Taxonomic Status, Length-Weight Relationship and Condition Factor of *Channa stewartii* (Playfair, 1867) in Morigaon District, Assam, India

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ABSTRACT

The Assamese snake-head fish, *Channa stewartii* (Playfair, 1867) is endemic to the Brahmaputra River Basin (upper, middle, and lower) in India and Bangladesh, as well as the Ganges River basin extending from southern Nepal south-eastward. This study aimed to examine the taxonomic status and the length-weight relationship and condition factor of *C. stewartii* populations from a newly found community in Assam, Morigaon district, India. Taxonomic assessment involves morphological examination and comparison with existing literature to confirm the species identity. The morphological observation clearly indicated that *C. stewartii* specimens were distinguished by their blue fins with black patches, red fin edges, and reddish-pink pectoral fins. The female fishes have slightly duller, yellowish colours than the males, although they still have brighter colours than the males. Phylogenetic analyses also showed that our recent collection was clustered with *C. stewartii* from Assam, India. Measurements of *C. stewartii* length-weight relationship exhibited that both male (b = 3.389) and female (b = 3.396) showed a positive allometric growth. Additionally, the condition factor (K) for both sexes exceeded 1, indicating the well-being and health of the fish in their habitat. The findings contribute to the understanding of the ecological characteristics and population dynamics of *C. stewartii* in Morigaon district, providing valuable information's for fisheries management and conservation efforts in the region.

Keywords: Condition factor, Endemic, Genetic identification, Length-weight relationship

INTRODUCTION

A freshwater fish of the family Channidae (Actinopterygii Perciformes), *Channa stewartii* was first described as an Assamese Snakehead in 1867 by Playfair from Cachar in Assam. The species is endemic to the Brahmaputra River Basin (upper, middle, and lower) in India and Bangladesh, as well as the Ganges River basin extending from southern Nepal south-eastward (Talwar and Jhingran, 1991; Borah *et al*., 2018; Kalita *et al*., 2018). Typically, the fish exhibits a dark violet to brown color on the back, gradually fading to dull white (tinged with purple) underneath. The back and flanks of the fish are adorned with large black blotches. The fins are blue with black spots, and the edges are red, while the pectoral fins display a reddish hue. Notably, the gular region of the head lacks patches of scales. In terms of sexual dimorphism, females lack the vibrant colors seen in males but are slightly duller and have a yellowish tint (Bhattacharya and Mahapatra, 2018).

C. stewartii is listed as a least-concern species in the IUCN Red List. It is native to the north-east Indian states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, and Tripura), Nepal, and Bangladesh (Chaudhry, 2010). The genetic diversity of the striped snakehead in India is not well-documented, particularly in terms of mitochondrial genes, especially 16S rRNA. Currently, there are no 16S mitochondrial sequence records for the striped snakehead from Assam, India.

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Length weight relationships can provide an easy alternative to estimating body weight using length measures that are less variable and easily collected in the field (Primavera *et al*., 1998). Assessing fish condition based on weight at a specific is considered a reliable indicator of energetic condition or energy reserves throughout various life cycle stages. The condition factor (K) reflects the well-being of the population during various life cycle phases (Lambert and Dutil, 1997). This factor is viewed as a mathematical expression of the well-being of fish species. Examining fish growth outcomes and the correlation between length and weight is crucial. The length-weight connection plays a significant role in fisheries management, providing insights into the size, structure, age, and overall health of the fish. The statistical relationship between these two parameters is extremely important for understanding their shape, biology, nutrition, condition, and growth rate.

Despite its importance to the economy, society and environment, data on the length-weight relationship and condition factor for *C. stewartii* are relatively limited. There exists a critical research gap in seamlessly integrating these approaches. Additionally, the lack of standardization in the combining morphological and genetic data, identifying universally applicable genetic markers, and recognizing taxonomic gaps in poorly studied species all necessitate further research. To achieve a comprehensive understanding of the diversity, taxonomy, and dynamics of the fish ecosystem, these gaps must be addressed. Consequently, this study focuses on determining the taxonomic status based on morphology and genetic data, as well as exploring the length-weight relationship and condition factor of *C. stewartii* from Tarani Kalbari in the Morigaon district of Assam. This research aims to contribute to species identification, fish growth assessment, health monitoring, and the management of the fish population in its natural environment, which is critical for a breeding purpose.

