformations like lordosis, kyphosis, scoliosis, fusion, and compression compared to the normal vertebrae. The total vertebrae were 30-34 and 52-58 in *P. chola* and *H. fossilis* respectively. Different environmental and genetic factors could influence the development of these abnormal short-bodied phenotypes in the fish population of this river. High anthropogenic stresses, deficiency of vitamin C and phosphorous, and poor quality of water and habitat could be associated with skeletal anomalies in collected individuals. However, stress throughout the embryonic and early life stages may potentially be the cause of these spinal abnormalities. This type of abnormality is very rare in natural ecosystems. This is the first report of such a monstrosity in the ichthyofauna of the Gomti river, Uttar Pradesh. Our research findings contribute to understanding the health and environmental conditions of the Gomti river and may have implications for conservation efforts and management.

Keywords: Gomti river, Integrative taxonomy, Radiography, DNA barcoding, Anthropogenic.

INTRODUCTION

Malformations of the vertebral column occur in several teleost groups [1] as well as in other taxa. In fish, various morphological deformities can occur, including dysplasia in the opercular bones, deformities in the maxillary and mandibular regions, fin and eye deformities, bone and skin neoplasms, and spinal deformities [2]. Spinal deformities in both farmed and wild fish populations have been reported [3]. These deformities can be caused by different factors, including bacterial and parasitic infections, deficiencies in vitamin C and phosphorus, elevated temperatures during the egg incubation period, rapid growth rates in the juvenile phase, inappropriate light regimes, vaccination, water flow, and quality issues, and aquatic pollution [1, 4]. In aquatic organisms, it could be complicated to determine the main contributor to a specific type of skeletal malformation because many of these defects are probably generated by multiple factors [5].

Congenital diseases in fish, similar to those in other animals, are conditions that are present at birth that result from genetic or developmental abnormalities. Some congenital diseases in fish include scoliosis, lordosis, deformed fins or tails, eye abnormalities, jaw

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deformities, swim bladder disorders, coloration abnormalities, organ malformations, and genetic disorders. These diseases can affect various aspects of anatomy, physiology, and behavior, including swimming ability, balance and movement patterns, the ability to find food and avoid predators, and the development or function of the swim bladder, which can lead to buoyancy issues. Macroscopically, vertebral deformations are classified into lordosis (dorsoventral curvature), kyphosis (upward spinal curvature), scoliosis (lateral curvature), fusion, and compression [6, 7]. Anatomically, they are attributed to dislocation, fusion, shortening, or deformation of the implicated vertebrae. Abnormal skeleton and scale sequences, pop-eye, bowed vertebral column, lack of fins, and other traits are seldom encountered in wild stocks but are more common in captive stock. These types of abnormalities in several fish have been reported in many studies [8, 9].

Diagnosis of vertebral abnormalities in fish can be performed through visual investigation, palpation (examination by pressing on the body surface to feel the organs or tissues beneath), X-ray of internal organs, clearing and staining techniques, computed tomography, and histopathological examination [4,10]. Confirmation of vertebral deformities in fishes and many other vertebrates, including humans, could be difficult based solely on physical observation. However, radiography is an emerging technique that is increasingly effective in resolving these issues.

Radiography (X-ray imaging) is a valuable tool in the fisheries sector for examining the internal anatomy of fish. It provides detailed information about the internal structure of any animal, aiding in disease diagnosis, identifying deformities, and detecting pathological changes. In the present study, we examined spinal vertebrae abnormalities in two species of teleost fish collected from their natural ecosystems. We use X-ray radiography as a valuable tool to visualize spinal abnormalities with precision. The collected fish were identified through integrative taxonomy, combining morphological traits with DNA barcoding.

MATERIALS AND METHODS

Sample collection and identification

During the experimental sampling in 2023, we randomly got two species of unusual specimens from two sampling sites in the river Gomti, Lucknow, Uttar Pradesh. We brought all suspected specimens and another five normal specimens of each species to the "Molecular phylogenetics lab (ICAR-NBFGR, Lucknow)" for further examination. All the collected fish were photographed. Morphometric measurements and meristic counts of both deformed and normal fish (N = 2-5) were made point-to-point using a digital caliper, with precision to the nearest 0.1 mm. Body part measurements are shown as proportions to the standard length (SL) (Tables 6 and 7). Identification of the collected fish was done through the integrative taxonomy.

