RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 4: 577-585 (April 2026)

Baseline Morpho-molecular Insights Into Indigenous Ornamental Fish Zebra Loach, *Botia striata* from Koyna River, Western Ghats, India

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10.18805/IJAR.B-5723

ABSTRACT

Background: *Botia striata* (zebra loach), an endangered ornamental species endemic to the Western Ghats, is threatened by habitat loss and restricted distribution. Limited baseline data exist on its morphology, growth and genetic identity. This study uses an integrative morpho-molecular approach to characterise Koyna River populations and support conservation planning.

Methods: A total of 360 specimens were collected from the Koyna River (August 2023-July 2024). Sixteen morphometric traits and five meristic counts were measured. Length-weight relationships and condition factor were estimated using log-transformed regressions. Muscle tissues were preserved for DNA extraction. The COI gene was amplified using universal FishF1-FishR1 primers and the purified PCR products were sequenced to generate consensus sequences. Bioinformatic analyses included BLASTn identification, nucleotide composition, genetic divergence and Maximum Likelihood phylogeny (T92+I model).

Result: Morphometric analysis revealed total length ranging from 3.2 to 5.6 cm, with strong positive correlations among major body dimensions. Meristic counts (D: I-9; A: I-6; C: 18; V: I-8; P: 13) were consistent with earlier descriptions for *Botia* spp. LWR analyses showed strong length-weight association (R^2 = 0.880-0.925) and negative allometric growth (b = 2.395-2.597). Condition factor values (1.19-2.799) indicated an overall healthy population status. COI barcoding generated a 662 bp sequence exhibiting low intraspecific divergence (0-0.6%) and formed a well-supported clade (bootstrap 96-97%) with authenticated *B. striata* sequences, validating taxonomic identity.

Key words: *Botia striata*, Conservation, DNA barcoding, Length-weight relationship, Meristic characters, Morphometry.

INTRODUCTION

Cypriniformes, the largest order of freshwater fishes (Nelson, 1998), comprises the superfamilies Cyprinoidea (carps) and Cobitoidea (loaches). Cobitids were historically regarded as a monophyletic group based on the presence of a movable, bifurcated suborbital spine, a defining feature of the spined loaches (Bohlen and Šlechtovα, 2016). Later studies, however, distinguished Cobitidae from Botiidae by differences in barbel arrangement and swim bladder ossification (Nalbant, 2002). The genus *Botia*, belonging to the family Botiidae, includes about 20 species (Dey *et al*., 2015) and nine genera (Kottelat, 2004) and is well known for its vibrant colouration, active behaviour and ornamental importance (Shaji *et al*., 2000). The Western Ghats of India, a recognised biodiversity hotspot, harbour *Botia striata*, a species listed as endangered and threatened on the IUCN Red List due to habitat degradation and restricted distribution (Dahanukar *et al*., 2004; Dahanukar, 2013; Keskar *et al*., 2014). Its narrow range and continuing habitat loss highlight the species' vulnerability and the urgent need for conservation attention.

Morphological assessment, including morphometric and meristic analyses, is fundamental to taxonomy, stock identification and understanding evolutionary patterns (Ujjania, 2003; Suyani *et al*., 2021). Such data support evaluations of species health and are essential for estimating key biological parameters related to growth, reproduction, life-history traits, population dynamics and overall stock resilience (Meshram *et al*., 2021). Specimen size, influenced by ecological and physiological factors, further enhances the value of morphometric studies

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How to cite this article: Ujjania, V.K., Sawant, P.B., Munilkumar, S., Biswas, G., Jaiswar, A.K., Rasal, K.D. and Sarma, D. (2026). Baseline Morpho-molecular Insights Into Indigenous Ornamental Fish Zebra Loach, *Botia striata* from Koyna River, Western Ghats, India. *Indian Journal of Animal Research*. **60(4):** 577-585. doi: 10.18805/IJAR.B-5723.