MATERIALS AND METHODS

Sample collection and length-weight relationship study

A total of 119 specimens of *Channa stewartii*, measuring between 20.5 and 26.0 cm in length and weighing between 80 and 200 g for both sexes, were randomly collected from Tarani Kalbari (Moijhali) in the Morigaon district of Assam, India, from January 2022 to February 2023 (Figure 1). Local fishermen assisted in capturing *C. stewartii* using fishing nets (Ghukijal) and bamboo traps (Sepa). Small plastic tarpaulin tanks were used for transfer during measurement, with length recorded in centimeters (nearest to 0.1 cm) and weight in grams (nearest to 0.1 g). Fish were promptly released into the water to prevent injury. Morphological identification of specimens was carried out following Talwar and Jhingran (1991) and Musikasinthorn (2000).

16S Mitochondrial sequencing and molecular identification

C. stewartii of four specimens procured from a newly identified population from Tarani Kalbari of Morigaon district, Assam, India (Figure 1) was used for molecular identification. Using sterile scissors, 50–100 mg of muscle tissue from each of the four samples were dissected and placed in a 1.5 mL sterile tube containing 99% ethanol. Subsequently, the tissue samples were stored at -20 °C in the laboratory of the Postgraduate Department of Zoology at Darrang College for further study.

Total genomic DNA was extracted from muscle tissue using a DNeasy tissue kit (QIAGEN, Valencia, USA) as directed by the manufacturer. The 16S mitochondrial gene was amplified from snakehead samples using the primers 16S ar (5’-CGCCTGTTTATCAAAAACAT-3’) and 16Sbr (5’-CCGGTCTGAACTCAGATCACGT-3’) (Palumbi, 1996). Each 25 µL PCR reaction consisted of 3 µL (10–100 ng) of genomic DNA, 12.5 µL of MyTaq HS Red Mix PCR Kit (Bioline), 2 mM $MgCl_2$, 0.6 µM of each primer, and 5.5 µL of double distilled water (ddH_2O).