Radiography of specimens

The X-rays of fresh specimens were taken in the radiography lab at NBFGR (Table 4). We have taken all measurable and countable traits of the skeletal system. Spinal anomalies were assessed on lateral radiograph images using standard protocols [1].

Tissue collection and submission of voucher specimens

After radiography, the fin clip and 50 mg of muscle tissues were kept in 95% ethanol for molecular studies. The voucher specimens were stored at the NBFGR lab in Lucknow.

DNA extraction and PCR amplification

Genomic DNA (gDNA) was extracted using the phenol:chloroform: isoamyl alcohol (PCI) method with minor modifications [11]. The concentration of isolated DNA was checked by a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA) and 0.7% agarose gel using a UV spectrophotometer, then stored at - 20 °C for further use. The estimated concentration of DNA was 90-95 ng/µL. The COI gene was amplified in a 50 µL volume with 5 µL of 10X Taq polymerase buffer (Thermo Scientific), 2 µL of $MgCl_2$ (50 mM) (Thermo Scientific), 0.25 µL of dNTP (0.05 mM) (Thermo Scientific), 0.5 µL of each primer (0.01 mM), 0.6 U of Taq polymerase (Thermo Scientific), and 5 µl of DNA template. The universal primers used for the amplification (Tm value: 54°C) of the COI gene are FishF1-5'TCAACCAACCACAAAGACATTGGCAC3', and FishR1-5'TAGACTTCTGGGTGGCCAAAGAA TCA3' [12]. The thermal regime consists of an initial step of 2 min at 95°C followed by 35 cycles of 40s at 94°C, 40s at 54°C and 1 min 10s at 72°C followed in turn by a final extension of 10 min at 72°C [11]. The PCR products were visualized on 1% agarose gel, and the most intense products were selected for DNA sequencing.

DNA sequencing

PCR products were sequenced by Sanger's method using the Big Dye Terminator V.3.1 Cycle Sequencing

Kit (Applied Biosystems, Inc.) and run on an ABI 3500 capillary sequencer following the manufacturer's instructions.

Bioinformatics and statistical analysis

All morphometric data were analyzed using MS Excel. The sequences were aligned using the CLUSTALW module integrated with MEGA 11 (Molecular Evolutionary Genetics Analysis) software [13]. The sequences were blasted in the National Center for Biotechnology Information (NCBI) for the nearest matches and submitted to GenBank, as given in Table 1. The COI sequences of *Balanoglossus aurantiaca* (OQ322732) were used as an outgroup species. A maximum likelihood (ML) tree was constructed, and bootstrapping analysis was performed using 1000 pseudo-replications.

RESULTS AND DISCUSSION

Identification of all specimens through integrative taxonomy

This study successfully identified the *P. chola* and *H. fossilis* using integrative taxonomy. We generated DNA barcodes of abnormal specimens (two barcodes of normal specimens of both species) of collected fish for identification using a partial COI sequence.

Phylogenetic analysis

The generated COI sequences were blasted in the NCBI for nearest neighbors to validate all the individuals to species level. A total of 7 sequences (5 from abnormal and 2 from normal fish) were generated from the two collected species. COI sequences were aligned using ClustalW in MEGA after trimming the primers. All generated DNA sequences showed simplicity and unambiguity, and no insertions, deletions, or stop codons were observed in any sequences. Data analysis of both deformed specimens and normal specimens revealed that the average Kimura two-parameter (K2P) distances in percentage between individuals of a species and between species were 0.3% (*H. fossilis*) and 1.2% (*P. chola*) (Table 2, 3). The figure represents a summary ML tree derived from the COI gene (Fig. 1). There were a total of 753 positions in the final dataset.

Radiography of abnormal and normal specimens

In the present study, we reported deformed *Puntius chola* and *H. fossilis* from the Gomti river, Lucknow. All morphometric traits of 2-5 normal and abnormal specimens were recorded for comparison. No significant morphological differences were observed, although the body shapes differed notably. Because externally unusual specimens have relatively more body width and a shorter

Table 1. Details of suspected specimens with coordinates of the sampling locations and GenBank accession numbers.