Submitted: 18-11-2025 **Accepted:** 20-01-2026 **Online:** 03-02-2026

(Kalayci *et al*., 2007). Morphometric relationships are widely recognised as critical tools in fisheries biology (Ujjania and Choudhary, 2021) and taxonomic investigations (Poria *et al*., 2013), particularly given the pronounced morphological plasticity exhibited by fish in response to environmental variability (Mir *et al*., 2014). Abiotic factors such as water physicochemical properties, habitat structure and substrate type, alongside biotic pressures including competition and predation, shape morphological expression and thereby influence stock assessments and taxonomic interpretations (Shah *et al*., 2024). Length-weight relationships (LWRs) further contribute to fisheries science by informing biomass estimation and growth assessment, especially for threatened species (Beverton and Holt, 1957; Froese, 2006; Guo *et al*., 2020). Anthro-pogenic impacts-such as overfishing, dam construction, habitat modification and biological pollution-continue to alter species composition and abundance (Shuman *et al*., 2022), while metrics like frequency of occurrence and length-weight distribution provide valuable insights into population structure and ecosystem functioning (Zhao *et al*., 2017).

Although isometric growth theoretically follows a cube law, where weight increases proportionally to the cube of length (Lagler, 1952), resulting in an exponent of 3 in the LWR equation, deviations from this idealised value, indicative of allometric growth, are frequently observed. These deviations are often attributed to environmental conditions and the physiological state of individual fish (Le Cren, 1951). Given the limited existing data on the biology and ecology of *B. striata* and its endangered status within the Koyna River region of the Western Ghats, this study was initiated to establish essential baseline data to inform conservation strategies for this ornamental freshwater fish. Biotechnological analysis, including DNA barcoding, has emerged as a reliable approach for species-level identification based on mitochondrial DNA sequences, facilitating the examination of evolutionary and taxonomic relationships among various taxa. This method relies on a standardised fragment of approximately 655 base pairs from the mitochondrial cytochrome oxidase subunit I gene, amplified using universal primers (Hebert *et al*., 2003). The global interest in fisheries and biodiversity conservation led to the establishment of the "Barcode of Life Project (iBOL)" (Hebert *et al*., 2003), which identified the COI gene as a robust molecular marker for fish species differentiation due to the rapid evolution of mitochondrial DNA, its strict maternal inheritance and haploid nature (Moore, 1995).

The zebra loach (*B. striata*) from the Koyna River is a prominent freshwater ornamental fish, for which limited data exist on its biology and ecology. This study employed an integrated approach combining morphological and genetic assessments to elucidate the relationships among morphometric parameters, meristic counts and DNA barcoding. Subsequent bioinformatic analyses will be beneficial for fish biologists in developing conservation strategies for freshwater ornamental fish that are naturally vulnerable.

MATERIALS AND METHODS

The study was conducted in the Koyna River, a major tributary of the Krishna River system in the Western Ghats, Maharashtra, India. Originating in the Mahabaleshwar hill ranges, the river flows southward for about 65 km before turning east near Helwak and subsequently joining the Krishna River at Karad (Jadhav *et al*., 2011). A total of 360 specimens of *Botia striata* were collected systematically from August 2023 to July 2024 from its major gene pool at Patan, Satara (17.33996°N, 73.95691°E) (Fig 1). The specimens were stored in sterilised ice-filled containers and transported to the Division of Aquaculture, ICAR-Central Institute of Fisheries Education, Mumbai, for further examination. Identification was carried out using standard taxonomic keys (Talwar and Jhingran, 1991). Sixteen morphometric variables-total length, standard length, fork length, head length, pre-dorsal length, dorsal fin length, post-dorsal length, caudal fin length, caudal height, snout length, eye diameter, pectoral fin length, pelvic fin length, pre-anal length, post-anal length and body width-were measured following standard procedures (Fig 2). Five meristic characters, including counts of dorsal, caudal, anal, ventral and pectoral fin rays, were recorded following Sharma *et al*. (2014); Menon (1992); Hossen *et al*. (2016) and

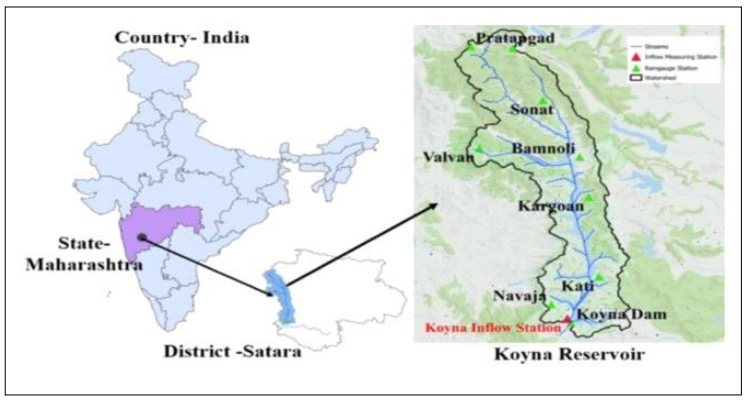


Fig 1: Geographic representation of the sampling site along the Koyna River, located within the Western Ghats, India.