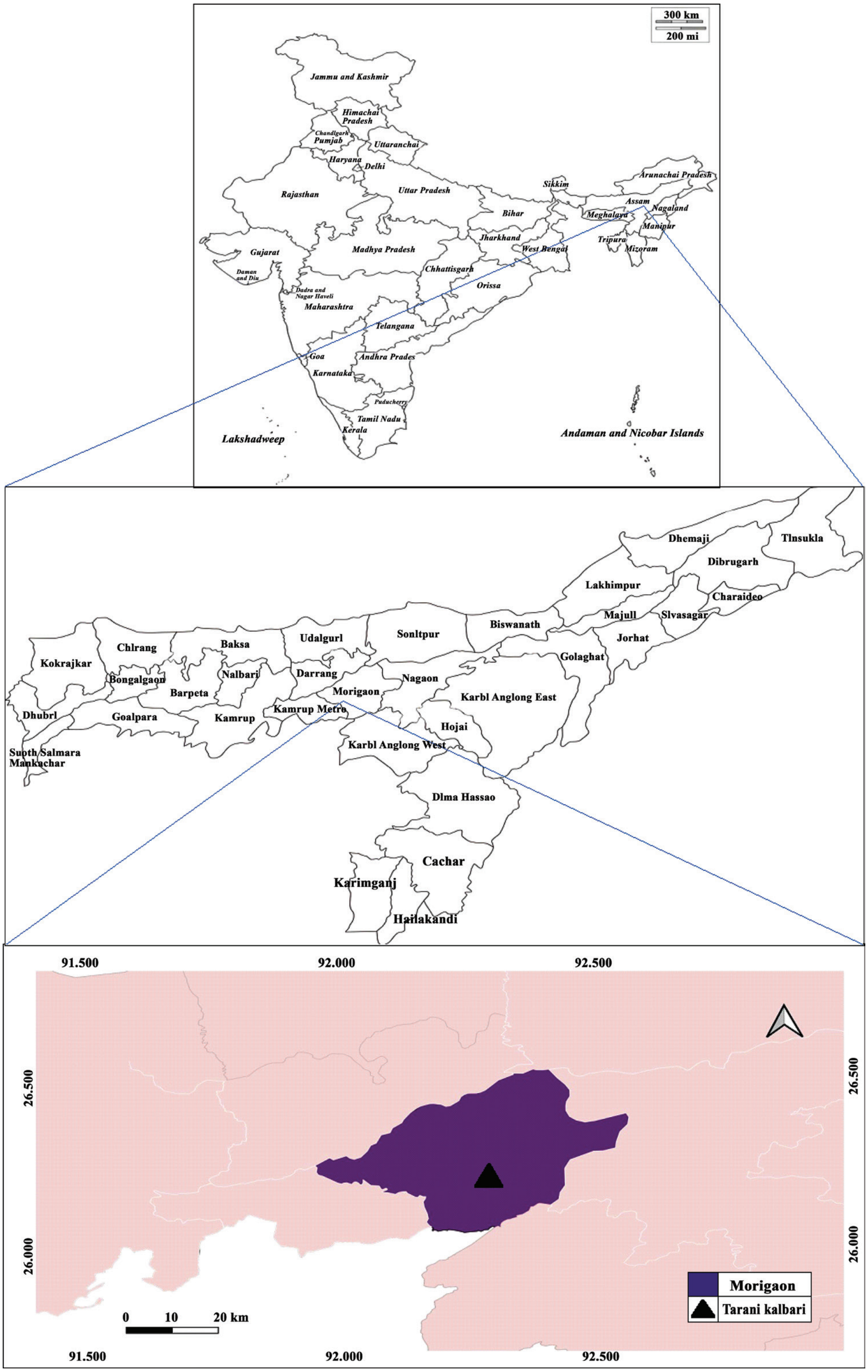

Figure 1. Map of study area and sample collection.

The amplification conditions included an initial denaturation at 95 °C for 2 min, 35 cycles of denaturation at 95 °C for 15 s, annealing at 50 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The reaction was then held at 4 °C. The PCR products were purified using ExoSAP-ITTM (Applied Biosystems) and analyzed on a 1% agarose gel and stained with Gel Red (Bioline). The PCR products were then sequenced in both directions on an ABI 3500 capillary sequencer using 16S universal primers as directed by the manufacturer (at Eurofins Genomics India Pvt Ltd, Bangalore). The nucleotide sequences were submitted to the public sequence repository GenBank (NCBI) after verification. The NCBI's Nucleotide BLAST tool was used to corroborate the snakehead sample data.

The dataset for phylogenetic analyses included multiple 16S rRNA sequences extracted from GenBank (NCBI). The phylogenetic tree was constructed using Maximum Parsimony, as implemented in MEGA11 (Tamura *et al*., 2021). The bootstrap consensus tree was derived from 1,000 replicates.

Length-weight relationship and condition factor

The following formula (Le Cren, 1951) was used to calculate the length-weight relationship:

$$W = aL^b$$

where W = weight in grams, L = length in centimeters, a = constant, and b = the exponent of the regression curve. Tesch (1971) transformed the equation into a logarithmic form to estimate the Log W = Log a + b. Log L

where a = constant (initial growth), b = growth coefficient, Log W = dependent variable, Log L = independent variable. The logarithmically converted total length and total weight data were used to determine the correlation coefficient (r) and coefficient of determination (r^2). The coefficient of correlation (r) was calculated to identify a characteristic of linear regression.

Following Fulton (1904), the condition factor (K_F) was calculated using the formula:

$$K_F = 100 \times W/L^3$$

where W = weight in g, L = length in cm; 100 = factor to bring the K_F nearest to unity. The t-test was used to determine if there is a significant difference between the length and weight of the fish, which compares the mean length and mean weight of the fish sample to assess if there is significant difference between these two variables. The comparison was conducted at a significant level of 0.05, meaning if the calculated p-value is less than 0.05 then the difference between the groups is considered statistically significant.

RESULTS AND DISCUSSION

Morphological identification

Sex identification was performed based on the secondary sexual characteristics, Males are distinguished from females by a high, sharply pointed head, a dorsal fin with vivid colour, and a body with spots. In comparison to males, females exhibit a shorter dorsal fin and a larger head. Additionally, females have pectoral fins with a 'U' shape, a rounded caudal fin, a slight deviation at the lower jaw, a wide mouth gap, and a thin lip (Figure 2) (Bhattacharya and Mahapatra, 2018).

Genetic identification

The amplification of Assamese snakehead samples from Tarani kalbari (Morigaon District) yielded 562 bp amplicon. Nucleotide BLAST results revealed a 99% homology between the snakehead samples and the complete mitochondrial genome (size: 16.765 kb) of *Channa stewartii* (retrieved GenBank accession number NC_071939). The sequences from four specimens of *C. stewartii* have been successfully deposited in GenBank with the accession numbers OR056418 OR618338, OR 618339, and OR618340. The novel sequence (OR 056418, OR618338, OR618339, and OR618340) have shown a clade with *C. stewartii* with strong bootstrap support (99%) (Figure 3).