Fish Species	Collection Date	Voucher Id	Sampling Site	Location (Daliganj, Lucknow)		NCBI Accession Number
				Latitude	Longitude	
Puntius chola	24.05.2023	NKGPC-1#	Site 1	26.884244	80.903764	PP064992
	24.05.2023	NKGPC-26A##	Site 1	26.884244	80.903764	PP064993
						KJ936779*
						KY682980*
						JQ713852*
Heteropneustes fossilis	14.09.2023	NKGHF-1#	Site 2	26.871274	80.916025	PP064987
	14.09.2023	NKGHF-2#	Site 2	26.871274	80.916025	PP064988
	14.09.2023	NKGHF-3#	Site 2	26.871274	80.916025	PP064989
	14.09.2023	NKGHF-4#	Site 2	26.871274	80.916025	PP064990
	14.09.2023	HFN##	Site 2	26.871274	80.916025	PP064991
						KT364787*
						KJ936724*
						KT896694*

[Site 1: Near Mehndi Ghat; Site 2: Pakka pul, Daliganj; #- Abnormal fishes; ##- Normal fishes; *- Sequence taken from NCBI database].

Table 2. Details of K2P genetic distance among individuals of *H. fossilis*.

	H. fossilis NKGHF-1	*H. fossilis* NKGHF-2	*H. fossilis* NKGHF-3	*H. fossilis* NKGHF-4	*H. fossilis* HFN	*H. fossilis* KT364787	*H. fossilis* KJ936724	*H. fossilis* KT896694
H. fossilis NKGHF-1								
H. fossilis NKGHF-2	0.00331							
H. fossilis NKGHF-3	0.00826	0.00661						
H. fossilis NKGHF-4	0.00661	0.00496	0.00496					
H. fossilis HFN	0.00165	0.00165	0.00661	0.00496				
H. fossilis KT364787	0.00661	0.00496	0.00496	0.00331	0.00496			
H. fossilis KJ936724	0.00661	0.00496	0.00496	0.00331	0.00496	0.00331		
H. fossilis KT896694	0.00496	0.00331	0.00331	0.00165	0.00331	0.00165	0.00165	0.00

Table 3. Details of K2P genetic distance among individuals of *P. chola*.

	P. chola NKGPC-1	*P. chola* NKGPC-26A	*P. chola* KJ936779	*P. chola* KY682980	*P. chola* JQ713852
P. chola NKGPC-1					
P. chola NKGPC-26A	0.0129				
P. chola KJ936779	0.0097	0.0032			
P. chola KY682980	0.0113	0.0048	0.0016		
P. chola JQ713852	0.0129	0.0064	0.0032	0.0048	0.00

body profile than normal individuals. So, through the radiography tool, we examined the internal anatomy of deformed specimens and found that radiography can detect abnormalities in fish (deformities). This simple tool can significantly improve the clinical evaluation of sick fish. X-ray images were used to examine and summarize all measurable and countable traits of the fish skeletal system, as detailed in Table 4.

The radiograph revealed that both the abdominal and caudal vertebrae were involved in the upward and downward arching of the vertebral column. All collected individuals were considered malformed based on visual examination of deviations in body shape from the normal phenotype. Externally, *P. chola* showed only a single hump located at the origin of the anal fin and near the caudal peduncle, while concavity shows the end of the dorsal fin and near the caudal peduncle region (Fig 2, 3, and 4). In the case of *H. fossilis*, each specimen exhibits at least 3-5 humps, as shown in figure 3 and 4. Vertebral column deformities (spinal curvature, SC) in fish were classified into three categories: platyspondyly (shortening of the vertebral column without curvature), lordosis (ventral curvature), and kyphosis (dorsal curvature). The following section provides a detailed description of each analyzed specimen.

Responsible factors for the development of phenotypic-anatomical abnormalities in fish

Based on a literature survey, there were no previous records of factors leading to such deformities in wild fish populations, except for a few studies in India [9,14,15]. In comparison, several studies have been reported on cultured salmonids [18, 19, 20]. These studies were also unable to figure out the exact reason and mentioned multiple factors [1, 16, 17], which are described below.