Paunikar and Panwar (2021). All measurements were taken from the snout tip to defined anatomical landmarks using a vernier caliper supported by a metal divider, with precision up to 0.1 cm. Body weight was recorded to the nearest 0.1 g using an electronic single-pan balance after removing excess water and debris. The collected morphometric and meristic data were used to compute minimum, maximum, range and percentage variation for each trait.

The length-weight relationship (LWR) of fish specimens was established using the logarithmic transformation approach proposed by Le Cren (1951). The association between total length (cm) and body weight (g) was quantified using the length-weight equation:

$$W = aL^b$$

Where

'a and b' = Represent the relationship's intercept and slope or growth coefficient, respectively.

To determine these parameters, a linear regression analysis was conducted on the logarithmically transformed equation:

$$\log (W) = \log (a) \pm b \log (L)$$

Where

"a" = Stood for the relationship's intercept.

"b" = Its slope or growth constant.

The values of the compiled growth condition were used for the calculation of the condition factor (K) was calculated by the formula:

$$\text{Condition factor (K)} = \frac{100\,W}{L^b}$$

Where,

W = Total body weight (g).

L = Total length (cm).

b = Growth constant.

Conservation management faces significant challenges in the accurate identification of fishery resources, particularly eggs, larvae and juvenile stages, due to pronounced morphological similarities among closely related taxa (Balaganesan *et al.*, 2025). DNA barcoding has emerged as a robust species categorization tool, enabling the assignment of unknown specimens to recognised species through the analysis of standardized DNA barcode sequences and has been successfully applied across a wide range of organisms (Schindel and Miller, 2005; Rathipriya *et al.*, 2022). In this context, molecular approaches, especially DNA barcoding, offer a reliable and precise means of species authentication. Accordingly, for biotechno- logical assessment, muscle tissue was dissected from the right side of each specimen and immediately preserved in 95% ethanol for subsequent genomic DNA extraction. Genomic DNA was extracted from approximately 25-40 mg of ethanol-preserved tissue using the standard phenol-chloroform-isoamyl alcohol protocol described by Sambrook and Russell (2001). The mitochondrial Cytochrome c Oxidase Subunit I (COI) gene was selected for molecular analysis and amplified using the universal primers FishF1 (5′ -TCAACCAACCACAAAGACATT GGCAC-3′) and FishR1 (5′ -TAGACTTCTGGGTGGCCAA AGAATCA-3′) (Ward *et al.*, 2005). PCR reactions were performed in a 25 µl volume containing 1× PCR buffer, 2 mM MgCl, 100 µM of each dNTP, 5 pmol of each primer, 2 U of Taq DNA polymerase and approximately 100 ng of template DNA. Thermal cycling conditions consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 50 s, annealing at 54°C for 30 s and extension at 72°C for 80 s, with a final extension at 72°C for 10 min. PCR products were purified with Exonucleasel and FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) and sequenced using the Sanger dideoxyn-ucleotide chain-termination method (Sanger *et al.*, 1977) on an ABI 3500 Genetic Analyser (Applied Biosystems, USA).

The obtained COI sequences were curated by visual inspection of chromatograms in FinchTV, with trimming of ambiguous bases and submitted to GenBank under accession number PV564526. Sequence identity was confirmed using BLASTn searches against the GenBank nucleotide database. Genetic divergence within and between species was estimated in MEGA 12 (Kumar *et al.*, 2024) and phylogenetic relationships were inferred using the maximum likelihood method.