Figure 2. Morphology of *Channa stewartii*: (a) a female and (b) a male.

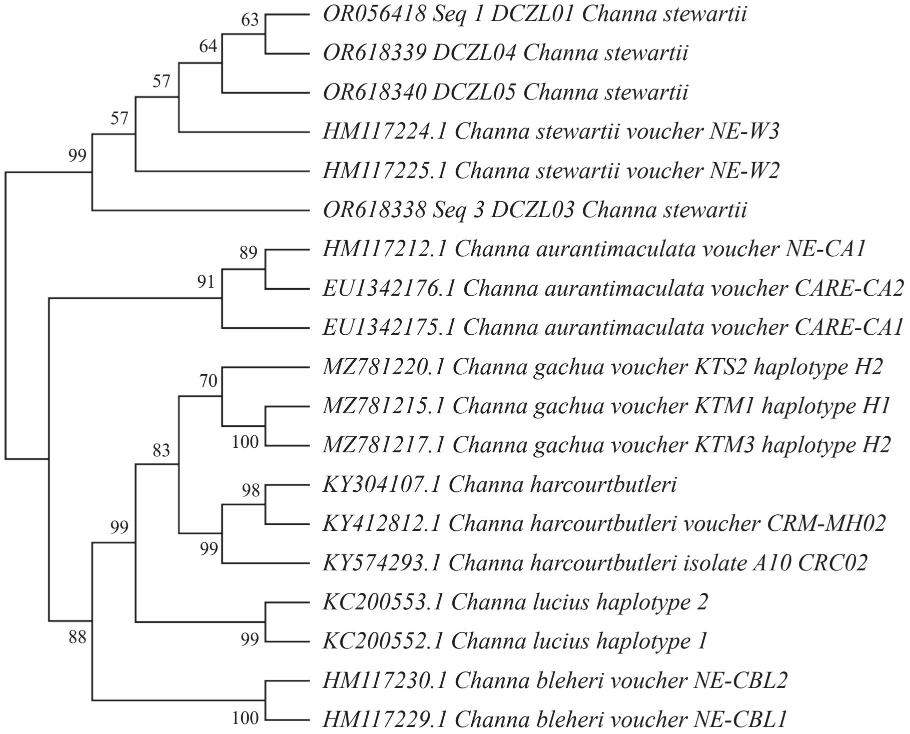

Figure 3. Maximum Parsimony analysis for 16 S rRNA-based evolutionary profile of *Channa stewartii* (GenBank IDs assigned to sequences from the samples of four specimens: OR056418, OR618338, OR618339, and OR618340).

Regression of total length to morphometric values and correlation

A total number of 119 fish samples were collected for this study, with lengths ranging from 20.5 to 26.0 cm and weights from 80 to 200 g. Table 1 shows the regression of total length on each characteristic. These equations are useful for predicting the value of each respective trait when only total length is available.

Length-weight relationship, relative condition factor

The b exponents for the length-weight relationship observed for males (+3.389) and females (+3.396) indicated positive allometric growth. In addition, the r^2 indicated that ~87% of the variation in weight is explained by length, hence indicating a well-fit regression model. Table 2 shows Length-weight relationship and condition factor (K_F) of *Channa stewartii*.

Growth is said to be positive allometric when the weight of an organism increases more than length ($b>3$) and negative allometric when length increases more than weight ($b<3$) (Morey *et al*., 2003; Datta *et al*., 2013) which indicates that fish's bodies are growing more quickly than their weight if $b<3$. In the research of *Channa punctatus*, similar positive allometric growth was observed (Singh and Serajuddin, 2017; Hossen *et al*., 2019). According to Hile (1936) and Martin (1949) the 'b' value usually ranges from 2.5 to 4.0 and for an ideal fish it remains constant (Allen, 1938).

Table 1. Regression equation of total length (X) on various morphometric characters (Y) and correlation coefficient between total length and each trait of *Channa stewartii* (measurement in cm).