Possible impact of predator-driven phenotypic abnormality

Riverine ecosystems are one of the most threatened habitats by several human-mediated sources of contaminants [21]. Extreme climatic conditions, such as floods during the monsoon season, are prevalent in natural water bodies, including the Gomti river. Exotic fish, which are currently widespread in almost all freshwater habitats, may escape from the culture system as a result of these occurrences. In our sampling, we found that six exotic fish, including *Clarias gariepinus, Cyprinus carpio, Oreochromis niloticus, Aristichthys nobilis, Hypophthalmichthys molitrix, Ctenopharyngodon idella*, are currently distributed across the Gomti river. These invasive species can outcompete native species for resources and alter and disrupt the food web balance overall in the ecosystem. They often thrive in disturbed habitats and can spread rapidly [21].

The accidental or deliberate introduction of exotics increases the predation stress of the indigenous fishes,

Table 4. All morphometric characters of the skeleton system of abnormal and normal specimens.

X-ray photo Id	Fish Species	Measurement					
		Skeleton length (cm)	Fused vertebrae	Trunk vertebrae	Caudal vertebrae	Total vertebrae	Vertebrae length (cm)
12072023-01	*P. chola*	8.7	3	13	14	30	6.6
12072023-02	*P. chola**	8.4	2	18	14	34	5.2
14092023-17	*H. fossilis**	13.5	2	2	52	56	10.7
14092023-17	*H. fossilis*	9.1	-	-	-	54	8
14092023-17	*H. fossilis*	11.4	-	-	-	57	10.7
14092023-17	*H. fossilis*	11.4	-	-	-	52	10.5
14092023-17	*H. fossilis*	10.7	-	-	-	58	11.2

* Normal specimens; (-): unable to count because of compressed vertebrae.