RESULTS AND DISCUSSION

Morphometric and meristic characterisation

The morphometric characterisation of *Botia striata* (Rao, 1920) was conducted and the traits are illustrated in Fig 2. The total length (TL) ranged from 3.2 to 5.6 cm and body weight (BW) varied between 0.600 and 3.800 g (Table 1). The morphometric parameters were described in Table 1, where fork length was 2.900-5.400 (4.649±0.032), noted the largest one contributing 80.952-96.875 (93.488±0.124%), while eye diameter was 0.200-0.400 (0.325±0.003), noted the smallest one that contributed 5.357-7.895 (6.543±0.047%). Furthermore, the multiple correlation for various morphometric variables was calculated and it was noted that the correlation coefficient (r) was maximum (0.983) and minimum (0.409) in total length v/s fork length and eye diameter v/s snout length, respectively (Fig 3). Comparative analysis revealed a close morphological resemblance

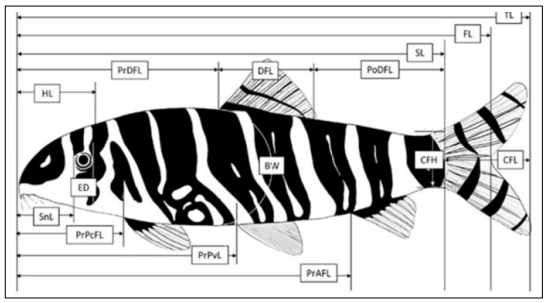


Fig 2: Morphometric measurements of *Botia striata* (Rao, 1920) from the Koyna River, Western Ghats, India.

Table 1: Observations and their percentages with different morphometric parameters of *Botia striata* (Rao, 1920) from the Koyna River, Western Ghats, India.

Parameter	Observation (cm) Min-max (Mean±SE)	Range difference	% of total length Min-max (Mean±SE)
Total length	3.200-5.600 (4.969±0.032)	2.400	
Standard length	2.300-4.600 (4.030±0.028)	2.300	67.647-88.235 (81.050±0.146)
Fork length	2.900-5.400 (4.649±0.032	2.500	80.952-96.875 (93.488±0.124)
Head length	0.600-1.200 (0.993±0.007)	0.600	16.981-21.818 (19.987±0.052)
Pre dorsal length	1.100-2.200 (1.847±0.013)	1.100	31.373-41.026 (37.157±0.101)
Dorsal fin length	0.800-1.400 (1.213±0.007)	0.600	20.755-30.233 (24.480±0.076)
Post dorsal length	0.600-1.200 (0.992±0.007)	0.600	16.667-23.529 (19.969±0.081)
Caudal fin length	0.500-1.100 (0.876±0.0060	0.600	14.706-20.513 (17.640±0.049)
Caudal height	1.100-2.100 (1.783±0.012)	1.000	30.189-38.889 (35.887±0.072)
Snout length	0.300-0.700 (0.552±0.004)	0.400	9.091-14.706 (11.146±0.050)
Eye diameter	0.200-0.400 (0.325±0.003)	0.200	5.357-7.895 (6.543±0.047)
Pectoral fin length	0.700-1.500 (1.161±0.008)	0.800	16.981-28.571 (23.442±0.117)
Pelvic fin length	1.400-2.400 (2.073±0.013)	1.000	38.889-46.154 (41.738±0.058)
Pre anal length	2.100-3.700 (3.268±0.021)	1.600	60.714-71.795 (65.787±0.100)
Post anal length	0.500-1.000 (0.801±0.006)	0.500	13.208-20.000 (16.132±0.058)
Body width	1.700-3.100 (2.701±0.017)	1.400	50.943-58.974 (54.397±0.083)
Weight	0.600-3.800 (2.602±0.034)	3.200	
Condition factor (K)	1.190- 2.799 (2.074±0.012)		

Table 2: Meristic counts of the *Botia striata* from the Koyna River.