Morphometric measurements	Range (cm)	Mean±SD (cm)	Regression Equation Log Y = a+b Log X	Correlation coefficient (r)
Total length (TL)	20.5 26.0	22.9±1.5		
Standard length (SL)	17.5 22.5	19.5±1.4	SL = -0.226+0.858TL	0.941
Body height (BH)	2.9 4.5	3.9±0.5	BH = -0.956+0.211TL	0.645
Head length (HL)	4.83 5.5	5.4±0.3	HL = 0.565+0.210TL	0.929
Eye diameter (ED)	0.72 4.9	2.2±1.9	ED = 3.0696-0.037TL	0.029
Pre dorsal length (PdL)	5.5 12.5	7.8±2.4	PdL = -2.497+0.449TL	0.275
Post orbital length (PoL)	3.0 4.3	3.7±0.4	PoL = 0.285+0.148TL	0.538
Dorsal fin base length (DBL)	10.9 13.8	11.7±0.9	DBL = 2.577+0.398TL	0.654
Anal fin base length (ABL)	6.8 8.1	7.5±0.4	ABL = 5.286+0.097TL	0.338
Pelvic fin base length (PvBL)	2.2 3.3	2.9±0.4	PvBL = 1.402+0.067TL	0.227
Pelvic fin length (PvL)	2.5 3.6	3.3±0.4	PvL = 2.325+0.041TL	0.168
Anal fin height (AH)	1.0 2.0	1.6±0.4	AH = 1.819+0.150TL	0.613
Anal fin base length (ABL)	3.0 8.1	6.9±1.6	ABL = 1.617+0.232TL	0.215
Dorsal fin height (DH)	1.3 2.9	2.1±0.5	DH = 1.449+0.153TL	0.475
Caudal fin length (CL)	3.0 4.4	3.8±0.4	CL = -1.562+0.235TL	0.811

Table 2. Length-weight relationship and condition factor (K_F) of *Channa stewartia*.

Sex	n	Length (cm)		Weight (g)		a	b	r^2	K_F	p-value
		Range	Mean±SD	Range	Mean±SD					
Male	51	20.5–26.0	22.8±1.6	20.5–26.0	22.8±1.6	0.003	3.389	0.885	1.005	$p<0.05$
Female	68	20.5–26.0	23.0±1.5	20.5–26.0	23.0±1.5	0.003	3.396	0.876	1.004	$p<0.05$

Note: n = sample size; SD = standard deviation; a = constant; b = exponent of the regression curve; r^2 = coefficient of determination; K_F = condition factor

In the current study, male and female scores are both greater than three, indicating good allometric growth. According to Saikia *et al*. (2011), the Assam *C. punctatus* developed allometrically. Higher 'b' values are associated with better feeding performance (Saikia *et al*., 2011). A similar higher correlation coefficient of Length and weight ‘r’ and high degree of exponential growth ‘b’ was observed in *Channa aurantimaculata* (Bhatta and Goswami, 2014). Positive allometric growth is observed due to the period of food availability and intensity of food consumed because of the favorable condition. As a result, it reflects on the Length-Weight relationship.

Both sexes were in good health in their natural environment, as shown by the estimated values of "K_F" for males and females being 1.005 and 1.004, respectively, which are greater than the value of 1 (Table 2). The Physical and biological circumstances of a fish can be known by the condition factor (K) of a fish. The changes obtained in condition factor are due to different feeding conditions, physiological factors and different interactions among other fishes (Le Cren, 1951). K-factor is an important metric for measuring fish feeding intensity and growth rate in general (Oni *et al*., 1983). By predicting interactions between biotic and abiotic components, the condition factor serves as a parameter to evaluate a fish's environmental adaptation (Froese, 2006). If the factor value is more than one (k>1), the fish is healthy and should preferably be in its natural habitat. K values exceeding one for both males and females indicate that the fish are in good health condition. High K-values for both species indicates that the condition factor grew as fish length and weight increased (Yousuf and Khurshid, 2008). Difference in the length-weight relationship implies the effects by geographical and environmental conditions (Damchoo *et al*., 2021).

CONCLUSION

According to the study mentioned above, the ‘b’ values for both sexes (male = 3.389 and female = 3.396) show a positive allometric growth pattern. The condition factors of both sexes are greater than one, indicating high health and habitat compatibility. These facts are essential for understanding the characteristics, breeding seasons, habitat suitability, feeding patterns, and native habitat of the species. They may be beneficial for management and conservation initiatives. The evolutionary analysis revealed that the new sequence obtained from the present study, which have been assigned GenBank accession numbers OR056418, OR618338, OR 618339, and OR618340, form a clade with GenBank Sequences of *Channa stewartii* with bootstrap support of 99%.

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