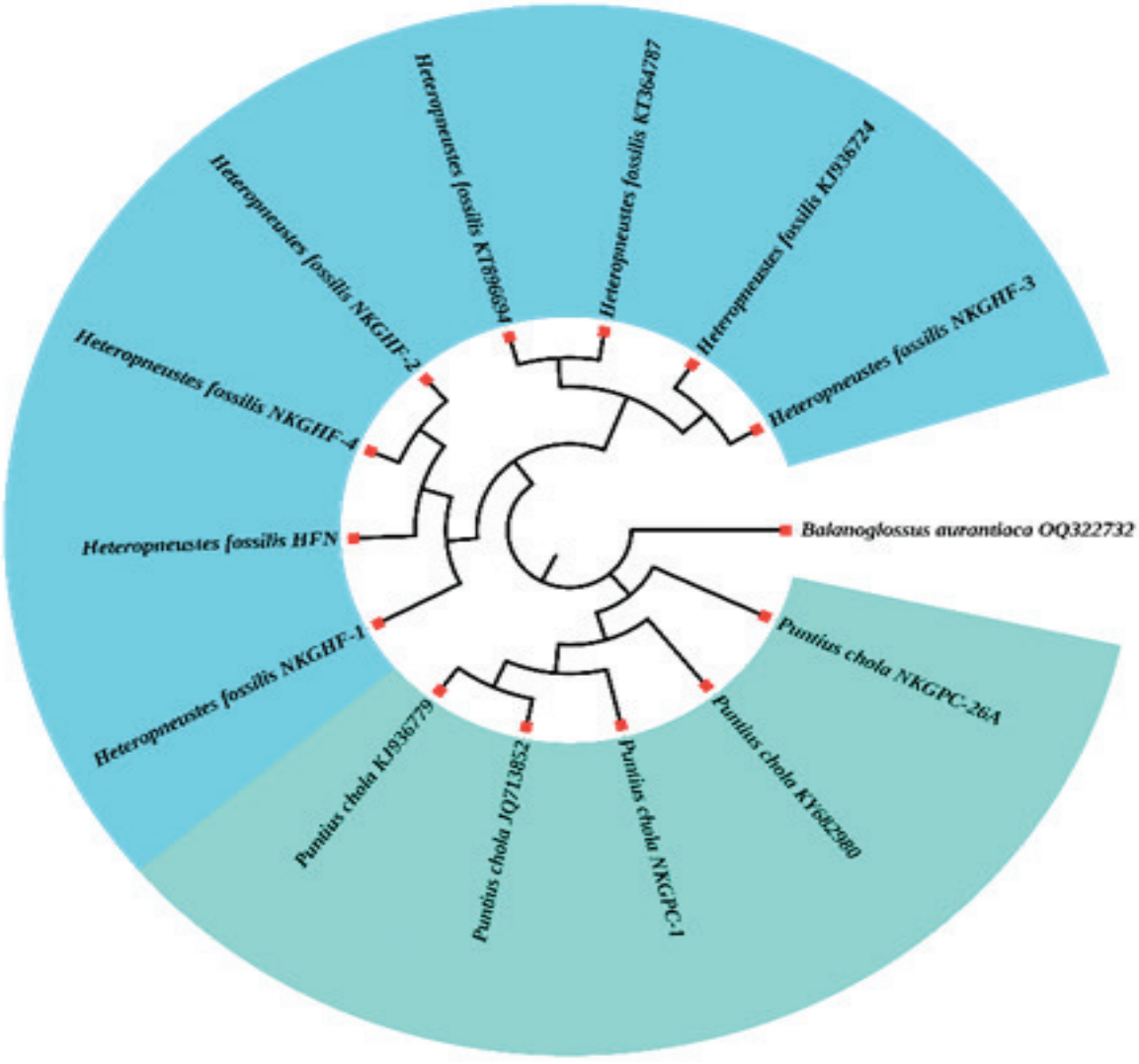

Fig. 1. ML tree based on COI sequences for normal and deformed individuals of both species.

Table 5. Types of abnormalities in *P. chola* and *H. fossilis*.

Types of abnormalities	Characters	Species	Figure
Platyspondyly	Short body & deformed tail regions	Both species	2, 3, 4
Tail region lordo-kyphosis	Normal trunk & curvature in tail region	*P. chola*	2
Tail region lordo-kyphosis	Curved trunk & tail	*H. fossilis*	4

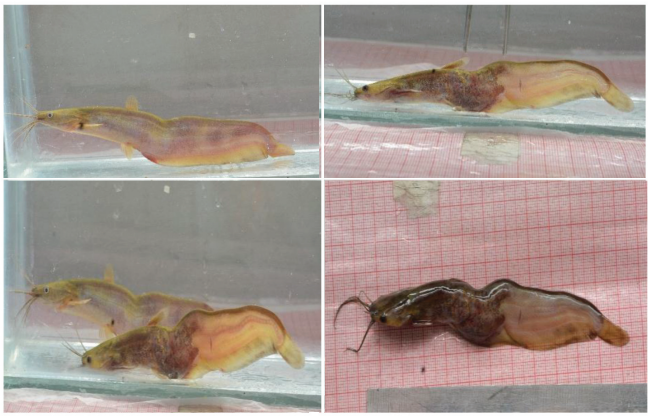

Fig. 3. All deformed individuals of *H. fossilis* collected from the Gomti river.

Table 6A. Descriptive statistics of collected specimens (N-6) with biometric data of the abnormal specimen of *Puntius chola* (Morphometric character).