Meristic characters	Range
Dorsal fin rays	I-9
Caudal fin rays	18
Anal fin rays	I-6
Ventral fin rays	I-8
Pectoral fin rays	13

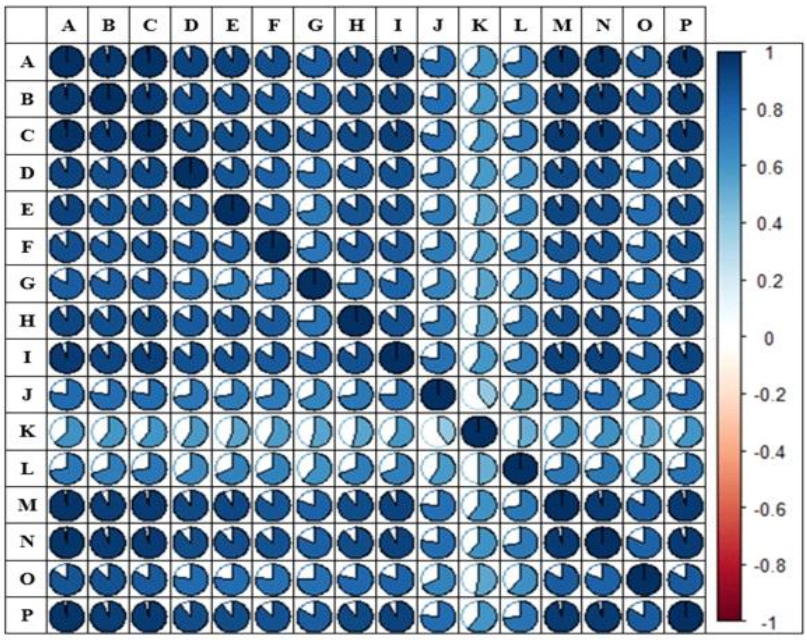
between *Botia striata* and *Nemacheilus triangularis*, as previously reported by Mercy *et al*. (2007) and with *Botia birdi*, as documented by Sharma *et al*. (2014). These findings highlight the taxonomic and morphological affinities of *Botia striata* within its related taxa, providing valuable insights into its structural and developmental characteristics.

The meristic characteristics of the examined specimens were recorded as follows: Dorsal fin rays (I-9), caudal fin rays (18.0), anal fin rays (I-6), ventral fin rays (I-8) and pectoral fin rays (13.0), as detailed in Table 2. These meristic counts exhibit notable similarity to the findings reported by Hossen *et al*. (2016) for *Botia lohachata* and by Paunikar and Panwar (2021) for *Nemacheilus botia*, indicating consistency in fin-ray patterns across related loach species. However, despite this meristic overlap, *Botia striata* can be distinguished in the field by its well-defined

vertical zebra-like bands, deeper laterally compressed body and the presence of a distinct movable suborbital spine, a diagnostic feature of botiid loaches.

Length-weight relationship (LWR) and condition factor (K)

The length-weight relationship of *Botia striata* was analysed based on measured total length (TL), standard length (SL) and weight (WT) variables and results were depicted that the intercept was 1.974 and 1.594, the correlation coefficient (R^2) was 0.880 and 0.925 and the growth constant (b) was 2.395 and 2.597 in total length v/s weight and standard length v/s weight, respectively (Fig 4 and 5). The correlation coefficient indicates a strong positive relationship between total length, standard length and weight. In contrast, the growth constant reveals negative allometric growth of the fish in the studied area. The 'b' values of the LWR for *B. striata* in this study were within the limits reported by Carlander (1969). Our findings align with those reported by Sui *et al*. (2015), Froese and Pauly (2021), Khan and Sabah (2013), Bashir *et al*. (2016) and Sheikh and Ahmed (2019), reinforcing the consistency of observed trends across various studies. The condition factor of zebra loach was



(A-Total Length, B-Standard length, C-Fork Length, D-Head length, E-Pre dorsal length, F-Dorsal Fin length, G-Post Dorsal length, H-Caudal Fin length, I-Caudal height, J-Snout length, K-Eye diameter, L-Pectoral fin length, M-Pelvic fin length, N-Pre anal length, O-Post anal length and P-Body Width).

Fig 3: Correlation among the different morphometric variables of *Botia striata* (Rao, 1920) from the Koyna River, Western Ghat of India.

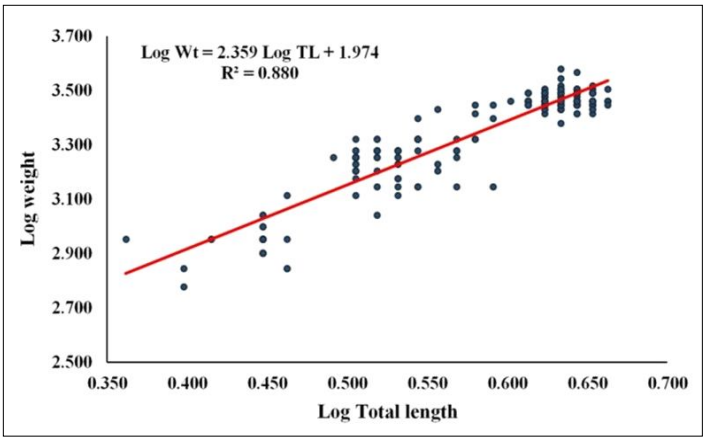


Fig 4: Length weight relationship (total length and weight) of *Botia striata* (Rao, 1920) from the Koyna River, Western Ghat of India.

analysed and found to range from 1.190 to 2.799, with a mean value of 2.074±0.012 (Table 1), which serves as an indicator of the overall health and well-being of the studied fish supported by Caldwell and Beyer (1987). These analyses provide valuable insights into the species' growth dynamics, morphological variation and physiological condition, contributing to a more comprehensive understanding of its ecological adaptations and population structure. To account for seasonal variations influencing the parameter b, fish specimens were categorised based on their capture period: cold season (fall and winter) and warm season (spring and summer) (Zaher *et al*., 2015). Wherever a significant seasonal effect was not evident, the evaluation of b proceeded at a broader temporal scale. Given the influence of environmental factors and the physiological state of fish, the value of b may diverge from the theoretical isometric growth coefficient of 3, as established by Ricker (1958). When b < 3, fish exhibit negative allometric growth, becoming progressively thinner as their length increases. Conversely, when b > 3, fish demonstrate positive allometric growth, signifying increased weight gain and optimal growth conditions.

This variation in growth patterns is further reflected in the seasonal fluctuations of the condition factor (K value) of *B. dario*, as reported by Haque and Biswas (2014), with values ranging from 1.03 to 1.11. These findings align closely with the results of the present study, reinforcing the role of environmental conditions in shaping fish physiology. Moreover, the consistency of these trends, as documented by previous researchers-including Dasgupta (1991) and Paswan *et al*. (2012)- highlights the broader applicability of these observations across different studies. Taken together, these insights emphasise the necessity of incorporating seasonal and environmental considerations when assessing fish condition and habitat quality, ensuring a more comprehensive understanding of aquatic ecosystems.

DNA barcoding

A total of 662 base pairs of the mitochondrial cytochrome c oxidase subunit I (COI) gene were analysed from *Botia striata* sequences. The overall nucleotide composition consisted of 57.3% adenine and thymine (AT) and 42.7% guanine and cytosine (GC) content. Codon position-specific analysis revealed an AT content of 56.56% at the 1st codon position, 71.04% at the 2nd position and 44.09% at the 3rd position, indicating notable codon bias typically observed in mitochondrial genes. Model selection for nucleotide substitution was conducted using the Bayesian Information Criterion (BIC) in MEGA software. The best-fit model identified was the Tamura 3-parameter model with invariant sites (T92+I). This model was subsequently employed for Maximum Likelihood (ML) phylogenetic analysis, providing a robust framework for evaluating evolutionary relationships among the sequences. The pairwise genetic distances (p-distance) among five *Botia striata* sequences ranged from 0 to 0.6%, indicating very low intraspecific variation. The sequence from the present study (KRCIFE01) showed 0-0.6% divergence compared to GenBank sequences. The Maximum Likelihood (ML) phylogenetic tree presented in Fig 6, derived from COI gene sequences, validated the identification of the *Botia striata* specimen from this study (KRCIFE01). It exhibited strong clustering with a bootstrap support of 97%, aligning closely with other *B. striata* sequences from GenBank (KF738186, KX384742). All *B. striata* sequences formed a distinct clade with 96% support, clearly separated from the outgroup species *Channa stewartii* (MK599531), indicating strong genetic relatedness and validating species-level identification. Hebert *et al*. (2003) introduced the concept that a short nucleotide sequence from the mitochondrial genome could serve as a DNA barcode for species identification, particularly in eukaryotic organisms, with a primary focus on animals. This approach has demonstrated its efficacy as a rapid and accurate method for identifying biological