Morphometrics character	Mean	% SL	Max	Min	SD	Abnormal specimen
Standard length (SL, cm)	6.66	-	7.04	6.1	0.34	6.89
In percent of standard length						
Total length (cm)	8.38	121.58	8.99	7.74	0.45	8.99
Forked length	7.37	106.92	8.16	6.65	0.50	8.16
Head length	1.75	25.42	1.89	1.6	0.11	1.88
Body width at dorsal-fin origin	2.19	31.76	2.77	0.24	0.97	2.77
Body width at anal-fin origin	1.76	25.57	1.89	1.46	0.16	1.85
Pre-dorsal distance	3.65	52.93	4.18	3.34	0.32	4.18
Post-dorsal distance	4.66	67.66	5.92	4.18	0.64	5.92
Pre-pelvic distance	3.31	48.06	3.79	2.98	0.30	3.79
Pre-anal distance	4.88	70.80	5.53	4.39	0.46	5.53
Pre-pectoral distance	1.84	26.68	2.09	1.66	0.15	2.09
Length of dorsal-fin base	1.38	20.05	1.67	1.05	0.26	1.57
Pectoral-fin length	1.28	18.63	1.45	1.15	0.10	1.45
Anal-fin length	1.04	15.05	1.12	0.93	0.06	1.12
Caudal-peduncle length	1.27	18.48	1.55	0.47	0.40	0.47
Caudal-peduncle depth	0.98	14.18	1.09	0.85	0.09	0.9
HL%						
Eye diameter	0.48	6.92	0.54	0.38	0.07	0.51
Pre-orbital length	0.56	8.10	0.95	0.42	0.20	0.52
Post-orbital length	0.54	7.81	0.98	0.1	0.39	0.83

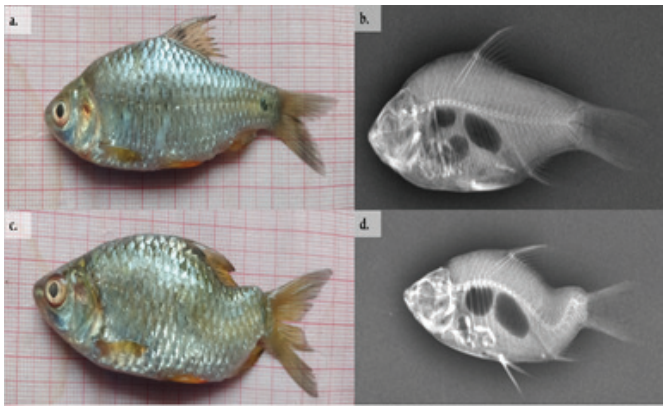

Fig. 2. Compression of normal and abnormal specimens of *P. chola* through radiography technique. [(a) Normal specimen, (b) Radiograph of normal specimen, (c) Abnormal specimen, (d) Radiograph of the abnormal specimen].

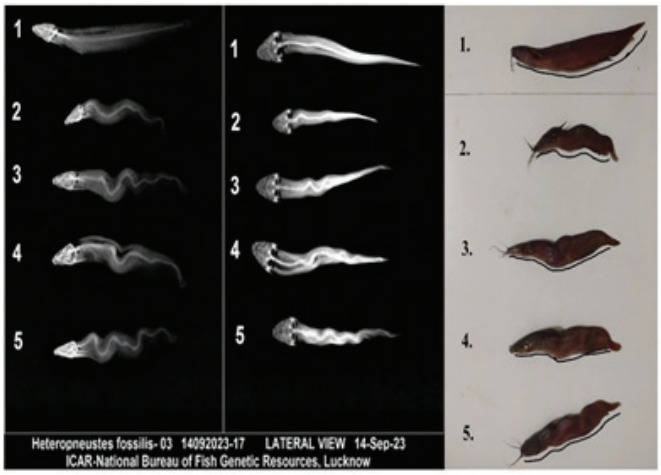

Fig. 4. Comparison of normal (No.1) and abnormal (2-5) specimens of *H. fossilis*. [Left side: lateral view X-ray radiograph; Middle: dorsal view X-ray radiograph; Right side: individuals].

Table 6B. Descriptive statistics of collected specimens (N-6) with biometric data of the abnormal specimen of *Puntius chola* (Meristic characters).

Meristics Characters	Normal specimens					Abnormal specimen
	N1	N2	N3	N4	N5	N6
Lateral line series scales	25	25	26	27	28	27
Pre-dorsal scales	8	9	8	10	11	8
Pre-pelvic scales	10	10	10	9	9	10
Pre-anal scales	9	8	8	7	8	7
Circum peduncular scales	5	6	6	6	6	4
Dorsal-fin ray	10	10	11	10	11	10
Pectoral-fin ray	13	15	15	14	15	13
Pelvic-fin ray	11	10	11	10	10	9
Anal-fin ray	7	7	6	7	7	7
Caudal-fin ray	28	27	29	28	29	29

Table 7A. Descriptive statistics of specimens (n=6) with Biometric data of the abnormal specimen of *H. fossilis*.

Morphometrics Character	Mean	% SL	Maxi	Mini	SD	Abnormal specimens (Means)
Standard length (SL, cm)	9.51	9.51	11.73	7.29	1.58	9.45
In percent of standard length						
Total length (cm)	10.30	108.29	13.51	8.09	1.89	10.34
Head length	1.00	10.48	1.98	0.21	0.87	1.15
Body width at dorsal-fin origin	1.27	13.30	1.56	1.03	0.21	1.31
Body width at anal-fin origin	1.59	16.67	1.82	1.23	0.21	1.56
Pre-dorsal distance	3.52	37.05	3.96	2.79	0.42	3.48
Post-dorsal distance	4.33	45.55	5	3.61	0.62	4.21
Pre-pelvic distance	2.87	30.13	4.11	1.83	0.96	2.79
Pre-anal distance	3.96	41.66	4.64	3.24	0.54	3.93
Pre-pectoral distance	1.71	17.98	1.91	1.31	0.22	1.79
Length of dorsal-fin base	0.36	3.79	0.41	0.32	0.03	0.36
Pectoral-fin length	1.30	13.62	1.44	1.16	0.11	1.31
Anal-fin length	5.35	56.27	7.1	4.39	0.97	5.44
Caudal-peduncle depth	CPD: unable to measure due to abnormality in the caudal region					
Caudal-peduncle depth	0.55	5.78	0.61	0.49	0.04	0.55
HL%						
Eye diameter	0.21	2.16	0.24	0.19	0.02	0.21
Pre-orbital length	0.62	6.55	0.75	0.42	0.13	0.60
Post-orbital length	0.93	9.78	1.1	0.75	0.13	0.90

Table 7B. Descriptive statistics of specimens (n=6) with biometric data of the abnormal specimen of *H. fossilis*.

Meristics characters	Abnormal specimens					Normal specimens
	N1	N2	N3	N4	N5	N6
Dorsal-fin ray	6	7	7	6	8	6
Pectoral-fin ray	9	8	9	9	10	10
Pelvic-fin ray	8	9	8	9	8	8
Anal-fin ray	70	69	68	67	69	70
Caudal-fin ray	18	22	22	23	23	18

particularly in the immature stages [22]. It is already known that smaller-sized organisms have more pressure due to predation during their fast-growing stages [23]. The accidental or deliberate implantation of exotics increases the predation stress of native aquatic life, particularly in their immature stages [22]. It is previously known that smaller organisms face additional pressure due to predation during their rapid growing stages [23]. Similarly, the existence of invasive predatory fish in the Gomti river could be the reason for the occurrence of aberrant fish species.

Environmental factors and nutrient deficiency

Several researchers have found skeletal anomalies in natural environments, however, the genuine cause of these deformities is unresolved.

Several environmental parameters, such as climate change, elevated temperatures, water quality, aquatic pollution, infections, bacterial and parasitic diseases, light regimes, genetic variance, inbreeding depression, and nutritional imbalance, are often recognized as the causative factors that contribute to the increased occurrence of skeletal deformities [4, 24, 25]. Environmental factors influence phenotypes either as individual parameters or as interactive variables, combining two factors, such as dissolved oxygen (DO) and water current [26]. Other factors include lack of micro and macronutrients, immunization, elevated temperatures in the early stages of embryogenesis, changes in photoperiod, etc [1]. Changes in riverine habitats offer novel selection pressures on native fish fauna, influencing their physiques as evidenced by genetic-based morphometric plasticity in reservoirs and hillstream fish [27]. Stress caused by pollution and unfavorable environmental factors could also lead to eggs and larvae developing deformed body shapes as they grow. In addition, the waters have been characterized by elevated temperatures and reduced DO levels [28]. Surface sediments and water in the river are found to have high concentrations of heavy metal pollutants, such as lead, which can severely affect the metabolic activities of riverine biota and disrupt community structures [29].