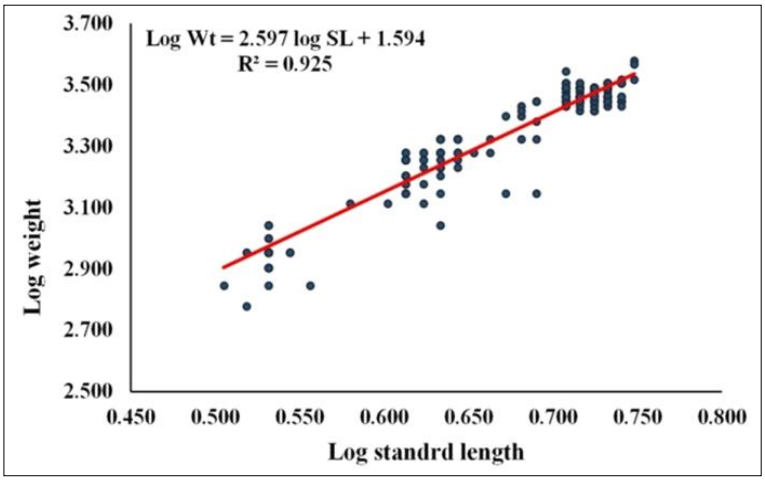
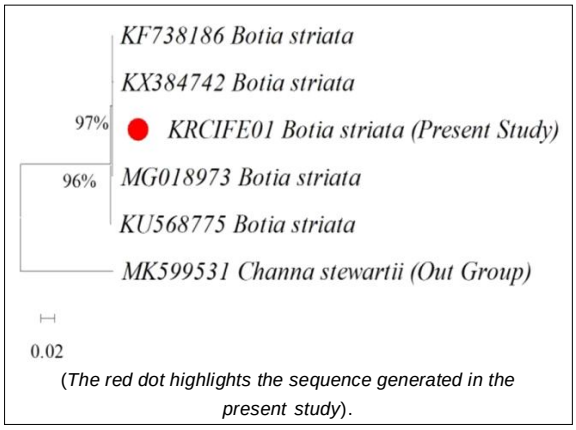


Fig 5: Length weight relationship (standard length and weight) of *Botia striata* (Rao, 1920) from the Koyna River, Western Ghat of India.



(*The red dot highlights the sequence generated in the present study*).

Fig 6: Maximum likelihood (ML) phylogenetic tree based on mitochondrial COI gene sequences.

specimens. Several studies have validated the efficacy of COI-based DNA barcoding in distinguishing closely related fish species (Steinke *et al*., 2005; Ward *et al*., 2005; Ratnasingham and Hebert, 2007; Hubert *et al*., 2008; Lakra *et al*., 2011; Chandra *et al*., 2012; Ambili *et al*., 2014; Alam *et al*., 2020; Goswami *et al*., 2022), reinforcing its utility in taxonomic classification and biodiversity assessment. These findings further establish the utility of mitochondrial markers in differentiating and classifying fish species with high precision.

CONCLUSION

The present investigation employed an integrative approach, combining morphological and molecular methodologies, to establish foundational reference data for *Botia striata* populations inhabiting the Western Ghats ecosystem of India. Morphometric analyses revealed distinct structural patterns, accompanied by consistent meristic counts and a negative allometric growth trajectory, collectively indicative of favourable population vitality. The condition factor further substantiated the overall physiological well-being of the species. Molecular validation *via* Cytochrome c oxidase I (COI) barcoding corroborated the taxonomic identity of *B. striata*, with negligible intraspecific genetic variation, suggesting a genetically cohesive population structure. These multidimensional insights offer a comprehensive baseline essential for targeted conservation initiatives and underscore the synergistic utility of classical taxonomy and molecular diagnostics in advancing biodiversity management frameworks.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Director and Vice-Chancellor of the ICAR–Central Institute of Fisheries Education, Mumbai, for providing the necessary research facilities to carry out this study. We also extend our sincere appreciation to Dr. N.C. Ujjania, Mr. Samad Sheikh and Dr. Mahendra Verma for their valuable support and guidance in specific components of the research. The first author further acknowledges the Indian Council of Agricultural Research (ICAR) for providing a doctoral fellowship.

Conflict of interest

The authors declare that there is no conflict of interest.

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