Both species collected from the Gomti River, Lucknow, are affected by several anthropogenic activities that may lead to such deformities. The collection sites of the deformed specimens were found to have relatively low dissolved oxygen (DO) levels and high biological oxygen demand (BOD). Khan *et al*. [30] assess and quantify six heavy metals (As, Fe, Cd, Pb, Mn, Cr) from the river Gomti. Untreated sewage and agricultural runoff discharge, including household and commercial wastewater, has been identified as a potential contributor to heavy metal pollution in river water, degrading water quality and endangering fish populations. The ecohydrology of this river is deteriorating while entering Lucknow city due to the discharge of huge quantities of sewage through different drains into the river Gomti [31]. The ichthyo-diversity of freshwater ecosystems is fragile to a wide range of stressors. Water quality has tremendous effects on population survival, growth, and reproduction [32, 34]. The native ichthyofauna of the Gomti river is seriously threatened by environmental pollutants such as metals and pesticides.

Toxins can affect physiological effects such as hormonal disbalances, and neurological, and metabolic

systems as well as eliminate behaviors that are necessary for survival and fitness in natural environments [33]. We also observed that several sewage drains discharge into the river, leading to degraded water quality and the destruction of spawning and nursing grounds for the fish fauna of the Gomti river.

The scoliosis condition in all collected fishes was visible externally, compared with the normal specimens. This kind of disease is caused by Vitamin C deficiency, which is associated with skeletal anomalies. Vitamin C deficiency can be responsible for the decrease in bone collagen content, which may result in the formation of spinal kyphosis, scoliosis, and lordosis. A fish skeleton is made up of complex, metabolically active tissue (composed of bones and cartilage) that continuously changes throughout the fish's life. Fish can absorb calcium directly from water, so calcium deficiency is rare in fish. At the same time, bone development and growth are influenced by phosphorus (P) concentration in the water and its availability in the diet. Slow growth, lethargic bone calcification, and the formation of skeletal abnormalities are symptoms of a phosphorus shortage in fish bodies.

The relatively low genetic variation in the least mutating gene between two species has prompted us to explore whether it is an evolutionary characteristic developed by humans or natural selection. We cannot confirm it as an evolutionary trait due to the small number of specimens. Without proof of an evolutionary aspect, we currently interpret these problems as anomalies caused by multiple unidentified natural or manmade sources [14].

The loss of ichthyofaunal diversity in riverine ecosystems can be attributed to various natural and human-induced factors. Therefore, it is crucial to identify the exact causes to ensure the protection of indigenous fish species. To conserve these species, anthropogenic activities must be minimized to safeguard the vital riverine habitats of the Gomti river in Uttar Pradesh [35].

CONCLUSION

In the current study, we reported for the first time the occurrence of vertebral deformities in *P. chola* and *H. fossilis* from the Gomti river ecosystem. We address three types of deformities, identified by the direction of their spinal curvature: lordosis (a condition that encompasses the abnormal ventral curvature of the vertebral, v shape), kyphosis (dorsal curvature, ^ shape), and scoliosis (lateral deformity, zig-zag shape). These types of vertebrae abnormities are sporadic in wild ichthyofauna stock. There are no previous records of this type of phenotypic abnormality in any fish from the Gomti river. Radiography is faster and easier to perform than traditional approaches for examining skeletal structures and provides considerable detail without sacrificing the animal. Additionally, X-rays can be used for quickly examining known mutants to identify skeletal irregularities. X-rays, sometimes called radiographs, are a useful diagnostic technique for a variety of fish diseases without affecting the specimens. Radiography can detect abnormalities or diseases in fish, such as tumors, fractures, deformities, or the presence of foreign objects. We found that different conditions were present such as; Platyspondyly: externally, short body, and deformed tail fin were observed in all specimens of both species. Tail region lordo-kyphosis: Morphologically, a normal trunk, and curved tail were observed in *P. chola*. Whole-body kypho-lordo-kyphosis: externally, curved trunk and tail in all specimens of *H. fossilis*. These findings contribute to our understanding of fish health and can aid in diagnosing and treating diseases in both wild and captive fish populations. Skeletal deformities in commercially valuable fishes have been recognized as a significant issue, impacting both the economy and animal welfare. We close by acknowledging that it is a good study with exciting and novel information about spinal deformities in wild fish species.

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